Abstract

The consumption of non-renewable fossil fuels has increased with the rapid development of modern industry, agriculture and transportation. The oil-based bio-refinery process based on photosynthetic microalgae has been the most promising for biofuel production. However, the production on a commercial scale is not yet economically viable. Biomass productivity plays an important role in achieving economical mass productivity of compounds produced by microalgae and this is a major factor for success in any new industrial scale venture. This thesis seeks to develop *Chloroparva pannonica* which has the potential for the bio-refinery process to grow rapidly, produce higher biomass and enhanced production of a type of carotenoid, lutein.

As the project involved the screening of a large number of mutants, rapid methods using relatively low levels of sample were developed. The lutein content was extracted and measured with a one-step closed-tube (OSCT) under dark conditions without the need for large amounts of samples and solvents.

The production medium was optimised via the formulation of new FU medium, to achieve high-density culture as the standard Guillard’s F/2 medium resulted in low biomass productivity. In the FU medium, urea was used as the main nitrogen sources. In addition, the concentration of ferric ions was increased twofold, cobalt, sodium molybdate and zinc were increased fourfold and manganese was increased eightfold.

The exposure of *Chloroparva pannonica* to different concentrations of copper resulted in the unexpected selection of *Chloroparva pannonica* Cu40 with a higher growth and lutein content than the *Chloroparva pannonica* wild type. *Chloroparva pannonica* Cu40 was been selected for the random mutagenesis though UV-C irradiation. The mutants were further screened for resistance to one of two herbicides, aclonifen and chlodinafop-propargyl. Mutants that exhibit in high lutein content and high biomass in small scale were chosen for the short-term laboratory experiments in

batch mode using 3 L flasks with 1% carbon dioxide supplied. 10 mutants that exhibit in high lutein content and high biomass were chosen then for the short-term laboratory experiments in 11 L rectangular inter-loop airlift photobioreactor. Three (3) of these mutants (Fu4, Fu5C and Fu41A) were selected for the taxonomy study along with the *Chloroparva pannonica* Cu40 and wild type to ensure that they belong to the same species and were not contaminants.

Based on the molecular analysis of the 18S rRNA gene sequence, *Chloroparva pannonica* wild type (WT), Cu40 parent culture (PC), Fu4, Fu5c and Fu41A were close to *Chloroparva* sp. ACT 0608 within Trebouxiophyceae, Chlorophyta. The molar guanine+cytosine (G+C mol%) of the genomic DNA ranged from 56.27 – 59.06%. The spherical cells ranged in size from 2–5 µm. The lipid content ranged from 22.94–25.59%. The predominant fatty acids were oleic acid, linoleic acid, linolenic acid and palmitic acid. The pigments were chlorophyll *a* and *b*, while the carotenoid was lutein. The lutein concentration ranged from 1864–5502 mg/kg of biomass. However, the lipid and lutein content could be manipulated based on their cultivation conditions. These strains were tolerant of salinity up to 7%, a pH ranging from 6–11 and temperature from 10–30°C.

As previously mentioned, 10 mutants that showed higher growth, lutein content and total lipid content than the Cu40 parent culture and wild type were selected to evaluate their growth, lutein and lipid content in batch mode in an 11 L rectangular inter-loop airlift photobioreactor. Of these, Fu5C was identified as the strain for the semi-continuous cultivation in the 11L photobioreactor. In order to improve the yields of lutein, it was decided in the current study, based on the growth and lutein content patterns, to harvest the culture every eight days when the lutein was at its highest point while maintaining the exponential phase of the growth. Having achieved very high lutein concentration the draw-fill cycles were continued for a 94-day period. In the long-term semi-continuous system, the performance (biomass productivity, lutein productivity and lipid productivity) of *Chloroparva pannonica* Cu40 parent culture was better than that of Fu5C and wild type. The maximum total dried biomass, biomass productivity, specific growth rate, lutein content and lutein productivity were 1.678 g/l, 0.15 g/l/day, 0.482 µ/day, 5768 mg/kg of biomass and 604.32 mg/kg of biomass/day, respectively. Due to the performance (high biomass productivity and lipid productivity) of Cu40 parent culture, the long-term semi-continuous 11 L rectangular inter-loop airlift photobioreactor system was continued for a further 96-day period to investigate the ability to produce sufficient biomass and lutein yield to be a feedstock for the microalgae based bio-refinery process. The maximum total biomass, biomass productivity and specific growth rate were 1.705 g/l, 0.1538 g/l/day and 0.4963 μ/day, respectively. The maximum lutein content was 6960 mg/kg of biomass while lutein productivity was 613.4 mg/kg of biomass/day. However, lipid content ranged from 10–22% while the lipid productivity averaged 2.5%/day. In the current study, lutein was successfully extracted and purified from *Chloroparva pannonica* Cu40 parent culture cultivated in the long-term semi-continuous mode in an 11 L rectangular inter-loop airlift photobioreactor.

In conclusion, this work has demonstrated that, by using the semi-continuous system, *Chloroparva pannonica* Cu40 and Fu5C have shown improved biomass productivity and enhanced lutein accumulation when cultivated using the FU medium. These strains could be considered as promising microalgae for the production of lutein in a large-scale system