Flinders University

Faculty of Science and Engineering

School of the Environment

# EXPLOITATION OF WASTEWATER GROWN MICROALGAE FOR THE PRODUCTION OF BIOGAS

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**"This thesis is presented as part of the requirements for the**

**award of the degree of Doctor of Philosophy**

**of the**

**Flinders University"**

**June 2016**

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# List of Acronyms, Symbols and Abbreviations

| | |
|---|---|
| % | Percentage |
| µ | Growth rate |
| A | ALBAZOD |
| ACCase | Acetyl CoA Carboxylase |
| AFDW | Ash free dry weight |
| AFOSR | Air Force Office of Scientific Research |
| ALBAZOD | Algae, Bacteria, Zooplankton, and Detritus |
| AOC | Algal organic carbon |
| AOM | Algogenic organic matter |
| ARPA-E | Advanced Research Projects Agency-Energy |
| ARPS | Algal Raceway Production System |
| ASP | Aquatic Species Programme |
| BOC | Bacterial organic carbon |
| $BOD_5$ | Biochemical oxygen demand, 5 days |
| C | Carbon |
| ℃ | Temperature Celsius |
| C/N | Carbon nitrogen ratio |
| CA | Carbon anhydrase |
| $CaCO_3$ | Calcium carbonate |
| CAL | Covered anaerobic lagoons |
| CCM | Carbon concentrating mechanism |
| $CH_4$ | Methane |
| Chla | Chlorophyll a |

| | |
|---|---|
| $CO_2$ | Carbon dioxide |
| $CO_3^{2-}$ | Carbonate |
| COD | Chemical oxygen demand |
| CWMS | Community Waste Management System |
| DAF | Dissolved air floatation |
| DM | Dry matter |
| DOC | Dissolved organic carbon |
| DOE | Department of Energy, U.S |
| DRP | Dissolved reactive phosphorous |
| FAS | Ferrous ammonium sulphate |
| FTS | Fischer-Tropsch Synthesis |
| FVW | Fruit, vegetable wastes |
| GFC | Glass fibre filter |
| GHG | Greenhouse gas |
| $HCO_3^-$ | Bicarbonate |
| HRAP | High Rate Algal Pond |
| HRP | High Rate Pond |
| HRT | Hydraulic retention times |
| HSCW | Hot standard carcase weight |
| I | Light Intensity |
| IC | Inorganic carbon |
| KoM | Kingston-on-Murray |
| L | Litre |
| L/D | Light and Dark |
| LCA | Life cycle assessment |

| | |
|---|---|
| MITI | Ministry of International Trade and Industry |
| MSW | Municipal solid waste |
| mtoe | Million tonnes of oil equivalent |
| N | Nitrogen |
| $NaHCO_3$ | Sodium bicarbonate |
| NEW | Nitrified wastewater effluent |
| $NH_3$-N | Nitrogen Ammonia |
| $NH_4$-N | Nitrogen Ammonium |
| $NO_2$-N | Nitrogen Nitrite |
| $NO_3$-N | Nitrogen Nitrate |
| NREL | National Renewable Energy Laboratory |
| OLR | Organic loading rate |
| OMW | Organic municipal waste |
| OTF | Outdoor Test Facility |
| P | Phosphorus |
| PE | Population equivalents |
| PFD | Photon flux density |
| pH | Power of hydrogen |
| PL | Person load |
| $PO_4$-P | Orthophosphate phosphorus |
| POC | Particulate organic carbon |
| PON | Particulate organic nitrogen |
| PorkCRC | The Cooperative Research Centre for High Integrity Australian Pork |
| PS | Pig slurry |
| Quad | Quadrillion |

| | |
|---|---|
| R&D | Research and development |
| $R^2$ | Correlation coefficients |
| S | Nutrients |
| SBIR | Small Business Innovative Research |
| SCOD | Soluble chemical oxygen demand |
| SD | Standard deviation |
| Si | Silicon |
| SPU | Standard pig unit |
| SS | Suspended solid |
| T | Temperature |
| TAN | Total ammonia nitrogen |
| TC | Total carbon |
| TCOD | Total chemical oxygen demand |
| THRT | Theoretical hydraulic retention time |
| TN | Total Nitrogen |
| TOC | Total organic carbon |
| TS | Total solid |
| TSS | Total suspended solid |
| UGPase | UDP Glucose pyrophosphorylase |
| UV | Ultraviolet |
| VFA | Volatile fatty acid |
| VS | Volatile solid |
| VSR | Volatile solids reduction |
| VSS | Volatile suspended solid |
| WAS | Waste activated sludge |

| | |
|---|---|
| WWTP | Wastewater Treatment Plant |

# Summary

There is a current need to question if $CO_2$ addition enhances algal production in all types of wastewater, due to the conflicting views exist within the literature, and limited comparative investigations have been undertaken. In addition, while other wastes are commonly co-digested e.g. industrial organic wastes, fruit and vegetable solid waste, olive wastes and farm wastes, there are limited studies on the digestion of algal biomass either as a sole substrate or co-digested with other wastes, significantly for this proposal, only limited studies are considered co-digestion with pig slurry.

In this current research, a laboratory approach was utilised to examine the effect of the addition of $CO_2$ on the growth of microalgae in wastewaters of three different $BOD_5$ strengths. Somewhat uniquely in this area of wastewater research and algal biomass production (ALBAZOD) a comparison was also made, between the outcomes for biomass production and treatment, of pH stasis using acid rather than $CO_2$.

Results of the research demonstrated that the addition of $CO_2$ did not increase biomass production since the native organic carbon pool, following bacterial mineralisation, within both wastewaters was sufficient to support optimal biomass production under the prevailing conditions of light and temperature. The corresponding statistically significance also suggested that the maintenance of pH stasis in the absence of carbon addition implies that the forcing of the carbonate bicarbonate equilibrium in favour of free $CO_2$ was of more likely importance to productivity than external carbon addition. The differential response of wastewaters to $CO_2$ addition, in terms of biomass production, reported here suggests that careful consideration is required before investing capital in infrastructure to support $CO_2$ addition to large scale systems. The results suggest that wastewaters with low $BOD_5$ content or a low available organic carbon pool or which have been extensively pretreated resulting in a recalcitrant organic carbon pool resistant to mineralisation are most *likely* to respond positively to $CO_2$ addition. In contrast, wastewaters which have not been extensively treated and which contain a large, readily mineralisable organic carbon pool are *unlikely* to respond positively to $CO_2$ addition.

Co-digestion of pig slurry and ALBAZOD resulted in a slightly higher methane yield under psychrophilic temperatures than pig slurry alone, however, the increase was not significantly different statistically and the results also suggested that the ratio should be carefully considered as the biodegradability of ALBAZOD was lower than the biodegradability of pig slurry. One of the challenges of this research was the low VS loading rate in low concentration of microalgae biomass present in large volume of water sample. However, this was considered a typical ALBAZOD substrate obtained following dissolved air flotation; a common and relatively low cost separation technology suitable for on-farm operation that is without the adoption of high energy – high capital cost concentrating systems such as centrifugation. Similar observations were also recorded when co-digested with waste activated sludge. It was observed that a much longer solid retention time was required for solo ALBAZOD anaerobic digestion. It was concluded overall conclusion, that the low biodegradability of algae cell wall which caused the extended period of digestion.

The research presented here provides a better understanding of how to achieve integration of algae and wastewater treatment by determining, whether it is necessary to supply external $CO_2$, and evaluating the outcome of anaerobic co-digestion of algal biomass with either pig slurry or waste activated sludge.

# Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Ngai Ning Cheng

# Acknowledgements

This journey has been really interesting, yet, without all the help I received among these people, this thesis would not be possible without your significant assistance. Here I would like to give my sincere thanks to all of you.

First and foremost, my sincere thanks goes to my principle supervisor Professor Howard Fallowfield. Howard, you have been an incredible supervisor and mentor. The way of your teaching is very unique, you have the ability to stimulate great ideas and great vibes, and I am deeply motivated by that. Knowing my parents and siblings are thousand miles away, you always invite me over for a Christmas lunch to make me feel like home. That 2 kg palm sugar salmon fish, I don't think that's something I will ever forget. I would also like to thank you for your patient and thank you for the opportunities you've given me. I particularly like the seminar you gave on how your career was developed. You mentioned about the term "following your internal compass" is definitely something I want to be true and be well principled to it. Through this opportunity, I would also like to thank you Professor Adrian Werner for your advices, time, and assistance.

Dr. Neil Buchanan - Thank you so much for your help and the opportunities you've given me, from the bottom of my heart. Your passion and commitment to science never cease to amaze me. Those character building moments or finding you were doing chlorophyll a at 11:30pm after a long trip to collect the samples or over the weekends, it makes me want to push myself harder.

Pork CRC – Thank you very much for the financial support for this project and very well organised workshops and conferences. Also, thank you Dr. Roger Campbell for this excellent opportunity. I got to learn a new range of challenges across the Australia pig industry, researches, networks, and new friends. I would also like to thank you, in no particular order, Alice, Alicia, Robyn, Jeremy, Fan, Belinda, Tanya, Rousset, Rebecca A, Diana, Lechelle, and Even. You guys made the workshop experience amazing.

Roseworthy Piggery – Andrew and Simon, thank you very much for your time and assistance in collecting the pig slurry samples.

Mount Barker CWMS – Mark Millington, Madhawa Rupasinghe, Ashley Coombe, and Arthur Woolcock, thank you very much for your assistance in collecting the wastewater and sludge. I also learned a lot about how things work in a wastewater treatment plant.

SAWater Bolivar WWTP – Dr. Ben van den Akker, thank you very much for your assistance in collecting the waste activated sludge.

Flinders University School of the Environment – Thank you very much for all your help! In no particular order, Nancy, Richard, John, Kirstin, Nick, Raj, Guaxin, Neil, Mary, Jess, Natalie, Harriet, Michael, Tara, Amy, Megan, Mira, Paul, Lida, Farah, Emmanuel, Sarah, Catherine and in particularly, Patrick! Patrick, thank you very much for your friendship and encouragement. You made the tough time goes a little bit easier and I am sure our paths will cross again!

I would also like to thank you the amazing support from my parents, brother, and sister. Thank you so much for being patient, understanding, and letting me experience this long journey. Without your support, definitely I won't be able to do what I love to do.

Last but not least, I would like to thank you our departments' coffee machine, my pair of running shoes, and distance-running in general. I found my first ever full Marathon running experience was in a way that was very similar, if not almost identical, to this journey. And it can be corresponded to six stages:

The starting line

Whenever we enter a new chapter of life, new challenge, or new experience, we're always excited on how this journey will change us for better and can't wait to see how it will be unfolded. You try to tell yourself: pace yourself, be patient, don't burn yourself out in the first few kms. But you can't help, the adrenaline is skyrocketing.

10 km

We should celebrate right? That is a huge accomplishment but now it feels like nothing. You remind yourself how far you've come, at the same time, you just realise you still have 32.2km to go. Never mind.

Half marathon point (21.1 km)

Constantly watching your watch is distracting and stresses you out. You tell yourself to make sure you are being well looked after, stopping at the water stations definitely helps and thanks for the remarkable spectators who woke up as early as you to cheer for the runners.

30km

You realise you have already used the "glass half-full" strategy. Your maths tells you that you just have another 10 km to go and I've done that so many times, no worries. The very next second you realise, it is 12.2 km to be exact. You start to dig up you old memories to distract and entertain yourself.

35km

Also known as "the wall". You don't care about how you look anymore. You have crystallised salt on your face and runny nose. Maybe you will just walk the last few kms and no one will find out? Too late now, you realise that you shouldn't have posted your bib number so everyone can track your progress. There is no going-back now, you just want to finish it.

42.2km

The finish line is finally in sight and waves of adrenalines are coming back in. You are fully present in here and now. You turn around to look back and realise, not everyone who started with you, are necessarily going to finish with you anymore. But that's ok. You raise both of your arms, try to look good as much as possible in front of the cameras. You cross the line, pick up your hard-earned banana and a slice of watermelon. At the 30km mark, you told yourself you will never do this again. On the same night, within 24 hours, you already signed up for another marathon in the coming months.

I hope when I look back in times, I will cherish every single moment of it.


RUN ADELAIDE
FLINDERS TEAM
1006
1006 Attach this tag to your clothing bag 1006
5420
TRIATHLONS
170
5092
COPPER COAST
162
5266
JACOB'S CREEK
2356
NGAI
21KM RUN
asics
1169
20TH ANNIVERSARY
5079
5557
Hills To Henley
60
RYAN
1137
330
MSS
2144
RYAN
9810
441
5269
SPORTS
448
asics
VICTOR HARBOR
1574
TRIATHLONS
2736
321
Ryan
BAY-CITY
1652
5496
0321
42.2
CLELAND
459
3174
0336
1187
2319
asics
VICTOR HARBOR
446
TRIATHLONS

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# CHAPTER 1

# GENERAL INTRODUCTION

# 1. GENERAL INTRODUCTION

## 1.1. The history of renewable biofuels research

In 2008, the primary annual energy consumption worldwide was estimated at 11,295 million tonnes of oil equivalent (mtoe) (Petroleum, 2009), which is about two thousand times more total energy use than a thousand years ago. Furthermore, with nearly seven billion people in the 21$^{st}$ century world, compared to roughly 300 million in the 19$^{th}$ century, the current worldwide energy consumption is even more intensive (OECD, 2008) and (USCB, 2009). Fossil fuels are our primary energy supply which accounted for 88% of the primary energy consumption, comprising oil (35% ), coal (29%) and natural gas (24%), with nuclear energy and hydroelectricity accounting for 5% and 6% respectively of the total primary energy consumption (Brennan & Owende, 2010). The current technological progress, potential reserves and increased exploitation leads to energy insecurity and climate change by increasing greenhouse gas (GHG) due to high fossil fuels usage (Singh et al., 2011). Although there is still a plentiful supply of fossil fuels at reasonably low cost, a rising use of fossil fuels is unlikely to be sustainable in the longer term due to the increase in anthropogenic GHG emissions and depleting limited fossil reserves (Pittman et al., 2011). Due to the large scale use and reliance on unsustainable fossil fuels for transportation, electricity and thermal energy generation, there is therefore significant interest in identifying alternative renewable energy sources which are capable of sequestering the atmospheric $CO_2$, to reduce the dependency on fossil reserves and also to maintain environmental and economic sustainability (Demirbas, 2009; Hill et al., 2006; Pittman et al., 2011; Prasad et al., 2007a; Prasad et al., 2007b; Rittmann, 2008; Singh et al., 2011; Singh et al., 2010a; Singh et al., 2010b).

Extensive research was undertaken to seek alternative energy sources following the oil crisis of the 1970s. However, most research and development efforts were unable to continue due to the oil prices remaining competitively low in mid-1990s. In recent years, the high worldwide energy demand has once again motivated scientists and technologists to search for various alternate sources of energy. Biofuels are an

attractive alternative to current petroleum based fuels because they can be easily utilized as transportation fuels with minor modification and have significant potential to improve sustainability and reduce GHG emissions (Singh et al., 2011). The majority of the current commercially available biofuels are bioethanol derived from sugar cane or corn starch or biodiesel derived from oil crops including soybean and oilseed rape, which are our first generation biofuels (Pittman et al., 2011).

### 1.1.1. First and second generation biofuels

First generation biofuels which have now attained economic levels of production, have been mainly extracted from food and oil crops including rapeseed oil, sugarcane, sugar beet, and maize (Nigam & Singh, 2011) as well as vegetable oils and animal fats using conventional technology (Brennan & Owende, 2010). Although first generation liquid biofuels production and consumption growth is increasing rapidly, their impacts towards meeting the overall energy demands in the transport sector will remain limited and unsustainable due to competition with food and fibre production for the use of arable land, regionally constrained market structures, lack of well managed agricultural practices in emerging economies, high cost of water and fertilizer equipment, and a need for conservation of bio-diversity (Brennan & Owende, 2010; IEA, 2007). The sustainability of many first generation biofuels has been increasingly questioned over concerns regarding their potential to replace fossil fuels and sustainability of their production. For examples, the high risk on food prices and security, the demand for biofuels could place substantial additional pressure on the natural resource base, and effects on the environment and climate change (Brennan & Owende, 2010; IEA, 2007; Singh et al., 2011). There is a growing concern that biofuel technologies must become more efficient in terms of net lifecycle GHG emission reduction while at the same time be socially and environmentally sustainable (Eisentraut, 2010; Singh et al., 2011). In 2010, about 1% (14million hectares) of the world's available arable land is used for the production of biofuels, providing 1% of global transport fuels (Brennan & Owende, 2010). In terms of providing 100% of world energy supply it is clearly impractical owing to the severe impact on the world's food supply and the large production land areas required (IEA, 2006).

The increasing criticism of the sustainability of many first generation biofuels has raised attention to the potential of second generation biofuels. The advent of second generation biofuels is intended to produce alternative fuels from the whole plant matter of dedicated energy crops or agricultural residues, forest harvesting residues or wood processing waste, rather than from food crops (Moore, 2008). Depending on the selection of feedstock and cultivation technique, second generation biofuels have great potential to provide additional advantages in consuming waste residues and making use of abandoned land, promoting rural development and improving economic conditions in emerging and developing regions (Singh et al., 2011). However, the technology for conversion in the most part has not been able to satisfy the necessary scale for commercial exploitation. This has limited any significant exploitation, since their sustainability is still dependant on whether producers comply with criteria like minimum lifecycle GHG reductions, land use change and social standards (Brennan & Owende, 2010; Eisentraut, 2010).

### 1.1.2. Major obstacles of first and second biofuel: competition for land and freshwater

On-going deforestation from first generation biofuel is a significant concern in many countries, particularly with the growth in palm oil plantations in south-east Asia. The continued development of first generation biofuel derived from starch or sugar crops might eventually lead to accelerated net deforestation as more land is converted to agriculture. Furthermore, the increase use of fresh water for irrigating energy crops is also another significant concern. The increasing use of freshwater for irrigating food crop production is already an arising challenge and therefore its usage for energy cropping may be unacceptable (Sims et al., 2008).

The ideal conditions for a technically and economically viable next generation biofuel should be: competitive or cost less than petroleum fuels; require low to no additional land use; enable air quality improvement by reducing GHG emissions such as $CO_2$ sequestration, and; require minimal water use (Brennan & Owende, 2010; Wang et al.,

2008). In recent years, many biofuel researches have been focused on examining the possibilities of using algae as a source of bio-oil and biogas for energy applications because microalgae could easily meet these conditions and therefore make a significant contribution to meeting the primary energy demand, while simultaneously providing environmental benefits such as $CO_2$ capture (Wang et al., 2008). There is also a growing interest in investigating marine and brackish water for algal biofuel cultivations which eliminates the need to use scarce freshwater.

## 1.2. Third generation biofuels: A history of algal biofuels

The purpose of this section is to present a comprehensive summary of development and evolution of many key concepts and research on algal biofuels. This will also highlight the feasibility and techno-economic challenges associated with commercial scaling up of processes.

### 1.2.1. The early years (1940s and 1950s)

Back in the 1940s, it was the time of discovery that many species of microalgae can produce large amounts of lipids as cellular oil droplets under certain growth conditions. In 1942, Harder and Von Witsch were the very first to propose the microalgae might be a suitable source of lipids which could then turn into food or fuels (Harder & von Witsch, 1942a; Harder & von Witsch, 1942b). In fact, the original idea of microalgae research was not biofuel production based because the need for liquid fuel alternatives was no longer a problem after World War II, despite the well-known capability of accumulating very high levels of lipids but with a very low actual lipid productivity. Therefore, the major application of microalgae was the potential protein and food source (Geoghegan, 1953; Spoehr & Milner, 1948; Spoehr & Milner, 1949). After World War II, large scale of algal research began to take place in the U.S., Germany, Japan, England and Israel on culturing techniques and engineering systems for growing microalgae.

In 1948-1950s, Cook (1950) and Burlew (1953a, b) were the very first working on large-scale culture and the engineering requirements for algae production systems at the Stanford Research Institute, USA (Burlew, 1953; Burlew, 1953a; Cook, 1950). In 1951, Milner (1951) considered the possibility of photosynthetic production of oils using algae (Milner, 1951). In 1952, the study of Aach (1952) indicated that *Chlorella pyrenoidosa* could accumulate up 70% dry weight as lipids (mainly neutral lipids) in stationary phase when nitrogen was limited. An internally-lit photobioreactor was first introduced to estimate of photosynthetic efficiency (Aach, 1952). In 1953 in Esseen, Germany, Gummert et al. (1953) studied the possibility of $CO_2$ utilization of waste gases from industry (Gummert et al., 1953). Mituya et al. (1953) also conduted similar research on circular algae ponds at the Japanese Microalgae Research Institute at Kunitachi-machi, Tokyo (Krauss, 1962; Mituya et al., 1953). In the same year, smaller scale studies were also carried out by Imperial Chemical Industries Ltd in England by Geoghegan (Geoghegan, 1953) and in Israel (Evenari et al., 1953). It is important to note that all these studies were based on strains of *Chlorella*.

In 1951, Anon was the first to develop significant outdoor pilot plant studies on the production of *Chlorella* at Arthur D. Little Inc. in Cambridge, Massachusetts, USA. Two types of closed tube reactors were used on the roof of the building which is now known as a closed photobioreactor. Both of the systems were provided with filtered air enriched with 5% $CO_2$ and pH was maintained at about pH 6 by the periodic addition of dilute nitric acid (Anon, 1953). At about the same time, Gummert et al. (1953) in Germany compared large-scale culture of *Chlorella pyrenoidosa* in 100 and 200L tanks (15-21 cm deep) in a glasshouse with plastic lined, inclined trenches (9 m long, 70 cm wide, 20-24 cm deep at low ends). The tanks and the trenches were aerated with 1 % $CO_2$ in air. The common issues of these pilot plants were contamination of cultures with other algae, protozoa, and greatly influenced by climatic conditions (Gummert et al., 1953).

In 1953, Oswald et al. described the oxygen-supplying role algal photosynthesis plays in sewage oxidation ponds (Oswald et al., 1953b). Laboratory and pilot-plant investigations of sewage treatment in open ponds by photosnythetically produced

oxygen have been well conducted during the years between 1951 and 1955 (Caldwell, 1946; Ludwig et al., 1951; Oswald et al., 1953a; Van Heuvelen & Svore, 1954). These studies have provided the fundamental principles which can later be utilised for the engineering design of the process as well as for the prediction of the operational performance of new or existing oxidation ponds. This ultimately led to another new application of microalgae which is the use of microalgae in wastewater treatment proposed by Oswald and Gotaas in 1957 (Oswald & Gotaas, 1957).

In 1955, Sasa et al. were the first to perform a detailed study of the seasonal variation in algae productivity over a whole 12 months period using a range of strains with different temperature tolerance and under natural light condition (Sasa et al., 1955). The study demonstrated the growth rate of *Chlorella ellipsoidea* agreed with the actual yield obtained from the open outdoor cultures in each season. Among other scientists in Japan, Sasa et al. (1955), Morimura et al. (1955), and Kanizawa et al. (1958) were the very first groups moving from the laboratory towards eventual commercial microalgae production and identified most of the key issues which commercial-scale microalgae production systems are still facing today (Kanazawa et al., 1958; Morimura et al., 1955; Sasa et al., 1955).

In the late 1950s, Meier (1955), Golueke et al. (1957), and Oswald and Golueke (1960) suggested the utilization of carbohydrate fraction of algae cells for the production of methane gas via anaerobic digestion (Golueke et al., 1957; Meier, 1955; Oswald & Golueke, 1960). Until then, only little work has been done on microalgal biomass fermentation rather than methane production from seaweed (Chen, 1987; Matsunaga & Izumida, 1984; Uziel, 1978). Recently, the topic of microalgal biomass anaerobic digestion has received increasing attention again.

These various studies during the 1940s and 1950s have indicated large-scale culture experiments, understanding of microalgae light capture and photosynthesis, culture manipulation using starvation of key nutrients, the concept of utilization the lipid stores as a source of energy, and anaerobic digestion of microalgae.

### 1.2.2. 1960s to 1996

In the mid-1950s, the initial phase of work on microalgae mass culture in the USA had largely ceased. In the early 1960s, William (Bill) Oswald and colleagues at the University of California, Berkeley who focused on the large-scale culture of algae for biomass production and for wastewater treatment continued in this research area (Golueke et al., 1957; Oswald & Golueke, 1960). A 2,700 $m^2$ (approximately $10^6$ L capacity) meandering pond was constructed at Richmond, California and the research eventually led to the construction of large-scale wastewater treatment ponds at several locations in California, which are still in operation to date (Oswald, 1969a; Oswald, 1969b; Oswald, 1988). In 1971, John H. Ryther and colleagues at Woods Hole Oceanographic Institution, Massachusetts, USA, started to work on the marine counterpart of Oswald's work with two small 4 $m^2$ (2,000 L) circular ponds (Goldman & Stanley, 1974) and culminating in outdoor experiments with six 150 $m^2$ (35,000 L) ponds which were mixed by small pumps (D'Elia et al., 1977; Goldman, 1979a; Goldman & Ryther, 1976). These studies represent the foundation understanding of nutrient requirements of the algae and limitation to growth, the effects of temperature and species succession in open ponds. In the 1960s, the commercial production of microalgae mainly for use as nutritional supplements and nutraceuticals began to develop across the globe (Borowitzka, 2013a).

From the beginning of 1960s, Oswald and Golueke (1960) had suggested the potential of algae as sources of energy by microalgae biomass fermentation to produce methane. Towards the end of 1970s, Oswald and Benemann (1977) and Benemann et al. (1977, 1978) summarised a critical assessment on the possibility of using algae for energy. With all these fundamental researches and proposals on algal biofuels on top of the oil embargo and oil price surges in the 1970s, this eventually led to the U.S. Department of Energy to initiate the Aquatic Species Programme (ASP) in 1978. This programme spent $25 million over 18 years (1978-1996) with initiative aim to develop algae as sources of liquid transportation fuel that would be price competitive with petroleum-

derived fuels (Sheehan et al., 1998). The most significant findings among the program were that rapid growth and high lipid production were “mutually exclusive” because the former required high nutrients and the latter required low nutrients (Sheehan et al., 1998). The major findings of the ASP will be discussed in Section 1.3 (below). The program successfully demonstrated that large-scale production of algae for fuel in outdoor ponds was feasible, however, the program failed to demonstrate the competitiveness with petroleum since oil prices sank in the 1990s. Sheehan et al. (1998a) estimated that unextracted algal oil would cost $59-186 per barrel while petroleum cost less than $20 per barrel in 1995. Therefore, the ASP program was abandoned under the budget pressure in 1996 (Sheehan et al., 1998). Figure 1.1 shows the chronology trend of ASP programme over the 18 years.

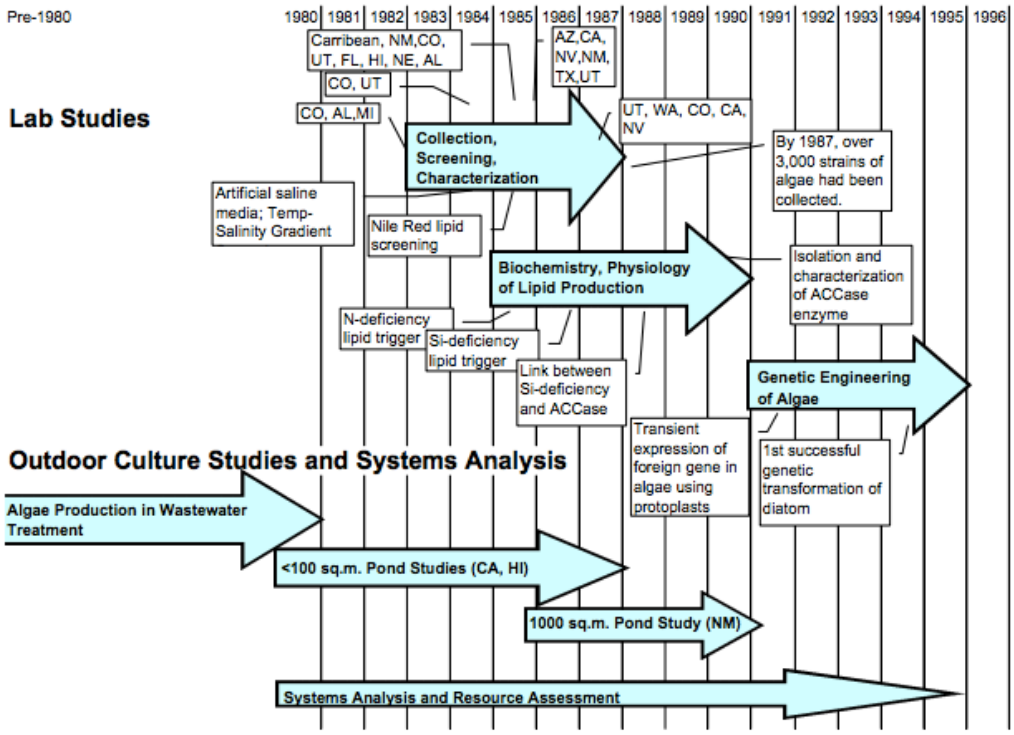


Fig. 1.1 Chronology trend of the Aquatic Species Program from 1978 to 1996 (Sheehan et al., 1998).

### 1.2.3. 1996 till current

After the ASP in 1996, the majority of U.S. federal funding for algal research has come from Department of Energy, the Department of Defense, the National Science Foundation, and the Department of Agriculture (DOE, 2010). It has also been

suggested that funding levels are beginning to increase with the recent initiatives such as a major Defense Advanced Research Projects Agency solicitation, the Air Force Office of Scientific Research (AFOSR) algal bio-jet program, and several DOE small business Innovative Research (SBIR) request for proposals. In addition to U.S. National Algal Biofuels Technology Roadmap (2010), DOE's Advanced Research Projects Agency-Energy (ARPA-E), Office of Science, Office of Fossil Energy, and Biomass Program are all funding research activities that include investigating microalgae, cyanobacteria, and macroalgae for biofuels and beneficial re-use of $CO_2$. From the roadmap report, it also points out a number of U.S. national labs are increasingly focusing on algal biofuels research. State funding programs and research support from private industry also make up a significant proportion of research funding. These trends represented private investment in algal biofuels has been increasing at a dramatic rate over the last years and significantly outpacing government funding (DOE, 2010).

### 1.3. Current algal biofuel opportunity and challenges

Abundant, affordable, and sustainable feedstocks are the key elements to be considered for a successful biofuel industry today. To develop a viable and sustainable algal biofuel industry, these elements should be investigated through R&D so it can be commercialized. In 1998, Sheehan et al. summarised the main findings of the ASP and a number of recommendations for future research (Sheehan et al., 1998). The major findings are listed below:

1. *Laboratory studies – collection, screening and characterization of algae*
   More than 3,000 strains of microalgae were collected over a seven-year period (1980 -1987) from various sites in the western, northwestern, and southeastern U.S. representing a diversity of aquatic environments and water types. After screening, isolation and characterization efforts, the collection was eventually narrowed down to around 300 most promising strains, primarily green algae (*Chlorophyceae*) and diatoms (*Bacillariophyceae*). These isolates were screened for their tolerance to variations in salinity, pH, and temperature, and also their ability to produce neutral lipids (DOE, 2010). In the last years of the

collection, the focus switched to finding algae that were tolerant to low temperature (Sheehan et al., 1998).

The challenge here is the ideal microalgal species candidate for each outdoor biofuel production facility will likely be different for each location at different seasons and unlikely to meet all of the needs of the technology. Therefore more than one strain will likely be used at a site in order to maximize the productivity at different times of the year. The logical approach will be to screen for a highly productive, oleaginous strains at a selected site, optimized growth conditions for large-scale culture, and optimize productivity and lipid production through genetic manipulation or biochemical manipulation of the timing of lipid accumulation in the selected strain (Sheehan et al., 1998). However, this also raised a practical concern of transferring from laboratory based studies to large scale systems.

The most significant observation here was that the conditions that promote high productivity and rapid growth (i.e. nutrient sufficiency such as nitrogen and phosphorus) and the conditions (e.g. light and temperature) that induce lipid accumulation (nutrient limitation) are mutually exclusive. This triggered the next movement of laboratory studies on the physiology and biochemistry of oil production in algae in hopes of learning how to improve the performance of existing organisms from 1985 to 1990 (Fig. 1.1).

2. *Laboratory studies - physiology and biochemistry of microalgae on nutrient deficiency and lipid production*

   Prior to the programme, limited work had been done to improve oil production in algal organisms (Sheehan et al., 1998). The focus quickly became on finding the elusive “lipid trigger”. It refers to the observation that deficiencies in nitrogen could lead to an increase in the level of oil present in many species of microalgae. However, this is a false observation due to the cessation of cell division. The accumulation of oil content in the microalgal cells is caused by the lower rate of production of all cell components under the nutrient starvation. Therefore, the oil production seems to remain higher. In addition, the increase oil content in microalgae cells does not lead to increase overall

productivity of oil. Higher levels of oil in the cells are more than offset by lower rates of cells growth. Therefore, it was concluded that overall rates of oil production are lower during period of nutrient deficiency. Both NREL researchers (National Renewable Energy Laboratory) and ASP program subcontractors concluded that no simple trigger for lipid production exists (Sheehan et al., 1998).

The depletion of silicon (Si), which is another environmental stress, has also been suggested as a way to increase oil levels in diatoms by NREL researches. Si is a component of the diatoms' cell walls. It was found that cell division slowed down when Si was used up. In addition, a study found in the diatom of *Cyclotella. cryptica*, the rate of oil production remained constant once Si depletion occurred while growth rate of the cells dropped. While the diatoms store carbon in either lipid form or in carbohydrate form, it was suggested that Si-depleted cells provide two factors: direct newly assimilated carbon towards more lipid production and less toward carbohydrate production; slowly convert non-lipid cell components to lipids, as shown in Fig. 1.2.

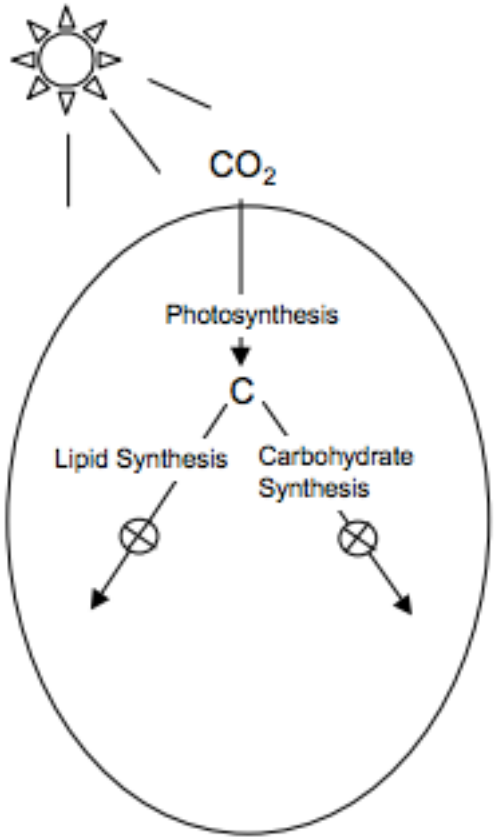


Fig. 1.2 The major two pathways of Si-depleted cells on utilisating carbon dioxide ($CO_2$) (Sheehan et al., 1998)

These two pathways consist of sequences of enzymes, each of which catalyses a specific reaction to direct carbon into different pathways. This ultimately drove the next direction of ASP programme which was the identification of key enzymes in fatty acid and carbohydrate (chrysolaminarin) pathways.

3. *Laboratory studies – molecular biology and genetic engineering*

In 1987 & 1988, Roessler was the first to isolate the enzyme Acetyl CoA Carboxylase (ACCase) from a diatom and also characterised UDP glucose pyrophosphorylase (UGPase) and chrysolaminarin synthase activities (Roessler, 1988; Roessler, 1987). By the end of the ASP program, both ACCase gene (Roessler & Ohlrogge, 1993) and UGPase gene (Jarvis & Roessler, 1999) were successfully cloned from *C. cryptica*. Researchers had also succeeded in developing the tools for expressing foreign genes in diatoms.

In the 1990s, there was a shift of focus in the area of genetic manipulation of algal strains to increase photosynthetic efficiency or to increase constitutive levels of lipid synthesis in algal strains. In 1998, Nedihardt et al. proposed the size of light harvesting antenna through mutation or genetic engineering can be reduced in order to maximized the photosynthetic productivity and light utilization in microalgae (Neidhardt et al., 1998). This approach has later shown possible by Melis et al. (1999) at the laboratory based studies (Melis et al., 1998).

4. *Transition to mass outdoor microalgal growth systems – photobioreactor and raceway pond*

   The ASP programme successfully demonstrated that some microalgal species could be cultivated reliably on a large scale. From 1980 to 1987, the program funded two parallel efforts to develop large scale microalgae culture systems. One effort was located at the University of Hawaii to investigate a patented "Algae Raceway Production System" (ARPS) with a configuration of 60 cm deep and sized 48 $m^2$ raceway with cover. Another effort was located at the University of California to investigate a "High Rate Pond" (HRP) system developed and based on a shallow & mixed raceway concept at UC Berkeley in 1963 with implementation of wastewater treatment operations (Sheehan et al., 1998). This design was selected for scale-up and an "Outdoor Test Facility" (OTF) was built at the site of an abandoned water treatment plant in Roswell, New Mexico. From 1988 to 1990, 1,000 square meter ponds were successfully operated at Roswell. This project demonstrated how to achieve very efficient (>90%) utilization of $CO_2$ in large ponds. The best results were obtained using native species of algae such as *Cyclotella, Monoraphidium, Amphora, Tetraselmis* that naturally took over in the ponds as opposed to previous focus on using laboratory cultures. Typical productivities from these two type systems were 15-25 $g/m^2$/day biomass over productive months. While Roswell's daily productivity did reach program target levels of 50 $g/m^2$/day, overall productivity was much lower at around 10 $g/m^2$/day due to low temperature (Sheehan et al., 1998).

These outdoor open pond studies indicated that there were no fundamental engineering and economic issues that would limit the technical feasibility of microalgae culture, either in terms of net energy input, nutrients such as $CO_2$ utilization, water requirements, harvesting technologies or general system designs (Sheehan et al., 1998). However, the main challenge here was the economic viability on running the systems. Although the productivities of the systems, in terms of total biomass and algal lipids (oils) achieved were high, they were still well below the theoretical potential and more importantly the requirements for economic viability (Borowitzka, 2013a).

5. *Sustainability concerns on commercial scale*

While the microalgal species studies looked very promising in the laboratory based conditions, they were not robust enough under conditions encountered in the field. There was a disconnection between the laboratory based studies and the field and the program has shown an important lesson that the outdoor testing of algae production systems is incapable of maintaining laboratory organisms in the field. The best approach suggested by the program was to successfully cultivate a consistent species of algae that would allow a contaminant native to the area to take over the ponds (Sheehan et al., 1998).

At 1982, the ASP program began to analyse the question of resource availability for algae technology. The major concerns were:

- Land area usage
- Freshwater usage
- Nutrients usage
- Urea (nitrogen) – linked with crude oil price
- Rock phosphorus – linked with crude oil price
- $CO_2$ supply costs and sources

In 1990, estimates of available $CO_2$ supplies in U.S. were examined and it was suggested that there was enough waste $CO_2$ available in the States where climate conditions were suitable to support 2 to 7 quads (quadrillion, $10^{15}$) of fuel production annually. The costs of supplying $CO_2$ was estimated to be between $9 to $90 per ton of $CO_2$ (Sheehan et al., 1998). The program also

pointed out the difficulties faced between access to land and water availability and consideration of available $CO_2$ supply sources.

## 1.4. Future directions on algae research: A shift interest in using wastewater as the algal growth medium and $CO_2$ biofixation

From the program, Sheehan et al. (1998) also stated that a more balanced approach was needed in which more near term opportunities could be used to launch the technology. The integration with wastewater treatment was the only plausible near- to mid-term application of microalgal biofuels production (Sheehan et al., 1998). This was due to the economic and resource constraints were much more relaxed and therefore it allowed for such processes to be considered with well below maximal productivities (Borowitzka, 2013a).

Prior to 1900, little works were carried out in this area until the introduction of Japanese RITE (Research Innovative Technologies of the Earth) Program for microalgae biofixation of $CO_2$, supported by MITI (Ministry of International Trade and Industry) from 1990 to 2000. This was a major R&D program with approximately US$250 million total funding in effort included the participation of over twenty private companies and several government research institutions, in parallel efforts to develop closed photobioreactor technologies for the production of high value products using power plant flue gas for $CO_2$ (Nakamura et al., 2001). However, this part of R&D efforts was not continued due to the unfavourable economic projections for such approaches.

## 1.5. Understanding of algal growth

### 1.5.1. Control factors of algal growth

In 1978, Goldman (1978) summarised two review papers on outdoor algal mass cultures in terms of their applications (part I) and photosynthetic yield limitations (part

II) (Goldman, 1979a; Goldman, 1979b). On part I of the review, it was shown that the common yield of algal mass culturing was 15-25 g dry wt $m^{-2}$ for 1-3 month periods and up to 30-40 g dry wt $m^{-2}$ for shorter periods. In part II of the review, Goldman (1978) further examined the important environmental parameters influencing algal growth rates (μ) such as light intensity (I), temperature (T), nutrients (S) and pH. The response to these parameters are distinctly species specific. These three parameters can be quantified by examining the shapes of the response curves to each of the parameters with one being a variable and the other two being constants as shown in Fig. 1.3.

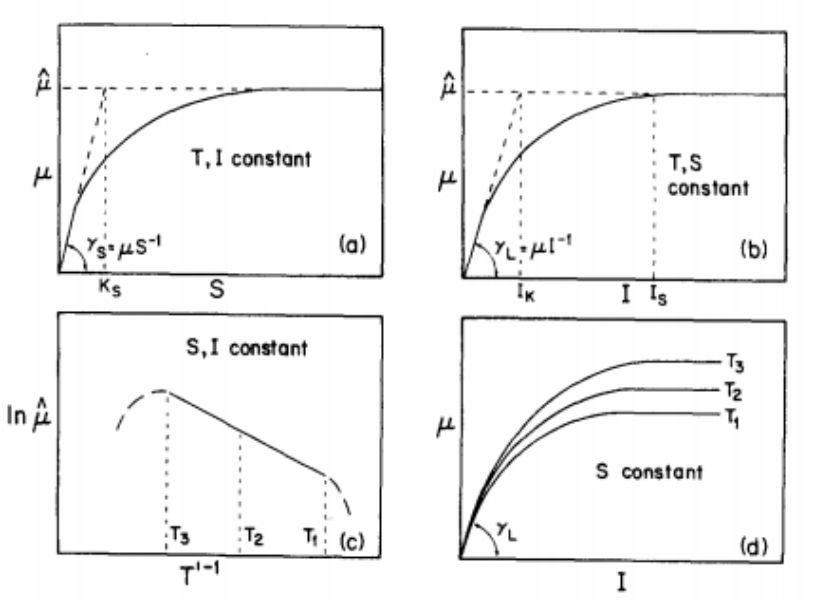


Fig. 1.3 General relationship between algal growth rates (μ) and environmental parameters (a.) limiting nutrient (S), (b.) light intensity (I), (c.) temperature (T) and (d.) light intensity for varying temperatures, adapted from (Goldman, 1979b)

### 1.5.2. Cultivation limiting factors on algal growth

#### 1.5.2.1. Sunlight and photo-inhibition

In mass cultivation of microalgae outdoor, the effective use and availability of light is one of the most important issues. This is due to the three main roles of light which it drives photosynthesis by algae, the production of oxygen and the pH (Lindström, 1984; Richmond, 2004; Smith, 1983). The production of oxygen inhibits algal growth in PBRS because it is involved in photorespiration and therefore reduction in biomass

production. However, in wastewater treatment, it is beneficial for providing disinfection function (EPA, 2002). Similarly for pH, high pH is unfavourable for algal cultivation because inorganic carbon equilibrium is shifted towards unavailable carbonate in defined media culture leading to $CO_2$ limitation. However, for wastewater treatment, high pH plays an important role in disinfection for water reuse.

The factors influencing availability of light include the function of time, seasonal variations, weather, time of day, latitude/longitude, and positon of a pond. The amount of light absorbed by an algal cell suspended in an algal cultivation system varies according to different factors including the specific position of the cell at a given instance, the density of the culture, and the pigmentation of the cells (Malone, 1982). Chlorophyll *a* is therefore often used as a key parameter in phytoplankton photophysiology and ecology, which represents an averaged optical absorption cross-section of an algal population (Marra & Heinemann, 1982). Light penetration is also an important function to facultative and maturation ponds which also known as light attenuation (Curtis et al., 1994). The intensity decays rapidly in both clean water and turbid water e.g. waste stabilisation ponds. The high productivity of algae influences the total light attenuation significantly on the surface, which often leads to growth limitation of the algae. This phenomenon is known as self-shading (Curtis et al., 1994).

During photosynthesis and growth, respiration and cell death occurs concurrently. There is a general agreement that dark respiration and photorespiration are mutually exclusive processes in algae (Stewart, 1974). At low light intensity, dark respiration is relatively more important than photorespiration. However, photorespiration increases and overshadows dark respiration with increasing light, oxygen concentration, and decreasing carbon dioxide concentration (Goldman, 1979b; Jackson & Volk, 1970; Zelitch, 1971). Fig 1.4 shows a common practice in trying to account for decay losses in algal growth models has been to assume a constant value for $k_d$. The point at which the combined effect of these influences equals the photosynthetic growth rate is commonly referred to as the compensation point, and this point varies widely with environmental conditions in outdoor cultures.

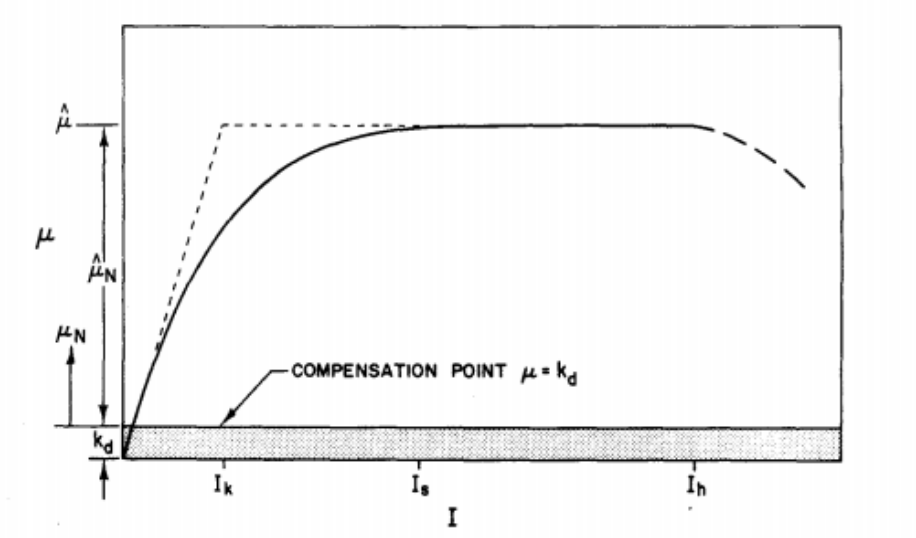


Fig. 1.4 Detailed general relationship between algal growth rate (μ) and light intensity (I) (Goldman, 1979b)

Fig. 1.4 also demonstrates there is a falling off in photosynthetic activity resulting from light inhibition ($I_h$). Ryther (1956) demonstrated that light inhibition for marine green algae, diatoms, and flagellates starts at ~0.07 cal $cm^{-2}$ $min^{-1}$, which is only ~10% of natural sunlight depending on the intensity, quality and duration of irradiance (Ryther, 1956).

Ratchford and Fallowfield (2003), showed that with both *Chlorella vulgaris* and *Synechoccus* the onset of photoinhibition occurred at irradiances >300 μmol/$m^2$ /s (65.7 W/$m^2$) at temperatures >15°C. They showed that oxygen evolution decreased rapidly when cells were continuously irradiated at 65.7, 109.5 & 164 W/$m^2$. However, *Chlorella vulgaris* irradiated at the same irradiances on a light:dark cycle of 60s:20s, 30s:60s, and 60s:120s respectively maintained a constant rate of oxygen evolution over a 24 hour incubation period suggesting that the adverse effects of photoinhibition could be ameliorated by periods in the dark. Exposure time rather than the total light dose appeared to determine the effect of light:dark cycle times on photosynthesis (Ratchford & Fallowfield, 2003). Mixing in HRAPs creates turbulent flow offering the potential of moving algae in and out of the 'light zone' and 'self-shaded zone' and therefore improving total algal productivity. However, these data point to the probability that photoinhibition is still an issue despite the potential for light dark cycling in the HRAP (Buchanan, 2015).

#### 1.5.2.2. Temperature

Microalgae are sensitive to environmental temperature and therefore an optimum growth temperature is required to provide an optimum biomass productivity. While most microalgae can easily tolerate a wide range of temperatures, exceeding the optimum by only 2 to 4$^{o}$C may result in the total loss of some microalgal culture (Borowitzka, 1998). The ranges are commonly divided into three categories which are psychrophilic, mesophilic, and thermophilic. Dependent upon the species, temperature variations can affect their nutritional requirements, metabolic rate and cell composition (Borowitzka, 1998).

For optimum algae productivity, temperature is often the second limiting factor besides light limitation for algal cultivation in open or closed systems. Considerations must be given to their annual variation in the temperature if the specific algal cultures are used for mass production over the whole year round. This also often involves a selection of species with a wide range of temperature tolerance in the designed location of the culture facilities or ponds. For example, Fan et al. (1994) and Moheimani & Borowitzka (2007) both stated that heating of the cultivation pond in the morning can increase the daily algal productivity by up to 20% (Fan et al., 1994; Moheimani & Borowitzka, 2007a). While temperature effects are well documented for many algal species in the laboratory, the effects on annual production of biomass outdoors is less documented thoroughly in the current literature.

#### 1.5.2.3. pH

When there is no additional $CO_2$ supplied, high-density algae production systems commonly reach up to pH 11 during the day under sufficient sunlight (Brewer & Goldman, 1976; Moheimani & Borowitzka, 2007a; Moheimani & Borowitzka, 2007b). This high pH level may cause a decrease productivity of most algae because most freshwater algae are markedly inhibited at > pH 8 as well as the limitation of $CO_2$ in this high pH level. Therefore, addition of $CO_2$ may be used to reduce the pH and make more $CO_2$ available for algal photosynthesis to achieve high biomass

productivity. Moheimani (2013) suggested that the pH can be adjusted using acid such as HCI for a short term solution to ensure that most of the inorganic carbon, as in the form of $CO_2$, is available in the pond (Moheimani, 2013). However, this method is relatively expensive and therefore it may not be a cost-effective method. For wastewater treatment, the pH values are also beneficial for pathogen removal despite of the reduction of algal productivity (Sampson et al., 2015).

The problem associated with ammonia toxicity at high pH will be discussed in the next section in nitrogen (N).

#### 1.5.2.4. Nutrients limitation – Nitrogen(N), Ammonia toxicity, Phosphorus (P), and Carbon (C)

Hill and Lincoln (1981) produced a mathematical model to describe the conditions for algal growth. In this model, algae were considered to require four substrates $PO_4$-P, $NH_4$-N, $CO_2$ and light; all of which could therefore limit growth. The substrates were amalgamated into a single simplified overall equation (Eq. 1.1) describing the final algal cell constituents in terms of the Redfield ratio of:

$C_{106}H_{180}O_{45}N_{16}P$ Equation 1.1

The three major components which are nitrogen (N), phosphorus (P), and carbon (C) will be discussed in this section, followed by the co-responding relationship of these elements and algal growth rate modelling will be discussed in the next section.

*Nitrogen (N)*

Nitrogen is a constituent of protein synthesis, an essential nutrient of all structural and functional protein in the algal cells and accounts for 7-20% of cell dry weight (Hu, 2004). Microalgae have a relatively high protein content ranged from 30 to 60% when compared to terrestrial plants (Becker, 1994). Therefore, nitrogen supply along with carbon supply for microalgae cultivation systems is one of the main nutrient expenses as well as an indirect energy input (Borowitzka, 2013b).

Nitrogen can not only be utilized as in inorganic form of $NO_3^-$, $NO_2^-$ or $NH_4^+$ and in some cases as $N_2$, but also in organic form like urea or amino acids (Flores & Herrero, 2005; Markou et al., 2014; Perez-Garcia et al., 2011). The process of uptake of nitrate is light energy dependent and therefore cyanobacteria prefers to use reduced nitrogen in the forms of ammonium or urea that are toxic at high concentrations (Converti et al., 2006). The excess of ammonium can lead to limited uptake of nitrate because ammonium represses the synthesis of nitrate reductase, while high nitrate concentration inhibits ammonia uptake (Darley, 1982; Meeks et al., 1983; Ohmori et al., 1977). One of the main factors affecting the toxicity is the pH of cultivation medium, which determines whether the toxic form of free ammonia is dominant or the no-toxic ammonium ion (Markou et al., 2014). When ammonia is used as the sole nitrogen source, the pH will drop due to the release of $H^+$ ions (Grobbelaar, 2004a).

*Ammonia toxicity*

The form in which ammonium nitrogen is present in a solution is pH and temperature dependent. According to Fig. 1.5, in pH values higher than 9.25 free ammonia begins to dominate over ammonium as shown here (Eq. 1.2):

$$NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O$$

Where, $pK_a = 9.25$ (25℃) Equation 1.2

Also, high temperatures favour the formation of free ammonia and it is generally toxic to photosynthetic organisms (Abeliovich & Azov, 1976).

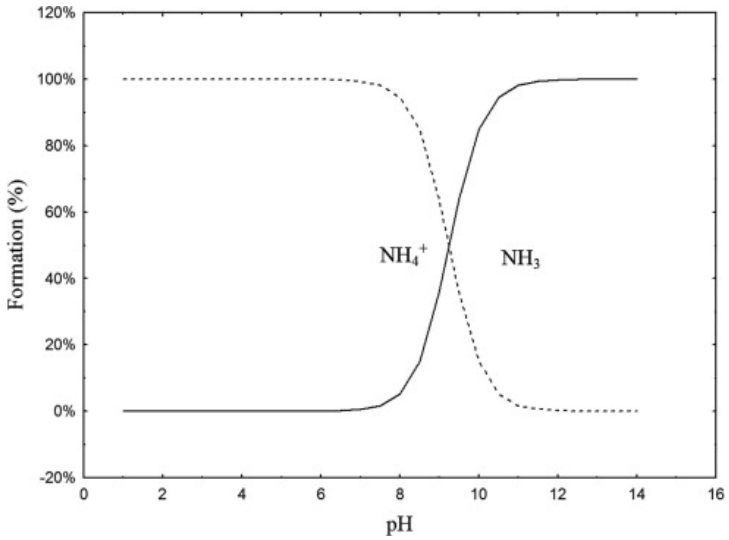


Fig. 1.5 Formation of ammonia/ammonium species as function of pH (Markou & Georgakakis, 2011).

Nitrogen deficiency in algal culture severely affects protein synthesis and reduces photosynthetic rates which results in enhanced biosynthesis and accumulation of lipids (Converti et al., 2009; Thompson Jr, 1996) and triglycerides (Stephenson et al., 2010; Takagi et al., 2000). Several studies have reported on high lipid accumulation under nitrogen deprived conditions in microalgal species such as *Neochloris oleoabundans*, *Nannochloris sp.*, *Scenedesmus* sp., and *Chlorella muelleri* (Courchesne et al., 2009; Gao et al., 2013; Radakovits et al., 2010).

Przytocka-Jusiak (1976) reported 50% and 100% inhibition of *Chlorella vulgaris* cell growth at 330 mg $NH_3$-N $L^{-1}$ and 700 mg $NH_3$-N $L^{-1}$ at pH 8-9 (Przytocka-Jsiak, 1975). Konig *et al.* (1987) also showed that both *Chlorella* and *Euglena* exhibited no ammonia toxicity at 560 mg $NH_3$-N $L^{-1}$ at pH 6.8 (100% ammonium ion). In this study, *Euglena* grew well at 17 mg $NH_3$- N $L^{-1}$ and pH 9.0, but was completely inhibited with 170 mg $NH_3$-N $L^{-1}$ and pH 9.0 (Konig et al., 1987). Azov and Goldman (1982) demonstrated 50% and 90% inhibition of *Scendesmus obliquus* photosynthesis at 34 and 51 mg $NH_3$-N $L^{-1}$ at pH 9.5 and 20 – 25°C. These reports would suggest that some species of sewage-associated algae, such as *Scendesmus*, are sensitive to the levels of ammonia and pH often encountered in HRAPs; others such as *Euglena* are tolerant of higher ammonia levels and *Chlorella* would not be affected by the levels found in these ponds (Azov & Goldman, 1982; Buchanan et al., 2013)

Performing wastewater treatment as well as microalgal growth in HRAP has been commonly suggested as a method for supplying external nitrogen. This does not only utilise the domestic wastewater effluents as a microalga growth media, but also associated with several other benefits such as reduction on wastewater nutrient release, leverage the currently existing infrastructure of treatment plants, provide oxygen for biological organic matter oxidation and nitrification, and contribute to biofuel production (Perez-Garcia et al., 2011).

*Phosphorus (P)*

Phosphorous is also an essential macro-nutrient for microalgae growth. Although cyanobacterial biomass do not need large amounts of phosphorus, range from 0.05% up to 3.3%, phosphorus is a primary growth limiting factor especially in natural environments, rather than nitrogen (Grobbelaar, 2004a; McKinney, 2004). Phosphorus limitation results in reduction in the synthesis and regeneration of substrates in the Calvin-Benson cycle and a consequential reduction in the rate of light utilization required for carbon fixation (Juneja et al., 2013). Similar to nitrogen, phosphorus limitation also leads to lipid accumulation. Studies have shown that the phosphorous deprived conditions increased the lipid content in *Phaeodactylum tricornutum*, *Chaetoceros* sp., *Isochrysis galbana* and *Pavlova lutheri* (Sharma et al., 2012). Liang et al. examined the effect of phosphorus on lipid accumulation in *Chlorella* sp. and observed an increase in lipid accumulation with decreasing phosphorus concentrations (Liang et al., 2013).

In natural environments and wastewater, phosphorus is present in various forms such as orthophosphate, polyphosphate, pyrophosphate, metaphosphate and their organic forms (Cembella et al., 1982; Yeoman et al., 1988). The form of phosphorus, which is utilized by microalgae, is the orthophosphate ($PO_4^{3-}$) form. Fig. 1.6 shows the formation of phosphate species as a function of pH. In aquatic systems phosphorus occurs in pentavalent form as a mixture of dissolved and particulate types and the available organic phosphorus is hydrolyzed to $PO_4^{3-}$ by extracellular enzymes (Correll, 1998).

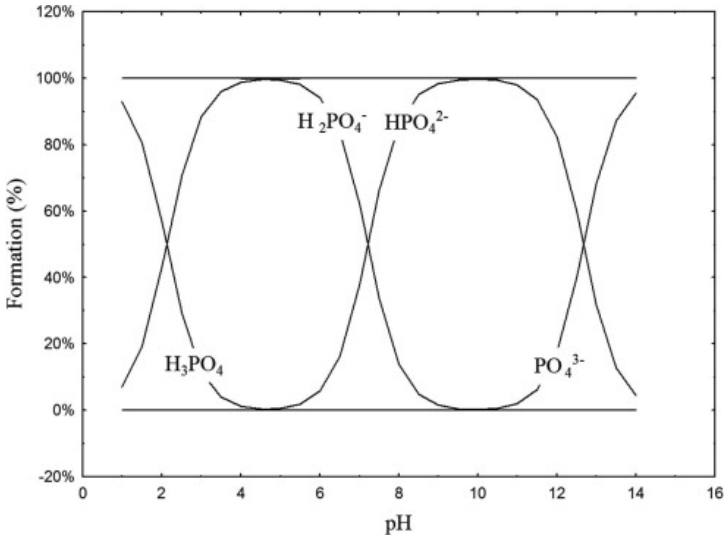


Fig. 1.6 Formation of phosphate species as a function of pH (Markou & Georgakakis, 2011).

*Carbon (C)*

The major process occurring between the algal and bacterial system for the treatment of wastewaters are embodied in Fig. 1.7. It depicts waste organic matter entering a cycle containing two groups of microorganisms, aerobic bacteria (as sludge) and micro algae which establishes an equilibrium between algal oxygen production and bacterial oxygen consumption, together with the relative composition of the biomass controlled via the organic carbon loading rate (Cromar & Fallowfield, 1997).

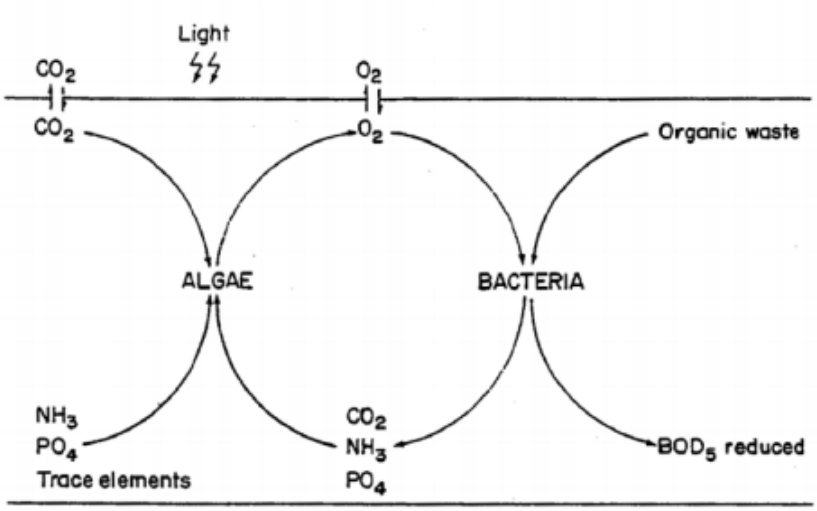


Fig. 1.7 The major process occurring within an algal – bacterial wastewater treatment system (Fallowfield & Garrett, 1985).

Referring to Fig. 1.7, the degradation of bacterial biomass releases the main nutrients $NH_3$ and $CO_2$ for algal photosynthesis. Cyanobacteria like microalgae have the ability to utilize both $CO_2$ and $HCO_3^-$ as their inorganic carbon source (Markou & Georgakakis, 2011). Carbon anhydrase (referred as CA) is a critical enzyme in catalyzing the hydration and dehydration of $CO_2$ in the reaction of:

$$CO_2 + H_2O \rightarrow HCO_3^- + H^+ \qquad \text{Equation 1.3}$$

The $CO_2$ dissolved in water forms a weak acid/base buffer system which is called the bicarbonate-carbonate buffer system. It provides carbon for photosynthesis through the following reactions:

$$2HCO_3^- \rightarrow CO_3^{2-} + H_2O + CO_2 \qquad \text{Equation 1.4}$$

$$HCO_3^- \rightarrow CO_2 + OH^- \qquad \text{Equation 1.5}$$

$$CO_3^{2-} + H_2O \rightarrow CO_2 + 2OH \qquad \text{Equation 1.6}$$

This buffer system naturally occurs in natural waters, anaerobically digested wastes and various organic acid acids buffer subsystems mixed with weak acid/base systems (Markou & Georgakakis, 2011). The formation of an inorganic carbon species is a function of pH and temperature. Figure 1.8, shows that bicarbonate ($HCO_3^-$) species dominate up to 10.5 pH value while carbonate ($CO_3^{2-}$) species dominate in higher pH value. Calcium carbonate ($CaCO_3$) is usually promoted under high pH values as well and generates minerals and protons:

$$Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H^+ \qquad \text{Equation 1.7}$$

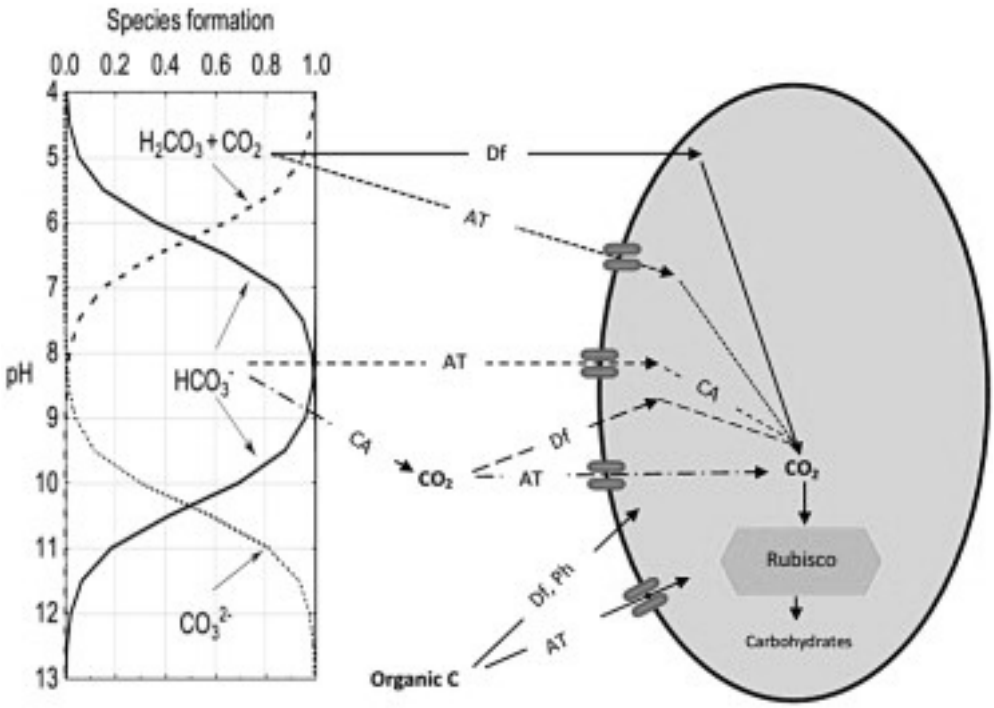


Fig. 1.8 Formation of inorganic carbon species as a function of pH and inorganic carbon uptake of Df: diffusion, AT: Active Transport, CA: Carbonic Anhydrase, Ph: Phosphorylation(Markou & Georgakakis, 2011).(Markou et al., 2014)

For some considerable time, carbon has been suspected of being a growth limiting factor in HRAPs treating wastewater, due to the high algal demand for it; whilst its concentration and bio-availability to algae is relatively low compared to other nutrients (Azov et al., 1982). According to Azov et al. (1982), about 48% of the incoming carbon will be in an inorganic form and 52% in organic form. The form of carbon preferred by most algal species for photosynthesis is unionised, dissolved $CO_2$. In the HRAP this will mostly come from daytime bacterial respiration. The degradation of bacterial biomass releases the main nutrients $NH_4^+$ and $CO_2$ for algal photosynthesis (Azov et al., 1982). This is quite a slow reaction rate, but has been calculated to proceed fast enough to supply $CO_2$ demand for algal photosynthesis in alkaline HRAP wastewater.

Azov et al. (1982) also determined that the conditions under which carbon could become limiting to algal productivity based on three major factors:

- Low inlet water organic carbon
- High algal concentrations when the inlet water has low alkalinity and
- Low retention times

In some studies, carbon has been considered a growth limiting factor in HRAPs treating wastewater due to the high algal carbon demand. It has been suggested that algal biomass production in organic carbon rich wastewaters may benefit from $CO_2$ enrichment. Park and Craggs (2010) suggest that $CO_2$ addition to a HRAP on a 4 day HRT nearly doubled algal production compared with one operated with $CO_2$ at an 8-day HRT in summer conditions. However, in this study there was no comparison of performance in the absence of $CO_2$ enrichment. Their further study using outdoor pilot-scale HRAPs also suggests that the proportion of algae in the algal/bacterial biomass in an HRAP with $CO_2$ addition was much higher in the 4-day HRT (80.5%) than in 8-day HRT (55.6%) (Park & Craggs, 2010; Park et al., 2011). However, the $CO_2$ enriched pond was not operated simultaneously with a control pond, receiving no additional $CO_2$ and the study compared data between two different years of pond operation. Furthermore, there has been little consideration of the effect of organic carbon content of the wastewater on the outcome of $CO_2$ addition. The authors also argue that the carbon:nitrogen (C:N) ratio of typical wastewater as limiting to algal growth, based on the stoichiometry of wastewater and algal biomass; however, there is uncertainty regarding the relative C:N ratios of wastewater and algal biomass. Park and Craggs (2010) argue that typical domestic wastewater has a C:N ratio of 7:1, while algal biomass is typically C:N 15:1 (Park & Craggs, 2010). Park et. al. (2011) stated that, by referencing of Benemann (2003) and Harmelen (2006), domestic sewage is typically between 3-7:1 C:N and algal biomass 6-15:1 C:N (Benemann, 2003; Harmelen, 2006; Park et al., 2011). Craggs et al. (2011) stated that, by referencing of Benemann (2003), facultative pond wastewater is 2:1 C:N and algal biomass between 5 and 10:1 C:N (Benemann, 2003; Craggs et al., 2011). Meanwhile Craggs et. al. (2012) stated that, by referencing of Benemann (2003), domestic wastewater is typically 3:1 C:N and algal biomass 6:1 C:N (Benemann, 2003; Craggs et al., 2012). To the best of our knowledge, considerable confusion surrounds these claims as unfortunately, there is no such stoichiometric data in the Benemann reference. To add to the confusion, even though they reference the same paper, they quote quite wide variations in stoichiometry which was also observed by Buchanan (Buchanan, 2015).

Although $CO_2$ addition to autotrophic (defined media) cultures is required to maximise algal growth since $CO_2$ diffusion from the atmosphere is rate limiting for

photosynthesis, it is also important to note that wastewater cultures which have an internal organic carbon pool can be utilised for conversion to $CO_2$. Oswald (1985) reported that *Chlorella* absorbs carbon dioxide principally in the undissociated forms ($CO_2$ or $H_2CO_3$) and little if any as $HCO_3^-$ or $CO_3^{2-}$. Early studies on effect of carbon dioxide concentration on photosynthesis indicate that carbon dioxide saturation is achieved at or below 0.1 per cent. Above about 5 per cent, toxic effects become operative, although the upper limit is not definitely known. He therefore expected that growth rate will be independent of carbon dioxide concentration between 0.1 and 5 per cent (Oswald, 1985).

The most widely quoted (Harris, 1986) stoichiometry for algal elements is using the Redfield ratio (Eq. 1.8):

$$C_{106}: H_{263}: O_{110}: N_{16}: P_{1}: S_{0.7} \text{ - by atoms}$$

$$C_{47}: N_{7}: P_{1} \text{ - by weight} \qquad \text{Equation 1.8}$$

which converts to a 6.6 C:N ratio. This raises a question whether carbon (from an external source such as $CO_2$) is the limiting factor for carbon rich (for internal carbon pool) wastewater growth medium. In order to fully understand the limiting nutrients for algal growth, the relationship between the key elements N, P, and C though a series of studies and modelling will be discussed in the next section.

### 1.5.2.5. Algal growth rate modelling

The purpose of this section is to understand the relationship between the key elements N, P, and C through a series of studies and modelling.

Nitrogen and phosphorus are often considered as the primary limiting nutrients in most aquatic systems. Healey (1973) demonstrated conclusive evidence that algae excrete extracellular phosphatases almost immediately upon the onset of P limited conditions (Healey, 1973). From a study of Grobbelaar (1983) on the availability to algae of N and P adsorbed on suspended solids in turbid waters of the Amazon River, he

demonstrated that algae can also excrete other compounds and change the pH of their surroundings, which in turn can render absorbed P available (Grobbelaar, 1983).

By using the Monod model (Monod, 1950), it has provided the most successful nutrient uptake kinetic mode for identifying limiting nutrients for algal growth, as defined in Equation1.9 (Goldman & Stanley, 1974):

$$\mu = \mu_{max}\left(\frac{S}{K_s + S}\right) \qquad \text{Equation 1.9}$$

Where

$\mu$ = specific growth rate, $d^{-1}$

$\mu_{max}$ = maximum specific growth rate, 1 $d^{-1}$

S = limiting nutrient concentration, mg $L^{-1}$

Ks = half-saturation coefficient (limiting nutrient concentration at $\mu_{max}/2$), mg $L^{-1}$

While the $K_S$ value is the upper nutrient concentration at which growth rate ceases to be proportional to that nutrient, therefore the nutrient concentration must be equal to or less than the $K_S$ value when it is limiting. Goldman et al. (1974) have successfully demonstrated the $K_S$ values for two green algae, *Selenastrum cornutum* and *Scendesmus quadricornum* at three pH ranges from 7.05 to 7.61, were so low that carbon would not be a limiting nutrient in natural waters until the pH reach very high levels (Goldman et al., 1974). A study by Hill and Lincoln (1981) indicated that the Ks for $CO_2$ in their model was only 0.105 mg inorganic C/L. They further state that at such high pH levels, precipitation of other essential nutrients such as phosphorous, iron and trace elements, and metabolic inhibition would become major factors limiting algal growth (Hill & Lincoln, 1981).

In addition, algae can store resources like P in excess of their immediate needs. In this case, by using Monod (1950) nutrient uptake kinetics which are only based on external resource concentrations, it does not truly represent the cellular nutrient content (Monod, 1950). Epply and Strickland (1968) concluded that the growth rate of phytoplankton is more closely related to the cellular nutrient content than to external

concentrations. It is, therefore, necessary to establish a relationship between the cell quota of a nutrient and the growth rate of an alga (Eppley & Strickland, 1968). Such a relationship was given by Droop (1968, 1983) and in a generalised form it is:

$$\mu = \mu_{max}\left(1 - \frac{K_q}{Q}\right) \qquad \text{Equation 1.10}$$

Where

$\mu$ = specific growth rate, $d^{-1}$

$\mu_{max}$ = maximum specific growth rate, 1 $d^{-1}$

Q = cell quota for the limiting resource, mg $L^{-1}$

Kq = the minimum cell quota for limiting resource or subsistence quota, mg $L^{-1}$

This model has been applied to a number of species and nutrients such as; P, N ($NO_3$, $NH_4^+$ and urea), Si, Vitamin B12 and Fe (Droop, 1968; Droop, 1983). However, it was also shown that the model did not work notably with $NH_4^+$ limited growth of *Monochrysis* and *Dunaliella* (Caperon & Meyer, 1972; Laws & Caperon, 1976).

In terms of the steady-state nutrient assimilation, Equation 1.10 can be written as (Droop, 1983):

$$\mu = \frac{\mu_{max}[S]}{K_s + [S]} \qquad \text{Equation 1.11}$$

In comparison to substrate concentration (Equation 1.10) and cell quota (Equation 1.11), their hyperbolic relation to specific growth rate is shown in Fig. 1.9 and 1.10 accordingly. In Fig. 1.9, it shows that the smaller μmax (i.e. the greater the quota flexibility) becomes, the steeper the initial slope of μ. The slope has a direct influence on the half-saturation constants, being high for low quota flexibility and low for high quota flexibility. Low half-saturation constants are typical of P and N, especially $NH_4^+$ (Glibert et al., 1982), whereas high half-saturation constants are typical for carbon (Turpin et al., 1985). While considering Fig. 1.10, it shows the variation in growth rate response to different Ks values at a specific μmax. It shows that low $K_S$ values are observed with increased growth rates at low substrate concentrations, and vice versa.

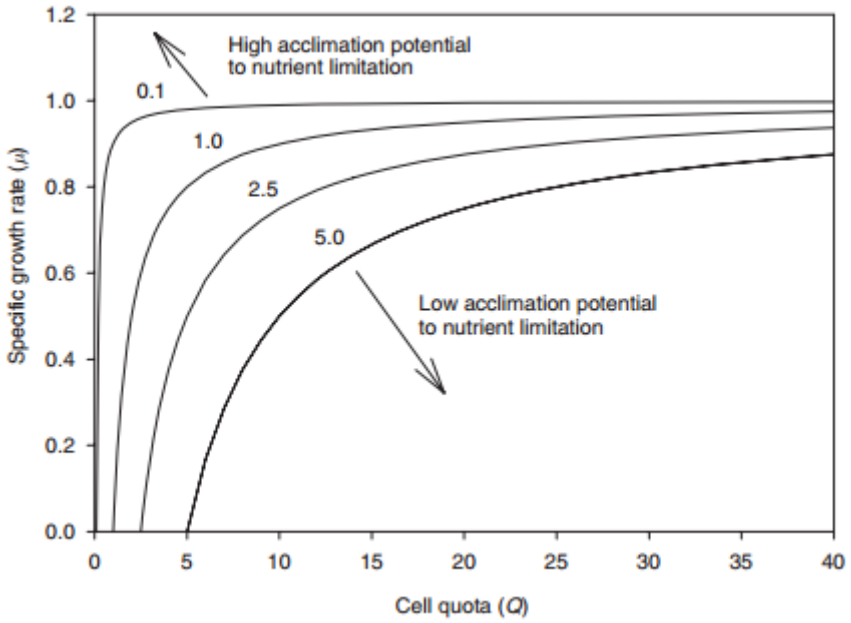


Fig. 1.9 Specific growth rate against the cell quota (Q), using equation 1.10 by Droop (1983), assuming a $\mu_{max}$ = 1 and varying kq values over a range of 0.1 to 5 for both the high and low acclimation potential to nutrient limitation (Grobbelaar, 2004b)

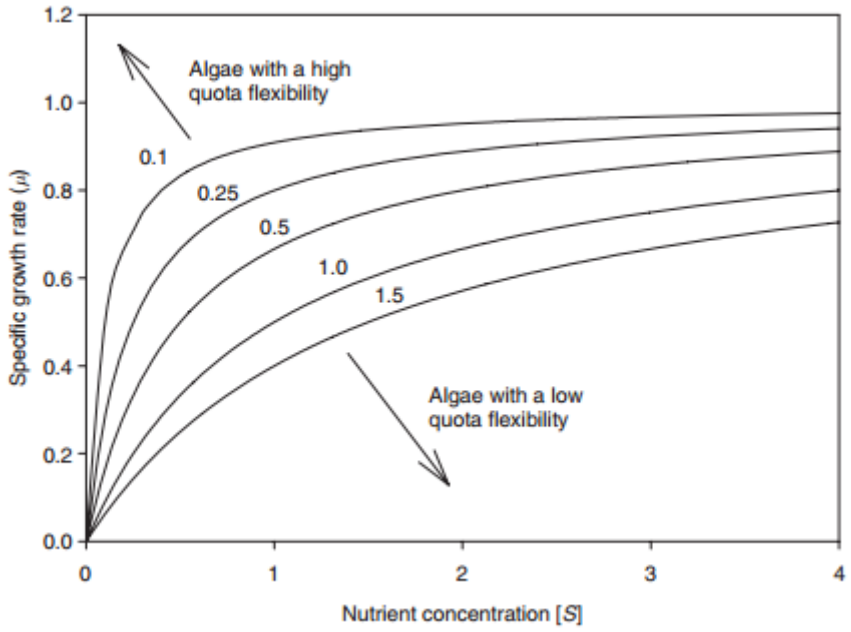


Fig. 1.10 A hypothetical example showing the specific growth rate (μ) of an alga against substrate concentrations (*S*) and the concept of algal quota flexibility adaptation. The Monod kinetics are shown as equation 1.11 and Ks vary from 0.1 to 1.5 (Grobbelaar, 2004b)

According to Rhee and Gotham (1980), the optimum nutrient ratio is the ratio at which a transition from one nutrient limitation to another occurs (thus both could be limiting),

or where the cellular ratio of resources required is such that the resource is not in short supply relative to another (Rhee & Gotham, 1980). Both of the limiting resource concentration and the consumption rate at the transition point where limitation occurs can be determined by using the internal concentration of nutrients and the uptake rates. For example, if the optimum N:P ratios for two species are 20 and 10 respectively, then both will be P limited when the ratio is >20. However, the second species will be more P-limited than the first. If they have similar μmax values, the first species will eliminate the second species at N:P ratios >20 (Grobbelaar & House, 1995). Since a limiting nutrient can be defined as the one with the smallest Q:kq ratio (Droop, 1974), transition between N and P limitation occurs when:

$$\frac{Q_N}{k_{qN}} = \frac{Q_P}{k_{qP}} \qquad \text{Equation 1.12}$$

The optimum ratio for N:P, showing the dependence of QN:QP on relative growth rates (Figure 1.11) (Turpin et al., 1988), can then be written as:

$$\frac{Q_N}{Q_p} = \frac{k_{qN}/\,(1-\,\mu/\,\mu_{maxN})}{k_{qP}\,/\,(1-\,\mu/\,\mu_{maxP})} \qquad \text{Equation 1.13}$$

On either side of the curve, either N or P limits growth. Note that the higher the growth rate, the more N pro rata is required and vice versa. Experimental support of this growth rate dependence of the optimum nutrient ratio was obtained by Terry et al. (1985) and Turpin (1986) (Terry et al., 1985; Turpin, 1986). An important detail is that the optimum N:P ratio varied between species and over the diurnal cycle (Rhee & Gotham, 1980). Ahlgren (1985) showed that algae were able to adapt to different N:P ratios at lower growth rates and that the ratio becomes more fixed at higher growth rates (Ahlgren, 1985).

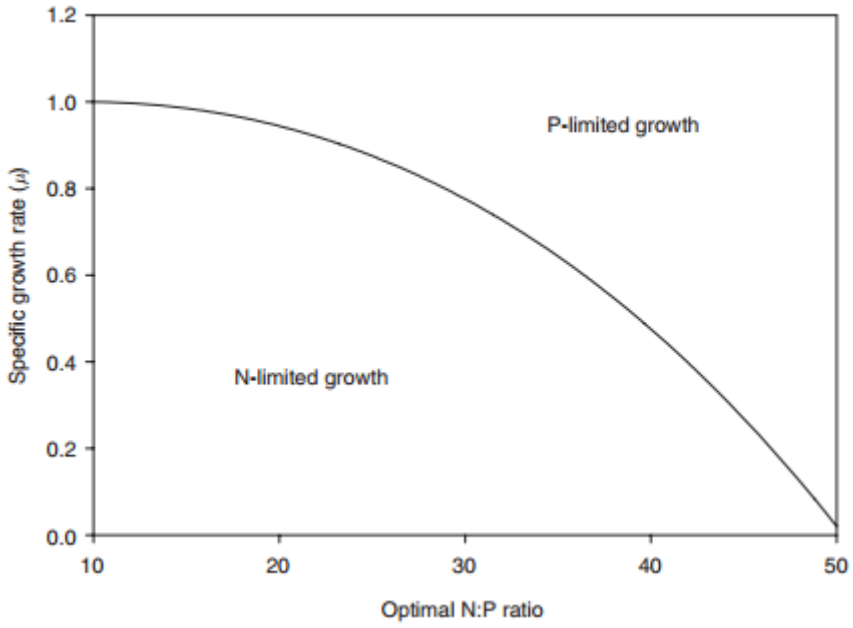


Fig. 1.11 Growth rate dependence for the optimal N:P ratio of an alga, showing the P- and N-limited growth regions with a $\mu_{max}$ = 1, a $k_{qN}$ = 5 mg $L^{-1}$ and a $k_{qP}$ = 0.5 mg $L^{-1}$ (Grobbelaar, 2004b)

Terr et al. (1985) also stated that the optimum N:P ratios vary only slightly, while Turpin (1986) studies shown the variations have been found for C:P. The optimum N:P ratio curves for different species could cross. For example, at low growth, one species might be P limited, another N limited. At growth rates higher than the crossover point, the situation would be reversed, which would influence the competition and dominance between species. At the crossover point, also termed the optimum ratio equivalence point, neither one of the species has an advantage over the other (Turpin et al., 1988).

On a note of carbon limiting in wastewater, it is also important to distinguish and describe the different characteristic of wastewater medium to be used in the cultivation system, as the levels of BOD and total carbon will vary accordingly to the prior treatment stages which will be discussed in the next section.

## 1.6. The characteristics of wastewater

### 1.6.1. Wastewater constituents

The quality of wastewater is described by a series of water quality parameters. These parameters (i.e., compositions) can be expressed in the concentration of individual compounds or it can be expressed in the concentration of a group of related

compounds, such as biochemical oxygen demand (BOD). The substances present in wastewater are either in solid, liquid, or gas form. As previously mentioned, whether carbon (from an external source such as $CO_2$) is the limiting factor for carbon rich (for internal carbon pool) wastewater growth medium is uncertain due to the fact that the contribution of wastewater constituents can vary strongly. According to Henze (2008), the constituents in wastewater can be divided into main categories in Tables 1.1 & 1.2 (Henze, 2008):

Table 1.1 Different types of wastewater (Henze, 2008)

| **Wastewater from society** | **Wastewater generated internally in treatment plants** |
|---|---|
| Domestic wastewater | Thickener supernatant |
| Wastewater from institutions | Digester supernatant |
| Industrial wastewater | Reject water from sludge dewatering |
| Infiltration into sewers | Drainage water from sludge drying beds |
| Stormwater | Filter wash water |
| Leachate | Equipment cleaning water |
| Septic tank wastewater | |

Table 1.2 Constituents present in domestic wastewater (Henze et al., 2001)

| **Wastewater constituents** | **Examples** | **Associated hazard** |
|---|---|---|
| Microorganisms | Pathogenic bacteria, virus and worms eggs | Risk when bathing and eating shellfish |
| Biodegradable organic materials | Oxygen depletion in rivers, lakes and fjords | Fish death, odours |
| Other organic materials | Detergents, pesticides, fat, oil and grease, colouring, solvents, phenols, cyanide | Toxic effect, aesthetic inconveniences, bio accumulation in the food chain |
| Nutrients | Nitrogen, phosphorus, ammonium | Eutrophication, oxygen depletion, toxic effect |
| Metals | Hg, Pb, Cd, Cr, Cu, Ni | Toxic effect, bioaccumulation |
| Other inorganic materials | Acids, for example hydrogen sulphide, bases | Corrosion, toxic effect |
| Thermal effects | Hot water | Changing living conditions for flora and fauna |
| Odour (and taste) | Hydrogen sulphide | Aesthetic inconveniences, toxic effect |
| Radioactivity | | Toxic effect, accumulation |

*Person equivalents (PE) and person load*

Person equivalents (PE) can be expressed in water volume or BOD. The two definitions used worldwide are (Henze, 2008):

$$1\ PE = 0.2\ m^3/d$$

$$1\ PE = 60\ g\ BOD/d$$

These two definitions are based on fixed nonchangeable values. The actual contribution from a person living in a sewer catchment, so-called the Person Load (PL), can vary considerably (Table 1.3). The reasons for the variation can be such as: working place outside the catchment, socio-economic factors, lifestyle, and type of household installation (Henze, 2008).

Table 1.3 Variations in person load (Henze et al., 2001)

| **Parameter** | **Unit** | **Range** |
|---|---|---|
| COD | g/cap.d | 25-200 |
| BOD | g/cap.d | 15-80 |
| Nitrogen | g/cap.d | 2-15 |
| Phosphorus | g/cap.d | 1-3 |
| Wastewater | $m^3$/cap.d | 0.05-0.40 |

The compositions of municipal wastewater also varies significantly from one location to another. This is mainly due to water consumption in households and infiltration and exfiltration during transport inside the sewage system. Table 1.4 shows the composition of typical domestic wastewater (Metcalf, 1991). The author catalogues untreated (i.e., raw) domestic wastewater based on the $BOD_5$ concentrations accordingly as low (110 mg $L^{-1}$), medium (190 mg $L^{-1}$), and high strength (350 mg $L^{-1}$).

In comparison, Table 1.5 shows typical effluent quality following various levels of treatment published in Australian guidelines for sewerage systems (ARMCANZ, 1997). By comparing the (A) pre-treatment and (D) nutrient removal wastewater, the

latter BOD is significantly lower at range 5-20 mg $L^{-1}$ compared to 140-350 mg $L^{-1}$. This supports the previous note discussed in the section above that, in terms of supplying an external carbon source such as $CO_2$ in wastewater for microalgal cultivation, it is important to distinguish and describe the different characteristic of wastewater medium to be used in the cultivation system, as the levels of BOD and total carbon will be vary according to the prior treatment stages. If the BOD and internal carbon content in the wastewater is already sufficient, the effects of $CO_2$ addition on algal growth in wastewater may not be a cost-effective for enhancing biomass production. A consequence of a presumed requirement for $CO_2$ addition (e.g. coming from a power plant) to an algal cultivation system with wastewater, is that it strictly limits the flexibility of the system to being built in a specific location i.e. next to a power plant. Without this requirement, it allows the systems (e.g., HRAPs) to be built in some remote areas.

Table 1.4 Physical-chemical characteristics of untreated (raw) domestic wastewater with different concentrations based on PEs (Metcalf, 1991)

| | | **Concentration[a]** | | |
|---|---|---|---|---|
| **Contaminants** | **Unit** | **Low strength** | **Medium strength** | **High strength** |
| $BOD_5$ at 20℃ | mg $L^{-1}$ | 110 | 190 | 350 |
| TOC | mg $L^{-1}$ | 80 | 140 | 260 |
| COD | mg $L^{-1}$ | 250 | 430 | 800 |
| Volatile organic compounds (VOCs) | mg $L^{-1}$ | <100 | 100-400 | >400 |
| TS | mg $L^{-1}$ | 390 | 720 | 1230 |
| Total dissolved solid (TDS) | mg $L^{-1}$ | 270 | 500 | 860 |
| Fixed | mg $L^{-1}$ | 160 | 300 | 520 |
| Volatile | mg $L^{-1}$ | 110 | 200 | 340 |
| TSS | mg $L^{-1}$ | 120 | 210 | 400 |
| Fixed | mg $L^{-1}$ | 25 | 50 | 85 |
| Volatile | mg $L^{-1}$ | 95 | 160 | 315 |
| Settleable solids | mg $L^{-1}$ | 5 | 10 | 20 |
| Nitrogen (total as N) | mg $L^{-1}$ | 20 | 40 | 70 |
| Organic | mg $L^{-1}$ | 8 | 15 | 25 |
| Free ammonia | mg $L^{-1}$ | 12 | 25 | 45 |
| Nitrites | mg $L^{-1}$ | 0 | 0 | 0 |
| Nitrates | mg $L^{-1}$ | 0 | 0 | 0 |
| Phosphorus (total as P) | mg $L^{-1}$ | 4 | 7 | 12 |
| Organic | mg $L^{-1}$ | 1 | 2 | 4 |
| Inorganic | mg $L^{-1}$ | 3 | 5 | 10 |
| Chlorides[b] | mg $L^{-1}$ | 30 | 50 | 90 |
| Sulfate[b] | mg $L^{-1}$ | 20 | 30 | 50 |
| Oil and grease | mg $L^{-1}$ | 50 | 90 | 100 |
| Total coliform | No./100 mL | $10^6$-$10^8$ | $10^7$-$10^9$ | $10^7$-$10^{10}$ |
| Fecal coliform | No./100 mL | $10^3$-$10^5$ | $10^4$-$10^6$ | $10^5$-$10^8$ |
| *Cryptosporidum oocysts* | No./100 mL | $10^{-1}$-$10^0$ | $10^{-1}$-$10^1$ | $10^{-1}$-$10^2$ |
| *Giardia lamblia cysts* | No./100 mL | $10^{-1}$-$10^1$ | $10^{-1}$-$10^2$ | $10^{-1}$-$10^3$ |

[a] Low strength is based on approximate wastewater flowrate of 750 L/capita.d (220 gal/capita.d)
Medium strength is based on an approximate wastewater flowrate of 460 L/capita.d (120 gal/capita.d)
High strength is based on an approximate wastewater flowrate of 240 L/capita.d (120 gal/capita.d)

[b] Values should be increase by amount of constituent present in domestic water supply

Note: mg $L^{-1}$ = g $m^{-3}$

Table 1.5 Typical effluent quality following various levels of treatment based on Australian Guidelines for Sewerage Systems: effluent management (ARMCANZ, 1997)

| **Treatment** | **BOD ($mg\ L^{-1}$)** | **TSS ($mg\ L^{-1}$)** | **TN ($mg\ L^{-1}$)** | **TP ($mg\ L^{-1}$)** | ***E.coli* (org/100ml)** | **Anionic Surfactants ($mg\ L^{-1}$)** | **Oil and Grease ($mg\ L^{-1}$)** |
|---|---|---|---|---|---|---|---|
| **Raw wastewater** | 150-500 | 150-450 | 35-60 | 6-16 | $10^7 – 10^8$ | 5 – 10 | 50 - 100 |
| **A** | 140-350 | 140-350 | | | | | |
| **B** | 120-250 | 80-200 | 30-55 | 6-14 | $10^6 – 10^7$ | | 30-70 |
| **C** | 20-30 | 25-40 | 20-50 | 6-12 | $10^5 – 10^6$ | < 5 | < 10 |
| **D** | 5-20 | 5-20 | 10-20 | < 2 | | | < 5 |
| **E** | | | | | $< 10^3$ | | |
| **F** | 2-5 | 2-5 | < 10 | < 1 | $< 10^2$ | | < 5 |

Note:

| Treatment process category: | Parameters to be removed: | Examples of treatment processes: |
|---|---|---|
| A: Pre treatment | Gross solids, some of the readily settleable solids | Screening |
| B: Primary treatment | Gross solids plus readily settleable solids | Primary sedimentation |
| C: Secondary treatment | Most solids and BOD | Biological treatment, chemically assisted treatment, lagoons |
| D: Nutrient removal | Nutrients after removal of solids | Biological, chemical precipitation |
| E: Disinfection | Bacteria and viruses | Lagooning, ultraviolet, chlorination |
| F: Advanced wastewater treatment | Treatment to further reduce selected parameters | Sand filtration, microfiltration |

Heubeck, Craggs and Shilton (2007) investigated the influence of $CO_2$ addition from biogas scrubbing on HRAP wastewater treatment performance (BOD, $NH_4$-N, dissolved reactive phosphorous (DRP) and *E.coli* removal) and algal production (growth and species composition). The preliminary findings of the study showed the potential to scrub $CO_2$ from biogas using HRAP without decreasing the effectiveness of wastewater treatment and enabling increased recovery of wastewater nutrients as algal biomass. However, the initial $BOD_5$ levels in the wastewater used in this experiment were adjusted by spiking with a homogenised solution of chicken egg (two separated doses, high and low) and deionised water to achieve two $BOD_5$ concentrations approximately 44 mg $L^{-1}$ (high egg dose) and 24 mg $L^{-1}$ (low egg dose) (Heubeck et al., 2007). Refer to Table 1.5 again, these $BOD_5$ concentrations fit in the C: Secondary Treatment ($BOD_5$ 20-30 mg $L^{-1}$) or D: Nutrient Removal ($BOD_5$ 5-20 mg $L^{-1}$) wastewater groups. This raises a concern that the wastewater was pre-treated and the internal carbon pool was low at the outset.

A study by Park and Craggs (2010) investigates the influence of $CO_2$ addition (to augment daytime carbon availability) on wastewater treatment performance and algal production of two pilot-scale HRAPs (West and East) operated with different hydraulic retention times (4 and 8 days) over a New Zealand summer in November-March 2007/08. These two HRAPs were part of an Advanced Pond System (ASP) treating domestic wastewater at the Ruakura Research Centre located at Hamilton, New Zealand. The study includes parameters such as total suspended solids (TSS), volatile suspended solids (VSS), total and soluble 5-day biochemical oxygen demand ($TBOD_5$, $SBOD_5$) and chlorophyll a. The proportion of algal biomass in the HRAPs was estimated from the chlorophyll a concentration using Raschke's (1993) equation:

[Algae biomass (mg/L)] = [chlorophyll a (mg/L)] x 100/1.5

This equation is based on an assumption that algal biomass has constant chlorophyll a content of 1.5% of the dry weight. The $TBOD_5$ concentrations for both HRAPs are 272.8 g/$m^3$ and $SBOD_5$ concentrations are 257.7 g/$m^3$, with a $SBOD_5$ loading rate 26.0 g/$m^3$/d for $HRAP_{8d}$ and 24.8 g/$m^3$/d for $HRAP_{4d}$.

The study showed that the wastewater treatment HRAPs (4d and 8d HRT) with $CO_2$ addition achieved a mean areal algal biomass (i.e., algal only) productivity of 16.7

g/$m^2$/d for the $HRAP_{4d}$ and 9.0 g/$m^2$/d for the $HRAP_{8d}$. Each of the HRAPs received anaerobic digester effluent (1 $m^3$/d) which was added at the pond bottom downstream of the paddlewheel. The influent to the West HRAP was diluted with 1 $m^3$/d of tap water. The proposed purpose of this was to simulate the recycling of treated effluent after complete algae and nutrient removal to give HRT of 4 and 8 days respectively for the West HRAP ($HRAP_{4d}$) and East HRAP ($HRAP_{8d}$). The study also shows a higher mean areal biomass (i.e., algal + bacterial) productivity of 20.7 g/$m^2$/d in $HRAP_{4d}$ than in the $HRAP_{8d}$ (15.8 g/$m^2$/d). However, no control study (i.e., no $CO_2$ addition) was provided in this study. In addition, the only comparison (i.e. without $CO_2$ addition) was based on values measured in previous individual HRAP researches in New Zealand (Craggs et al., 2003; Heubeck et al., 2007).

In summary, with the previous discussions on

- Redfield ratio ($C_{106}$: $H_{263}$: $O_{110}$: $N_{16}$: $P_1$: $S_{0.7}$ – b atoms) which converts a 6:6 C: N ratio,
- Uncertainty regarding the relative C:N ratios of wastewater and algal biomass in some literature studies,
- And variety strengths on domestic wastewater based on their treatment stages, existing internal carbon contents, and $BOD_5$ levels

This raises a question whether carbon (from an external source such as $CO_2$) is the limiting factor for carbon rich (for internal carbon pool) wastewater growth medium. The effect of $CO_2$ addition to wastewater grown algae cultures demonstrated higher algal photosynthetic efficiencies and productivities cannot be simply applied to all types of wastewater, especially for studies involved with raw wastewater with higher BOD.

## 1.7. Maximising the sustainability of algal growth systems – energy extraction

### 1.7.1. Remove the dependency on using fossil fuel

As previously discussed in this chapter, a number of studies have argued that biofuel production (especially for biodiesel) from algae is both economically and environmentally sustainable compared to the first and second biofuel generations (Brune et al., 2009; Chisti, 2008; Huntley & Redalje, 2007; Pittman et al., 2011; Stephens et al., 2010), there has been a growing concern regarding the long term viability and economics of biofuels from harvested algal cake, especially learning from the Aquatic Species Program from 1978 to 1996 (Pittman et al., 2011; Reijnders, 2008; van Beilen, 2010; Walker, 2009).

One of the major criticisms of algal biofuels production process is the dependence on using fossil fuels. Processes such as the construction of algal growth facilities, supply of nutrients for algal growth, harvesting of algae and biomass processing are still heavily reliant on the use of fossil resources, this would in fact give rise to a net negative energy output (Pittman et al., 2011). The major carbon and energy advantages from using algae biofuels involve two separate displacements – direct and process-related.

The first displacement refers to fuel derived from algae displacing fossil fuel i.e., leaving the fossil fuel in the ground. It is important in determining the overall carbon balance from this displacement in order to be able to calculate all of the carbon (both direct and indirect) involved in the lifecycle of algae production, including end use. It is only appropriate to discuss the negative carbon balance of algae biofuels relative to petroleum if these emissions are fewer compared to the overall lifecycle carbon balance of extracting and burning fossil fuel. It is also important to understand that from the perspective of net impact on atmospheric greenhouse gas concentrations, it does not actually matter if $CO_2$ is first released to the atmosphere from an industrial facility and then captured by algae or captured directly from the facility flue gas by the algae. According to Ryan (2009), if the energy needed to utilize flue gas directly into

the algae cultivation system does not produce sufficiently more incremental algae than what would have occurred using atmospheric carbon, then the carbon balance will be better if the two systems are not coupled (Ryan, 2009).

The second process related is displacement which involves the displacement of carbon emission by the co-products or byproducts of the algae biofuels process. For example, growing algae in a process that also treats wastewater displaces the carbon that would have been generated in conventional wastewater treatment processes. Referring to this point, Harmelen (2006) explains that approximately one ton of algae biomass produced during wastewater treatment reduces the equivalent of one ton fossil $CO_2$ derived from the algal biomass and the greenhouse gas reductions compared to conventional wastewater treatment processes, as well as fertilizers and other potentials co-products, currently derived from fossil fuels (Harmelen, 2006). However, Harmelen (2006) also stated that this is highly variable because it is based on factors such as the biofuel production, the fossil fuel displaced, and the energy savings realised in the production of co-products or wastewater treatment compared to current fossil fuel-based technologies. Using an example in Europe, one ton of $CO_2$ avoided is currently worth about € 20-30/tonne. However, in developing countries or the USA, the value is currently much lower which is well below € 5/ton. Harmelen (2006) also predicts the price could rise up to € 50/ton $CO_2$ avoided by the year 2020 due to strictly regulated climate policies. In a stand-alone microalgae system, where biofuel is the only product, the revenues would be only the biofuel output and GHG abatement.

In addition, the use of wastewater resources may be a viable solution to enhance the sustainability of algal biofuel production by providing not only an effective growth medium for algal cultivation but also a freely available nutrient input such as N and P (Pittman et al., 2011).

### 1.7.2. Processing of “whole” algae

In order to reduce the production cost associated with the extraction process, which is one of the major expenditures of mass microalgal cultivation, it is possible to process whole algae into fuels instead of first extracting oils and/or starch and post-processing. In combination with using wastewater as grow medium, turning algal biomass into a more sustainable biofuel becomes more achievable using this approach. Currently, there are four major conversion technologies that are capable of processing whole algae: anaerobic digestion, supercritical processing, pyrolysis and gasification (Fig. 1.12) (Harun et al., 2010; Luisa, 2011). Although some form of dewatering is still required in the processing, these methods provide benefits on cost reduction associated with the extraction process and added benefit of being amenable to process a diverse consortium of algae. However, Harun et al. (2010) also stated that each of these processes should be economically evaluated on specifically designed industries in order to economically maximize the entire process of using algae.

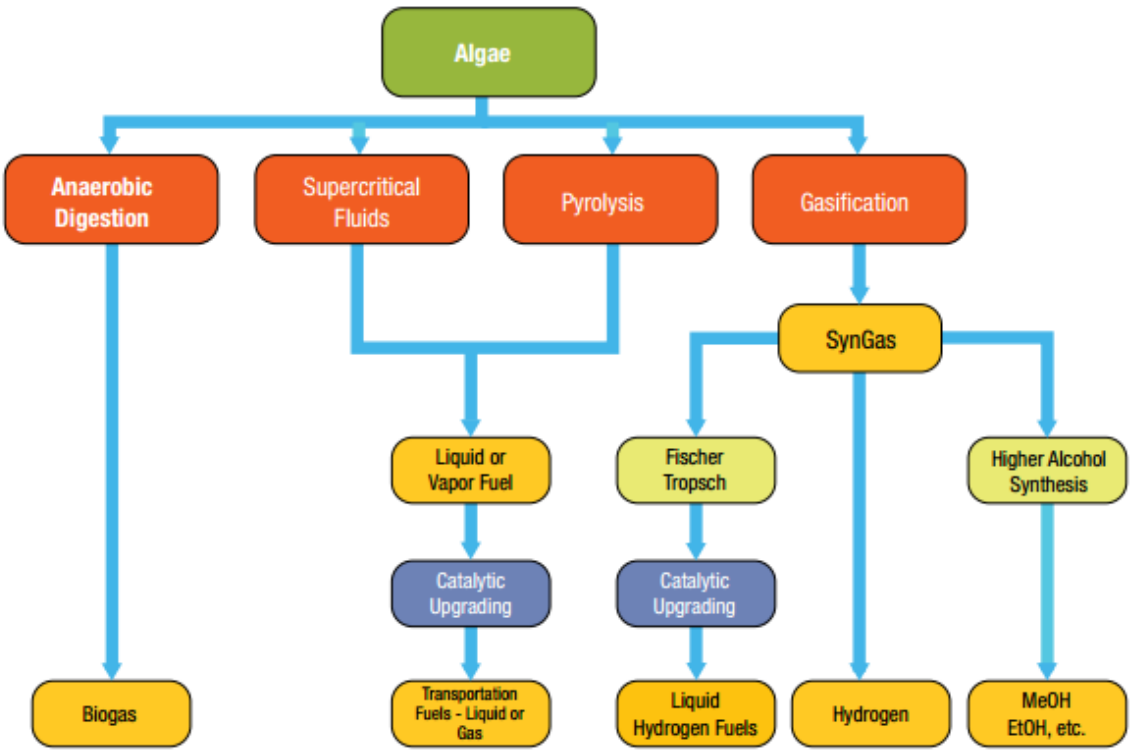


Fig. 1.12 Schematic of the potential conversion routes for whole algae into biofuels (DOE, 2010)

*Supercritical fluids*

Supercritical processing is a recent addition to the potential conversion routes which is capable of simultaneously extracting and converting oils into biofuels (Demirbas, 2006). The advantage of using this technique on algal oil extraction is far more efficient than traditional solvent separation methods and extremely powerful in the extraction of other component within algae (Mendes, 2007). This is because supercritical fluids are selective and therefore they provide high purity and product concentrations. Another benefit of using the method is that it excludes organic solvent residues in the extract or spent biomass (Demirbas, 2009). Extraction is efficient at modest operating temperatures, for example, at less than 50˚C, ensuring maximum product stability and quality (DOE, 2010). In addition, this method can be used on whole algae without dewatering and therefore the efficiency of the process is also significantly increased.

A major roadblock of this technology is the ability to demonstrate similar yields and efficiencies at a level that can be scaled to commercial production when is applied in processing of algae, either whole or its oil extract. To be considered viable, this method must be able to demonstrate the ability to tolerate the complex compositions that are often found in raw, unprocessed algae and indicate no negative impact due to the presence of other small metabolites (DOE, 2010).

*Pyrolysis*

Pyrolysis is the chemical decomposition of a condensed substance by heating. It does not involve reactions with oxygen or any other reagents but can frequently take place in their presence (DOE, 2010). This method can be used in a wide range of products, including algae and other biomass, depending on the reaction parameters. The major advantage of pyrolysis over other conversion methods is that it is extremely fast, with reaction times of the order of seconds to minutes. Huber et al. (2006) also stated that liquid product yield from using this method tends to favour short residence times, fast heating rates, and moderate temperature (Huber & Dumesic, 2006).

However, a major roadblock of using this method for algae conversion is moisture content and significant dehydration must be performed upstream for the process to work efficiently. No comprehensive and detailed side-by-side comparison is available in the scientific literature and therefore it is difficult to estimate this method on converting algae into a bio-oil compared to other biomass due to uncertainties in the ability to dehydrate the feedstock. This method is considered not to be cost-competitive over the short-term unless an inexpensive dewatering or extraction process is also developed. In addition, this technology is a relatively mature process and it is expected that only incremental improvements will occur and a breakthrough in conversion efficiency appears unlikely (DOE, 2010). While algal bio-oil may be similar to bio-oil from other biomass sources, it may have a different range of compounds and compositions depending on the type of algae and upstream processing conditions (Bing et al., 1994). Miao and Wu (2004) demonstrated the bio-oil produced by pyrolysis of algae can be tailored, if the conditions of algal growth is carefully controlled (Miao & Wu, 2004). Unfortunately, there are also significant gaps in the information available about the specifications for converting algal bio-oil and the resulting products, for example, the optimal residence time, temperature, and understanding of detailed molecular composition to produce different algal bio-oils from different feedstocks. In addition, further studies are also required to understand the catalytic conversion of the resulting algal bio-oils. Another area of interest is the development of stabilizers for the viscosity of the bio-oil and acid neutralizing agents, so the bio-oil may be more easily transported throughout the upgrading process (DOE, 2010).

*Gasification*

The primary pathways of algal biomass gasification are through Fischer-Tropsch Synthesis (FTS), hydrogen, or mixed alcohol synthesis of the resulting syngas. The synthesis of mixed alcohols using gasification of lignocellulose is relatively mature and reasonable to expect that once water content is adjusted for, the gasification of algae to these biofuels would be comparatively straightforward (Philip et al., 2007; Yung et al., 2009). FTS is also a relatively mature technology where the syngas components (CO, $CO_2$, $H_2O$, $H_2$, and impurities) are cleaned and upgraded to usable liquid fuels through a water-gas shift and CO hydrogenation (Balat, 2006; Okabe et

al., 2009; Srinivas et al., 2007). The major advantage of using gasification is the ability to provide a wide variety of fuels with acceptable and known properties, making the process more flexible. In addition, it is also possible to feed algae into a coal gasification plant to reduce the capital investment requirement. This provides several benefits such as integrating an algal feedstock into an existing thermochemical infrastructure, addressing the issues of availability for dedicated biomass plants, and improving the process efficiency through economy of scale. Regenerative heat from FTS (i.e., exothermic process) is possible to be recovered for drying the algae during a harvesting or dewatering process (DOE, 2010).

The roadblocks of FTS for algae are similar to those for coal (Yang et al., 2005), with the exception of any upstream process steps that may be a source of contaminants which will need to be removed prior to reaching the Fischer-Tropsch (FT) catalyst. A very large scale production of FTS is also required to make the process efficient overall. The major roadblock of this method is associated with high cost of clean-up and tar reforming. This is due to tars having high molecular weight and can develop during the gasification process. The tars cause coking of the synthesis catalyst and any other catalysts used in the syngas cleanup process and must be removed (DOE, 2010). In a study by Hallgren et al. (1993), it was demonstrated that tar formation was minimized or avoided via entrained-flow gasification at high temperature. This method requires sub-millimeter sized particles and it is often difficult to reach such a small size with other biomass sources or pretreatment is required. Algae, however, may have a unique advantage of this process because certain algal species may not require pretreatment due to their inherent small size (Hallgren et al., 1993). Even though FTS is a mature technology, there are still several areas that should be investigated and require R&D. For example, it is important to determine the optimum conditions for indirect gasification of algae, the feasibility of using the oxygen generated by algae for use in the gasifier to reduce or eliminate the need for a tar reformer, and leveraging ongoing syngas-to-ethanol research using cellulosic (DOE, 2010).

*Anaerobic digestion*

In 1987, Hansen et al. (1987) demonstrated the production of biogas from the anaerobic digestion of macroalgae *Laminaria hyperbore* and *Laminaria saccharina*. The study received scant attention in the United States (Hanssen et al., 1987). The major advantage of this technology eliminates several of the key obstacles that are responsible for the current high costs associated with algal biofuels, including drying, extraction, and fuel conversion, making it economically viable and cost-effective when compared to the other methods above. A detailed discussion will be presented in the next section.

### 1.7.3. Anaerobic digestion of "whole" algae

Anaerobic digestion is a controlled process of microbial decomposition under anaerobic conditions (i.e., no or limited oxygen). Organic matter is converted by a consortium of microorganisms into biogas such as methane and carbon dioxide, inorganic nutrients and humus (Chynoweth & Pullammanappallil, 1996). This process is naturally occurring in anaerobic environments such as lake and ocean sediments, animal digestive tracts and where organic matter has accumulated and involved in microbial methanogenesis (Chynoweth, 1996). Adaptation of these processes into the management and treatment of wastewater biosolid and livestock manures was driven by the requirements for finding new sources of renewable energy as well as to decrease the pollution potential of manures.

Anaerobic digestion of wastewater sludge is a well-known technology and is widely used in many modern wastewater treatment processes (Gunaseelan, 1997). Creating a controlled anaerobic environment, such as a covered pond or tank, allows the methane to be captured and combusted for energy production. In the biochemical process of anaerobic digestion, the waste goes through three steps as it is converted to methane: hydrolysis, fermentation, and methanogenesis. The potential for using microalgae as an alternative to biofuel crops together with their abilities for $CO_2$ fixation is a promising technology. Some eukaryotic microalgae and prokaryotic (cyanobacteria) microorganisms can synthesize significant amount of lipids under certain environmental conditions, which are the important compounds in making biofuels (Metting & Pyne, 1986). The use of this conversion technology eliminates several of

the key obstacles that are responsible for the current high costs associated with algal biofuels, including drying, extraction, and fuel conversion, and as such may be a cost-effective methodology.

Golueke, Oswald and Gotaas (1957) initiated the very first investigation of the anaerobic digestion of microalgae under different conditions such as temperature, alum in the algal feed, detention period, and loading on digestion performance. The study demonstrated microalgae, grown on domestic sewage and separated either by centrifugation or by coagulation with filter alum, digest readily when placed under the proper environmental and operating conditions. The microalgal culture, consisting principally of *Scenedesmus spp*. and *Chlorella spp.,* was concentrated to a paste having 15 % solids, and then diluted with water in a Waring Blendor to a slurry having a solids content of 8 to 9 %. The best digester performance was obtained at 50 ℃ with a detention period of 11 to 30 days. The maximum permissible loading rate was not determined, but it is greater than 0.18 lb of volatile matter per cu ft of culture volume per day. Under normal conditions, each pound of algal volatile matter introduced into a digester yielded approximately 8 cu ft of gas, of which approximately 2.5 cu ft was $CO_2$, 5.0 cu ft $CH_4$, 0.5 cu ft $H_2$, $N_2$, and other gases. The study also concluded that digestion of microalgae was characterized by a tolerance of sudden and wide variations in the environmental conditions under which the process was operating (Golueke et al., 1957).

Several studies have been carried out that demonstrate the potential of this approach. A recent study indicated that biogas production levels of 180.4 ml $g^{-1}$ $d^{-1}$ of biogas can be realized using a two-stage anaerobic digestion process with different strains of algae, with a methane concentration of 65% (Vergara-Fernández et al., 2008). If this approach can be modified for the use of microalgae, it may be very effective for situations like integrated wastewater treatment, where microalgae are grown under uncontrolled conditions using strains not optimized for lipid production.

Anaerobic digestion studies on algae are scarce when compared to other organic substrates. Studies considering anaerobic digestion of microalgae are less common

than those considering macroalgae (Sialve et al., 2009). In general research utilising unicellular algae can be separated into two main approaches: either a multispecific biomass is harvested from a wastewater treatment pond or a monospecific biomass grown in the laboratory. Sialve (2009) has summarized the experimental conditions and the corresponding methane conversion yield on some reported studies on microalgae anaerobic digestion (Table 1.6). The methane yield varies from 0.09 to 0.45 L g $VS^{-1}$ among these studies. The overall methane yield performance, however, is species and culture conditions dependant, irrespective of whether mixed or single algae cultures are used.

Table 1.6 Experiments with anaerobic digestion of microalgae species and algal sludge: substrate characteristics, methane yield and process conditions (Sialve et al., 2009)

| **Reactor** | **Substrate** | ***T*[a] (°C)** | **HRT[b] (d)** | **Loading rate (g VS $L^{-1}$ $j^{-1}$)** | **Methane yield (L $CH_4$g $VS^{-1}$)** | **$CH_4$(% vol)** | **References** |
|---|---|---|---|---|---|---|---|
| Batch 11 L | Algae sludge (*Chlorella–Scenedesmus*) | 35–50 | 3–30 | 1.44–2.89 | 0.17–0.32 | 62–64 | (Golueke et al., 1957) |
| | Algal biomass | 35 | 28 | 1 | 0.42 | 72 | (Chen, 1987) |
| | *Spirulina* | 35 | 28 | 0.91 | 0.32–0.31 | | |
| | *Dunaliella* | 35 | 28 | 0.91 | 0.44–0.45 | | |
| CSTR[c] 2–5 L | *Tretraselmis* (fresh) | 35 | 14 | 2 | 0.31 | 72–74 | (Marzano et al., 1982) |
| | *Tretraselmis* (dry) | 35 | 14 | 2 | 0.26 | 72–74 | |
| | *Tretraselmis* (dry) + NaCl 35 g/L | 35 | 14 | 2 | 0.25 | 72–74 | |
| Batch 5 L | *Chlorella vulgaris* | 28–31 | 64 | – | 0.31–0.35[d] | 68–75 | (Hernández & Córdoba, 1993) |
| Semi-continuous (daily fed) 10 L | *Spirulina maxima* | 35 | 33 | 0.97 | 0.26 | 68–72 | (Samson & Leduy, 1982) |
| Fed batch 2 L | *Spirulina maxima* | 15–52 | 5–40 | 20–100 | 0.25–0.34 | 46–76 | (Samson & Leduyt, 1986) |
| CSTR[c] 4L | *Chlorella–Scenesmus* | 35 | 10 | 2–6 | 0.09–0.136 | 69 | (Yen & Brune, 2007) |

a Temperature.

b Hydraulic Retention Time.

c Continuous Stirred-Tank Reactor.

d Estimated from data given in L $CH_4$ $gCOD^{-1}$ using a COD/VS ratio of 1.5 (where COD is the Chemical Oxygen Demand).

### 1.7.3.1. Operational conditions effecting anaerobic digestion

The process of anaerobic digestion can be effected by a number of environmental factors, operating conditions, and the waste characteristics. Monitoring these environmental conditions is an important procedure for evaluating the stability of the anaerobic digestion process. The following considers some of the main factors influencing the anaerobic digestion process.

*Temperature*

There are two temperature ranges are commonly used: mesophilic between 35-37ºC and thermophilic between 55-60ºC. These temperature ranges mostly affect methanogens since acidogenic bacteria are not as temperature sensitive (Yu & Fang, 2003). Operation at thermophilic temperature ranges offer potential benefits. Mackie and Bryant (1995) noted that the loading rates of thermophilic systems have been shown to be more robust and can cope with higher loading rates than mesophilic systems. Svoboda (2003) found that biogas production was effectively increased when the digestion temperature of pig slurry was increased from 33°C to 39° to 42°C (Svoboda, 2003). Similarly Feilden (1981) recommended the optimal temperature for maximum gas production for livestock wastes was 40° to 44°C (Feilden, 1981). Recently, in some of the centralised anaerobic digestion plants in Denmark, the treatment temperature has been maintained in the thermophilic range of between 55° and 62°C (Buchanan et al., 2013).

Also, thermophilic digestion provides an advantage by greatly reduce pathogens present within the waste stream. In contrast, there are also studies showing the digestion performance at thermophilic temperature was more unstable than at mesophilic temperatures with no significant improvement on the degradation rate (Archer, 1983). Comparison of using mesophilic temperatures with thermophilic temperatures suggested that the necessary increased energy input may not be warranted based on methane yields. Ghosh et al. (2000) reported that methane yield in thermophilic laboratory-scale digesters has been shown to be only 7% higher than in the equivalent mesophilic system (Ghosh et al., 2000). Practically, temperatures used

for large scale digesters are usually selected on economics due to the higher cost associated with thermophilic digestion. In some cases, additional energy may be required to heat the digester to maintain the specific temperatures (Fang & Chung, 1999; Kim et al., 2002; Maibaum & Kuehn, 1999). For example, a part of the $CH_4$ generated from the thermophilic digesters are used to maintain the temperature and therefore decreasing the amount of $CH_4$ available for more beneficial use. Alternatively, heat is also recovered from combustion or electricity generators nearby.

When anaerobic digestion occurs in covered anaerobic lagoons (CAL), it is important to keep in mind that there is no temperature control. This process can be significantly affected by temperature, with biogas production at lower temperatures such as in the winter months. Pond covers are usually made of a black material which will absorb heat and help to maintain the pond temperature during winter months. Light-colour materials are also used and these may have better resistance to UV degradation due to the lower surface temperature. Therefore, studies performed in control temperature environments in laboratory-scale anaerobic digesters are not necessary to represent the performance of $CH_4$ production in outdoor and no temperature control CALs.

*pH*

Optimal pH is different for each group of microorganisms active within the different phases of the anaerobic digestion. Many studies have concluded that pH was the one parameter that microbial communities had the most difficulty in adapting to if it is below a certain threshold (Callaghan et al., 1999; Dearman, 2005; Ghaly et al., 2000; Lay et al., 1997).

McCarty & McKinney (1961) stated the optimal pH for anaerobic digestion, particularly in the methanogenesis phase is between 6.5 and 7.5. At these levels the volatile fatty acids (VFA) have no significant toxic effects upon methanogenic bacteria at concentrations up to 1,000 mg/L (McCarty & McKinney, 1961). Since the acetogenic phase of the digestion has a higher reaction rate than the methanogenic phase, accumulation of organic VFA can occur in the reactor causing a decrease in pH and a further increase in VFA concentration. This can also be a consequence of

overloading the biomass with organic material or from the effect of inhibitors like antibiotics or disinfectants. When the process is not corrected and the concentration of VFA (volatile fatty acid) is not reduced to tens or hundreds of mg VFA $L^{-1}$, the production of methane can stop and only carbon dioxide is produced. VFA in their protonated form are toxic to microbial cells. When the pronated VFAs enter the cells because of the similarity of intercellular pH being around 7.0, these VFAs then become ionised releasing the hydrogen ion and causing a decrease in intercellular pH and exerting toxicity (McCarty & McKinney, 1961).

In Callaghan et al.'s study (1999), cattle slurries were mixed with range of solid wastes and allowed to co-digest in 1-1 (manure is diluted with water to 7.5% and 15% solids due to a high total solids content of 27.2% in chicken manure) batch digesters. The native pH levels on each solid wastes vary. In terms of the specific methane yield ($m^3$ $CH_4$ $kg^{-1}$ $VS_{removed}$), the study found that the co-digestions containing fish offal and the brewery sludge gave higher values in methane yield ($m^3$ $CH_4$ $kg^{-1}$ $VS_{removed}$) than the control digestion with cattle slurry alone. Compared with their control (cattle slurry alone), both co-digestions with poultry manure (7.5 and 15% TS) gave higher cumulative productions of methane and the system with the lower concentration of poultry manure gave a higher specific methane yield. However, free ammonia concentration was found in both the digestions with chicken manure suggests that it causes inhibition on the digestions (Callaghan et al., 1999).

In Lay et al.'s study (1997), a series of organic waste including sludge cake, meat, carrot, rice, potato and cabbage were examined to determine how environmental factors effect methane productions. The study found that the methanogenic activity of these digesters decreased with a decrease in the moisture content. The moisture content threshold limit, at which the methanogenic activity dropped to zero, was found to be 56.6% for the sludge cake, but greater than 80% for meat, carrot and cabbage. In the high-solids sludge digestion, the relative methanogenic activity dropped from 100% to 53% when the moisture content decreased from 96% to 90%. The study also found that the rate of methane production at moisture contents of 90% to 96% functioned in a pH range between 6.6 and 7.8, but optimally at pH 6.8, and the process may fail if the pH was lower than 6.1 or higher than 8.3. The authors also observed

that the methanogenic activity was dependent on the level of ammonium, $NH_4^+$, but not free ammonia, $NH_3$ , indicating that the $NH_4^+$ was the more significant factor rather than the $NH_3$ in affecting the methanogenic activity of a well-acclimatized bacterial system. In the wide pH range of 6.5 to 8.5, the methanogenic activity decreased with the increase in the $NH_4^+$; dropped 10% at the $NH_4^+$-N concentration of 1670-3720 mg $L^{-1}$, 50% at 4090-5550 mg $L^{-1}$ and dropped to zero at 5880-6600 mg $L^{-1}$ (Lay et al., 1997).

Ghaly et al. (2000) investigated the effects of reseeding and using sodium bicarbonate for controlling the pH on the performance of a two-stage mesophilic anaerobic digester. The study found that it was necessary to control the pH of the digester during the anaerobic digestion of acid cheese whey. Without pH control, the very low pH (3.3) inhibited the methanogenic process and as a result the gas produced contained little or no methane. The pH inhibition of methanogens was irreversible and the digester did not recover (no methane production) when the pH was restored to 7 (without reseeding). Restoring the pH to 7 without reseeding only increased the gas production which was a false indication of recovery as the gas was mainly carbon dioxide (Ghaly et al., 2000).

Dinamarca et al. (2003) examined the influence of the pH in the first stage, the hydrolytic stage, of the anaerobic digestion of the organic fraction of urban solid waste in a two phase anaerobic reactor at three controlled pHs 6, 7, and 8, and one with free pH, the temperature was keep at 37 °C. The higher degradation of TSS and VSS was obtained in the reactors operated at pH 7 and 8; 75% degradation of TSS and 85% degradation of VSS. The volatile fatty acids were determined at the different pH conditions, no significant differences were found, and as was expected, the acetic acid was found at the higher value among them (from 25 to 29 g/L). It was suggested that in the case of the hydrolytic stage of the anaerobic digestion of the organic fraction of urban solid waste, it was not necessary to control the pH because it was kept stable by the buffer effect of the protein residues and other macromolecules present in the residue (Dinamarca et al., 2003).

*Ammonia*

Both ammonia (unionised, $NH_3$-N) and ammonium (ionised, $NH_4^+$-N), are rapidly formed in a digester during the decomposition of proteins. Their relative concentrations vary in response to pH. At higher pH values, the more toxic unionised form ($NH_3$) dominates in anaerobic systems (Mata-Alvarez et al., 2000). Particularly in pig and poultry livestock slurry, the ratio of carbon to nitrogen can be as high as 100:5 and therefore the ammoniacal nitrogen concentration can reach levels of 2 to 6 g $NH_4^+$ -N $L^{-1}$. Although some studies have shown to successfully operate at $NH_3$-N levels of 3500 mg $L^{-1}$ and as high as 4000 mg $L^{-1}$, free $NH_3$-N levels should be maintained below 80 mg $L^{-1}$ while ammonium ion can generally be tolerated up to 1500 mg $L^{-1}$ as $NH_4^+$ - N (Angenent et al., 2002; Buchanan et al., 2013; Dearman, 2005; Van Velsen et al., 1979; Wang et al., 1997).

In addition to Lay et al's. study (1997), it was found that the methanogenic activity was dependent on the level of ammonium, $NH_4^+$, but not free ammonia, $NH_3$ , indicating that the $NH_4^+$ was the more significant factor rather than the $NH_3$ in effecting the methanogenic activity of a well-acclimatized bacterial system. In the wide pH range of 6.5 to 8.5, the methanogenic activity decreased with the increase in the $NH_4^+$; dropping 10% at the $NH_4^+$-N concentration of 1670–3720 mg $L^{-1}$, 50% at 4090–5550 mg·$L^{-1}$ and to zero at 5880–6600 mg $NH_4^+$-N ·$L^{-1}$. However, the lag phase time was dependent on the $NH_3$ 1evel, but not on $NH_4^+$, and when $NH_3$-N was higher than 500 mg $L^{-1}$, a notable shock was observed. This suggests that the $NH_3$ level was the more sensitive factor than the $NH_4^+$level for an unacclimatized bacterial system (Lay et al., 1997).

*Sulphides*

The degradation of proteins in anaerobic reactors also causes the accumulation of sulphides as well as the reduction of sulphates presented in the influent. The total concentration of soluble sulphides depends upon the pH of the liquid phase, the presence of heavy metals, and the composition of the gas phase (Lawrence et al., 1966). To avoid inhibition of methanogenic bacteria metabolic activity, the concentration of

soluble sulphides should not be exceed 200 mg $L^{-1}$. The addition of metal such as iron can be used to reduce the soluble sulphide concentration in anaerobic digestion due to their highly insoluble sulphide precipitates (Buchanan et al., 2013; Lawrence et al., 1966)

*Organic loading rate*

Hydraulic residence time (HRT) and the organic loading of the digester affect the reduction of organic matter in the treated slurry (stabilisation) and the production of biogas. Slurry solids concentration used in anaerobic digestion plants is assumed to be between 4 to 6 % in dry matter (Svoboda, 2003).

Using the fermentation kinetics model developed by Chen & Hashimoto (1978), the effects of $S_0$ (influent VS concentration), HRT and temperature can be concluded. *B*, which is expressed as $m^3$ $CH_4$ per kg VS fed at infinite retention, can be described by Equation 1.14 (Chen, 1987):

$$B = B_0 \left[1 - \frac{K}{\theta_{\mu m} - 1 + K}\right] \quad \text{Equation 1.14}$$

Where:

$S_0$ =influent VS concentration (g $L^{-1}$);

$B_0$ = ultimate $CH_4$ yield (litres of $CH_4$ per gram of VS added) as $\theta \rightarrow \infty$;

$\theta$ = HRT (days);

$\mu m$ = maximum specific growth rate of microorganisms per day (for 35 °C, $\mu m$ = 0.33);

$K$ = kinetic parameter (dimensionless)

*K* was calculated using the values of *B* from this study (Fischer et al., 1984) and from Fischer *et* al. (1975), $B_0$ = 0.49 litre $CH_4$ per gram of VS added as determined by Hashimoto (1984) and $\mu m$ =0.33 $day^{-1}$ at 35°C as reported by Hashimoto et al. ( 1983). Figure 1.13 is a plot of calculated *K* values versus $S_0$, and an empirical relationship described by (Hashimoto, 1984; Hashimoto, 1983):

$K = 0.5 + 0.03 \exp(0.05 * S_0)$ Equation 1.15

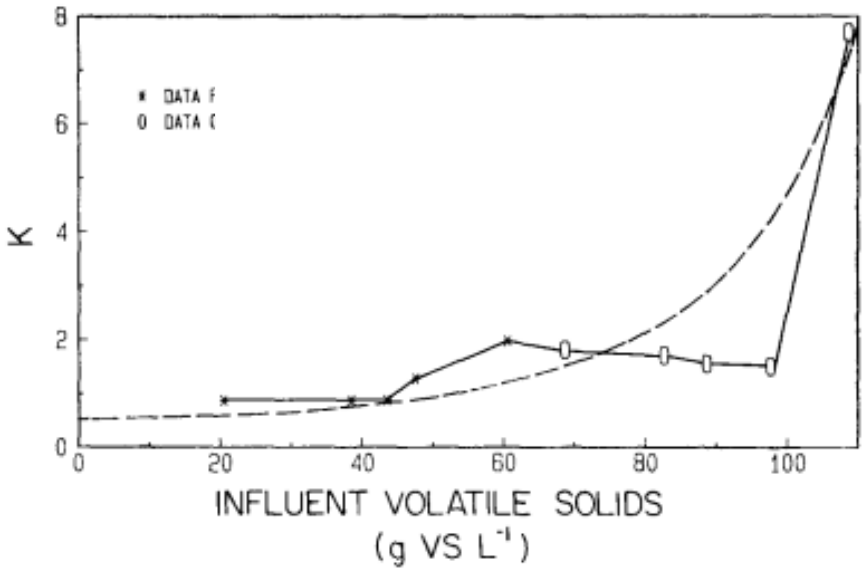


Fig. 1.13 The relationship between the dimensionless parameter, K, and influent VS concentration. (x) as data adapted from (Fischer et al., 1974); (0) as data adapted from (Fischer et al., 1984)

Using the *K* values shown in Fig. 1.13 and Equation (1.15), *B* can be calculated for various HRTs (0). The equation can be used to compare with other data and to indicate trends. Fig. 1.13 shows a three-dimensional plot of HRT and $S_0$ versus *B*. This figure shows as HRT increases, B increases. This is based an observation that the bacteria have more time to break down macronutrients from the manure into usable substrates for methane formation. However, this figure also shows as $S_0$ increases, *B* decreases. This indicates that the bacteria seem to become overwhelmed by nutrients and toxic byproducts of digestion. At short HRT, these nutrients are flushed out of the digester before the bacteria can utilize them, which can be observed when comparing a 5-day HRT with a 30-day HRT in Fig. 1.14. Therefore, Fischer (1984) stated that a digester can be loaded at an $S_0$ of 70 to 80g of VS $L^{-1}$ without seriously reducing *B* if the HRT is longer (Fischer et al., 1984).

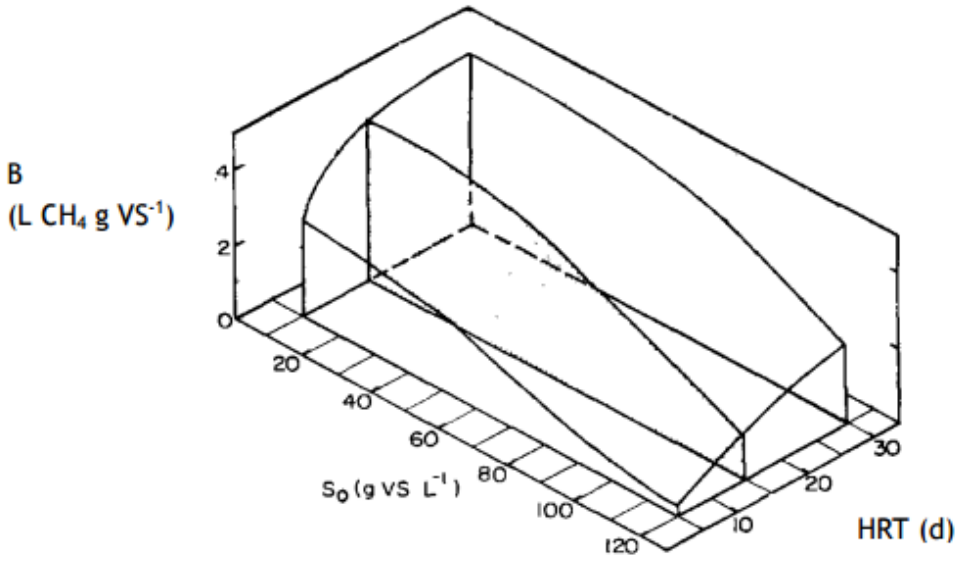


Fig. 1.14 Theoretical relationship between B ($CH_4$ production g $VS^{-1}$ added) and HRT (d) and influent VS concentration (Fischer et al., 1984)

### 1.7.4. Application of algae on piggery wastewater treatment

In previous sections, it was suggested the integration with wastewater treatment is the only plausible near- to mid-term application of microalgal biofuels production (Sheehan et al., 1998). This is due to the economic and resource constraints are much more relaxed and therefore it is allowing for such processes to be considered with well below maximal productivities (Borowitzka, 2013a). Apart from treating municipal wastewater, microalgae are also capable of treating different types of wastewater.

There are substantial literature reports demonstrating the effectiveness of HRAPs for combined biomass production from pig wastes albeit diluted to varying degrees and improved effluent treatment. Pond and lagoon systems are familiar technology to the pork industry in Australia since the majority of producers currently use lagoons for wastewater treatment. With the high effectiveness of HRAPs previously described, they can be used as a more efficient variant of current pig effluent treatment technology offering improved treatment and added value from biomass production.

#### 1.7.4.1. Integrated piggery wastewater treatment and microalgae – a review by Pork CRC

*Piggery anaerobic lagoons*

The majority of Australian pork producers currently use a series of anaerobic and facultative lagoons to treat piggery manure. They are most commonly used for livestock waste treatment because they provide a convenient, economical and simple method for stabilising organic matter into less reactive compounds and gases in the process of treating effluent. They are also able to store, dilute and treat high strength effluent rather inexpensively with minimal labor and maintenance. The land area required to construct such a lagoon is also relatively small, making them practical for many operations. However, pond malfunctioning does occur. A common fault is caused a result of over-production of volatile fatty acids (VFAs) which is gradually developed by overloading with organic matter, either continuously i.e. the pond is undersized or needs desludging, or due to shock loading. In addition, the desludging processes can be very expensive and potentially release offensive odours as well (Tucker, 2010).

Standard anaerobic lagoons are 4-6 m deep with a length to width ratio of 2-3:1. As a general guideline, 6 - 8 $m^3$ of pond volume should be provided per standard pig unit (SPU), although lesser volumes e.g. 4 - 6 $m^3$ or less are possible with efficient solids removal (Kruger et al., 1995). To avoid hydraulic short-circuiting influent should be added at a point as far away as possible from the outlet of the treated. Furthermore, Kruger *et al.* (1995) also noted the use of organic loading rate method to size the treatment capacity plus an allowance for sludge storage to provide a more accurate method for sizing an anaerobic pond which is based on VS and *K* factors (Kruger et al., 1995). By using Murray Bridge area as an example (rural city located about 60 km east of Adelaide, mean annual rainfall is 347 mm), Kruger et al. (1995) suggest a K factor of 0.73, which translates to a VS loading rate of about 73 g VS $L^{-1}$ /d (refer to Equation 1.15). The authors also suggested a further 25-40% of pond volume should be added for sludge storage (Kruger et al., 1995).

Most primary piggery effluent treatment ponds are anaerobic. A properly functioning anaerobic pond can reduce the VS content of effluent by up to 70%. However, any anaerobic pond will require periodic desludging to maintain effective treatment capacity at designed HRTs, due to accumulation of stabilised sludge at the bottom of the pond at a rate proportional to the amount of total solids treated (Hudson et al., 2004). Table 1.7 shows suggested details for large anaerobic ponds in different climatic zones, desludging frequencies, and pre-treatment options of the effluent stream.

Table 1.7 Sizing details for large anaerobic ponds in three broad climatic zones, with different desludging frequencies, with or without pre-treatment of the effluent stream (Hudson et al., 2004)

| **Climate** | **Desludging frequency** | **Effluent treatment & desludging frequency ($m^3$/SPU)** | |
|---|---|---|---|
| | | **No pre-treatment** | **Pre-treatment[a]** |
| **Cool[b]** | Annually | 4.6 | 3.5 |
| | 5 yearly | 6.0 | 4.6 |
| | 10 yearly | 7.7 | 5.9 |
| **Warm[c]** | Annually | 3.5 | 2.7 |
| | 5 yearly | 4.9 | 3.8 |
| | 10 yearly | 6.6 | 5.1 |
| **Hot[d]** | Annually | 2.9 | 2.2 |
| | 5 yearly | 4.3 | 3.3 |
| | 10 yearly | 6.0 | 4.6 |

SPU = standard pig unit

[a] Assume a screen that removes 20% of the TS and 25% of the VS (e.g. a stationary run-down screen).

[b] Based on a treatment capacity loading rate of 60 g VS/$m^3$/day/ Examples of localities with cool climates are Armidale New South Wales, southern and central Victoria, southern South Australia, and Tasmania.

[c] Based on treatment capacity loading rate of 80 g VS/$m^3$/day. Examples of localities with warm climates are most of inland New South Wales, South-East Queensland, South Australia and southern Western Australia.

[d] based on a treatment capacity loading rate of 100 g VS/$m^3$/day. Examples of localities with hot climates are central to northern Queensland, Moree and Goondiwindi.

*HRAP with piggery wastewater*

Previous laboratory work in the USA (Barlow et al., 1975; Boersma et al., 1975) Ireland (Wilson & Houghton, 1974), Canada (Waygood et al., 1980) and Germany (Baumgarten et al., 1999) showed that algal growth could be substantial in the diluted

liquid phase of pig slurry. Subsequent work (Allen & Garrett, 1977; Garrett & Allen, 1976; Garrett et al., 1978; Garrett et al., 1976) selected *Chlorella vulgaris* as a candidate species, which was nutritionally valuable and that algal culture adversely affected the survival of bacterial pathogens. Gonzalez, et al (1997) and Travieso, et al., (2006) have similarly conducted laboratory based research to determine the performance of *Chlorella* cultures grown on diluted pig slurry (González-Fernández et al., 2010; Travieso et al., 2006a; Travieso et al., 2006b). Fallowfield & Garrett (1985) conducted a pilot plant study in Northern Ireland with the objective of establishing an energy budget for the growth of algal biomass in high rate algal ponds on pig slurry liquid phase. The raw slurry was pre-treated by rotary press screen separation, polymer flocculation and sedimentation, however, the decanted liquid phase required 1:9 dilution with water to enable algal growth (Fallowfield & Garrett, 1985). The hydraulic retention time varied between 12.8 d (autumn) and 4.4 d summer at pond operating depths of 0.2 m. The mean long term productivity, over the limited 153 d growing season in Northern Ireland, corrected for incoming solids, was 18.1 g DM $m^{-2}$ $d^{-1}$ equivalent to 26.7 T $ha^{-1}$ ; the gross dry matter output (not corrected for incoming solids) was estimated at 41.5 T $ha^{-1}$ over 153 days. Groeneweg et al (1980) growing *Scenedesmus spp.* and *Coelastrum sp.* in even more dilute pig slurry in Germany reported algal productivities between 2.5 and 14.0 g DM $m^{-2}$ $d^{-1}$ (Groeneweg et al., 1980). Other measured productivities in piggery wastewaters include; De Pauw et al. (1978) reported mean productivities of 12 g DM $m^{-2}$ $d^{-1}$ for *Scenedesmus acutus* grown in filtered diluted pig slurry (De Pauw et al., 1978), Boersma et al. (1975) 22 g DM $m^{-2}$ $d^{-1}$ in Oregon (Boersma et al., 1975), 30 g DM $m^{-2}$ $d^{-1}$ in Florida (Lincoln & Hill, 1980) and 25 g DM $m^{-2}$ $d^{-1}$ in Singapore (Lee & Dodd, 1980).

Initial laboratory studies by Garrett et al. (1978) suggested 1:1 dilution of slurry was suitable for algal growth, however, the 1:9 dilution of slurry liquid phase necessary to achieve outdoor culture this increased the estimated area required to treat effluent from 100 pigs from 10 $m^2$ to 100 $m^2$ (Garrett et al., 1978). Under Australian conditions, heavy dilution will require large amounts of fresh water, which is often in short supply. It also could make the HRAP pond area so large as to be too costly to construct. Therefore, dilution should be avoided when HRAP is integrated into piggery wastewater treatment. The proposed option by PorkCRC was to pre-treat anaerobically digested effluent by one of two methods aerobic pre-treatment or biological filtration.

Overall there is a substantial body of literature which demonstrates the effectiveness of HRAPs for the integrated treatment of, and biomass production from, animal wastes albeit diluted to varying degrees. Furthermore, HRAPs have been integrated with aerobic treatment and operated at pilot scale on a piggery. Pond and lagoons are familiar technology to the pork industry since the majority of producers currently use lagoons for wastewater treatment and biogas production. HRAPs may be seen as a more efficient variant of current technology offering improved treatment and 'added value' biomass production; the research challenge is to manage the issues associated with light attenuation and ammonia toxicity which adversely affect algal growth, without using dilution.

#### 1.7.4.2. Anaerobic co-digestion on piggery manure and microalgal biomass– a review by Pork CRC

Combination of various substrates is an effective way to enhance the performance of a digester by ensuring an optimal influent composition, C:N ratio and effect on biogas yields (Sialve et al., 2009).

*C:N ratio and biogas yields*

The low C:N ratio of algal sludge is a challenge for anaerobic digestion. Although, an optimum C:N range in feedstock for the anaerobic digestion is still debatable in the literature, 20–30 is a most acceptable range (Parkin & Owen, 1986). The C:N ratio in algal sludge is about 6, which is too low for the digestion.

Since the optimum C:N ratio is between 20 and 30 (Parkin & Owen, 1986) and general sewage sludge has a C:N ratio of between 6 and 16, co-digestion with other organic waste with a higher C:N ratio could improve the nutrient balance, increasing the amount of degradable carbon, and most importantly the biogas yield (Silvestre et al., 2011; Sosnowski et al., 2007). Low C:N ratio feedstock could also result in high total ammonia nitrogen (TAN) released and high volatile fatty acid (VFA) accumulated in the digesters. When C:N ratio is lower than 20, there is an imbalance between carbon and nitrogen requirement for the anaerobic microflora (Speece, 1996) leading to

nitrogen ($NH_3$) release, which can become inhibiting and results in an accumulation of VFAs (Sialve et al., 2009). The TAN and VFAs are both important intermediates and potential inhibitors in the anaerobic digestion process. High concentration of TAN and VFAs in the digester would decrease the methanogen activity and further accumulation could cause the failure of anaerobic digestion (Yen & Brune, 2007).

Yen and Brune (2007) reported a significant enhancement of the methane production following the addition of a series of 25%, 50%, 75%, and 100% (based on volatile solid) of waste paper in algal sludge feedstock. In mesophilic conditions operated at 4g VS $L^{-1}$ $d^{-1}$, 35ºC and 10 days retention time, the methane production rate (1.17 L $CH_4$ $L^{-1}$ $d^{-1}$) at 50% of volatile waste paper solids added to an algal sludge feedstock was double that recorded for the anaerobic digestion of algal biomass alone (0.57 L $CH_4$ $L^{-1}$ $d^{-1}$). Furthermore, the study also found the maximum methane production rate of 1.61 L $CH_4$ $L^{-1}$ $d^{-1}$ was observed at a combined 5 g VS $L^{-1}$ $d^{-1}$ loading rate, with 60% of volatile waste paper solids added to an algal sludge feedstock. The optimum C:N was observed to be between 20 and 25:1 (Sialve et al., 2009; Yen & Brune, 2007).

One method to avoid excessive ammonia accumulation is to adjust low feedstock C:N ratios by adding high carbon content materials, and therefore improving the digestion performance (Yen & Brune, 2007). Such approach was confirmed by Sosnowaki *et al.* (2003) who combined sewage sludge and municipal solid waste (MSW) for codigestion. Most MSW consists of paper material including office and newspaper has a C:N ratio ranging from 173:1 to greater than 1000:1 while typical sewage sludge has a C:N ratio ranging from 6:1 to 16:1 (Sosnowski et al., 2007; Stroot et al., 2001). Another example of codigestion with high C:N and low C:N feedstocks was the mixture of cattle manure slurry with fruit, vegetable wastes (FVW) and chicken manures which also improved digester performance (Callaghan et al., 2002). The study found that by increasing the proportion of FVW from 20% to 50% improved the methane yield from 0.23 to 0.45 $m^3$ $CH_4$ $kg^{-1}$ VS added, and caused the VS reduction to decrease slightly. Co-digestion of sisal pulp and fish wastes has shown a 59%-94% increase in the methane production yield as compared to single substrate anaerobic digestion (Mshandete et al., 2004; Yen & Brune, 2007). This study found that co-digestion with 33% of fish waste and 67% of sisal pulp representing 16.6% of TS, a

methane yield of 0.62 $m^3$ $CH_4$ / kg VS added was obtained. This was also supported by other studies that involved using some microalgae species such as *S. maxima* and protein-extracted algae to optimise the codigestion C:N ratio between 25 and 35 (Angelidaki et al., 2003; Chen, 1987; Samson & LedDy, 1983).

As previously discussed, on the basis of an average composition of microalgae given by $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ (Grobbelaar, 2004b), the nitrogen and phosphorus requirement per unit of surface and per year can be estimated. This leads to a nitrogen amendment that varies from 8 to 1 T N/ha/year. This figure is in a range of 55 to 111 times greater than for rapeseed (Halleux et al., 2008). Anaerobic digestion is not only a biotechnological process that can mineralize algal waste containing organic nitrogen and phosphorus, but also the biomass after lipid extraction can be transformed into methane to maximize the economical balance (OlguÌn, 2000; Phang et al., 2000). Therefore, if anaerobic digestion is used to process algal waste, it will not only recycle nitrogen and phosphorus but also potentially lead to an energetic balance of the microalgae to biofuel process.

Although some processing is possibly required to provide an optimum solid:VSS ratio before feeding into a digester, the use of algal anaerobic digestion eliminates several of the key obstacles that are responsible for the current high costs associated with algal biofuels, including drying, extraction, and fuel conversion, and as such may be a cost-effective methodology. Several studies have been carried out that demonstrate the potential of this approach. According to Sialve et al. (2009), the methane content of the biogas from microalgae is 7 to 13% higher when compared with the biogas from maize (Sialve et al., 2009). A recent study indicated that biogas production levels of 180.4 mL/g /day of biogas can be realized using a two-stage anaerobic digestion process with different strains of algae, with a methane concentration of 65% (Vergara-Fernández et al., 2008). Microalgae biomass composition is directly related to growth conditions and most microalgae have the capacity, under certain conditions, to accumulate important quantities of carbon in the form of starch or lipids (Qiang, 2007). A common circumstance to stimulate this accumulation of lipid is nitrogen deficiency. Calorific value is directly correlated with the lipid content, and nitrogen starvation results in a significant increase in the caloric value of the biomass with a decrease in

the protein content and a reduction in the growth rate (Illman et al., 2000). Based on the data of these authors, Sialve et al. (2009) evaluated the energetic content (normal and N-starvation growth) of the microalgae *C. vulgaris*, *C. emersonii* and *C. protothecoides*, in two scenarios, such as the anaerobic digestion of the whole biomass and of the algal residues after lipids extraction. From the latter process, biodiesel and methane could be obtained with a higher energetic value (kJ $g^{-1}$ VS), calculated with a methane calorific value of 35.6 kJ $L^{-1}$. However, the energetic cost of biomass harvesting and lipid recovery was probably higher than the recovery energy, especially because most of the techniques involve biomass drying (Carlsson et al., 2007). When the cell lipid content is < 40%, anaerobic digestion of the whole biomass appears to be the optimal strategy on an energy balance basis, for the energetic recovery of cell biomass and a next return on energy invested (Sialve et al., 2009).

Mussgnug et al. (2010), screened several microalgae for their biogas production potential including, *Chlamydomonas reinhardtii, Chlorella kessleri, Euglena gracilis, Spirulina* (*Arthrospira*) *platensis, S. obliquus* and *Dunaliella salina*. It was demonstrated that the quantity of biogas was strongly dependent on the species and on the pre-treatment (drying at 105 ℃ for 24h in this study). *C. reinhardtii* was the more efficient with a biogas production of 587 mL (±8.8 SE) biogas/g volatile solids (Mussgnug et al., 2010). For biogas production, the microalgae species should have a high degree of degradation and low amount of indigestible residues (Mussgnug et al., 2010). The substrates should be concentrated but drying process should be avoided, as it results in a general decrease in the biogas production potential in around 20% from this study. Microalgae are grown in liquid medium for mass cultivation and the dry matter content usually is below 15 g $L^{-1}$ culture, although up to 84 g $L^{-1}$ have been previously reported by a photoautotrophic ultrahigh-cell-density culture of *Chlorococcum littorale* with significantly high (16.7 g $CO_2$ $L^{-1}$ 24 $h^{-1}$) in a photobioreactor (Hu et al., 1998). Efficient biogas production will therefore require a concentration step, e.g. by filtration or centrifugation. Depending on the concentration method, the fresh algal biomass may still contains a high degree of water. The transportation of the wet biomass should also be avoided. The algal production facility and the biogas fermentation plant should be as close as possible (Mussgnug et al., 2010).

Anaerobic digestion well explored in the past, will probably re-emerge in the coming years either as a mandatory step to support large-scale microalgal cultures or as a standalone bioenergy-producing process (Sialve et al., 2009). This technology could be very effective for situations such as integrated wastewater treatment, where algae are grown under uncontrolled conditions using strains that are not optimized for lipid production.

Compared to existing covered anaerobic lagoon, Pork CRC proposes an integrated treatment option to minimise the TS, colour, and oxidising the ammonia to nitrate in piggery effluent prior to feeding an HRAP. Figure 1.15 shows the proposed conditioning of piggery slurry for algal growth. It proposes a combination of pre-treatment by existing covered anaerobic lagoon (CAL) or in an engineered anaerobic reactor, followed by treatment in a close aerobic reactor operated to maximise nitrification. By combining the ability of integrated microalgae treatment options on piggery slurry and bioenergy production, a life cycle assessment (LCA) by Pork CRC suggests this is the least beneficial option in terms of greenhouse gas such as $CO_2$ equivalents (Buchanan et al., 2013).

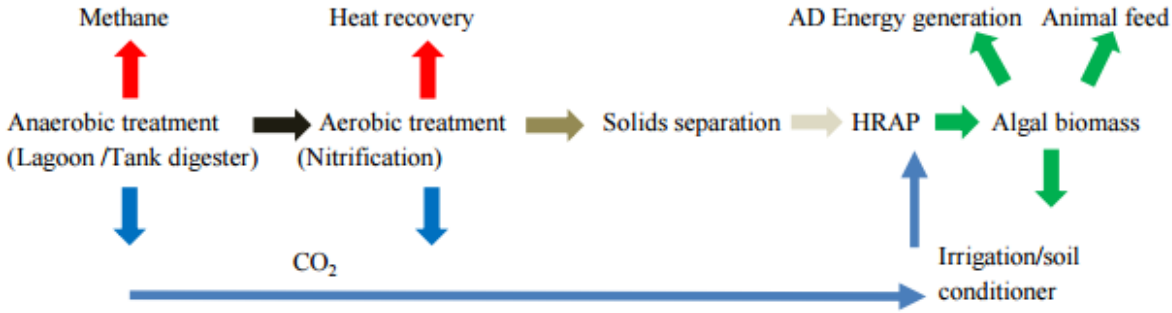


Fig. 1.15 Conditioning piggery slurry for algal growth under the integrated system (Buchanan et al., 2013)

The LCA shows that this this configuration occurs only a small penalty compared with using only a CAL to treat the effluent but has the benefit, by oxidising ammonia to nitrate, of enabling algal biomass production which could be used to generate further methane and resultant electricity. The LCA suggests that this option has the potential to abate over 100kg $CO_2$ -e 60kg HSCW (Hot standard carcase weight) (Fig. 1.16).

The algal biomass (ALBAZOD) is then used for further energy generation by either anaerobic digestion or anaerobic co-digestion with pig slurry into the existing lagoons.

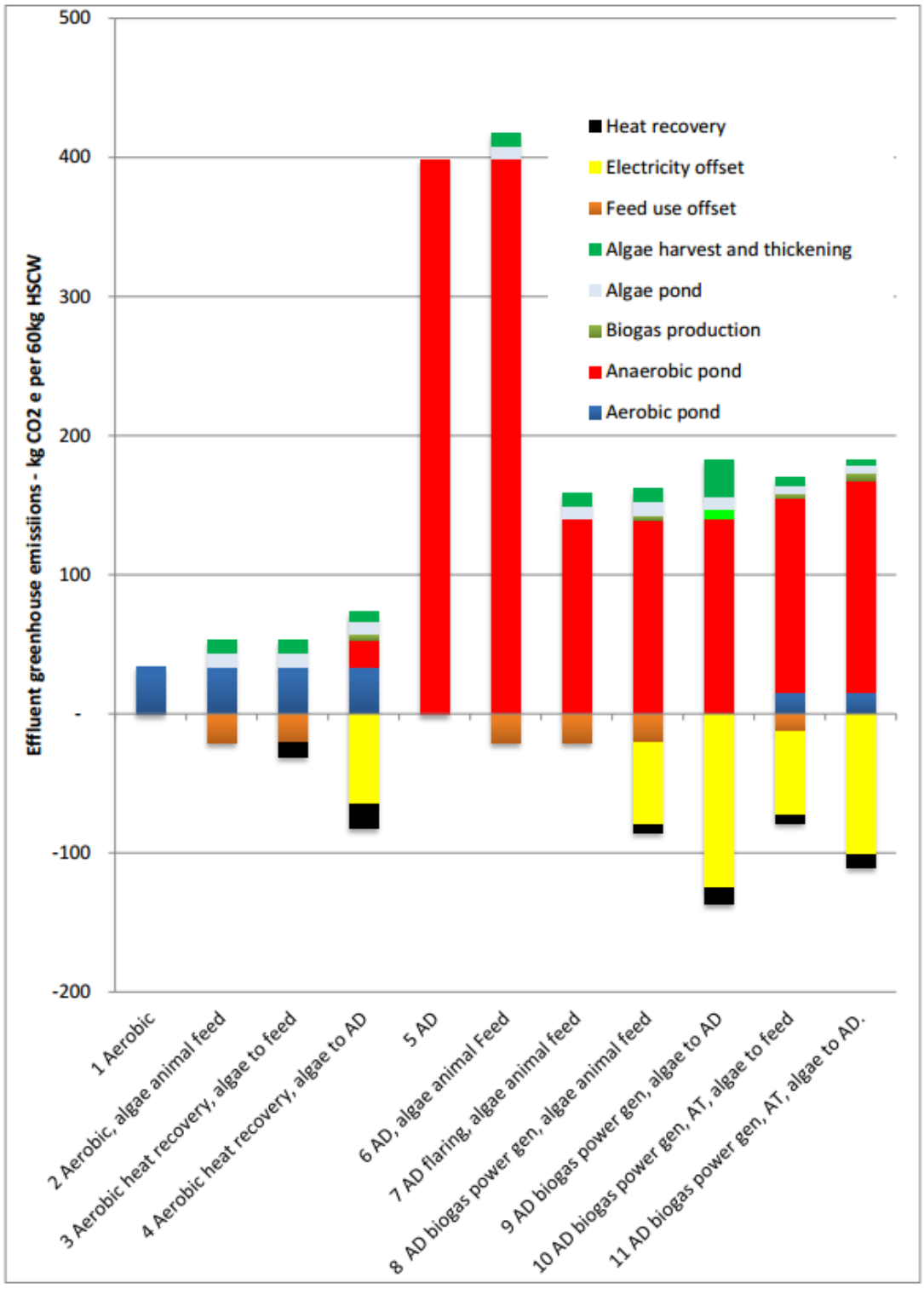


Fig. 1.16 Comparison of 11 scenarios –greenhouse gas results by process contribution from effluent treatment for 1 60kg HSCW, values in kg of $CO_2e$ (Buchanan et al., 2013)

## 1.8. Research objectives

There is a current need to question if $CO_2$ addition enhances algal production in all types of wastewater, due to the conflicting views exit within the literature, and limited comparative investigations have been undertaken. In addition, while other wastes are commonly co-digested e.g. industrial organic wastes, fruit and vegetable solid waste, olive wastes and farm wastes, there are limited studies on the digestion of algal biomass either as a sole substrate or co-digested with other wastes, significantly for this proposal, only limited studies are considered co-digestion with pig slurry.

Based on the summary of this chapter, this research program has four essential research areas to determine:

- The determination of the influence of wastewater strength on the outcome of $CO_2$ addition for algal biomass (ALBAZOD) production
- The evaluation of the performance of anaerobic co-digestion of algal biomass (ALBAZOD) with pig slurry
- The effects of thermal pretreatment of algal biomass (ALBAZOD) on the outcome of anaerobic co-digestion with pig slurry (PS)
- The evaluation of the performance of the anaerobic co-digestion of algal biomass (ALBAZOD) with waste activated sludge (WAS)

This project builds on the outcomes of *Project 4A-101 Algae for Energy & Feed: a wastewater solution* (Buchanan et al., 2013) which reviewed options for the industry to integrate algal biomass production for energy and feed into pig slurry treatment.

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# CHAPTER 2

# MATERIAL AND METHODS

# 2. MATERIAL AND METHODS

This chapter describes the general methods and specialized apparatus used throughout this thesis.

## 2.1. Identification and enumeration of microalgae

The method described below was mainly utilised for collection of data from two sites, Mount Barker WWTP and Kingston-on-Murray HRAP, which is presented in Chapter 3. Samples were taken to identify the species presented in the lagoon and HRAP.

Representative algal samples were collected from each site for both qualitative and quantitative assessment of algal presence. Accumulations of algae, such as scums within the water column, were avoided when collecting quantitative samples. Samples were collected 3 m from the bank of the facultative pond Mount Barker. A 250mL polypropylene bottle attached to a sampling pole (The Mighty Gripper) was submerged, initially with the bottle neck downwards, the water sample at approximately 25cm depth was then obtained by rotating the bottle to enable it to fill. Several samples were collect from the lagoon since the microalgae were not evenly dispersed. The samples were then mixed in a bucket to provide a composite sample.

Wastewater samples for microscopic analysis were stored in a cold room ($3^{o}C$) and analysed within 24 - 48hrs. Extended storage (> a week), required addition of Lugol's iodine solution (final concentration 0.04%) to preserve the samples.

Generally no concentration was required to enumerate algal cells, however, for low concentration samples with fewer cells present, a ten-fold concentration step was required. This was achieved by pouring 100 mL of the preserved sample into a 100 mL measuring cylinder, and waiting $\geq$ 24 hours (Longer duration may be required for smaller sized cells which take longer to sediment). The top 90 mL of the settled sample

was then carefully removed using a pipette and the remaining 10ml of sample used for enumeration.

A standard compound microscope (Olympus BH2 Microscope) was used for algal identification and enumeration. A Sedgwick-Rafter Counting chamber (1000 $mm^2$, Sedgwick-Rafter Cell – SRC REF S51) was used when cell densities were in the range of 30 - $10^4$ cells/mL. For method once concentrated, this needs to integrate the concentration factor = final count x. The 10 fold concentrated sample was mixed and 1.0 mL withdrawn by a pipette. The algal cells in were allowed to settle within the SRC (5 min) before counting. To obtain a counting precision of at least ± 20%, at least 100 units of each dominant species needs to be counted, a unit being either a single cell, a colony or a filament, depending on the morphology of the algal species. A minimum of 30 fields of view were randomly selected and counted. The procedure was repeated to obtain duplicate determinations. The number of cells/mL was then calculated Equation 2.1:

No of cells/mL = (cells counted x 1000 $mm^3$) / (number of fields of view counted x the volume within each field of view in $mm^3$ x (settled volume / initial volume))

**Equation 2.1**

The algal species present were compared with micrograph catalogues of known genera. A range of taxonomic texts were also consulted for genus and species identification (Baker, 1991; Baker, 1992; Baker & Fabbro, 2002; Bellinger & Sigee, 2010; McGregor, 2007; McGregor & Fabbro, 2001).

## 2.2. Chlorophyll a

Chlorophyll *a* concentration was determined using the 90% acetone extraction method of Jeffery and Humphrey (1975). A know volume of sample was filtered through a glass fibre filter (GFC-exclusion pore size 0.22 µm 47mm; Whatman Ltd) and the filter was then immersed in a scintillation vial containing 10 mL of 90% (v/v) acetone/water. The vial was stored at 4ºC for 24 hours in the dark and then 1 mL of

the acetone extract was transferred into a 1 cm Eppendorf micro-centrifuge tube. The sample was then centrifuged at 3000 g for 5 minutes to remove particular mater. The light absorbances at 664, 647 and 630 nm were measured spectrophotometrically (UV-1700 Spectrophotometer Shimadzu) against a 90% (v/v) aqueous acetone blank. The chlorophyll a concentration (µg/ L) was calculated using Equations 2.2. and 2.3 (Jeffery and Humphrey, 1975):

Chl *a* absorbance = 11.85 ($OD_{664}$) – 1.54 ($OD_{647}$) – 0.08 ($OD_{630}$)

**Equation** 2.2

Where: $OD_{664}$, $OD_{647}$ and $OD_{630}$ were the absorbance at the respective wavelength (nm).

Chl. *a* (µg / L) = Chl. *a* absorbance x (volume of acetone (ml)/sample volume (L))

**Equation 2.3**

## 2.3. Solids

### 2.3.1. Total solids (TS)

A known volume of water withdrawn from the well mixed sample (magnetic stirrer) was evaporated in a pre-dried (105$^{o}$C / 24 h) and weighed dish and dried to constant weight (105$^{o}$C / 24 h). The increase in weight over that of the empty dish represents the total solids in the sample volume which was subsequently corrected (mg TS/L). Duplicate determinations should agree to within 5% of their average (APHA, 1992)

### 2.3.2. Suspended solids (SS)

A well-mixed sample of known volume was filtered through a pre-weighted, pre-dried (overnight at 105$^{o}$C) glass-fibre filter (0.45 µm 110 mm GFC; Whatman Ltd.) and the residue retained on the filter dried to a constant weight (105$^{o}$C for 24 h). The increase in weight of the filter represents the total suspended solids which, following calculation were presented as mg SS/L (APHA, 1992).

### 2.3.3. Fixed and volatile solids (VS or VSS)

The residue from determination of either the TS or SS was ignited in a pre-ignited (550$^{o}$C) and weighed silica crucible to constant weight. The remaining solids represented either the fixed total (mg TS/L) or suspended solids (mg SS/L) while the weight lost on ignition was the volatile solids (VS or VSS) (APHA, 1992).

## 2.4. Freeze drying

Biomass in the wastewater was freeze dried prior to compositional analysis. The wastewater (50mL in Polylab Centrifuge Tube, Conical Bottom) was centrifuged (Phoenix, Orbital 400 Clements) at 3000g for 10 mins with a 3 min cool-down. The pellet was washed following resuspension in distilled water (10mL) and centrifuged (3000 g for 10 mins). The pellet was store4d frozen at -80$^{o}$C (Revco Ultima II, Thermo Electron Corporation) for at least 24-72 h. The centrifuge tubes were transferred to the freeze dryer (VirTis BenchTop 2k Freeze Dryers, SP industries) which was pre-conditioned (20-30min) to ensure the condenser had attained -40$^{o}$C and the vacuum system was > 100 mTorr before freeze drying commenced at -67.8$^{o}$C at 30 mTorr. The samples were stored in a desiccator at room temperature for later measurements.

## 2.5. Biological oxygen demand ($BOD_5$)

The five day BOD test was performed as described by APHA (1995) Test 5210 B (5-day BOD analysis) using the OxiTop® BOD measuring system as described by the manufacturer. This respirometric system is incorporated with the OxiTop® OC 100 Controller, OxiTop®-C measuring heads, an inductive stirring system in a temperature controlled cabinet, and dark brown sample bottles. Briefly, an appropriate volume of wastewater was placed within the OxiTop® bottle, two pellets of NaOH were placed inside a rubber quiver which was positioned inside the neck of the bottle to absorb $CO_2$. The bottle sealed by the OxiTop® measuring head and measurement initiated with the OC100 controller. The bottles were placed in the temperature controlled

(20℃) light-sealed cabinet for 5 days. As the $O_2$ level in the bottle decreased the pressure reduction was recorded by strain sensors attached to a rubber diaphragm in the measuring head. The difference in pressure from start to finish was then converted to a $BOD_5$ value (mg $BOD_5$/L) by the inbuilt software in the OC100 controller.

## 2.6. Chemical oxygen demand (COD) and soluble COD

### 2.6.1. Closed reflux, colorimetric method

COD determination (APHA, 1992) was used to obtain results reported in Chapters 4, 5 and 6. For total COD determination a whole mixed liquor sample was used whereas for soluble COD determination, the mixed liquor sample was centrifuged (3,000g for 5 mins) and the supernatant decanted for soluble COD determination. A 5 mL diluted sample (1:1000), for whole or soluble COD analysis, was placed in a tube and mixed with 3 mL of potassium dichromate (0.01667 M $K_2Cr_2O_7$) digestion solution. 3.5 mL sulphuric acid reagent was added down inside of the tube so an acid layer was formed under the sample digestion solution layer, the tube capped and inverted several times. The well mixed sample was digested in a block digester preheated to 150$^o$C and close refluxed for 2h inside the fume cupboard. The digestate was cooled for a minimum of 15 minutes. 0.05 mL Ferroin indicator (Ferrous Ammonium Sulfate, FAS) was added to the cooled sample and titrated with standardized 0.1 M FAS to the end-point as shown by a colour change from blue-green to reddish-brown. A distilled water blank was prepared in the same manner, refluxed and titrated as per the wastewater mixed liquor (APHA, 1992). The COD was calculated using Equation 2.4

$$COD\ (mg\ O_2\ /L) = [(A-B) \times M \times 8000) / (V_{sample})$$

**Equation 2.4**

Where:

A = volume of FAS used for blank (mL)

B = volume of FAS used for sample (mL)

M = molarity of FAS

8000 = milli equivalent weight of oxygen (8) ×1000 mL/L.

### 2.6.2. Carbon analysis

Total carbon comprises inorganic carbon (IC), total organic carbon (TOC) which may be further defined as comprising dissolved organic carbon (DOC) and particulate organic carbon (POC) and dependent upon sample handling prior to presentation for analysis (Table 2.1).

**Table 2.1** Definitions of various terms and parameters according to EN 1484 "Guidelines for determination of total organic carbon (TOC) and dissolved organic carbon (DOC)"

| Fraction of total carbon | Descriptions |
|---|---|
| Total carbon (TC) | The sum of organically bound and inorganically bound carbon present in water, including elemental carbon |
| Inorganic carbon (IC) | The carbonate, bicarbonate, and dissolved $CO_2$ |
| Total organic carbon (TOC) | All carbon atoms covalently boned in organic molecules; |
| Dissolved organic carbon (DOC) | The fraction of TOC that passes through a 0.22 µm filter |
| Particulate organic carbon (POC) | Also referred to as non-dissolved organic carbon, the fraction of TOC retained by a 0.22 µm filter |

The TOC, and its constituents, was analysed using a Total Organic Carbon / Total Nitrogen Analyser – (Shimadzu TOC-LCSH). The TOC was calculated from the difference between TC and IC. The POC was determined from the difference between TOC of filtered (0.22 µm 47 mm GFC; Whatman Ltd.) and whole wastewater samples, with the DOC determined by analysis of the 0.22 µm filtrate (when applicable) (Shimadzu, 2011).

## 2.7. Determination of nitrogen

In waters and wastewater the forms of nitrogen nitrate, nitrite, ammonia and organic nitrogen (in order of decreasing oxidation state) are of interest. Total oxidized nitrogen is the sum of nitrate and nitrite-nitrogen. Prior to analysis of nitrate, nitrite and ammonia, water samples were filtered through a glass fibre filter (GFC, Whatman Ltd.) to eliminate interference by suspended organic matter.

### 2.7.1. Determination of nitrate ($NO_3$-N) and nitrite ($NO_2$-N)

An automatic direct spectrophotometric method for the simultaneous was used to determinate of nitrite and nitrate by flow-injection analysis (APHA, 1992). Briefly, $NO_2$-N reacts with 3-nitroaniline in the presence of 1 M HCl to form a diazonium cation, which reacts with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a stable reddish purple azo dye. The absorbance was measured spectrophotometrically at 540 nm (Shimadzu UV-2550) against a distilled water blank. $NO_3$-N was reduced by copper sulphate in the presence of hydrazine sulphate to nitrite which was then reacted as above. The concentration in the samples was determined from standard lines of known concentrations of $NO_2$-N and $NO_3$-N. The $NO_3$-N concentration was determined by difference between pre and post reduction concentrations. This standard manual method was used for Chapter 4, 5, and 6 (APHA, 1992). Each test was performed in triplicate.

For managing a large quantity of sample analysis in Chapter 3, the Colorimetric Method described in Test 4500-$NO_2^-$ (Nitrogen-Nitrite $NO_2$-N) on pages 4-85/6 of APHA (1992) was performed by the FIAStar 5000 analyser (Foss, Sweden). Similarly, the principle behind this test is the formation of a reddish purple azo dye at pH 2.0 to 2.5 by coupling diazotised sulphanilamide with N-(1-naphthyl)-ethylenediamine dihydrochloride (NED reagent). Photometric measurement was made at 540 nm wavelength (Shimadzu UV-2550). For Nitrogen-Nitrite ($NO_3$-N), the Automated Cadmium Reduction Method described in Test 4500-$NO_3^-$ F (Automated Cadmium Reduction Method) on pages 4-91/2 of APHA (1992) was also performed by the FIAStar 5000 analyser (Foss, Sweden). The principle behind this method is nitrate ($NO_3^-$) is reduced to nitrite ($NO_2^-$ , as described) in the presence of cadmium (ISO_13395, 1996). Each test was performed in triplicate.

### 2.8. Determination of ammonium ($NH_4$-N)

Ammonium was determined by direct nesslerization. This method was applicable for samples with ammonium concentrations up to 10 mg $L^{-1}$. Dilutions were used for any higher concentrations.

A series of standard solutions of $NH_4$-N ($NH_4Cl$), or samples were mixed with Nessler Reagent, following 20-30 minutes for colour development, the absorbance was read at 630 nm using a spectrophotometer (Shimadzu UV-2550) against a distilled water blank (APHA, 1992). The concentration of $NH_4$-N in the samples was determined from the equation of the standard line. This standard manual method was used for Chapter 4, 5, and 6 (APHA, 1992). Each test was performed in triplicate.

For managing a large quantity of sample analysis in Chapter 3, the Automated Phenate Method described in Test 4500-$NH_3$ H (Automated Phenate Method) on pages 4-84/5 of APHA (1992) was performed by the FIAStar 5000 analyser (Foss, Sweden). Similarly, the principle behind this test is alkaline phenol and hypochlorite react with ammonia to form indophenol blue in proportion to the ammonia concentration. The blue colour is intensified with sodium nitroprusside. Photometric measurement was made at 630 nm. Each test was performed in triplicate (ISO_11732, 2005).

Note: N = Organic Nitrogen (ON) + Inorganic Nitrogen (IN)

ON = Particulate Organic Nitrogen (PON) + Dissolved Organic Nitrogen (DON)

while PON = Algal Organic Nitrogen (AON) + Bacterial Organic Nitrogen (BON)

IN = NH4+-N + NO3-N + NO2-N

## 2.9. Determination of phosphorus

The principle of this test method is based on conversion of phosphorus to dissolved orthophosphate which is then determined spectrophotometrically.

### 2.9.1. Soluble phosphorous ($PO_4$-P)

Filtered samples were mixed with a solution of 200 µL ammonium molybdate reagent and stannous chloride reagent, to produce the intensely coloured molybdenum blue. The concentration of phosphate was then determined spectrophotometrically by measuring the absorbance of the samples at 720 nm. Standard solutions ($K_2HPO_4$) in distilled water were used to produce a standard line from which the concentration of $PO_4$-P within the sample was determined (APHA, 1992). This standard manual method was used for Chapter 4, 5, and 6 (APHA, 1992). Each test was performed in triplicate.

For managing a large quantity of sample analysis in Chapter 3, the Stannous Chloride Method described in Test 4500-P D (Stannous Chloride Method) on page 4-114 of APHA (1992) was performed by the FIAStar 5000 analyser (Foss, Sweden). Similarly, the principle behind this test is the formation of molybdophosphoric acid and subsequent reduction to intensely coloured molybdenum blue by stannous chloride. Photometric measurement was made at 720 nm. Each test was performed in triplicate (ISO_15681-1, 2003).

### 2.10. Statistical analysis

Statistical analysis of data was performed using SPSS (PASW Statistics 18, USA). Graphical data was analysed using Microsoft Excel 2010 (Microsoft Corporation, USA). Statistical tests utilised included independent sample T-test for Equality of Means and Levene's Test for Equality of Variances. Statistical significance was accepted at >95% confidence ($p \leq 0.05$). Results were presented as the mean ± standard deviation (SD) of duplicate or triplicate analysis.

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# CHAPTER 3

# THE INFLUENCE OF WASTEWATER STRENGTH ON THE OUTCOME OF $CO_2$ ADDITION FOR ALGAL BIOMASS PRODUCTION

# 3. THE INFLUENCE OF WASTEWATER STRENGTH ON THE OUTCOME OF $CO_2$ ADDITION FOR ALGAL BIOMASS (ALBAZOD) PRODUCTION

## 3.1. Introduction

An increase in atmospheric carbon dioxide, resulting from the combustion of fossil fuels, for energy is a major cause of the global climate change and ocean acidification (Chi et al., 2011). The urgent need for substantive net reductions in atmospheric $CO_2$ can be achieved by the transition to more extensive use of renewable energy sources. Biological $CO_2$ mitigation through microalgae, followed by their exploitation for biomass energy, has recently attracted much attention as a strategic alternative that offers both environmental and economic benefits (Chisti, 2007; Tredici, 2010; Wang et al., 2008). Usual sources of $CO_2$ for microalgae include atmospheric $CO_2$, $CO_2$ from industrial exhaust gases (e.g. flue gas and flaring gas), and $CO_2$ chemically fixed in the form of soluble carbonates (e.g. $NaHCO_3$ and $Na_2CO_3$) (Kumar et al., 2010).

As previously discussed in Chapter 1, in terms of supplying an external carbon source such as $CO_2$ in wastewater for microalgal cultivation, it is important to distinguish and describe the different characteristic of wastewater medium to be used in the cultivation system. This is due to the levels of BOD and total carbon varying accordingly to the prior treatment of the wastewater used. If the BOD and internal carbon content in the wastewater is already bio-available, the effects of $CO_2$ addition on algal growth in wastewater may not be a cost-effective method. It is recognised that the organic carbon in wastewaters, following bacterial mineralisation, is an important source of inorganic carbon for algal photosynthesis. Although $CO_2$ addition to autotrophic (defined media) cultures is required to maximise algal growth since $CO_2$ diffusion from the atmosphere is rate limiting for photosynthesis, it is also important to note that wastewater cultures which have an internal organic carbon pool which can be utilised for conversion as a carbon source through alternating CCM ($CO_2$ concentrating mechanism) and/or accessible forms of POC. Oswald (1985) reported that *Chlorella* absorbs carbon dioxide principally in the undissociated forms ($CO_2$ or $H_2CO_3$) and little if any as $HCO_3^-$ or $CO_3^{2-}$. Early studies on effect of carbon dioxide concentration on photosynthesis indicate that carbon dioxide saturation is achieved at or below 0.1 per

cent. Above about 5 per cent, toxic effects become operative, although the upper limit is not definitely known. It is therefore expected that growth rate will be independent of carbon dioxide concentration between 0.1 and 5 per cent (Oswald, 1985).

To revisit the discussions from Chapter 1, in some studies, carbon has been considered a growth limiting factor in HRAPs treating wastewater due to the high algal carbon demand. It has been suggested that algal biomass production in organic carbon rich wastewaters may benefit from $CO_2$ enrichment. It was argued that the carbon:nitrogen (C:N) ratio of typical wastewater was limiting to algal growth, based on the stoichiometry of wastewater and algal biomass. However, there is uncertainty regarding the relative C:N ratios of wastewater and algal biomass. Park and Craggs (2010) argue that typical domestic wastewater has a C:N ratio of 7:1, while algal biomass is typically C:N 15:1 (Park & Craggs, 2010). Park et. al. (2011) stated that, by referencing of Benemann (2003) and Harmelen (2006), domestic sewage is typically between 3-7:1 C:N and algal biomass 6-15:1 C:N (Benemann, 2003; Harmelen, 2006; Park et al., 2011). Craggs et. al. (2011) stated that, by referencing of Benemann (2003), facultative pond wastewater is 2:1 C:N and algal biomass between 5 and 10:1 C:N (Benemann, 2003; Craggs et al., 2011). Meanwhile Craggs et. al. (2012) stated that, by referencing of Benemann (2003), domestic wastewater is typically 3:1 C:N and algal biomass 6:1 C:N (Benemann, 2003; Craggs et al., 2012). To the best of our knowledge, considerable confusion surrounds these claims as unfortunately, there is no such stoichiometric data in the Benemann reference (2003). To add to the confusion, even though the same paper was referenced, the authors quoted quite wide variations in stoichiometry which was also observed by Buchanan (Buchanan, 2015).

In summary, it can be concluded that:

- Redfield ratio ($C_{106}$: $H_{263}$: $O_{110}$: $N_{16}$: $P_{1}$: $S_{0.7}$ – b atoms) which converts a 6:6 C: N ratio,
- Uncertainty regarding the relative C:N ratios of wastewater and algal biomass in some literature studies,
- And variety strengths on domestic wastewater based on their treatment stages, existing internal carbon contents, and $BOD_5$ levels

This raises a question whether carbon (from an external source such as $CO_2$) is the limiting factor for carbon rich (for internal organic carbon pool) wastewater growth medium. The addition of $CO_2$ to wastewater grown algae cultures with the anticipated outcome of higher algal photosynthetic efficiencies and productivities may not apply to all types of wastewater, and may be dependent on the size of the organic carbon pool or BOD.

In this chapter, the effect of $CO_2$ addition to wastewaters with different biochemical oxygen demand ($BOD_5$), which is a surrogate indicator of available organic carbon, on algal biomass production was examined. In nature and in an open system such as wastewater treatment ponds, microbes live in a diverse community of algae, bacteria, zooplankton and detritus. This combined microbial biomass is referred to as ALBAZOD (algae-bacteria-zooplankton-detritus) and it is therefore used in this study and not “algal biomass”.

## 3.2. Methods

### 3.2.1. Wastewater sources and CO2 input

The wastewater came from two sources in South Australia. Secondary treated septic tank effluent wastewater from an aerated lagoon operated at a theoretical hydraulic retention time (THRT) of 22d was collected from Mount Barker Community Waste Management System (CWMS) WWTP South Australia (35°04'08.4"S 138°52'31.9"E) and represented the low $BOD_5$ strength wastewater. Septic tank treated effluent (high strength $BOD_5$) and septic tank effluent further treated in a facultative pond (THRT 6d, mid strength $BOD_5$) were both collected from a HRAP at Kingston on Murray (KoM), South Australia (34.2167°S, 140.3333°E). The KoM septic tank effluent originated from well-maintained systems with a minimum THRT time of 24h. All wastewaters were stored at 3$^{o}$C immediately prior to use. Four experiment groups were used in this experiment to determine the effect of pH controlled $CO_2$ addition to wastewaters, of varying $BOD_5$ concentration, on algal growth and wastewater treatment:

1. Mount Barker aerated lagoon effluent – low strength $BOD_5$ (+$CO_2$ addition)
2. KoM Facultative effluent – mid strength $BOD_5$ (+$CO_2$ addition)
3. KoM Septic tank raw effluent - high strength $BOD_5$ (+$CO_2$ addition)
4. KoM Facultative effluent – mid strength $BOD_5$ (+acid HCl 0.1M addition)

(Low, mid, and high BOD is based on Australian treatment process category (ARMCANZ, 1997). The classification of "low" is based on the extended treatment similar to secondary treatment cited in Table 1.5; while "high" is similar to raw wastewater or minimum pre-treatment, "mid" is being in between)

### 3.2.2. Laboratory microalgae culture system

The microalgal culture system comprised of two, open, PVC culture vessels each with a 3 L working volume and a surface area of 8.24 $cm^2$ (Fig. 3.1). The vessels were irradiated from above at a photon flux density (PFD) of 170 µmol PAR (400- 700nm) $m^{-2}$ $s^{-1}$ (Energy Saver 100 W, Mirabella, Australia) on diurnal light: dark cycle of 15:9 h. Temperatures were recorded ranging from 23-32 ℃ during the light cycle (15h) and

a lower range from 18-26 ℃ during the dark cycle (9h). Day 0 initial culture temperature (time = 0) was the same as the inlet wastewater which was maintained at 8-10 ℃. The reactors were magnetically stirred at 60 rpm.

A peristaltic pump (Watson Marlow 503S) was connected to each reactor to provide a continuous feed of wastewater to provide a THRT of 4 days including compensation for evaporative losses. The inlet wastewater was maintained at 8-10 ℃ by a temperature controller and it was refilled with stock inlet wastewater stored at 3 ℃ after each HRT cycle. The outlet and sampling point was via an overflow port in the sides of the vessels (Fig. 3.1). Experiments were performed simultaneously; the control reactor was dependent upon the organic and inorganic carbon pool of the wastewater influent and atmospheric diffusion for its inorganic carbon supply for photosynthesis. The second reactor was sparged from the base with air enriched with air + 5% $CO_2$ (v/v, 0.5 L $min^{-1}$) and it was attached to an air stone (Aqua Nova Aquarium Air Stone Cylinder 15x25mm length) to generate fine bubbles. The pH in this reactor was continuously monitored by a pH transmitter controller (ABB Ltd) with $CO_2$ addition activated at ≥ pH8.0 and stopped when ≤pH 7.5, while there was no pH control for the comparative control studies. It is also important to note that, due to the pH transmitter controller (ABB Ltd) machine epsilon (i.e., relative error due to rounding in floating point arithmetic), the $CO_2$ or acid firing range includes a ± 1% error for the pH range 7.50 to 8.00.

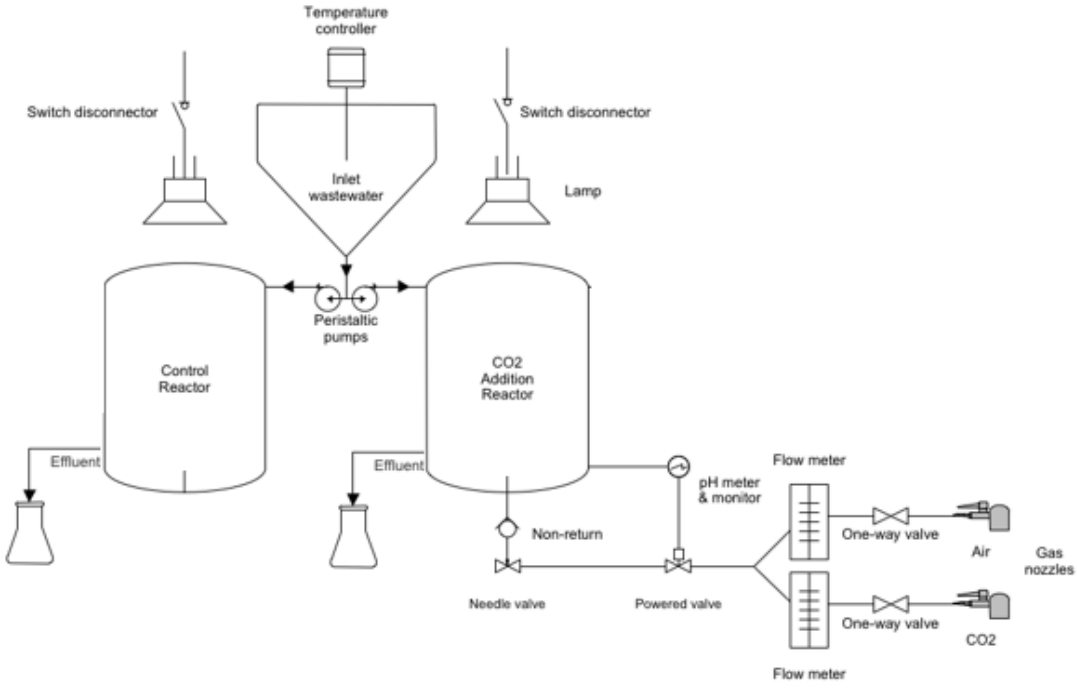



Fig. 3.1 Reactor for continuous wastewater influent and $CO_2$ injection under pH control. Simultaneously a similarly constructed reactor but without pH controlled gas injection was operated as a control.

### 3.2.3. Analysis

Samples were collected twice daily at the end of the dark cycle (9am) and mid-way through the light cycle (4pm). The culture outlet samples were filtered (0.22 μm; Whatman Ltd) and stored in a -80$^{o}$C freezer for subsequent analysis of total organic carbon (TOC) and inorganic carbon (IC). Particulate organic carbon (POC) was determined as the difference between pre- and post-filtration analysis of TOC. The analysis of IC was based on no filtration to avoid adding atmospheric $CO_2$ into the sample. $BOD_5$ was measured in every retention cycle over 24 days. The pH was continuously monitored by a chart recorder and enabled the determination of $CO_2$ addition frequency to the reactor over the incubation period. All results presented for each parameter were average values with their corresponding analytical standard deviation from a series of individual measurements: independent sample T-test for Equality of Means and Levene's Test for Equality of Variances ($\pm$SD, n=3).

## 3.3. Results

### 3.3.1. Wastewater composition

The composition of the wastewaters used in the study are shown in Table 3.1. In general domestic wastewater, N concentrations vary between 15 and 90 mg $L^{-1}$ and P concentrations between 4 and 20 mg $L^{-1}$ (Abdelaziz et al., 2013; Cai et al., 2013; Christenson & Sims, 2011). Due to the different sources of N and P in wastewater, N and P concentrations often vary independently from each other. Human excreta are a source of both P and N while detergents, soaps and personal care products contain P but little N (Smil, 2000; Tjandraatmadja et al., 2010). The analysis in this current study shows that the concentrations of nitrogen (TN: 17.7 – 73.6 mg $L^{-1}$) and phosphorus ($PO_4$-P: 14.1 – 27.9 mg $L^{-1}$) within the respective wastewaters, with a range of 6.7 – 28.9 mg $L^{-1}$ in $NH_4$-N concentrations.

The wastewaters in this current study were selected on the basis of their $BOD_5$ concentrations. This varied from 120 to 15mg $L^{-1}$ $BOD_5$ in the high strength and low strength wastewater respectively. Similarly, the total carbon varied from 138.01 to 30.43 mg $L^{-1}$ in the high and low strength wastewaters. The highest TOC was observed in high $BOD_5$ strength wastewater at 116.37 mg $L^{-1}$, while the lowest 24.42 mg $L^{-1}$ in the low $BOD_5$ strength aerated lagoon wastewater. The aerated lagoon effluent had a low $BOD_5$ and a low IC suggesting that the TOC was recalcitrant and unavailable for mineralisation, with a POC of 2.84 mg $L^{-1}$ (11.63 % of the TOC). The septic tank effluent, however, had both a high $BOD_5$ and a high IC content suggesting that the TOC was available for respiration by the biota. Interestingly, POC (7.61 mg $L^{-1}$) only makes up a small part (6.54%) of the TOC (116.37 mg $L^{-1}$) pool meaning much more of the organic carbon was in a soluble form (DOC, dissolved organic carbon) and therefore by inference more available for mineralisation to IC than in the other wastewaters. The IC concentrations, within the KoM facultative pond effluent (mid $BOD_5$), were much higher (45-50 mg $L^{-1}$) compared to both septic tank and aerated lagoon effluent. The POC percentage in TOC was amongst the highest (27~29%).

Since the wastewaters were sourced from two separated treatment systems (i.e., septic tank effluent and lagoon based), consequently, there was a significant difference in chlorophyll *a* concentrations within the respective influents to the wastewater cultures. The highest chlorophyll *a* concentration was observed from the KoM facultative lagoon (mid strength $BOD_5$) effluent. In Mount Barker aerated lagoon, the least chlorophyll *a* concentration was observed whereas the septic tank effluent was as anticipated (Table 3.1). The small amount chlorophyll *a* concentration from the KoM septic tank effluent could be due to background chlorophyll *a* presented or extraction of non-specific absorption at measurement wavelengths.

Table 3.1. The composition, mean (± SD), of the wastewaters used in the $CO_2$ addition experiments and in the experiment where pH was maintained by controlled acid addition.

| | Mount Barker Aerated lagoon effluent – with $CO_2$ (Low strength $BOD_5$) | KoM[a] Facultative effluent – with $CO_2$ (Mid strength $BOD_5$) | KoM Septic tank effluent – with $CO_2$ (High strength $BOD_5$) | KoM Facultative effluent – with acid (HCl 0.1M) (Mid strength $BOD_5$) |
|---|---|---|---|---|
| $BOD_5$ (mg/L) | 15 (0.46) | 78 (3.12) | 120 (3.66) | 72 (2.88) |
| TC (mg/L) | 30.43 | 134.62 | 138.01 | 104.38 |
| IC (mg/L) | 6.01 (0.12) | 45.64 (0.92) | 21.64 (0.43) | 50.63 (2.82) |
| TOC (mg/L) | 24.42 (1.17) | 88.98 (1.80) | 116.37 (2.34) | 53.75 (3.43) |
| POC (mg/L) | 2.84 (0.55) | 23.68 (1.27) | 7.61 (1.41) | 15.55 (0.79) |
| POC/TOC (%) | 11.63 | 26.61 | 6.54 | 28.93 |
| TN (mg/L) | 40.54 (0.81) | 31.87 (0.64) | 73.58 (1.48) | 17.67 (0.36) |
| $NH_4$-N (mg/L) | 28.94 (0.03) | 10.16 (0.20) | 28.4 (0.57) | 6.67 (0.13) |
| $NO_2$-N (mg/L) | 0.57 (0.02) | 1.53 (0.03) | 2.70 (0.05) | 0.82 (0.02) |
| $NO_3$-N (mg/L) | 0.06 (0) | 1.31 (0.03) | 3.60 (0.07) | 2.55 (0.05) |
| $PO_4$-P (mg/L) | 14.10 (0.56) | 23.61 (0.47) | 27.92 (0.56) | 18.72 (0.38) |
| SS (mg/ml) | 0.22 (0.01) | 0.26 (0.01) | 0.19 (0.01) | 0.22 (0.01) |
| VSS (mg/ml) | 0.10 (0.01) | 0.15 (0.02) | 0.08 (0.01) | 0.13 (0.02) |
| Chl a (mg/L) | 0.287 (0.15) | 1.949 (0.23) | 0.087 (0.04) | 1.191 (0.38) |
| pH | 7.41 (0.15) | 8.31 (0.17) | 8.22 (0.17) | 8.36 (0.17) |

[a]KoM: Kingston on Murray Community Wastewater Management Scheme, Adelaide, South Australia

### 3.3.2. pH, $CO_2$ injection volumes and inorganic carbon concentrations in wastewater cultures.

#### 3.3.2.1. pH regime in wastewater cultures receiving additional $CO_2$

The change of pH increased more rapidly at the early stage due to the significant increase in chlorophyll *a* and associated increase in photosynthesis. It was observed that the culture pH was lower than the pH of respective inlet wastewater during the dark cycle, and an increase of ciliate population was also observed (data not shown) (Fig. 3.2-3.5). It is also important to note that due to the pH transmitter controller's (ABB Ltd) relative error due to rounding in floating point arithmetic - epsilon, the $CO_2$ or acid activation range includes a ± 1% error for the pH range 7.50 to 8.00. For example, as shown in Fig. 3.2 – 3.5, the activation might start at approximately pH 7.90 ~ 7.95. For the controls, the overall uncontrolled pH ranged from 10.50 to 6.70 in the absence of $CO_2$ or acid addition. The similar pattern was observed over the whole period for all controls and the frequency of the pattern gradually decreased when chlorophyll *a* concentration decreased.

Low strength $BOD_5$ wastewater with an initial chlorophyll *a* concentration at 0.29 mg $L^{-1}$ (Fig. 3.2) – a lower pH value in the dark phase was observed at about pH 6.8 when respiration predominated. After 192 hours the pH reached below 6.5, a longer recovery time was observed before the pH levels increased back to the designed experiment pH range 7.50 – 8.00. The $CO_2$ addition was commenced frequently at the first 168 hours, however, gradually limited injections of $CO_2$ were observed (i.e. when pH exceeded 8.00) in the light period (this will be discussed in further sections).

Mid strength $BOD_5$ wastewater with an initial chlorophyll *a* concentration at 1.95 mg $L^{-1}$ (Fig. 3.3) - a higher initial chlorophyll *a* concentration was observed when it was compared to both low strength and high strength wastewater. Therefore, higher photosynthetic rates may be inferred and consequently pH >8.00 occurred more frequently resulting in more $CO_2$ injections. The pH at the end of the dark period maintained at about 7.00 – 7.10, except at time 360 hours when the pH value was 6.70.

High strength $BOD_5$ wastewater with an initial chlorophyll *a* concentration at 0.09 mg $L^{-1}$ (Fig. 3.4) – an initial chlorophyll *a* content similar to the low strength culture. Initially, the pH at the end of dark phase maintained at approximately 7.25. However, beginning from time 264 hours, a lower pH was observed (pH 6.78) at the end of dark period which a similar observation was also found in low strength BOD wastewater. The $CO_2$ injection occurred more frequently than for the low BOD wastewater as shown in Table 3.2.

Mid strength $BOD_5$ wastewater with acid addition and an initial chlorophyll *a* concentration at 1.19 mg $L^{-1}$ (Fig. 3.5) – at the end of the dark period, pH level maintained at approximately 7.10. A lower initial chlorophyll *a* level was observed when compared to the mid BOD strength culture with $CO_2$ addition. In addition, the acid additions occurred more frequently compared to the $CO_2$ addition which is shown in Table 3.2.

Table 3.2 Time course of $CO_2$ or acid injection frequency and daily volume injected into different $BOD_5$ strength of wastewaters: (A) Mount Barker low $BOD_5$ with $CO_2$, (B) KOM mid $BOD_5$ with $CO_2$, (C) KOM high $BOD_5$ with $CO_2$, and (D) KOM mid $BOD_5$ with acid (HCl 0.1M) addition.

| Day | Number of $CO_2$ injection/ day | | | Number of acid Injection/ day | Daily volume | | | | Accumulative sum | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | A (L) | B (L) | C (L) | D (ml) | A (L) | B (L) | C (L) | D (ml) |
| 0 | 7 | 10 | 6 | 7 | 52.5 | 50.0 | 30.0 | 87.5 | 52.5 | 50.0 | 30.0 | 87.5 |
| 1 | 4 | 8 | 5 | 4 | 30.0 | 20.0 | 25.0 | 50.0 | 82.5 | 70.0 | 55.0 | 137.5 |
| 2 | 4 | 7 | 5 | 6 | 30.0 | 17.5 | 25.0 | 75.0 | 112.5 | 87.5 | 80.0 | 212.5 |
| 3 | 4 | 6 | 5 | 6 | 30.0 | 60.0 | 25.0 | 75.0 | 142.5 | 147.5 | 105.0 | 287.5 |
| 4 | 6 | 5 | 5 | 4 | 45.0 | 50.0 | 25.0 | 50.0 | 187.5 | 197.5 | 130.0 | 337.5 |
| 5 | 5 | 9 | 6 | 4 | 37.5 | 90.0 | 30.0 | 50.0 | 225.0 | 287.5 | 160.0 | 387.5 |
| 6 | 4 | 7 | 4 | 4 | 30.0 | 70.0 | 20.0 | 50.0 | 255.0 | 357.5 | 180.0 | 437.5 |
| 7 | 3 | 8 | 6 | 7 | 22.5 | 80.0 | 30.0 | 87.5 | 277.5 | 437.5 | 210.0 | 525.0 |
| 8 | 3 | 5 | 6 | 10 | 22.5 | 62.5 | 30.0 | 125.0 | 300.0 | 500.0 | 240.0 | 650.0 |
| 9 | 2 | 7 | 6 | 6 | 15.0 | 87.5 | 30.0 | 45.0 | 315.0 | 587.5 | 270.0 | 695.0 |
| 10 | 3 | 9 | 6 | 5 | 22.5 | 112.5 | 75.0 | 37.5 | 337.5 | 700.0 | 345.0 | 732.5 |
| 11 | 3 | 5 | 5 | 5 | 22.5 | 75.0 | 75.0 | 37.5 | 360.0 | 775.0 | 420.0 | 770.0 |
| 12 | 3 | 8 | 4 | 3 | 22.5 | 200.0 | 50.0 | 22.5 | 382.5 | 975.0 | 470.0 | 792.5 |
| 13 | 4 | 8 | 4 | 2 | 60.0 | 120.0 | 50.0 | 35.0 | 442.5 | 1095.0 | 520.0 | 827.5 |
| 14 | 4 | 8 | 4 | 3 | 60.0 | 200.0 | 40.0 | 52.5 | 502.5 | 1295.0 | 560.0 | 880.0 |
| 15 | 5 | 10 | 4 | 4 | 75.0 | 25.0 | 40.0 | 70.0 | 577.5 | 1320.0 | 600.0 | 950.0 |
| 16 | 2 | 7 | 3 | 4 | 30.0 | 175.0 | 30.0 | 70.0 | 607.5 | 1495.0 | 630.0 | 1020.0 |
| 17 | 2 | 7 | 3 | 4 | 50.0 | 105.0 | 30.0 | 70.0 | 657.5 | 1600.0 | 660.0 | 1090.0 |
| 18 | 2 | 4 | 3 | 4 | 50.0 | 40.0 | 30.0 | 100.0 | 707.5 | 1640.0 | 690.0 | 1190.0 |
| 19 | 1 | 8 | 3 | 3 | 25.0 | 80.0 | 30.0 | 112.5 | 732.5 | 1720.0 | 720.0 | 1302.5 |
| 20 | 1 | 5 | 2 | 3 | 27.5 | 50.0 | 20.0 | 75.0 | 760.0 | 1770.0 | 740.0 | 1377.5 |
| 21 | 1 | 4 | 2 | 4 | 27.5 | 40.0 | 20.0 | 100.0 | 787.5 | 1810.0 | 760.0 | 1477.5 |
| 22 | 0 | 3 | 2 | 4 | 0.0 | 30.0 | 20.0 | 50.0 | 787.5 | 1840.0 | 780.0 | 1527.5 |
| 23 | 0 | 3 | 2 | 3 | 0.0 | 75.0 | 20.0 | 37.5 | 787.5 | 1915.0 | 800.0 | 1565.0 |
| 24 | 0 | 0 | 0 | 0 | 0.0 | 0.0 | 0.0 | 0.0 | 787.5 | 1915.0 | 800.0 | 1565.0 |

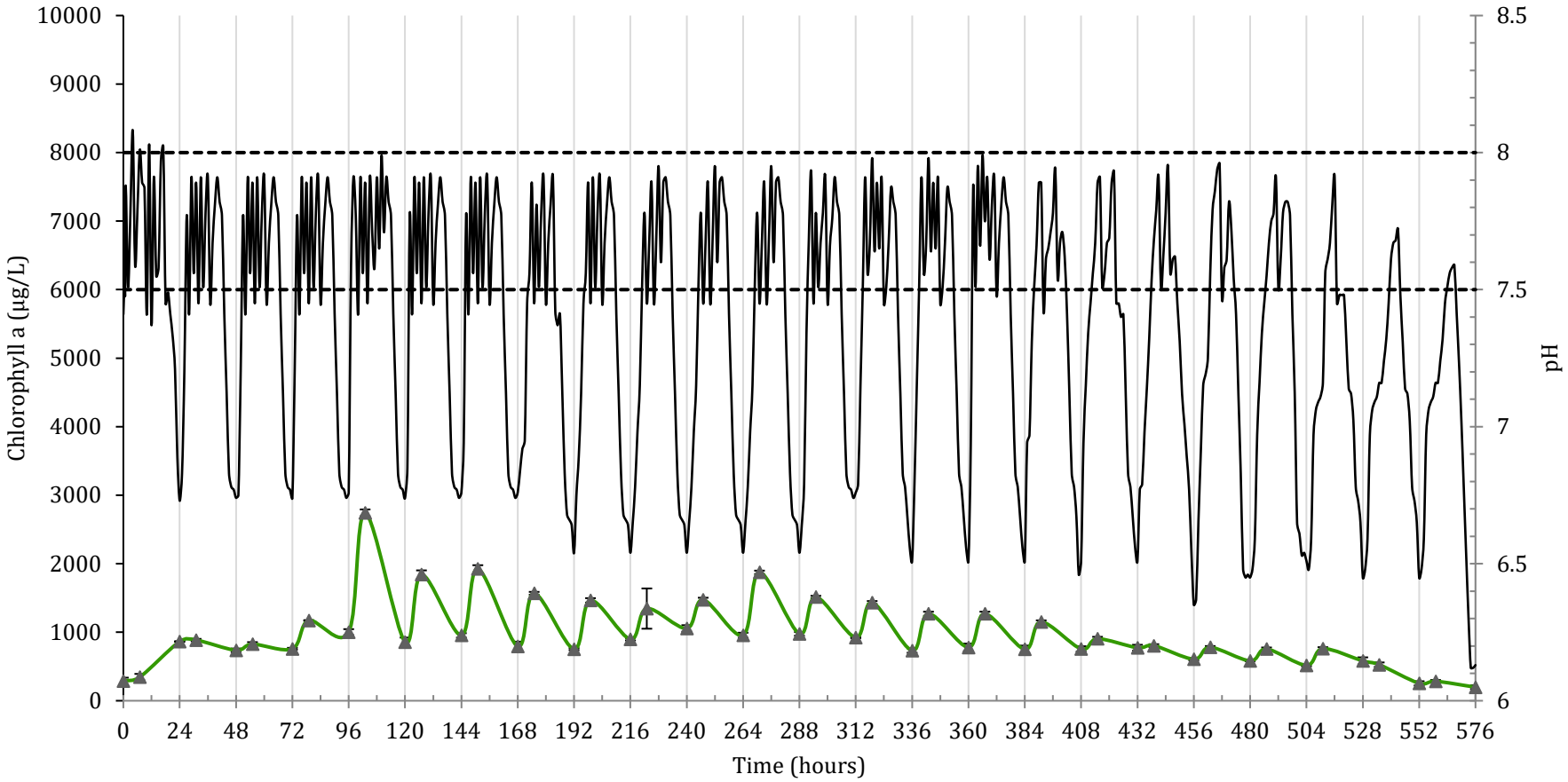



Fig. 3.2 Low strength $BOD_5$ wastewater (influent 15mg $BOD_5$/L): Relationship between chlorophyll *a* (▲) and pH (–) between pH 8 (onset of $CO_2$ addition) and pH 7.5 (cessation of $CO_2$ addition) during the 24 days culture period in Mount Barker aerated lagoon effluent.

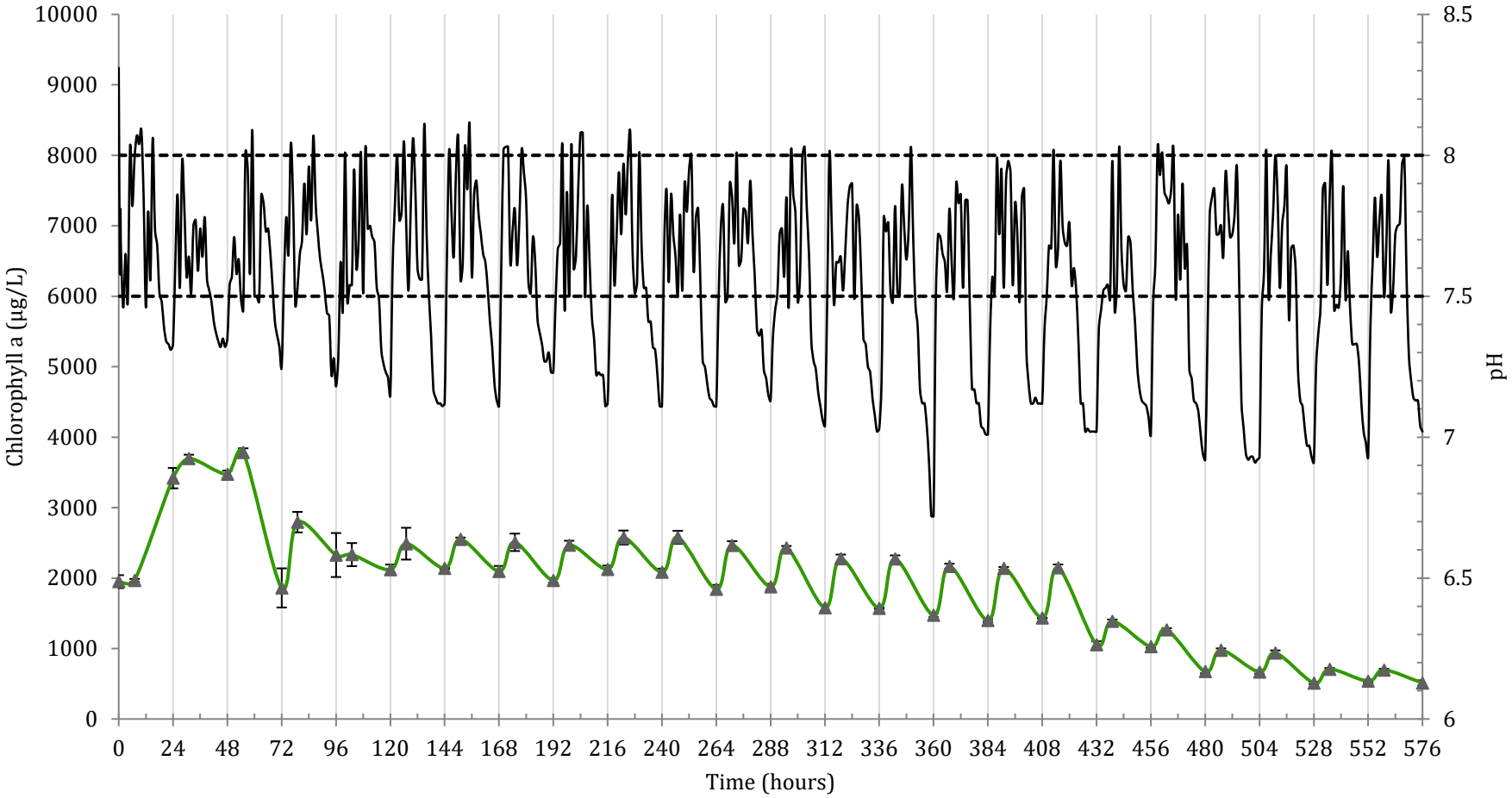



Fig. 3.3 Mid strength $BOD_5$ wastewater (influent 78 mg$BOD_5$/L). ): Relationship between chlorophyll *a* (▲) and pH (–) between pH 8 (onset of $CO_2$ addition) and pH 7.5(cessation of $CO_2$ addition) during the 24 days culture period in KoM facultative pond effluent.

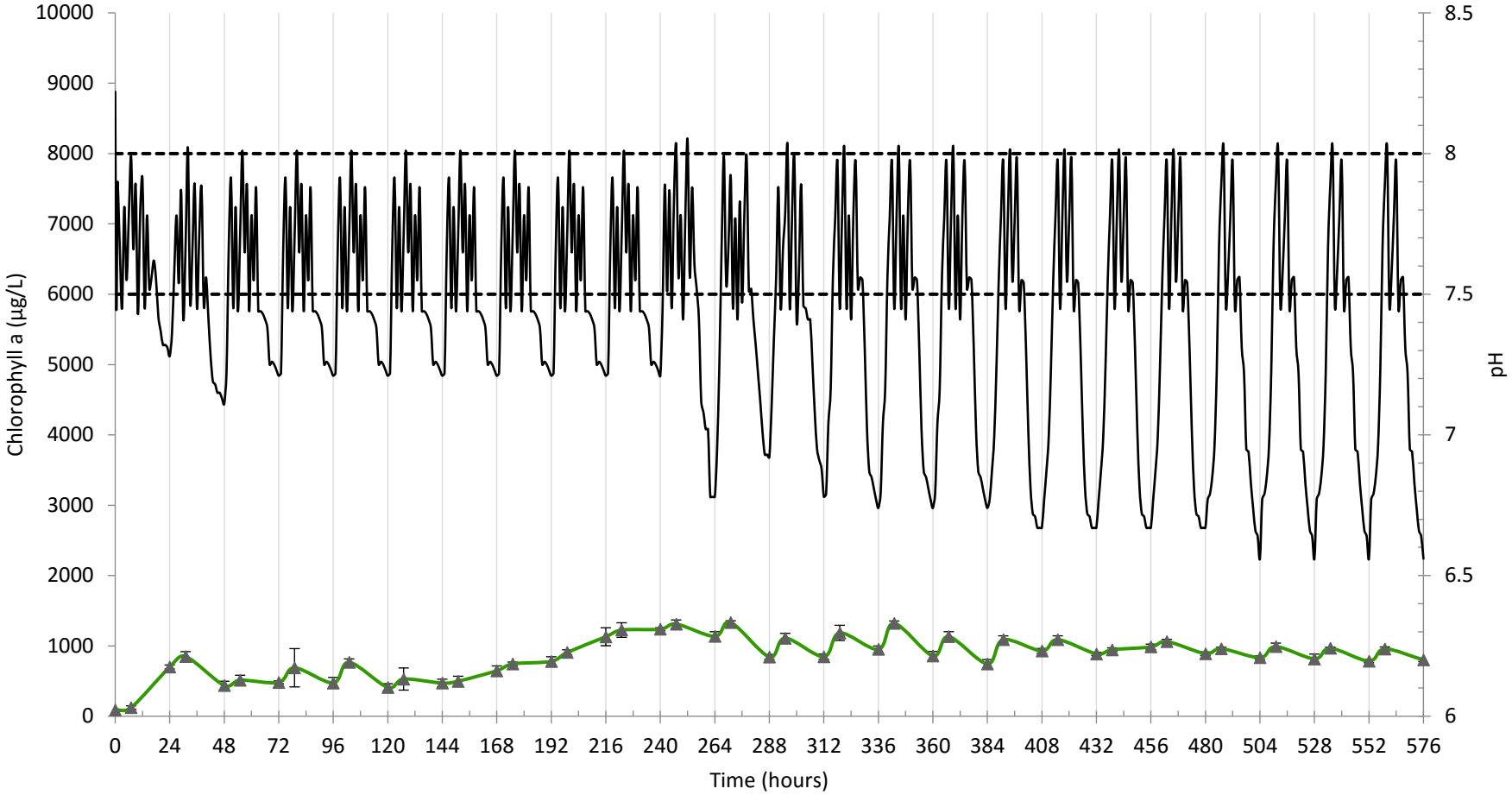



Fig. 3.4 High strength $BOD_5$ wastewater (influent 120 mg $BOD_5$/L): Relationship between chlorophyll *a* (▲) and pH (–) between pH 8 (onset of $CO_2$ addition) and pH 7.5 (cessation of $CO_2$ addition) during the 24 days culture period in KoM septic tank raw effluent.

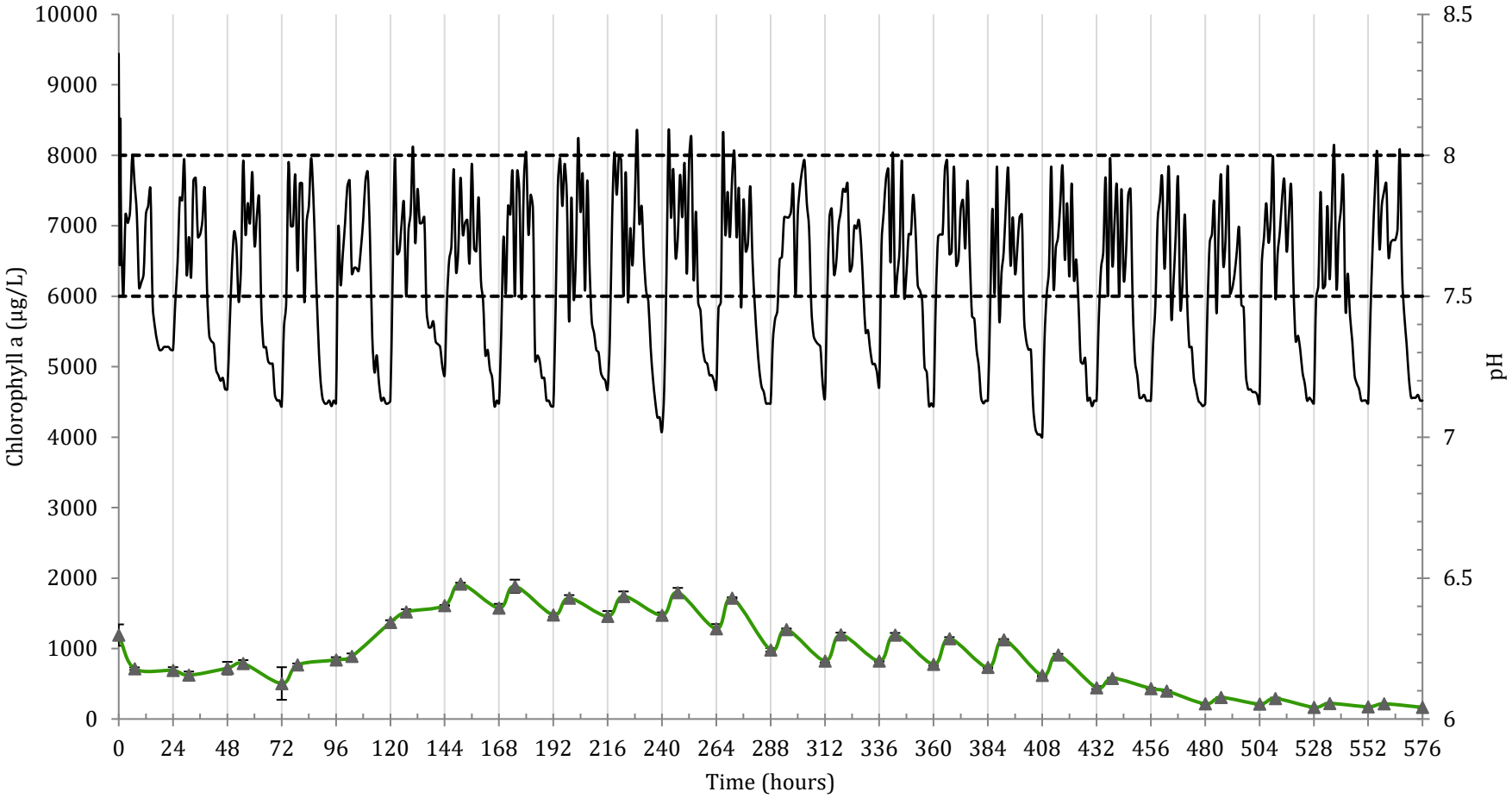



Fig. 3.5 Mid strength $BOD_5$ wastewater (influent 78 mg$BOD_5$/L). ): Relationship between chlorophyll *a* (▲) and pH (–) between pH 8 (onset of HCL addition) and pH 7.5(cessation of HCl 0.1M addition) during the 24 days culture period in KoM facultative pond effluent

3.3.2.2. Total $CO_2$/Acid injection

The carbon dioxide injection flow rate was 5 L $min^{-1}$ with an air + 5% $CO_2$ concentration (v/v). The total injection volumes over the period of culture were:

| | |
|---|---|
| Mount Barker low $BOD_5$ + $CO_2$: | 787.5 L |
| KOM high $BOD_5$ + $CO_2$: | 800 L |
| KOM mid $BOD_5$ + $CO_2$: | 1915 L |
| KOM mid $BOD_5$ + acid HCI (0.1M): | 1.57 L (acid) |

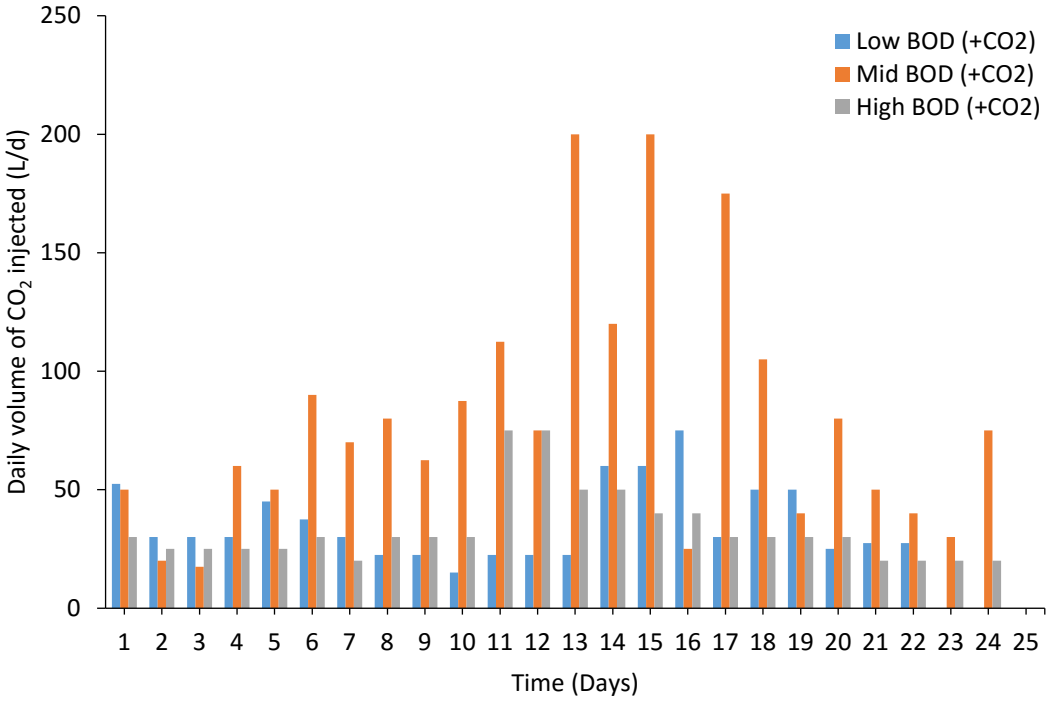



Fig. 3.6 Daily $CO_2$ injection volume into the three different $BOD_5$ strength wastewater during the 24-day experiments.

Figure 3.6 shows the daily volume of $CO_2$ injected into the three wastewaters with different $BOD_5$ concentrations over the 24-day experiment. The frequency of injection gradually increased corresponding to the chlorophyll *a* concentrations. Table 3.2 shows a detailed time course of $CO_2$ or acid (HCI 0.1M) injection frequency and daily volume injections into different $BOD_5$ strength wastewaters. The low $BOD_5$ wastewater with $CO_2$ (Table 3.2 - A) responded similarly to the high $BOD_5$ wastewater with $CO_2$ (C), with similar total $CO_2$ injection volumes of 787.5 L and 800 L respectively. As it was discussed in the section above that, in low $BOD_5$ wastewater, the observations of pH level below 6.50 were more frequently after time 192 hours

(Day 8). Therefore, a longer recovery time was observed for the pH increased back to the designed experiment pH range 7.50 – 8.00. This was also correlated with less injections of $CO_2$ per day. Similar results were also observed in high $BOD_5$ wastewater which started at about day 8-10. However, in mid $BOD_5$ wastewater with $CO_2$, an approximately 2.4 times higher in the total $CO_2$ injection number was observed (1915 L).

Referring to Table 3.1, the initial chlorophyll *a* level in the inlet mid $BOD_5$ wastewater was 1.95 mg $L^{-1}$, which was significantly higher than the levels in the inlet low $BOD_5$ wastewater at 0.29 mg $L^{-1}$ and the inlet high $BOD_5$ wastewater at 0.09 mg $L^{-1}$. This explains a higher total $CO_2$ injection number observed in the mid $BOD_5$ wastewater. Therefore, the higher initial microalgal inoculum results of a more active photosynthetic population which causes the pH to rise more frequently > pH 8.00 resulting in injection of more $CO_2$ into the culture.

In addition, a high frequency of acid addition was also observed in the mid $BOD_5$ wastewater with an initial chlorophyll *a* level of 1.19 mg $L^{-1}$. This leads to an interesting outcome of the pH based experiment by only using acid (HCI 0.1M) to adjust the designed pH range 7.5 - 8. It is understood that the mid $BOD_5$ wastewater with $CO_2$ addition exceed pH 8.0 resulting in significant $CO_2$ volume injection. Equally, the experiment with pH controlled by acid has achieved a similar outcome when compared to the pH controlled by $CO_2$ addition studies. This is possibly caused by the continuous photosynthesis from using a carbon source derived from both diffusion and internal IC/TOC (mineralised to IC) and different forms of carbon (aq) at different pH levels. This suggests that there was a sufficient internal pool of TOC derived IC to maintain a high photosynthetic rate, even without additional of external $CO_2$. The difference in biomass levels (SS and VSS) between the four experiments will be discussed in section 3.3.3.4.

3.3.2.3. Inorganic carbon (IC)

Figures 3.7 – 3.10 show that the characteristic changes of IC in the wastewater cultures when $CO_2$ or acid was injected over a period of 120 or 30 seconds respectively. During the $CO_2$ injection period (120 seconds), it was observed in low, mid and high $BOD_5$ wastewater that the IC values ranged from 1 -1.5 mg $L^{-1}$. As previously described in the method section, while the method of POC was determined from the difference between pre- and post-filtration (0.22 μm filter paper) of TOC analysis, the analysis of IC was based on no filtration to avoid adding atmospheric $CO_2$ into the sample.

Fig. 3.10 shows the interval change of IC in KoM facultative influent (mid $BOD_5$) by using only HCl addition. Similar change in IC was also observed with approximately 1 mg $L^{-1}$ over the 30 seconds. The reason of a shorter data collection period was because the acid (HCl 0.1M) addition was more effective and faster in changing the culture pH levels. It was observed that it took approximately 120-180 seconds for the $CO_2$ addition experiment to achieve a 0.5 pH decrease, from pH 8 to pH 7.5.

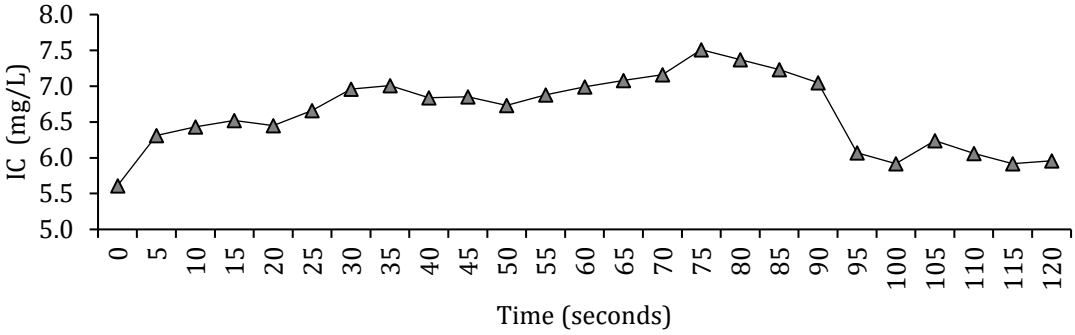



Fig. 3.7 The interval change of IC (▲) when $CO_2$ was injected over 120 seconds into Mount Barker aerated facultative pond influent - low $BOD_5$ wastewater

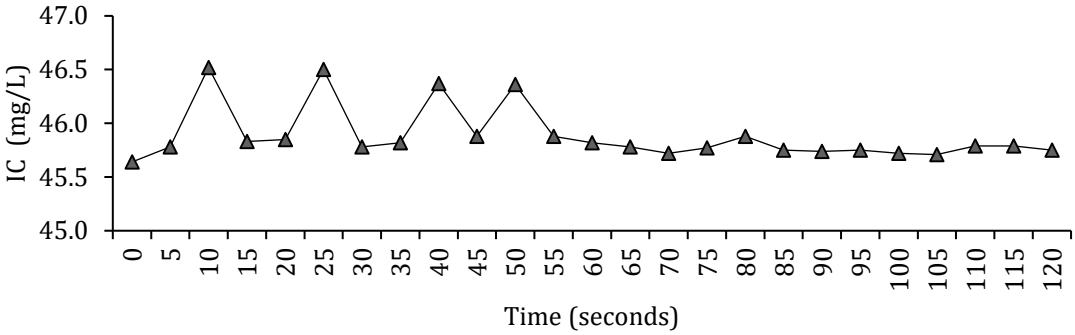



Fig. 3.8 The interval change of IC (▲) when $CO_2$ was injected over 120 seconds into KoM facultative influent - mid $BOD_5$ wastewater

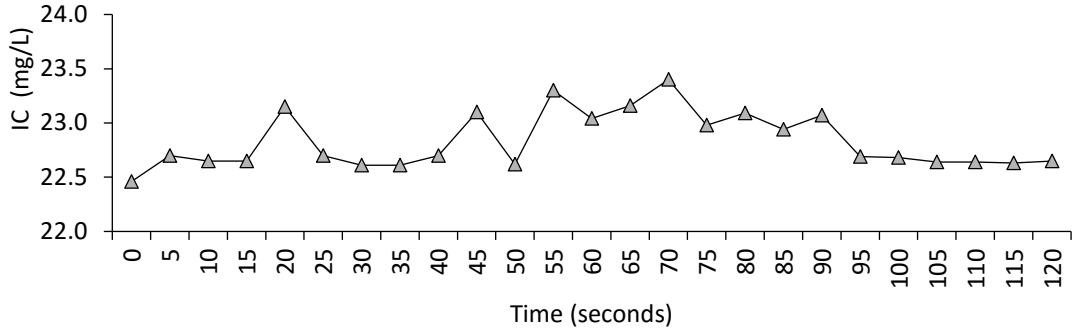



Fig. 3.9 The interval change of IC (▲) when $CO_2$ was injected over 120 seconds into KoM septic tank raw influent - high $BOD_5$ wastewater

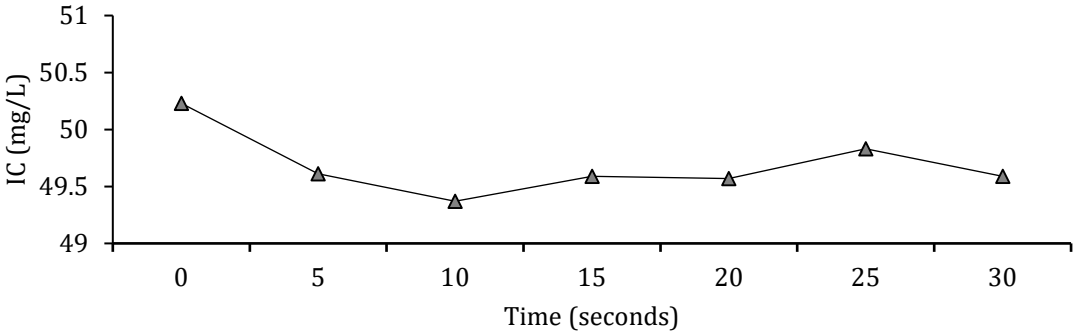



Fig. 3.10 The interval change of IC (▲) in KoM facultative influent with HCl addition - mid $BOD_5$ wastewater

In low and mid strength $BOD_5$ wastewater cultures, significant differences were found in IC concentration when $CO_2$ addition was injected. However, no significant difference was found in IC levels from high $BOD_5$ strength wastewater (Table 3.3).

Table 3.3 Significant differences in inorganic carbon (IC) levels (mg/L) of wastewaters of different strengths over the 24 days period ($P < 0.05$). (a) A summary on independent sample T-test for Equality of Means and Levene's Test for Equality of Variances of control and cultures to which $CO_2$ was added under pH control

| (a) IC (mg/L) | **Mount Barker (Low $BOD_5$)** | **KOM (Mid $BOD_5$, + $CO_2$)** | **KOM (High $BOD_5$)** | **KOM (Mid $BOD_5$,+ Acid)** |
|---|---|---|---|---|
| Are means significantly different ($P < 0.05$)? | Yes | Yes | No | No |
| P value | 0.00 | 0.00 | 0.53 | 0.25 |
| Mean ± SD (mg/L) | 10.02 (4.66) | 32.01 (10.97) | 32.62 (4.19) | 30.95 (9.91) |
| Mean (control) ± SD (mg/L) | 5.57 (2.23) | 24.62 (8.99) | 32.03 (5.30) | 28.52 (10.85) |
| N | 50 | 50 | 50 | 50 |
| F | 75.99 | 1.80 | 4.86 | 0.43 |
| df | 70.38 | 98.00 | 93.07 | 98.00 |
| t | 6.10 | 3.68 | 0.63 | 1.17 |
| Mean difference (%) | +79.89 | +30.02 | +1.87 | +8.52 |
| Total injected $CO_2$ or acid (HCl 0.1M) volume (L) | 787.50 | 1915.00 | 800.00 | 1.57 |
| Initial inlet chlorophyll *a* (mg/L) | 0.29 | 1.95 | 0.09 | 1.19 |
| Initial inlet POC (mg/L) | 2.84 | 23.68 | 7.61 | 15.55 |

Figures 3.11 – 3.14 show the changes in inlet IC concentrations and the IC concentrations within the respective wastewaters for both the control and cultures with $CO_2$ or acid addition.

From Fig. 3.11 (low $BOD_5$ wastewater), it was observed there was an increase of IC concentration from day 3-5, and day 15-24. This corresponds to Table 3.2 where, there was an increase of daily $CO_2$ injection volume on those particular days. From example, the volume increased from 30 L to 45 L from day 3 to 4, and 22.5 L to 60 L from day 12 to 15. The daily IC concentration maintained at 13-15 mg $L^{-1}$ from day 16 to 24, with no significant increase in $CO_2$ injection volume (except day 17-18, 50L daily volume; day 22-24, 0 L daily volume). Overall, there was a significant difference ($p < 0.05$) found in mean IC concentration between control and $CO_2$ addition cultures with a total 787.5 L $CO_2$ volume injected.

From Fig. 3.12 (mid $BOD_5$ wastewater), an association between $CO_2$ volume injected (Table 3.2) and IC was observed. The injection volume was significantly increased between day 8 and 17 which reflects the maintenance of a higher IC concentration over this period. This relationship continued as the $CO_2$ injection volume declined from day 20 to 24 and the decrease of IC concentrations was observed. Over the 24 days period, it was observed that the IC of $CO_2$ injected culture was consistently higher than control. Overall, there was a significant difference found in IC levels ($p < 0.05$), with an approximately 2.4 times higher of total $CO_2$ injected volume (1915 L) when compared to the low $BOD_5$ wastewater.

From Fig. 3.13 (high $BOD_5$ wastewater), an association between $CO_2$ volume injected (Table 3.2) and IC concentration was observed at day 10 – 13, with a steady injection volume averaging 20-30 L per day for the 24 days period. However, the IC concentrations observed in Fig. 3.13 were not correlated to the $CO_2$ volume injected. The IC of control culture was consistently higher than the inlet IC levels, even without injecting $CO_2$. It is possibly due to the evidence of organic carbon in the inlet being converted to IC in the culture.

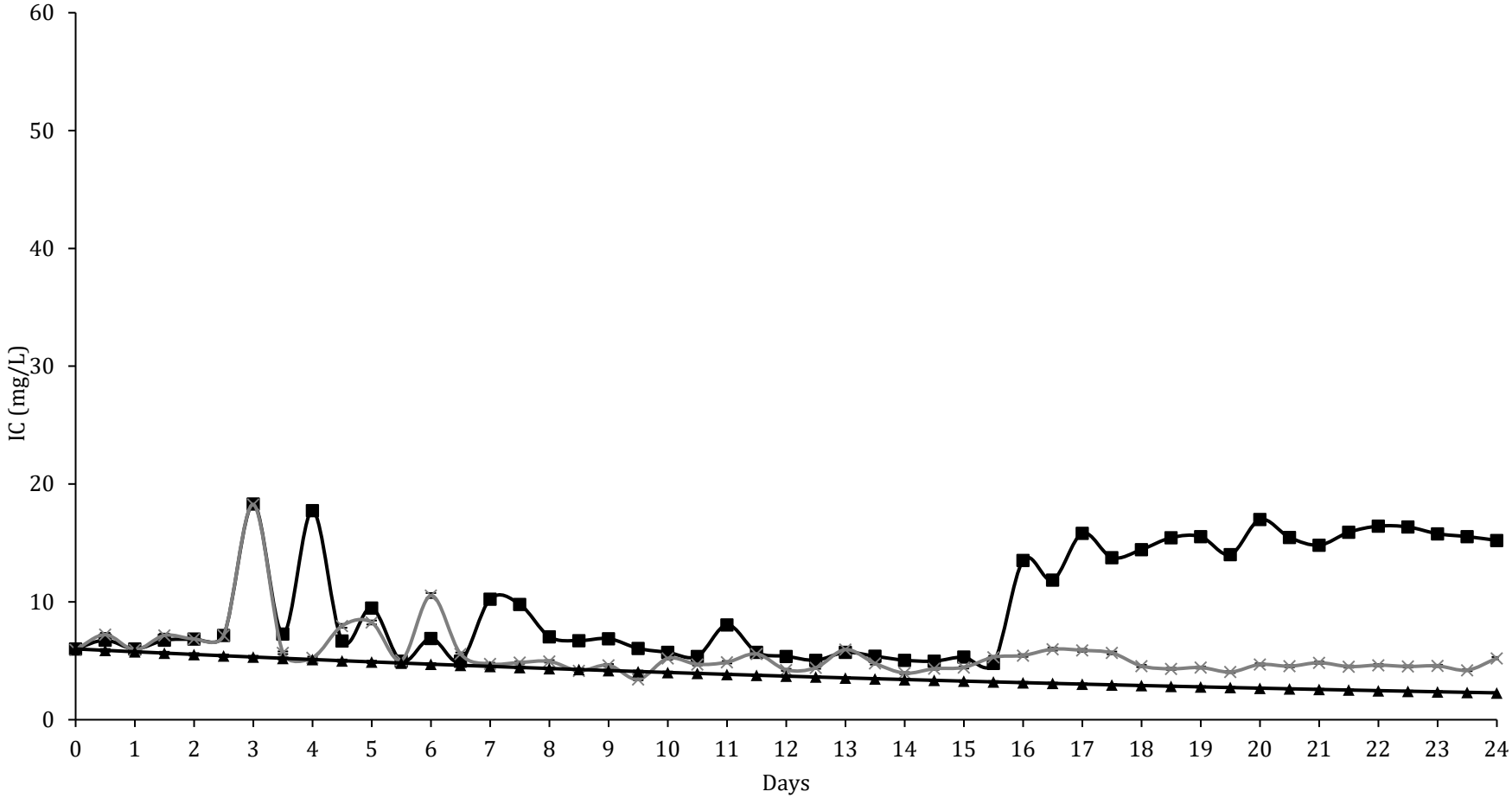



Fig. 3.11 Low $BOD_5$ strength wastewater from Mt Barker aerated lagoon effluent; time series of inorganic carbon concentration in culture with $CO_2$ addition (■), control culture without $CO_2$ addition (X) and inlet IC concentration (▲). Values are means ± SE (n =3).

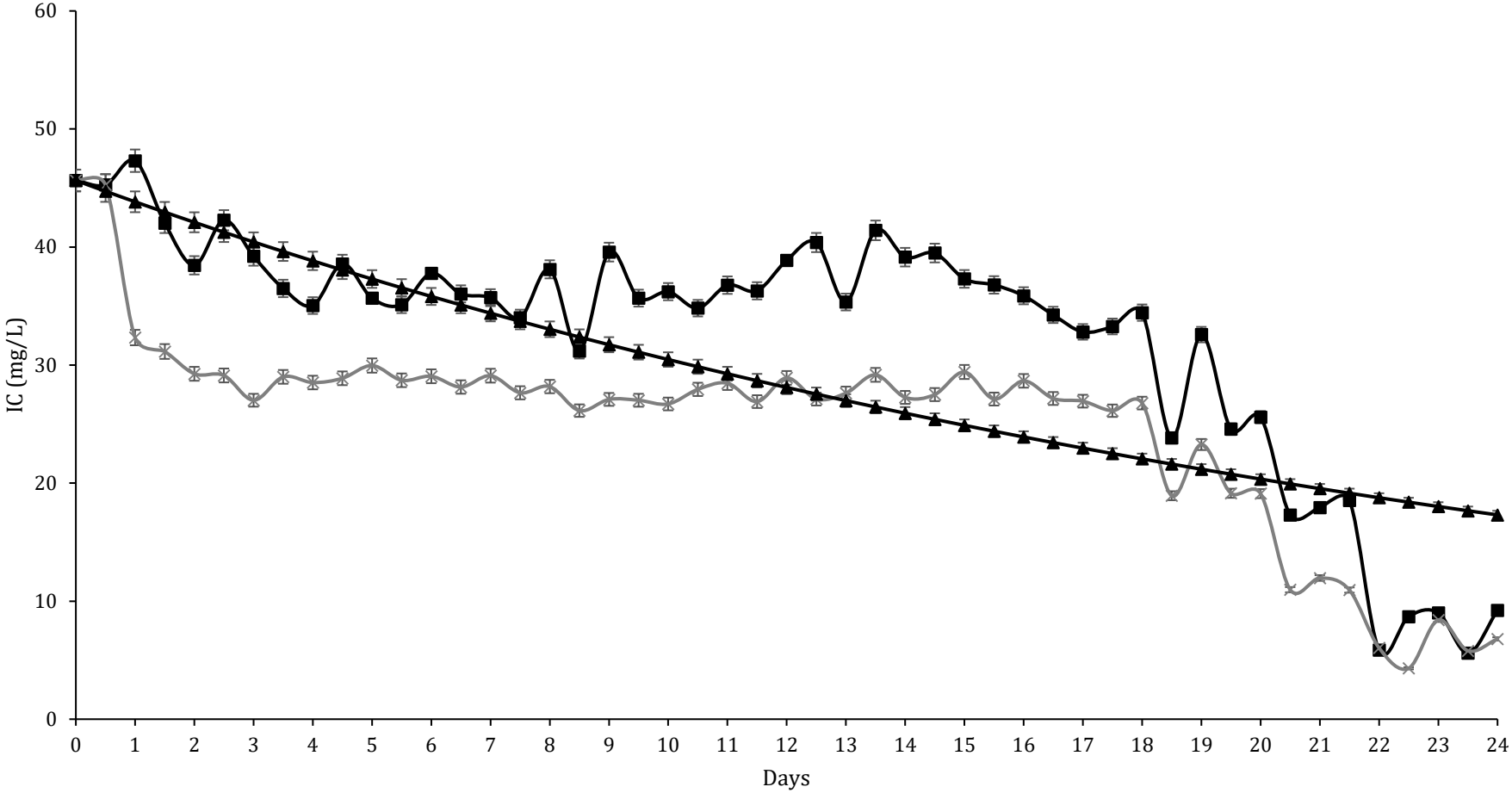



Fig. 3.12 Mid strength $BOD_5$ KoM facultative pond effluent; time series of inorganic carbon concentration in culture with $CO_2$ addition (■), control culture without $CO_2$ addition (X) and inlet IC concentration (▲). Values are means ± SE (n =3).

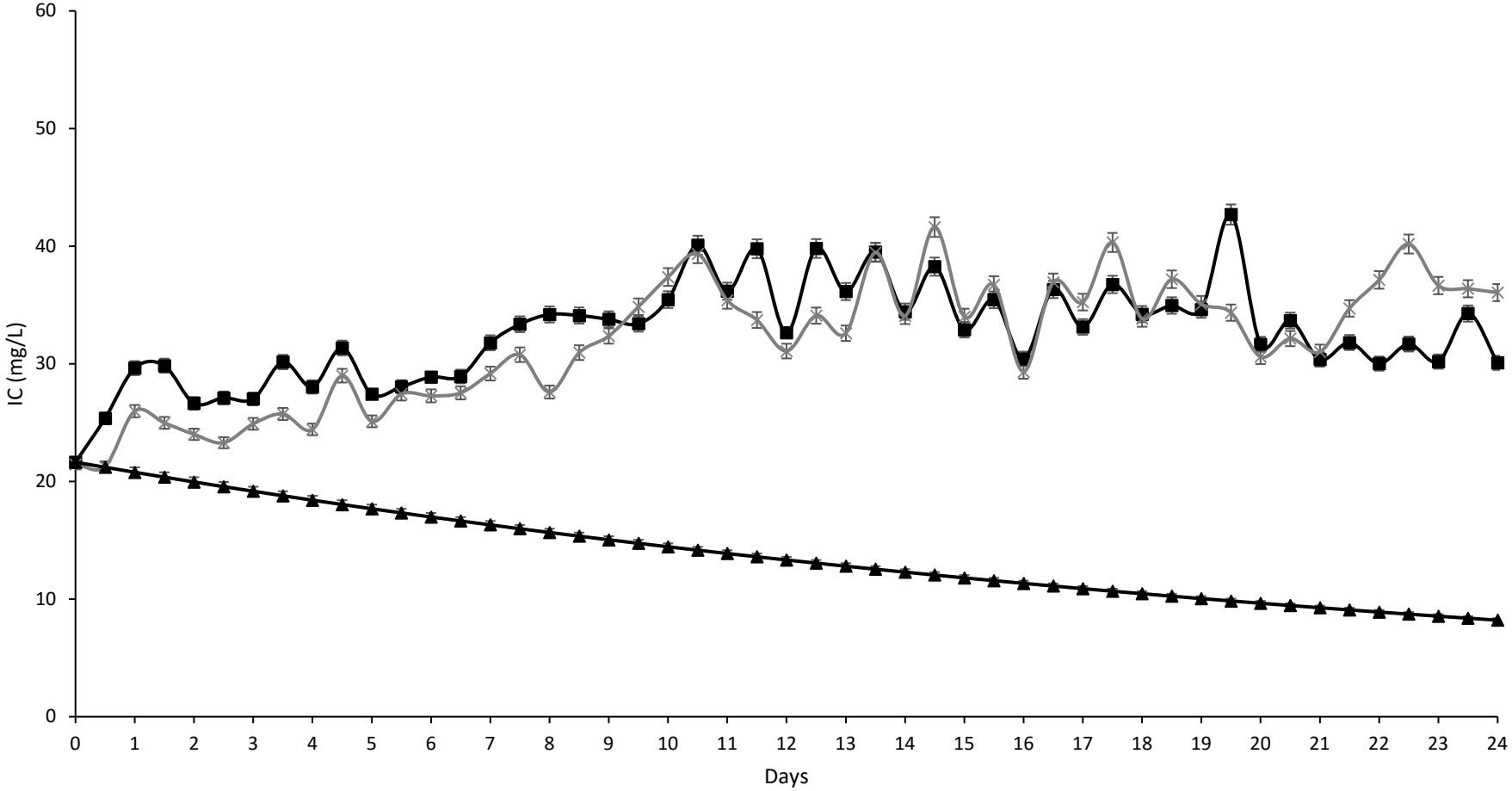



Fig. 3.13 High-strength $BOD_5$ KoM septic tank effluent; time series of inorganic carbon concentration in culture with $CO_2$ addition (■), control culture without $CO_2$ addition (X) and inlet IC concentration (▲). Values are means ± SE (n =3).

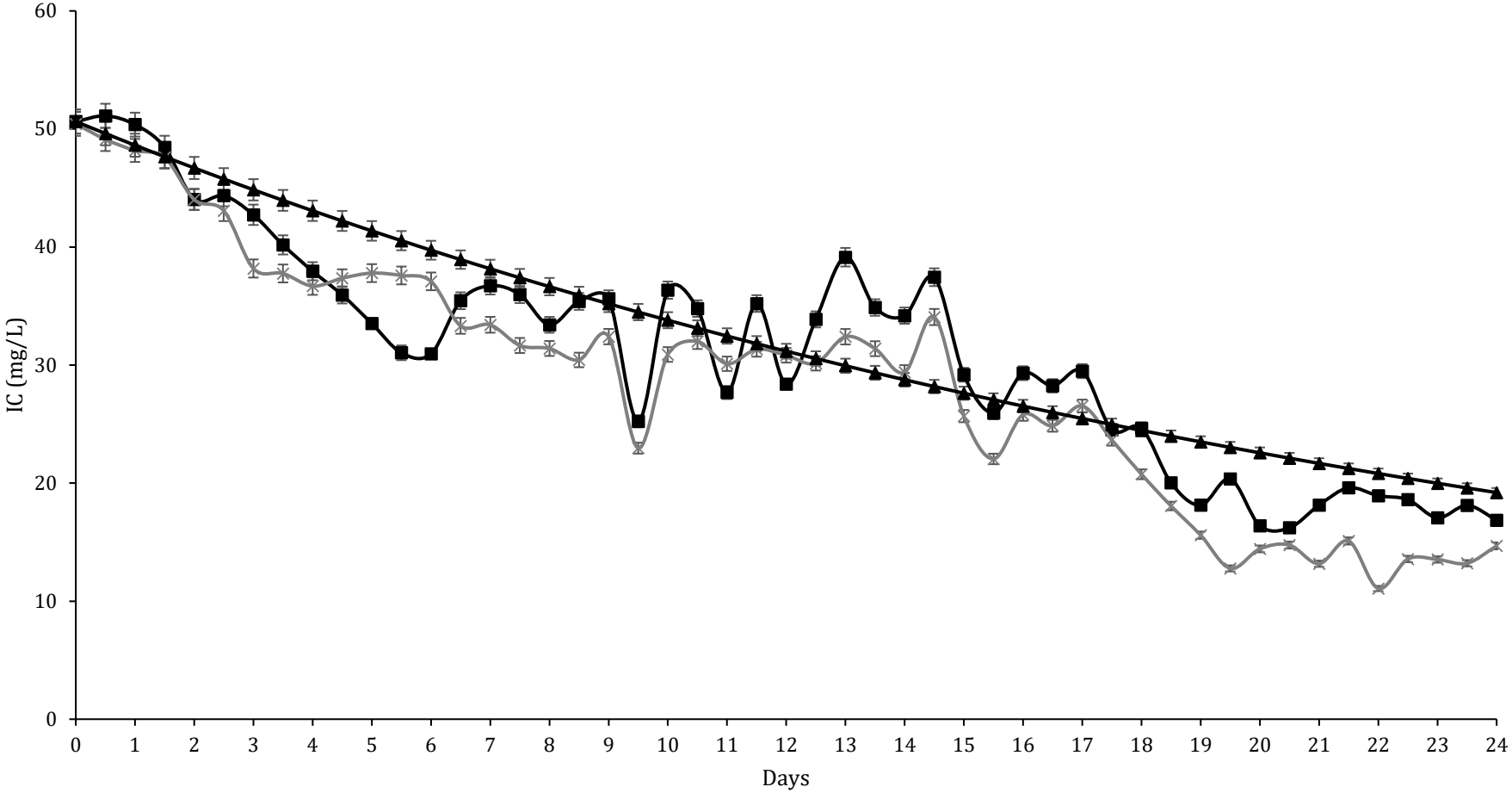



Fig. 3.14 Mid strength $BOD_5$ KoM facultative pond effluent; time series of inorganic carbon concentration in culture with HCl addition (■), control culture without HCl addition (X) and inlet IC concentration (▲). Values are means ± SE (n =3).

### 3.3.3. The effect on algal growth of $CO_2$ addition to wastewaters with differing $BOD_5$ concentrations

The biomass produced within the various cultures was mixed comprising algae, bacteria and, towards the end of some experiments, zooplankton. Biomass production was determined by measurement of chlorophyll *a* concentration, particulate organic carbon (POC; >0.22µm), particulate organic nitrogen (PON; >0.22µm), suspended solids (SS) and volatile suspended solids (VSS). Results were presented for a 24d continuous culture period with cultures operated with a THRT of 4d. In low and mid $BOD_5$ wastewater, zooplankton were presented after day 17-18 in the $CO_2$ addition cultures (indicated as blue arrows in Fig. 3.16 and 3.17). This was also observed in the acid addition culture at day 12 (Fig. 3.19), interestingly this was not observed in the high $BOD_5$ wastewater (Fig. 3.18).

#### 3.3.3.1. Chlorophyll *a*

Figures 3.16 – 3.18 present the chlorophyll *a* contents of the inlet wastewater and clearly show the diurnal variation in chlorophyll *a* content of the control and $CO_2$ amended cultures. The initial chlorophyll *a* contents of represented inlet wastewater was different between all experiments. From the KoM facultative pond (mid $BOD_5$), the inlet wastewater used to determine the biomass production in control and $CO_2$ amended cultures contained 1949 µg Chl*a* $L^{-1}$ throughout the culture period, whereas chlorophyll *a* was, as would be expected, low (87 µg Chl*a* $L^{-1}$ ) within the KoM septic tank (high $BOD_5$) effluent (Fig 3.18). The presence of chlorophyll *a* in the inlet wastewater was similar to what might be expected in HRAPs operating in series with other lagoon based treatment systems as polishing/maturation ponds (Buchanan, 2015).

Chlorophyll *a* productivity was observed in all cultures irrespective of the chlorophyll a concentration of the inlet wastewater. The chlorophyll *a* levels increased rapidly usually within 3 to 4 days in all four experiments. Zooplankton e.g. ciliates, typically present in wastewater treatment lagoons, was observed about day 17, which was indicated by the sudden decrease in chlorophyll *a* levels, in all cultures except the high $BOD_5$ strength wastewater from KOM septic tanks (Fig. 3.18). In the low $BOD_5$

strength experiment, the chlorophyll *a* levels in control were lower than the inlet at around day 17 and the level was unable to maintain above of inlet chlorophyll a levels (Fig. 3.16). In the mid $BOD_5$ strength wastewater experiment using acid to maintain pH stasis, zooplankton was observed after day 12, however, the concentration of chlorophyll *a* was unable to maintain above of inlet value until day 18 in the acid amended culture, and day 15 in the control culture respectively (Figure 3.19).

The addition of $CO_2$ resulted in a significant increase in chlorophyll *a* in all wastewater cultures compared to the controls. Comparison of the differences in mean chlorophyll *a* concentrations between $CO_2$ amended and control cultures showed increases in chlorophyll *a* concentrations following $CO_2$ addition which were inversely related to the initial $BOD_5$ strength of the wastewater: chlorophyll *a* increase of 56.6% (low strength wastewater), 19.3% (mid strength wastewater) and 17.7% (high strength wastewater) compared to the respective control cultures. Interestingly, the control chlorophyll *a* concentration in mid strength wastewater + acid addition, where pH stasis was maintained by the addition of acid rather than $CO_2$, also recorded a 36.1% increase in the mean chlorophyll *a* concentration compared to control. Statistical analysis (Table 3.4) confirmed that there was a significant ($p < 0.05$) difference in chlorophyll *a* concentration between all cultures (low, mid, and high $BOD_5$) amended with $CO_2$ compared to their respective control cultures. Furthermore, maintenance of pH stasis using 0.1M HCl rather than $CO_2$, also resulted in statistically significant increase ($P < 0.05$) in chlorophyll *a* (mid $BOD_5$) compared to control.

Table 3.4 Significant differences in chlorophyll *a* levels (μg/L) in wastewater of different strengths over the 24 days period (P < 0.05). (a) A summary on independent sample T-test for Equality of Means and Levene's Test for Equality of Variances between control and cultures to which $CO_2$ was added under pH control

| (a) Chla (μg/L) | **Mount Barker (Low $BOD_5$)** | **KOM (Mid $BOD_5$, + $CO_2$)** | **KOM (High $BOD_5$)** | **KOM (Mid $BOD_5$,+ Acid)** |
|---|---|---|---|---|
| Are means significantly different (P < 0.05)? | Yes | Yes | Yes | Yes |
| P value | 0.00 | 0.045 | 0.03 | 0.01 |
| Mean ± SD (μg/L) | 948.19 (493.59) | 1880.43 (835.77) | 847.15 (286.34) | 917.63 (534.19) |
| Mean (control) ± SD (μg/L) | 605.19 (406.05) | 1576.72 (650.02) | 719.79 (288.80) | 674.10 (302.94) |
| N | 50 | 50 | 50 | 50 |
| F | 0.27 | 1.97 | 0.33 | 19.20 |
| df | 98 | 98 | 98 | 98 |
| t | 3.80 | 2.32 | 2.03 | 2.80 |
| Mean difference (%) | +56.64 | +19.26 | +17.70 | +36.13 |
| Total injected $CO_2$ or acid (HCl 0.1M) volume (L) | 787.50 | 1915.00 | 800.00 | 1.57 |
| Initial inlet chlorophyll a (μg/L) | 287.00 | 1949.00 | 87.00 | 1191.00 |
| Initial inlet POC (mg/L) | 2.84 | 23.68 | 7.61 | 15.55 |

### 3.3.3.2. Particulate organic carbon (POC) & POC/chlorophyll *a* ratio

POC represents algal organic carbon (AOC) plus bacterial organic carbon (BOC), providing an insight into both primary and secondary productivity. While chlorophyll *a* levels only represent the increase or decrease of AOC, POC also gives an insight to the bacterial productivity and an overall biomass increase.

It is generally understood that the increase of algal biomass is also indicated by the increase of POC. Conventionally, the content of algal chlorophyll is often considered as a reliable and standard index for algal biomass measurement in literature (Boyce et al., 2010; Bricaud et al., 2002; Kasprzak et al., 2008; Moore & Schindler, 2008; Ramaraj et al., 2013; Wiltshire et al., 1998). In this section, the relationship between POC and chlorophyll *a* (Chla) was examined. The values used in the comparison of POC and Chla were from the 4pm sample points. Each wastewater will be discussed individually regarding to their internal carbon pool (i.e. TOC, POC, and DOC) and the effect of additional $CO_2$ as an inorganic source (i.e. IC).

In Table 3.5 (b), high $BOD_5$ inlet, a relatively much lower POC/TOC percentage was observed at 6.69 % which confirms a large part of internal carbon pool in this wastewater was reserved in a high DOC (TOC = POC + DOC). In addition, a high POC/Chla ratio was observed (38.36) which also confirms this POC was contributed largely from another parts of ALBAZOD (possibly mostly from bacteria; and zooplankton and detritus) and a relatively small portion came from algae, due to the fact that this wastewater was collected from a septic tank raw effluent and therefore a low Chla level (Table 3.5 (b)). In Table 3.5 (a), a much lower POC/Chla ratio was observed (6.61 in treatment, 6.86 in control) when compared to the inlet (38.36), indicated that there was a relatively larger portion of algae contributed to the POC. Interestingly, the percentages of POC/TOC in both study groups remained similar (8.90% in treatment, 9.62 in control) when compared to inlet (6.69%). This shows, although there is a slightly increase of POC in their TOC after the 24d experiment, the concentration of DOC remained similar which still contributed a large portion of the TOC in high $BOD_5$ wastewater, with or without $CO_2$ addition. This demonstrated that high $BOD_5$ wastewater with a high internal DOC pool, the effect of $CO_2$ addition as an inorganic carbon source was minimal, due to the fact that the increase of POC and Chla was more likely to be coming from the readily and assessable source i.e. the high internal DOC pool.

In Table 3.5 (b), both mid $BOD_5$ inlets, although they were both collected from the same facultative effluent in KoM at different times, it is important to note that their POC/Chla ratios were different (7.92 for the $CO_2$ experiment; 16.27 for the acid experiment). This is in agreement with their individual POC and Chla values. The inlet Chla concentration was approximately 3 times higher (1.91 mg $L^{-1}$) for the $CO_2$ experiment group than the acid experiment group (0.61 mg $L^{-1}$). However, their percentages of POC/TOC were similar (27.17 % and 28.47%) which demonstrated a similar portion of internal DOC pool and it was confirmed by the similar $BOD_5$ concentrations (78 and 72 mg $L^{-1}$). Interestingly, their POC/Chla ratios (~ 14) were similar after the 24d experiment which means a similar balance of POC and Chla was achieved by either $CO_2$ or acid addition into the wastewater (Table 3.5 a). As previously discussed, the $CO_2$ injection volumes were significant higher in mid $BOD_5$ strength wastewater, compared to low and high $BOD_5$ strength wastewater which both were observed with a similar daily injection volume (Table 3.2). This shows, although

there is an increase (approximately +10%) of POC in their TOC after the 24d experiment with or without $CO_2$ addition, the effect of $CO_2$ addition was not significant in increasing of POC/TOC percentage i.e. in increasing of ALBAZOD (this will be discussed further in Table 3.6). On the other hand with the acid addition experiment in mid $BOD_5$ wastewater, the percentage of POC/TOC in both treatment (26.43%) and control (23.82%) groups were lower than inlet (28.47%). This may due to the lower inlet Chla concentration (0.61 mg $L^{-1}$) and therefore a lower POC in contributing to the overall POC/TOC percentages. However, the addition of acid has successfully demonstrated a higher POC/TOC percentage (26.43%) than control (23.82%). This suggests, using acid to adjust culture pH, the internal DOC pool can be utilised for a higher production of ALBAZOD based on the increase of POC.

In Table 3.5 (b), low $BOD_5$ inlet, a relatively lower POC/TOC percentage was observed at 11.84 % which confirms a large part of internal carbon pool in this wastewater was reserved in a high DOC. However, it is important to note that the TOC was 15.37 mg $L^{-1}$ and it was the lowest among all $BOD_5$ wastewater especially compared to the highest 73.24 mg $L^{-1}$ in high $BOD_5$ wastewater. Therefore, the DOC level (TOC – POC = 13.55 mg $L^{-1}$) was also the lowest. There were significant increases in POC/TOC percentages in both $CO_2$ treatment (39.97%) and control (29.38%) when using this low DOC concentration wastewater (11.84%). In addition, the percentage was 10% higher when $CO_2$ was injected. This demonstrated that low $BOD_5$ wastewater with a low Chla and internal DOC pool, the effect of $CO_2$ addition as inorganic carbon source was substantial, due to the fact that there was not enough internal DOC to support the expanded algal growth. In this case, this low $BOD_5$ wastewater may be considered as carbon limited (Table 3.5 (a))

Figure 3.15 (a, b, and d) shows a strong, positive linear relationship between POC and Chla, for low $BOD_5$ wastewater for, mid + $CO_2$, and mid + acid $BOD_5$ wastewater (including the controls) with all $R^2 > 0.93$. However, the correlation coefficients ($R^2$) in the linear regression of POC with Chla in high $BOD_5$ wastewater were significantly lower at $R^2$ 0.09 (+ $CO_2$) and $R^2$ 0.21 (control). Based on the regression analysis of POC and Chla concentrations in the different $BOD_5$ wastewaters, the POC/Chla ratios indicated at range ~14:1, except ~7;1 in the high $BOD_5$ wastewater. The data suggests

that the change in the ratio was brought about by an increase in chlorophyll relative to POC. In addition, in the low BOD + $CO_2$ experiment, it was the only group with a higher POC/Chla ratio (13.43) than control (13.38). This implies that supplying additional $CO_2$ to all $BOD_5$ strength wastewater, in perspective of algal growth, was beneficial based on the observations of significantly increases in Chla. However, with the observations based on the increase of POC, supplying additional $CO_2$ was only beneficial when $BOD_5$ was low (~15 mg BOD $L^{-1}$) when considering ALBAZOD (algae, zooplankton, detritus and bacteria). (Table 3.5).

Table 3.5 (a) Ratio of POC (mg/L) and chlorophyll *a* (Chl*a*) (mg/L) on different wastewater $BOD_5$ strengths, using the 4pm (i.e. the mid-point of light cycle) sample points only. The relative TOC values and POC/TOC percentages (%) were also indicated. (b) Inlet, using the 4pm sample points only.

| | | Mount Barker (Low $BOD_5$) | KOM (Mid $BOD_5$) | KOM (High $BOD_5$) | KOM (Mid $BOD_5$,+ Acid) |
|---|---|---|---|---|---|
| $BOD_5$ (mg/L) | | 15 (0.46) | 78 (3.12) | 120 (3.66) | 72 (2.88) |
| Treatment (+$CO_2$ or acid) | Mean POC (mg/L) | 15.61 | 27.98 | 6.14 | 13.46 |
| | Mean Chla (mg/L) | 1.16 | 2.09 | 0.93 | 1.01 |
| | Mean TOC (mg/L) | 39.05 | 78.15 | 69.00 | 50.93 |
| | **POC/TOC (%)** | **39.97** | **35.69** | **8.90** | **26.43** |
| Control | Mean POC (mg/L) | 9.81 | 24.47 | 5.53 | 10.28 |
| | Mean Chla (mg/L) | 0.73 | 1.74 | 0.81 | 0.75 |
| | Mean TOC (mg/L) | 33.39 | 65.05 | 57.48 | 43.16 |
| | **POC/TOC (%)** | **29.38** | **37.62** | **9.62** | **23.82** |
| **POC/Chl*a* ratio** | Treatment (+$CO_2$ or acid) | **13.43** | **13.37** | **6.61** | **13.39** |
| | Control | **13.38** | **14.05** | **6.86** | **13.67** |

**(b)** Inlet, using the 4pm sample points only

| | Mount Barker (Low $BOD_5$) | KOM (Mid $BOD_5$) | KOM (High $BOD_5$) | KOM (Mid $BOD_5$,+ Acid) |
|---|---|---|---|---|
| Mean POC (mg/L) | 1.82 | 15.22 | 4.90 | 9.99 |
| Mean Chla (mg/L) | 0.33 | 1.91 | 0.13 | 0.61 |
| Mean TOC (mg/L) | 15.37 | 56.01 | 73.24 | 35.09 |
| **POC/TOC (%)** | **11.84** | **27.17** | **6.69** | **28.47** |
| **POC/Chl*a* ratio** | **5.52** | **7.97** | **38.36** | **16.27** |

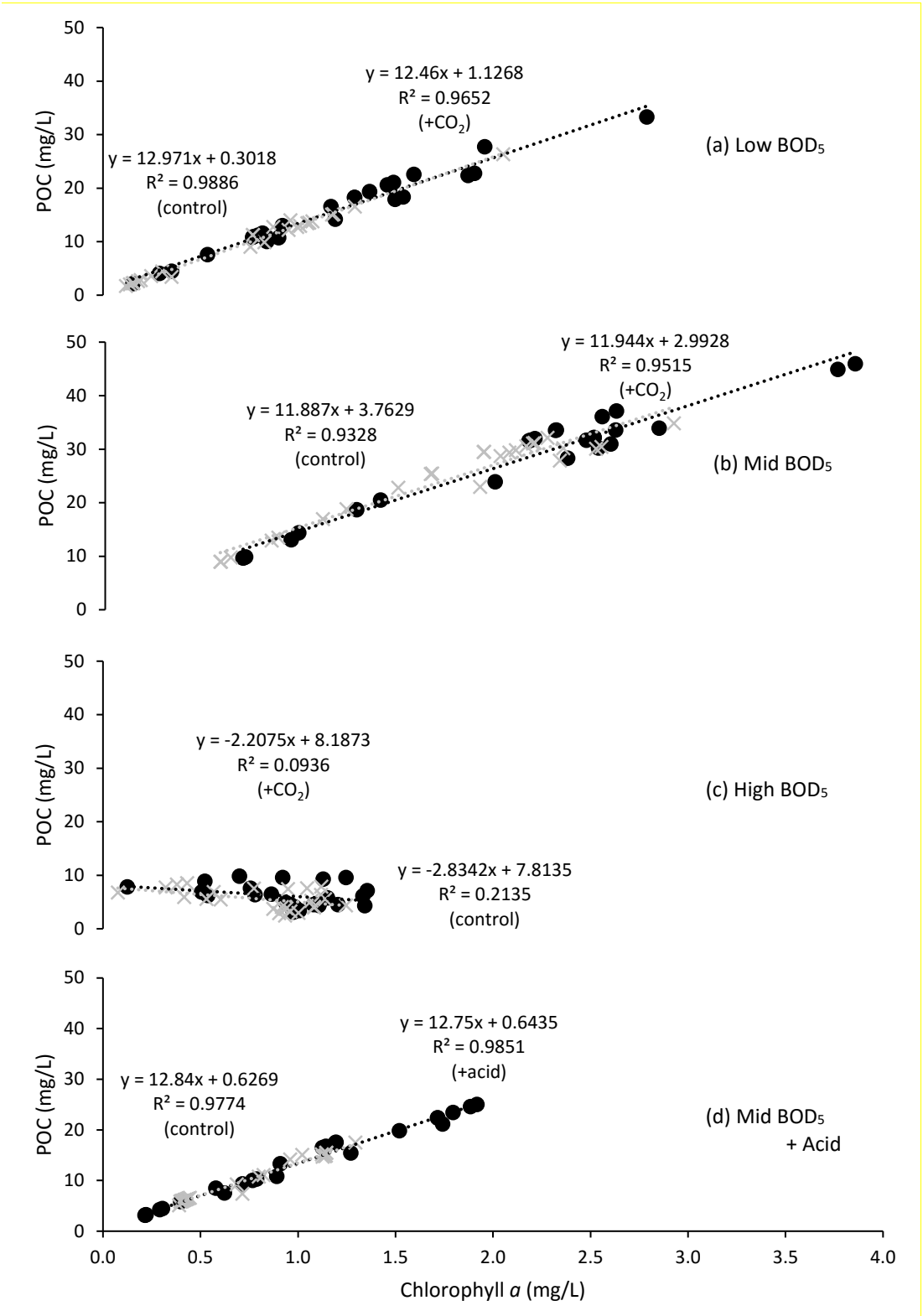



Fig. 3.15 Relationships between POC and chlorophyll *a* observed in (a) low, (b) mid, (c) high, and (d) mid $BOD_5$ with acid addition wastewater by using 4pm (i.e. the mid-point of light cycle) sample points only; with $CO_2$ or acid addition (●) and control (X).

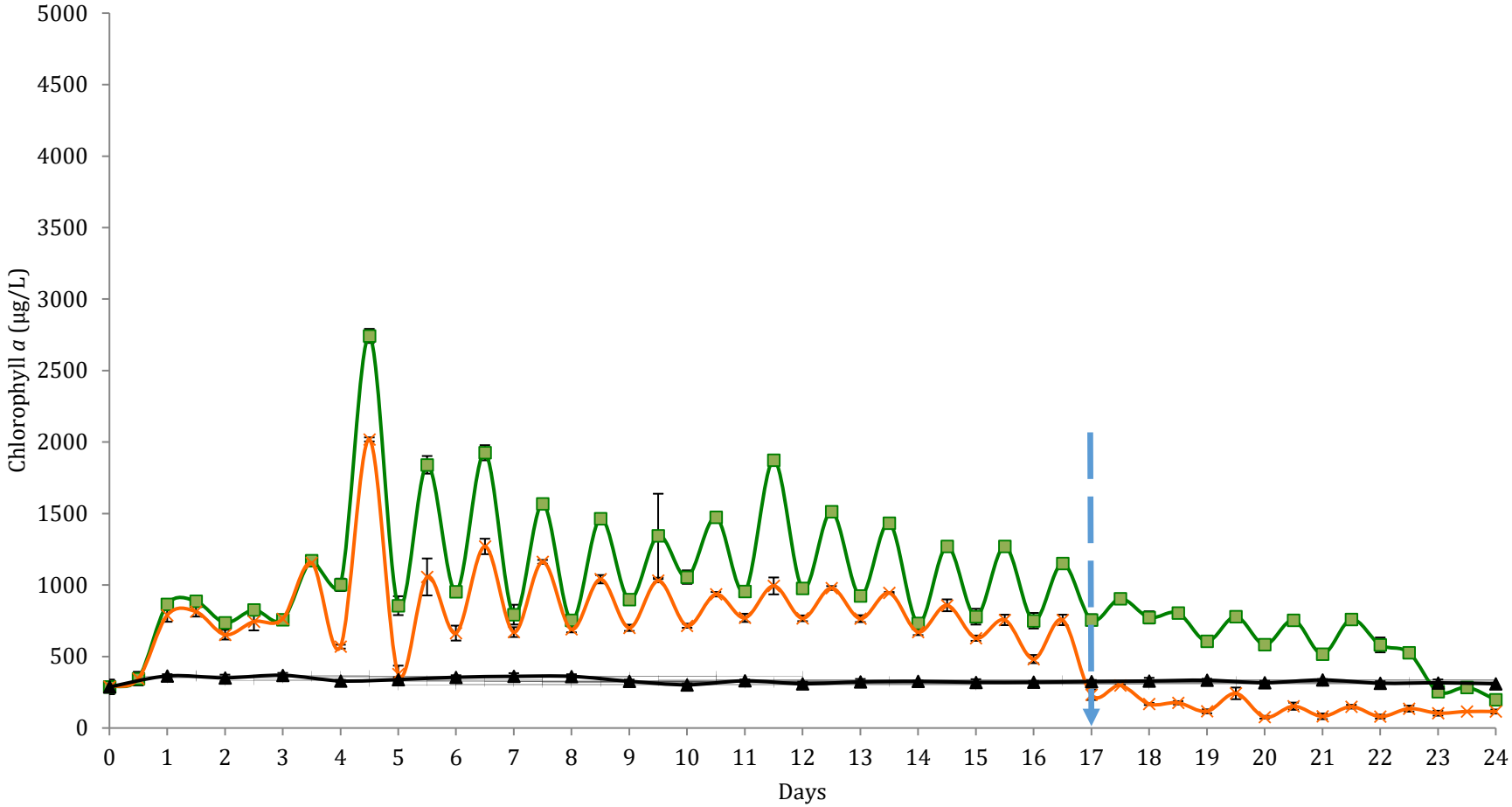



Fig. 3.16 Low strength $BOD_5$ wastewater from Mt Barker aerated lagoon effluent; chlorophyll *a* concentration in culture with $CO_2$ addition (■), control culture without $CO_2$ addition (X) and inlet chlorophyll a concentration (▲). Values are means ± SE (n =3); onset of the presence of zooplankton (→).

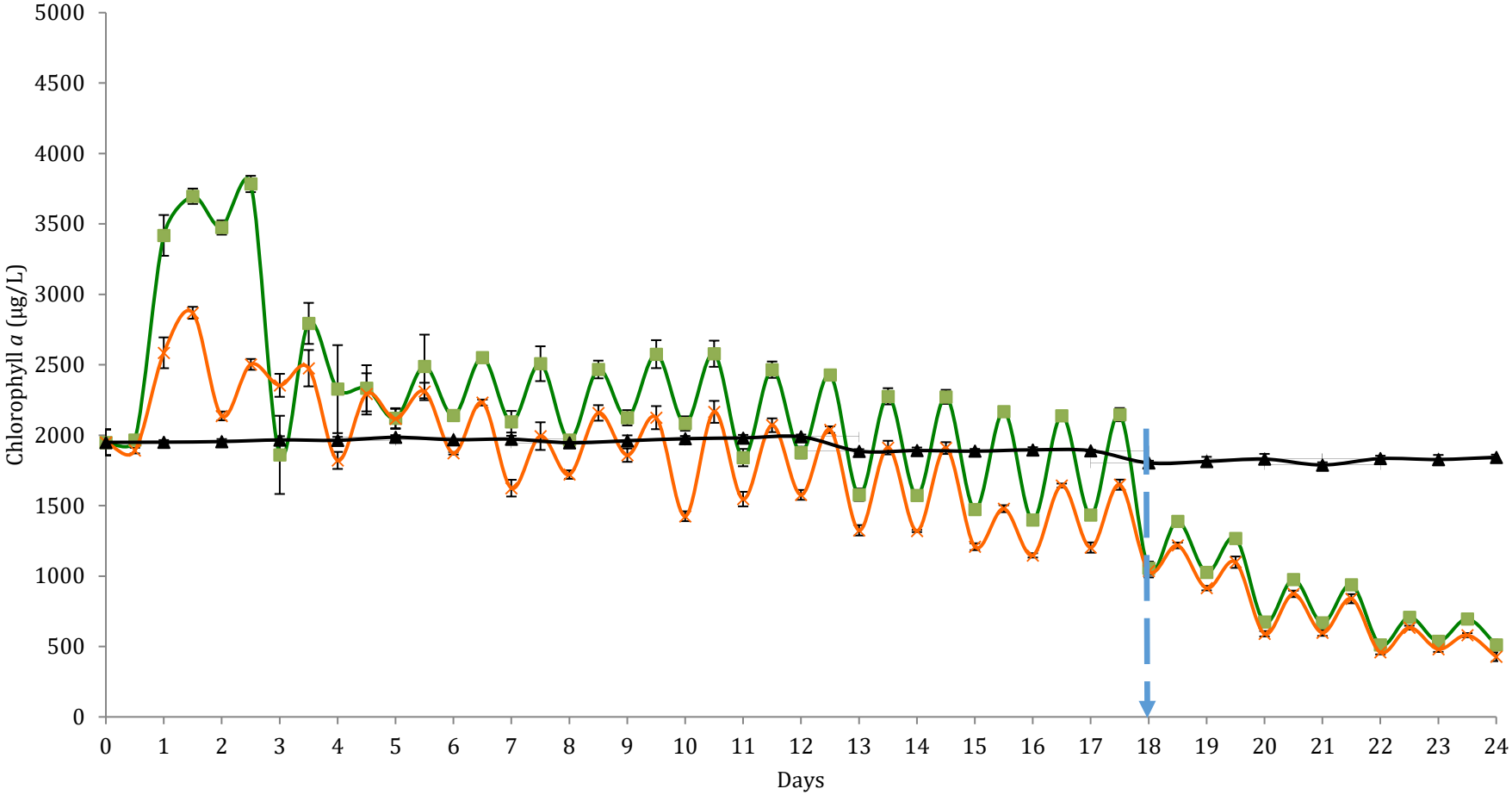



Fig. 3.17 Mid strength $BOD_5$ KoM facultative pond effluent; chlorophyll *a* concentration in culture with $CO_2$ addition (■), control culture without $CO_2$ addition (X) and inlet chlorophyll a concentration (▲). Values are means ± SE (n =3); onset of the presence of zooplankton (→).

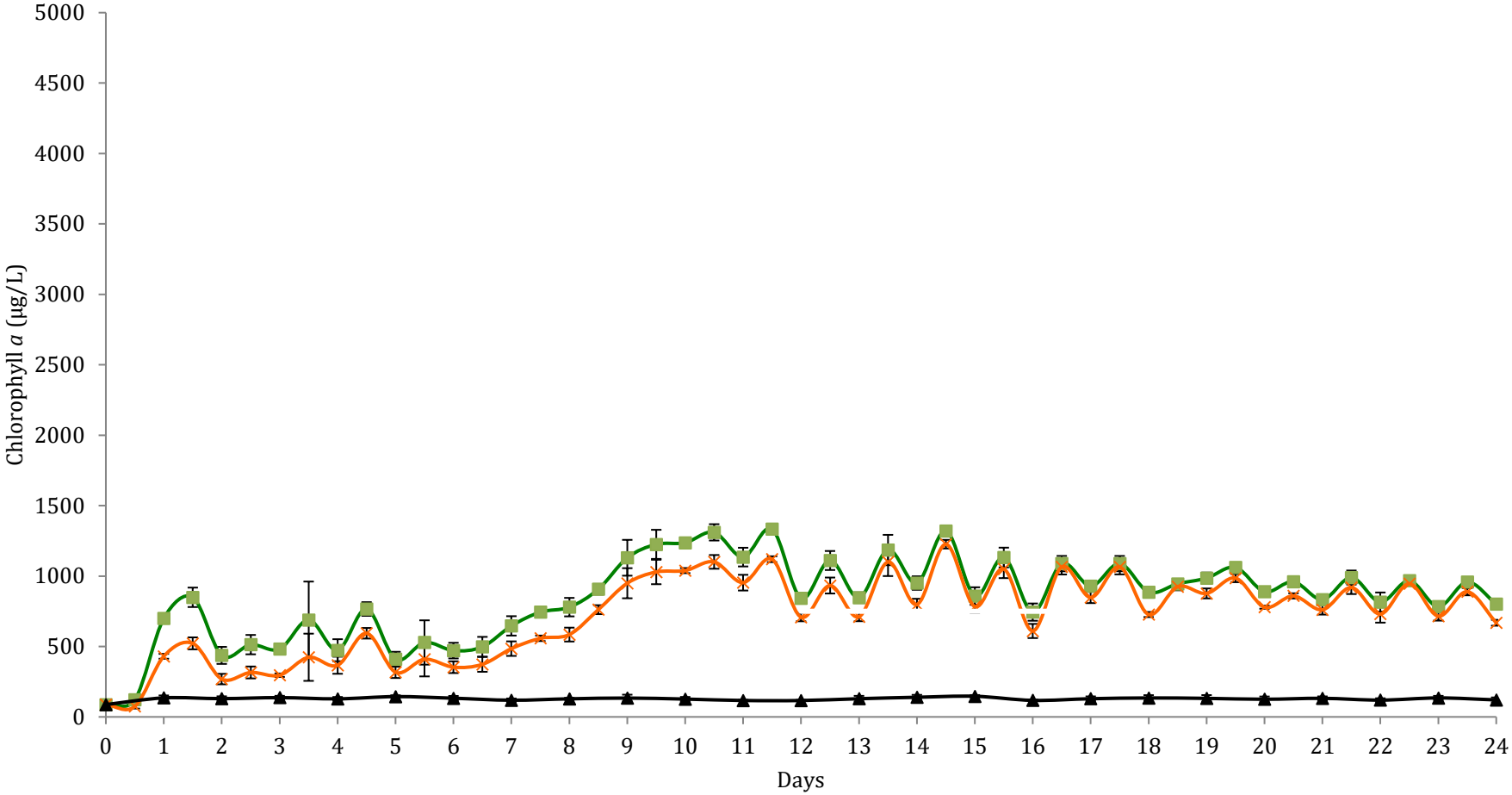



Fig. 3.18 High strength $BOD_5$ KoM septic tank effluent; chlorophyll *a* concentration in culture with $CO_2$ addition (■), control culture without $CO_2$ addition (X) and inlet chlorophyll a concentration (▲). Values are means ± SE (n =3).

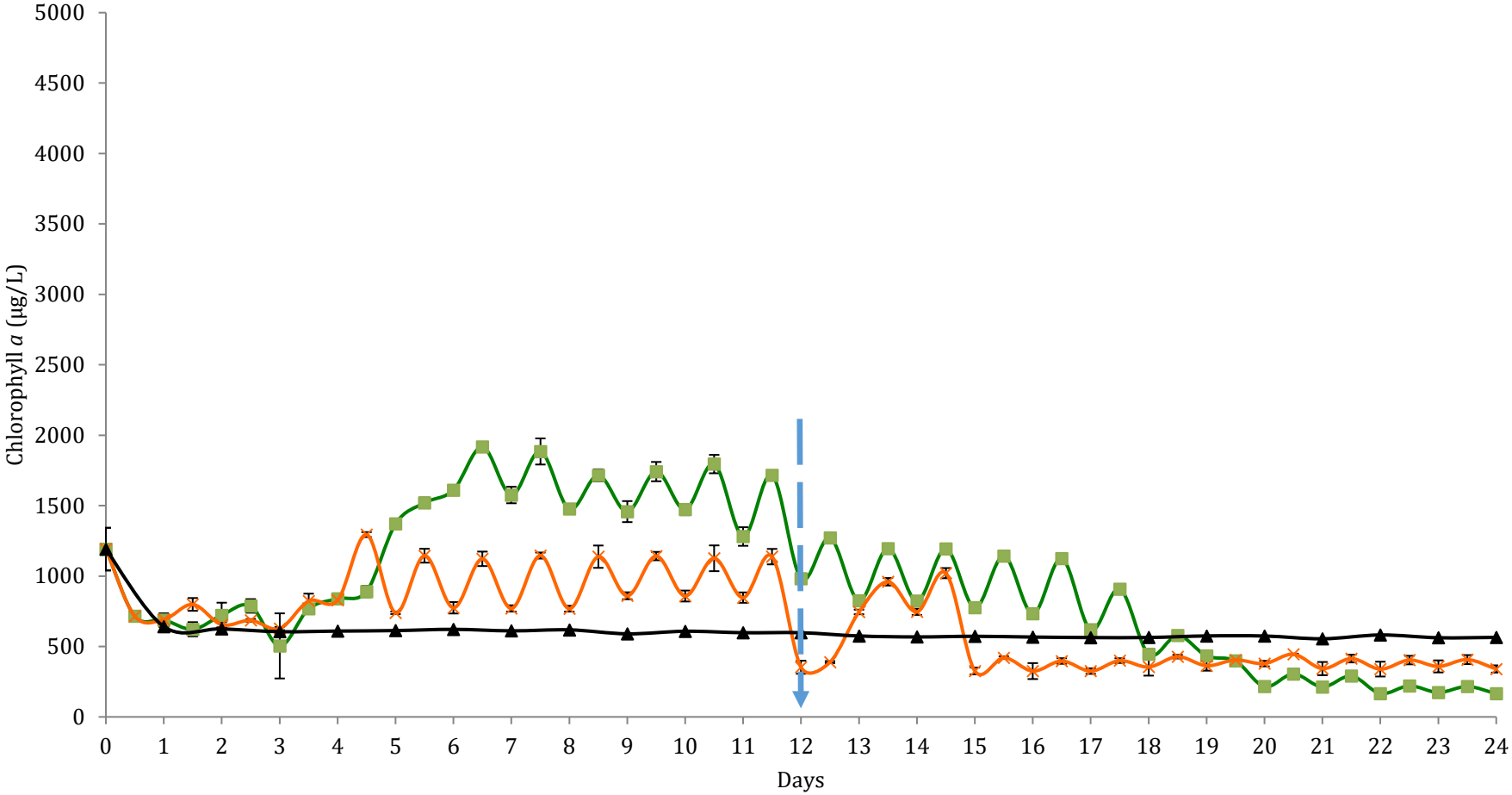



Fig. 3.19 Mid strength $BOD_5$ KoM facultative pond effluent; chlorophyll *a* in culture with HCl addition (■), control culture without HCl addition (X) and inlet chlorophyll a concentration (▲). Values are means ± SE (n =3); onset of the presence of zooplankton (→).

Figs 3.20 – 3.23 show the time series of POC concentration in the control culture and the $CO_2$ amended cultures in the respective $BOD_5$ strength wastewaters together with the comparison of the effect of acid addition to the mid-strength wastewater rather than $CO_2$ ( Fig. 3.22). Table 3.6 shows that overall changes in the mean POC contents were very similar to those changes recorded for chlorophyll *a*. In Mount Barker low $BOD_5$ strength wastewater, a 56.31% increase in mean POC (12.62 mg $L^{-1}$) was recorded compared to the control (8.03 mg $L^{-1}$). Interestingly, a similar increase (+33.73%) was also observed in the mid $BOD_5$ strength KOM wastewater with acid addition (12.21 mg $L^{-1}$) when compared to control (9.13 mg $L^{-1}$). This implies that merely operating the wastewater culture as a pH stat without addition of any external inorganic carbon source substantially increased POC. Moreover the increase was greater than in the mid $BOD_5$ wastewater supplemented with $CO_2$ (24.92 mg $L^{-1}$ → 21.76 mg $L^{-1}$, +14.52%). There was a comparatively small mean increase (+13.48%) in the POC (6.23 mg $L^{-1}$) of the high $BOD_5$ wastewater with $CO_2$ addition compared to control (5.49 mg $L^{-1}$).

Table 3.6 also shows that there were statistically significant differences between the wastewater with additional $CO_2$ and the respective control culture. Significantly differences in chlorophyll *a* were found in all $BOD_5$ strengths wastewater following $CO_2$ addition, however, corresponding, statistically significant increases in both POC and Chla levels following $CO_2$ addition were only found in low $BOD_5$ and mid $BOD_5$ with acid. This implies that supplying additional $CO_2$ to wastewater for algal growth (ALBAZOD) was only beneficial when $BOD_5$ was low (~15 mg $L^{-1}$) in reflects to the significantly increase of POC.

Table 3.6 Significant differences and mean increase (%) between POC (mg/L) and chlorophyll *a* (Chla) (mg/L) from cultures on different wastewater strength ($P < 0.05$). A summary on independent sample T-test for Equality of Means and Levene's Test for Equality of Variances

| | | **Mount Barker (Low $BOD_5$)** | **KOM (Mid $BOD_5$, + $CO_2$)** | **KOM (High $BOD_5$)** | **KOM (Mid $BOD_5$,+ Acid)** |
|---|---|---|---|---|---|
| Are means significantly different? ($P < 0.05$) | | **Yes** | **No** | **No** | **Yes** |
| P value | **POC (mg/L)** | 0.00 | 0.09 | 0.06 | 0.01 |
| Mean ± SD | | 12.62 (6.38) | 24.92 (10.27) | 6.23 (1.99) | 12.21 (6.80) |
| Mean (control) ± SD | | 8.08 (5.32) | 21.76 (7.93) | 5.49 (1.82) | 9.13 (3.82) |
| N | | 50 | 50 | 50 | 50 |
| F | | 0.28 | 2.97 | 0.60 | 21.48 |
| df | | 98 | 98 | 98 | 98 |
| t | | 3.87 | 1.72 | 1.94 | 2.80 |
| Mean difference (%) | | +56.31 | +14.52 | +13.48 | +33.73 |
| Are means significantly different ($P < 0.05$)? | | **Yes** | **Yes** | **Yes** | **Yes** |
| P value | **Chla (mg/L)** | 0.00 | 0.045 | 0.03 | 0.01 |
| Mean ± SD | | 0.95 (0.49) | 1.88 (0.84) | 0.85 (0.29) | 0.92 (0.53) |
| Mean (control) ± SD | | 0.61 (0.41) | 1.58 (0.65) | 0.72 (0.29) | 0.67 (0.30) |
| N | | 50 | 50 | 50 | 50 |
| F | | 0.27 | 1.97 | 0.33 | 19.20 |
| df | | 98 | 98 | 98 | 98 |
| t | | 3.80 | 2.32 | 2.03 | 2.80 |
| Mean difference (%) | | +56.64 | +19.26 | +17.70 | +36.13 |
| Total injected $CO_2$ or acid (HCl 0.1M) volume (L) | | 787.50 | 1915.00 | 800.00 | 1.57 |
| Initial inlet chlorophyll a (mg/L) | | 0.29 | 1.95 | 0.09 | 1.19 |
| Initial inlet POC (mg/L) | | 2.84 | 23.68 | 7.61 | 15.55 |

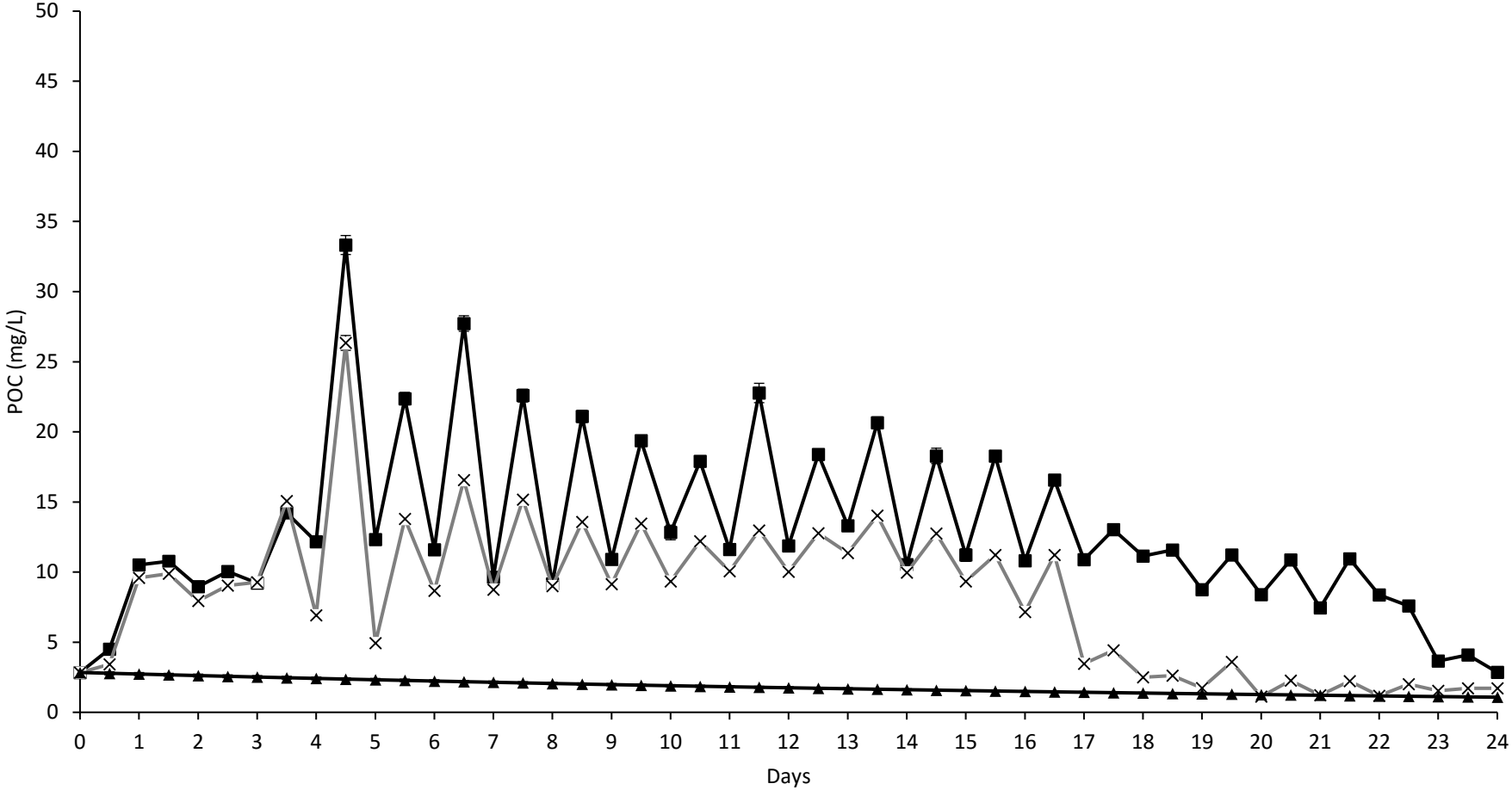



Fig. 3.20 Low $BOD_5$ strength wastewater from Mt Barker aerated lagoon effluent; time series of particulate organic carbon (POC; >0.22μm) concentration in culture with $CO_2$ addition (■), control culture without $CO_2$ addition (X) and inlet concentration (▲). Values are means ± SE (n =3).

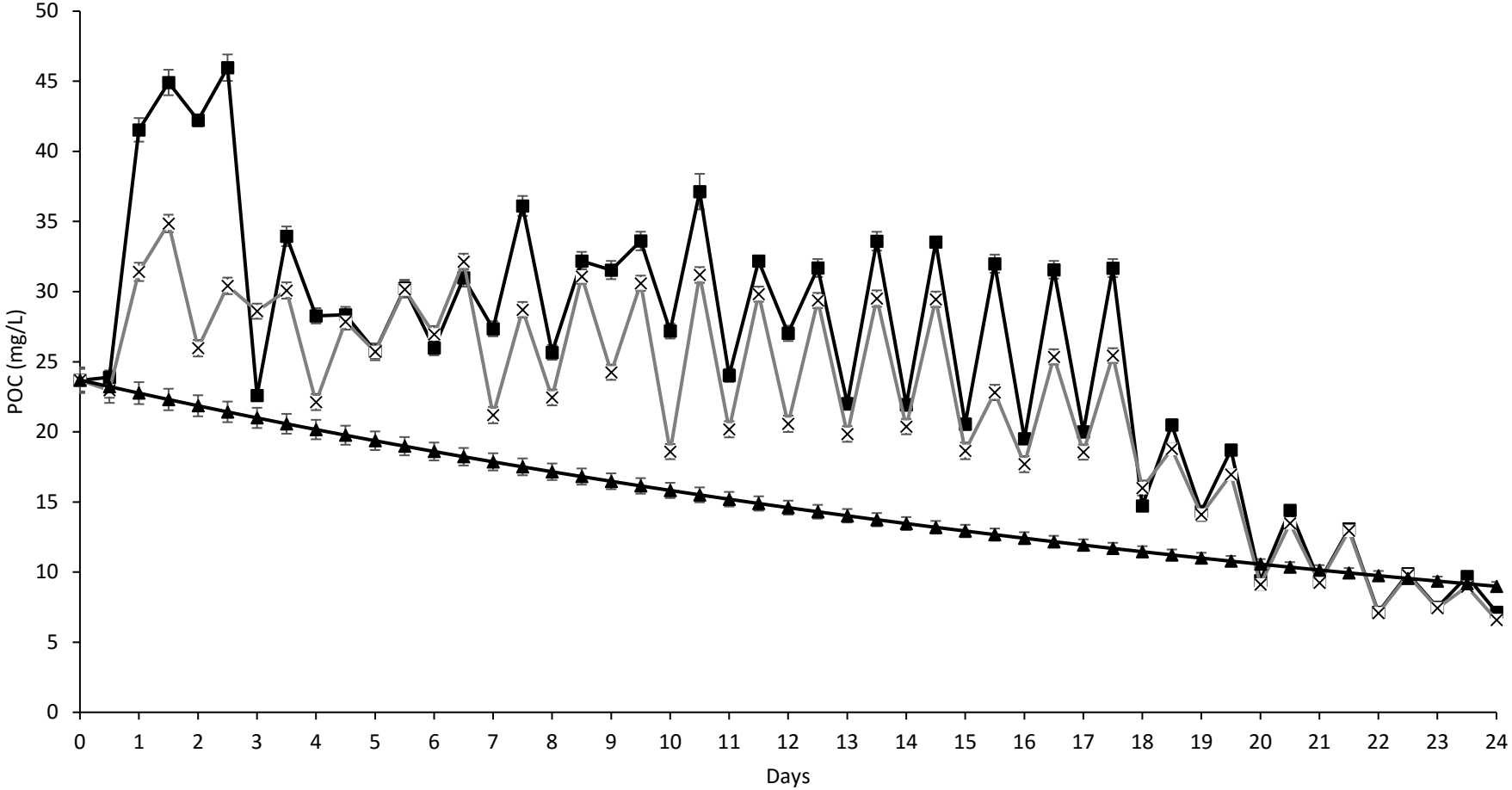



Fig. 3.21 Mid strength $BOD_5$ KoM facultative pond effluent; time series of particulate organic carbon (POC; >0.22µm) concentration in culture with $CO_2$ addition (■), control culture without $CO_2$ addition (X) and inlet concentration (▲). Values are means ± SE (n =3).

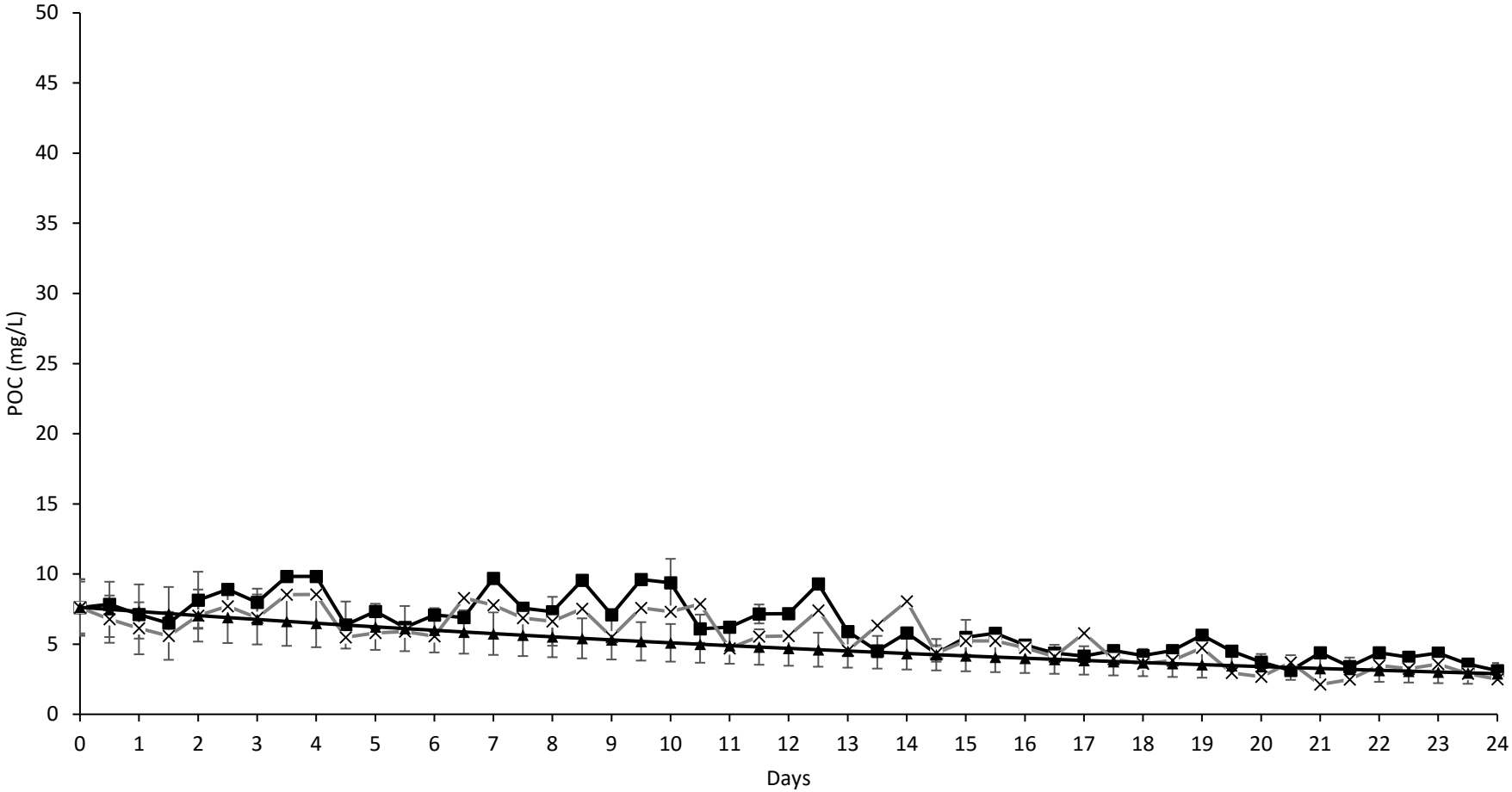



Fig. 3.22 High strength $BOD_5$ KoM septic tank effluent; time series of particulate organic carbon (POC; >0.22µm) concentration in culture with $CO_2$ addition (■), control culture without $CO_2$ addition (X) and inlet concentration (▲). Values are means ± SE (n =3).

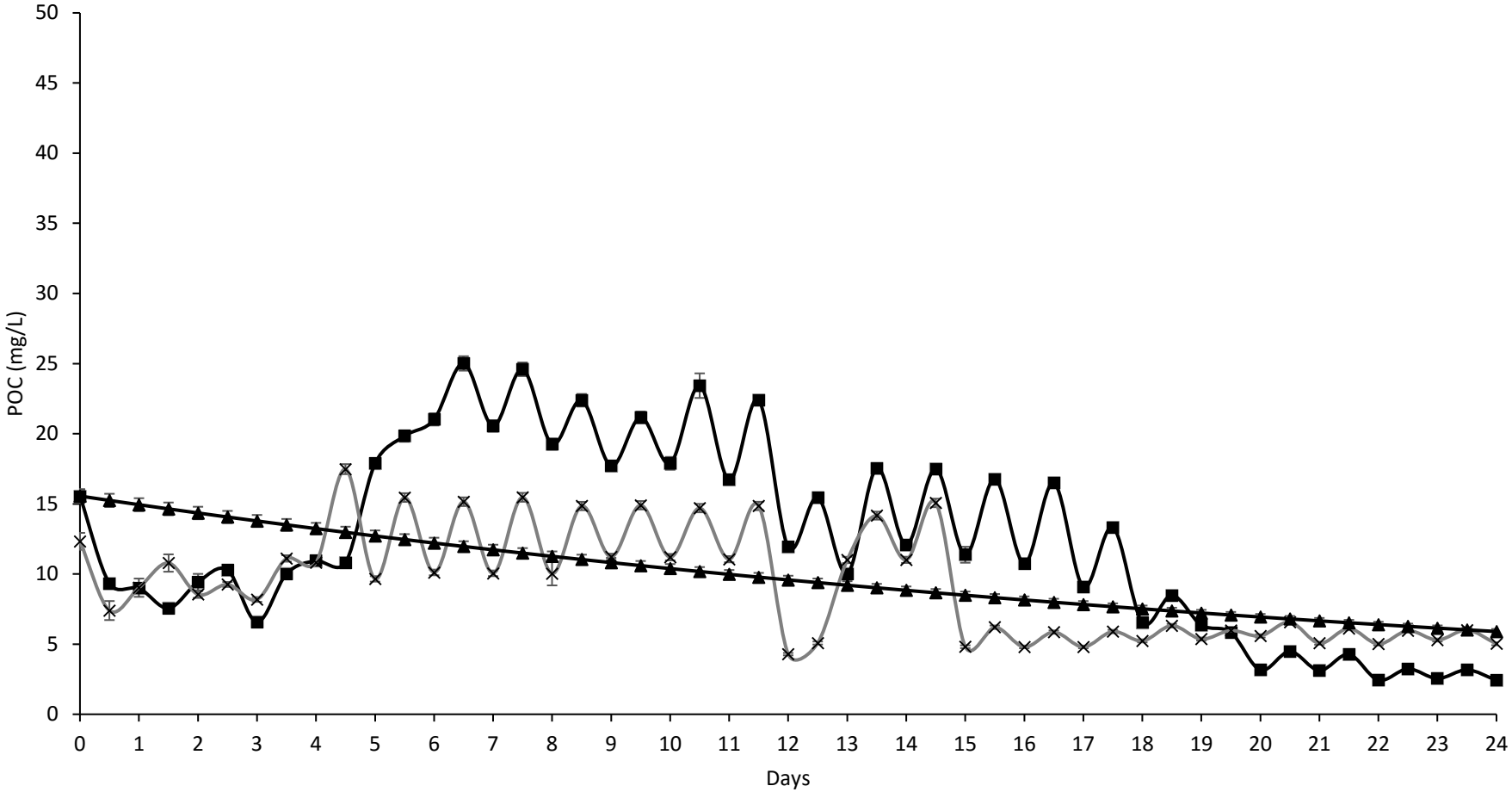



Fig. 3.23 Mid strength $BOD_5$ KoM facultative pond effluent; time series of particulate organic carbon (POC; >0.22µm) concentration in culture with HCl addition (■), control culture without HCl addition (X) and inlet concentration (▲). Values are means ± SE (n =3).

### 3.3.3.3. Particulate organic nitrogen (PON)

The measurement of TN consists of organic nitrogen ($N_{org}$) and inorganic nitrogen ($N_{inorg}$). The $N_{org}$ is separated into particulate organic nitrogen (PON) and dissolved organic nitrogen (DON). The measurement of PON consists both algal organic nitrogen (AON) and bacterial organic nitrogen (BON). The rest of TN is coming from $N_{inorg}$ which is separated into $NH_4$–N + $NO_3$–N + $NO_2$–N. The relationship between TN, PON, DON, and IN is shown in Equation 3.1:

$$TN = PON + DON + NH_4\text{-}N + NO_3\text{-}N + NO_2\text{-}N \quad \textbf{Equation 3.1}$$

Uptake of $NH_4$, $NO_3$, and $NO_2$ has important implications in relation to $CO_2$ assimilation for two reasons – competition for photosynthetic reducing power and alteration of $CO_2$ availability in the surrounding microenvironment. Overall, the results show that there was a statistically significant increase (Table 3.7) in the PON levels in all wastewaters when additional $CO_2$ was supplied (Fig. 3.24 – 3.27). The mean increase of PON in the cultures to which $CO_2$ was added compared to the control cultures was 36.5%, 35.5% and 48.7% in the low, mid, and high strength $BOD_5$ wastewaters. However, the mid $BOD_5$ strength wastewater to which only acid was added to maintain pH (Fig 3.27) also showed an increase in the mean PON of 34.6% compared to control (Table 3.7).

Table 3.7 Significant differences of different wastewater strength on particulate organic nitrogen (PON mg/L) over the 24 days period ($P < 0.05$). (a) A summary on independent sample T-test for Equality of Means and Levene's Test for Equality of Variances

| (a) PON (mg/L) | **Mount Barker (Low $BOD_5$)** | **KOM (Mid $BOD_5$, + $CO_2$)** | **KOM (High $BOD_5$)** | **KOM (Mid $BOD_5$,+ Acid)** |
|---|---|---|---|---|
| Are means significantly different ($P < 0.05$)? | Yes | Yes | Yes | Yes |
| P value | 0.00 | 0.00 | 0.00 | 0.00 |
| Mean ± SD (mg/L) | 6.32 (3.32) | 9.60 (3.35) | 10.38 (4.83) | 6.39 (0.91) |
| Mean (control) ± SD (mg/L) | 4.63 (2.35) | 7.09 (2.64) | 6.98 (3.26) | 4.74 (0.85) |
| N | 50 | 50 | 50 | 50 |
| F | 5.89 | 1.23 | 8.18 | 0.19 |
| df | 98 | 98 | 98 | 98 |
| t | 2.94 | 4.17 | 4.12 | 9.34 |
| Mean difference (%) | +36.50 | +35.54 | +48.71 | +34.60 |
| Total injected $CO_2$ or acid (HCl 0.1M) volume (L) | 787.50 | 1915.00 | 800.00 | 1.57 |
| Initial inlet chlorophyll a (mg/L) | 0.29 | 1.95 | 0.09 | 1.19 |
| Initial inlet POC (mg/L) | 2.84 | 23.68 | 7.61 | 15.55 |

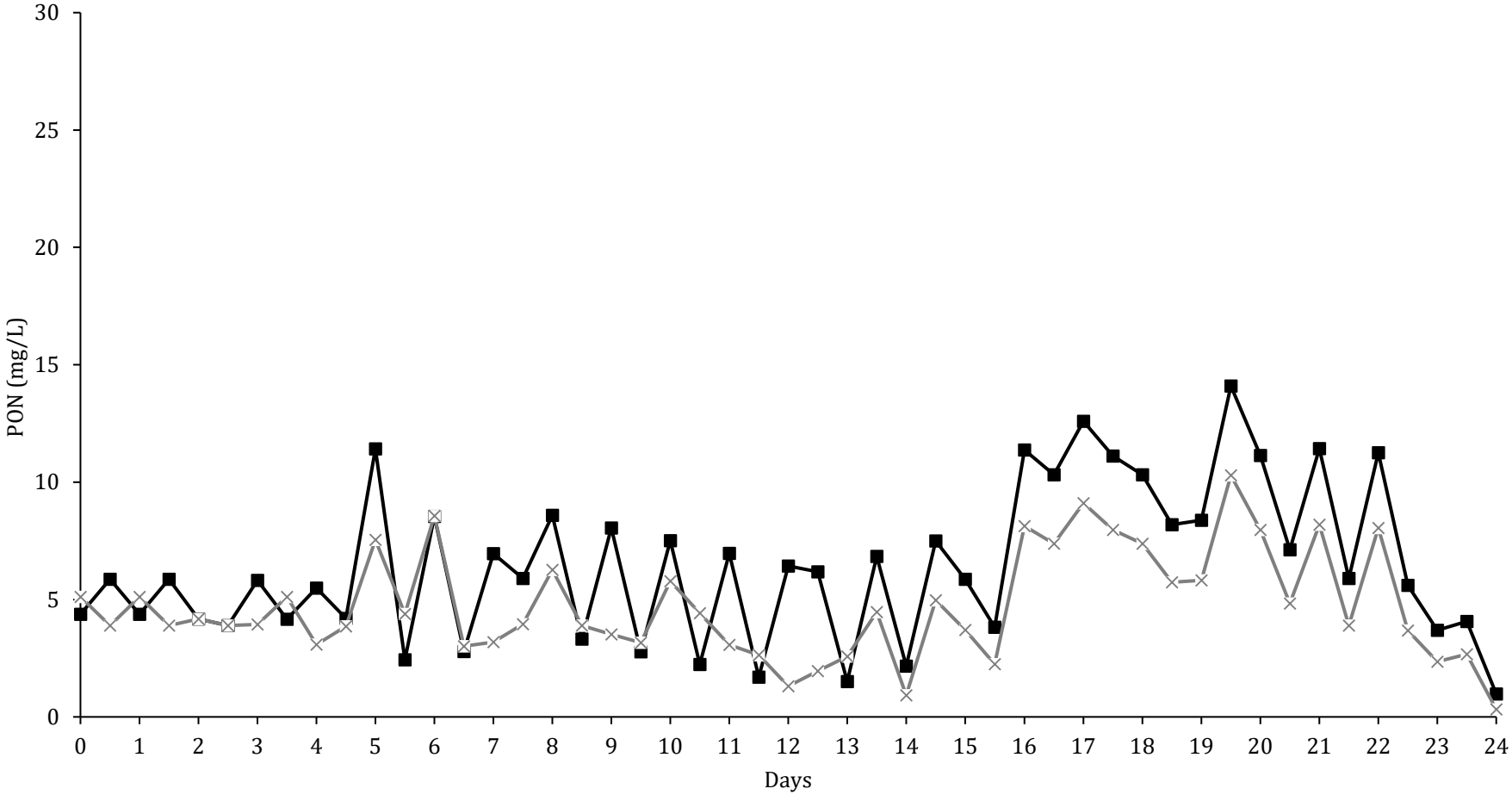



Fig. 3.24 Low $BOD_5$ strength wastewater from Mt Barker aerated lagoon effluent; time series of particulate organic nitrogen (PON) concentration in culture with $CO_2$ addition (■) and control culture without $CO_2$ addition (X).

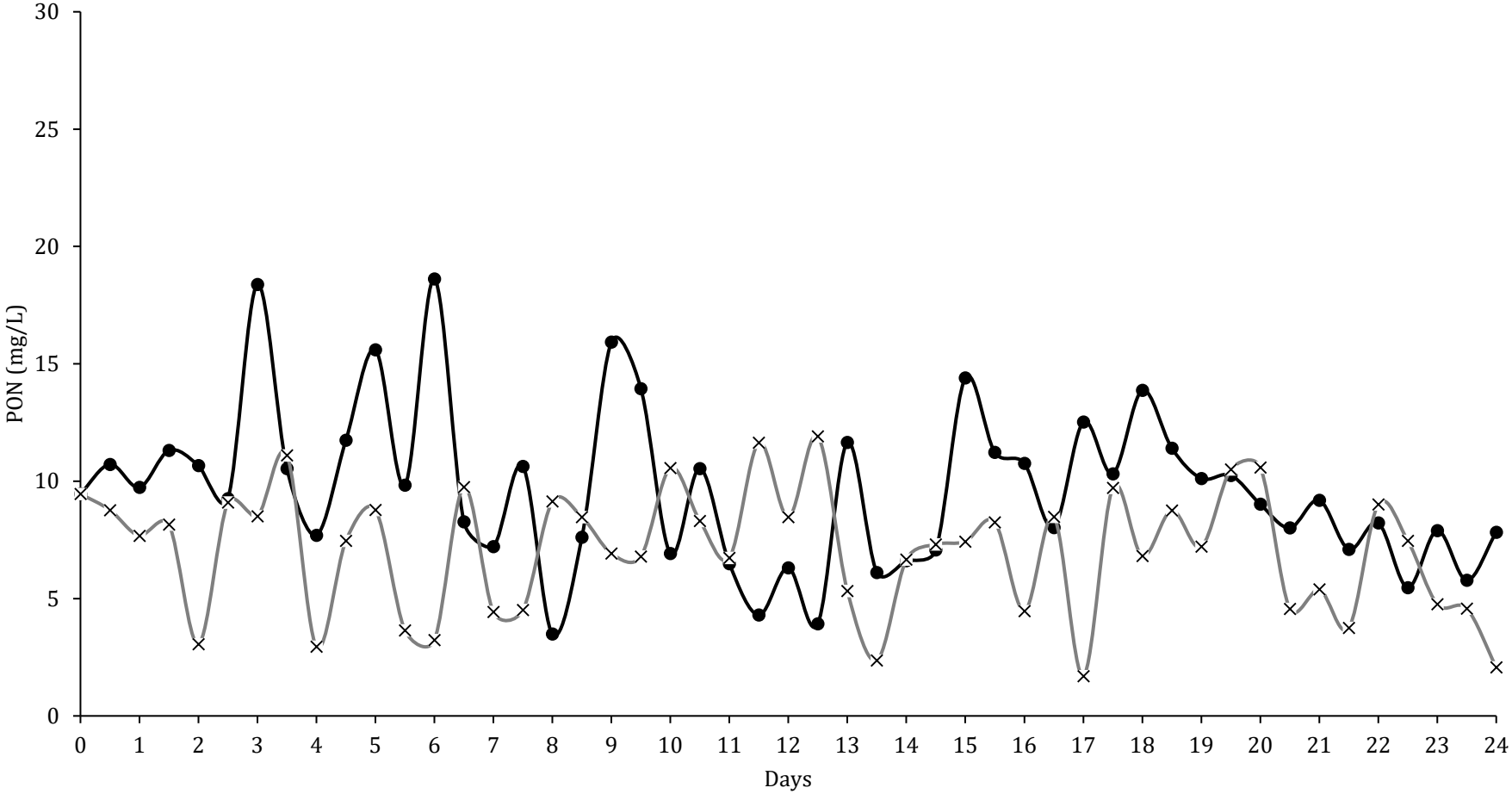



Fig. 3.25 Mid strength $BOD_5$ KoM facultative pond effluent; time series of particulate organic nitrogen (PON) concentration in culture with $CO_2$ addition (■), control culture without $CO_2$ addition (X) and inlet chlorophyll a concentration (▲).

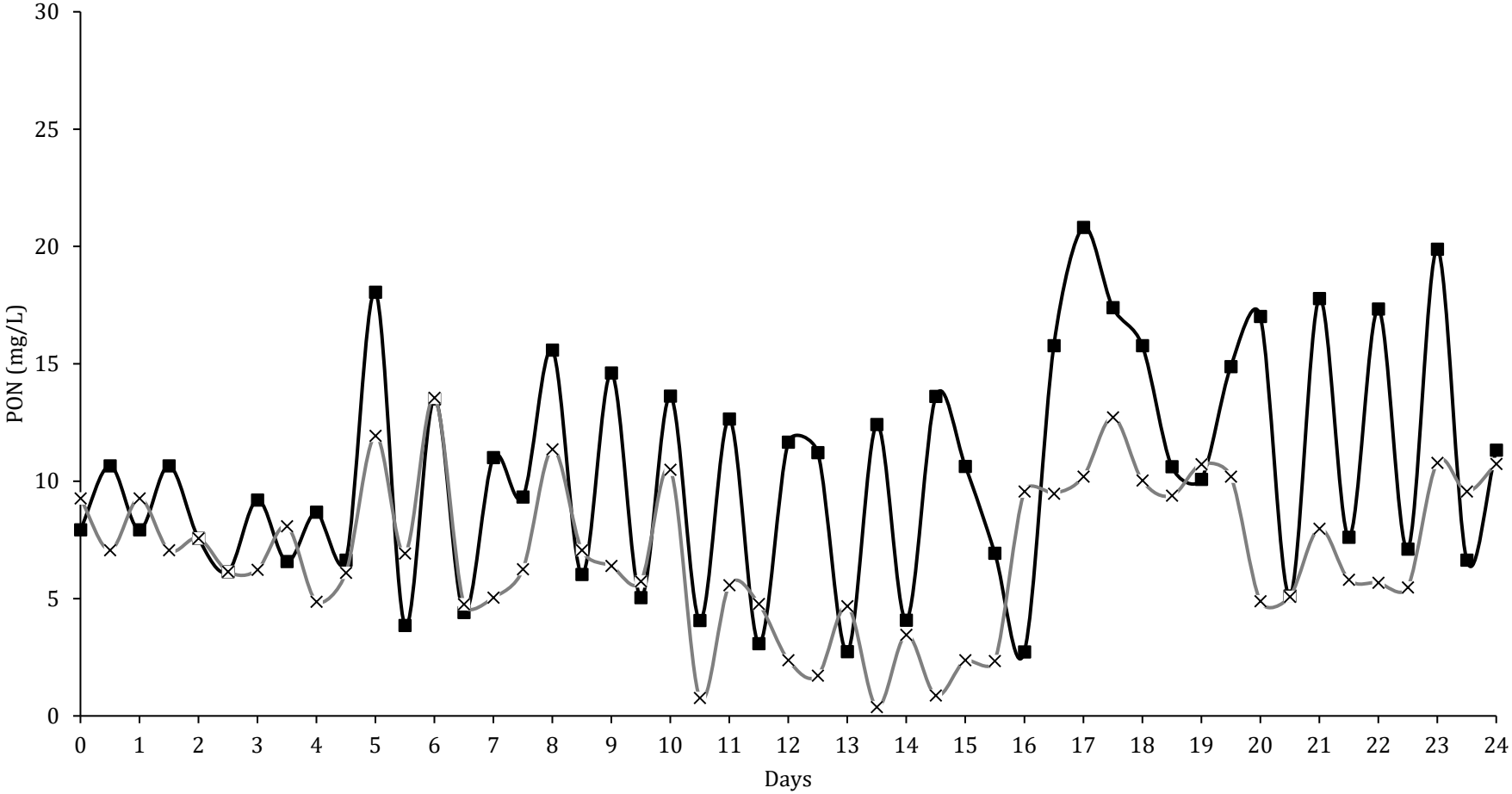



Fig. 3.26 High strength $BOD_5$ KoM septic tank effluent; time series of particulate organic nitrogen (PON) concentration in culture with $CO_2$ addition (■) and control culture without $CO_2$ addition (X).

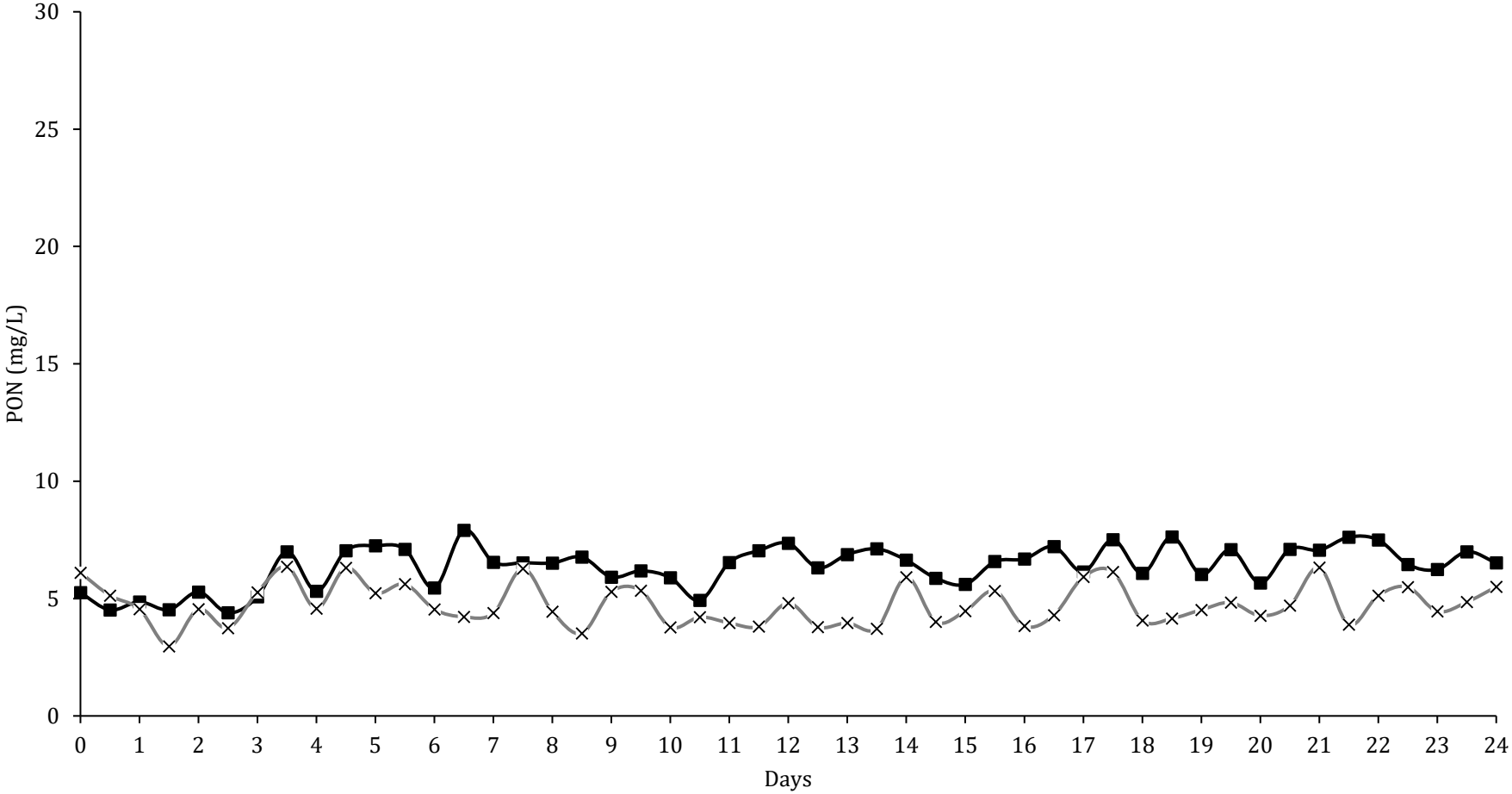



Fig. 3.27 Mid strength $BOD_5$ KoM facultative pond effluent; time series of particulate organic nitrogen (PON) concentration in culture with HCl addition (■) and control culture without HCl addition (X).

### 3.3.3.4. Suspended solid (SS) and volatile suspended solid (VSS)

Suspended solids is used to determine areal productivities in mass algal cultures on wastewater. No significant difference (Table 3.8 (a) & (b)) was found between control and amended cultures ($CO_2$ or acid) for any of the three wastewaters studied. The mean increases of SS and VSS are very similar in mid strength and high strength wastewater range from 15 to 18 % increase (Fig 3.29 & 3.30). Exceptionally in the low BOD strength wastewater (Fig. 3.28), the increase of SS was among the highest with + 0.10 SS mg/ml (+ 21.59%). Although there was no significant difference found in the VSS in low $BOD_5$ strength wastewater, the mean increase of VSS was also among the highest with + 0.10 VSS mg/ml (+ 47.67%) which is approximately 3 times higher than the mid and high BOD groups. The mean increase in both SS and VSS in the mid strength wastewater to which acid was added was similar to that for high and mid strength wastewater cultures to which pH was maintained by $CO_2$ addition (Fig 3.31).

**Table 3.8** Significant differences of different wastewater strength on suspended solid (SS, mg/ml) and volatile suspended solids (VSS, mg/ml) over the 24 days period ($P < 0.05$). A summary of independent sample T-test for Equality of Means and Levene's Test for Equality of Variances of (a) SS and (b) VSS

| **(a) SS (mg/ml)** | **Mount Barker (Low $BOD_5$)** | **KOM (Mid $BOD_5$, + $CO_2$)** | **KOM (High $BOD_5$)** | **KOM (Mid $BOD_5$,+ Acid)** |
|---|---|---|---|---|
| Are means significantly different ($P < 0.05$)? | No | No | No | No |
| P value | 0.50 | 0.38 | 0.42 | 0.24 |
| Mean ± SD (mg/ml) | 0.58 (0.35) | 0.39 (0.13) | 0.48 (0.18) | 0.23 (0.06) |
| Mean (control) ± SD (mg/ml) | 0.48 (0.17) | 0.33 (0.10) | 0.40 (0.14) | 0.19 (0.05) |
| N | 7 | 7 | 7 | 7 |
| F | 1.91 | 0.42 | 1.02 | 0.17 |
| df | 12 | 12 | 12 | 12 |
| t | 0.71 | 0.85 | 0.92 | 1.23 |
| Mean difference (%) | +21.59 | +17.16 | +18.08 | +17.65 |
| Total injected $CO_2$ or acid (HCl 0.1M) volume (L) | 787.50 | 1915.00 | 800.00 | 1.57 |
| Initial inlet chlorophyll a (mg/L) | 0.29 | 1.95 | 0.09 | 1.19 |
| Initial inlet POC (mg/L) | 2.84 | 23.68 | 7.61 | 15.55 |

| **(b) VSS (mg/ml)** | **Mount Barker (Low $BOD_5$)** | **KOM (Mid $BOD_5$, + $CO_2$)** | **KOM (High $BOD_5$)** | **KOM (Mid $BOD_5$,+ Acid)** |
|---|---|---|---|---|
| Are means significantly different ($P < 0.05$)? | No | No | No | No |
| P value | 0.19 | 0.39 | 0.59 | 0.24 |
| Mean ± SD (mg/ml) | 0.24 (0.16) | 0.24 (0.08) | 0.16 (0.09) | 0.23 (0.06) |
| Mean (control) ± SD (mg/ml) | 0.14 (0.07) | 0.21 (0.06) | 0.14 (0.07) | 0.19 (0.05) |
| N | 7 | 7 | 7 | 7 |
| F | 4.00 | 0.45 | 0.30 | 0.17 |
| df | 12 | 12 | 12 | 12 |
| t | 1.42 | 0.89 | 0.58 | 1.23 |
| Mean difference (%) | +47.67 | +15.56 | +17.90 | +17.65 |
| Total injected $CO_2$ or acid (HCl 0.1M) volume (L) | 787.50 | 1915.00 | 800.00 | 1.57 |
| Initial inlet chlorophyll a (mg/L) | 0.29 | 1.95 | 0.09 | 1.19 |
| Initial inlet POC (mg/L) | 2.84 | 23.68 | 7.61 | 15.55 |

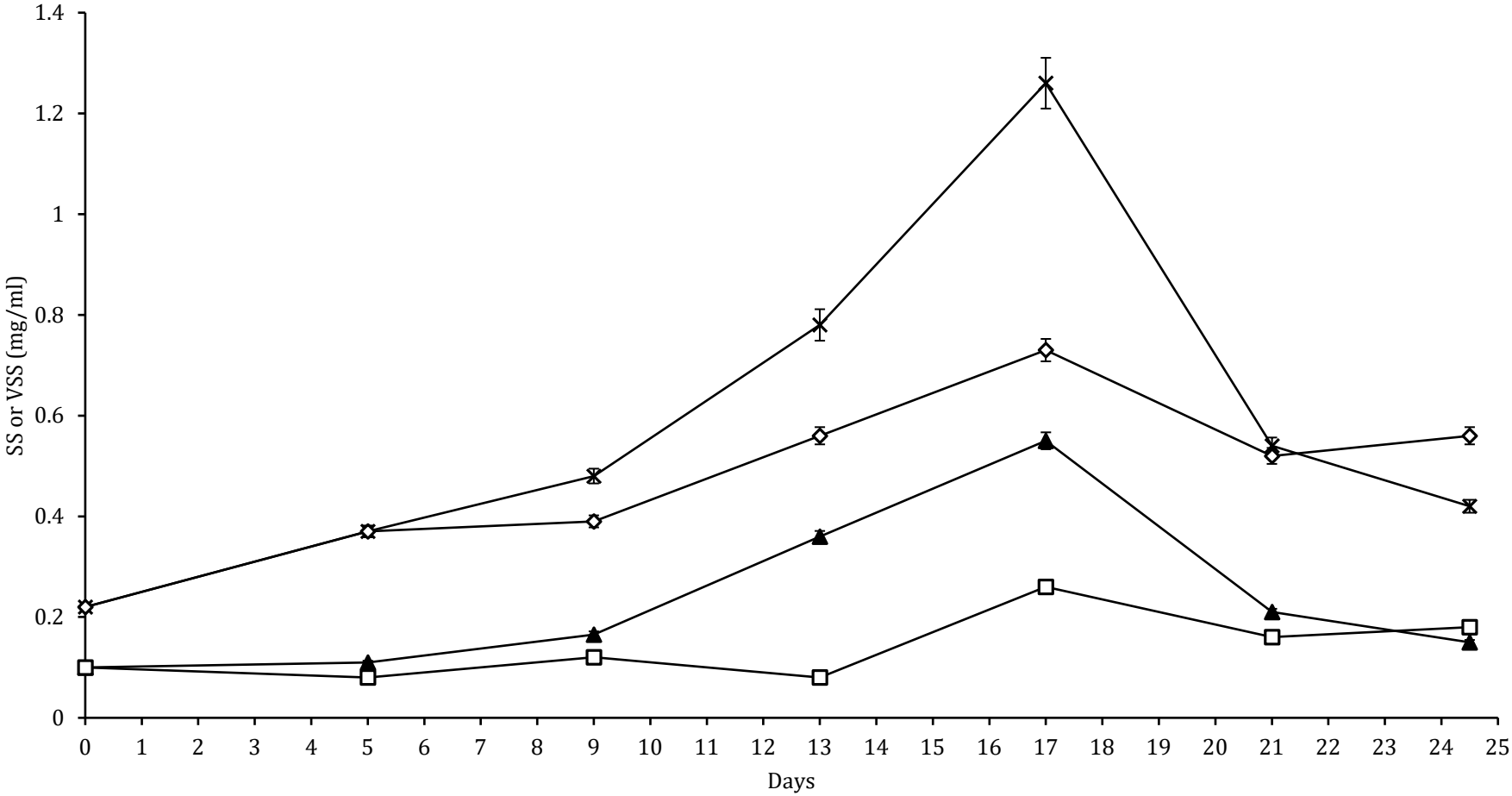



Fig. 3.28 Low $BOD_5$ strength wastewater from Mt Barker aerated lagoon effluent; time series of suspended solid (SS; X) and volatile suspended solid (VSS; ▲) concentrations in culture with $CO_2$ addition and in the control culture without $CO_2$ addition (◊ - SS; □ - VSS). Values are means ± SE (n =3).

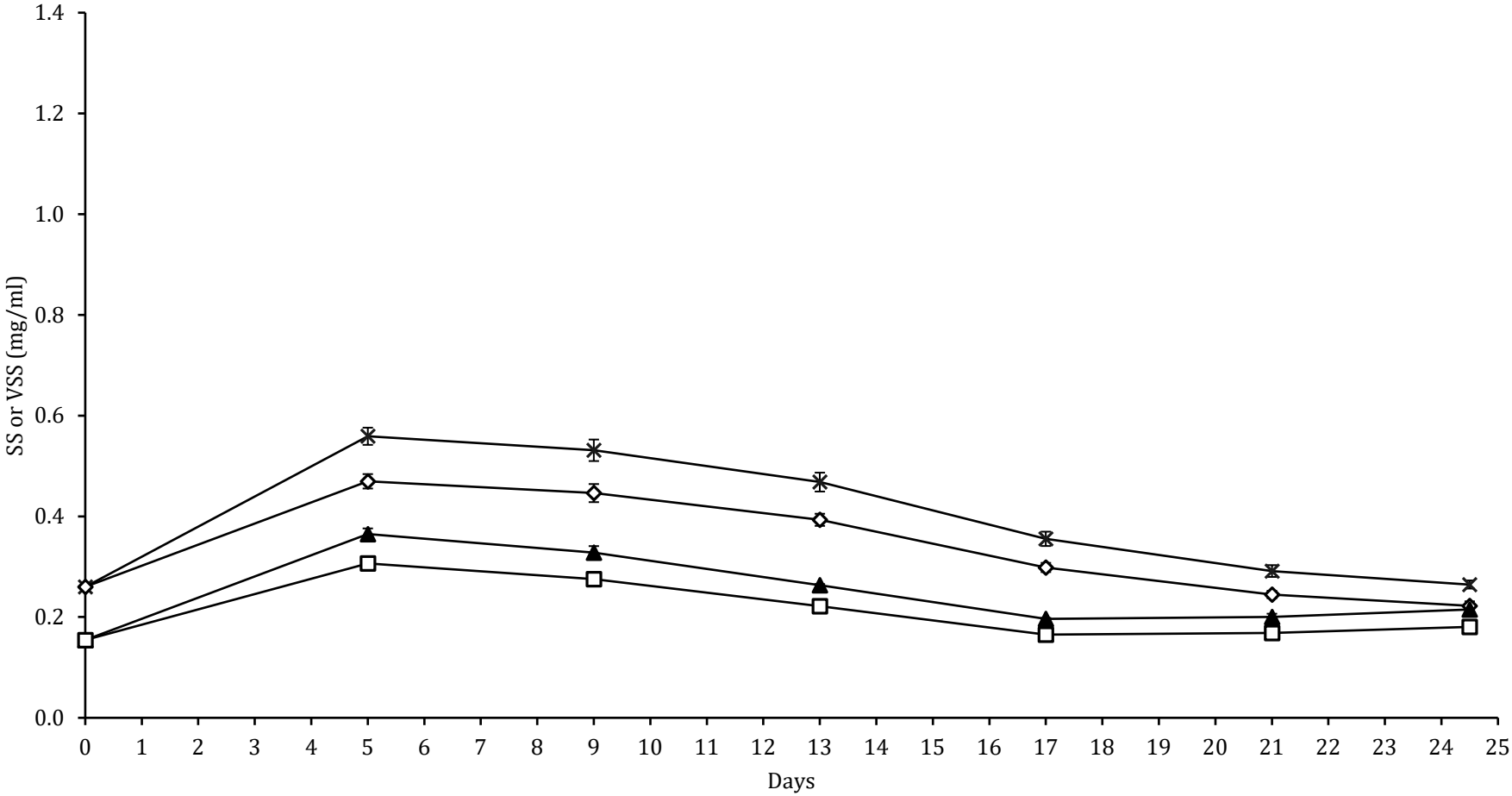



Fig. 3.29 Mid strength $BOD_5$ KoM facultative pond effluent; time series of suspended solid (SS; X) and volatile suspended solid (VSS; ▲)) concentrations in culture with $CO_2$ addition and in the control culture without $CO_2$ addition (◊ - SS; □ - VSS). Values are means ± SE (n =3).

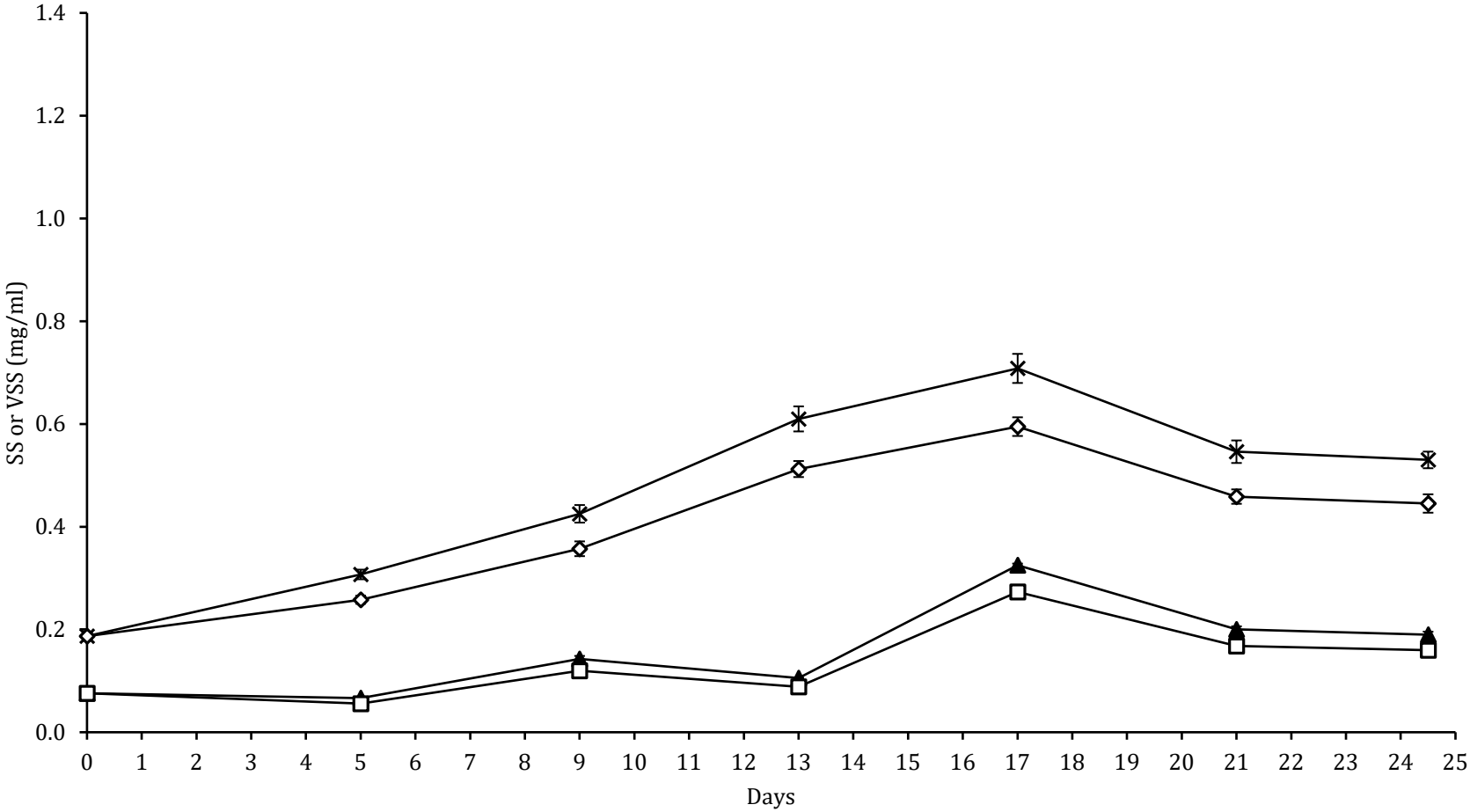



Fig. 3.30 High strength $BOD_5$ KoM septic tank effluent; time series of suspended solid (SS; X) and volatile suspended solid (VSS; ▲) concentrations in culture with $CO_2$ addition and in the control culture without $CO_2$ addition (◊ - SS; □ - VSS). Values are means ± SE (n =3).

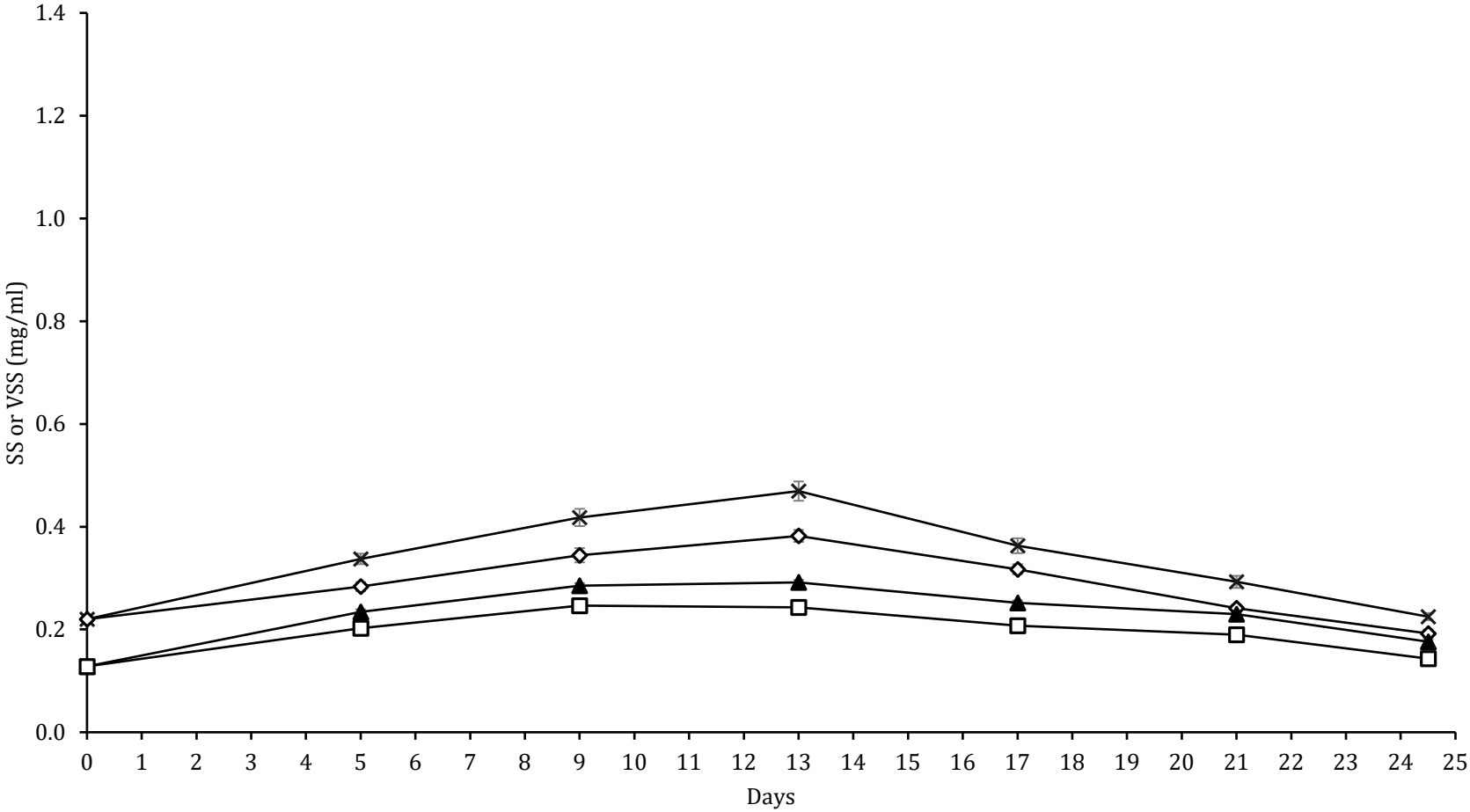


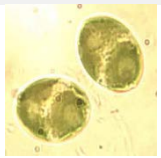
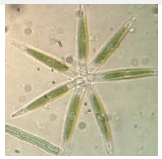
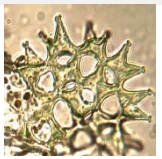

Fig. 3.31 Mid strength $BOD_5$ KoM facultative pond effluent; time series of suspended solid (SS; X) and volatile suspended solid (VSS; ▲) concentrations in culture with $CO_2$ addition and in the control culture without $CO_2$ addition (◊ - SS; □ - VSS). Values are means ± SE (n =3).1

3.3.3.5. Microalgae species

Microalgae population changed accordingly with the pH levels and the population changed from multi-species to single-species. The population of ciliates were not visible until day 17-18 in low $BOD_5$ Mount Barker aerated lagoon effluent.

Table 3.9 shows the common species found in Mount Barker facultative pond during the summer (January 2012). *Chlorella vulgaris* and *Scenedesmus quadricauda* were the dominated species at the early stage (day <12) due to the lower pH while *Pediastrum boryanum* and *Actinstrum gracillimum* were the less dominant species. The common microalgae species were also found in KOM septic tank raw influent with *Chlorococcum euchlorum* and *Scenedesmus quadricauda* as the dominant species in late stage (day ≥ 12).

Table 3.9 Neurtal and dominant microalgae speices present in different BOD strength wastewater (a) Mount Barker Facultative low BOD (January 2012) & (b) KOM Septic tank raw influent (June 2012).

| | | **Name** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *Chlorella pyrenoidosa* | *Staurastrum paradoxum* | *Arthrospira jenneri* | *Chlorella vulgaris* | *Chlorococcum euchlorum* | *Scenedesmus quadricauda* | *Actinstrum gracillimum* | *Pediastrum boryanum* | *Oocystis borgei* |
| (a) Mount Barker Facultative low BOD (January 2012) | Microalgae species |  |  |  |  |  |  |  |  |  |
| | | *Staurastrum paradoxum* | *Arthrospira jenneri* | *Chlorella vulgaris* | *Chlorococcum euchlorum* | *Scenedesmus quadricauda* | | | | |
| (b) KOM Septic tank raw influent (June 2012) | Microalgae species |  |  |  |  |  | | | | |

### 3.3.4. Wastewater composition in control cultures and cultures receiving $CO_2$ addition

#### 3.3.4.1. Inorganic nitrogen - Ammonia ($NH_4$ -N)

The $NH_4$-N concentration of inlet wastewater to all cultures decreased over the time course of the experiments (Figs 3.32 - 3.35). Similarly, the $NH_4$-N concentration in the control and amended cultures was greater than that of the inlet irrespective of the wastewater being studied. This suggests ammonification of organic nitrogen was occurring in all cultures, irrespective of $CO_2$ or acid amendment.

In low $BOD_5$ strength wastewater, no significant difference was found in $NH_4$-N concentration between control and $CO_2$ amended culture (Fig. 3.32). The $NH_4$-N concentration was significantly higher (Table 3.10) in the $CO_2$ amended culture compared to the control in both mid and high strength $BOD_5$ wastewater (Fig. 3.33 and 3.34). The $NH_4$-N concentration was also higher in the culture where acid was added to control pH compared to the control culture (Fig.3.35). It may be implied from the finding that the $NH_4$-N concentration was consistently lower in the control cultures that the higher, unchecked, pH associated with photosynthesis resulted an equilibrium shift towards $NH_4$-N formation and a consequential increase in $NH_4$-N volatilization when compared with cultures where pH was controlled within pH 7.5 – 8.0.

Table 3.10 Significant differences of different wastewater strength on Ammonia ($NH_4$ -N) over the 24 days period ($P < 0.05$). (a) A summary of independent sample T-test for Equality of Means and Levene's Test for Equality of Variances

| (a) $NH_4$ -N (mg/L) | **Mount Barker (Low $BOD_5$)** | **KOM (Mid $BOD_5$, + $CO_2$)** | **KOM (High $BOD_5$)** | **KOM (Mid $BOD_5$,+ Acid)** |
|---|---|---|---|---|
| Are means significantly different ($P < 0.05$)? | No | Yes | Yes | Yes |
| P value | 0.47 | 0.00 | 0.00 | 0.00 |
| Mean ± SD (mg/L) | 19.31 (3.42) | 10.53 (1.00) | 22.79 (2.96) | 5.96 (0.43) |
| Mean (control) ± SD (mg/L) | 18.78 (3.78) | 8.29 (1.62) | 19.62 (2.55) | 5.62 (0.54) |
| N | 50 | 50 | 50 | 50 |
| F | 1.11 | 14.04 | 1.71 | 2.12 |
| df | 98 | 98 | 98 | 98 |
| t | 0.73 | 8.33 | 5.74 | 3.49 |
| Mean difference (%) | +2.77 | +26.99 | +16.16 | +6.05 |
| Total injected $CO_2$ or acid (HCl 0.1M) volume (L) | 787.50 | 1915.00 | 800.00 | 1.57 |
| Initial inlet chlorophyll a (mg/L) | 0.29 | 1.95 | 0.09 | 1.19 |
| Initial inlet POC (mg/L) | 2.84 | 23.68 | 7.61 | 15.55 |

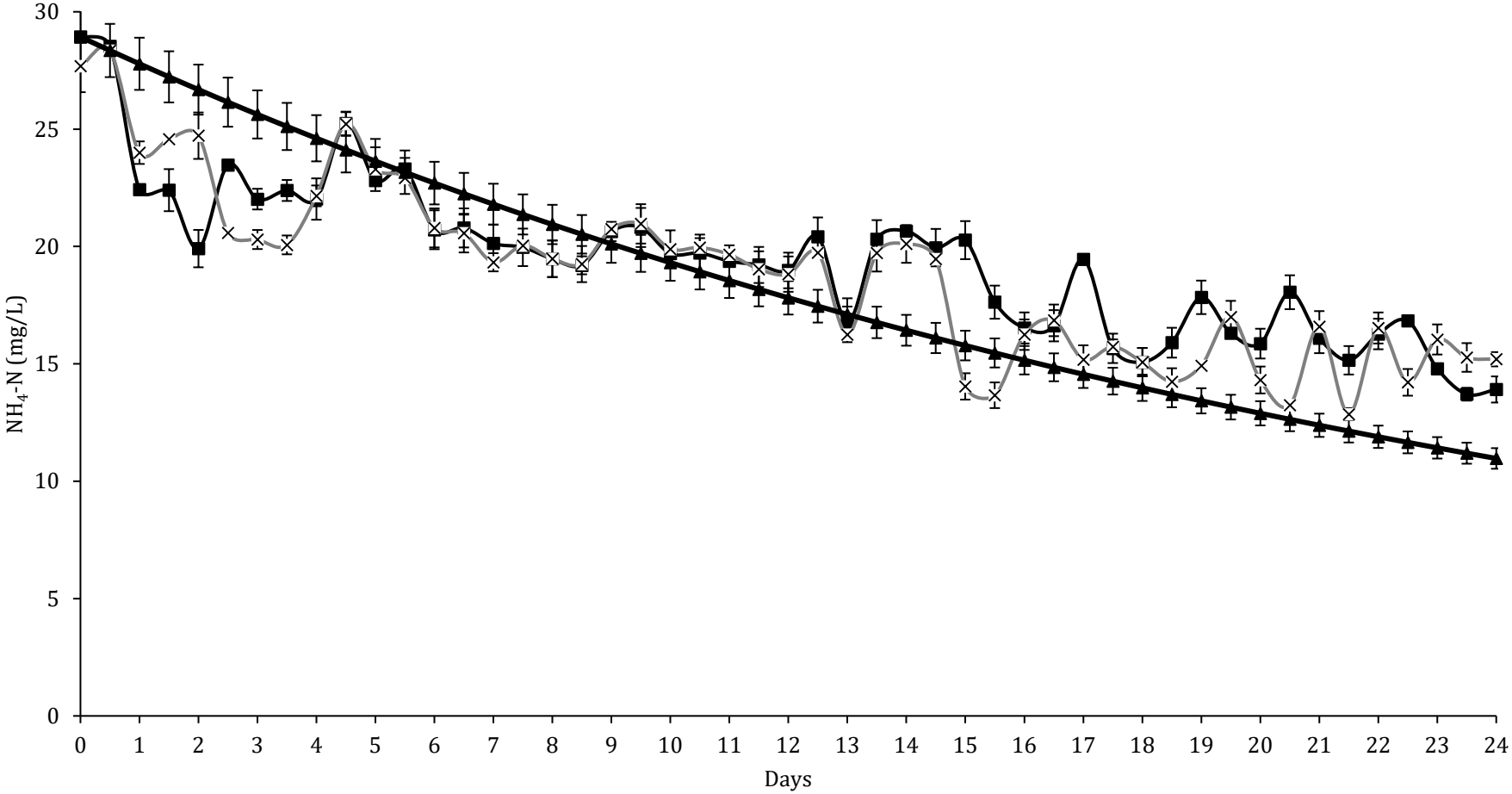



Fig. 3.32 Low $BOD_5$ strength wastewater from Mt Barker aerated lagoon effluent; time series of ammonia ($NH_4$-N) concentration in culture with $CO_2$ addition (■), control culture without $CO_2$ addition (X) and inlet concentration (▲). Values are means ± SE (n =3).

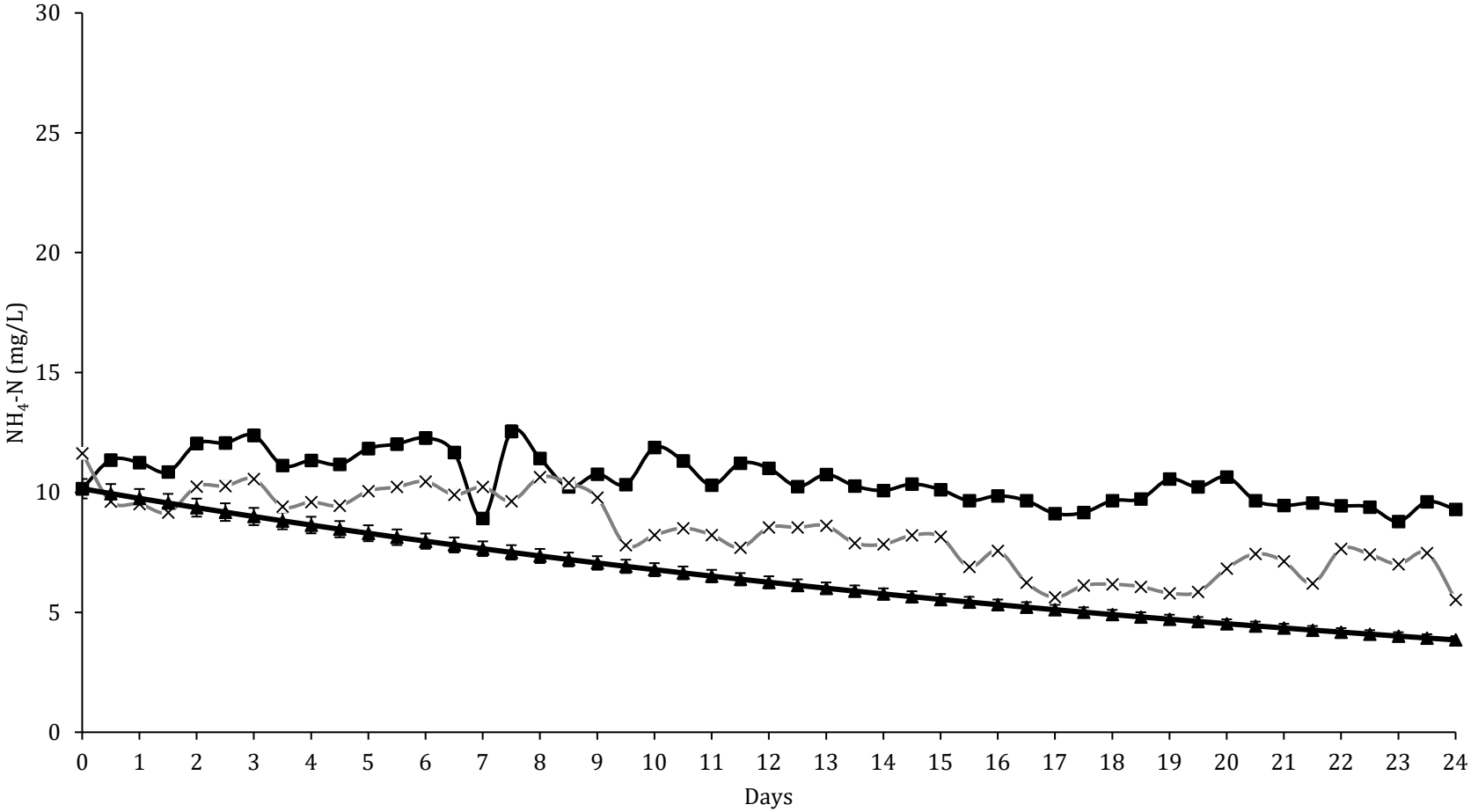



Fig. 3.33 Mid-strength $BOD_5$ KoM facultative pond effluent; time series of ammonia ($NH_4$-N) concentration in culture with $CO_2$ addition (■), control culture without $CO_2$ addition (X) and inlet concentration (▲). Values are means ± SE (n =3).

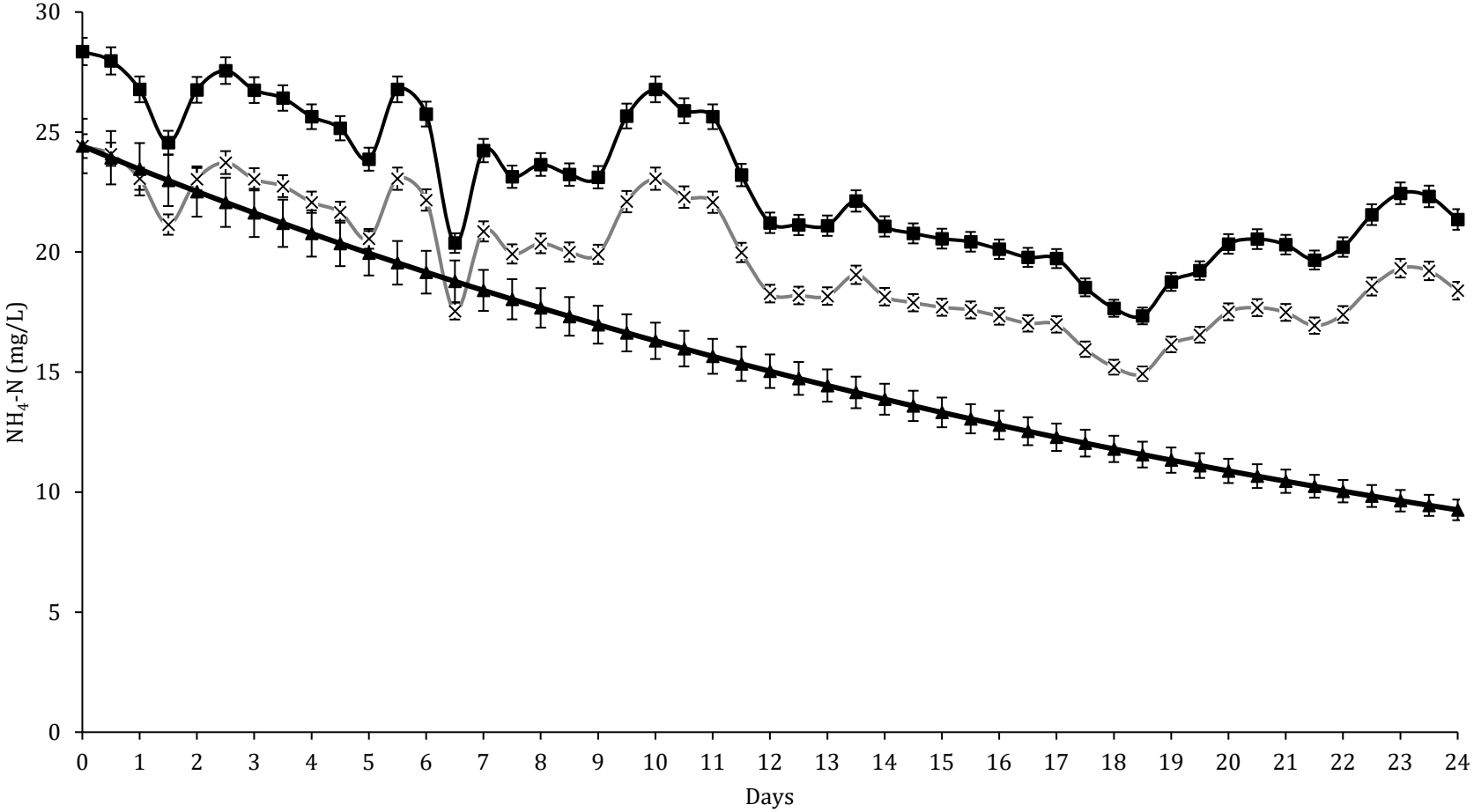



Fig. 3.34 High-strength $BOD_5$ KoM septic tank effluent; time series of ammonia ($NH_4$-N) concentration in culture with $CO_2$ addition (■), control culture without $CO_2$ addition (X) and inlet concentration (▲). Values are means ± SE (n =3).

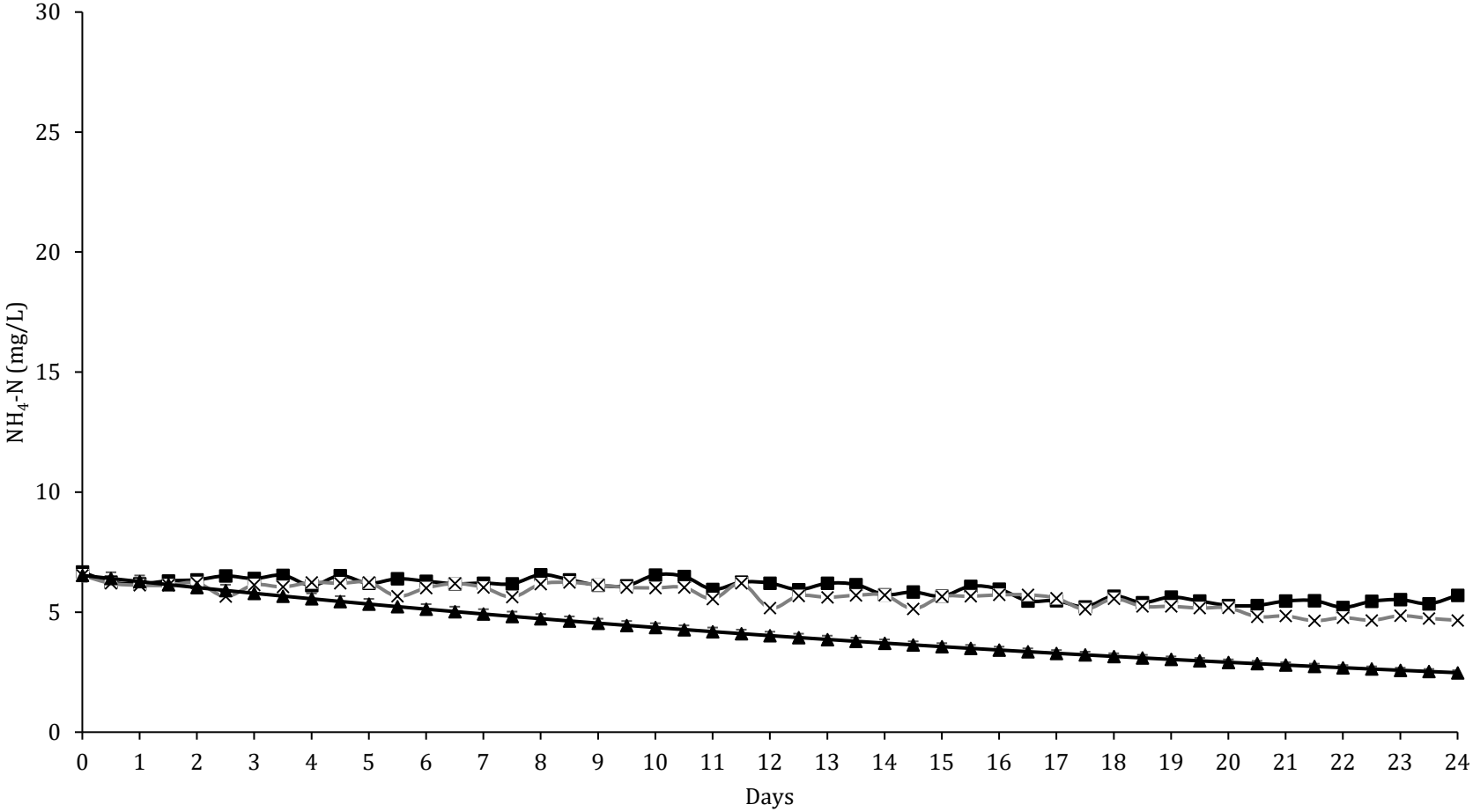



Fig. 3.35 Mid strength $BOD_5$ KoM facultative pond effluent; time series of ammonia ($NH_4$-N) concentration in culture with HCl addition (■), control culture without HCl addition (X) and inlet concentration (▲). Values are means ± SE (n =3).

3.3.4.2. Oxidised inorganic nitrogen - Nitrite ($NO_2$-N) and Nitrate ($NO_3$-N)

The inorganic, oxidised nitrogen profiles varied considerably between the inlet wastewaters. The low $BOD_5$ strength wastewater from the aerated lagoon had a low concentration of $NO_2$-N (<0.5 mg $NO_2$-N $L^{-1}$; Fig. 3.35). The $NO_3$-N concentration was below the limit of detection (Fig 3.40). These results, together with the high concentration of $NH_4$-N (Fig. 3.32), show that this was a poorly nitrified effluent from an aerated lagoon. The $NO_2$-N concentration increased in both control and $CO_2$ amended cultures, however, it was significantly higher in the culture to which $CO_2$ was added (Fig. 3.36). Interestingly there was a rapid onset on nitrification, evidenced by the rapid increase in $NO_3$-N concentration, (Fig 3.40) in both control and $CO_2$ amended cultures. However, there was no statistically significant difference between the $NO_3$-N concentration of the cultures (Table 3.11) suggesting there was sufficient $CO_2$ available in both cultures to satisfy the demand for both photosynthesis and autotrophic nitrification.

The inlet wastewater to the mid-$BOD_5$ strength control and $CO_2$ amended cultures had low concentrations (< 2 mg $L^{-1}$) of both $NO_2$-N and $NO_3$-N (Fig. 3.37 and 3.41 respectively). The onset of nitrification between days 5-7 was evident in both cultures from the rise in $NO_2$-N and $NO_3$-N concentrations. The means of both $NO_2$-N and $NO_3$-N concentrations were significantly higher in the culture to which $CO_2$ was added compared with controls (Tables 3.11 and 3.12 respectively).

The $NO_2$-N and $NO_3$-N concentrations (Fig. 3.38 and 3.42 respectively) in the high – $BOD_5$ strength inlet wastewater where higher than might be expected for a wastewater emanating from septic tanks. Significant nitrification was evident in both control and $CO_2$ amended cultures after 2 days incubation. The concentrations of both $NO_2$-N and $NO_3$-N were relatively more consistent over the incubation period in the $CO_2$ amended culture suggesting there was more stability in the equilibria associated with nitrification in this culture. There was a statistically significant differences in both the means of $NO_2$-N and $NO_3$-N concentrations between $CO_2$ amended and control cultures (Table 3.11 and 3.12)

The oxidized inorganic nitrogen composition of the mid-strength inlet wastewater used to compare the performance of acid addition to that of a control culture was similar to that used for $CO_2$ amendment study on the same wastewater (Fig. 3.39 and 3.43). However, nitrification was less in both control and acid amended cultures compared to the control and $CO_2$ amended cultures in the same wastewater. Statistical analysis showed that the mean concentrations of both $NO_2$-N and $NO_3$-N were significant higher in the acid amended cultures compared to the control (Table 3.11 and Table 3.12).

The results suggest that pH stasis, effected either by $CO_2$ addition or acid addition, benefitted nitrification in all cultures irrespective of wastewater strength.

Table 3.11 Significant differences of different wastewater strength on nitrite ($NO_2$ -N) over the 24 days period ($P < 0.05$). (a) A summary of independent sample T-test for Equality of Means and Levene's Test for Equality of Variances

| (a) $NO_2$-N (mg/L) | **Mount Barker (Low $BOD_5$)** | **KOM (Mid $BOD_5$, + $CO_2$)** | **KOM (High $BOD_5$)** | **KOM (Mid $BOD_5$,+ Acid)** |
|---|---|---|---|---|
| Are means significantly different ($P < 0.05$)? | Yes | Yes | Yes | Yes |
| P value | 0.03 | 0.00 | 0.00 | 0.00 |
| Mean ± SD (mg/L) | 0.57 ( 0.25) | 1.72 (0.53) | 2.34 (0.42) | 0.85 (0.20) |
| Mean (control) ± SD (mg/L) | 0.46 (0.21) | 1.43 (0.33) | 1.83 (0.51) | 0.66 (0.05) |
| N | 50 | 50 | 50 | 50 |
| F | 0.42 | 6.53 | 4.18 | 44.95 |
| df | 98 | 98 | 98 | 98 |
| t | 2.29 | 3.35 | 5.44 | 6.34 |
| Mean difference (%) | +23.91 | +20.98 | +27.87 | +28.79 |
| Total injected $CO_2$ or acid (HCl 0.1M) volume (L) | 787.50 | 1915.00 | 800.00 | 1.57 |
| Initial inlet chlorophyll a (mg/L) | 0.29 | 1.95 | 0.09 | 1.19 |
| Initial inlet POC (mg/L) | 2.84 | 23.68 | 7.61 | 15.55 |

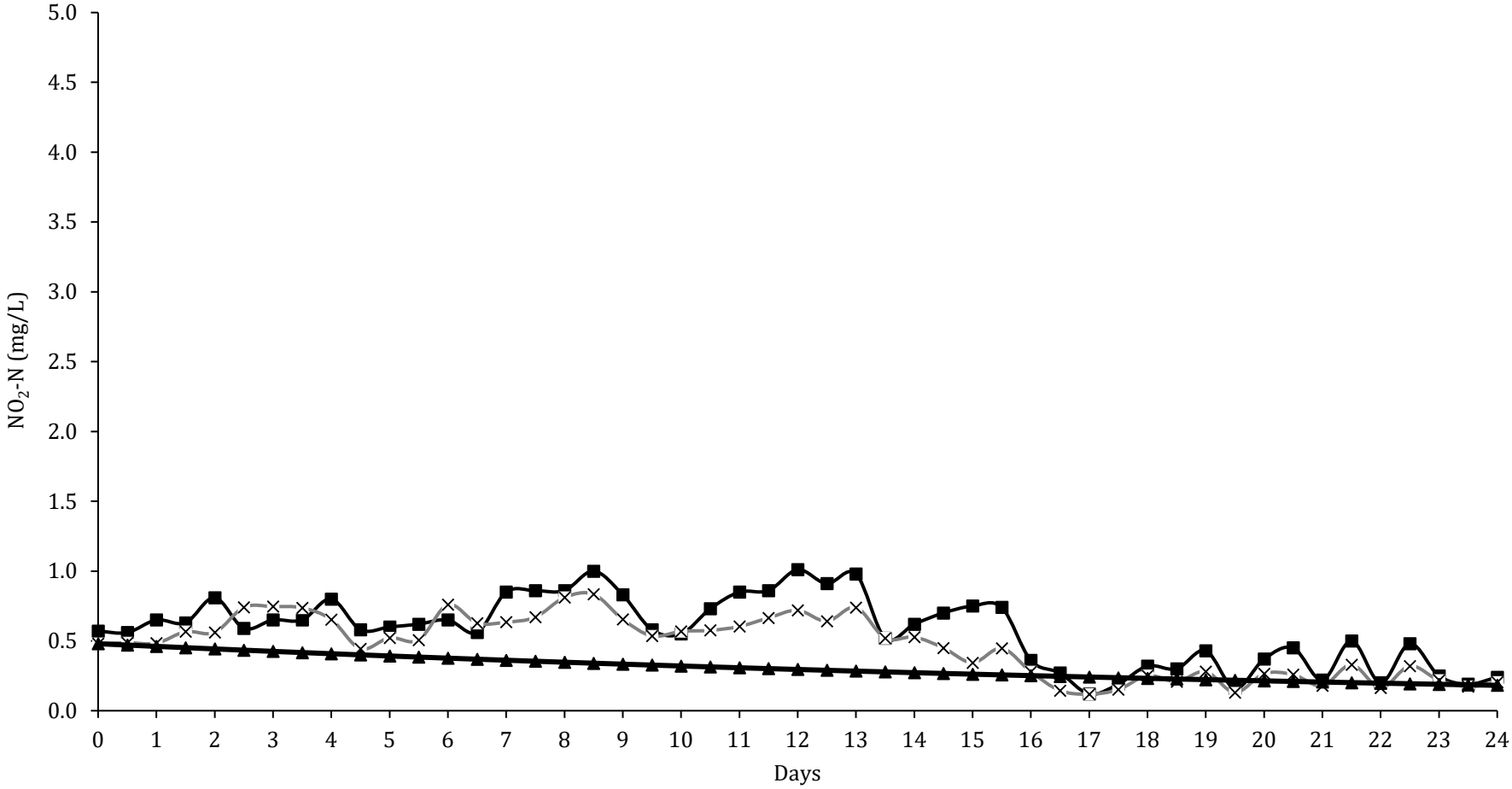



Fig. 3.36 Low $BOD_5$ strength wastewater from Mt Barker aerated lagoon effluent; time series of nitrite ($NO_2$ -N) concentration in culture with $CO_2$ addition (■), control culture without $CO_2$ addition (X) and inlet concentration (▲). Values are means ± SE (n =3).

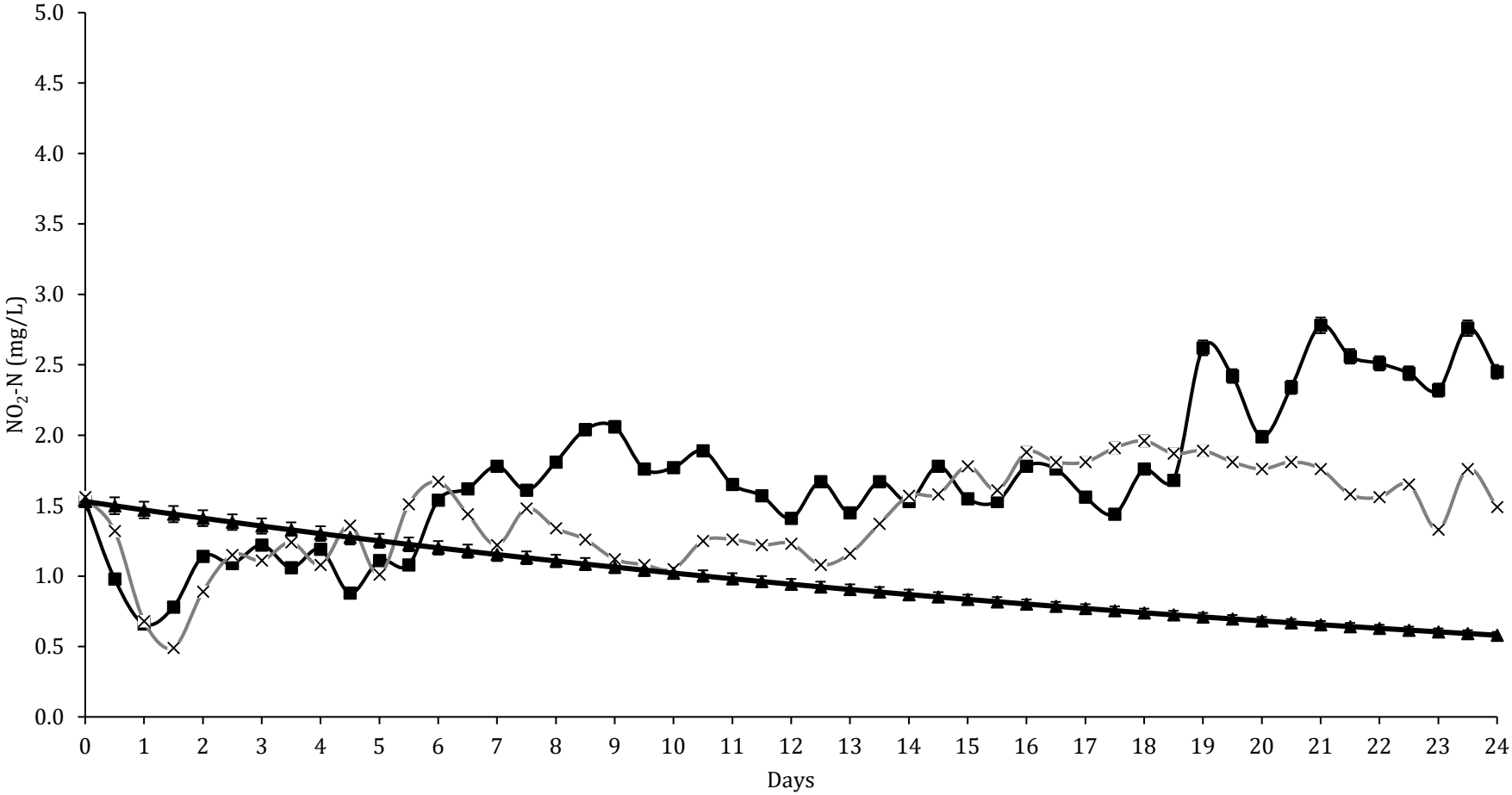



Fig. 3.37 Mid strength $BOD_5$ KoM facultative pond effluent; time series of nitrite ($NO_2$-N) concentration in culture with $CO_2$ addition (■), control culture without $CO_2$ addition (X) and inlet concentration (▲). Values are means ± SE (n =3).

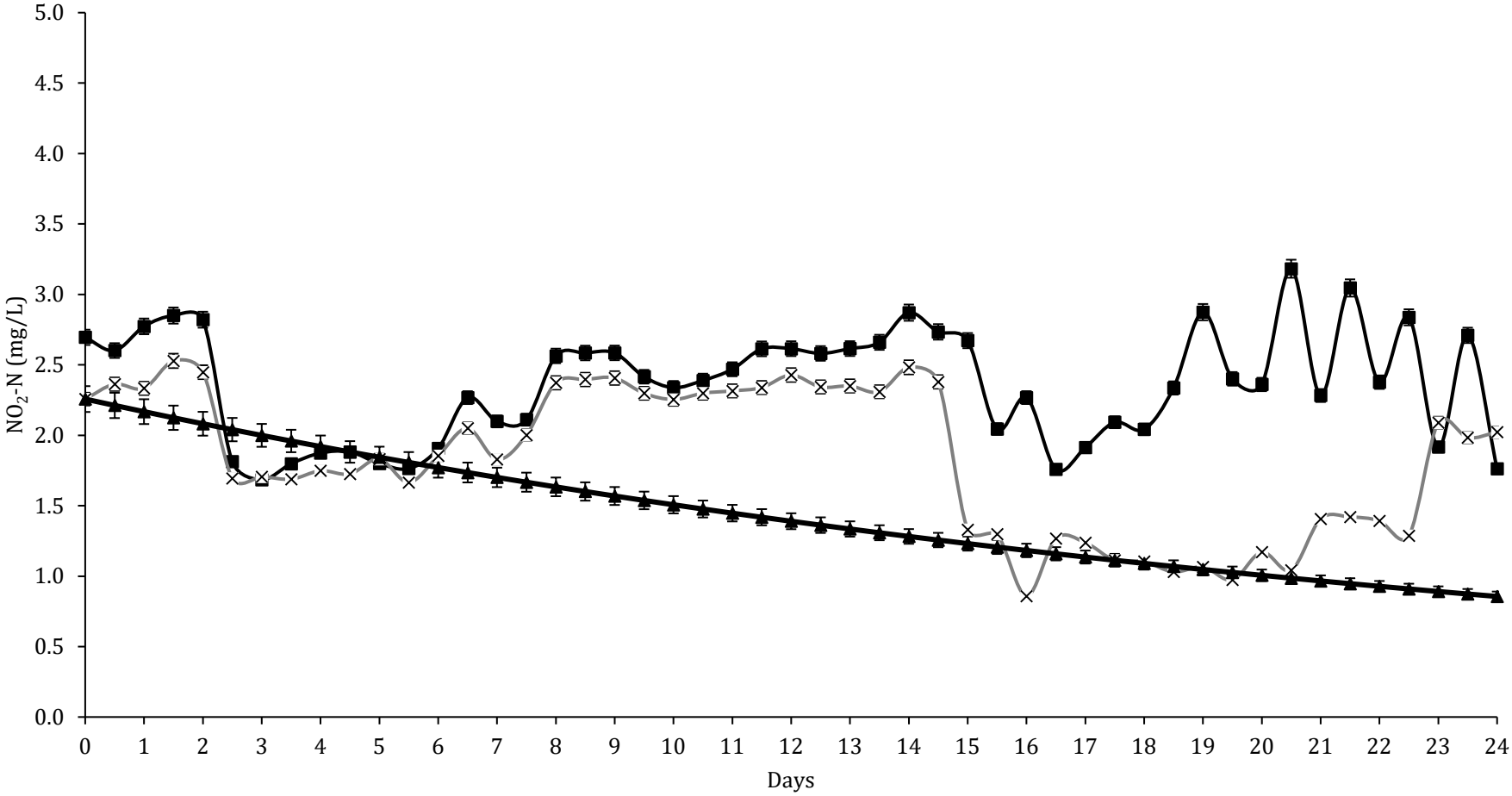



Fig. 3.38 High strength $BOD_5$ KoM septic tank effluent; time series of nitrite ($NO_2$-N) concentration in culture with $CO_2$ addition (■), control culture without $CO_2$ addition (X) and inlet concentration (▲). Values are means ± SE (n =3).

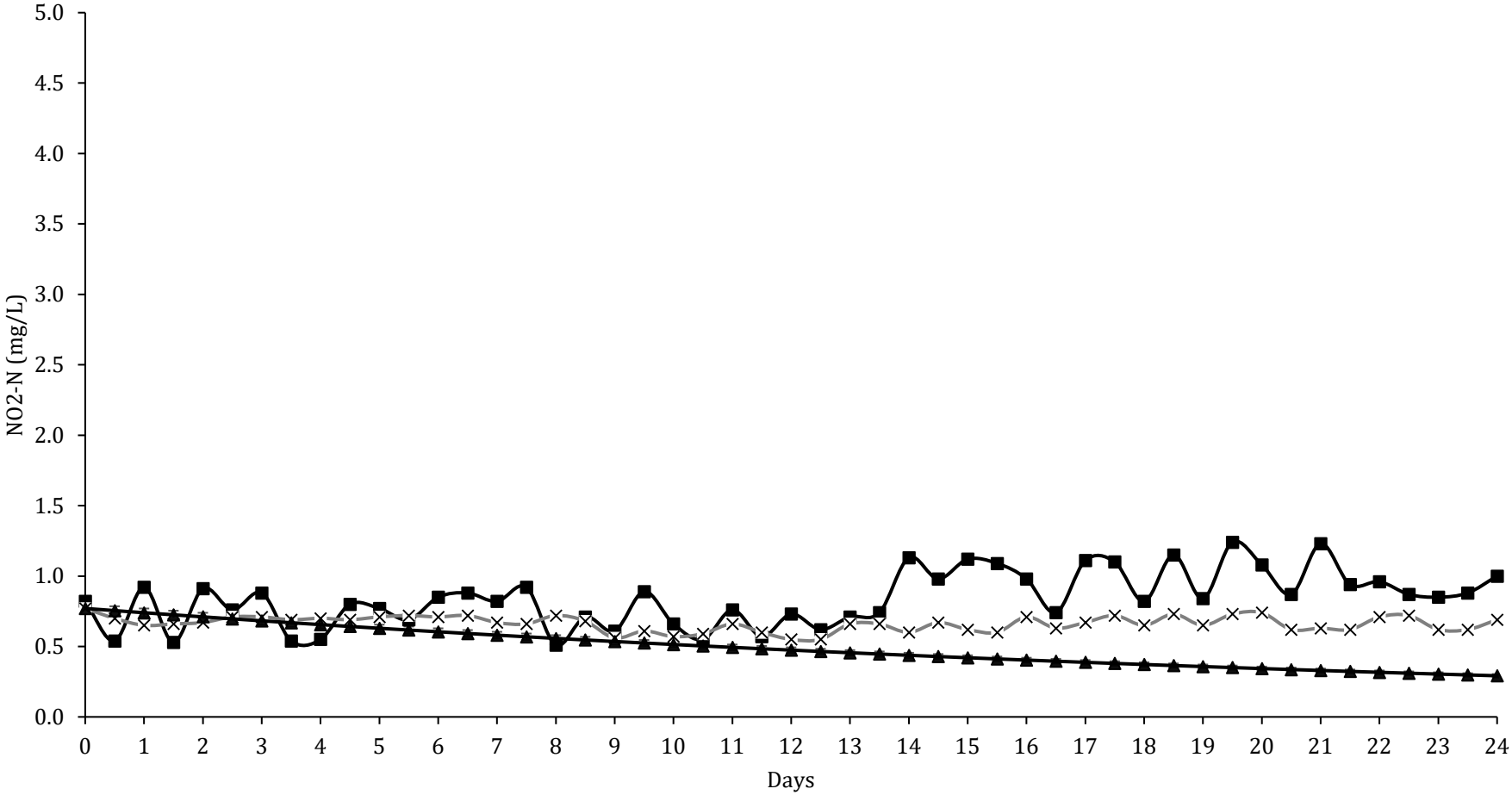



Fig. 3.39 Mid strength $BOD_5$ KoM facultative pond effluent; time series of nitrite ($NO_2$ -N) concentration in culture with HCl addition (■), control culture without HCl addition (X) and inlet concentration (▲). Values are means ± SE (n =3).

Table 3.12 Significant differences of different wastewater strength on nitrate ($NO_3$ -N) over the 24 days period ($P < 0.05$). (a) A summary of independent sample T-test for Equality of Means and Levene's Test for Equality of Variances

| (a) $NO_3$-N (mg/L) | **Mount Barker (Low $BOD_5$)** | **KOM (Mid $BOD_5$, + $CO_2$)** | **KOM (High $BOD_5$)** | **KOM (Mid $BOD_5$,+ Acid)** |
|---|---|---|---|---|
| Are means significantly different ($P < 0.05$)? | Yes | Yes | Yes | Yes |
| P value | 0.15 | 0.25 | 0.00 | 0.00 |
| Mean ± SD (mg/L) | 5.42 (2.04) | 4.04 (1.97) | 10.74 (3.28) | 2.48 (0.19) |
| Mean (control) ± SD (mg/L) | 4.87 (1.76) | 3.60 (1.87) | 7.61 (1.84) | 2.12 (0.17) |
| N | 50 | 50 | 50 | 50 |
| F | 0.31 | 0.49 | 15.12 | 0.35 |
| df | 98 | 98 | 98 | 98 |
| t | 1.45 | 1.16 | 5.87 | 9.91 |
| Mean difference (%) | +11.30 | +12.50 | +41.13 | +16.51 |
| Total injected $CO_2$ or acid (HCl 0.1M) volume (L) | 787.50 | 1915.00 | 800.00 | 1.57 |
| Initial inlet chlorophyll a (mg/L) | 0.29 | 1.95 | 0.09 | 1.19 |
| Initial inlet POC (mg/L) | 2.84 | 23.68 | 7.61 | 15.55 |

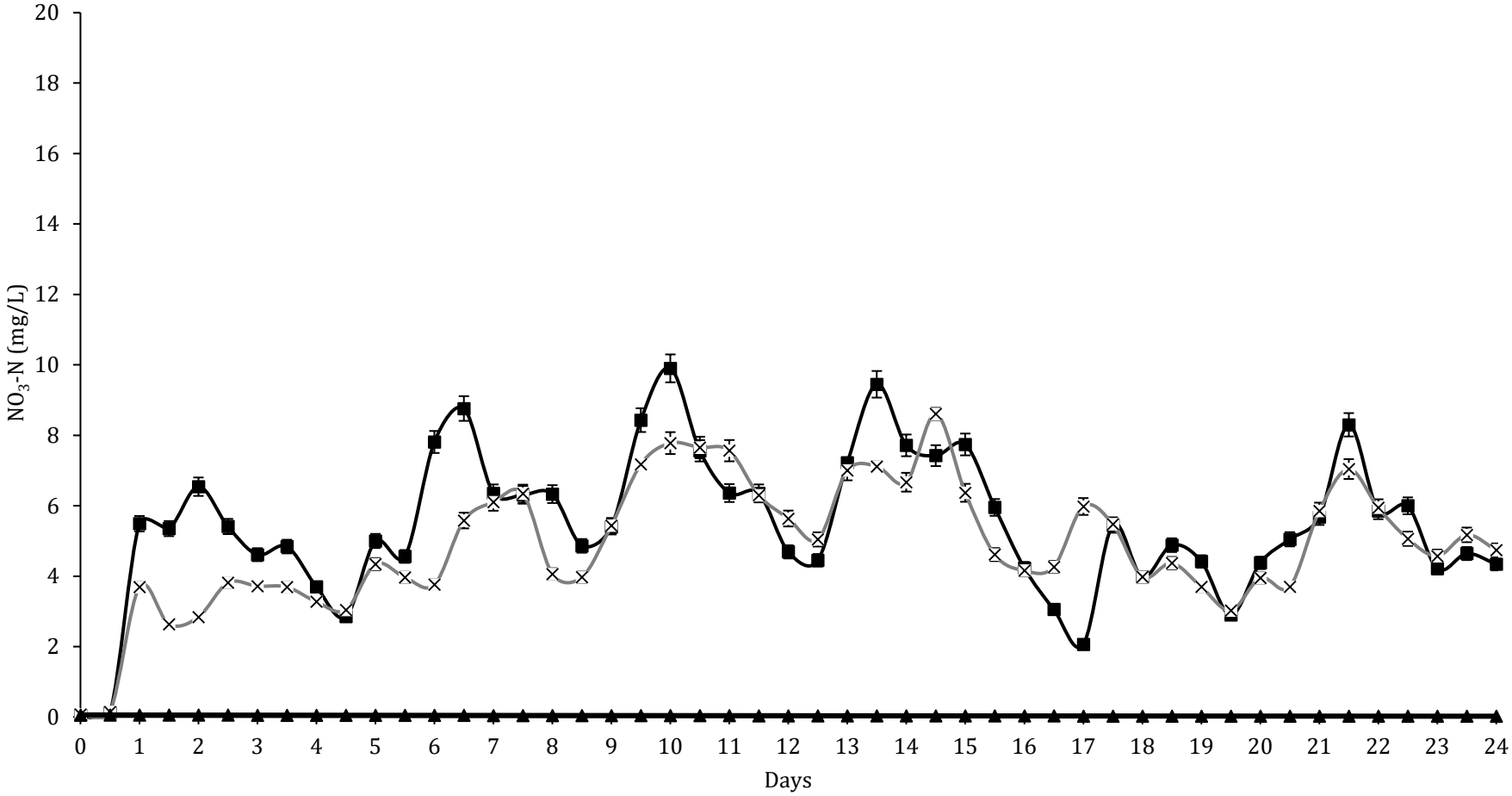



Fig. 3.40 Low $BOD_5$ strength wastewater from Mt Barker aerated lagoon effluent; time series of nitrate ($NO_3$-N) concentration in culture with $CO_2$ addition (■), control culture without $CO_2$ addition (X) and inlet concentration (▲). Values are means ± SE (n =3).

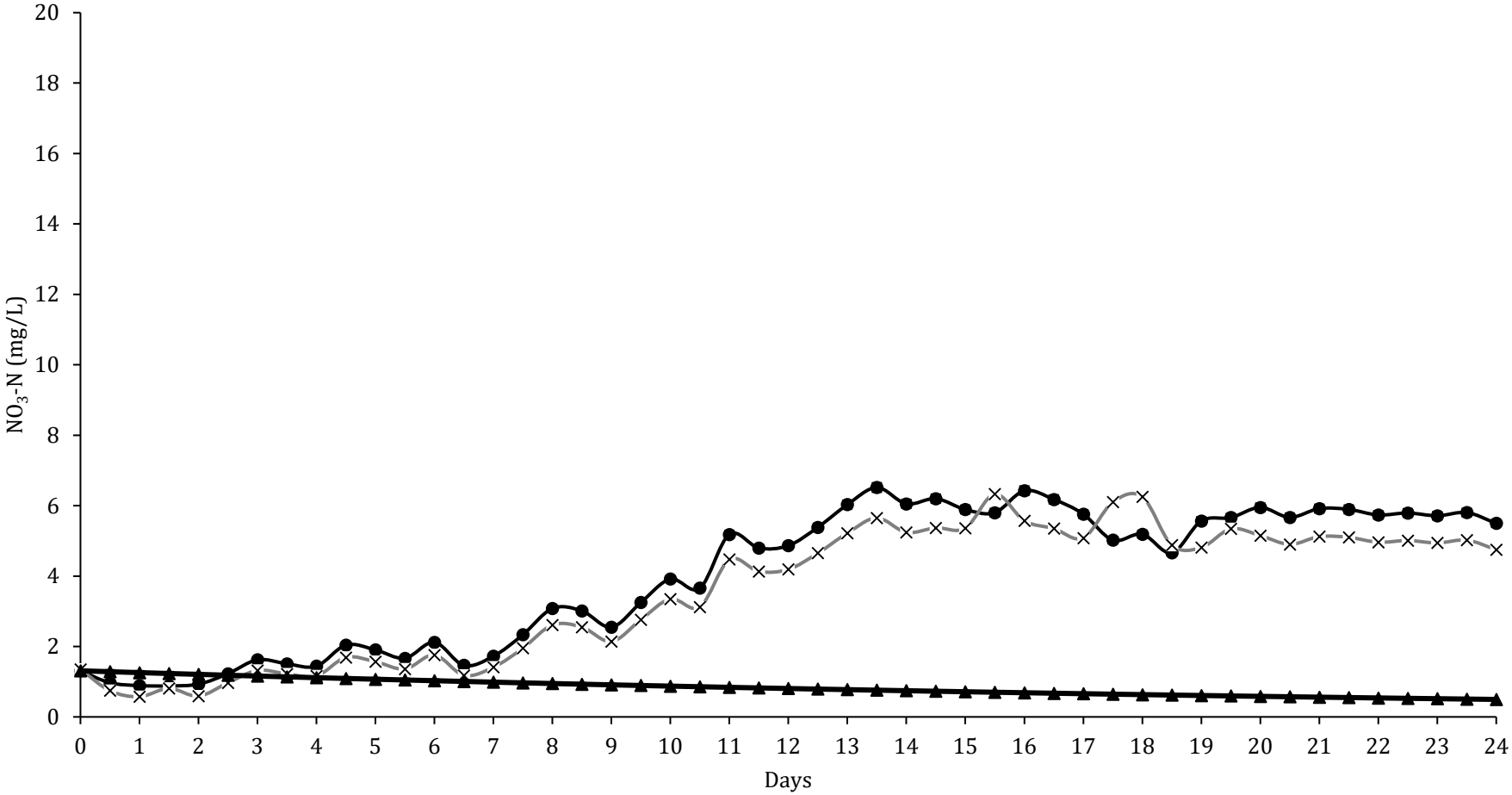



Fig. 3.41 Mid strength $BOD_5$ KoM facultative pond effluent; time series of nitrate ($NO_3$-N) concentration in culture with $CO_2$ addition (■), control culture without $CO_2$ addition (X) and inlet concentration (▲). Values are means ± SE (n =3).

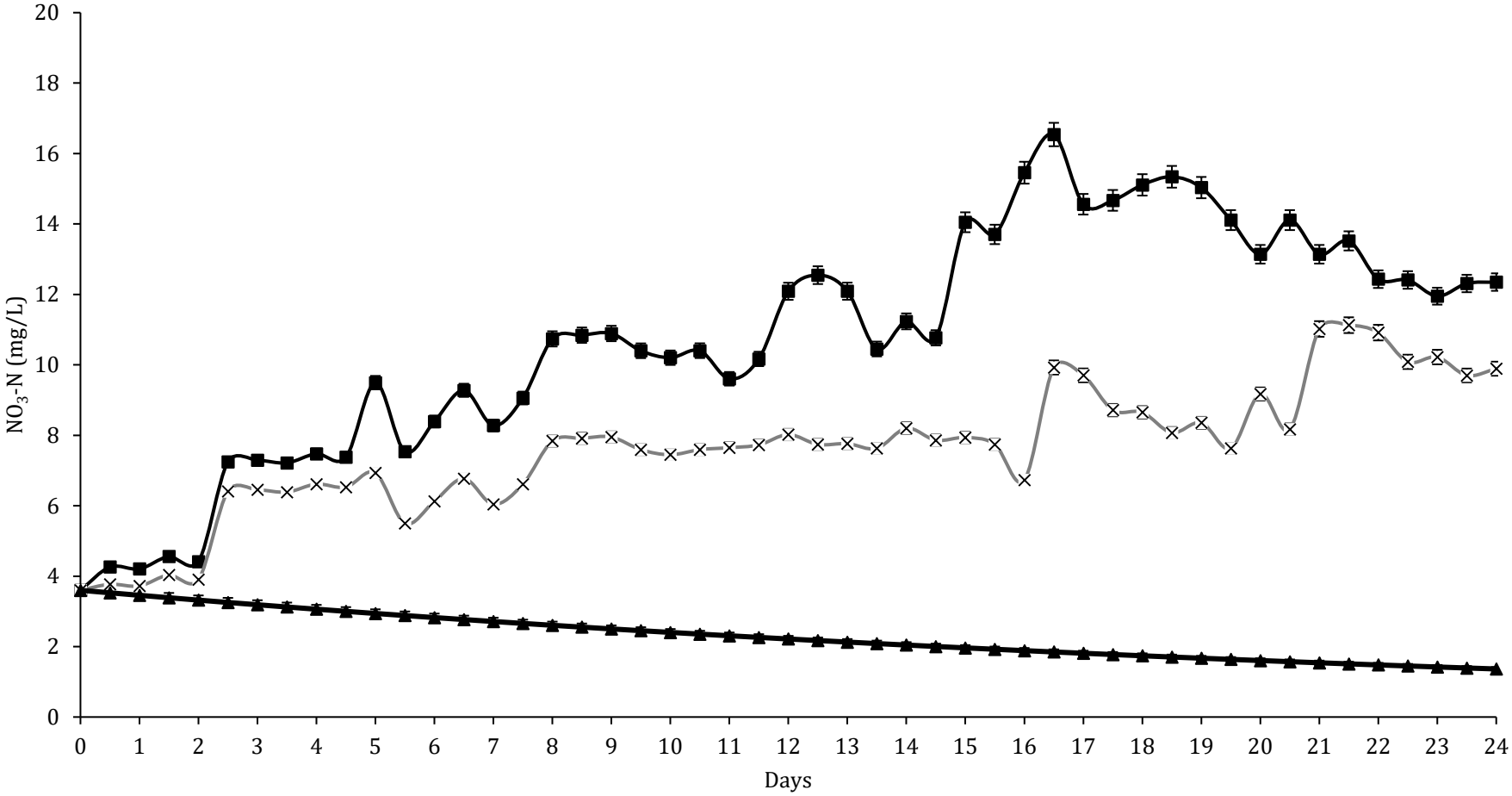



Fig. 3.42 High strength $BOD_5$ KoM septic tank effluent; time series of nitrate ($NO_3$ -N) concentration in culture with $CO_2$ addition (■), control culture without $CO_2$ addition (X) and inlet concentration (▲). Values are means ± SE (n =3).

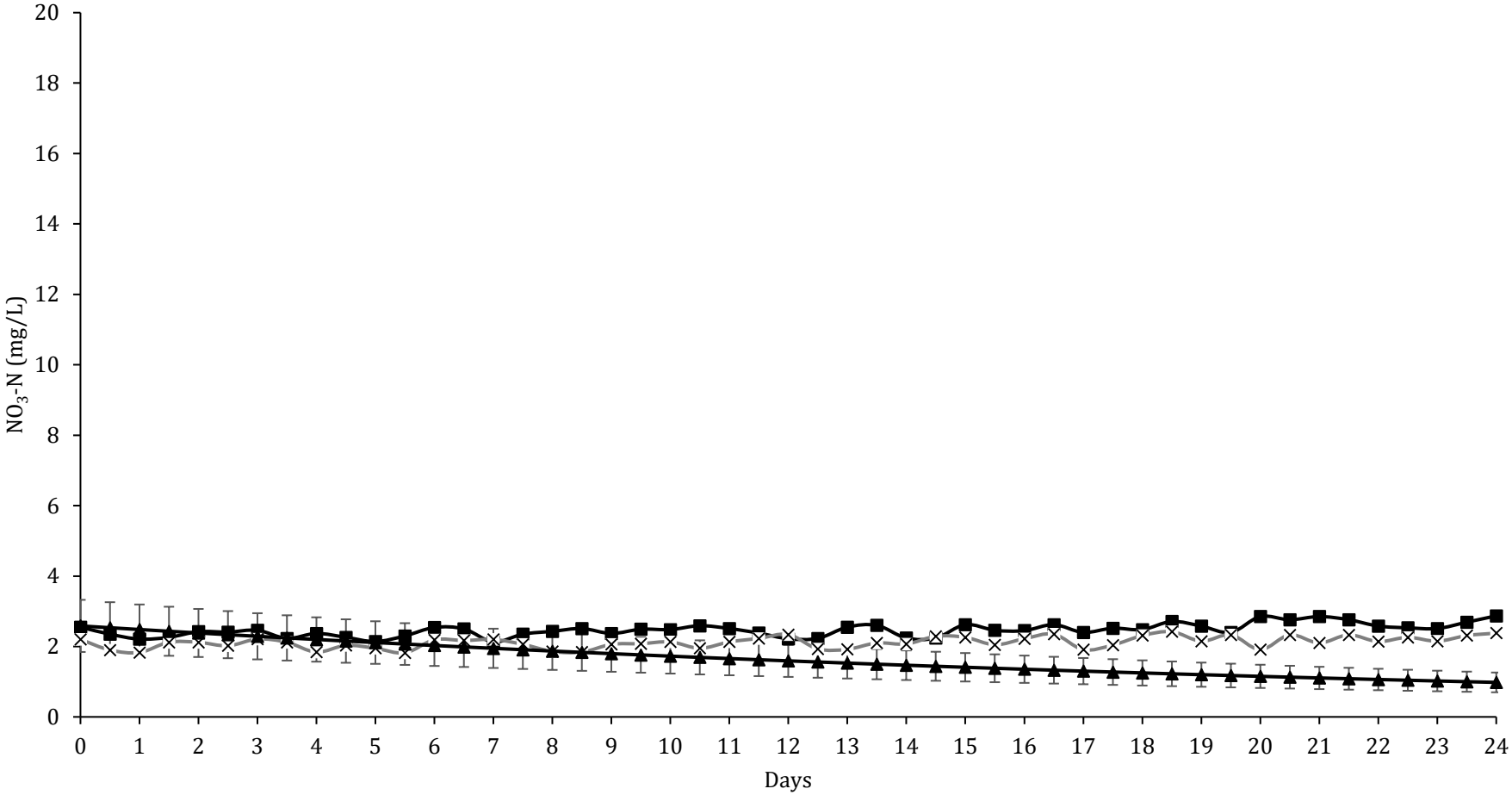



Fig. 3.43 Mid-strength $BOD_5$ KoM facultative pond effluent; time series of nitrate ($NO_3$-N) concentration in culture with HCl addition (■), control culture without HCl addition (X) and inlet concentration (▲). Values are means ± SE (n =3).

### 3.3.4.3. Soluble Phosphate ($PO_4$-P)

Significant difference was found except when additional $CO_2$ was supplied to low BOD strength wastewater (Table 3.13). The highest increase was observed once again in high BOD strength wastewater with a mean increase of 1.64 mg $L^{-1}$ (+7.31%). Similar increment was also observed in both low and mid BOD strength wastewater with a mean increase of 0.40 mg $L^{-1}$ (+3.75%) and 0.49 mg $L^{-1}$ (+2.79%) (Table 3.13). Note that a much higher $PO_4$-P concentrations were observed in all experiment throughout the whole incubation than controls (Fig. 3.44 – 3.47).

Table 3.13 Significant differences of different wastewater strength on Phosphate ($PO_4$-P) over the 24 days period ($P < 0.05$). (a) A summary of independent sample T-test for Equality of Means and Levene's Test for Equality of Variances

| (a) $PO_4$-P (mg/L) | **Mount Barker (Low $BOD_5$)** | **KOM (Mid $BOD_5$, + $CO_2$)** | **KOM (High $BOD_5$)** | **KOM (Mid $BOD_5$,+ Acid)** |
|---|---|---|---|---|
| Are means significantly different ($P < 0.05$)? | No | Yes | Yes | Yes |
| P value | 0.06 | 0.00 | 0.00 | 0.00 |
| Mean ± SD (mg/L) | 11.08 (1.13) | 21.63 (1.27) | 24.06 (1.25) | 18.03 (0.48) |
| Mean (control) ± SD (mg/L) | 10.68 (1.00) | 20.46 (1.01) | 22.43 (1.16) | 17.54 (0.34) |
| N | 50 | 50 | 50 | 50 |
| F | 1.13 | 5.99 | 0.14 | 10.01 |
| df | 98 | 98 | 98 | 98 |
| t | 1.88 | 5.10 | 6.79 | 5.95 |
| Mean difference (%) | + 3.75 | + 5.72 | + 7.31 | + 2.79 |
| Total injected $CO_2$ or acid (HCl 0.1M) volume (L) | 787.50 | 1915.00 | 800.00 | 1.57 |
| Initial inlet chlorophyll a (mg/L) | 0.29 | 1.95 | 0.09 | 1.19 |
| Initial inlet POC (mg/L) | 2.84 | 23.68 | 7.61 | 15.55 |

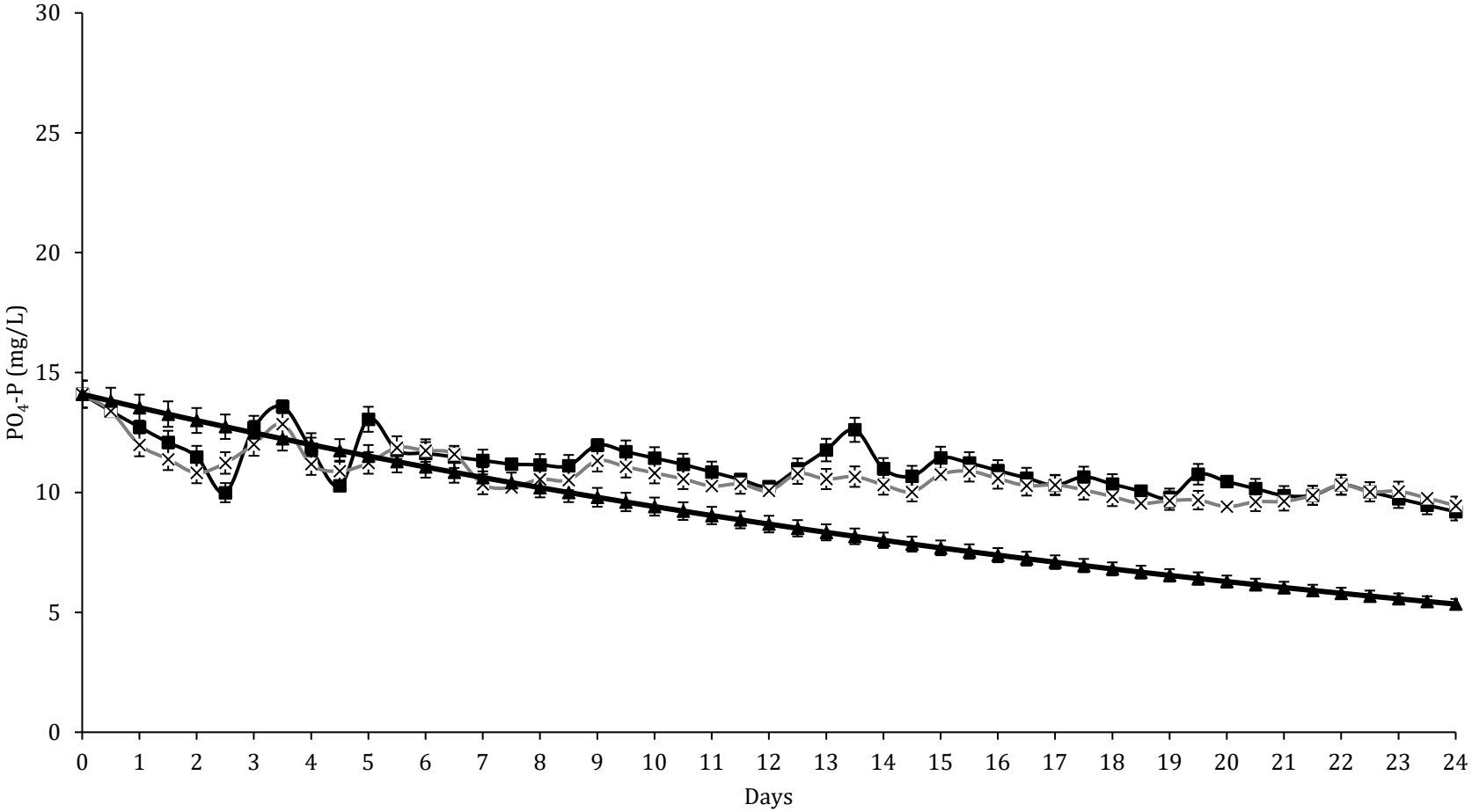



Fig. 3.44 Low $BOD_5$ strength wastewater from Mt Barker aerated lagoon effluent; time series of phosphate ($PO_4$-P) concentration in culture with $CO_2$ addition (■), control culture without $CO_2$ addition (X) and inlet concentration (▲). Values are means ± SE (n =3).

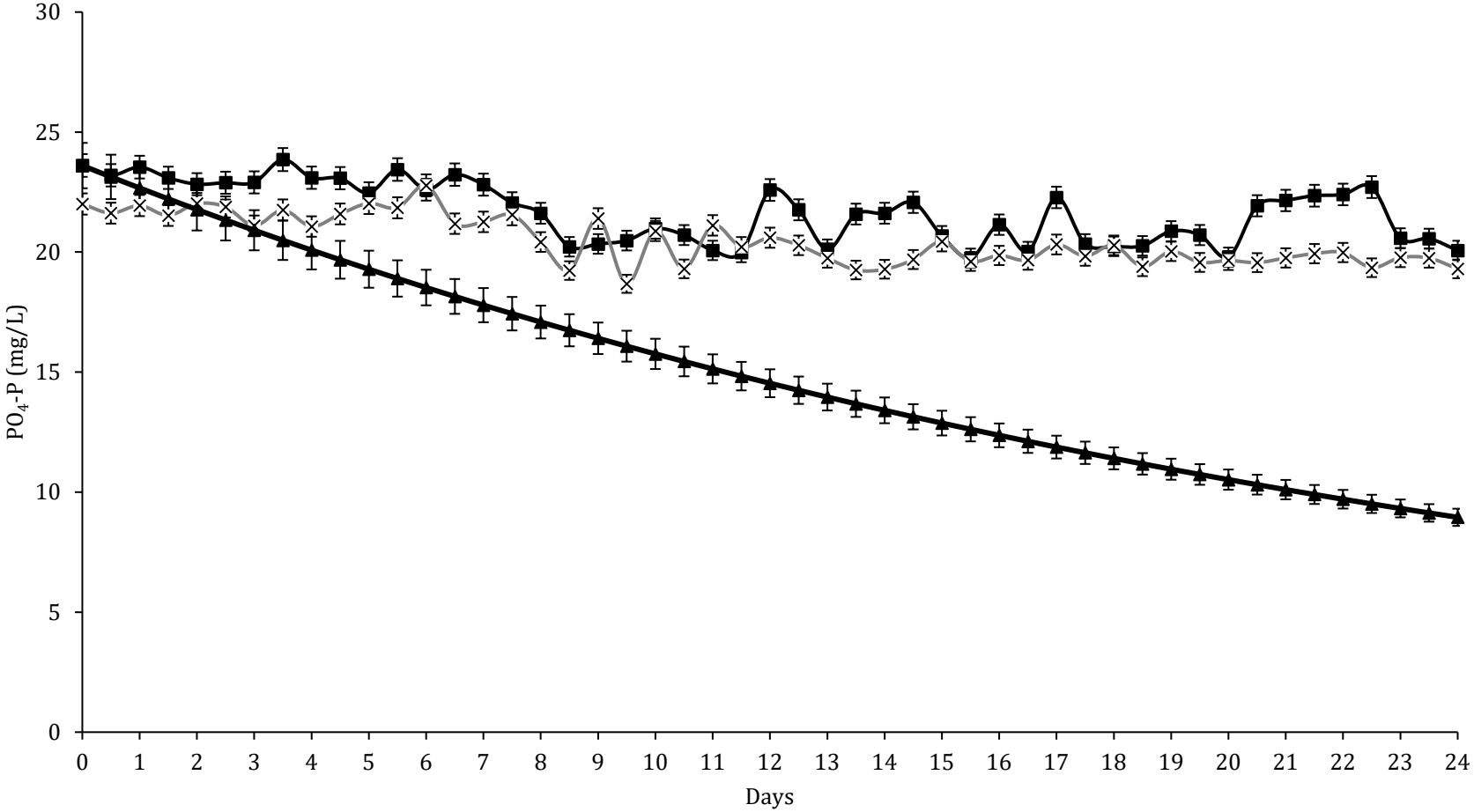



Fig. 3.45 Mid strength $BOD_5$ KoM facultative pond effluent; time series of phosphate ($PO_4$-P) concentration in culture with $CO_2$ addition (■), control culture without $CO_2$ addition (X) and inlet concentration (▲). Values are means ± SE (n =3).

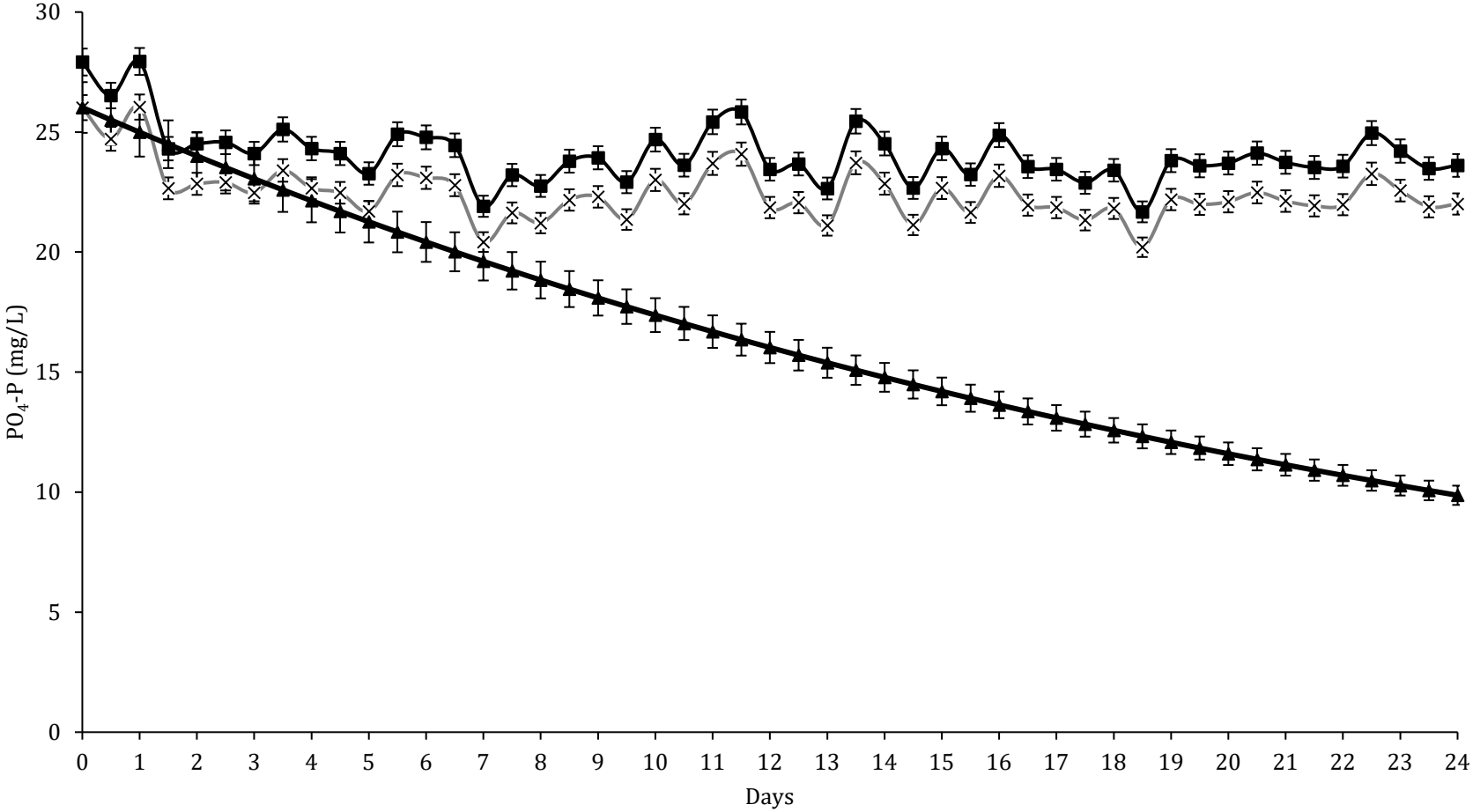



Fig. 3.46 High strength $BOD_5$ KoM septic tank effluent; time series of phosphate ($PO_4$-P) concentration in culture with $CO_2$ addition (■), control culture without $CO_2$ addition (X) and inlet concentration (▲). Values are means ± SE (n =3).

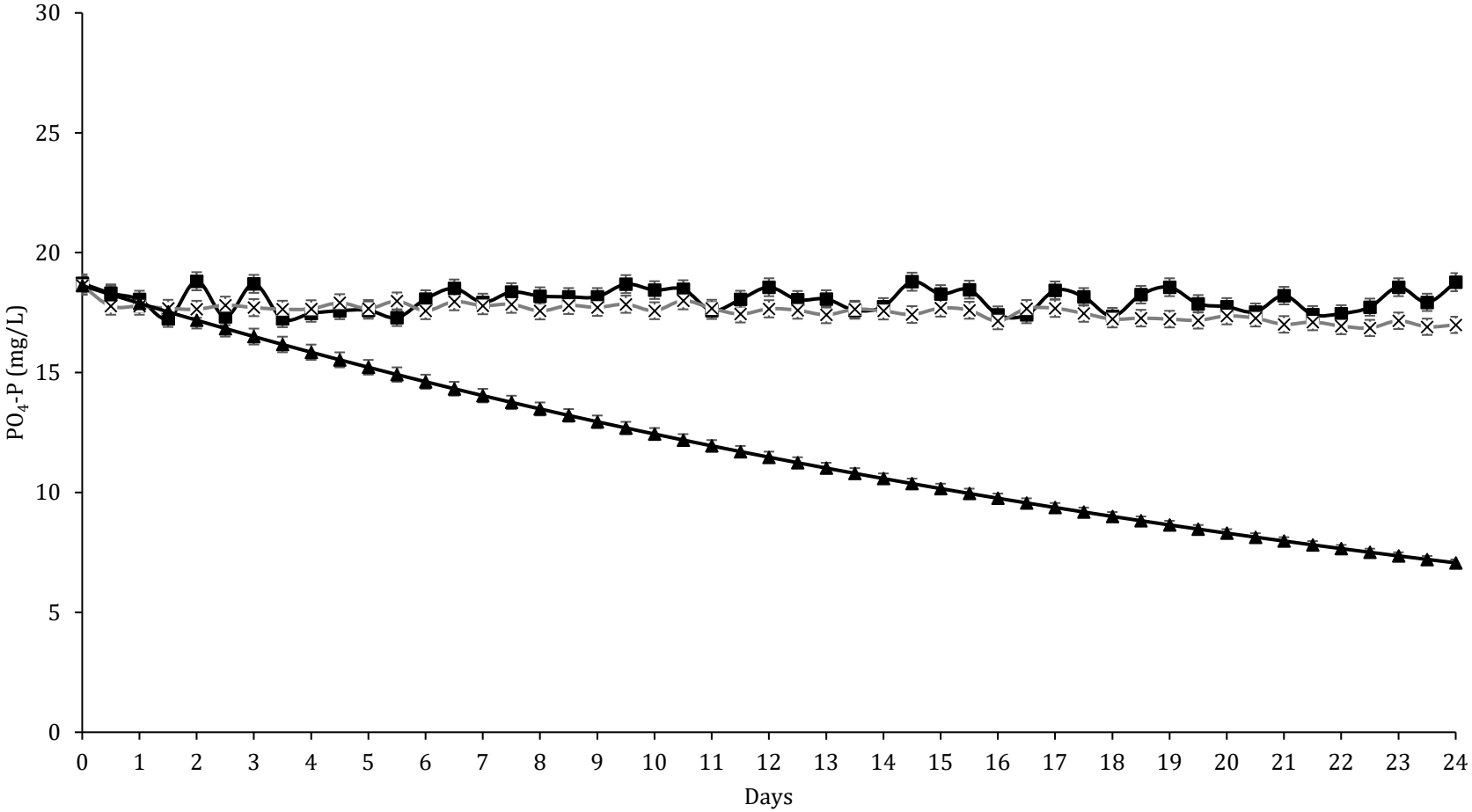



Fig. 3.47 Mid strength $BOD_5$ KoM facultative pond effluent; time series of phosphate ($PO_4$-P) concentration in culture with HCl addition (■), control culture without HCl addition (X) and inlet concentration (▲). Values are means ± SE (n =3).

### 3.3.5. N:P:C ratios

Most algae require substrate in N:P:C ratio of 8:1:50 as suggested by Lundquist (2007) and similarly some authors used the Redfield N:P:C ratio of 16:1:106 (Grobbelaar, 2004; Lundquist, 2007). The carbon component is very high and hence the assumption on the needs of $CO_2$ enriched algal culture to achieve optimal growth conditions is questionable in wastewater cultures. Based on the Redfield ratio, it is in fact likely to be limited by either N or P first, rather than carbon. Lundquist (2007) suggested that artificial $CO_2$ addition significantly improves nutrient removal and growth rate of algae. However, he also added that the ideal N:P:C ratio has not yet been found (Lundquist, 2007). Table 3.14 and 3.15 are summaries of substrate in mean N:P:C & mean C:N ratios comparison in different wastewater strength experiment. Overall, the N:P remained in a similar ratio at all times, with or without the $CO_2$ addition. Although it is widely suggested the artificial $CO_2$ addition significantly improves the growth rate of algae, the mean increase of C:N ratio was only observed in the low BOD strength wastewater when compared to inlet C:N. The additional of carbon dioxide improves the growth of algae but it only provides a significant benefit when the carbon source from the wastewater is already low (i.e. $BOD_5$ < 15 mg $L^{-1}$ in this study) (Table 3.15).

Table **3**.14 Substrate mean N: P: C ratio in different strength $BOD_5$ wastewaters in comparison with controls; N was calculated from unfiltered mean TN (mg/L); P was calculated from unfiltered mean soluble $PO_4$-P; C was calculated from unfiltered mean TC (TOC + IC, mg/L), over the 24-day experiment.

| | | Ratio | | |
|---|---|---|---|---|
| | | N | P | C |
| Mount Barker (Low $BOD_5$) | With $CO_2$ | 3.5 | 1 | 4.1 |
| | Control | 3.2 | 1 | 3.5 |
| KoM (Mid $BOD_5$) | With $CO_2$ | 1.3 | 1 | 5.0 |
| | Control | 1.3 | 1 | 4.3 |
| KoM (High $BOD_5$) | With $CO_2$ | 3.0 | 1 | 4.4 |
| | Control | 2.8 | 1 | 4.2 |
| KoM (Mid $BOD_5$, + Acid) | With $CO_2$ | 1.0 | 1 | 4.5 |
| | Control | 0.8 | 1 | 4.0 |

Table **3**.15 Substrate mean C:N ratios in different strength $BOD_5$ wastewaters and their sources; N was calculated from unfiltered mean TN (mg/L); C was calculated from unfiltered mean TC (TOC + IC, mg/L), over the 24-day experiment. Inlet C:N was calculated from unfiltered TC (TOC + IC, mg/L) and unfiltered TN (mg/L) at day 0.

| | Source | $BOD_5$ (mg/L) | | C:N | Inlet C:N |
|---|---|---|---|---|---|
| Mount Barker (Low $BOD_5$) | Aerated lagoon effluent | 15 | With $CO_2$ | 1.2 | 0.8 |
| | | | Control | 1.1 | |
| KoM (Mid $BOD_5$) | Facultative effluent | 78 | With $CO_2$ | 3.7 | 4.2 |
| | | | Control | 3.2 | |
| KoM (High $BOD_5$) | Septic tank raw effluent | 120 | With $CO_2$ | 1.5 | 1.9 |
| | | | Control | 1.5 | |
| KoM (Mid $BOD_5$ + Acid) | Facultative effluent | 72 | With $CO_2$ | 4.7 | 6.0 |
| | | | Control | 4.9 | |

## 3.4. Discussion

In this Chapter, the effect of $CO_2$ addition to wastewaters with different $BOD_5$ was examined by mainly comparing to the surrogate indicator of available organic carbon as POC, and the surrogate indicator for algae as Chla.

While POC is a measure of particulate organic matter in the wastewater environment, it was defined as suspended organic matter that remained on 0.22 μm pore size filter following the filtration of different $BOD_5$ strength wastewater. Therefore, POC consists of phytoplankton (in this case, microalgae) and zooplankton cells, detritus and bacteria. While Chla is widely used and it has been a common indicator in algal research studies, however, it is only a measure of phytoplankton. It is important to note that, referring to Chapter 1, while studies on microalgal production often looked very promising in laboratory conditions, they were not borne out under conditions encountered in the field. There was a disconnection between the laboratory based studies and the field and the ASP program demonstrated an important lesson that outdoor algae production systems were often incapable of maintaining organisms successfully cultured in the laboratory in the field. The best approach suggested by the program was to successfully cultivate a native species of algae that would allow a contaminant native to the area to take over the ponds such as with the integration of wastewater growth medium (Sheehan et al., 1998). In this growth medium, where the "algal biomass" is now represented as ALBAZOD, which consists of algae, bacteria, zooplankton and detritus, POC may be considered a more important surrogate indicator of biomass compared to Chla. Two parallel effects were examined, namely, using $+CO_2$ or acid (HCl 0.1M) to maintain a constant pH and comparing both POC and Chla changes to control cultures with naturally variable pH.

As discussed in Chapter 1, several factors affect algal growth and these include, in no particular order, temperature, light, pH, and nutrients such as N, P and C. Optimum conditions were supplied in order to focus on investigating the two key biomass parameters: POC and Chla. The temperature used this study was ranged at 23-32 ℃. The irradiance used in these experiments, 170 μmol $m^{-2}$ $s^{-1}$ PFD with an L/D cycle 15:9, was above those accepted as causing light limitation since Zondervan (2002)

stated that POC production was co-limited by $CO_2$ concentration and light at intensities below 150 µmol $m^{-2}$ $s^{-1}$ (Zondervan et al., 2002). Microalgae species have optimal pH ranges for growth with some *Chlorella* sp. can tolerate pH below 4. Higher percentages of $CO_2$ e.g. 10-20% (v/v) were not used because the pH of medium can be greatly reduced reaching pH5.5 (Chen et al., 2014; Zeng et al., 2011; Zhao & Su, 2014). This maybe counterbalanced by $CO_2$ uptake by microalgae which, depending on the buffering capacity of the medium, will generally cause the pH to rise and shift the balance of $CO_2$ concentrating mechanism (CCM) i.e. the inorganic carbon equilibrium shifts due to pH which then influences the form of C available and taken up by the algae. Therefore, pH 7.5 and 8 were used a 'set points' in this experiment. In addition, microalgae biomass production is greatly affected by cultivation conditions and each species has a different tolerance to $CO_2$. Chen *et al.* (2014) and Rahaman *et al.* (2011) both reported *Chlorella* sp. were able to grow in 40% (v/v) $CO_2$, at pH 5.5-6.0, 30 °C. In contrast, Lam *et al.* (2012) stated that *Chlorella* sp. could only grow in up to 2% (v/v) of $CO_2$ and further increases in $CO_2$ would inhibit their growth. Generally, it is well accepted that $CO_2$ concentration above 5% (v/v) is considered to be toxic to some microalgae growth (Ramanan et al., 2010; Zhao & Su, 2014). With the microalgae species observed in this study, they were mainly a mixed culture of *Chlorella sp.* and *Scenedesmus sp.*. Therefore, 5%, v/v of $CO_2$ mixed with air was used this current study and it is significant for the mixed culture (Ho et al., 2010; Lam et al., 2012; Li et al., 2011; Rinanti et al., 2014; Sydney et al., 2010).

Although the requirement for P by freshwater organisms is considerably less than N (Redfield ratio), it is normally P which is growth-limiting in fresh water systems. Nitrogen fixation by blue-green algae is able to compensate for existing deficiencies in nitrogen concentration, leaving P as the limiting nutrient. Unlike nitrogen, there is no gas phase for phosphorus, with no 'P fixation' – either inorganic (within the atmosphere) or biotic (within the aquatic system). There is equally no loss of P to the atmosphere. The analysis in this current study shows that the concentrations of nitrogen (TN: 17.7 – 73.6 mg $L^{-1}$) and phosphorus ($PO_4$-P: 14.1 – 27.9 mg $L^{-1}$) within the respective wastewaters, with a range of 6.7 – 28.9 mg $L^{-1}$ in $NH_4$-N concentrations. In general domestic wastewater, N concentrations vary between 15 and 90 mg $L^{-1}$ and P concentrations between 4 and 20 mg $L^{-1}$ (Abdelaziz et al., 2013; Cai et al., 2013;

Christenson & Sims, 2011). However, their concentrations were not a major investigation in this current study.

The research question to be answered here was:

Does $CO_2$ addition enhance algal (ALBAZOD) production in all wastewater?

For some considerable time, carbon has been suspected of being a growth limiting factor in HRAPs treating wastewater, due to the high algal demand for it, whilst its concentration and bio-availability to algae is relatively low compared to other nutrients (Azov et al., 1982). According to Azov et al. (1982), about 48% of the incoming carbon will be in an inorganic form and 52% in organic form. The form of carbon preferred by most algal species for photosynthesis is unionised, dissolved $CO_2$. In the HRAP this will mostly come from daytime bacterial respiration. The degradation of bacterial biomass releases the main nutrients $NH_3$ and $CO_2$ for algal photosynthesis (Azov et al., 1982). This is quite a slow reaction rate, but has been calculated to proceed fast enough to supply $CO_2$ demand for algal photosynthesis in alkaline HRAP wastewater. Azov et al. (1982) determined that the conditions under which carbon could become limiting to algal productivity were low inlet water organic carbon, high algal concentrations when the inlet water has low alkalinity and long retention times.

In this current study, it showed that there were statistically significant differences between the wastewater with additional $CO_2$ and the respective control culture. Significantly, differences in Chla were found in all wastewaters irrespective of $BOD_5$ strength following $CO_2$ addition, however, corresponding, statistically significant increases in both POC and Chla levels following $CO_2$ addition were only found in low $BOD_5$ (15 mg $L^{-1}$) and mid $BOD_5$ (72 mg $L^{-1}$) wastewater when acid was used to maintain pH. This implies that supplying additional $CO_2$ to all $BOD_5$ strength wastewater, with regards algal growth, was beneficial based on the observations of significantly increases in Chla. However, the observations based on the increase of POC, supplying additional $CO_2$ was only beneficial when $BOD_5$ was low (~15 mg $L^{-1}$) when considering ALBAZOD (algae, zooplankton, detritus and bacteria). The results also suggested that mid BOD (72 mg $L^{-1}$) wastewater provided enough carbon

resource (i.e. the internal DOC pool) for algal growth if the dissolved organic carbon availability was regulated by pH. In addition, no significant difference in either SS or VSS was found between control and amended cultures ($CO_2$ or acid) for any of the wastewaters studied. The mean increases in SS and VSS were very similar in mid strength and high strength wastewater ranging from 15 to 18 %. Exceptionally, in the low $BOD_5$ strength wastewater, the increase of SS and VSS were among the highest with + 0.10 SS mg $ml^{-1}$ (+ 21.59%) and + 0.10 VSS mg $ml^{-1}$ (+ 47.67%), which is approximately 3 times higher than the mid and high BOD wastewater. This suggested that although there was an increase in biomass productivity with $CO_2$ supplement, the increase was not significant in low $BOD_5$ wastewater. In fact, no significant difference was found on SS or VSS in any $BOD_5$ strength wastewater.

Chapter 1, considered the study by Heubeck, Craggs and Shilton (2007) who investigated the influence of $CO_2$ addition from biogas scrubbing on HRAP wastewater treatment performance (BOD, $NH_4$-N, dissolved reactive phosphorous (DRP) and *E.coli* removal) and algal production (growth and species composition). The preliminary findings of the study showed the potential to scrub $CO_2$ from biogas using a HRAP without decreasing the effectiveness of wastewater treatment and enabling increased recovery of wastewater nutrients as algal biomass. Two parallel batch culture experiments were conducted in both laboratory microcosms (2L) and in outside mesocosms (20L). The lab-batch culture was described as a mixture of microcosms (2L glass jars) containing anaerobically digested sewage (1.5L) and a HRAP water algal inoculum (0.5L) i.e. the algal inoculum presented 25% of the microcosms; no $BOD_5$ concentration was indicated for this HRAP culture (Heubeck et al., 2007). The term of microcosms would suggest, as discussed above in the current study, the biomass comprised of ALBAZOD (algae, bacteria, zooplankton, and detritus). The outside-batch culture was described as a mixture of mesocosms (20L HDPE buckets: surface area: 750 $cm^2$; depth: 27cm) with continuous mixing (magnetic stirrer bar) filled with 19L of HRAP water. Two $fBOD_5$ (filtered $BOD_5$) strengths of wastewater (high and low) were synthesised by spiking with a homogenised solution of chicken egg and deionised water. The $fBOD_5$ concentrations were approximately 44 mg $L^{-1}$ (high egg dose) and 24 mg $L^{-1}$ (low egg dose; (Heubeck et al., 2007). According to Australia Guideline for sewerage systems – effluent management (refers back to Chapter 1, Table 1.5), these $BOD_5$ concentrations fit in categories C:

Secondary Treatment ($BOD_5$ 20-30 mg $L^{-1}$) or D: Nutrient Removal ($BOD_5$ 5-20 mg $L^{-1}$) wastewater groups (ARMCANZ, 1997). In comparison to the wastewaters used in the research reported in this current study, the low-egg-dose $fBOD_5$ concentration would be comparable to the low-$BOD_5$ (15 mg $L^{-1}$) wastewater and the high-egg-dose $fBOD_5$ concentration would be between the low-$BOD_5$ and mid-$BOD_5$ (72-78 mg $L^{-1-}$) wastewater. This suggests that the wastewater was pre-treated and the internal carbon pool was low at the outset. Heubeck et al. (2007) found that the initial TSS level (~0.34 mg $ml^{-1}$) in the outside mesocosms was higher than that of the laboratory microcosms (0.10-0.14 mg $ml^{-1}$) due to the larger volume of HRAP water used as the inoculum for the mesocosms. In addition, increases in TSS levels (algal biomass) were higher in cultures with $CO_2$ addition than those in control algal cultures without $CO_2$ addition. Outside mesocosms cultures with higher initial $fBOD_5$ levels (42 mg $L^{-1}$) initially had the highest TSS increase ( ~0.40 mg $ml^{-1}$) compared with ~ 0.37 mg $ml^{-1}$ of those with low initial $fBOD_5$ levels (24 mg $L^{-1}$), in both $CO_2$ addition and control groups in the two $fBOD_5$ levels . It is also important to note that this was based on culture data obtained between Day 0 to Day 3 of the experiment. It was observed in the current study reported here that a much higher concentration of Chla and POC was found in the initial 3-4 days of culture. For example, the Chla concentration increased up to 2 fold within 24h in the low $BOD_5$ experiment, from 1.0 mg $L^{-1}$ at 9am to 2.4 mg $L^{-1}$ at 4pm at day 4 with $CO_2$ addition; from 0.57 mg $L^{-1}$ at 9am to 2.0 mg $L^{-1}$ at 4pm at day 4 without $CO_2$ addition. This observation was generally not found once the culture became stabilised. Interestingly, the authors suggested that this indicated that $CO_2$ released by bacterial break-down of the organic carbon may have initially promoted algal growth in these cultures, which is in agreement with the observation reported here regarding to the high percentage of available organic carbon (DOC) pool in mid to high $BOD_5$ wastewater in this current study. A high percentage of DOC was also found in low $BOD_5$, however, it is important to note that the TOC was 15.37 mg $L^{-1}$ and it was the lowest among all $BOD_5$ wastewater especially compared to the highest 73.24 mg $L^{-1}$ in high $BOD_5$ wastewater furthermore the DOC level (13.55 mg $L^{-1}$) was also the lowest.

Furthermore, Heubeck et al. (2007) performed no test of statistical significance on this TSS comparison and therefore it is unclear whether the differences between cultures were significantly different or not (Heubeck et al., 2007). In this current study there

were large differences between $CO_2$ amended cultures and control cultures, however, they were not statistically significant. The largest difference (21.59%), for instance, in SS between control (0.48 mg $ml^{-1}$) and $CO_2$ amended culture (0.58 mg $ml^{-1}$) was observed in the low $BOD_5$ wastewater (15 mg $L^{-1}$), however, the difference was not statistically significant. Similarly, in mid $BOD_5$ wastewater (78 mg $L^{-1}$), the 17.16% increase in SS (0.39 mg $ml^{-1}$) apparent in the $CO_2$ amended culture compared to the control culture (0.33 mg $ml^{-1}$), was also not statistically significantly different. This suggests two conclusions: firstly, although there was an increase in SS with the $CO_2$ addition, however, no significant increase in difference was suggested. Secondly, the apparent increase became less as the $BOD_5$ level in the wastewater increased i.e. the higher $BOD_5$ strength of the wastewater, the less beneficial effect in terms of SS productivity with $CO_2$ addition.

In addition, the daily $CO_2$ injection volumes were significant higher in mid $BOD_5$ strength wastewater compared to low and high $BOD_5$ strength wastewater. However, according to the POC/Chla value, this does not suggest the significant daily $CO_2$ injection was due to insufficient carbon content presented in the wastewater. Possible explanations may be, firstly, this wastewater was collected from a facultative pond effluent which comes with a developed microalgae population to begin with (1.95 mg $L^{-1}$). This means from day 1, this wastewater was ready to utilize light and $CO_2$ addition, while in low and high BOD strength wastewater samples, their chlorophyll *a* levels were initially 0.29 and 0.09 mg $L^{-1}$ respectively. Secondly, a sudden growth of microalgae population was observed in low and mid BOD strength wastewater when additional $CO_2$ was suppled. This occurred at around day 3-4 when chlorophyll *a* levels suddenly increased up to 2 to 3 times higher in a 24-hour period. This was also observed in mid BOD strength wastewater experiment with acid addition and high BOD strength wastewater. However, the effects were relatively smaller and their chlorophyll *a* levels reminded higher even after the 9h dark period. i.e. they have an overall more stable increase of chlorophyll *a* levels over the period as well as higher chlorophyll *a* levels to start with every day when compared to low and high BOD strength wastewater experiment.

To continue the discussion from Chapter 1, a study by Park and Craggs (2010) investigated the influence of $CO_2$ addition (to augment daytime carbon availability) on wastewater treatment performance and algal production of two pilot-scale HRAPs (West and East) operated with different hydraulic retention times (4 and 8 days) over a New Zealand summer in November-March 2007/08. These two HRAPS were a part of an Advanced Pond System (ASP) treating domestic wastewater at the Ruakura Research Centre located at Hamilton, New Zealand. The study included determination of parameters such as TSS, VSS, total and soluble 5-day biochemical oxygen demand ($TBOD_5$, $SBOD_5$) and chlorophyll *a*. In the $CO_2$ addition experiment, pH was controlled between 8.0 and 7.8 by opening a solenoid vale and bubbling $CO_2$ into the ponds (2L $min^{-1}$). The percentage of $CO_2$ was not specified and no control study (i.e. no $CO_2$ addition) was conducted simultaneously. The $TBOD_5$ was 272.8 g $m^{-3}$ and $SBOD_5$ was 257.7 g $m^{-3}$. The overall pH range observed in this study was 7.9 to 6.2 in the HRAP with $CO_2$ addition. Since no simultaneous, control study was performed, the data from the HRAP control with pH between 10.2 and 7.2 was used from Heubeck et al. (2007) to perform a comparison on the effect of $CO_2$ addition. These pH ranges were similar to study reported here. The daily variation in pond water pH and volumetric $CO_2$ addition rates were also recorded. Similarly to this current study, a wide variation in $CO_2$ addition rates over time and between the ponds (west-4d and east-8d) was also observed in their study (Fig. 3.48). The authors suggested that this wide variation in $CO_2$ addition rates over time and between the ponds was probably due to a combination of variation in weather, total algal concentration and algal photosynthesis and consequent variation in bacterial degradation of organic matter. This is in agreement the observations in this current study. However, reluctantly, this seems not to be re-emphasised before the authors drew the final conclusion. Two main conclusions were drew on biomass productivity: firstly, mean areal biomass (algal/bacterial) productivity (20.7 g/$m^2$/d as VSS) was greater in the shorter retention time $HRAP_{4d}$ than in the $HRAP_{8d}$ (15.8 g/$m^2$/d); secondly, mean areal algal productivity (16.7 g/$m^2$/d) in the shorter retention time $HRAP_{4d}$ was nearly twice that of the $HRAP_{8d}$ (9.0 g/$m^2$/d). However, there are four uncertainties from this paper: 1). with all the parameters were provided for each pond, unfortunately influent Chla (g/$m^3$) for both HRAPs were not provided. Effluent Chla for $HRAP_{4d\ (west)}$ was 3.7 g/$m^3$ and 4.2 for $HRAP_{8d\ (east)}$; 2). The results showed algae percentage was 80.5% for $HRAP_{4d\ (west)}$ and 55.6% for $HRAP_{8d\ (east)}$; 3). The equation of algae biomass concentration was calculated by chlorophyll a concentrations, [Algae biomass (mg/L)]

= [chlorophyll a (mg/L)] x100/1.5; 4). A wide variation in $CO_2$ addition rates over time and between the ponds (west-4d and east-8d) was also observed in their study (Fig. 3.48). With no control study was provided, the authors suggested the mean areal biomass (algal/bacterial) productivity (20.7 g/m$^2$/d as VSS) was high when compared with values measure from previous HRAP research Craggs et al. (2003) and Heubeck et al. (2007) in New Zealand (Craggs et al., 2003; Heubeck et al., 2007). Also, the authors suggested the peak summer production (24.7 g VSS/m$^2$/d and 30.8 g TSS/m$^2$/d for the HRAP$_4$d was similar to annual maximum literature values (~30 g/m$^2$/d measured as TSS, (Weissman & Goebel, 1987). Therefore, the authors proposed that $CO_2$ addition to HRAPs enhanced algal production by augmenting daytime carbon availability (Park & Craggs, 2010). Based on the results observed in this Chapter study and those variables in their papers as described, we strongly disagree that $CO_2$ addition enhances algal (ALBAZOD) production in all wastewater.

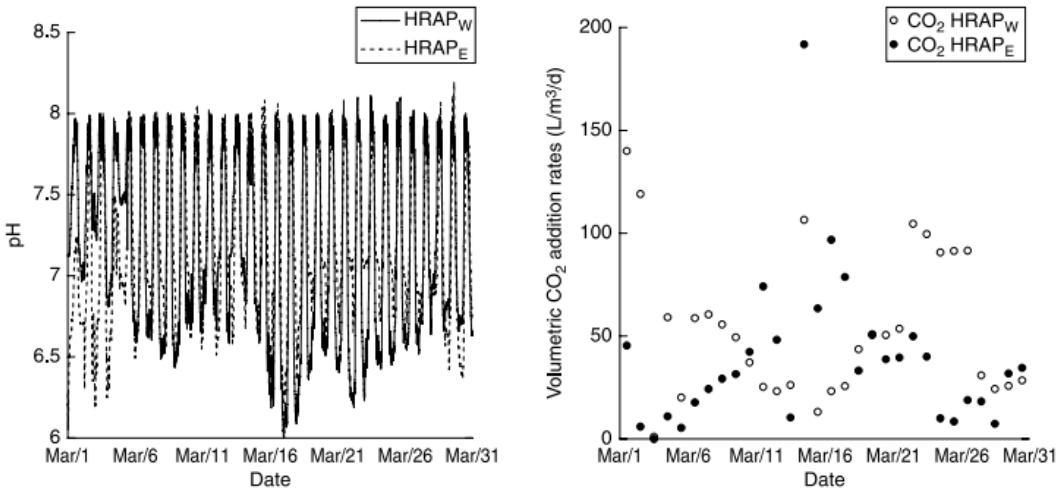



Fig. 3.48 Example of pH control and volumetric $CO_2$ addition rates for the HRAPS (Park & Craggs, 2010)

The study examined in this Chapter here, provides two conclusions: firstly, a developed microalgae population used as an algal culture medium provides an advantage from the rapid development of the microalgae population with minimal to no-lag phase as the results suggested. This may affect the interpretation of the final algal or ALBAZOD productivity especially for a short-period experiment. Secondly, this also suggests that the significant daily $CO_2$ injection was not due to insufficient carbon content present in the wastewater. As the authors from Park and Craggs (2010) already suggested, this was due to a combination of variation in weather, total algal

concentration and algal photosynthesis (i.e. initial Chla concentration) and consequent variation in bacterial degradation of organic matter (i.e. the availability of internal readily accessible DOC pool). Unfortunately, these variables were not included or discussed in depth before the final conclusion was drew.

Another concern is regarding the actual duration of injecting $CO_2$ bubbles into the wastewater. Supplying $CO_2$ to shallow open suspensions can also lead to large $CO_2$ losses to the atmosphere, since $CO_2$ bubbles need a sufficient residence time in order to be absorbed into the water or algae. An 85% $CO_2$ transfer efficiency was reported at a depth of only 20-25 cm in raceway ponds, when the carbon dioxide was sparged with a fine bubble diffuser, activated by a pH regulated solenoid (Benemann, 2008). The level of absorption is pH dependent, and controlling $CO_2$ losses was reported as most difficult at a near neutral pH (Mata et al., 2010).

In summary, while the addition of $CO_2$ increases the growth of microalgae, it is still uncertain whether this is simply due to the need of increase availability of carbon in wastewater coming from the external $CO_2$ source. In the four experiments of this current study, the POC levels ranged from 10-30 mg/L, with a range of inlet TOC from 88.98 (mid $BOD_5$), 116.37 (high $BOD_5$), and 53.75 (mid $BOD_5$ for acid addition), which provided sufficient carbon for the growth of microalgae or ALBAZOD without $CO_2$ addition; except in the low $BOD_5$ wastewater which had a much lower TOC 24.42 mg $L^{-1}$. The experiment with an acid addition provided a higher and statistically significant mean increase in POC ($p < 0.05$, 9.13 mg $L^{-1}$ increased to 12.21 mg $L^{-1}$, +33.73%) than the $CO_2$ addition in mid BOD strength wastewater ($p > 0.05$, 21.76 mg $L^{-1}$ increased to 24.92 mg $L^{-1}$, +14.52%). This raises questions whether the addition $CO_2$ will provide a significant and substantial improvement in microalgae growth when the addition costs of implementing $CO_2$ injection equipment are also considered. In terms of supplying an external carbon source such as $CO_2$ in wastewater for microalgal cultivation, it is also important to distinguish and describe the different characteristic of wastewater medium to be used in the cultivation system, as the levels of BOD and total carbon will be varied accordingly to the prior treatment stages. If the BOD and internal carbon content in the wastewater is already sufficient, the effects of $CO_2$ addition on algal growth in wastewater may not be a cost-effective for enhancing

biomass production. A consequence of a presumed requirement for $CO_2$ addition (e.g. coming from a power plant) to an algal cultivation system with wastewater, is that it strictly limits the flexibility of the system to being built in a specific location i.e. next to a power plant. Without this requirement, it allows the systems e.g. HRAPs to be built in some remote areas. This is particularly useful for remote communities as the population may not be large enough to be considered for the use of waste stabilisation ponds (WSP).

The final conclusions were discussed in Chapter 7 (General discussions).

A brief summary of observations from this chapter:

- Although there was an increase in SS and VSS with the $CO_2$ addition, however, no significant increase in difference was suggested. This also raises a concern whether the additional cost of artificial $CO_2$ is benefit to the harvesting cost.
- The apparent increase became less as the $BOD_5$ level in the wastewater increased of i.e. the higher $BOD_5$ strength of the wastewater, the less beneficial effect in terms of SS productivity with $CO_2$ addition.
- A developed microalgae population used as an algal culture medium provides an advantage from the rapid development of the microalgae population with minimal to no-lag phase as the results suggested. This may affect the interpretation of the final algal or ALBAZOD productivity especially for a short-period experiment.
- This also suggests that the significant daily $CO_2$ injection was not due to insufficient carbon content present in the wastewater. This was due to a combination of total algal concentration and algal photosynthesis (i.e. initial Chla concentration) and consequent variation in bacterial degradation of organic matter (i.e. the availability of internal readily accessible DOC pool).
- In this current study, it showed that there were statistically significant differences between the wastewater with additional $CO_2$ and the respective control culture. Significantly, differences in Chla were found in all wastewaters irrespective of $BOD_5$ strength following $CO_2$ addition, however, corresponding, statistically significant increases in both POC and Chla levels following $CO_2$ addition were only found in low $BOD_5$ (15 mg $L^{-1}$) and mid $BOD_5$ (72 mg $L^{-1}$) wastewater when acid was used to maintain pH. This implies that supplying additional $CO_2$ to all $BOD_5$ strength wastewater, with regards algal growth, was beneficial based on the observations of significantly increases in Chla. However, the observations based on the increase of POC, supplying additional $CO_2$ was only beneficial when $BOD_5$ was low (~15 mg $L^{-1}$) when considering ALBAZOD (algae, zooplankton, detritus and bacteria). The results also suggested that mid BOD (72 mg $L^{-1}$) wastewater provided enough carbon resource (i.e. the internal DOC pool) for algal growth if the dissolved organic carbon availability was regulated by pH.

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# CHAPTER 4

# PSYCHROPHILIC ANAEROBIC CO-DIGESTION OF PIG SLURRY WITH ALGAL SLUDGE ORIGINATING FROM MUNICIPAL WASTEWATER (ALBAZOD)

# 4. PSYCHROPHILIC ANAEROBIC CO-DIGESTION OF PIG SLURRY WITH ALGAL SLUDGE ORIGINATING FROM MUNICIPAL WASTEWATER (ALBAZOD)

## 4.1. Introduction

Anaerobic digestion was originally designed for single substrate and single purpose treatment. For example, many sewage sludges are treated using a variety of digestion techniques in order to reduce the amount of organic matter and the number of potential disease-causing microorganisms presented in the solids. In South Australia, the digested sewage sludge is mechanically filtered and centrifuged, and then dried under sunlight. These thickened, dried, and nutrient rich biosolids are then provided to farmers free-of-charge to use as a natural fertiliser. Although this method has greatly reduced the amount of biosolids going into landfill every year, as today the limits and the possibilities of anaerobic digestion are better known such as the maximum potential of methane generation, the long time required for the process (30 days or above), and the high capital and maintenance cost; alternatives are urged to replace the standard technology such as mono-substrate anaerobic digestion in activated sludge plants

Co-digestion utilises feedstock in which each digestant has an attribute that is in itself not only beneficial, but also when placed with another digestant, the simultaneous digestion of the homogenous mixture of two or more substrate enable a successful process (Braun, 2002). Generally, co-digestion is applied when a major amount of a main basic substrate (e.g., manure, sewage sludge or mature municipal sludge waste MSW) is mixed and digested together with a high putrescible organic waste such as fruit and vegetables, animal or fish offal (Angelidaki et al., 2003; Mshandete et al., 2004). The putrescible waste acts as the main contributor of methane yield, while the other co-digestants provide either a large and diverse microbial biomass or mainly act as pH buffers (Callaghan et al., 1999; Callaghan et al., 2002; Lin et al., 2011; Sosnowski et al., 2007; Wang et al., 2010). Depending on the country, strict regulation for the application of co-digestion may apply, particularly legislation prevented the

application of digested co-substrates on agricultural fields such as in Netherlands (Schomaker, 1987) and Australia.

In Australia, the fact that renewable energy is poorly valued and organic waste can still be readily and cheaply landfilled are obvious barriers to anaerobic co-digestion (Iacovidou et al., 2012; Mata-Alvarez et al., 2000). Yet, wastewater treatment plant operators are still interested in becoming an integrated component of organic municipal waste (OMW) management. Yarra Valley Water's co-digestion proposal for their Aurora site, and Sydney Water's joint research with the Office of Environment and Heritage provide two clear examples. The need to add capacity to treating OMW immediately, particularly in New South Wales and Victoria where landfill levies are higher, seems to be the main driver for co-digestion. Wastewater utilities have identified they can gain a gate fee whilst neutralising their own power bill. While co-digestion has been used in Europe, the US and Asia for some time - SUEZ environnement and its partners were the first to bring this emerging technology to Australia (Edwards, 2014; Edwards et al., 2015).

Anaerobic digestion is a well-established process for treating many types of organic wastes, both solid and liquid. Anaerobic digestion of sludge is often employed to reduce both the mass of solids and pathogen load and produce energy in the form of methane gas (Mata-Alvarez et al., 2014). Increasingly, covered anaerobic lagoons are being considered by the pork industry to manage GHG emissions and recover the methane for energy production. Algal biomass produced in HRAPs treating piggery wastewaters removes $CO_2$, contributing to GHG mitigation, and is an additional source of biomass energy which could be released via co-digestion with pig slurry (Buchanan, 2014).

My previous Honours research examined the proximate composition and anaerobic digestion potential of algal solids generated from a small community waste stabilization pond system treating sewage in South Australia. This study examined the seasonal variation in the composition of microalgal biomass grown on domestic wastewater i.e., chlorophyll *a*, carbohydrate, protein and lipid and the potential for methane production. The results showed there were significant differences in

chlorophyll *a* and carbohydrate concentrations over the year. However, the protein and lipid concentrations remained constant over the four seasons, suggesting that the biomass would potentially be a reliable biomass energy source. Preliminary experiments investigating anaerobic digestion performance in terms of gas composition showed that the digestion of algal solids on their own was relatively poor; however, when the algal solids were co-digested with pre-digested wastewater sludge (50:50) the peak level of methane significantly increased (Cheng, 2010).

While other wastes are commonly co-digested e.g., industrial organic wastes, fruit and vegetable solid waste, olive wastes and farm wastes. There are limited studies on the digestion of algal biomass either as a sole substrate or co-digested with other wastes, significantly for this proposal, none consider co-digestion with pig slurry except a study by Astals et al. (2015). These authors investigate anaerobic co-digestion of pig manure and algae (*Scenedesmus sp.*) with and without extraction of intracellular algal co-products, with a view towards the development of a biorefinery concept for lipid, protein and/or biogas production. The experiment demonstrates a synergy between pig manure and raw algae that increased raw algae methane yield from 0.163 to 0.245 L $CH_4$ $g^{-1}$ $VS_{added}$ (Astals et al., 2015). Algal biomass is relatively high in nitrogen, which results in the production of high concentrations of ammonia upon digestion which may inhibit the microorganisms involved in the anaerobic digestion process, additionally this elevates the pH which may further inhibit the digestion. Methane production from swine slurry has been reported as relatively low due to several factors such as the high quantity of water, unbalanced carbon/nitrogen (C/N) ratio or high solids content which requires a long hydrolysis time (Mata-Alvarez et al., 2014). Two major strategies have been suggested to overcome these limitations namely pre-treatment of manure or co-digestion with other substrates (Mata-Alvarez et al., 2014). In addition, to overcome low C/N ratio problem in microalgae, it has been suggested by González-Fernández et al. (2011) and Shouquan et al. (2009) that using the addition of pig manure to microalgae to aid digestion (González-Fernández et al., 2011; Wang et al., 2009). Therefore, the addition of algal biomass to pig slurry entering covered anaerobic lagoons is likely beneficial for methane/energy production via co-digestion (Birchall, 2010; Buchanan et al., 2013).

The life cycle assessment conducted for the Pork CRC Review (*Project 4A-101 Algae for Energy & Feed: a wastewater solution*), suggests that the anaerobic co-digestion of slurry grown microalgal biomass with pig slurry was novel and was a primary route to achieve significant GHG abatement. The review proposes a combination of pre-treatment by existing covered anaerobic lagoon or in an engineered anaerobic reactor, followed by treatment in a closed aerobic reactor operated to maximise nitrification, as described in Chapter 1 (Fig. 1.14). In the literature, the majority of full-scale applications and research effort has been concentrated on anaerobic digestion within the mesophilic (25-45 ℃) or thermophilic (45-65 ℃) temperature ranges (Connaughton et al., 2006). This was largely due to the belief that sub-ambient or psychrophilic (<20 °C) anaerobic digestion was not viable because of low microbial activity and biogas production rates under low temperature conditions (Lettinga et al., 2001; Lin et al., 1987). Therefore, anaerobic digestion at psychrophilic temperature has not been as extensively explored. In addition to the anaerobic co-digestion, we believe the approach of using existing covered anaerobic ponds would be more approachable by Australia pig farmers rather than using an engineered anaerobic reactor. This is due to the concept of covering an anaerobic lagoon for the purpose of biogas recovery from pig manure has emerged and floating covers have been successfully installed, since many facilities already utilize hydraulic flushing for manure collection and anaerobic lagoons for treatment (Safley & Westerman, 1990). However, Safley (1990) also emphases that very limited methodology has been proposed for the design of covered lagoon digesters which would naturally function at psychrophilic temperatures. Additionally, the pig effluents are discharged at low-ambient temperatures due to the hydraulic flushing. As a consequence, one of the main advantages of psychrophilic anaerobic wastewater treatment would be increased cost-efficiency, as the need to heat influent wastewaters or to direct anaerobic-digestion-produced energy back into system maintenance (e.g., bioreactor heating) is reduced or eliminated (Connaughton et al., 2006). This approach also reduces or eliminates any additional energy which may be required to heat the digester to maintain at the specific temperature. For example, in cold climate seasons, a certain amount of $CH_4$ or heat recovered from combustion is required to maintain the specific mesophilic and thermophilic temperature.

The objective of the research presented in this was to conduct a comparative study on psychrophilic anaerobic co-digestion of pig slurry and ALBAZOD (algae-bacteria-zooplankton-detritus). In nature and in an open system such as wastewater treatment ponds, microbes live in a diverse community of algae, bacteria, zooplankton and detritus. This combined microbial biomass is referred to as ALBAZOD (algae-bacteria-zooplankton-detritus) and it is therefore used in this study and not “algal biomass”.

This research was previously presented as an oral presentation: *Co-digestion of wastewater grown algae with pig slurry or activated sludge in laboratory scale anaerobic digesters. 22nd European Union Biomass Conference and Exhibition, Hamburg, Germany, 23-26 June, 2014.*

## 4.2. Methods

Co-digestion of algal biomass with pig slurry is the focus of this research, to determine the optimum ratio of algal biomass to pig slurry which maximises methane production in quantity and quality. This research determines the quantity and quality of the biogas ($CO_2$ and $CH_4$) using gas monitor together with key process parameters including pH, COD, total solid (TS), volatile solid (VS), and $NH_4$-N.

### 4.2.1. Feedstock

Algae rich sludge was collected from Mount Barker CWMS DAF (dissolved air floatation) plant in South Australia (Fig. 4.1). In natural, open systems such as wastewater treatment ponds the biomass comprises of a diverse community of algae, bacteria, zooplankton and detritus (ALBAZOD).

Fig. 4.1 ALBAZOD sample obtained from Mount Barker CWMS DAF plant in South Australia

Pig slurry sample was collected from the under floor drains of the finishing sheds at Roseworthy Piggery Pty/Ltd, South Australia (Fig. 4.2). The animals were in the finishing phase and fed diets formulated to meet the requirements of this phase. The composition of feedstocks was analysed upon arrival and samples were stored in a refrigerator at 0-4ºC.

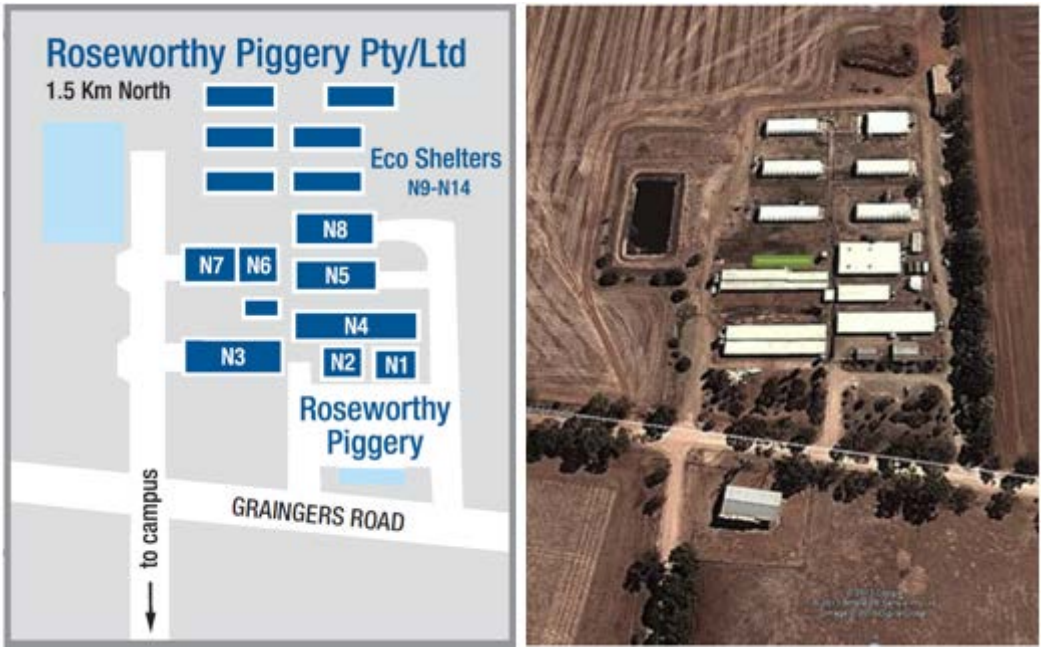



Fig. 4.2 Roseworthy Piggery, South Australia. Raw pig slurry was collected from the underfloor drains located in the pig shed (N3).

### 4.2.2. Batch anaerobic digesters

Experiments were set up in 30 L capacity, plastic, batch anaerobic digesters, which were seeded with 20 L of anaerobically digested sludge obtained from the two feedstock sites. The reactors were purged with $N_2$ gas and operated at ambient psychrophilic temperature (17- 25 °C) for 3 months with manual mixing once per day. The temperature was recorded through a thermometer inside the reactor. There were two valves on each of the reactors, one for solid and liquid sampling (at the base) and the other one for gas sampling (at the top). The top valve contains two external valves one of which was permanently connected to a central manifold for gas venting to avoid the build-up of biogas inside the reactors when gas sampling was not performed. The other external valve was used for biogas sampling when it was required. The reactors were setup as shown below (Fig, 4.3 & 4.4)

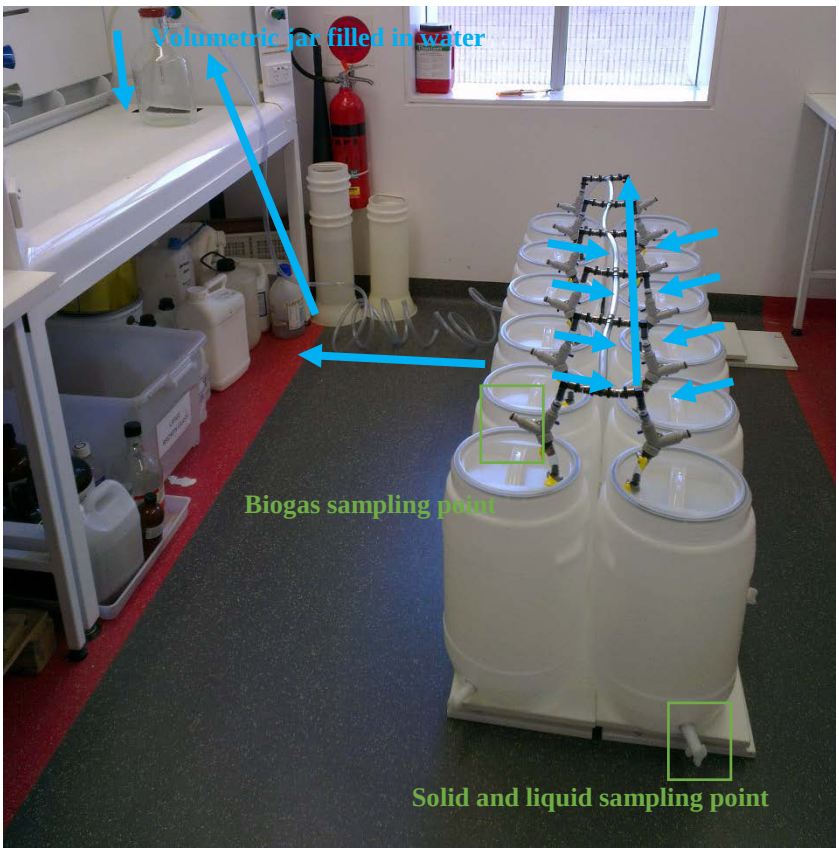


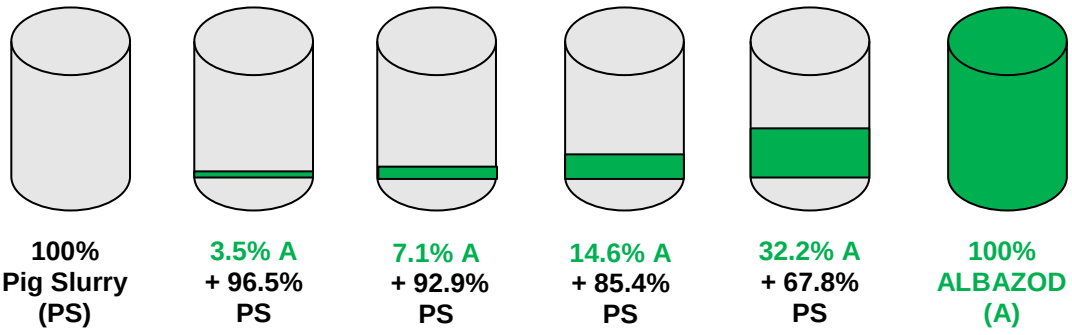

Fig. 4.3 The setup of anaerobic digesters as described with two sampling valves. The central tubing was designed for biogas evacuation as indicated in blue lines. It was connected to a volumetric jar filled in water before the biogas was discharged to the fume hood.


Fig. 4.4 The anaerobic digesters with different algal sludge (ALBAZOD)/pig slurry mixtures. The working volume was 20L.

Six experiment groups (Table 4.1 & Fig. 4.5) were studied as following (VS, w/w): 100% pig slurry (PS), 96.5% PS + 3.5% ALBAZOD (A), 92.9% PS + 7.1% A, 85.4% PS + 14.6% A, 67.8% PS + 32.2% A, and 100% A; all ratios were calculated on dry weight VS (w/w) . All experiments were performed in duplicate reactors, each with triplicate analysis (n=6).

Table 4.1 Mixtures of ALBAZOD (A) and Pig Slurry (PS); compositional ratio characterised by volatile solid (VS, w/w) and by volume (v/v)

| | Mixture | | | | | |
|---|---|---|---|---|---|---|
| Ratio by VS (w/w) | 100% PS | 96.5% PS<br>3.5% A | 92.9% PS<br>7.1% A | 85.4% PS<br>14.6% A | 67.8% PS<br>32.2% A | 100% A |
| Ratio by volume (v/v) | 100% PS | 95% PS<br>5% A | 90% PS<br>10% A | 80% PS<br>20% A | 60% PS<br>40% A | 100% A |




**a.**

**b.** **c.**

Fig. 4.5 Illustrations of batch anaerobic digesters a. different ratio in VS (w/w) as described in Table 4.1 b. Pig slurry sample (100%PS v/v) c. Algal sample – ALBAZOD (100% A v/v).

### 4.2.3. Analytical methods

The composition of the mixed liquor within the anaerobic digester was analyzed before and after digestion for the following parameters: total solids TS (g $L^{-1}$), VS (g $L^{-1}$), dry matter (%), moisture (%), pH, temperature ($^{o}$C), $NH_4$-N (mg $L^{-1}$), TCOD (g COD $L^{-1}$), gas production (L $d^{-1}$), $CH_4$ (%), and $CO_2$ (%). TS, DM, moisture, and VS were measured according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1992). Temperature and pH were measured using glass pH probe (Jenway portable Model 370 pH/mV meter). COD and $NH_4$-N were analysed using the method previously described (Chapter 2).

### 4.2.4. Quantitative and Qualitative Assessment of the Biogas Produced

The digesters were connected to a gas collecting jar (a 5L capacity measuring cylinder) inverted over a water solution. The gas was collected by "upward delivery downward displacement" of water displacement, and the volume of the gas* produced was recorded per hour, interpreted into daily volumes accordingly over a three months period (91 days), by using the water displacement method as described in (Callaghan et al., 1999). The digesters were kept at ambient temperature range at 17-25 ℃. The percentage of methane ($CH_4$) and carbon dioxide ($CO_2$) in the biogas was determined using an LMSx landfill gas analyser (Anri Instruments and Controls Pty Ltd, Melbourne, Australia). The volume of each gas was determined by the ratio presented in the total biogas volume.

* due to the low solubility of $CO_2$ in water and the equilibrium constant for this reaction: $CO_2$ (aq) + $H_2O \leftrightarrow H_2CO_3$ (aq) is about 1.6 x $10^{-3}$ around room temperature, which means that most of the dissolved $CO_2$ is presented as hydrated $CO_2$ (aq) instead of $H_2CO_3$ (aq). Therefore, an assumption was made that the volume of biogas collected represents the true volumes of $CH_4$ and $CO_2$.

### 4.2.5. Cumulative specific methane yield (L $CH_4$ $g^{-1}$ $VS_{removed}$)

By using the method described in Callaghan (1999), the specific gas yield (L $CH_4$ $g^{-1}$ $VS_{removed}$) was calculated by the generation of biogas volume and reduction in VS concentration of the solids on the specific day over the experiment period. According to Linke (1997), the digestion of pig slurry with other organic waste showed that the yield was inversely related to the solids loading rate (Linke, 1997). However, it is not possible in this current study, which was based on batch studies, to define loading rates, but the applied VS loads were calculated in Table 4.2.

## 4.3. Results

### 4.3.1. Characteristic of substrates

The characteristic of the pig slurry (PS) and ALBAZOD (A) mixtures are shown in Table 4.2. Pig slurry has about a 10 times higher COD content (35.09 g COD $L^{-1}$) than ALBAZOD (3.82 g COD $L^{-1}$). The volatile solid (VS) of control 100% pig slurry was about 75%, while a lower percentage was recorded for control 100% ALBZAOD (52% VS). The dry matter was about 4% for pig slurry and 1% for ALBAZOD. The initial pH of pig slurry was more alkaline than ALBAZOD, pH 8.42 and pH 7.70 respectively. No temperature control was used in this study as ambient psychrophilic temperature varied between 17 and 25 °C. Pig slurry contained a much higher ammonia level (1361.22 mg $NH_4$-N $L^{-1}$) compared to 12.73 mg $NH_4$-N $L^{-1}$ in the ALBAZOD.

Table 4.2 Characteristics of the pig slurry (PS) and ALBAZOD (A) mixtures used in the batch anaerobic digestions at ambient psychrophilic temperature (17-25˚C). Analytical standard errors are also indicated (n=6).

| **Ratio by VS (w/w)** | **100% PS** | **96.5% PS 3.5% A** | **92.9% PS 7.1% A** | **85.4% PS 14.6% A** | **67.8% PS 32.2% A** | **100% A** |
|---|---|---|---|---|---|---|
| **Ratio by volume** | 100% PS | 95% PS 5% A | 90% PS 10% A | 80% PS 20% A | 60% PS 40% A | 100% A |
| **TS (g/L)** | 40.11 (± 0.21) | 35.20 (± 0.71) | 26.71 (± 2.31) | 20.74 (± 1.33) | 16.78 (± 1.29) | 9.59 (± 0.56) |
| **VS (g/L)** | 30.26 (± 0.55) | 25.16 (± 0.20) | 17.37 (± 0.10) | 12.78 (± 0.13) | 9.22 (± 0.17) | 4.98 (± 0.10) |
| **VS (%)** | 75.44 | 71.49 | 65.05 | 61.60 | 54.91 | 51.90 |
| **DM (%)** | 3.94 | 3.74 | 3.64 | 3.32 | 2.74 | 1.03 |
| **Moisture (%)** | 96.06 | 96.26 | 96.36 | 96.68 | 97.26 | 98.97 |
| **pH** | 8.42 (± 0.10) | 8.36 (± 0.10) | 8.34 (± 0.05) | 8.26 (± 0.05) | 8.13 (± 0.10) | 7.70 (± 0.10) |
| **$NH_4$-N (mg/L)** | 1361.22 (± 42.26) | 1295.19 (± 13.07) | 1225.81 (± 17.16) | 1089.76 (± 53.43) | 819.73 (± 57.78) | 12.73 (± 0.24) |
| **TCOD (g COD/L)** | 35.09 (± 2.15) | 20.38 (± 1.11) | 10.77 (± 2.25) | 6.78 (± 0.27) | 4.33 (± 0.13) | 3.82 (± 0.10) |

### 4.3.2. pH and $NH_4$-N

The mixtures pH in all reactors remained relatively constant during the experiment with values approaching pH 8.0 to 7.5, except pH 7.09 in 100% A at day 91. A sudden decline in pH value and minimal or nil methane production in the low biogas yields

were observed at the beginning of all digestions within first 10-20 days at a ratio of ALBAZOD above 32.2%, the reductions of $NH_4$-N between groups 100% PS, 3.5% A, 7.1% A, and 14.6% A were similar, reaching 600-650 mg $L^{-1}$ $NH_4$-N at day 91. The initial $NH_4$-N in 100% A was 12.73 mg $L^{-1}$ and rapidly reduced to 1.93 mg $L^{-1}$ in day 7 and 0.88 mg $L^{-1}$ in day 14 (Fig. 4.6).

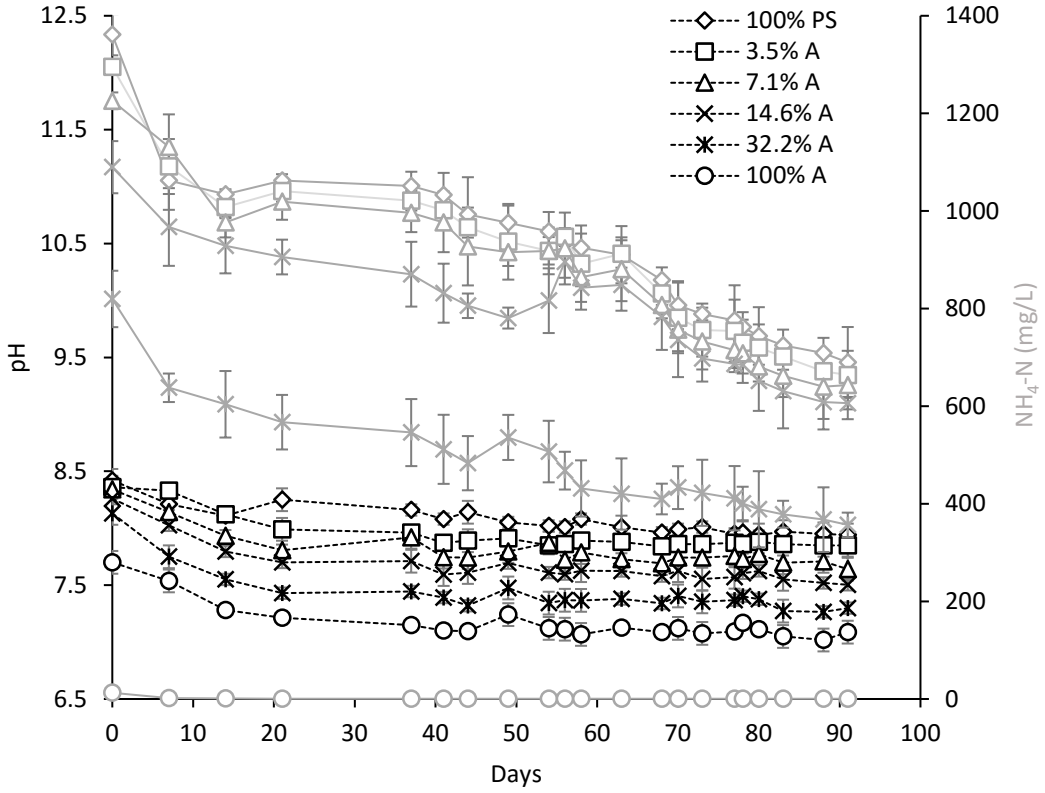



Fig. 4.6 Time course of pH and $NH_4$-N concentrations attained during anaerobic co-digestion between pig slurry (PS) and ALBAZOD (A) in different ratios (w/w) over 91 day period. Grey line is $NH_4$-N (mg/L) and broken black line is pH. With ± analytical standard error (n=6).

### 4.3.3. Biogas (L) accumulation

The accumulated biogas production from the 100% ALBAZOD (A), 100% pig slurry (PS) and co-digestions of pig slurry with various percentages of ALBAZOD (%VS w/w of ALBAZOD, A) of 3.5% A, 7.1% A, 14.6% A, and 32.2% A are shown in Fig. 4.7. No stationary phase was observed in either 100% PS or 3.5% A treatments as indicated by the continuous biogas accumulation over the 91 day incubation period. The final accumulation of biogas for 100%PS and 3.5% A was 156.70 L and 144.6 L respectively. The remaining four mixtures of pig slurry and ALBAZOD slowly

attained stationary phase at about 58-63 days. Co-digestion with 7.1% A accumulated a third of the biogas (55.40 L) compared to co-digestion of pig slurry with 3.5% A (144.60 L). The accumulation of biogas was less as the ratio of ALBZAOD increased, with 26.70L (14.6% A), 8.30L (32.2% A), and 5.20L (100% A).

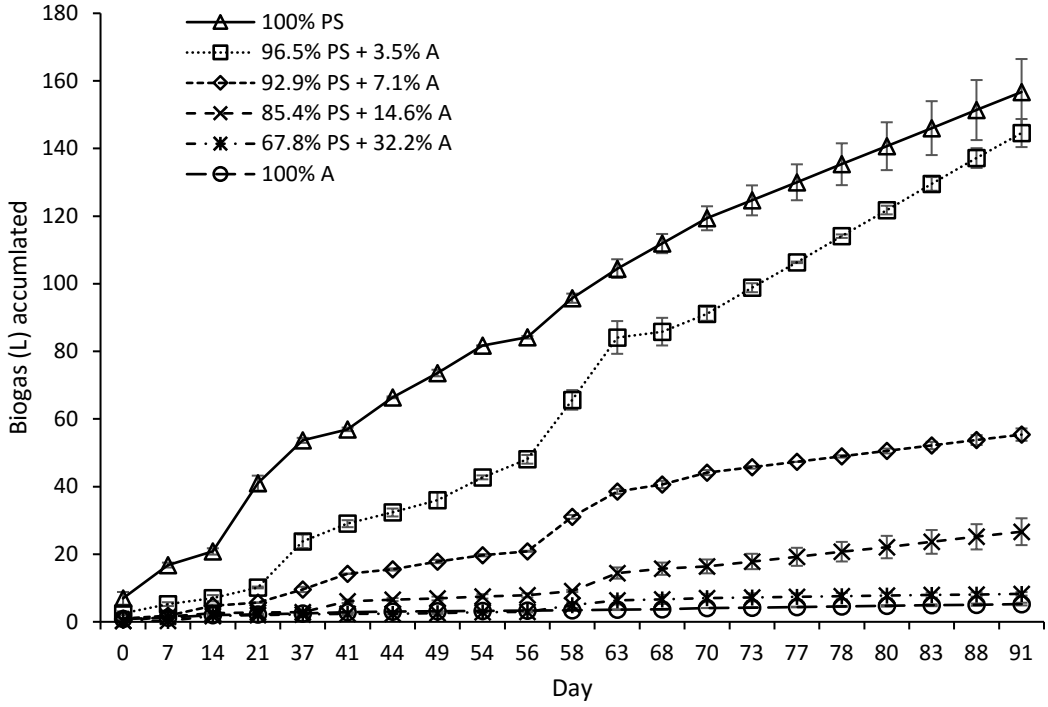



Fig. 4.7 The accumulated biogas production from co-digestion of pig slurry (PS) and ALBAZOD (A) over 91 day period. With ± analytical standard error (n=6).

### 4.3.4. Mean methane ($CH_4$) content (%)

The trends in the methane content were similar for all six digestion experiment groups. Peak methane contents were observed in about 7-14 days: 59.8 % (100% PS), 55.0 % (3.5% A), 38.5% (7.1% A), 35.2% (14.6% A), 30.7% (32.2% A), and 31.5% (100% A). The pig slurry alone produced the highest percentage methane content while its percentage decreased as the ALBAZOD percentage increased (Fig. 4.8). Following the peak, the methane contents remained stationary until about 56 days when it attained a 2nd peak methane content: 74.9 % (100% PS), 72.1 % (3.5% A), 60.3 % (7.1% A), 56.2 % (14.6% A), 55.1 % (32.2% A), and 50.1 % (100% A). Overall, the methane content from the digestions remained relatively stable over the 91 day period.

### 4.3.5. Mean carbon dioxide ($CO_2$) content (%)

While the carbon dioxide content from the six digestions was less stable over the period, peak carbon dioxide contents were observed in about 7.-14 days: 19.1 % (100% PS), 17.7 % (3.5% A), 26.5 % (7.1% A), 29.1 % (14.6% A), 20.4 % (32.2% A), and 23.5 % (100% A). The carbon dioxide contents became unstable over the period after day 14 to day 54-63. Stationary phases were observed from day 58: 16.0 % (100% PS), 11.7 % (3.5% A), 23.8 % (7.1% A), 29.3 % (14.6% A), 13.8 % (32.2% A), 23.2 % (100% A). The 85.4% PS + 14.6% A mixture produced the highest percentage $CO_2$ content overall and the percentage of $CO_2$ gradually increased as the ALBAZOD percentage increased (Fig. 4.9).

### 4.3.6. Methane ($CH_4$, L) accumulation

Similar results were observed for methane accumulation production to those for biogas accumulation. The final accumulation of methane for both 100% PS and 3.5% A groups are 98.52 L and 73.54 L, with a continuous methane accumulation over the period (Fig. 4.10). The stationary phases were observed at about 58-63 days for the rest of four mixtures. A half quantity of the methane was accumulated (35.94 L) by the 7.1% A compared to 3.5% A group (73.54 L). The accumulation of methane appeared to be less as the ratio of ALBZAOD increased in the substrates, with 16.47 L (14.6% A), 10.30 L (32.2% A), and 8.38 L (100% A).

### 4.3.7. Carbon dioxide ($CO_2$, L) accumulation

The final accumulation of carbon dioxide (Fig. 4.11) for both 100% PS, 3.5% A, 7.1% A groups were similar with a total volume of 17.63 L, 13.22 L, and 12.72 L respectively; the accumulated volumes were much less than observed for methane production. The onset of the stationary phases were gradually observed from about 58-63 days. The accumulation of carbon dioxide volumes appeared to be less as the ratio of ALBZAOD increased in the substrates, with 6.74 L (14.6% A), 2.04 L (32.2% A), and 2.57 L (100% A) over the 91 day period.

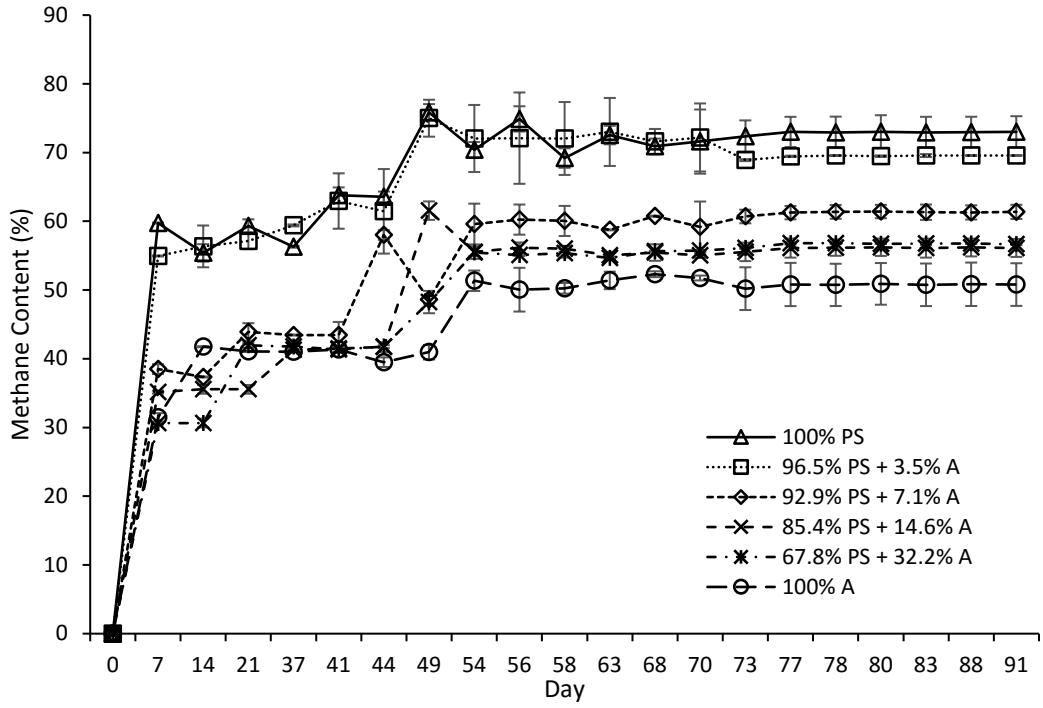



Fig. 4.8 Mean methane content (%) from co-digestion of pig slurry (PS) and ALBAZOD (A) over 91 day period. With ± analytical standard error (n=6).

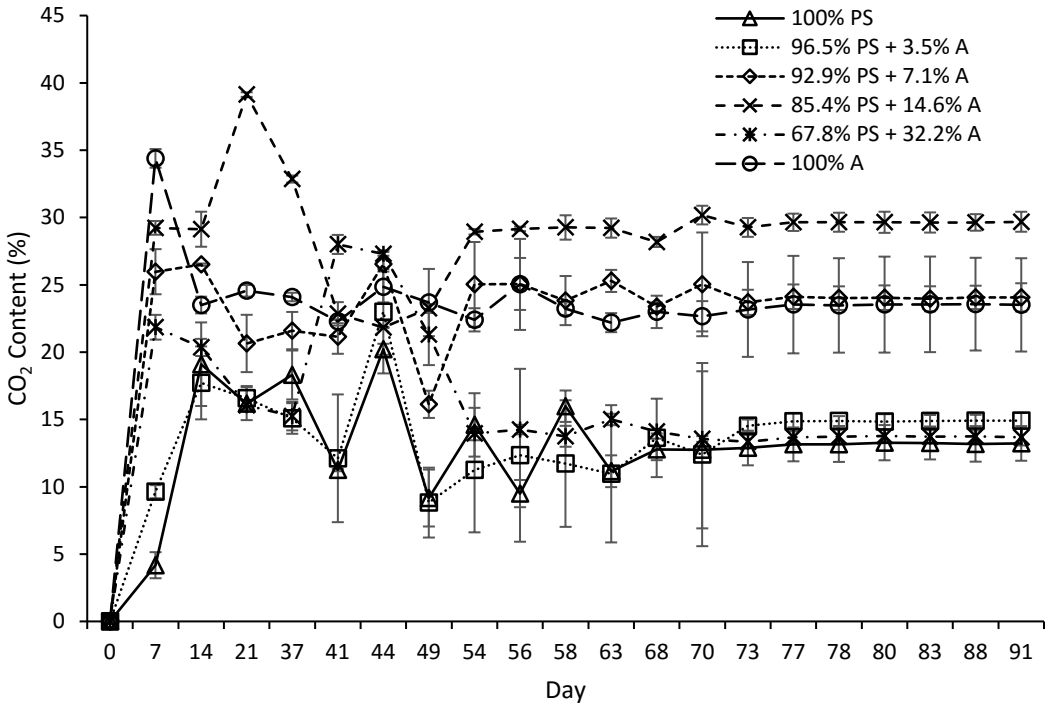



Fig. 4.9 Mean carbon dioxide content (%) from co-digestion of pig slurry (PS) and ALBAZOD (A) over 91 day period. With ± analytical standard error (n=6).

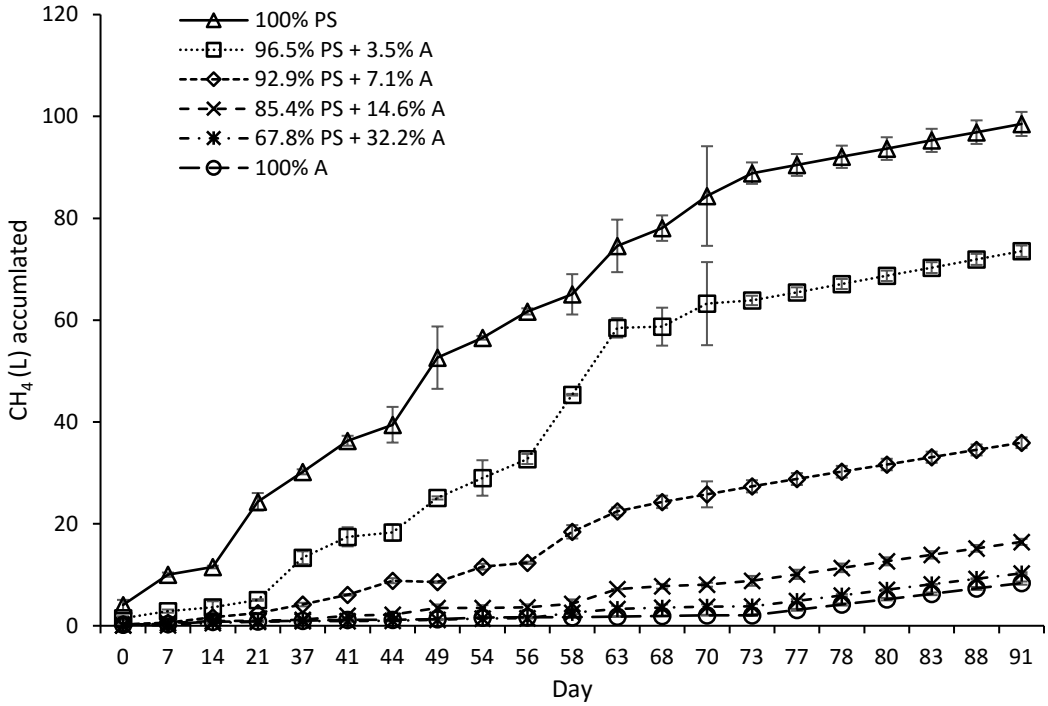



Fig. 4.10 The accumulative methane production ($CH_4$, L) from co-digestion of pig slurry (PS) and ALBAZOD (A) over 91 day period. With ± analytical standard error (n=6).

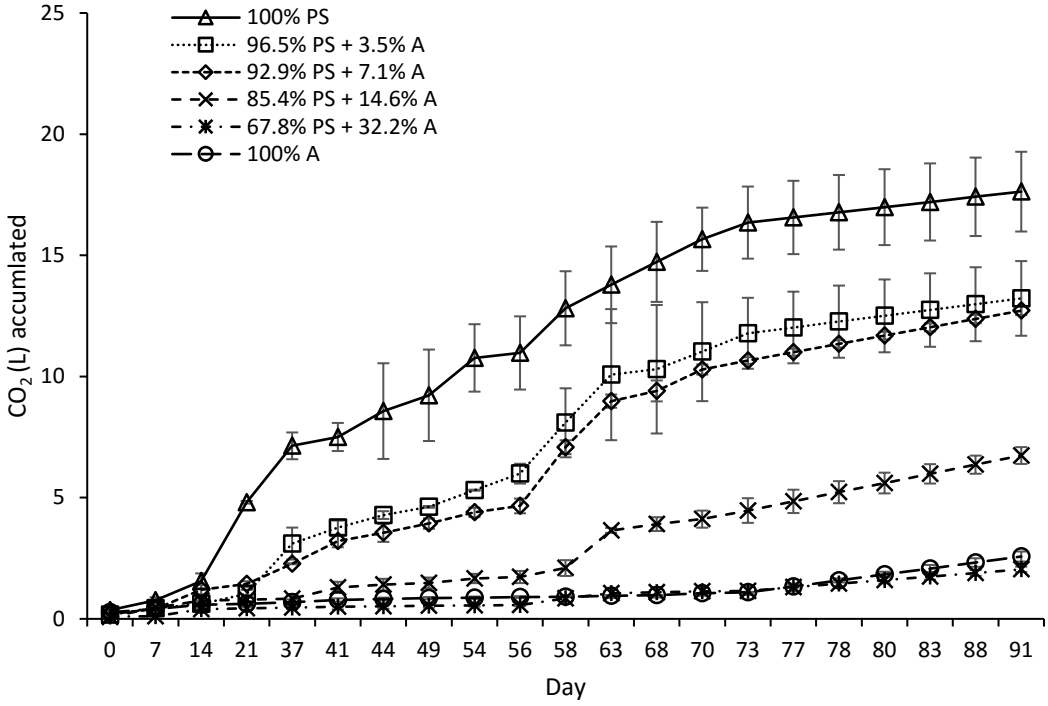



Fig. 4.11 The accumulative carbon dioxide $CO_2$ production (L) from co-digestion of pig slurry (PS) and ALBAZOD (A) over 91 day period. With ± analytical standard error (n=6).

### 4.3.8. Cumulative specific methane production in VS removed (L CH4 $g^{-1}VS_{removed}$)

Fig. 4.12 shows the cumulative methane production per gram of volatile solid removed. Highest methane production (0.344 L $CH_4$ $g^{-1}$ $VS_{removed}$) was observed from 96.5% PS + 3.5% A mixture at day 91. A slightly lower production of 0.339 L $CH_4$ $g^{-1}$ $VS_{removed}$ was recorded for the 100% PS. The methane production decreased as the ALBAZOD ratio increased in the mixture. When the ALBAZOD ratio was beyond 7.1% A, the methane production decreased to below 0.200 L $CH_4$ $g^{-1}$ $VS_{removed}$. The lowest $CH_4$ (L $CH_4$ $g^{-1}$ $VS_{removed}$) was observed from the 100% A digestion with a mean of 0.040 L $CH_4$ $g^{-1}$ $VS_{removed}$ over the first 73 day period, which then rapidly increased to 0.174 L $CH_4$ $g^{-1}$ $VS_{removed}$ at the day 91. The results also suggest reasonable methane yields was possible beyond 91 days. This is in line with Safley and Westerman (1990) observations. They suggest reasonable methane yields can be expected at low temperatures if digester loading rates are reduced appropriately by extending the detention time (θ) to the 100-300 day range (Safley & Westerman, 1990). Therefore, extending the detention time simply makes them extremely lightly loaded “process” anaerobic digesters. Another explanation may possibly due to there was a lag phase before more recalcitrant components which once made accessible were than rapidly digested e.g. cellulose.

### 4.3.9. Cumulative specific methane production in COD removed (L $CH_4$ $g^{-1}$ $TCOD_{removed}$)

Fig. 4.13 shows the cumulative methane production per gram of total COD removed. The highest methane production (0.103 L $g^{-1}$ $TCOD_{removed}$) was again observed from 96.5% PS + 3.5%A co-digestion, which was slightly lower than the 0.075 & 0.095 L $g^{-1}$ $TCOD_{removed}$ from 100% PS & 92.9% PS + 7.1% A respectively. The methane production decreased as the ALBAZOD ratio increased in the co-substrates. When the ALBAZOD ratio was beyond 7.1% A, the methane production decreased to below than 0.060 L $g^{-1}$ $TCOD_{removed}$. The lowest $CH_4$ (L $g^{-1}$ $TCOD_{removed}$) was once again observed from the 100% A experiment (0.04 L $g^{-1}$ $TOCD_{removed}$) with average 0.018 L $g^{-1}$ $TCOD_{removed}$ over the first 73 day period.

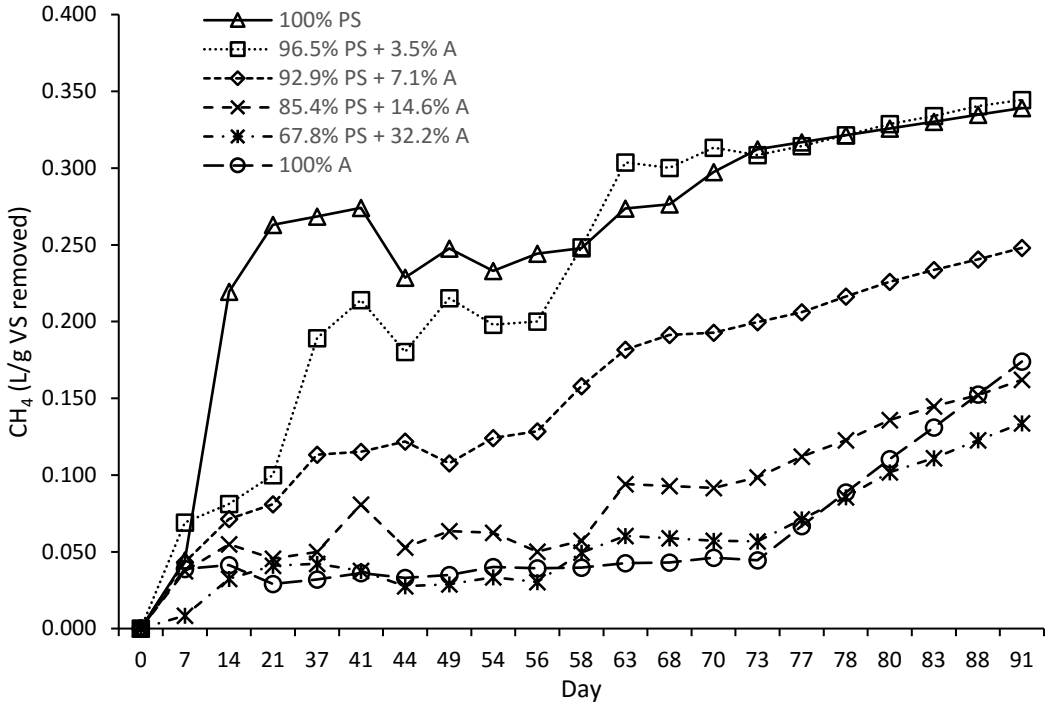



Fig. 4.12 Cumulative methane production calculated based on per gram of volatile solid removed (L $CH_4$ $g^{-1}$ VS removed) from co-digestion of pig slurry (PS) and ALBAZOD (A) over 91 day period. With ± analytical standard error (n=6).

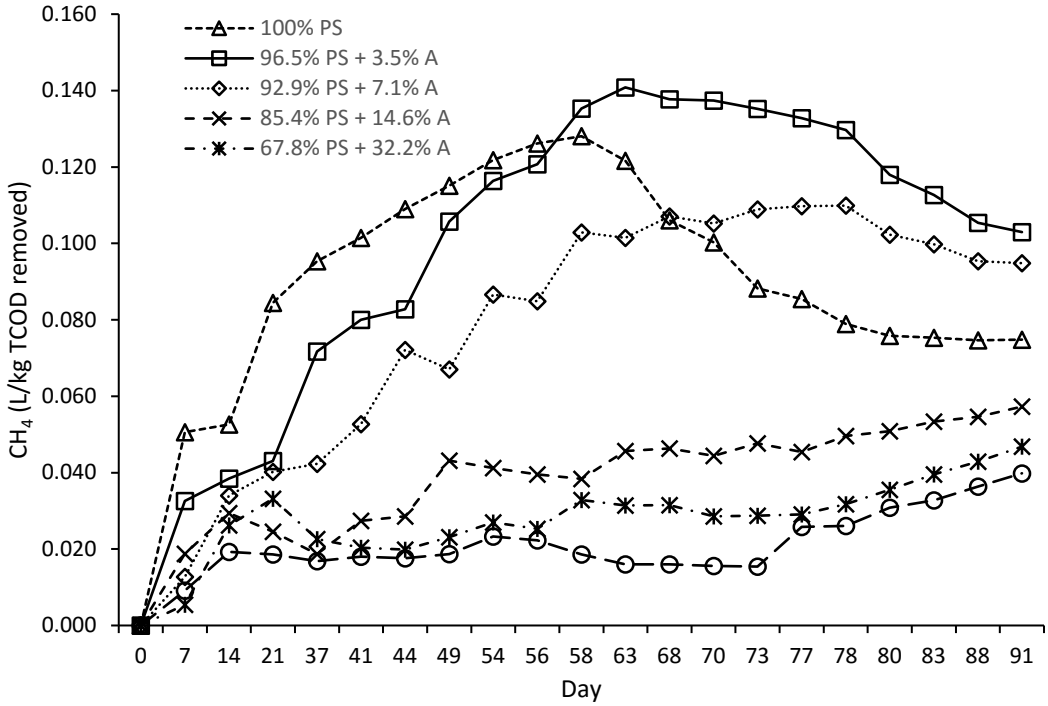



Fig. 4.13 Cumulative methane production calculated based on per gram of total COD removed (L $CH_4$ $g^{-1}$ TCOD removed) from co-digestion of pig slurry (PS) and ALBAZOD (A) over 91 day period. With ± analytical standard error (n=6).

### 4.3.10. TS reduction

As expected, the TS concentrations decreased gradually over the 91 day period (Fig. 4.14). The reduction of TS was based on the difference in TS content measured on day 1 of the test, corresponding to the start of methanogenesis, and at the end of process. The methane production resulted from the partial TS reduction. The increase percentage of ALBAZOD not only decreased methane yield based on $VS_{removed}$ but also resulted in the general decrease of TS reduction, except the cases of 96.5% PS + 3.5%A and 100% A (Table 4.3, Fig. 4.12 & 4.14). The highest TS reduction was 89.25% when 100% ALBAZOD was digested, which was about 15% high than that of the 100% pig slurry at 74.54% TS reduction. In this current study, it is important to note that it was observed the ability of ALBAZOD to be able to pass through an anaerobic digester remained intact and undigested partially after the 91 days (data not shown).

Table 4.3 Methane yields (L $CH_4$ $g^{-1}$ VS removed), and percentage TS & VS removal for the batch co-digestion of pig slurry (PS) and ALBAZOD (A) over 91 day period.

| **Digestate composition** | **Methane yield (L $CH_4$ $g^{-1}$ $VS_{removed}$)** | **TS reduction %** | **VS reduction %** |
|---|---|---|---|
| **100% PS** | 0.339 (± 0.026) | 74.54 | 63.69 |
| **96.5% PS + 3.5% A** | 0.344 (± 0.020) | 75.77 | 26.82 |
| **92.9% PS + 7.1% A** | 0.248 (± 0.018) | 74.18 | 23.28 |
| **85.4% PS + 14.6% A** | 0.162 (± 0.014) | 71.26 | 17.82 |
| **67.8% PS + 32.2% A** | 0.134 (± 0.003) | 70.77 | 23.93 |
| **100% A** | 0.174 (± 0.026) | 89.25 | 36.37 |

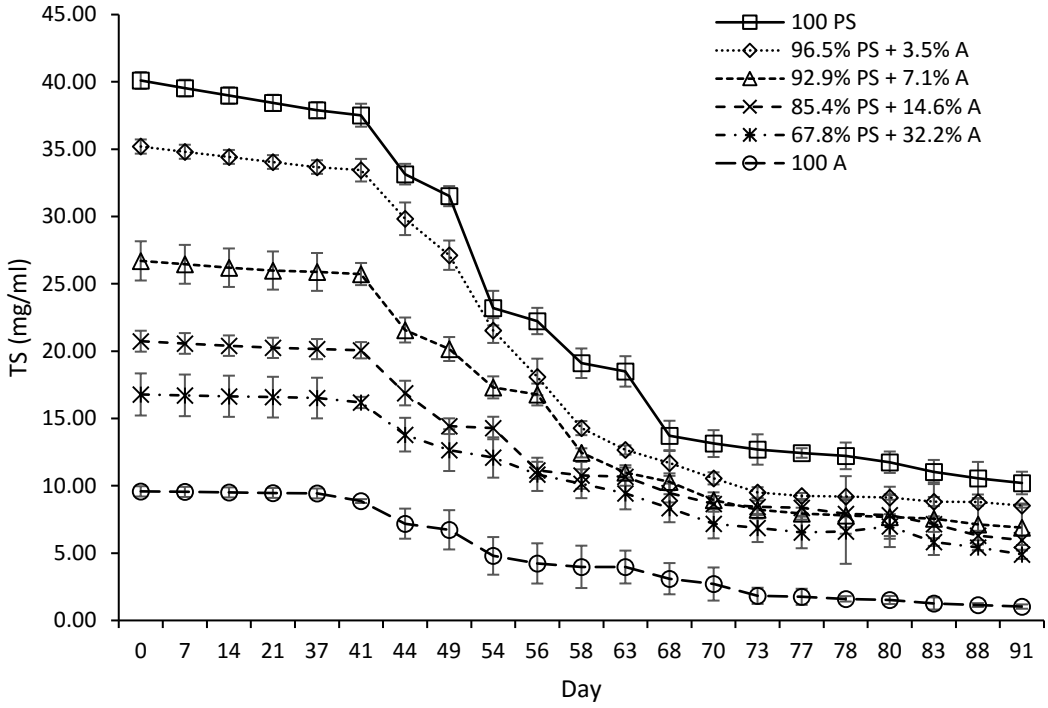



Fig. 4.14 Reduction of TS (g/L) from co-digestion of pig slurry (PS) and ALBAZOD (A) over 91 day period. With ± analytical standard error (n=6).

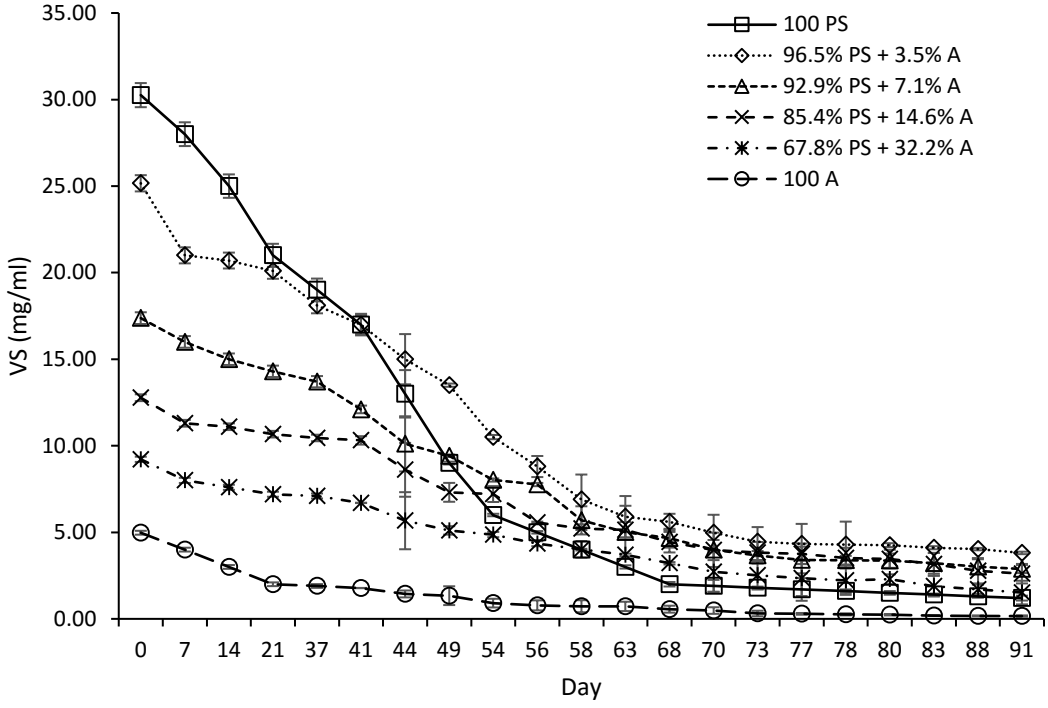



Fig. 4.15 Reduction of VS (g/L) from co-digestion of pig slurry (PS) and ALBAZOD (A) over 91 day period. With ± analytical standard error (n=6).

### 4.3.11. VS reduction

The VS concentrations decreased gradually over the 91 day period (Fig. 4.15). The reduction of VS was based on the difference in VS content measured on day 1 of the test, corresponding to the start of methanogenesis, and at the end of process. The methane production resulted from the partial VS reduction. An increase in the percentage of ALBAZOD decreased methane yield based on $VS_{removed}$ rate (Table 4.3, Fig. 4.14 & 4.15). The highest VS reduction was 63.69% when 100% pig slurry was digested, which was about 27% high than that of the 100% ALBAZOD at 36.37% VS reduction (Fig. 4.16).

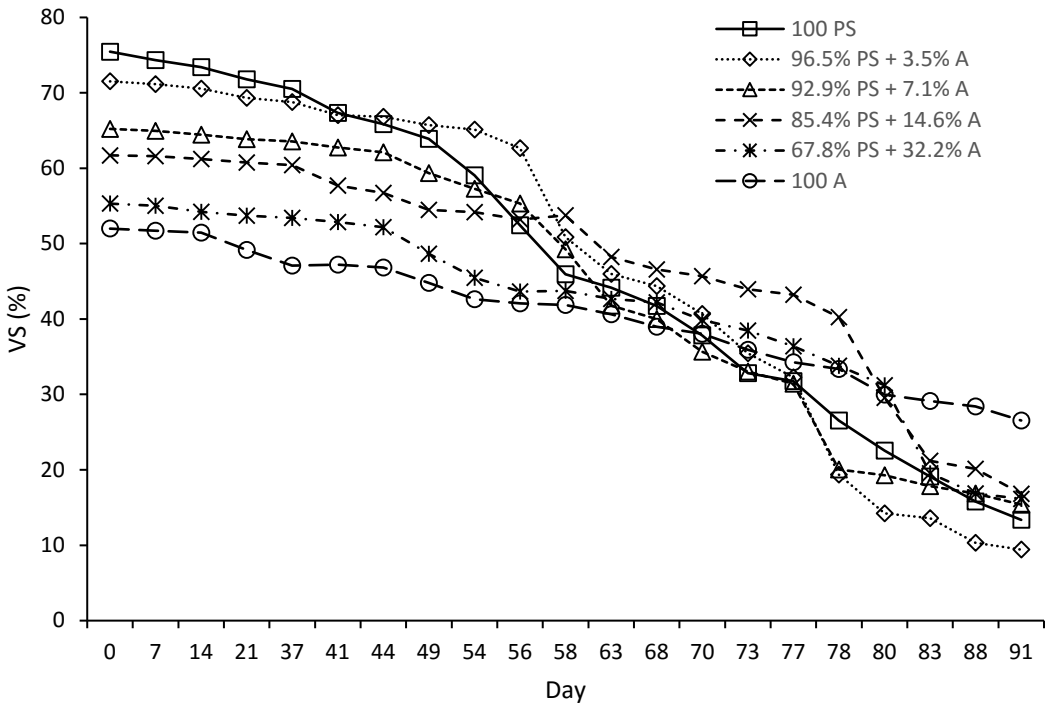



Fig. 4.16 The percentage of volatile solid from co-digestion of pig slurry (PS) and ALBAZOD (A) over 91 day period. With ± analytical standard error (n=6).

### 4.3.12. COD reduction

The TCOD concentrations decreased gradually (Fig. 4.17) over the 91 day period but overall the declines were much less steep than those recorded for TS & VS removals. The destruction of TCOD was based on the difference in TOCD content measured on day 1 of the test, corresponding to the start of methanogenesis, and at the end of process. The methane production also resulted from the partial TCOD reduction which is an indicator for theoretical methane production. An increased percentage of ALBAZOD not only decreased methane yield based on $VS_{removed}$ but also resulted in a general decrease of TCOD reduction (Table 4.4, Fig. 4.17). The highest TCOD removal was 55.01% when 100% ALBAZOD was digested, which was about 17% high than that of the 100% pig slurry at 37.54 TCOD reduction.

Table 4.4 Methane yields (L $CH_4$ $g^{-1}$ TCOD removed), TS & VS removal rates for the batch co-digestion of pig slurry (PS) and ALBAZOD (A) over 91 day period.

| **Composition of digestate** | **Methane yield (L $CH_4$ $g^{-1}$ $TCOD_{removed}$)** | **TCOD reduction (%)** |
|---|---|---|
| **100% PS** | 0.075 (± 0.005) | 37.54 |
| **96.5% PS + 3.5% A** | 0.103 (± 0.033) | 35.04 |
| **92.9% PS + 7.1% A** | 0.095 (± 0.012) | 35.20 |
| **85.4% PS + 14.6% A** | 0.057 (± 0.010) | 42.39 |
| **67.8% PS + 32.2% A** | 0.047 (± 0.003) | 50.78 |
| **100% A** | 0.040 (± 0.005) | 55.01 |

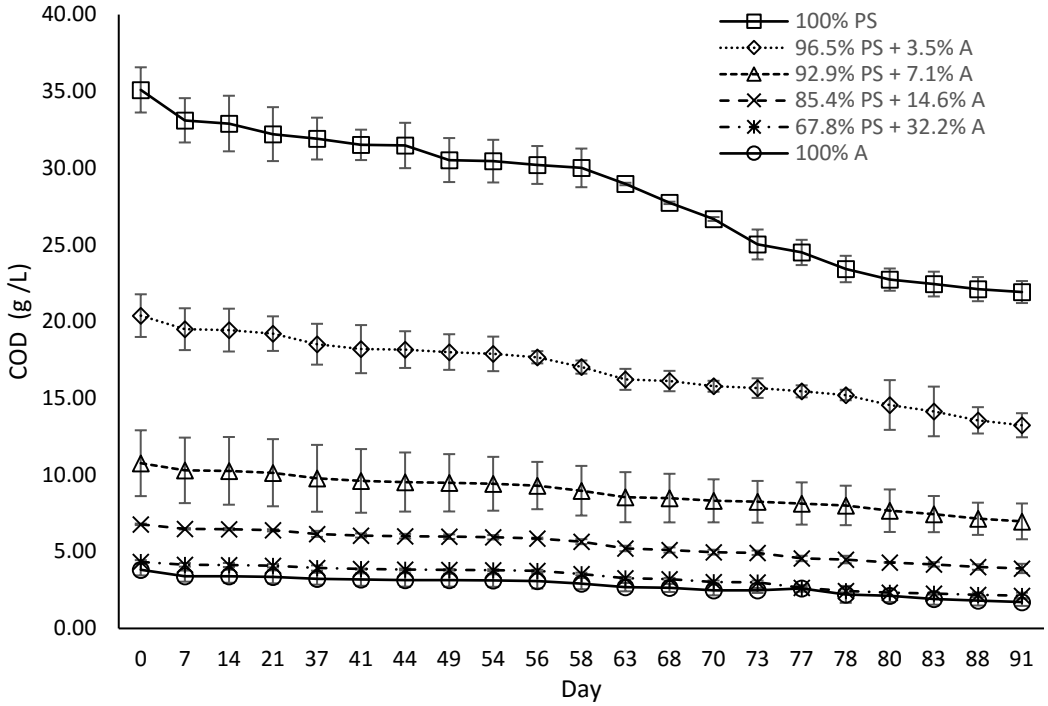



Fig. 4.17 Reduction of TCOD (g $O_2$/L) from co-digestion of pig slurry (PS) and ALBAZOD (A) over 91 day period. With ± analytical standard error (n=6).

## 4.4. Discussion

### 4.4.1. Acclimatisation phase, pH and $NH_4$-N

All reactors experienced a stable start-up irrespective of the composition of pig manure and ALBAZOD being digested. The mixtures pH in all reactors remained relatively constant during the experiment with values approaching pH 8, which is commonly found in pig manure or animal wastes digesters (Angelidaki & Ahring, 1993) due to their high bicarbonate and ammonia contents (Pind et al., 2003). This is particularly useful for them to tolerate marked changes in volatile fatty acid (VFA) concentrations during its build in in acidogenesis and acetogenesis processes which result in inhibition of methane production (Braun et al., 2010).

The sudden decline in pH value and minimal or nil methane production in the low biogas yields at the very beginning of some of the co-digesters were probably also explained by the accumulation of VFAs during this phase. The conditions probably resulted in the biochemical thermodynamics within the microbial populations favouring the fermentative acidogenic phase of anaerobic digestion (Angelidaki & Sanders, 2004; Monou et al., 2009; Pind et al., 2003). The methanogenic population was observed quickly within the day 7-14 with the co-digestion of pig slurry and ALBAZOD based on the observation of rapid increase in methane percentages.

A study by McCarty (1964) demonstrated that when total ammonia nitrogen (TAN) concentration exceeds 3000 mg $L^{-1}$ $NH_4$–N, the anaerobic digestion processes were inhibited at any pH (McCarty, 1964). Hobson and Shaw (1976) also reported a similar range of TAN concentration at 2500 mg $L^{-1}$ $NH_4$-N which resulted in some inhibition of methane production and a complete inhibition of methanogenesis at a concentration of 3300 mg $L^{-1}$ $NH_4$-N (Hobson & Shaw, 1976). For an adapted process, Angelidaki and Ahring (1993) demonstrated that an ammonia nitrogen tolerance of up to 3000-4000 mg $L^{-1}$ $NH_4$-N in livestock waste (Angelidaki & Ahring, 1993). In support to this, Sung and Liu (2003) and Procházka et al. (2012) also demonstrated a higher TAN concentration (>4000 mg $L^{-1}$) could result in obvious inhibition of methanogenesis (Procházka et al., 2012; Sung & Liu, 2003). Sawayama et al. (2004) and Lauterböck

et al. (2012) further extended this approach in which they have demonstrated an inhibition when the TAN concentration exceeds 6000 mg $L^{-1}$ $NH_4$-N (Lauterböck et al., 2012; Sawayama et al., 2004). On the other hand, low ammonia nitrogen concentration (500 mg $L^{-1}$) can cause low methane yield, loss of biomass (as volatile suspended solids VSS) and loss of the aceticlastic methanogenic activity (Procházka et al., 2012).

The results suggested that anaerobic co-digestion of pig slurry and ALBAZOD (i.e. a lower $NH_4$-N concentration) provided a benefit on neutralising the high $NH_4$-N concentration on the pig slurry, by reducing from "Inhibition (especially at higher pH values)" $NH_4$-N range i.e.1500-3000 mg $L^{-1}$ (Angelidaki and Ahring (1993)) to "No antagonistic effect" $NH_4$-N range i.e. 200-1000 mg/ $L^{-1}$ (Hobson and Shaw (1976)). In addition, Rajagopal (2013) stated that low temperature anaerobic systems are particularly well adapted to manure treatment because of lower free ammonia nitrogen (FAN) level than in mesophilic or thermophilic process (Rajagopal et al., 2013). The effects of psychrophilic anaerobic digestion are discussed in the following section.

Massé et al. (2003) and (2010) described that psychrophilic anaerobic systems may be more tolerant than mesophilic or thermophilic anaerobic digesters to high ammonia concentrations because the ratio of FAN to TAN decreases with temperature (Massé et al., 2010; Massé et al., 2003). While treating swine manure in a low temperature sequencing batch reactor, Massé et al. (2003) recorded a FAN concentration of 62 mg $L^{-1}$ at 10 °C. Whereas, FAN level increased almost threefold from 62 (pH, 7.89) to 185 mg $L^{-1}$ (pH, 8.03), when they increased the temperature from 10 to 20 °C. They also concluded that FAN concentrations would have ranged from 304 to 448 mg $L^{-1}$ at 35 °C with similar pH and TAN levels. Therefore, the authors also stated that low temperature anaerobic systems are particularly well adapted to manure treatment because of lower FAN level than in mesophilic or thermophilic process (Massé et al., 2010; Massé et al., 2003). This may explain the psychrophilic temperature ranges used in this current study may in fact provide a benefit on the high $NH_4$-N concentrations in the pig manure anaerobic digestion and co-digestion with ALBAZOD.

### 4.4.2. Microalgae species

As previously described, the most dominant microalgae species found from Mount Barker CWMS wastewater or ALBAZOD was generally *Chlorella* spp. and *Scenedesmus* spp. The latter is known to have a rigid cell wall because of its poorly biodegradable carbohydrates composition (Ramos-Suárez & Carreras, 2014; Ward et al., 2014).

The variability of methane production on anaerobic digestion of microalgae or ALBAZOD is related to two main aspects which are the macromolecular composition and the cell wall characteristics of each microalgae species (Pandey et al., 2014). Sialve (2009) also added that the theoretical methane yield in microalgae cells was lipid (1.014 L $g^{-1}$ VS), carbohydrates (0.415 L $g^{-1}$ VS) and protein (0.851 L $g^{-1}$ VS). Note the protein formula was calculated with the average composition in amino acid weights by their frequency in *Chlorella vulgaris*. Therefore, its potential anaerobic biodegradability depends on the different organic compounds in each microalgae cells (Sialve et al., 2009). Research conducted with carbohydrate-enriched cyanobacteria *Arthrospira platensis* following phosphorus limitation attained a methane yield of 0.203 L $g^{-1}$ COD when biomass had 60% of carbohydrates in respect to 0.123 L $g^{-1}$ COD when the carbohydrate content was 20% (Markou et al., 2013).

A study by Sialve et al. (2009) showed that the theoretical microalgae methane yield was estimated in the range of 0.48-0.80 L $CH_4$ $g^{-1}$ VS (Sialve et al., 2009). Results from experiments by González-Fernández et al. (2012), however, have demonstrated yields limited to 0.05-0.31 L $CH_4$ $g^{-1}$ VS (González-Fernández et al., 2012). A study on *S. obliquus* anaerobic digestion reported 0.13 L $CH_4$ $g^{-1}$ VS (Zamalloa et al., 2011). Another study on the anaerobic digestion of *Chlorella vulgaris* achieved 0.24 L $CH_4$ $g^{-1}$ VS and 51% COD removal at 28 days HRT (Ras et al., 2011). Microalgal biomass cultivated in wastewater treatment raceway ponds attained 0.17 L $CH_4$ $g^{-1}$ VS and 31 % COD removal at 20 days HRT (Passos et al., 2014) compared to the results found from this chapter study at day 90 (0.174 L $CH_4$ $g^{-1}$ $VS_{removed}$, 55% COD removal), given the difference in units for methane yield.

### 4.4.3. ALBAZOD methane production

The methane production (L $CH_4$ $g^{-1}$ VS removed) from ALBAZOD mono-digestion in this study was relatively low ranging from 0.050 to 0.174 L $CH_4$ $g^{-1}$ $VS_{removed}$, when compared to pig manure mono-digestion, 0.339 L $CH_4$ $g^{-1}$ $VS_{removed}$. This is in agreement with the gas yield reported in the literature which ranged from 0.150 to 0.450 L $CH_4$ $g^{-1}$ VS for the similar microalgae species, although there were differences in reactor configurations and operating modes (Table 4.5). Table 4.6 shows a summary of biogas and methane yield of various algal biomass in different reactor configurations and operating modes (Prajapati et al., 2013). The first authors to report on anaerobic digestion using microalgae biomass were Golueke et al. in 1957 (Golueke et al., 1957). They investigated some commonly grown microalgae species such as *Chlorella vulgaris* and *Scenedesmus* as part of a wastewater treatment process including anaerobic digestion. Table 4.5 also highlights the difference in units and terminology used to report gas production from microalgae. Units range from gas production per gram of chemical oxygen demand (COD) destroyed, gas produced per gram of volatile solids loaded and gas produced per gram of total solids loaded. It is essential to standardise the units in reporting biogas productivities to enable comparison of microalgae with other digestible substrate. The unit used in this study was based on the removal of per gram of volatile solids, which is commonly used as well as ash free dry weight (AFDW) of microalgae (APHA, 1992; Zhu & Lee, 1997). Note that although the unit AFDW is used extensively by phycologists to report quantities of microalgae biomass, the variation in AFDW (i.e. indigestible component) and VS (i.e. digestible component) can be up to 50% between species and therefore can significantly affect predicting and comparing the theoretical biogas production potential for the anaerobic digestion of microalgae.

Table 4.5 Reported methane production from the anaerobic digestion of microalgae biomass reported (NR = Not reported), adapted from (Ward et al., 2014)

| Microalgae species | C/N Ratio | Methane yield | Loading rate | Reference |
|---|---|---|---|---|
| *Arthrospira maxima* | 4.3–5.33 | 173 mL/ g VS | 500 mg/TS/L | (Inglesby & Fisher, 2012) |
| *Arthrospira platensis* | N/R | 481 mL/ g VS | 2000 mg/TS/L | (Mussgnug et al., 2010) |
| Blue green algae | N/R | 366 mL/ g VS | 281.96 mg/VS/L | (Rui et al., 2009) |
| *Chlorella kessleri* | N/R | 335 mL/ g VS | 2000 mg/TS/L | (Mussgnug et al., 2010) |
| *Chlorella* sp., *Pseudokirchneriella* sp. and *Chlamydomonas* sp. | N/R | 0.28–0.60 $m^3$/kg/VS | 402 mg VS | (De Schamphelaire & Verstraete, 2009) |
| *Chlorella* sp., *Scenedesmus*, *Euglena* and *Oscillatoria* | N/R | 300–800 mL/ g VS | N/R | (Golueke & Oswald, 1959) |
| *Chlorella* sp., *Scenedesmus* | N/R | 170–320 mL/ g VS | 1.44–2.89 g/VS/L | (Golueke et al., 1957) |
| *Chlorella sorokiniana* | N/R | 212 mL $g^{-1}$ VS | N/A | (Polakovičová et al., 2012) |
| *Chlorella vulgaris* | N/R | 403 mL/ g VS | 2 g/VS/L | (Lu et al., 2013) |
| *Chlorella vulgaris* | N/R | 286 mL/ g VS | 5000 mg/VS/L | (Lakaniemi et al., 2011) |
| *Chlorella vulgaris* | 6 | 240 mL/ g VS | 1000 mg/VS/L | (Ras et al., 2011) |
| *Chlorella vulgaris* | N/R | 189 mL/ g VS | N/R | (Polakovičová et al., 2012) |
| *Chlorella vulgaris* | N/R | 0.40–0.45 L | 2677–6714 mg (COD) | (Sánchez Hernández & Travieso Córdoba, 1993) |
| *Scenedesmus obliquus* | N/R | 287 mL/ g VS | 2000 mg/TS/L | (Mussgnug et al., 2010) |
| *Scenedesmus obliquus* | N/R | 240 mL/ g VS | 2000 mg/VS/L | (Zamalloa et al., 2012) |
| *Scenedesmus* sp. | N/R | 170 mL / g COD | 1000 mg/COD/L | (González-Fernández et al., 2012b) |
| *Scenedesmus* sp. (single stage) | N/R | 290 mL/ g VS | 18,000 mg/VS/L | (Yang et al., 2011) |
| *Scenedesmus* sp. (two stage) Note: 46 mL/g/VS Hydrogen | N/R | 354 mL/ g VS | 18,000 mg/VS/L | (Yang et al., 2011) |
| *Scenedesmus* sp. and Chlorella sp. | N/R | 16.3–15.8 $ft^3$ | 7.8–9.2 ft3/lb (VS) | (Golueke et al., 1957) |
| *Scenedesmus* sp. and Chlorella sp. | 6.7 | 143 mL/ g VS | 4000 mg/VS/L | (Yen & Brune, 2007) |
| Waste water grown community | N/R | 497 mL/ g VS | 2.16 g/L/TS | (Salerno et al., 2009) |

Table 4.6 Biogas and methane yields of various algal biomass in different reactor configurations and operating mode (Prajapati et al., 2013).

| Algal biomass | Rector | C/N ratio | VS [a]/loading[b] rate | HRT (days) | Temp. (°C) | Gas yield[c] | $CH_4$ yield[c] | Reference |
|---|---|---|---|---|---|---|---|---|
| *Chlorella*, *Scenedesmus* sp. | Batch (11 L) | | 1.44–2.89 [b] | 30 | 35–50 | 0.24–0.43 | 0.17–0.32 | Golueke et al. (1957) |
| *Spirulina maxima* | Semi-continuous (10 L) | | 0.97[b] | 33 | 35 | – | 0.26 | Samson and Leduy (1982) |
| Marine green macroalgae | Semi-continuous(2 L) | | 1.1–2.6 [b] | 11–27 | 35 & 55 | 0.4–0.6 | 0.25–0.35 (0.35–0.48)[d] | Hansson (1983) |
| *S.maxima* | Fed-batch (2 L) | | 20–100 | 5–40 | 15–52 | | 0.25–0.34 | Samson and Leduy (1986) |
| *L.hyperborea*, *L. saccharina* *A. nodosum* | Batch/semi-continuous (10 L) | | 1.65[b] 1.65[b] 1.75[b] | 24 | 35 | 0.53 0.45–0.22 | 0.280.23–0.11 | Hanssen et al. (1987) |
| *Ulva rigida* & *G. confervoides* | Semi-continuous (3 digester, total 180 L) | | 81.2[a] 85.1[a]/1[b] | 20 | 35 | 0.347 | 0.212 | Rigoni-Stern and Rismondo (1990) |
| *Chlorella* & Scenedesmus paper waste | Semi-continuous(4 L) | 6.7–27.2 | 2–6 | 10 | 35 | 0.13–0.20 | 0.09–0.14 | Yen and Brune (2007) |
| *Laminaria* sp. & *Ulva* sp. with milk waste | Semi-continuous (1 L & 30 $m^3$) | 9.15–10.67 | | 21–44 | 35–55 | – | 0.153–0.23[e] | Matsui and Koike (2010) |
| *Chlorella* residues with glycerol | CSTR (5 L) | 5–25 | 94.6[a] 5–40[f] | 10–15 | 35 | 0.29–0.445 | 0.188–0.308 | Ehimen et al. (2010) |
| *C. reinhardtii* *A. platensis* *S. obliquus* *C.kessleri* *D.salina* *E.gracilis* | Batch (0.25 L) | – | – | 32 | 38 | 0.587 0.481 0.287 0.335 0.505 0.485 | 0.387 0.293 0.178 0.208 0.323 0.325 | Mussgnug et al. (2010) |
| *C.vulgaris* | | – | 80–90[a] | 16/28 | | – | 0.24 | Ras et al. (2011) |
| Taihu blue algae with corn straw | Batch (0.15 L) | 20 | 71.4 [a] / 20[f] | 30 | 35 | – | 0.325 | Zhong et al. (2012) |
| *S. obliquus* *P. triconutum* | Batch (1.15 L) & Hybrid reactor (2.3 L) | | 71.8[a] 82.7[a] /2.0[f] | 2–2.2 | 33–54 | | 0.24 0.36 | Zamalloa et al. (2012) |
| *Chroococcus* spp. | Batch (0.5 L) | 7.44–8.11 | 77–80 | 30 | 36 | 0.40–0.49 | | Prajapati et al. (2013) |

[a] Volatile solid (% of TS).
[b] Loading rate in kg VS $m^{-3}d^{-1}$.
[c] Gas and methane yield in $m^3$ $kg^{-1}$ VS added.
[d] During batch fermentation.
[e] Estimated from data given in $m^3CH_4$ $kg^{-1}$COD using a COD/VS ratio of 1.3 (adapted from (Zamalloa et al., 2012)).
[f] Substrate concentration (kg VS $m^{-3}$).

### 4.4.4. Major inhibitions associated with anaerobic digestion of microalgae

Most of the studies noted in Table 4.5 conclude a mechanism of concentrating or harvesting of microalgae biomass is desirable. This presents a fundamental problem to the financial viability of an energy system using microalgae biomass as a sustainable substrate for anaerobic digestion or alternative biofuel production. By using whole ALBAZOD directly coming from wastewater treatment plant or microalgae incubation ponds, it may potentially provide benefits such as minimal intensive energy, concentrating of harvesting microalgal biomass substrate and recovering nutrients from wastewater. Engineering issues associated with microalgae production with harvesting, dewatering, and further concentrating for biofuel are well discussed by many researchers such as (Benemann et al., 1977; Chen et al., 2011; Klein-Marcuschamer et al., 2013; Lee et al., 2013; Molina Grima et al., 2003; Pahl et al., 2013). The low concentration of microalgae biomass present in large volume of water means the low VS loading rate when it is used as a digestible substrate, as noted by Gouleke et al. (Golueke et al., 1957). To avoid this, highly concentrated microalgae are required. Sánchez Hernández and Travieso Córdoba used algal biomass with a high chlorophyll *a* concentration which ranged from 2.87 mg $L^{-1}$ to 9.62 mg $L^{-1}$ which required no concentrating step (Sánchez Hernández & Travieso Córdoba, 1993). The problem of low VS was therefore not evident in Sánchez Hernández and Travieso Córdoba experiment. Based on the observations in Chapter 3, it is suggested microalgae from facultative HRAP can achieve a chlorophyll *a* range similar to that of (Sánchez Hernández & Travieso Córdoba, 1993) with or without the additional of $CO_2$ (1.949 mg $L^{-1}$ increased up 3.5~4 mg $L^{-1}$ with $CO_2$ addition at certain days).

The low VS loading rate due to the low concentration of microalgae biomass present in a large volume of water sample may also explain the reduction of methane production as the ALBAZOD ratio increased in the pig slurry co-digestion. Although it is theorized that the improvement of methane production was related to an optimized C/N ratio due the introduction of ALBAZOD in the mixture, based on the findings from this current study, the optimum ratios are between 3.5% -7.1% in VS (w/w) of ALBAZOD when it is anaerobic co-digested with pig slurry. In fact, only the experiment with a ratio of 3.5% VS (w/w) ALBAZOD indicated a slightly increase of methane production when compared to mono-digestion of pig slurry.

In De Schamphelaire and Vertracete (2009) study, they suggested that a concentrating step would be essential for optimal performance of the anaerobic digestion process involved in microalgae biomass in order to solve the potential low VS loading rate (De Schamphelaire & Verstraete, 2009). Their results indicated that the digester failed once during the experimental period due to the required VS loading rate, comprising of microalgae biomass, being too dilute and containing excessive water, eventually leading to the washout of the anaerobic bacteria community. The bacterial washout was the consequence of the hydraulic retention time within the digester being shortened to less than the bacterial generational time. Therefore, the bacterial population decreased (McCarty, 1964; Parkin & Owen, 1986).

It should also be noted that the ALBAZOD sample obtained from the DAF plant at the Mount Barker CMWS contained aluminium as a result of using alum as the flocculent. There was no evidence of a decrease in digester performance due to the use of alum as a flocculent. The use of chemical coagulation, flocculation and centrifugation as a method of harvesting, concentrating and dewatering microalgae and the influence on anaerobic digestion has been considered by others (Benemann et al., 1977; Golueke & Oswald, 1965; Harun et al., 2010). Golueke and Oswald (1963) identified that digester performance was unaffected by the centrifugation or by alum addition as flocculent. It was suggested that there was no effect on digester stability or gas production with concentrations of aluminium in sludge up to 4% (Golueke & Oswald, 1963). Ward et al. (2014) and others (Barford et al., 1986; Campos et al., 2008; Krishnan et al., 2006; Ward et al., 2014) note that many new commercially formulated coagulants exist and are comprised of cationic and anionic polyelectrolytes, synthetic polyacrylamide polymers and starch-based polymer flocculants. Krishnan (2006) and Campos et al. (2008) stated that most of these flocculants which are currently utilised in the wastewater treatment industry have shown very few detrimental effects on digester stability or gas production (Campos et al., 2008; Krishnan et al., 2006). Indeed, it was reported that anaerobic digester performance was improved when commercially available chemical coagulants were utilised as flocculants. Kalyuzhnyi et al. (1998) and Callander (1983) both claimed that the increase in performance was due to better solid retention times of particulate matter which allowed more complete digestion of solids and resulted in higher conversions to biogas (Callander & Bearford, 1983;

Kalyuzhnyi et al., 1998). This was also in agreement with Barford et al. (1985) who used chemical flocculants which resulted in an increased biomass concentration in the digester compared to the control that did not utilise a flocculent (Barford et al., 1986). However, the authors also noted that the higher concentration of biomass could result in ammonia inhibition due to the much high loading rates that could be applied to a digester with a flocculated biomass. This may also be an explanation of the reduction in methane production as the ALBAZOD ratio increased of in the co-digestions reported in this chapter.

Co-digestion of 96.5% PS + 3.5% A resulted in a slightly higher methane yield than 100% PS alone (0.344 vs 0.339 $CH_4$ L $g^{-1}$ $VS_{removed}$). However, the results were not significantly different. In other cases, the introduction of ALBAZOD into pig slurry led to a reduction of the methane yield since the biodegradability of ALBAZOD was lower than the biodegradability of pig slurry. Synergistic mechanisms when co-digesting algae and pig manure were reported by Astals et al. (2015) and Gonzalez-Fernandez et al. (2011). In the study by Astals et al. (2015), anaerobic co-digestion of pig manure and algae (*Scenedesmus sp.)* with and without extraction of intracellular algal co-products was investigated. Astals et al. (2015) concluded that raw algae biodegradability increased from 0.163 to 0.245 L $CH_4$ $kg^{-1}$ VS due to synergistic mechanisms (Astals et al., 2015). Astal et al. (2015) theorized that the enhancement of the raw algae biodegradability in the presence of pig manure was related to the addition of specific microbes within the pig manure able to disrupt algal cell wall rather than in relation to an optimized C/N ratio.

The difficulties of using microalgae biomass for anaerobic digestion due to the low carbon to nitrogen ratio present in microalgal species were well identified by Vergara-Fernandez et al. (2008), Sialve et al. (2009) and Yen and Brune (2007) (Sialve et al., 2009; Vergara-Fernández et al., 2008; Yen & Brune, 2007). Sialve et al. (2009) stated that there was an imbalance between carbon and nitrogen requirements for the anaerobic bacterial community or consortia when the C/N ratio was below 20. To overcome this low C/N ratio problem in microalgae, it has been suggested by González-Fernández et al. (2011) and Shouquan et al. (2009) that using the addition of pig manure to microalgae to aid digestion (González-Fernández et al., 2011; Wang

et al., 2009). However, González-Fernández et al. (2011) also added that the C/N ratio of the digestion medium would be only balanced once the microalgae cell wall was broken.

From a nutrient balancing perspective, Mata-Alvarez et al. (2011) stated that anaerobic co-digestion of algae and manure does not seem obviously attractive, because both substrates are characterised by a relatively low carbon-to-nitrogen (C/N) ratio (<10) (Mata-Alvarez et al., 2011). In addition, González-Fernández et al. (2011) and Ramos-Suárez and Carreras (2014) also addressed that synergism is not always linked to the C/N ratio of the mixture when using algae as co-substrate (González-Fernández et al., 2011; Ramos-Suárez & Carreras, 2014). Another study by Mata-Alvarez et al. (2014) also stated that previous algae anaerobic co-digestion studies mainly linked the synergistic improvement in methane yield to the nutrient balance (Mata-Alvarez et al., 2014). Astal et al. (2015) are also in agreement that similar synergy behaviour should be observed when co-digesting pig manure and algal residues, if the improvement was based on an optimised C/N ratio. However, in their study, no synergies were observed for co-digestion of pig manure and algal residues. It is therefore theorised that the enhancement of the raw algae biodegradability in the presence of pig manure was related to other factors, such as the addition of specific microbes within the pig manure able to disrupt algal cell wall (Astals et al., 2015).

As mentioned in Chapter 1, previous algae anaerobic digestion and/or co-digestion studies on microalgae/ALBAZOD are limited and therefore the main objective of this Chapter is to provide critical insights on anaerobic co-digestion of algae and pig manure in the application of biogas production performances. C/N ratio was not a major investigation in this Chapter.

#### 4.4.5. Microalgae cell wall degradability and pre-treatment options for enhancing methane production

Microalgal cells are well known to be able to effectively resist bacterial attack and intact microalgae cells have been identified in digestate leaving a digester after an

extensive HRT (Golueke et al., 1957). In this current study, it was found that of ALBAZOD was able to pass through the anaerobic digestion and remained intact and partially digested after the 91 days period (data not shown). In this regard, *Scenedesmus sp.* cell wall has been described as a rigid wall of cellulose and hemicellulose, which together with the sporopollenin-like biopolymer provides great resistance to enzymatic degradation (Mendez et al., 2014; Mussgnug et al., 2010). The factors of pig age and diet should also be considered when co-digesting with pig manure and algae. Some studies have demonstrated that *Lactobacillus* and *Clostridia* in pig manure are very effective at degrading cellulosic organic matter (Calderon Santoyo et al., 2003; Li & Liu, 2012; Mussatto et al., 2008; Sethi & Scharf, 2013).

Table 4.7 summarises the various mechanical, physical, thermal and chemical methods used to improve microalgae methane production potential. These methods are energy intensive and their energy consumption is equal to or higher than the energy gained from the microalgal cell. The potential of using thermal pre-treatment of microalgae to improve anaerobic co-digestion with pig manure will be discussed in Chapter 5.

Table 4.7 Pre-treatment methods reported to improve the outcome of the anaerobic digestion of microalgae.

| Pre-treatment | Method | Improvement | Reference |
|---|---|---|---|
| **No cell wall or cell wall made from protein** | High degree of decomposition and low amount of indigestible residues (*Chlamydomonas reinhardtii*, *Chlorella kessleri*, *Dunaliella salina*, *Euglena gracilis*) | Efficient*: Chlamydomonas reinhardtii* 0.587 L biogas $g^{-1}$ VS<br>Inefficient: *Scenedesmus obliquus* 0.287 L biogas $g^{-1}$ VS | (Mussgnug et al., 2010) |
| **Thermal** | Raise above thermal limit of the microalgae species, | Resulting cell disruption, converted light energy into chemical energy of methane | (Golueke & Oswald, 1959) |
| **Thermal-chemical** | Heating to 100ºC for 8h, without an increase in pH using addition of sodium hydroxide | Increased gas productivity by 33%; up to 60% of the untreated microalgae biomass added to the anaerobic digester remain undigested | (Chen & Oswald, 1998) |
| | Thermal pre-treatment of *Scenedesmus* sp. | Pre-treatment at 70 °C, a 9% increase methane production, which increased to 57% when pre-treated at 90 °C when compared to untreated microalgae biomass | (González-Fernández et al., 2012b) |
| | Investigated the effect of the organic loading rates and the thermal pre-treatment of biomass at 90 °C for 1 h | A 2.9 and 3.4 fold increase in methane production for organic loading rates of 1 and 2.5 kg COD m− 3 day respectively | (González-Fernández et al., 2013) |
| | Thermal hydrolysis | An increase of 46% to 62% in methane productivity | (Alzate et al., 2012) |
| | Heat and sodium hydroxide addition | Pre-treatment at a temperature of 50 °C there was a 20% increase in substrate solubilisation and pre-treatment at 150 °C there was a 43% increase in substrate solubilisation | (Samson & Leduy, 1983) |
| **Ultrasonic disintegration** | ultrasonic treatment | Relatively short and only took 10 min compared to 1 h for the thermal pre-treatment | (Samson & Leduy, 1983) |
| | A frequency of 20 Hz but at varying power levels | The highest microalgae biodegradability of 44% was recorded for the longest sonication treatment as compared to 23% for un-sonicated biomass. | (González-Fernández et al., 2012a) |
| **Freezing** | Due to the disruption of the microalgae cell wall by ice crystals | A 26% increase in the solubilisation of microalgal substrates | (Samson & Leduy, 1983) |
| **High pressure thermal hydrolysis (HPTH) & Lipid-extraction** | HPTH processes heated substrate to approximately 160 °C at a pressure of approximately 6 bars. After these conditions had been maintained for 20–30 min the contents were suddenly reduced in pressure via a flash drum whereby the pressure change caused the cells to rupture and release the cell contents | The process substantially increased methane potential for lipid extracted and non-lipid extracted algae; when both lipid extraction and HPTL were combined an increase in the digestibility of the lipid extracted and HPTL microalgae biomass of 110% was recorded compared to untreated microalgae biomass | (Keymer et al., 2013) |
| **Enzymatic & bacterial** | By treating *Rhizoclonium* biomass with the addition of an enzymatic mixture | The greatest increase in gas production resulted from the addition of the single enzyme cellulase | (Ehimen et al., 2013) |
| | Bacterial cell disruption | An increase of 17–24% in biogas production by adding the bacterium *Clostridium thermocellum* to *C. vulgaris* biomass | (Lu et al., 2013) |

## 4.5. Reference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# CHAPTER 5

# THE EFFECT OF THERMAL PRE-TREATMENT OF ALGAL SLUDGE (ALZABOD) ON PSYCHROPHILIC ANAEROBIC CO-DIGESTION WITH PIG SLURRY

# 5. THE EFFECT OF THERMAL PRE-TREATMENT OF ALGAL SLUDGE (ALZABOD) ON PSYCHROPHILIC ANAEROBIC CO-DIGESTION WITH PIG SLURRY.

## 5.1. Introduction

To continue from Chapter 4, the literature identified a several of key role for pretreatment of algal biomass towards optimization of biogas production from microalgae. Several pre-treatment technologies are employed in anaerobic digestion and co-digestion, which combine physico-chemical modifications of substrates to enhance biogas yields (Golueke & Oswald, 1959) (Chen & Oswald, 1998) (González-Fernández et al., 2012b) (Alzate et al., 2012). Pre-treatments are generally recommended for substrates including lignin rich biomass, cellulose rich herbaceous materials, grasses, hydrophytes, agricultural biomasses, municipal solid waste and manure (Chandra et al., 2007). The rationale is that the amount of lignin, access of cellulase to cellulose and cellulose crystallinity decide the overall digestibility of the substrate in anaerobic digestion. Generally, plant biomass consists 40-50% cellulose, 20-40% hemicelluloses, 20-30% lignin by weight (Chandra et al., 2007; McKendry, 2002). (Shah et al., 2015), presented a summary of various types of pre-treatment to biomasses used in anaerobic digestion (Fig. 5.1).

Microalgal biomass or ALBAZOD is a good source of organic carbon. The assimilated inorganic-carbon becomes organic carbon and is stored inside the cellular components of biomass. During their growth, microalgae can accumulate carbohydrate, lipids and proteins over a short time period (John et al., 2011). Although the proportion of the different components depends on the growth environment conditions such as temperature, pH, irradiance and nitrogen depletion (Chen et al., 2013), these biodegradable components (i.e., carbohydrates, lipids and proteins) represent most of the cellular composition of the microalgal biomass, which contributes to more than 70% of the dry cell mass and contains approximately 50% carbon by dry weight (Chen et al., 2013; Chisti, 2007; Prajapati et al., 2013; Sánchez Mirón et al., 2000).

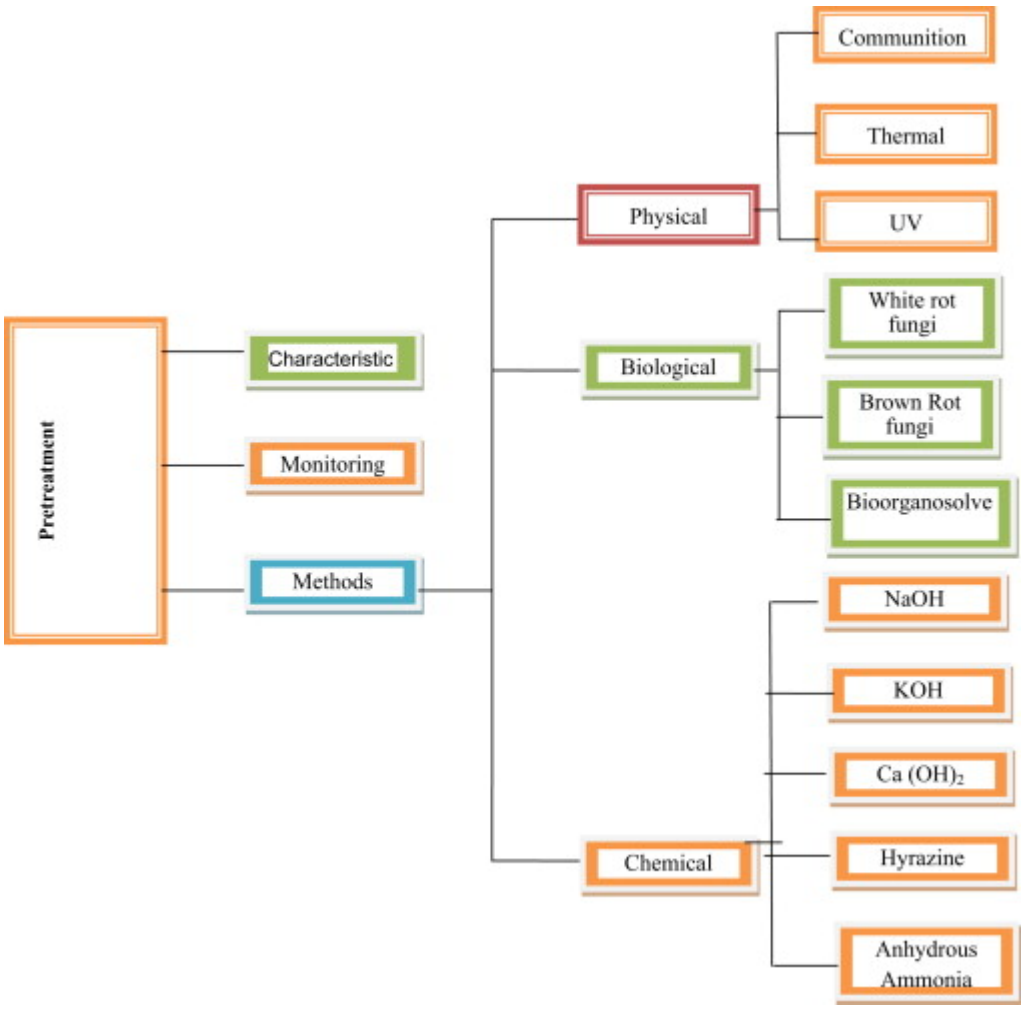


Fig. 5.1 A summary of pre-treatment methods applied to various biomasses for greater biogas yields (Shah et al., 2015).

Thermal treatment has been demonstrated to be a most effective method amongst the various pretreatment methods used prior to anaerobic digestion, notwithstanding, the high energy input which may make it unfeasible at large scale and economically unviable in long term applications (Alzate et al., 2012; González-Fernández et al., 2012a; González-Fernández et al., 2012b; Prajapati et al., 2013). The major challenge associated with microalgae anaerobic digestion is their low concentration of digestible substrate and high resistance cell wall.

For example, Gouleke et al (1957) reported the low volatile solids loading rate that was associated with microalgae when used as a digestible substrate (Golueke et al., 1957). De schamphelaire and Verstraete (2009) suggested that a concentrating step would be required for optimal performance of the anaerobic digestion process based on their observation that the required volatile solids loading rate comprising of

microalgae biomass was too dilute and contained excessive water which led to the washout of the anaerobic bacteria community and hence the digester completely failed once during the experimental period (De Schamphelaire & Verstraete, 2009). Mussgnug et al. (2010) noted the low digestibility of different algal biomasses with respective to their cell wall composition (Mussgnug et al., 2010). Alzate et al. (2012) further suggested that other recalcitrant compounds such as polyaromatics, heteropolysaccharides, algaenan, silica, uronic acid and lignin slow down the digestibility of algal biomass (Alzate et al., 2012).

To address this problem, one such hydrothermal treatment is autoclaving, where water is in fact used as a reagent at increased temperature and pressure, to hydrolyse and solubilise sugars, starch, proteins and hemicellulose. Thermal and hydrothermal pre-treatments have been widely suggested as a means of hydrolyzing recalcitrant components in a wide range of wastes to make them easier to degrade (Papadimitriou, 2010; Ren et al., 2006; Takashima & Tanaka, 2008). A several of studies on materials pre-treated by autoclaving under various conditions have shown increased methane production in batch tests. For example, digested swine slurry autoclaved at 120 °C showed an increase in $CH_4$ yield production of 115% (Menardo et al., 2011) and autoclaving of mixed kitchen garbage (175 °C, 40 bar, 1 h) increased $CH_4$ yield production by 30% (Sawayama et al., 1997).

The purpose of the research reported in this chapter was to examine the impact of thermal pre-treatment of algal sludge (ALZABOD) on psychrophilic anaerobic co-digestion with pig slurry on biogas yield. The energy balance will also be examined to determine if enhanced biogas production offsets the energy cost of thermal pretreatment.

The results of this research were delivered as an oral presentation: *Co-digestion of wastewater grown algae with pig slurry or activated sludge in laboratory scale anaerobic digesters. 22nd European Union Biomass Conference and Exhibition, Hamburg, Germany, 23-26 Jun*.

## 5.2. Methods

### 5.2.1. Algal sludge and pig slurry preparation

Refer to Chapter 4 methods.

### 5.2.2. Thermal pre-treatment (120 °C) at variable heating time (1h, 2h, and 3h)

ALBAZOD was thermally pretreated in an autoclave (Atherton cyber series, Chinchilla) at 120°C for 1h, 2h, and 3h. Soluble COD (SCOD) was measured after filtration through a 0.45-µm membrane filter after standard COD measurement (see Chapter 2). Two separate studies were performed:

1) For an initial investigation of TCOD and SCOD after thermal pre-treatment, 100% ALBAZOD was pretreated for 1, 2, and 3h at constant temperature 120 ℃. Based on the observations concluded in Chapter 4 and this study scenario 1, a separate experiment was investigated for the biogas volumes and compositions specifically for the case of 3.5% A (after thermal pre-treatment) + 96.5% PS for 30 days. The biogas volumes which were pretreated at 120 ℃ for 1, 2, and 3h were also used for calculating an energy balance of thermal pretreatment (case for 3.5% A only). Means of VS, TCOD, and SCOD were compared via t-test using equal or unequal variances, which was then determined by an f-test for significance ($p < 0.05$).

2) Based on the best scenario from study 1 (120 ℃, 3h), Six experiment groups were studied as following (VS, w/w): 100% pig slurry (PS), 96.5% PS + 3.5% ALBAZOD (A), 92.9% PS + 7.1% A, 85.4% PS + 14.6% A, 67.8% PS + 32.2% A, and 100% A; all ratios were calculated on dry weight VS (w/w) and all substrates were pretreated at 120 ℃ for 3h. All experiments were performed in duplicate reactors, each with analytical triplicate analysis (n=6). Biogas volume and composition were sampled in three months (91 days) at ambient psychrophilic temperature range 17 – 25 ℃. For full details, see Chapter 4 for the general methods.

### 5.2.3. Anaerobic digesters

Refer to Chapter 4 methods.

### 5.2.4. Analytical methods

The performance of cumulative specific gas yield (L $CH_4$ $g^{-1}$ $VS_{removed}$) at day 91 between the pretreated experiments at 120 ℃ for 3h and controls (i.e., no thermal pre-treatment) were compared.

## 5.3. Results

### 5.3.1. Study 1: Variable heating time at constant temperature (120 °C)

Changes in volatile solids, total and soluble COD as function of thermal pretreatment are shown in Table 5.1. There was a ten-fold increase in VS, TCOD and SCOD following thermal treatment at 120°C for 1h when compared with the unheated control. The thermal from 2h to 3h is less significant with an overall ~1 % increase in SCOD/TOCD only. There were statistically significant differences ($p < 0.05$) in VS concentrations between thermal treatment times at 120°C for pairwise combinations at 1, 2, and 3h. However, the difference was much less (2 g $L^{-1}$) in VS concentrations between thermal treatment times at 120°C for pairwise combinations at 2 and 3h. The increase of TCOD was possibly caused by removing the excess water content in the high moisture percentage in the ALZABOD samples (moisture percentage was 93.77 %). The increases in SCOD between all preheating times and control were similar to VS and TCOD. There was a significantly increase ($P < 0.05$) in SCOD for all preheating times vs. the control. After 3h at 120°C, the percentage of SCOD/TCOD increased from 24.78% to 46.19%.

Table 5.1 Change in volatile solids, total COD, soluble COD and SCOD/TOCD ratio of ALBAZOD as a function of thermal pretreatment at 120°C for 1, 2 or 3h in. With ± analytical standard errors (n=6).

| Condition | VS (g/L) | TCOD (g COD/L) | SCOD (g COD/L) | SCOD/TCOD (%) | Biogas (L/L ALBAZOD) | $CH_4$ (%) |
|---|---|---|---|---|---|---|
| Control | 4.28 ± 0.14 | 3.35 ± 0.25 | 0.83 ± 0.05 | 24.78 | 14.7 ± 2.2 | 40.8 ± 5.2 |
| 120°C (1h) | 40.26 ± 2.12 | 30.02 ± 1.32 | 10.91 ± 1.04 | 36.34 | 16.1 ± 1.8 | 42.3 ± 2.2 |
| 120°C (2h) | 45.27 ± 2.29 | 32.87 ± 1.26 | 14.94 ± 1.22 | 45.45 | 18.3 ± 1.1 | 46.5 ± 3.2 |
| 120°C (3h) | 48.27 ± 3.07 | 34.75 ± 1.22 | 16.05 ± 1.73 | 46.19 | 19.5 ± 1.6 | 50.1 ± 3.1 |

### 5.3.2. Study 1: Microalgae cell disruption following thermal pretreatment (120 °C)

The intracellular algogenic organic matter (AOM) release by thermal pretreatment at 120 °C was observed in 1h, 2h and 3h accordingly (Fig. 5.2 & 5.3). In all cases, it was observed that the preheating only applied preferentially on cell wall components at 1h on both *Chlorella. vulgaris* and *Scenedesmus sp.* After 2h, more AOM release was observed with damaged and expanded cell wall degradation. At 3h, the images clearly showed that expanded and partially disaggregated cell wall structure causing further release of internal AOM in *C. vulgaris.* It also showed the appearance of more empty and clear areas inside the cells boundaries (Fig. 5.2). Although such effect was not shown in *Scenedesmus sp.*, more release of AOM was observed in the patchy loss of cell turgidity (Fig. 5.3).

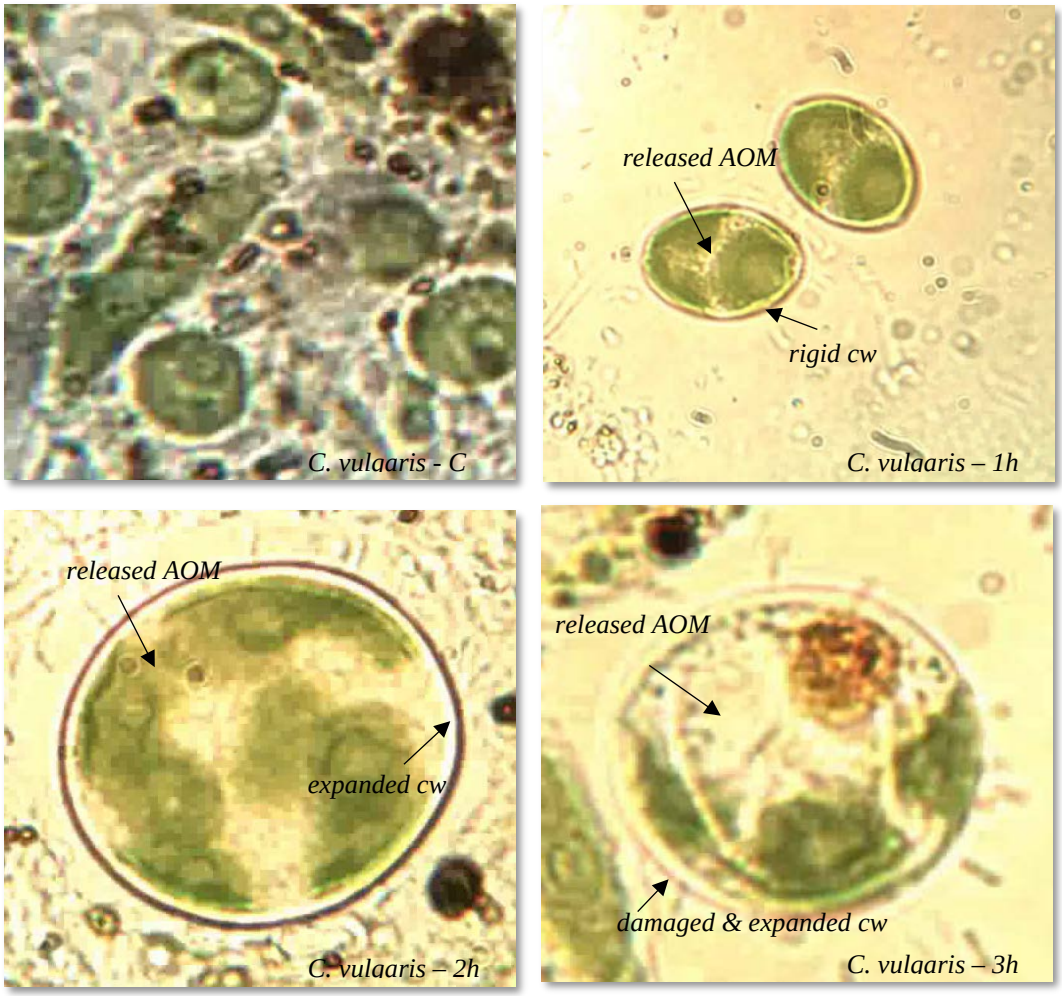


Fig. 5.2 Compound microscope picture of *Chlorella vulgaris* in ALBAZOD after thermal pretreatment at 120 °C for 1h, 2h, and 3h. cw = cell wall; c = control ; AOM = intracellular algogenic organic matter. Magnification x 1000.

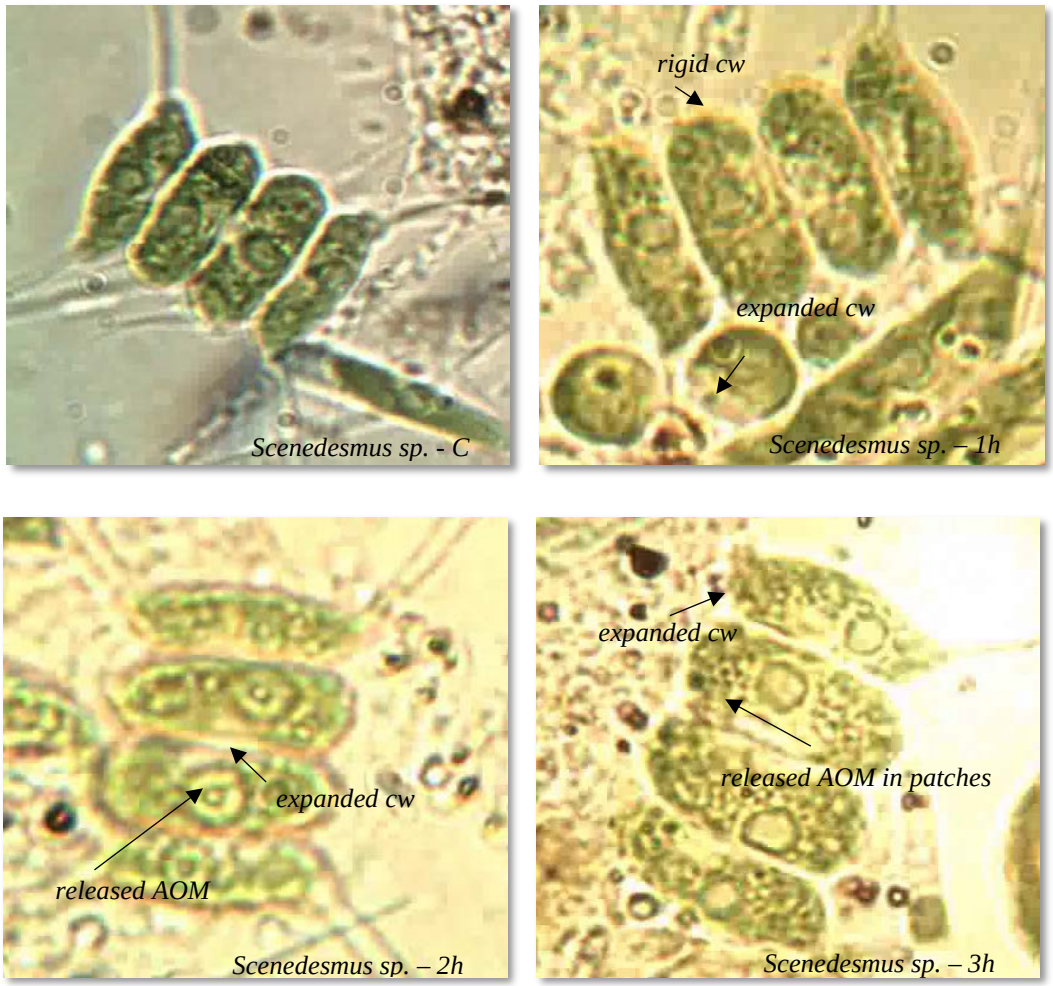


Fig. 5.3 Compound microscope picture of *Scenedesmus sp.* in ALBAZOD after thermal pretreatment at 120 °C for 1h, 2h, and 3h. cw = cell wall; c = control ; AOM = intracellular algogenic organic matter. Magnification x 1000.

The characteristic of thermally pretreated (120°C for 3h) pig slurry and ALBAZOD mixtures in anaerobic co-digestion are shown in Table 5.2. The TCOD (38.91 g TCOD $L^{-1}$) in pig slurry was very similar to ALBAZOD (34.35 g COD $L^{-1}$) after receiving 3h 120 °C thermal pretreatment. The volatile solid of 100% pig slurry was about 74% VS, whereas that of 100% ALBZOD was 68 % VS. The dry matter was about 4% DM in 100% pig slurry compared to 6.23% DM in 100% ALBAZOD which was a good indication of a lower volume based sample. The initial pH of pig slurry (pH 8.12) was more alkaline than ALBAZOD (pH7.61). Pig slurry contained a much higher ammonia level (1457.11 mg $NH_4$-N $L^{-1}$) compared than in the ALBAZOD (15.61 mg $NH_4$-N $L^{-1}$). Anaerobic co-digestion was then performed at ambient psychrophilic temperature (17 to 25°C).

Table 5.2 Characteristics of pig slurry (PS) and thermally pretreated (120°C for 3h) ALBAZOD (A) mixtures used in the batch anaerobic digestion at ambient psychrophilic temperature (17 to 25°C). With ± analytical standard errors (n=6).

| Co-digestion substrates based on percentages of VS (w/w) | 100% PS | 96.5% PS 3.5% A | 92.9% PS 7.1% A | 85.4% PS 14.6% A | 67.8% PS 32.2% A | 100% A |
|---|---|---|---|---|---|---|
| Relative percentage by volume (v/v) for references | 100% PS | 95% PS 5% A | 90% PS 10% A | 80% PS 20% A | 60% PS 40% A | 100% A |
| TS (g/L) | 47.25 (± 3.70) | 40.17 (± 5.21) | 32.81 (± 2.12) | 28.61 (± 3.43) | 26.24 (± 1.77) | 70.89 (± 1.11) |
| VS (g/L) | 34.74 (± 0.91) | 27.78 (± 0.11) | 20.89 (± 0.30) | 17.18 (± 0.22) | 15.25 (± 0.13) | 48.28 (± 0.11) |
| VS (%) | 73.52 | 69.16 | 63.67 | 60.05 | 58.12 | 68.10 |
| DM (%) | 3.73 | 3.58 | 3.45 | 3.08 | 2.57 | 6.23 |
| Moisture (%) | 96.27 | 96.42 | 96.55 | 96.92 | 97.43 | 93.77 |
| pH | 8.12 (± 0.20) | 8.03 (± 0.10) | 7.93 (± 0.10) | 7.85 (± 0.10) | 7.74 (± 0.10) | 7.61 (± 0.10) |
| $NH_4$-N (mg/L) | 1457.11 (± 125.71) | 1326.76 (± 81.26) | 1292.89 (± 105.39) | 1177.81 (± 74.55) | 920.16 (± 116.89) | 15.61 (± 2.13) |
| TCOD (g /L) | 38.91 (± 1.52) | 30.62 (± 2.53) | 25.71 (± 1.19) | 18.19 (± 2.39) | 9.85 (± 0.91) | 34.35 (± 2.17) |

### 5.3.3. Study 2: Cumulative specific methane yield (L $CH_4$ $g^{-1}$ $VS_{removed}$) on anaerobic co-digestion performance between pig slurry and ALBOAZOD under thermal pretreatment (120°C for 3h)

Fig. 5.4 shows the specific methane production (L $CH_4$ $g^{-1}$ $VS_{removed}$) in various pig slurry and ALBAZOD mixtures after thermally pretreatment. The highest methane production (0.400 L $CH_4$ $g^{-1}$ $VS_{removed}$) was observed when 3.5% (VS w/w) ALBAZOD was co-digested with pig slurry, compared with 0.368 L $CH_4$ $g^{-1}$ $VS_{removed}$ recorded from 100% pig slurry at day 91. As shown, the methane production decreased with the increase of ALBAZOD ratios in the mixtures. When the ALBAZOD ratio was beyond 7.1% A, the methane production decreased to 0.280 L $CH_4$ $g^{-1}$ $VS_{removed}$ at day 91. The lowest $CH_4$ production (L $CH_4$ $g^{-1}$ $VS_{removed}$) was observed from the 100% ALBAZOD experiment with 0.036 L $CH_4$ $g^{-1}$ $VS_{removed}$ over day 21, and then gradually increased to 0.053 L $CH_4$ $g^{-1}$ $VS_{removed}$ at day 72, and rapidly increased to 0.183 L $CH_4$ $g^{-1}$ $VS_{removed}$ at day 91.

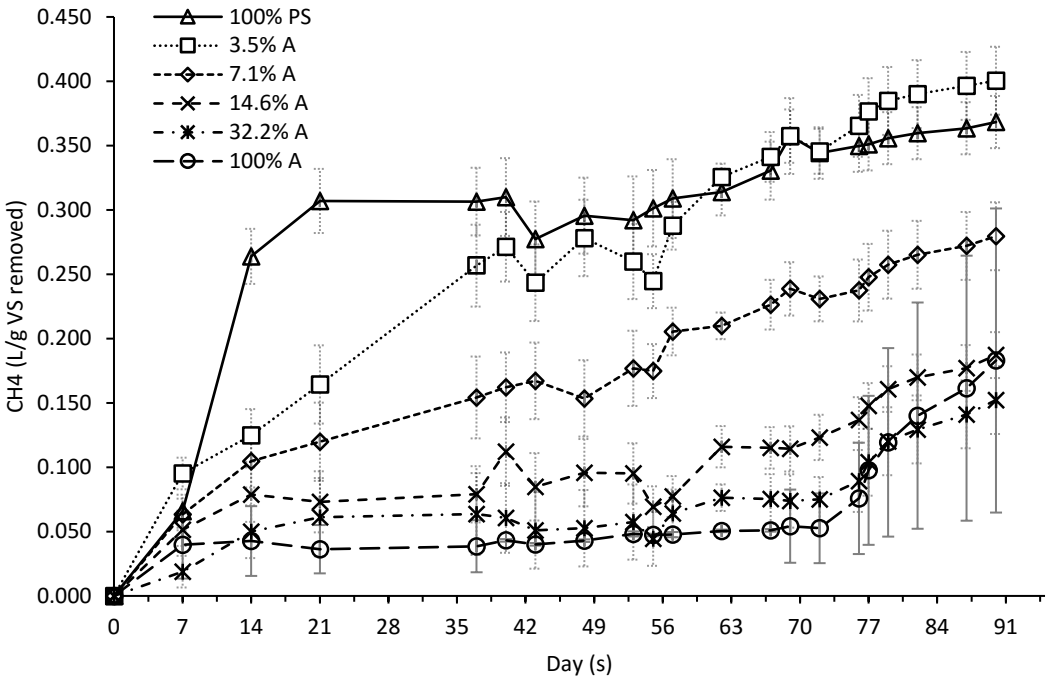


Fig. 5.4 Specific methane production ($CH_4$ L $g^{-1}$ VS removed) from co-digestion of pig slurry (PS) and mixtures of thermally pre-treated (120 °C for 3h) ALBAZOD (A) over 91 day period. With ± analytical standard errors (n=6)

Fig. 5.5 specifically shows the comparisons between the mixtures of both co-substrates under thermally treated (120 °C for 3h) and untreated controls in specific methane rate ($CH_4$ L $g^{-1}$ $VS_{removed}$) at day 91. There was no significant difference ($p > 0.05$) in the methane production between all mixtures comprising thermally treated and untreated controls, except there was a significant increase ($P < 0.05$) found on 3.5% A (0.400 $CH_4$ L $g^{-1}$ $VS_{removed}$) to control (0.344 $CH_4$ L $g^{-1}$ $VS_{removed}$) after the thermal pre-treatment at 120 °C for 3h. Table 5.3 summarises a comparison between the thermally pretreated substrate(s) and the corresponding controls with no pre-treatment.

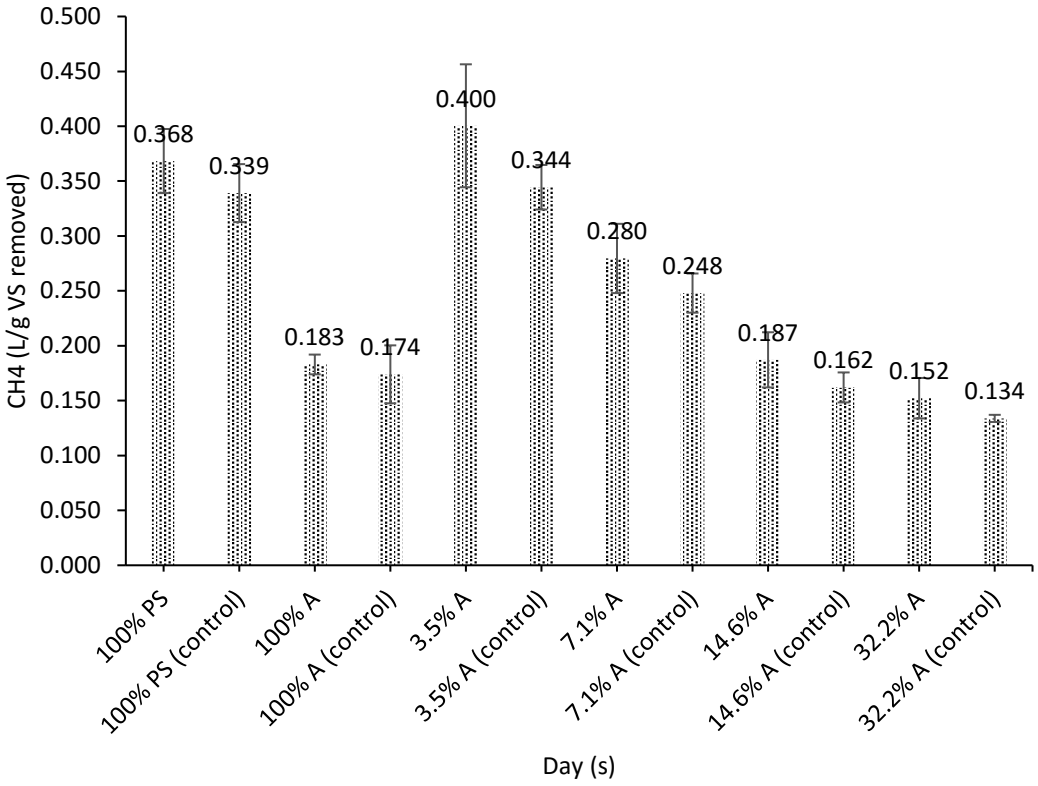


Fig. 5.5 Specific methane production ($CH_4$ L $g^{-1}$ VS removed) of the mixtures of both co-substrate(s) under thermally treated (120 °C for 3h) and untreated controls at 91 day. With ± analytical standard errors (n=6).

Table 5.3 Cumulative specific methane yield (L $CH_4$ $g^{-1}$ $VS_{removed}$) at 91 days: Comparison between thermally pretreated (120 °C for 3h) pig slurry with ALBAZOD mixtures and untreated controls ALBAZOD. With ± analytical standard errors (n=6).

| PS:ALBAZOD VS (w/w) | Biogas composition $CH_4$ (%) | Control (L $CH_4$ $g^{-1}$ $VS_{removed}$) | Thermally pre-treated at 120 °C for 3h (L $CH_4$ $g^{-1}$ $VS_{removed}$) | Increase in yield from thermal pre-treatment (L $CH_4$ $g^{-1}$ $VS_{removed}$) | Increase (%) |
|---|---|---|---|---|---|
| 100% PS | 74.9 (± 5.6) | 0.339 (± 0.026) | 0.368 (± 0.029) | + 0.029 | + 8.55 |
| 100% A | 50.1 (± 2.3) | 0.174 (± 0.026) | 0.183 (± 0.009) | + 0.009 | + 5.17 |
| 3.5% A | 72.1 (± 7.5) | 0.344 (± 0.020) | 0.400 (± 0.056) | + 0.056 | + 16.28 |
| 7.1% A | 60.3 (± 1.4) | 0.248 (± 0.018) | 0.280 (± 0.032) | + 0.032 | + 12.90 |
| 14.6% A | 56.2 (± 2.5) | 0.162 (± 0.014) | 0.187 (± 0.025) | + 0.025 | + 15.43 |
| 32.2% A | 55.1 (± 6.1) | 0.134 (± 0.003) | 0.152 (± 0.018) | + 0.018 | + 13.43 |

### 5.3.4. Energy balance of thermal pretreatment on ALBAZOD (120℃ 3h), based on study 1

The aim of this section was to determine if the enhanced methane production from thermal pre-treatment of ALBAZOD compensated for the energy expended.

Energy consumption in thermal pretreatments was estimated using Equation. 5.1

$$Q = cm\Delta t \qquad \text{Equation. 5.1}$$

Where, Q is the energy required, c is the specific heat capacity (J $g^{-1}$ $t^{-1}$), m is the mass, and $\Delta t$ is the difference between the original and the final temperature. The calculations were based on the specific heat capacity for wastewater sludges (4.186 J $g^{-1}$ $t^{-1}$), which is based on an assumption by Techobanoglous and Burton (1991) (Tchobanoglous & Burton, 1991). This calculation was based on a best-case scenario in which it does not include heat loss during preheating or for inefficiencies in utilizing the biogas. In this case, 394 kJ of energy are required for pretreatment per litre of ALBAZOD (based on dry matter = 6.23%) (Table 5.2 & 5.4).

Table 5.4 Energy balance of thermal pretreatment

| Assumed ALBAZOD density | Temperature change ($\Delta t$) | Heat capacity for water (c) | Energy cost (Q) per litre of ALZABOD |
|---|---|---|---|
| 0.938 (Based on dry matter = 6.23%) | 100 °C (from 20 to 120 °C) | 4.186 J $g^{-1}$ $t^{-1}$ | 393.585 kJ |

While the energy value of the biogas was calculated using 55.5 MJ/kg $CH_4$ (Lide, 2004), 24.4 L/mol at standard conditions, 16.04246 g/mol for molar mass of methane and 50.1 % methane in the biogas (100% ALBAZOD), this gives:

55.5 (MJ/kg $CH_4$) x 1/24.4 (mol/L) x 16.04246 (g/mol) x 0.501 ($CH_4$ %) = 18.28 kJ/L

Therefore, it gives a value of 18.28 kJ/L for the biogas collected from 100% ALBAZOD (based on dry matter = 6.23%). In the four conditions of variable time scenario, i.e. control, 1h, 2h, and 3h at 120 °C produced 14.7 (±2.2), 16.1 (±1.8), 18.3 (±1.1), 19.5 (± 1.6) L biogas per litre of sample respectively. The energy produced in

the biogas were 268.72, 294.31, 334.52, 356.46 kJ based on the heating value calculated above. When accounting the 393.59 kJ required for the thermal pretreatment, the final net energy produced are 295 (control), 124.87 (1h), 99.28 (2h), 37.13 (3h) kJ for the four conditions respectively. Therefore in all study cases, the additional methane production achieved from thermal pretreatment was not sufficient to balance the energy required to pretreat the biomass. The no thermal pretreatment is the best option in terms of net energy production in biogas, however, note that this calculation does not represent the synergy between pig slurry and ALBAZOD anaerobic co-digestion.

## 5.4. Discussion

### 5.4.1. Variable preheating time (1, 2, 3h) at constant temperature (120 °C)

The increase of TCOD after thermal pretreatment was possibly caused by removing the excess water content in the high moisture percentage in the ALZABOD samples (moisture percentage was 93.77 %). This may possibly be improved if some forms of de-watering (e.g. V-belt press is a common practice for some wastewater treatment plants) or centrifugation was performed after the ALBAZOD was obtained from the DAF plant (see Chapter 2 for method). However, the main objective of this experiment was to investigate an on-farm scenario which ALBAZOD would be integrated with piggery wastewater for anaerobic digestion in either covered anaerobic lagoon and engineered anaerobic digester, which requires minimal operating (energy costs) and capital costs for acceptance of the technology.

In literature, it is generally suggested that there was little oxidation occurred during thermal pretreatment and therefore a significant increase on TCOD was generally not observed. Marsolek *et* al. (2014) observed that there was no change in TCOD in centrifuged microalgal biomass (141 g TCOD $L^{-1}$ algae suspension to 136 g TCOD $L^{-1}$ algae suspension after 3.5h at 90 ℃) (Marsolek et al., 2014). However, the microalgae was thickened by suspension prior the experiment. Samson & Leduy (1983) also observed that there was no changes in TCOD when heated for ten minutes between 50 and 150 °C on *Spirulina maxima* algal biomass. However, the algal biomass was harvested by centrifugation and frozen until used (Samson & Leduy, 1983). In the study by Marsolek et al. (2014), SCOD increased significantly from the control to the 90 °C sample correlating with increased biogas production which similar effect was also observed in this Chapter results by using 120 °C after 1-3h. This could due the effect of extended heat on releasing intracellular AOM may possibly be associated with the increase of SCOD. Similar data was observed from Gonzalez-Gernandez *et al.* (2013) with an increase of SCOD with preheating at 90 °C (González-Fernández et al., 2013). With considerations to address on the significantly increase in TCOD, the thermal pre-treatment at 120℃ 3h was therefore only performed on study 2 which was based on the observation of thermal pre-treatment from 2h to 3h is less significant with

an overall ~1 % increase in SCOD/TOCD only. This is to assure the study 2 was more accurately based on the calculations of VS ratios rather than the solo benefit on reducing the moisture percentage in the substrates.

Cho *et al.* (2013) reported a 5.5 fold SCOD increase after autoclaving a mixture of *Scenedesmus* sp. and *Chlorella* sp. for 30 min at 120 °C (Cho et al., 2013). A 10 fold SCOD increase following heating (170 °C ) a mixture of natural algae was noted by (Keymer et al., 2013), which in this current Chapter study, a similar ~10 fold SCOD increase was also observed after 1h at 120 °C and ~20 fold SCOD after 3h by using thermal pre-treatment. Ometto *et al.* (2014) reported that at temperatures lower than 150 °C, *Chlorella sorokiniana* and *Scenedesmus obliquus* released similar amounts of SCOD to those treated using thermal hydrolysis pre-treatments. While at temperatures higher than 150 °C, the two algae species released significantly more SCOD with the thermal hydrolysis (by steam injection) pretreatment. This study suggested that the rapid change of high temperature/pressure caused by steam injection was only effective at pressures and temperatures higher than 4 bar and 150 °C to single cell algae characterised by the presence of carbohydrates polymers/cellulose/acetolysis resistant biopolymers (ARB). He suggested that lower temperature/pressure combinations were sufficient to produce cell damage to cellulose free filamentous algae (Ometto et al., 2014).

The results in this current study (study 1) have successfully demonstrated the significantly increase of SCOD and slightly increase of biogas production after 120 °C thermal pre-treatment at 3h, the research questions in here is if this pre-treatment method should be considered as practical. It was mentioned that due the high moisture content in ALZABOD substrate, some sort of concentration steps should be involved before co-digestion with pig slurry. However, as observed by literature results and this current study, thermal pre-treatment or complete drying at high temperatures should be avoided since the total biogas potential decreases significantly. It may also provide difficulties to introduce this idea to pig farmers since it is unavoidably to increase their infrastructure investment costs.

### 5.4.2. Efficiency and cell wall breaking at thermal pretreatment

The compound microscope pictures (magnification 1000) clearly showed the main microalgae cell components such as cell wall, the nucleus, chloroplasts inside the cytoplasm of untreated cells. The thermal pretreatment clearly disrupted the cell wall structure, causing the release of internal AOM into the media. It was observed that a more disaggregated cell wall structure and empty pouches were more clearly visible on *Chlorella vulgaris* than *Scenedesmus sp.* after a 3h treatment at 120°C. Based on the Chapter 4 results and controls, it was observed that ALBAZOD was able to pass through an anaerobic digestion and remained intact and undigested partially after 91 days. This was also observed in this current study. Golueke *et al.* (1957) demonstrated that microalgae cells are able to effectively resist bacterial attack and remained intact cell structures after 30 days hydraulic retention time in anaerobic digestion (Golueke et al., 1957). Zhou *et al*. (2009) also demonstrated intact microalgae cell structures in digestate from an anaerobic digester for a period of 45 days (Qing et al., 2009). Sanchez-Hernandez and Trvieso-Cordoba (1993) also reported the presence of chlorophyll *a* from the addition of *C. vulgaris* to a digester was still detectable after 64 days of the experiment (Sánchez Hernández & Travieso Córdoba, 1993). Mussgnug *et al.* (2010) reported intact microalgae cell structures and viable *Scenedesmus* cells after 6 months in an anaerobic digester with (Mussgnug et al., 2010). In the study, they further studied that the effect of heat pretreatment on changes in cell wall chemistry and its influence on substrate degradability on a variety of microalgae species. However, their study suggested that drying (at 105 °C for 24h) as a pretreatment decreases the fermentative potential of the substrates. They concluded the decreased biogas production are the loss of volatile organic compounds of high fermentation potential and/or a decreased accessibility of the dried organic compounds for the bacterial biocenosis within the fermenter sludge. Therefore, they suggested that the most energy efficient way of using algal biomass for fermentation is to use fresh biomass and avoid transportation if possible since drying of the biomass would require some sort of energy consumption (Mussgnug et al., 2010; Ras et al., 2011), in which correlated to the findings of energy balance calculations in this current study.

### 5.4.3. Performance of pig slurry co-digestions with ALBAZOD disrupted by thermal pretreatment (120 °C for 3h.)

The methane production (L $CH_4$ $g^{-1}$ $VS_{removed}$) of thermally pre-treated 100% ALBAZOD (120 °C for 3h) was relatively low (0.183 L $CH_4$ $g^{-1}$ $VS_{removed}$), when compared to thermally pre-treated 100% pig manure anaerobic digestion (0.368 L $CH_4$ $g^{-1}$ VS removed). In comparisons to the untreated controls, 0.174 (A) and 0.339 (PS) L $CH_4$ $g^{-1}$ $VS_{removed}$ were observed respectively. However, there was no significant difference found in both studies. In thermally pre-treated 3.5% A), a highest specific methane production rate (0.400 L $CH_4$ $g^{-1}$ $VS_{removed}$) was observed and significantly increased ($P < 0.05$) compared to control (0.344 L $CH_4$ $g^{-1}$ $VS_{removed}$). In all cases, the introduction of ALBAZOD into the pig slurry has demonstrated a reduction of specific methane production rate, except the case of 3.5% A. This is possibly due to the methane production rate of ALBAZOD is much lower than the rate of pig slurry. In addition, these such reductions in methane production rates were not in proportional to the amount of ALBAZOD and pig slurry in the mixtures. The best scenario observed in this study was 3.5% A mixture and the specific methane production rate was significantly decreased beyond this ratio. It is therefore concluded that high percentages (> 3.5%) of ALZABOD mixtures may possibly interrupt the synergy with pig slurry due to their much lower biodegradability than pig slurry.

In addition, the methane productivity of each single substrate was increased by +6.35% for ALBAZOD and +15.19% pig slurry after the thermal pre-treatment. The highest methane productivity increase (+30%) was observed in the 32.2% A. However, the percentage of methane (55.1% $CH_4$) as well as the biogas volume produced were relatively much lower when compared to 3.5% A (72.1% $CH_4$) and only slightly higher than 100% A (50.1% $CH_4$). This is possibly due to a higher percentage of untreated ALBAZOD was remained intact in the anaerobic co-digester in 32.2% A mixture. This is in agreement to Chen and Oswald's (1998) demonstration of thermal pretreatment combined with chemical pre-treatment using sodium hydroxide and variable exposure times. Their results demonstrated that the most efficient pretreatment for microalgal biomass required heating up to 100 °C for at least 8h without an increase of pH by using the addition of NaOH. The biogas productivity was increased by 33% after the thermal pretreatment. The study also indicated that up to 66% of the untreated

microalgal biomass was undigested due to the protection of intact cell wall throughout the digestion period (Chen & Oswald, 1998). Gonzalez-Fernandez et al. (2013) demonstrated a 9 and 57% increase in methane production following thermal pretreatment of *Scenedesmus sp* at 70 and 90 °C respectively (González-Fernández et al., 2012b). Their further work observed a 2.9 and 3.4 fold increase in methane production for organic loading rates of 1 and 2.5 kg COD $m^{-3}$ day respectively following thermal pretreatment of microalgal biomass at 90 °C for 1h compared with untreated controls (González-Fernández et al., 2013). In a study by DeSchamphelaire and Verstraete (2009), a batch anaerobic digestion tests were performed in a thermostatic room for 34 ℃ and in a thermostatic hot water bath of 41 ℃ placed in the same room, with samples of mixing mesophilic sludge and concentrated algal suspension. In addition, thermal pretreatment of algae involved a heating to 80 ℃ for 2.5h. However, it was reported that there was no significant different found in methane production when pretreating a mixture of *Chlorella*, *Pseudokirchneriella* and *Chlamydomonas* microalgae species at 80 °C for 2.5h in the study (De Schamphelaire & Verstraete, 2009). Based on the summary of literatures included in this Chapter, it is generally recommended for a temperature range of 90 – 150 °C and under a variable of time (30mins – 3h) to be effectively increase SCOD percentages in ALBAZOD and correlated biogas productions.

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# CHAPTER 6

# PSYCHROPHILIC ANAEROBIC CO-DIGESTION OF WASTE ACTIVED SLUDGE (WAS) WITH MUNICIPAL WASTEWATER DERIVED ALGAL SLUDGE (ALBAZOD)

# 6. PSYCHROPHILIC ANAEROBIC CO-DIGESTION OF WASTE ACTIVED SLUDGE (WAS) WITH MUNICIPAL WASTEWATER DERIVED ALGAL SLUDGE (ALBAZOD)

## 6.1. Introduction

Apart from pig slurry, which was considered in Chapters 4 and 5, there are also studies which have shown that algal sludge co-digestion with other carbon-rich cosubstrates such as primary and secondary sludge, oil-greases, waste papers a various food organic wastes can increase the anaerobic digestibility of algae by improving the total substrate composition, increasing the C/N ratio, and reducing the chance of ammonia toxicity. For example, Yen and Brune (2007) reported that the co-digestion of algae with waste paper increased the gas yield by more than 50% when compared with algae mono-digestion by (Yen & Brune, 2007).

Sludge is an attractive co-substrate because a large amount is produced on a daily basis at wastewater treatment plant and often associated with anaerobic digesters (Wang et al., 2013). This is in support with promising early studies with these two substrates. For example, Samson and Leduy (1983) demonstrated that co-digestion with cyanobacteria *Spirulina maxima* and primary sludge (50% by VS) increased the biogas yield by 2 fold (Samson & LeDuy, 1983). In addition, Cecchi et al. (1996) demonstrated that anaerobic co-digestion of macroalgae (around 30% algae, TS basis) from a lagoon in Venice with sewage sludge achieved a similar result for methane production when compared to control under mesophilic conditions (37℃ which operated at 11 to 15 day hydraulic retention times and 1.7 – 4.4 kg TVS/$m^3$/day organic loading rates). As previously mentioned in Chapter 1, the studies of anaerobic co-digestion with algae or ALBAZOD is limited, with either pig slurry or WWTP sludge. A study by Wang et al. (2013) demonstrated the effect of *Chlorella* in the modified Zarrouk medium with waste activated sludge (WAS). The results suggest that anaerobic co-digestion of algae and sludge improves the digestibility of microalgae and could also bring synergistic effects on the dewaterability of digested products for existing anaerobic digesters. The study also demonstrated that the biogas yield of

microalgae improved with a faster gas phase achieved when algae were co-digested with varying amounts of WAS (59–96% in mass).

To address the low methane productivities (0.174 L $CH_4$ $g^{-1}$ $VS_{removed}$ at day 91 from Chapter 4) when using 100% ALBAZOD observed previously, thermal pre-treatment was examined in Chapter 5. This method is classified as mechanical, however, lower cost and energy consumption method such as biological pre-treatment can also be performed. For example, Carrère et al. (2010) demonstrated that biological pretreatments based on increasing the bacterial hydrolytic activity enhanced methane productivity by 86% when applied to activated sludge (Carrère et al., 2010). A study by Alzate et al. (2012) investigated the anaerobic digestion of three microalgae mixtures at different substrate to inoculum (S/I) ratios (0.5, 1 and 3), biomass concentrations (3, 10 and 20 g TS $kg^{-1}$) and pretreatments (thermal hydrolysis, ultrasound and biological treatment). The study demonstrated that an S/I ratio of 0.5 and 10 g TS/kg resulted in the highest final methane productivities regardless of the microalgae tested (ranging from 188 to 395 mL CH4 $g^{-1}$ $VS_{added}$) (Alzate et al., 2012). Tampio et al. (2013) demonstrated that stable digestion of untreated and autoclaved food waste was possible in trace element supplemented mesophilic reactors at (organic loading rate) OLRs up to 6 kg VS $m^{-3}$ d, with yields of 0.435 and 0.393 L $CH_4$ $g^{-1}$ VS respectively. In addition, the study also demonstrated that using an acclimated inoculum allowed rapid increases in OLR without process disturbance (Tampio et al., 2014). Although statistically significant differences were identified at 3.5% ALBAZOD + pig slurry mixture with 120 $^oC$ 3h in autoclaving thermal pretreatment, overall energy balance on thermal pretreatment was not favoured under this scenario in terms of net energy production in biogas. Therefore, thermally pre-treated ALBAZOD was not considered in this current Chapter.

From observations in Chapter 5, it was also suggested that reasonable methane yields was possibly beyond 91 days with the applied VS loads in the batch anaerobic digestion. This is in line with Safley and Westerman (1990) observations. They suggest reasonable methane yields can be expected at low temperatures if digester loading rates are reduced appropriately by extending the detention time ($\theta$) to the 100-

300 day range (Safley & Westerman, 1990). Therefore, extending the detention time simply makes them extremely lightly loaded “process” anaerobic digesters.

In this Chapter, anaerobic co-digestion of ALBAZOD and WAS was reported under psychrophilic temperatures. The research investigated the effect of the addition of WAS on the digestibility of ALBAZOD, as well as using inoculum from a previous anaerobic digestion to determine if this would enhance digestion and reduce the digestion period.

## 6.2. Methods

### 6.2.1. Inoculum

Inoculum was collected following the digestion of 100% ALBAZOD sludge (Chapter 5) at ambient psychrophilic temperature (17-25°C) under batch operating conditions. It was then stored in a digester and fed ALBAZOD sludge daily following effluent withdrawal, to maintain a similar TS and DM of inoculum content. Prior to the anaerobic co-digestion experiment, the inoculum had been degassed by incubation at a constant room temperature without feeding in order to release any residual biodegradable organic material. Daily methane production was continuously monitored until no significant methane was being produced prior to use as inoculum. In this experiment, approximately 4% of this inoculum by mass of VS (g VS $L^{-1}$ w/w) or 10% (v/v) by volume. The influence of including the inoculum was assessed by comparing the results of digestions comprising 100% (VS, w/w) WAS or ALBAZOD with or without the inoculum.

### 6.2.2. Anaerobic digesters

The anaerobic digesters were used as described in Chapter 4. The waste activated sludge was collected from Bolivar WWTP SAWater, South Australia (34°45'40.8"S 138°34'39.8"E). The experiment groups were studied shown in Table 6.1. All ratios were calculated on dry weight VS (w/w) and performed in duplicate, each with triplicate analysis (n=6).

Table 6.1 Description of substrate mixtures (20L) used for the psychrophilic anaerobic co-digestion (17-25°C) of ALBAZOD (A), waste activated sludge (WAS), and inoculum expressed as percentage volatile solid (VS, w/w).

| Mixture number | ALBAZOD (A) (%) | WAS (%) | Inoculum (%) |
|---|---|---|---|
| 1 | - | 100 | - |
| 2 | - | 96 | 4 |
| 3 | 3 | 93 | 4 |
| 4 | 6 | 90 | 4 |
| 5 | 11 | 85 | 4 |
| 6 | 16 | 80 | 4 |
| 7 | 26 | 70 | 4 |
| 8 | 56 | 40 | 4 |
| 9 | 76 | 20 | 4 |
| 10 | 93 | 3 | 4 |
| 11 | 96 | - | 4 |
| 12 | 100 | - | - |

### 6.2.3. Analytical methods, quantitative and qualitative assessment of the biogas produced, and cumulative specific gas yield (L $CH_4$ $g^{-1}$ $VS_{removed}$)

Refer to Chapter 4 for relevant methods. Note the only difference was, a 90-day experiment was performed in this Chapter rather than the previously 91 days.

## 6.3. Results

### 6.3.1. Characteristics of ALBAZOD and WAS

The characteristic of ALBAZOD and WAS mixtures are shown in Table 6.2. The ALBAZOD TCOD content (3.82 g TCOD $L^{-1}$) was about 8 times higher than the WAS (0.48 g TCOD $L^{-1}$). The volatile solid of pure WAS was approximately 82% (26.19 g VS $L^{-1}$), with a lower volatile solids percentage (52%) recorded in 100% ALBAZOD which indicated the ALBAZOD exhibited a large organic fraction. The dry matter was 4.57% in WAS and 1.03% in ALBAZOD. The initial pH of the WAS (pH 8.55) was more alkaline than ALBAZOD (pH 7.70). The concentration of ammonium in the WAS (32.45 mg $NH_4$-N $L^{-1}$) was approximately 2.5 times that of the ALBAZOD (12.73 mg $NH_4$-N $L^{-1}$).

The TS of the inoculum (4.51 g TS $L^{-1}$) was approximately half that of the ALBAZOD. The VS of the inoculum was 1.44 g VS $L^{-1}$. The initial pH of the inoculum (pH 8.02) was more alkaline than 100% ALBAZOD (pH 7.70). The TCOD of the inoculum (1.16 g TCOD $L^{-1}$) was approximately 30% of the TCOD in 100% ALBAZOD (3.82 g TCOD $L^{-1}$).

### 6.3.2. The effect of an anaerobic inoculum on the digestion of waste activated sludge and ALBAZOD.

The WAS and ALBAZOD was digested with and without the addition of an inoculum from a previous anaerobic digestion (Chapter 5). The results presented in Fig. 6.3 show that the addition of the inoculum to both WAS and ALBAZOD solids resulted in small improvements in both TS and VS reduction by comparison to controls. The reductions in TS and VS will be discussed in details for each mixtures.

Table 6.2 Characteristics of substrate mixtures with ALBAZOD (A), waste activated sludge (WAS), and inoculum used in the batch anaerobic digestions. With ± analytical standard error (n=6).

| Mixture number | A (%) | WAS (%) | Inoculum (%) | TS (g/L) | VS (g/L) | VS/TS (%) | DM (%) | Moisture (%) | pH | $NH_4$-N (mg/L) | TCOD (g /L) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | - | 100 | - | 31.95 (± 2.94) | 26.19 (± 2.06) | 82 | 4.57 | 95.43 | 8.55 (± 0.10) | 32.45 (± 2.90) | 0.48 (± 0.10) |
| 2 | - | 96 | 4 | 30.85 (± 2.56) | 25.20 (± 1.92) | 80.00 | 4.42 | 95.58 | 8.53 (± 0.10) | 31.50 (± 3.07) | 0.51 (± 0.02) |
| 3 | 3 | 93 | 4 | 30.18 (± 1.86) | 24.56 (± 0.63) | 79.09 | 4.31 | 95.69 | 8.50 (± 0.10) | 30.90 (± 0.89) | 0.61 (± 0.12) |
| 4 | 6 | 90 | 4 | 29.51 (± 0.79) | 23.93 (± 0.98) | 78.19 | 4.21 | 95.79 | 8.48 (± 0.10) | 30.31 (± 1.11) | 0.71 (± 0.11) |
| 5 | 11 | 85 | 4 | 28.39 (± 0.63) | 22.87 (± 1.24) | 76.69 | 4.03 | 95.97 | 8.44 (± 0.05) | 29.33 (± 0.58) | 0.87 (± 0.03) |
| 6 | 16 | 80 | 4 | 27.27 (± 1.82) | 21.81 (± 1.77) | 75.18 | 3.85 | 96.15 | 8.39 (± 0.05) | 28.34 (± 0.52) | 1.04 (± 0.04) |
| 7 | 26 | 70 | 4 | 25.04 (± 2.41) | 19.69 (± 0.69) | 72.17 | 3.50 | 96.50 | 8.31 (± 0.10) | 26.37 (± 0.40) | 1.38 (± 0.01) |
| 8 | 56 | 40 | 4 | 18.33 (± 0.76) | 13.32 (± 0.43) | 63.14 | 2.44 | 97.56 | 8.05 (± 0.10) | 20.45 (± 0.23) | 2.38 (± 0.30) |
| 9 | 76 | 20 | 4 | 13.86 (± 0.21) | 9.08 (± 1.77) | 57.12 | 1.73 | 98.27 | 7.88 (± 0.05) | 16.51 (± 0.71) | 3.05 (± 0.07) |
| 10 | 93 | 3 | 4 | 10.06 (± 0.55) | 5.47 (± 0.25) | 52.00 | 1.13 | 98.87 | 7.74 (± 0.05) | 13.16 (± 0.22) | 3.61 (± 0.11) |
| 11 | 96 | - | 4 | 9.39 (± 0.22) | 4.84 (± 0.24) | 51.10 | 1.02 | 98.98 | 7.71 (± 0.05) | 12.57 (± 0.19) | 3.71 (± 0.10) |
| 12 | 100 | - | - | 9.59 (± 0.56) | 4.98 (± 0.10) | 51.90 | 1.03 | 98.97 | 7.70 (± 0.10) | 12.73 (± 0.24) | 3.82 (± 0.10) |
| Inoculum | | | | 4.51 (± 0.23) | 1.44 (± 0.11) | 31.93 | 0.83 | 99.17 | 8.02 (± 0.10) | 8.61 (± 0.13) | 1.16 (± 0.05) |

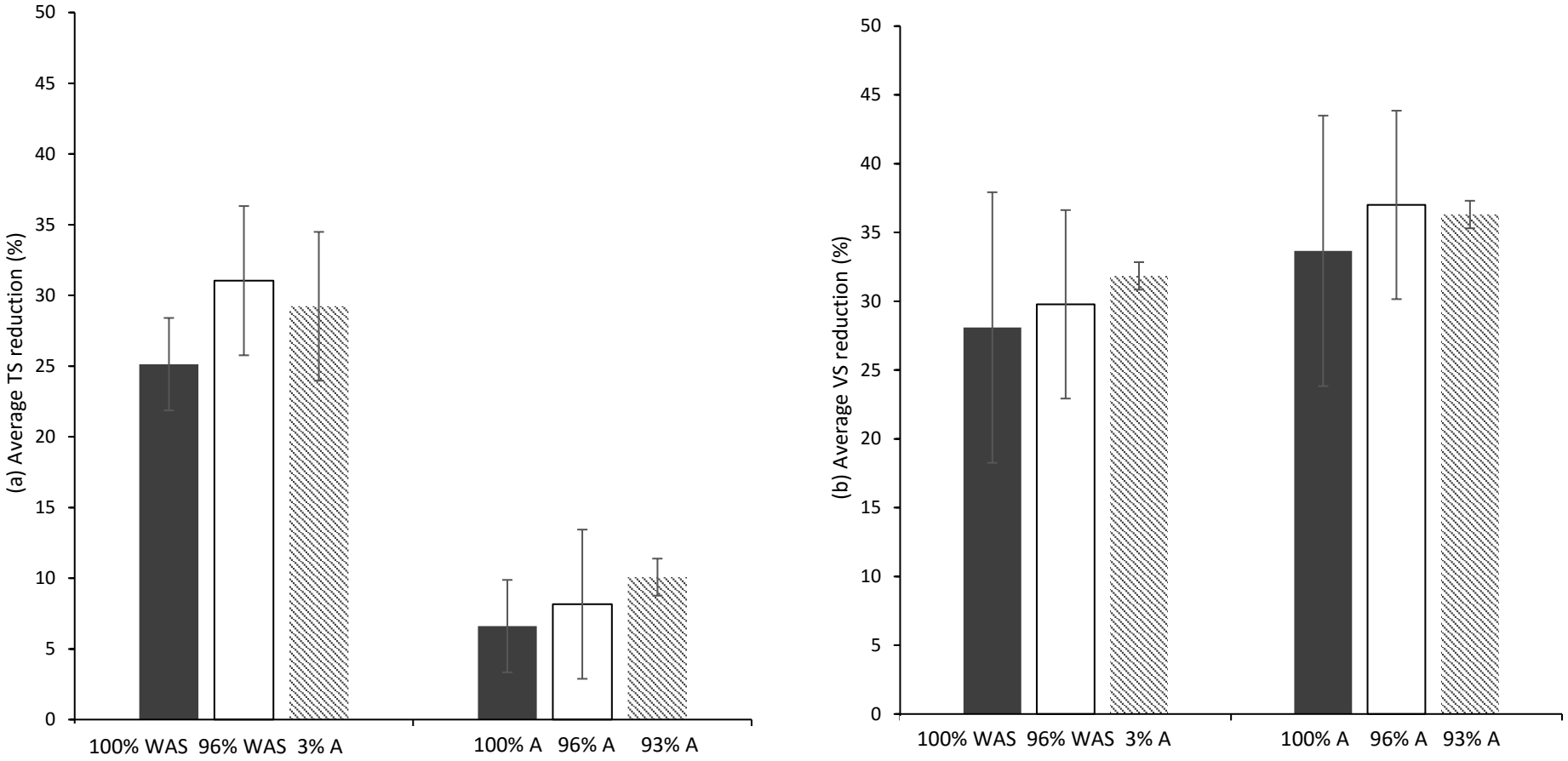


Fig. 6.3 Percentage reduction of (a) total solid (TS) and (b) volatile solid (VS) in digestions of 100% waste activated sludge (WAS) or 100% ALBAZOD (A) (■); in 96% WAS+4% inoculum (96% WAS) and 96% A+4% inoculum (96%A) (□) and for co-digestions comprising 93%WAS + 4% inoculum + 3% A (3% A) and 93% A + 4% inoculum + 3% WAS (93%A) (hatched). With ± analytical standard error (n=6).

### 6.3.3. Efficiency of total and volatile solid reductions in anaerobic co-digestion with varying amounts of ALBAZOD and WAS

Anaerobic co-digestions with varying amounts of ALBZAOD and WAS were performed for 90 days. The average total solid reductions are shown in Fig. 6.4. The 96% WAS with inoculum digestion showed the highest percentage reduction in TS (31.04%) while the 100% ALBAZOD (A) digestion showed the lowest (6.61%). The 3% A digestion which comprised 93% WAS and 4% inoculum, showed a slightly lower TS reduction percentage at 29.23%. As ALBAZOD percentages increased in the co-digestion, the average percentages TS reduction decreased (Fig. 6.4.). A significant decrease in TS reduction ($p < 0.05$) was only observed when the ALBAZOD percentage in the co-digestion with WAS > 26%. The average TS reduction percentage halved from 31.04% in 96% WAS to 15.35% in the co-digestion comprising and 56% A.

The average volatile solid reductions are shown in Fig. 6.5. The 96% A with 4% inoculum digestion showed the highest percentage VS reduction (37.01%) while the 100% WAS digestion showed the lowest (28.09%). Comparison of the 93% A (4% inoculum + 3%WAS), with 3% WAS (4%inoculum +93% A), showed a slightly lower VS reduction percentage at 36.31%. In contrast to the observations of TS reduction, as ALBAZOD percentages increase in the WAS co-digestions, the average percentage reduction in VS increased (Fig. 6.5). No significant difference ($p > 0.05$) in VS reduction was, however, identified between the co-digestions experiments.

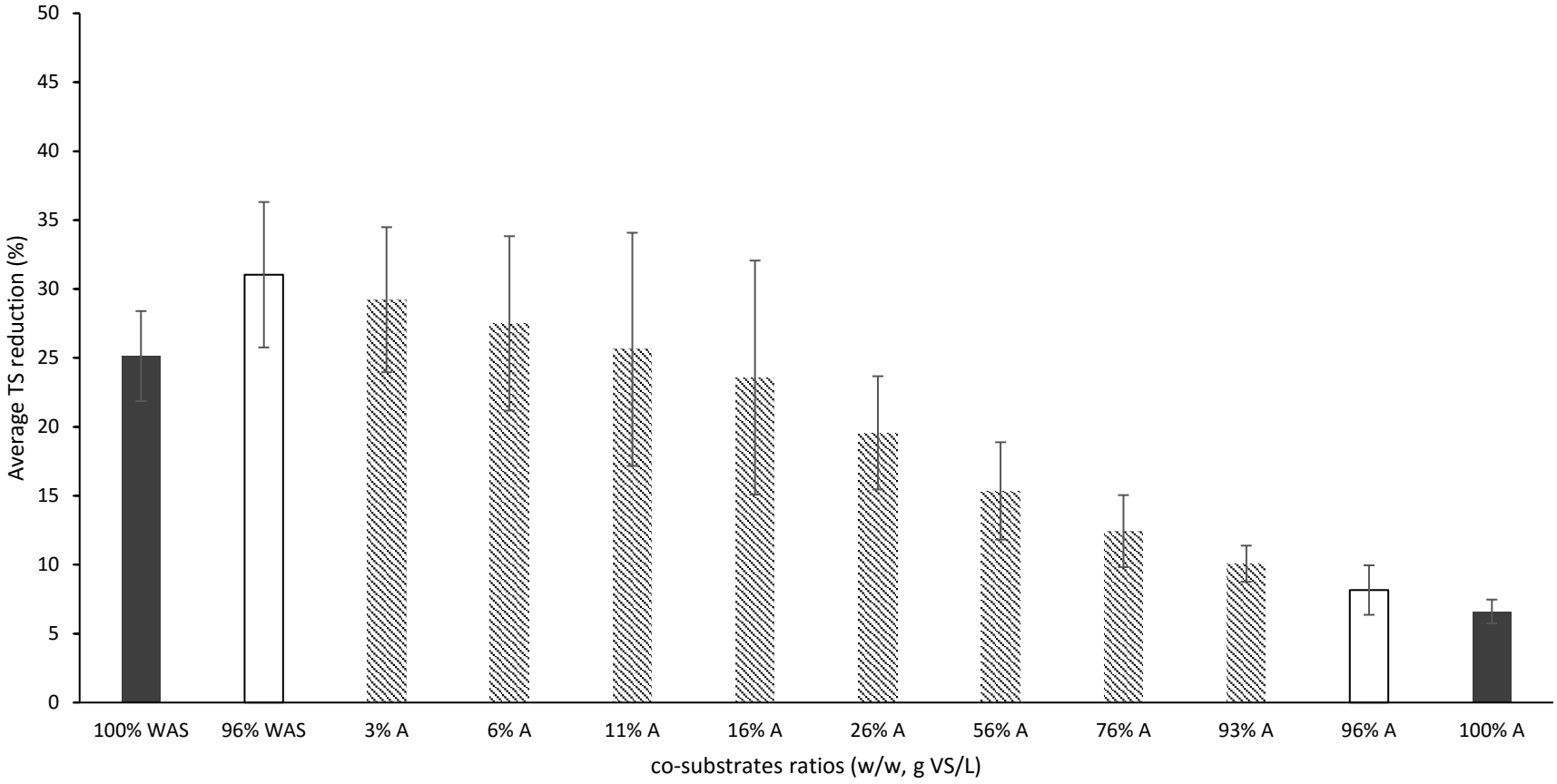


Fig. 6.4 Percentage reduction (%) in total solids (TS) in anaerobic co-digestions with varying percentages of ALBAZOD (A) and waste activated sludge (WAS). 100% WAS and A were both controls without inoculum (4%, w/w, g VS/L) (■); 96% WAS and A were both controls with inoculum (4%, w/w, g VS/L) (□); the rest co-substrate ratios indicated as grey hatching included the same inoculum (4%, w/w, g VS/L). With ± analytical standard error (n=6).

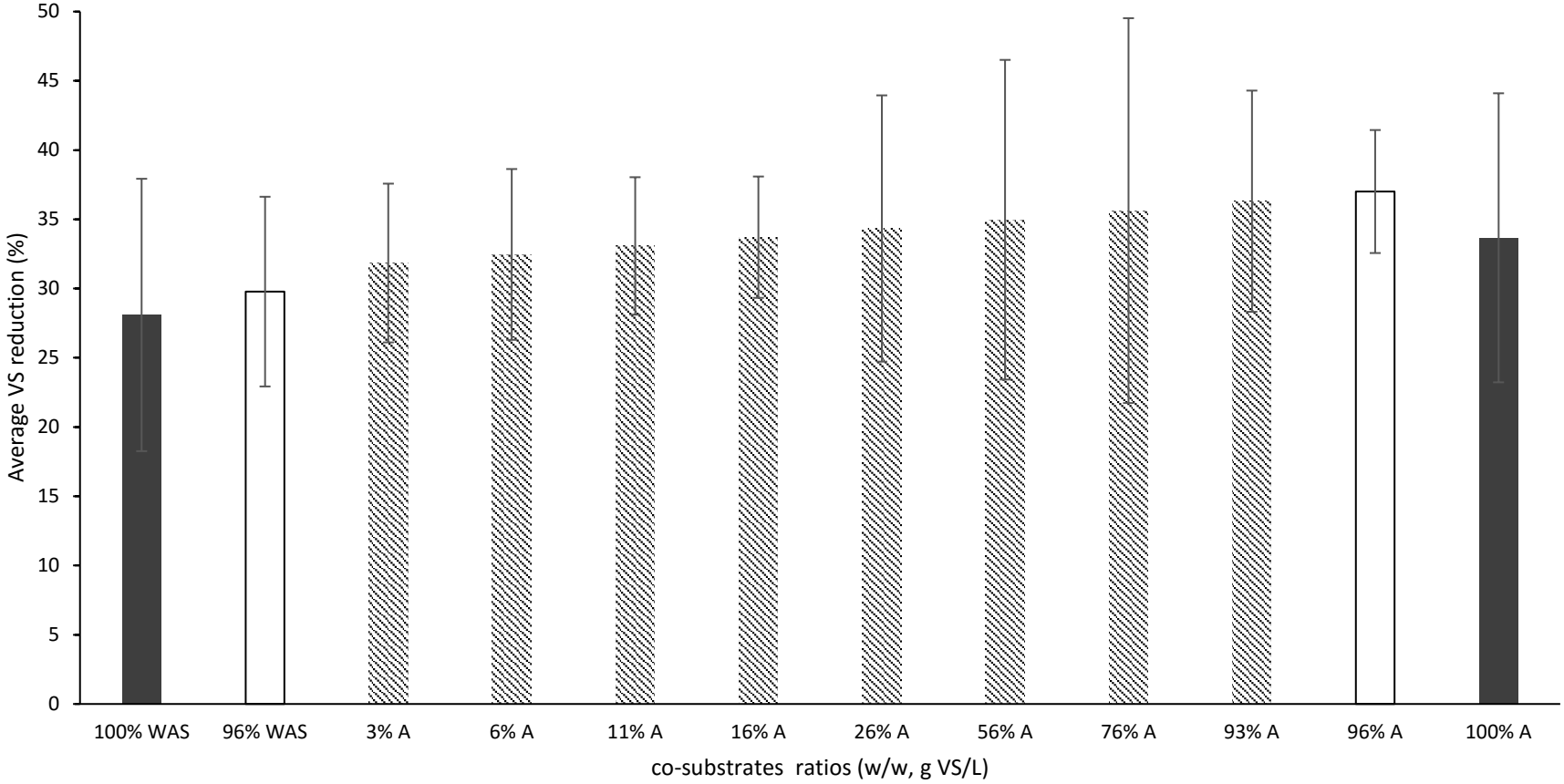


Fig. 6.5 Percentage reduction (%) in volatile solids (VS) in anaerobic co-digestions with varying percentages of ALBAZOD (A) and waste activated sludge (WAS). 100% WAS and A were both controls without inoculum (4%, w/w, g VS/L) (■); 96% WAS and A were both controls with inoculum (4%, w/w, g VS/L) (□); the rest co-substrate ratios indicated as grey hatching included the same inoculum (4%, w/w, g VS/L). With ± analytical standard error (n=6).

### 6.3.4. Biogas (L) accumulation

The accumulated biogas production is shown in Fig. 6.6. No stationary phase was observed when the ALBAZOD percentage were ≥ 56%. The highest biogas accumulated was in the 96% WAS experiment with a total of 70.93 L. Similar volumes of accumulated biogas were also observed in the 100% WAS and 3% A experiments with a total of 68.86 and 69.51 L respectively (Fig. 6.6). From the 3% A to 26% A co-digestions, the accumulated biogas decreased as the ALBAZOD percentage increased. However, no significant difference was found ($P > 0.05$).

The accumulated biogas was halved from 70.93 L to 35.37 L when the ALBAZOD percentage was increased to 56%. The accumulated biogas volumes gradually decreased with the increasing ALBAZOD percentage in the digestion. The lowest accumulated biogas volume was observed in the 100% A digestion with 14.70 L.

Overall the first 60 days, there was only very limited amount of accumulated biogas volume observed in the three high ALBAZOD percentage digestion sets (93% A, 96% A and 100% A), with under 10L of biogas accumulated. A rapid release of biogas was observed after 60 day in the 93% A co-digestion in which the accumulated biogas volume tripled from day 60 to day 73 (7.42 L → 21.05 L).

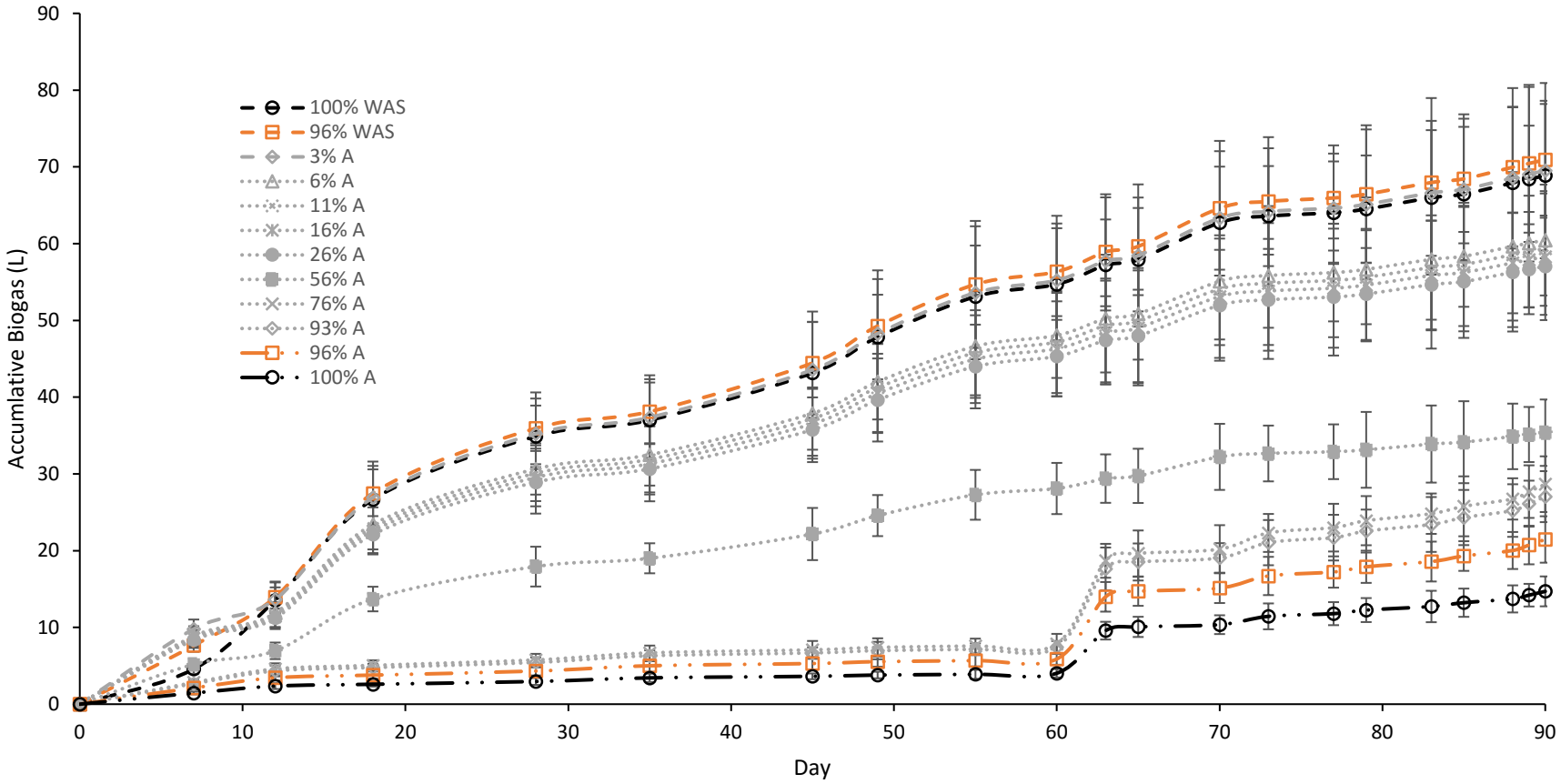


Fig. 6.6 The accumulation of biogas production (L) from co-digestion of waste activated sludge (WAS) and ALBAZOD (A) over 90 day period. 100% WAS and A were both controls without inoculum (4%, w/w, g VS/L) indicated as black lines; 96% WAS and A were both controls with inoculum (4%, w/w, g VS/L) indicated as orange lines; the rest co-substrate ratios indicated as grey dot lines were mixed with the same inoculum (4%, w/w, g VS/L). With ± analytical standard error (n=6).

### 6.3.5. Mean methane (% $CH_4$) and carbon dioxide (% $CO_2$) content in biogas

Percentage of methane in the biogas (% $CH_4$)

Fig. 6.7 shows the average of $CH_4$ percentage over the 90 day digestion period. Similar mean methane percentages were observed within the experiment groups 100% WAS, 96% WAS, 3% A, 93% A and 96% A which was generally 60% in average. The highest mean $CH_4$ percentage (62.59%) was observed in the 96% WAS digestion, which contained 4% inoculum, and the lowest (40.60%) was observed in 100% A digestion. Peak methane contents amongst all the experiment groups were achieved in around day 50. Afterward, the methane contents remained stationary to day 90 (Fig. 6.7).

Percentage of carbon dioxide (% $CO_2$)

Fig. 6.8 shows the average of $CO_2$ percentage over the 90 day digestion period. Similar mean $CO_2$ percentages were observed within the experiment groups 100% WAS, 96% WAS, and 3% A which was about 12% in average. The highest mean $CO_2$ percentage was observed in the 100% A digestion (27.93%) and the lowest was observed in 56% A digestion (8.48%). Peak $CO_2$ contents among all the experiment groups was achieved at around day 20. However, stationary phases were not observed due to the constantly changing of $CO_2$ percentages (Fig. 6.8).

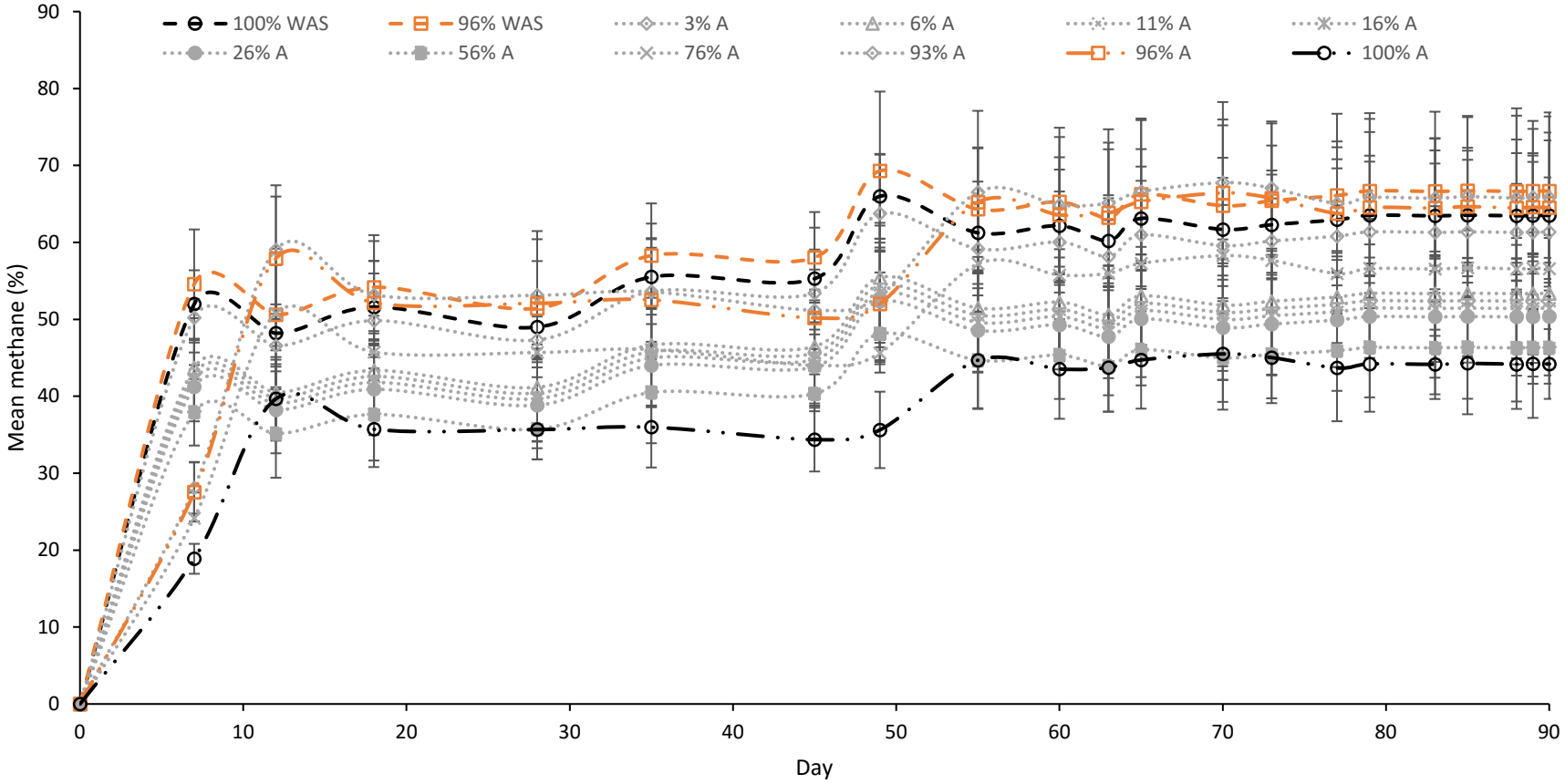


Fig. 6.7 Methane content (%) from co-digestion of waste activated sludge (WAS) and ALBAZOD (A) over 90 day period. 100% WAS and A were both controls without inoculum (4%, w/w, g VS/L) indicated as black lines; 96% WAS and A were both controls with inoculum (4%, w/w, g VS/L) indicated as orange lines; the rest co-substrate ratios indicated as grey dot lines were mixed with the same inoculum (4%, w/w, g VS/L). With ± analytical standard error (n=6).

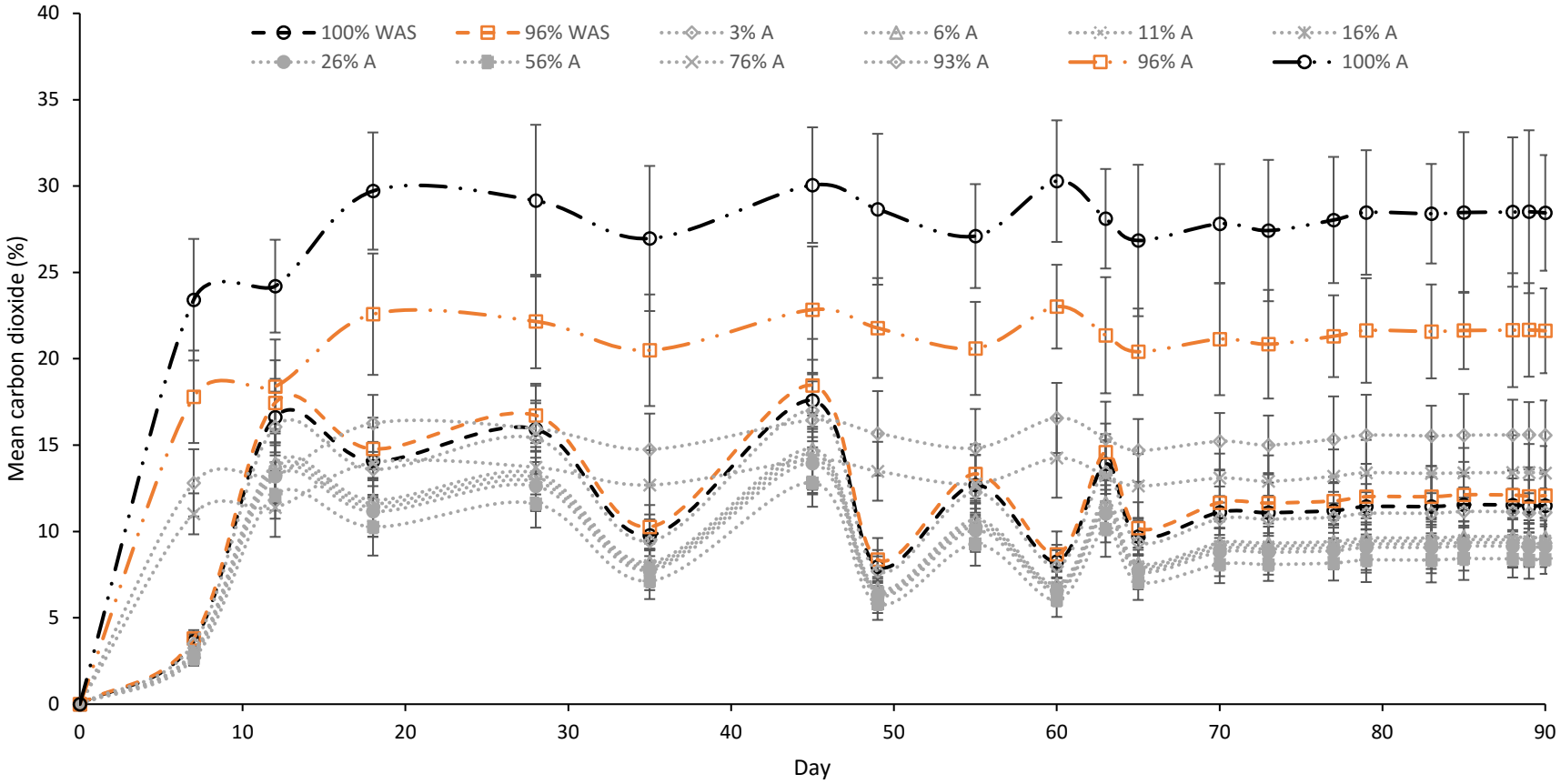


Fig. 6.8 Carbon dioxide content (%) from co-digestion of waste activated sludge (WAS) and ALBAZOD (A) over 90 day period. 100% WAS and A were both controls without inoculum (4%, w/w, g VS/L) indicated as black lines; 96% WAS and A were both controls with inoculum (4%, w/w, g VS/L) indicated as orange lines; the rest co-substrate ratios indicated as grey dot lines were mixed with the same inoculum (4%, w/w, g VS/L). With ± analytical standard error (n=6).

### 6.3.6. Cumulative specific methane production in $VS_{removed}$ (L $CH_4$ $g^{-1}$ $VS_{removed}$)

Fig. 6.9 shows the cumulative methane production (L $CH_4$ $g^{-1}$ $VS_{removed}$). The highest methane production of 0.276 L $CH_4$ $g^{-1}$ $VS_{removed}$ was observed for the 96% WAS co-digestion mixture, which gives an approximately 8% increase when compared to the slight lower production of 0.255 L $CH_4$ $g^{-1}$ $VS_{removed}$ recorded in the 100% WAS on day 90. The methane production decreased as the ALBAZOD ratio increased in the co-digestions. The methane production of 0.249 L $CH_4$ $g^{-1}$ $VS_{removed}$ was observed for the 3% A co-digestion mixture, a 10% decrease compared to 96% WAS and a 2.4% decrease compared to 100% WAS digestions at day 90. When the ALBAZOD ratio was beyond 6% A, the methane production decreased to below than 0.200 L $CH_4$ $g^{-1}$ $VS_{removed}$. The lowest $CH_4$ (0.010 L $CH_4$ $g^{-1}$ $VS_{removed}$) was observed for the co-digestion comprising 100% A accumulated over the first 60 days which rapidly increased up to 0.038 L $CH_4$ $g^{-1}$ $VS_{removed}$ at the day 90 (Fig. 6.9).

In order to study the biodegradability by using inoculum, $CH_4$ productions were compared over the first 45 days (Fig. 6.10). The period represents the pre-steady phase of the $CH_4$ production before most of the anaerobic digestions reached their steady phases. In Fig. 6.10, 96% WAS digestion had noticeably higher $CH_4$ production with approximately 75% increase (from 0.014 at day 1 to 0.024 L $CH_4$ $g^{-1}$ $VS_{removed}$ at day 7) with the use of 4% inoculum in the first 7 days. Interestingly, the 3% A co-digestion indicated a slightly higher $CH_4$ production (0.029 L $CH_4$ $g^{-1}$ $VS_{removed}$) with the use of 4% inoculum and mixed with 93% WAS at the first 7 days than 96% WAS digestion (0.024 L $CH_4$ $g^{-1}$ $VS_{removed}$). At day 45, the methane production of 0.151 L $CH_4$ $g^{-1}$ $VS_{removed}$ in the 96% WAS digestion and 0.136 L $CH_4$ $g^{-1}$ $VS_{removed}$ in the 3% A (Fig. 6.9). In 93% A digestion, although it performs better than 96% A, only a slightly increase of $CH_4$ production was observed (0.02 vs 0.016 L $CH_4$ $g^{-1}$ $VS_{removed}$). However, when compared 96% WAS to 100% WAS at day 90 (Fig. 6.9), the methane production increased significantly from 0.04 to 0.08 L $CH_4$ $g^{-1}$ $VS_{removed}$. This may indicate the use of inoculum provides a much higher and stable methane production increase in the high ALBAZOD mixture anaerobic co-digestion but only at an extended detention time (> 60-90 days). For example, up to the 100-300 day range was suggested by Safley and Westerman especially when psychrophilic temperature was used (Safley & Westerman, 1990).

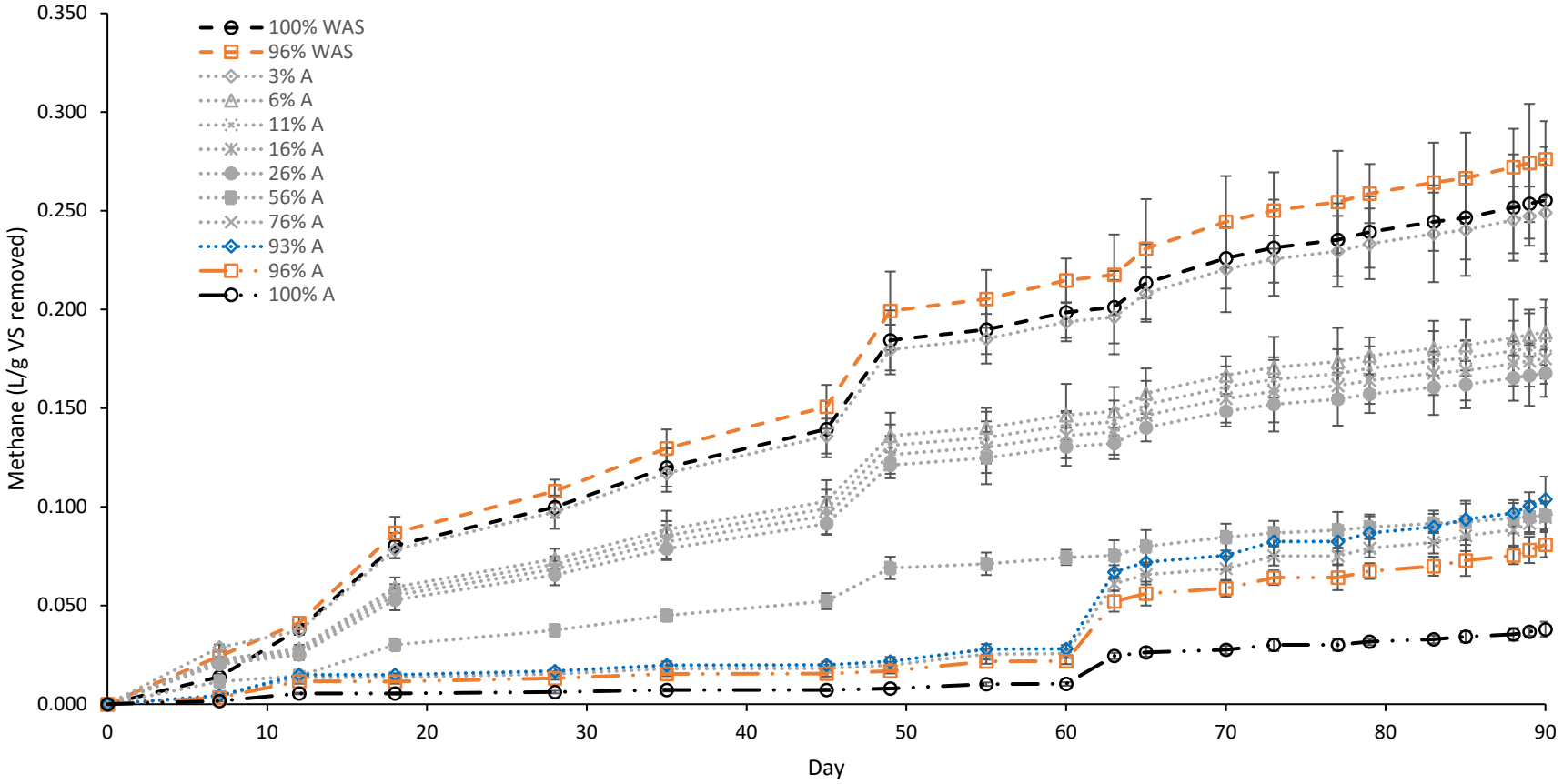


Fig. 6.9 Cumulative methane production ($CH_4$ L/g $VS_{removed}$) from co-digestion of waste activated sludge (WAS) and ALBAZOD (A) over 91 day period. 100% WAS and A were both controls without inoculum (4%, w/w, g VS/L) indicated as black lines; 96% WAS and A were both controls with inoculum (4%, w/w, g VS/L) indicated as orange lines; the rest co-substrate ratios indicated as grey dot lines were mixed with the same inoculum (4%, w/w, g VS/L) and 93% A indicated as blue. With ± analytical standard error (n=6).

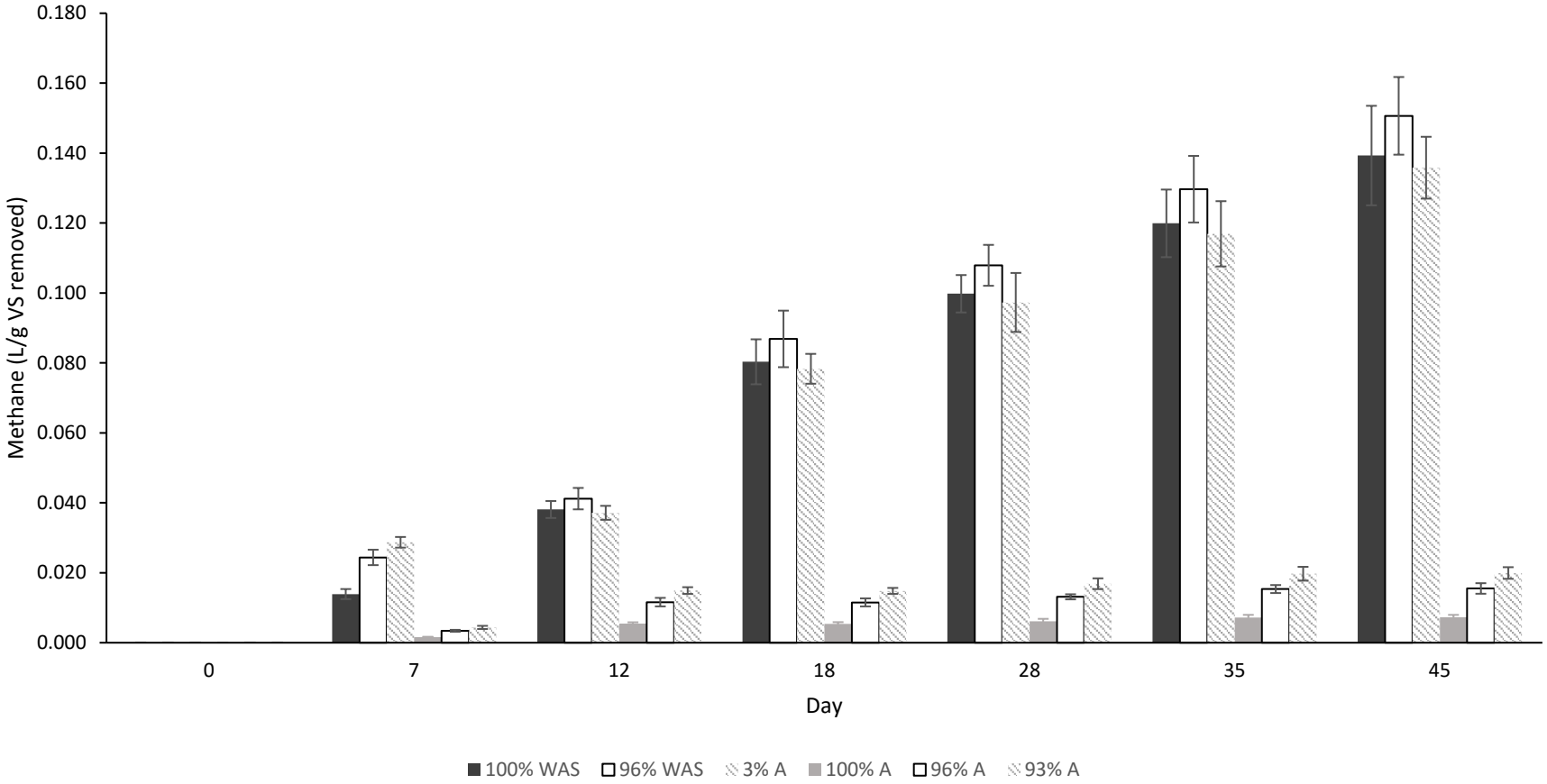


Fig. 6.10 Efficiency of methane production w/ inoculum vs. w/o inoculum on the anaerobic co-digestion of WAS and ALBAZOD in first 45 days; 100% WAS and A were both controls without inoculum (4%, w/w, g VS/L) (■); 96% WAS and A were both controls with inoculum (4%, w/w, g VS/L) (□); 3% A, mixed with 93% WAS and inoculum (4%, w/w, g VS/L) indicated as grey cross line; 93% A, also indicated as grey cross line were mixed with the same inoculum (4%, w/w, g VS/L) and 3% WAS. With ± analytical standard error (n=6)

## 6.4. Discussion

It was clear that the inclusion of a 4% inoculum from a previous anaerobic digestion reduced the pronounced lag period in both average TS and VS reduction, when compared to single substrate anaerobic digestion alone. This effect was associated with a benefit of higher biogas yield which will be discussed below.

### 6.4.1. TS and VS reduction

Anaerobic digestion was performed for 90 days and the average volatile solids reductions were shown in Fig. 6.5. The 96% A with inoculum digestion showed the highest VS reduction percentage while the 100% WAS digestion set showed the lowest. This is in agreement with the findings by Wang et al. (2013) and Yuan et al. (2012). Wang et al. (2013) studied mesophilic anaerobic co-digestion of algae and WAS for 45 days. The average volatile solids reduction using pure algae (*Chlorella sp.*, originally collected from a Amherst WWTP, MA, USA) and inoculated in a laboratory culture digestion set showed the highest reduction in VS with 100% WAS recording the lowest VS reduction. However, the author also stated that the lowest biogas production was observed from pure algae digestion, which was an indication of high volatile solids reductions in the digestion. It was suggested that this was not mediated via $CH_4$ production but likely due to volatilization of volatile organics produced in the digesters during solids measurement. They also suggested that some addition of algae into existing anaerobic digesters at WWTP could provide a benefit of high solids reduction, which in agreement with this current study (Wang et al., 2013).

In this current study, the average volatile solid reductions were shown in Fig. 6.5. The 96% A with 4% inoculum digestion showed the highest percentage VS reduction (37.01%) while the 100% WAS digestion showed the lowest (28.09%). This is in agreement with a study by Samson and LeDuy (1983). The authors studied the performance of anaerobic digestion of *Spirulina maxima* algal biomass by addition of three types of carbon-rich wastes: primary domestic sewage sludge (SEW), peat

hydrolysate (PHY) and spent sulfite liquor (SSL). The sewage sludge came from Valcartier wastewater treatment plant in Quebec, Canada. All substrate mixtures comprised different volumes of SEW, PHY and SSL to a constant VS concentration of *S. maxima* algal biomass (40 kg VS $m^{-3}$). By using a mixture of half-half of *S. maxima* algal biomass and SEW ($S_{to}$ 77 kg VS $m^{-3}$) , a 2.1 fold increase in the methane yield corresponding to 0.36 L $CH_4$ $g^{-1}$ VS and a 2.3 fold increase in methane productivity corresponding to 1.41 $m^3$ $CH_4/m^3$ $d^{-1}$. In addition, the highest VS reduction (48.1 %) was observed in this mixture (Samson & LeDuy, 1983). These results are also in a similar agreement with this current study.

It is also important to note that the ALBAZOD was well digested by itself under anaerobic digestion in this current study. The digestion of 100% ALBAZOD resulted in approximately 33.66% volatile solids reduction (VSR) which was greater than the 28.09% VSR observed in the 100% WAS mono-digestion, without the use of inoculum. This is also in agreement with a study by Yuan et al. (2012). In their study, two species, *Spirulina platensis* (cyanobacteria) and *Chlorella sp.* were grown on sludge centrate and a nitrified wastewater effluent (NWE) and centrate mixture. Harvested algae were co-digested with NWE at varying ratios. The volumetric ratios of algae : NWE were, 100%, 70%, 50%, 30%, and 0%. As the authors also noted, due to the algal biomass concentration being lower than that of NWE, the resulting algae:NWE mass ratios were 100%, 52%, 32%, 17%, and 0%. The authors demonstrated that the digestion of pure algal biomass resulted in approximately 57% VSR which was greater than the 47% reduction of VS observed from the NWE alone, which is also in a similar relationship to this current study although their reductions of VS were much higher. Interestingly, an overall VSR generally increased with increasing algal composition in the digester was also observed from their study. They reported that the reduction of VS value did not change once the algal mass fraction was > 32%. The digestion sets with 52% and 32% algae performed much better than the set with NWE only and slightly better than 100% algae, indicating that addition of algae to existing anaerobic digesters can improve overall digestion efficiency and potentially generate more biogas (Yuan et al., 2012). In this current study, however, significant decrease in TS reduction ($p < 0.05$) was only observed when the ALBAZOD percentage in the co-digestion with WAS was >26%. The average TS reduction percentage was halved from 31.04% to 15.35% in the co-digestion

comprising 96% WAS and 56% A. In contrast to the observations of TS reduction, as ALBAZOD percentages increased in the WAS co-digestions, the average percentage reduction in VS increased (Fig. 6.5). No significant difference ($p > 0.05$) in VS reduction was, however, identified between the co-digestions experiments.

### 6.4.2. Biogas accumulation yields

The data in Fig. 6.6 show that the lowest accumulated biogas volume (14.70 L) was observed in the 100% A digestion. This was also observed by Wang et al. (2013) where a pure algae digestion showed the highest VS reduction with the lowest biogas production (Wang et al., 2013). Wang et al. (2013) explained this was a good indication of the high VS reduction in the digestion was not mediated via $CH_4$ production but more likely due to volatilization of volatile organic production in the digester during solids measurement. As the overall higher VS reductions were observed in various co-digestion sets with the addition of ALBAZOD (when < 26% of the mixture), some addition of ALBAZOD into the WAS digestion indicates a benefit of both TS and VS reduction.

The percentage of $CH_4$ from 100% ALBAZOD was also substantially less with an average of 40.60% over the 90 days. In contrast, most digestions when ALBAZOD < 26% of the mixture showed similar $CH_4$ content compared to digestion with WAS only. These data strongly indicate that the $CH_4$ gas phase was rapidly reached in WAS + ALBAZOD co-digestion. Therefore, it suggests that an extended solid retention time (SRT) should be considered when co-digesting with algae or ALBAZOD in order to research the potential of higher biogas yield at late stage (e.g. day > 60).

The highest biogas accumulation (70.93 L) was observed in the 96% WAS experiment. Similar volumes of accumulated biogas were also observed in the 100% WAS and 3% A experiments with a total of 68.86 and 69.51 L accordingly. This study suggests that co-digestion of WAS and ALBAZOD increased the gas yield of ALBAZOD while maintaining similar gas yield from WAS. Although there was no significant difference observed, these results suggest that the addition of ALBAZOD provides the benefits

of both increased TS, VS reductions, and increased of OLR (organic loading rate). However, synergistic effects were undetermined from these sets of experiment.

### 6.4.3. Cumulative specific methane production (L $CH_4$ $g^{-1}$ $VS_{removed}$)

It was observed that a much longer SRT was required for solo ALBAZOD anaerobic digestion. It was suggested in previous chapters that it was the low biodegradability of algae cell wall which caused the extended period of digestion. The time of a pure ALBAZOD digestion was extended up to approximately 60 days. Belong day 60, the $CH_4$ yield increased more rapidly from 0.010 L $CH_4$ $g^{-1}$ $VS_{removed}$ to 0.038 L $CH_4$ $g^{-1}$ $VS_{removed}$ at day 90. However, the VS reduction increased only 15% (day 63: 40.09% VS compared with day 90: 54.81%). This is a strong indication that most of acid hydrolysis occurred early in this digestion and more hydrolyzed products converted to biogas during later digestion period hence the rapid increase of $CH_4$ yield at the late stage and the much extended digestion period than typical WAS (Wang et al., 2013).

Although it was shown ALBAZOD anaerobic biodegradability was higher than WAS in this study based on the higher VS reduction percentage, the challenge is on how to optimise the co-digestion conditions in order to improve its low methane production yield. From Fig. 6.7, it was clearly shown that by only adding a relatively ratios of WAS (3%), with or without inoculum, a significant increase of methane and decrease of carbon dioxide percentages were observed. In addition, the ALBAZOD used in this study mainly represents *Chlorella vulgaris* and *Scenedesmus sp.* as shown in Chapter 5. Some literature has shown wide variability between different micro-algal species with respect to their potential as substrates for methane production through anaerobic digestion. For example, Roberts et al. (2016) performed a comparative study of energy yields for a variety of microalgae species including *Isochrysis galbana*, *Thalassiosira pseudonana*, *Nannochloropsis occulata*, *Dunaliella sp.*, freshwater *Chlorella vulgaris* and *Scenedesmus spp.* Their study showed a broad range from 0.161 to 0.435 L $CH_4$ $g^{-1}$ VS observed in VS conversion to methane among the species. (Roberts et al., 2016). Additionally, it is generally reported that *Scenedesmus sp.* has poor degradability and a lower VS/TS ratio in its biomass (Frigon et al., 2013;

Lakaniemi et al., 2013; Roberts et al., 2016; Ward et al., 2014) which was also observed in this current study.

## 6.5. Reference


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# CHAPTER 7

# GENERAL DISCUSSIONS

# 7. GENERAL DISCUSSIONS

In depth discussion was incorporated to each relevant chapter. This section consolidates the conclusions from the research and also includes proposed future research directions.

This thesis presented four essential research areas:

- The determination of the influence of wastewater strength on the outcome of $CO_2$ addition for algal biomass (ALBAZOD) production
- The evaluation of the performance of anaerobic co-digestion of algal biomass (ALBAZOD) with pig slurry
- The effects of thermal pretreatment of algal biomass (ALBAZOD) on the outcome of anaerobic co-digestion with pig slurry (PS)
- The evaluation of the performance of the anaerobic co-digestion of algal biomass (ALBAZOD) with waste activated sludge (WAS)

The research presented in this thesis also builds on the outcomes of the High Integrity Australia Pork CRC's *Project 4A-101 Algae for Energy & Feed: a wastewater solution* (Buchanan et al., 2013) which reviewed options for the pork industry to integrate pig slurry treatment with growth of algal biomass. Conversion of $CO_2$ from biogas and from the mineralisation of organic carbon in pig slurry (as part of a waste treatment process) to more stable algal biomass would contribute to the pork industry by reducing $CO_2$ emissions. In addition, algal biomass produced in HRAPs treating piggery wastewaters removes $CO_2$, contributing to GHG mitigation, and is an additional source of biomass energy which could be released via co-digestion with pig slurry.

Recently it has been suggested that $CO_2$ addition to wastewater may enhance algal growth. The addition of $CO_2$ for algal growth could be sourced by stripping and capture of $CO_2$ from biogas produced from anaerobic digestion of piggery slurry in covered lagoons and/or from flue gases following combustion of $CH_4$ or fossil fuels from power stations.

A laboratory approach was utilised to examine the effect of the addition of $CO_2$ on the growth of microalgae in wastewaters of three different $BOD_5$ strengths. Somewhat uniquely in this area of wastewater research and algal biomass production a comparison was also made, between the outcomes for biomass production and treatment, of pH stasis using acid rather than $CO_2$.

The research presented here provides a better understanding of how to achieve integration of algae and wastewater treatment by determining, whether it is necessary to supply external $CO_2$, and evaluating the outcome of anaerobic co-digestion of algal biomass with either pig slurry or waste activated sludge.

**Aim 1 - Determination of the influence of wastewater strength on the outcome of $CO_2$ addition for algal biomass (ALBAZOD) production**

For some considerable time, carbon has been suspected of being a growth limiting factor in HRAPs treating wastewater, due to the high algal demand for it, whilst its concentration and bio-availability to algae is relatively low compared to other nutrients (Azov et al., 1982). According to Azov et al. (1982), about 48% of the incoming carbon will be in an inorganic form and 52% in organic form. The form of carbon preferred by most algal species for photosynthesis is unionised, dissolved $CO_2$. In the HRAP this will mostly come from daytime bacterial respiration. The degradation of bacterial biomass releases the main nutrients $NH_3$ and $CO_2$ for algal photosynthesis (Azov et al., 1982). This is quite a slow reaction rate, but has been calculated to proceed fast enough to supply $CO_2$ demand for algal photosynthesis in alkaline HRAP wastewater. Azov et al. (1982) determined that the conditions under which carbon could become limiting to algal productivity were low inlet water organic carbon, high algal concentrations when the inlet water has low alkalinity and long retention times. It is also recognised that the organic carbon in wastewaters, following bacterial mineralisation, is an important source of inorganic carbon for algal photosynthesis (Cromar & Fallowfield, 1997; Fallowfield & Garrett, 1985). Park and Craggs (2010) suggest that $CO_2$ addition to a high rate algal pond (HRAP) on a 4 day HRT nearly doubled algal production compared with one operated with $CO_2$ at an 8-day HRT in

summer conditions. However, in this study there was no comparison of performance in the absence of $CO_2$ enrichment (Park & Craggs, 2010).

In Chapter 3 the effect of external $CO_2$ addition, to wastewaters containing different amounts of organic carbon ($BOD_5$), on algal growth (ALBAZOD) and wastewater treatment was considered. There was an inconsistent response between the biomass indicators chlorophyll *a* and POC to $CO_2$ addition. The difference in chlorophyll *a* concentration between control and $CO_2$ amended cultures was statistically significant for all wastewaters irrespective of $BOD_5$ concentration. Furthermore, the difference in chlorophyll *a* concentration between the control culture and a culture where pH stasis was maintained by acid, rather than $CO_2$, addition was also statistically significant. In wastewater cultures the biomass comprises not only of algae but also bacteria, zooplankton and detritus (ALBAZOD). The objective of $CO_2$ addition is to increase inorganic carbon available for photosynthetic conversion to organic carbon, which is to increase primary productivity. Comparing the response of particulate organic carbon (POC) to $CO_2$ addition was considered more relevant in these systems since it includes changes in both primary and secondary productivity in response to carbon addition.

Considering the response of POC, only the low $BOD_5$ (15mg/L) wastewater cultures showed statistically significant increases in POC following $CO_2$ addition compared to the respective control wastewater culture. There was also a corresponding, statistically significant increase in chlorophyll *a* in this wastewater following $CO_2$ addition. This suggests that the supplementation of low $BOD_5$ wastewater with $CO_2$ increases biomass production. The low concentration of organic carbon decreases the concentration of inorganic carbon both produced by bacterial mineralisation and subsequently available for available for biomass production. Interestingly, corresponding statistically significant increases in both POC and chlorophyll *a* were only recorded in the mid strength $BOD_5$ (72 mg/L) wastewater where pH stasis was maintained by acid addition. The maintenance of pH stasis in the absence of carbon addition implies that the forcing of the carbonate bicarbonate equilibrium in favour of free $CO_2$ was of more likely importance to productivity than external carbon addition.

In contrast to the statistically significant positive response of chlorophyll *a* to $CO_2$ addition to both high $BOD_5$ (120 mg/L) and mid strength $BOD_5$ (78 mg/L) wastewaters there was no similar statistically significant positive response of POC, in either wastewater, to $CO_2$ addition. These results suggest that the addition of $CO_2$ did not increase biomass production since the native organic carbon pool, following bacterial mineralisation, within both wastewaters was sufficient to support optimal biomass production under the prevailing conditions of light and temperature.

This raises questions whether the addition $CO_2$ will provide a significant and substantial improvement in microalgae growth when the addition costs of implementing $CO_2$ injection equipment are also considered. In terms of supplying an external carbon source such as $CO_2$ in wastewater for microalgal cultivation, it is also important to distinguish and describe the different characteristic of wastewater medium to be used in the cultivation system, as the levels of BOD and total carbon will be varied accordingly to the prior treatment stages. If the BOD and internal carbon content in the wastewater is already sufficient, the effects of $CO_2$ addition on algal growth in wastewater may not be cost-effective for enhancing biomass production

The differential response of wastewaters to $CO_2$ addition, in terms of biomass production, reported here suggests that careful consideration is required before investing capital in infrastructure to support $CO_2$ addition to large scale systems. The results suggest that wastewaters with low $BOD_5$ content or a low available organic carbon pool or which have been extensively pretreated resulting in a recalcitrant organic carbon pool resistant to mineralisation are *most* likely to respond positively to $CO_2$ addition. In contrast, wastewaters which have not been extensively treated and which contain a large, readily mineralisable organic carbon pool are *unlikely* to respond positively to $CO_2$ addition.

The infrastructure required to manage addition of $CO_2$ to algal based wastewater treatment systems to increase biomass production is a significant additional capital cost. Data from Park et al. (2016) showed that inclusion of infrastructure for $CO_2$ addition to enhance biomass production in two hectare scale HRAPs treating

wastewater in Cambridge and Christchurch, New Zealand contributed to between 30 to 34% of the capital costs of the system for only relatively minor percentage gains in production and wastewater treatment, the significance of which was uncertain since no statistical analysis was provided. It is recommended that prior to investing in additional infrastructure to support $CO_2$ that extensive pre-screening of the wastewater and its response to $CO_2$ addition be conducted (Park et al., 2016).

Furthermore, a consequence of a presumed, ill-considered, requirement for $CO_2$ addition to an algal wastewater treatment system, is that it has the potential to limit the adoption of these systems by decision makers, who may erroneously conclude that they need to be built in a specific location adjacent to a power plant to be effective. This is clearly not the case. Additionally, the misconception may also reduce adoption of these systems in remote and rural communities where they have been shown to be effective in the absence of $CO_2$ addition (Young et al., 2016).

The proposed future directions can be summarized as follows:

- Assessment of a wider range of BOD strength from different types of wastewater.
- Assessment of a comparison study between $+CO_2$ vs acid on low $BOD_5$ wastewater.
- Assessment of different $CO_2$ concentrations on different microalgae species in the wastewater cultivation system.
- Build on the complexity identified in this thesis by assessing the effect of supplying $CO_2$ in larger algal cultivation systems or HRAPs

**Aim 2 - The evaluation of the performance of anaerobic co-digestion of algal biomass (ALBAZOD) with pig slurry**

Chapter 4 reported the anaerobic, co-digestion of algal biomass (ALBAZOD) and pig slurry under ambient psychrophilic temperatures (17-25 ℃). Algal biomass is relatively high in nitrogen, which results in the production of high concentrations of

ammonia upon digestion which may inhibit the microorganisms involved in the anaerobic digestion process, additionally this elevates the pH which may further inhibit the digestion. Methane production from swine slurry has always been reported as relatively low due to several factors such as the high quantity of water, unbalanced carbon/nitrogen (C/N) ratio or high solids content which requires a long hydrolysis time. Two major strategies have been suggested to overcome these limitations namely pre-treatment of manure or co-digestion with other substrates. From this chapter, the optimum ratio of algal biomass to pig slurry which maximises methane production (quantity and quality) were examined. The quantity and quality of the biogas ($CO_2$ & $CH_4$) was reported, together with key process parameters including pH, COD, TS, VS, and $NH_4$-N.

The major finding of this research was that methane production from ALBAZOD mono-digestion was relatively low, ranging from 0.040 L CH4 $g^{-1}$ $VS_{removed}$ at day 73 to 0.174 L CH4 $g^{-1}$ $VS_{removed}$ at day 91, when compared to pig manure mono-digestion (0.339 L $g^{-1}$ $VS_{removed}$). This is in agreement to gas yield in the literature which ranges from 0.150 to 0.450 L $g^{-1}$ VS (note units were L $g^{-1}$ $VS_{add}$) for similar microalgae species, although they are in different reactor configurations and operating modes. The results suggested that anaerobic co-digestion of pig slurry and ALBAZOD (i.e. a lower $NH_4$-N concentration) provided a benefit on neutralising the high $NH_4$-N concentration on the pig slurry.

One of the challenges of this research was the low VS loading rate in low concentration of microalgae biomass present in large volume of water sample. However, this was considered a typical ALBAZOD substrate obtained following dissolved air flotation; a common and relatively low cost separation technology suitable for on-farm operation, that is without the adoption of high energy – high capital cost concentrating systems such as centrifugation.

Co-digestion of 96.5% (VS w/w) pig slurry (PS) + 3.5% ALBAZOD (A) resulted in a slightly higher methane yield than 100% PS alone (0.344 vs 0.339 L $CH_4$ $g^{-1}$ $VS_{removed}$), however, the increase was not significantly different statistically. In other cases, the introduction of ALBAZOD into the pig slurry led to a reduction of the methane yield since the biodegradability of ALBAZOD was lower than the biodegradability of pig

slurry. This is in agreement with the conclusion of Astals et al. (2015) and Gonzalez-Fernandez et al. (2011) who also identified synergistic mechanisms when co-digesting algae and pig manure. Astals et al. (2015) concluded that raw algae biodegradability increased from 0.163 to 0.245 L $CH_4$ $kg^{-1}$ VS due to synergistic mechanisms (Astals et al., 2015). This was also in an agreement with Gonzalez-Fernandez (2011) who noted improved algal (a mixture of *Chlorella vulgaris* and *Scenedesmus obliquus*) biodegradability when co-digested with pig manure (Araujo et al., 2011). Astal et al. (2015) theorized that the enhancement of the raw algae biodegradability in the presence of pig manure was related to the addition of specific microbes within the pig manure able to disrupt algal cell wall rather than in relation to an optimized C/N ratio.

To overcome this low C/N ratio problem in microalgae, Gonzalez-Fernandez et al. (2011) and Shouquan et al. (2009) suggested the addition of microalgae to pig manure prior to digestion (González-Fernández et al., 2011; Wang et al., 2009). However, it was observed in the study reported here that ALBAZOD was able to pass through the anaerobic digestion and remained intact and partially digested after the 91 days period. In this regard, *Scenedesmus sp.* cell wall has been described as a rigid wall of cellulose and hemicellulose, which together with the sporopollenin-like biopolymer provides great resistance to enzymatic degradation (Mendez et al., 2014; Mussgnug et al., 2010). Gonzalez-Gernandez et al. (2011) also noted that the C/N ratio of the digestion medium would be only balanced once the microalgae cell wall is broken, which leads to the investigation of using microalgae for anaerobic digestion after thermal pre-treatment.

The proposed future directions are as follows:

- Assessment of the co-digestion under mesophilic and thermophilic temperature ranges to enable comparison with this psychrophilic study.
- Assessment of pig slurry collected from different periods of year, associated with the diets used in the particular farm – which can change the composition of pig slurry.
- Assessment of ALBAZOD collected from different seasons and species.
- Assessment of extended anaerobic digestion period with suggestions up to 100-300 days.

- Build on the complexity of this thesis by assessing the effect of ALBAZOD anaerobically co-digest with pig slurry in a larger scale. For example, investigation of anaerobic co-digestion with ALBAZOD from HRAPS and pig slurry in an anaerobic lagoon under outdoor conditions.

**Aim 3 – The effects of thermal pretreatment of algal biomass (ALBAZOD) on the outcome of anaerobic co-digestion with pig slurry (PS)**

In Chapter 5 the impact of thermal pretreatment by autoclaving on biogas yields was reported. ALBAZOD from wastewater was pretreated by autoclaving at 120 °C for 1, 2, and 3h. The thermally pretreated ALBAZOD was anaerobically co-digested with pig slurry under ambient psychrophilic temperatures (17-25 ℃). Poor digestion of ALBAZOD during the early period of digestion while gas yield increased slowly after 73 days of digestion, suggesting that long solid retention time is needed for solo algae-based anaerobic digestion. A higher yield in methane production was observed following pretreatment. The thermal pretreatment clearly disrupted the cell wall structure, causing the release of internal algal organic matter (AOM). The disruption was more effective for *Chlorella vulgaris* than *Scenedesmus sp.* with, after 3h treatment, a more visible disaggregated cell wall structure. As a consequence of thermal treatment (120 °C for 3h) there was a statistically significant increase of SCOD.

The methane production via anaerobic codigestion of ALBAZOD and pig slurry was substantially increased following thermal pretreatment at 120 °C for 3h. The highest methane production (0.400 $CH_4$ L $g^{-1}$ $VS_{removed}$) was observed when 3.5% (VS w/w) ALBAZOD was co-digested with pig slurry, compared with 0.368 $CH_4$ L $g^{-1}$ $VS_{removed}$ recorded for 100% pig slurry at day 91. However, the methane production decreased with an increase of ALBAZOD ratios in the mixtures. When the ALBAZOD ratio was beyond 7.1% A, the methane production decreased to 0.280 $CH_4$ L $g^{-1}$ $VS_{removed}$ at day 91. The lowest $CH_4$ production (0.036 $CH_4$ L $g^{-1}$ $VS_{removed}$) was observed from the 100% ALBAZOD digestion after 21d, and then gradually increased to 0.053 $CH_4$ L $g^{-1}$ $VS_{removed}$ at day 72, and rapidly increased to 0.183 $CH_4$ L $g^{-1}$ $VS_{removed}$ at day 91. There was no significant difference ($p > 0.05$) in the methane production between all

mixtures comprising thermally treated and untreated controls The exception was a significant increase ($P < 0.05$) in the thermally pretreated 3.5% A co-digestion (0.400 L $CH_4$ $g^{-1}$ VS $_{removed}$) when compared to control (0.344 L $CH_4$ $g^{-1}$ VS $_{removed}$).

These results are in agreement with Chen and Oswald's (1998) demonstration of thermal pretreatment combined with chemical pre-treatment using sodium hydroxide and variable exposure times. Their study reported all pretreatments produced better results than untreated control comparisons. Their results also demonstrated that the most efficient pretreatment for microalgal biomass required heating up to 100 °C for at least 8h without an increase of pH by using the addition of sodium hydroxide. The biogas productivity was increased by 33% after the thermal pretreatment. The study also indicated that up to 66% of the untreated microalgal biomass was undigested due to the protection of intact cell wall throughout the digestion period (Chen & Oswald, 1998).

However, the results reported here also imply that thermal pretreatment is energy intensive and the no thermal pretreatment was the best option in terms of net energy production in biogas, since the additional methane production was insufficient to balance the energy required to thermally pretreat the biomass. There are conditions under which it may still be beneficial to carry out the pretreatment at a relatively lower temperature. For example, in conditions where excess heat can be captured from associated processes near or in the anaerobic digestion infrastructure; including the heat for the surrounding of digester itself, water heating, and power generators in the algae-to biogas infrastructure or near to the piggery. These systems can compensate a portion of the energy requirement to pretreat the digester feedstock.

Further studies are required to investigate the synergy between pig slurry and ALBAZOD under different conditions and/or different pre-treatments. The proposed future directions can be summarized as follows:

- Although it was clear that the no-pretreatment option was favoured under the best case scenario, some excess heat can be captured from associated processes near or in the anaerobic digestion infrastructure. Therefore, assessment on

using the heat generalised from anaerobic digestion for the surrounding of digester itself, water heating, and power generators in the algae-to biogas infrastructure or near to the piggery are required in extended researches. These systems can compensate a portion of the energy requirement to pretreat the digester feedstock.

- Assessment of different pre-treatment methods with low energy intensity, including different aspects such as using chemicals or enzymes to degrade the substrates.

**Aim 4 - The evaluation of the performance of the anaerobic co-digestion of algal biomass (ALBAZOD) with waste activated sludge (WAS)**

Mata-Alvarez et al. (2014) reviewed the achievements and perspectives of anaerobic co-digestion within the period 2010-2013 (Mata-Alvarez et al., 2014). It was noted that anaerobic co-digestion between sewage sludge and the organic fraction of the municipal solid waste are traditionally the most reported co-digestion mixture, while the studies of anaerobic co-digestion with algae or ALBAZOD are still limited.

The outcomes of the anaerobic co-digestion of waste activated sludge (WAS) with ALBAZOD at ambient psychrophilic temperatures (17-25 ℃) were determined. Additionally the effect of using an inoculum from a previous anaerobic digestion on the outcome of digestion was also evaluated.

The digestion with 96% A (VS, w/w) and 4% inoculum showed the highest VS reduction percentage while the 100% WAS digestion showed the lowest. This is in agreement with the findings by Wang et al. (2013) and Yuan et al. (2012). Highest methane production 0.276 L $CH_4$ $g^{-1}$ $VS_{removed}$ was observed from 96% WAS with inoculum. The digestion with 96% WAS and inoculum showed the highest percentage reduction in TS (31.04%) while the 100% ALBAZOD (A) digestion showed the lowest (6.61%). A significant decrease in TS reduction ($p < 0.05$) was only observed when the ALBAZOD percentage in the co-digestion with WAS > 26%. No significant

difference ($p > 0.05$) in VS reduction was identified between the co-digestions experiments.

The methane production decreased as the ratio of ALBAZOD increased in the co-digestion mixtures. When the ALBAZOD ratio was > 6% A, the methane production decreased to below than 0.200 L/g $VS_{removed}$. The lowest $CH_4$ production was observed from the 100% A experiment with only 0.010 L $CH_4$ $g^{-1}$ $VS_{removed}$ accumulated over the first 60 days and then rapidly increased up to 0.038 L $CH_4$ $g^{-1}$ $VS_{removed}$ at the day 90 (Fig. 6.9). It was observed that a much longer solid retention time was required for solo ALBAZOD anaerobic digestion. It was concluded overall conclusion, that the low biodegradability of algae cell wall which caused the extended period of digestion. The digestion time of a pure ALBAZOD digestion was extended up to approximately 60 days.

The proposed future directions can be summarized as follows:

- Assessment of a wide range of sludges that is available near to wastewater treatment systems including agriculture and winery waste.
- Assessment of different pre-treatment methods with low energy intensity, including different aspects such as using chemicals or enzymes to degrade the substrates.
- Assessment of different ALBAZOD on different microalgae species from different cultivation systems such as waste stabilisation ponds and HRAPs.

**Final thoughts**

The potential for wastewater grown microalgae to be a source of energy and feedstock was recognized following the oil crisis of the 1970's, with now a considerable interest in microalgae as sources of liquid biofuels due to their high biomass productivities. However, there is a growing realisation that the grow microalgal biomass for fuel may compete indirectly with food production due to the concerns of land availability, costs of water, and nutrient supplies. With the increasing restrict environmental regulation requires wastewater treatment incorporating enhanced nutrient removal systems, particularly for nitrogen, which results in the potentially valuable commodity being emitted to the atmosphere as nitrogen gas or nitrous oxide. This increases the carbon footprint of the pig industry with direct emissions, as well as the increasing industry energy consumption with greenhouse gas by-product $CO_2$. The production of microalgal biomass via the integration of piggery wastewater treatment is a potential pathway to remove these carbon footprints, along with anaerobic co-digestion for biogas production. Supplying inorganic carbon in the form of $CO_2$ from the wastewater treatment processes or other sources could possibly overcome any limitations to algal growth as some suggest, while further reducing the carbon footprint. The significant of this research was to understand the perspective of net impact on using microalgae or ALBAZOD. While one may ask, if the energy needed to utilise $CO_2$ directly into the algae cultivation system does not produce sufficiently more incremental algae than what would have occurred by just using atmospheric carbon or internal carbon pool from wastewater, then the carbon balance should be better if the two systems are not coupled.

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