Chemical Engineering Journal 151 (2009) 73–78

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Microalgal growth characteristics and subsequent influence on dewatering efficiency

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ARTICLE INFO

Article history:
Received 8 October 2008
Received in revised form 27 January 2009
Accepted 29 January 2009

Keywords:
Microalgal cultivation
Intercellular interactions
Dewatering
Tangential flow filtration

ABSTRACT

Dewatering of microalgal culture is a major bottleneck towards the industrial-scale processing of microalgae for bio-diesel production. The dilute nature of harvested microalgal cultures poses a huge operation cost to dewater; thereby rendering microalgae-based fuels less economically attractive. This study explores the influence of microalgal growth phases and intercellular interactions during cultivation on dewatering efficiency of microalgae cultures. Experimental results show that microalgal cultures harvested during a low growth rate phase (LGRP) of 0.03 d^{-1} allowed a higher rate of settling than those harvested during a high growth rate phase (HGRP) of 0.11 d^{-1}, even though the latter displayed a higher average differential biomass concentration of 0.2 g L^{-1} d^{-1}. Zeta potential profile during the cultivation process showed a maximum electronegative value of -43.2 ± 0.7 mV during the HGRP which declined to stabilization at -34.5 ± 0.4 mV in the LGRP. The lower settling rate observed for HGRP microalgae is hence attributed to the high stability of the microalgal cells which electrostatically repel each other during this growth phase. Tangential flow filtration of 20 L HGRP culture concentrated 23 times by consuming 0.51 kWh/m^3 of supernatant removed whilst 0.38 kWh/m^3 was consumed to concentrate 20 L of LGRP by 48 times.

1. Introduction

Contemporary media commentaries on increasing petrol prices resulting from the depletion of fossil fuels and the alarming rate of global warming issues has resulted in bio-diesel becoming an attractive alternative transport fuel [1–3]. Bio-diesel is a fuel source obtained from fats and oils contained in renewable biological resources such as grains and algae [1]. It is mainly produced via trans-esterification of fats and oils, where vegetable oil and animal fats are currently used as raw materials. Animal fat and vegetable oils are typically made of triglycerides which are esters of free fatty acids with trihydric alcohol. In the trans-esterification process, methyl/ethyl alcohol is deprotonated with a base which turns it into a stronger nucleophile to convert the triglycerides to methyl/ethyl-esters which make up the bio-diesel [1–3]. Vegetable oils are a renewable source of raw materials for bio-diesel production and have the potential to reduce carbon dioxide emissions through photosynthesis [2,4]. However, a major problem associated with the utilisation of vegetable oil as raw materials is its capacity as a valuable food commodity for human consumption, thus increasing the demand and cost for this resource as large areas of land, capital and manpower are required for cultivation, and this makes bio-diesel production an economically challenging process [5]. Also, used vegetable oils are a finite resource and require extra downstream processing, which affects the amount of bio-diesel that can be produced and the economics of the process.

Microalgae have been identified as a potential raw material alternative for bio-diesel production as it does not require a large area of land for cultivation, possesses a high growth rate and accumulates a satisfactory amount of lipid for bio-diesel production. Microalgal lipids are mostly neutral lipids due to their lower degree of unsaturation and their accumulation in the microalgal cell at the early or late end of growth stage depending on the strain. This makes microalgal lipids a potential diesel fuel substitute [6,7]. However, fresh microalgal cultures are usually very dilute suspensions with concentrations less than ~1 g/L, so effective culture concentration or dewatering mechanisms are required to facilitate maximum lipid extraction [8,9]. Whilst dilute cultures may be ideal for sufficient light penetration, high-density microalgal cultures can provide the needed biomass for production processes without extensive dewatering. For example, a concentration of 15% (w/v) is the minimum microalgal solid concentration required by Bio-Fuels, Pty Ltd. (Laverton, Victoria, Australia) for lipid extraction. The cost of dewatering of microalgal culture for lipid extraction can make up to 20–30% of the total cost of production of lipid concentrate [9,10].

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doi:10.1016/j.cej.2009.01.047

Therefore, finding a sustainable dewatering method is an important factor for consideration in order to make microalgae a commercially viable source for the production of bio-diesel. Several techniques for the dewatering of microalgal cultures have been developed [11]. These include flocculation, flotation, centrifugation, sedimentation, electro-flocculation and filtration. Most of these techniques present several disadvantages under un-optimised conditions not only because of the high costs of operation but also the frequently low separation efficiencies and the intolerable product quality. An efficient and versatile microalgal separation process should be workable for all microalgal strains, yield a product with a high dry biomass weight percentage and require moderate cost of operation, energy and maintenance. Although many works applying different dewatering technologies have been reported, reports on exploiting the natural growth mechanism and intercellular interaction during microalgal cultivation to enhance dewatering performances are limited. This limitation has been a contribution factor to the high economics of microalgal dewatering. The following study explores the influence of intercellular interactions and growth characteristics during cultivation on the dewatering efficiency of microalgae. This body of work is perceived to have the capacity to increase the ease of dewatering of microalgae under reduced economics irrespective of the dewatering technique, whilst maintaining the microalgal biomass and lipid levels. An energy consumption and energy saving analysis employing tangential flow filtration is presented.

2. Experimental

2.1. Materials

Potassium nitrate (KNO_3) (BDH Chemicals Pty Ltd., MW 101.10, 99.0%), sodium phosphate monobasic (NaH_2PO_4) (Merck Pty Ltd., MW 119.98, 99.5%), manganese chloride ($MnCl_2$) (BDH Chemicals Pty Ltd., MW 125.84, 98.0%), zinc sulphate ($ZnSO_4$) (BDH Chemicals Pty Ltd., MW 161.47, 99.5%), cobalt nitrate ($Co(NO_3)_2$) (BDH Chemicals Pty Ltd., MW 182.94, 97.5%), copper sulphate ($CuSO_4$) (Prolabo Pty Ltd., MW 159.61, 98.5%), sodium molybdate ($Na_2MoO_4{\cdot}2H_2O$) (AJAX Chemicals Pty Ltd., MW 241.95, 98.0%), sodium metasilicate (Na_2SiO_3) (AJAX Finechem Pty Ltd., MW 122.06, 99.7%) and glycerol ($C_3H_5(OH)_3$) (Merck Pty Ltd., MW 92.10, 99.5%) were used for growth medium preparation for microalgae cultivation.

2.2. Microalgal strain description

The microalgae used throughout this body of work was a multi-strain *Tetraselmis suecica/Chlorococum* sp. culture cultivated in bag photobioreactors located at Bio-Fuels Pty Ltd., Laverton, Victoria, Australia. The microalgal specie was obtained from CSIRO Microalgae Research Centre (Hobart, Australia) as *T. suecica* in pacific artificial seawater. The culture was maintained with a modified F/2 medium [12] in 100 L bag photobioreactors cultivated outdoor and kept under semi-continuous conditions by 20% (v/v) dilution with fresh medium after harvesting equal volume of culture on a daily basis. *Chlorococum* sp., as a result of the weather conditions arising from the switch from winter to summer, also became established in the photobioreactors. Generally, one would expect a mixed culture in an open system. The *Chlorococum* sp. grows very well in a high temperature environment thus becomes dominant in the summer whilst *T. suecica* grows well at moderately low temperature conditions, hence becomes dominant in the winter. The presence of multi-strain microalgal systems in outdoor photobioreactors has been previously reported [13,14].

2.3. Tangential flow filtration (TFF) unit

The TFF unit is a 4 GPM Pellicon cassette system (Millipore, DUOBLOC TM, USA) consisting of a 0.22 μm Pellicon 2 filter with manifold plates, a positive displacement pump with variable speed and a retentate diaphragm valve. The pump is configured with a wattmeter for measuring energy consumption. A feed tank, filtrate tank, feed line, filtrate line and retentate line are connected to the unit. The retentate was fed back into the feed tank in order to recycle and concentrate the microalgal culture.

2.4. Simple outdoor cultivation of microalgae in bag photobioreactor

10% (v/v) culture was established in a 100 L open outdoor bag photobioreactor containing growth medium with compositions dissolved in sterile filtered natural seawater: KNO_3 (250 mg L^{-1}), NaH_2PO_4 (30 mg L^{-1}), $MnCl_2$ (7.2 mg L^{-1}), $ZnSO_4$ (0.4 mg L^{-1}), $Co(NO_3)_2$ (2 mg L^{-1}), $CuSO_4$ (9.8 mg L^{-1}), $Na_2MoO_4{\cdot}2H_2O$ (0.126 mg L^{-1}), Na_2SiO_3 (22.7 mg L^{-1}) and glycerol (20 mg L^{-1}). The temperature of the culture was recorded to be between 14 and 21 °C, corresponding to ambient temperatures between 17 and 25 °C. The culture was aerated with compressed air at 34.5 kPa (gauge) as a source of CO_2 and mixing. Cell density, dissolved CO_2 concentration and culture pH were monitored offline by taking daily samples in the early mornings before sunrise and afternoons before sunset. The water volume evaporated a day was less than 60 mL. The cultivation was terminated after the stationary growth phase was obtained.

2.5. Determination of dry cell weight of microalgae

The microalgal culture was mixed thoroughly before collecting each sample. 50 mL aliquot of the culture was pipetted and transferred into a 50 mL centrifuge tube. Centrifugation (Heraeus, multifuge 3S-R, Germany) was performed at 4500 × *g* for 20 min. The supernatant was carefully poured off from the tube in order not to disturb the pellet or pour off any unsettled material. The pellet from the centrifugation was rinsed with 10 mM HCl solution, transferred to a pre-weighed heat-resistant crucible and dried at 105 °C in an oven overnight. The sample was removed from the oven, kept in a desiccator and weight. This weight minus the weight of the empty crucible gives the dry cell weight. Samples were generally run in triplicate for each data point.

2.6. Measurement of microalgal zeta potential and size

The zeta potential and average cell size of the microalgal cells were monitored during the cultivation process by using Zetasizer Nano ZS series (Malvern, ZEN 3600, Australia) and Mastersizer (Malvern 2000, Australia) respectively. Microalgal culture samples were uniformly suspended, 1.0 mL was pipetted into a cuvette and inserted into the units for zeta potential and size measurements. Zeta potential and cell size measurements were performed in triplicate.

2.7. Determination of microalgal settling rate

10 L of microalgal culture was harvested at different growth phases (high growth rate phase and low growth rate phase) during cultivation and placed in a transparent glass bottle. The cultures were allowed to settle by gravity under different conditions of daylight and complete darkness at room temperature. The degree of settling after every hour of exposure was measured by determining the dry cell weight concentration at different points in the top (4 cm deep) and bottom of the culture suspension.

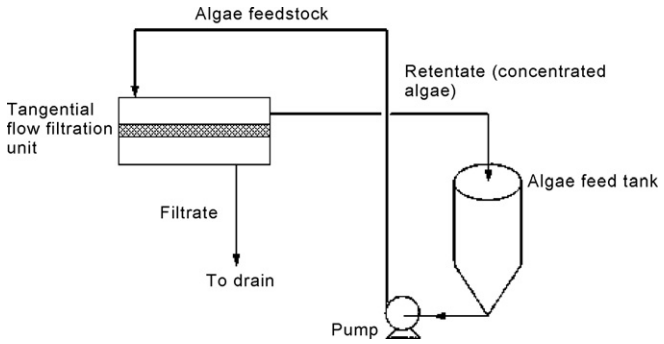

Fig. 1. Schematic description of tangential flow filtration process for concentrating 20 L microalgal culture harvested at HGRP and LGRP.

2.8. Tangential flow filtration of microalgal culture

Preliminary runs were performed to determine the optimal transmembrane pressure by examining filtrate fluxes at various transmembrane pressures. Regular measurements were taken to monitor the filtrate flow rates at each transmembrane pressure and the amount of energy consumed read from the wattmeter. 20 L of cultures harvested at different growth phases were concentrated in the TFF unit at the optimum transmembrane pressure of 30 psi (207 kPa). Fig. 1 shows a schematic flow diagram of this set up. The set up consist of 100 L sump tank to hold the algae feed, a removable plastic lid, and a feed pipe with a constant diameter which connects to the bottom. The retentate pipe was fitted to the top of the feed tank. The performance of the dewatering process after 25 min of continuous filtration was evaluated by measuring the dry cell concentration of the concentrated algae and the amount of energy consumed. The retentate valve was kept open at all times during the experiment. In between runs, the TFF unit was cleaned with tap water, DI water and 100 mM NaOH as outlined in the operator's manual.

3. Results and discussion

3.1. Biomass growth kinetics during microalgal cultivation

The biomass growth kinetics of the microalgal cells and the daily temperature profile of the culture suspension during cultivation are shown in Fig. 2. The results show a general increase in

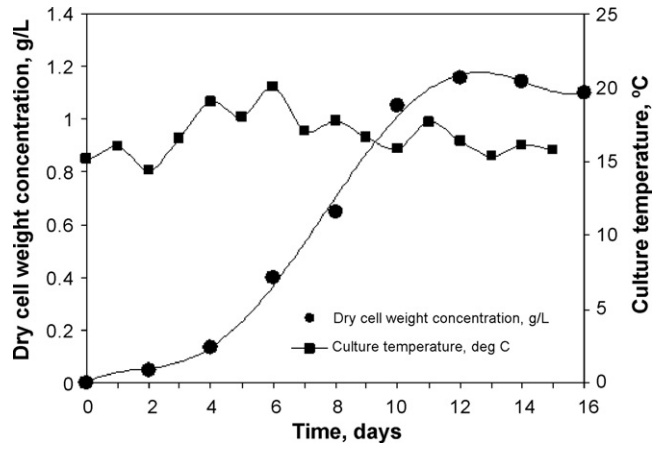

Fig. 2. Dry cell weight concentration and temperature profiles for 100 L outdoor cultivation of microalgae in a bag photobioreactor. Culture temperature ranged from 14 to 21 °C. The results shown represent the average values of 3 replicates ($n = 3$, $\sigma \leq 0.02$).

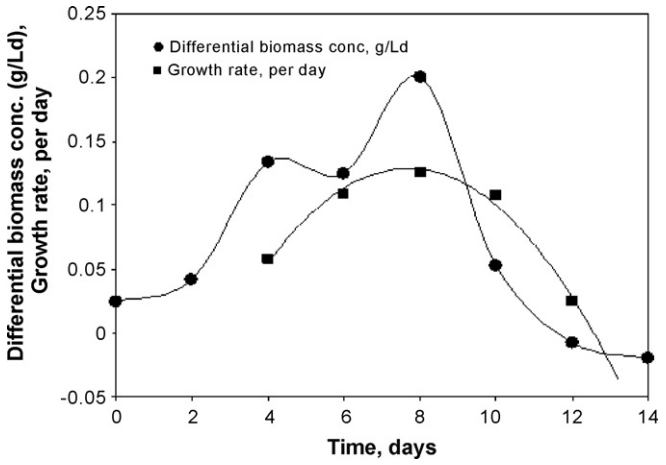

Fig. 3. Differential biomass growth and growth rate during microalgal cultivation. The results show a maximum differential biomass growth and microalgal growth rate of 0.2 g/L d and 0.11 d^{-1} respectively occurring within the exponential phase.

the biomass concentration up to 1.15 g/L in day 12 and declined to 1.1 g/L in day 16 where cultivation was halted. The exponential phase of the microalgal cultivation process exists from day 4 to day 10, where the biomass concentration increased from 0.13 to 1.05 g/L. This is the region with the highest differential biomass growth per unit time as shown in Fig. 3, and is termed as the High Growth Rate Phase (HGRP). The late exponential phase, that is after day 10, where the biomass concentration begins to enter the stationary phase and through to day 12 which is the beginning of the growth declining stage is termed as the Low Growth Rate Phase (LGRP). The average growth rate determined for the HGRP is ~0.11 d^{-1} and the maximum growth rate determined for LGRP is ~0.03 d^{-1}. The declination of biomass growth after day 12 is attributed to the depletion of active nutrients for microalgal growth.

3.2. Effect of microalgal growth on pH and dissolved CO_2 profiles

According to Fig. 4, the daily (afternoons) pH of the culture increased rapidly from 6.6 to a maximum value of ~8.5 after the introduction of the microalgal culture. This is due to the initial uptake of CO_2 by the microalgal cells during the day. The daily pH value of the culture decreased slightly and stabilised at ~8.1

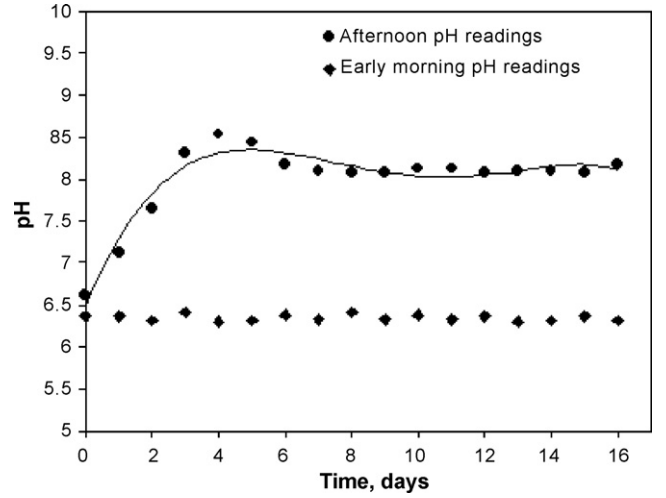

Fig. 4. Early mornings and afternoons pH profiles during the cultivation of microalgae in outdoor bag photobioreactor. The results represent the average values of 3 replicates ($n = 3$, $\sigma \leq 0.04$).

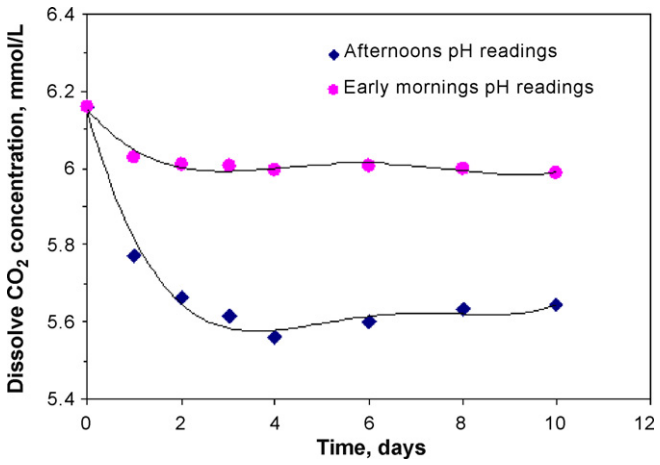

Fig. 5. Early mornings and afternoons dissolved CO_2 concentration profiles during the cultivation of microalgae in outdoor bag photobioreactor. The results represent the average values of 3 replicates ($n = 3$, $\sigma \leq 0.03$).

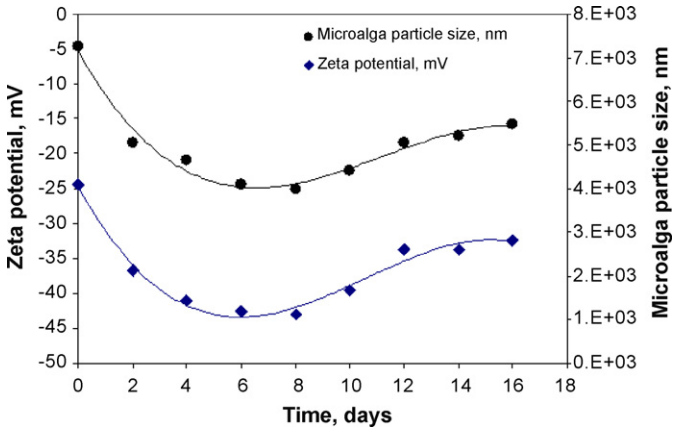

Fig. 6. Zeta potential and microalgal cell size profile during the cultivation process. Results show a maximum electronegative zeta potential and cell size of -43.2 ± 0.7 mV and ~4.1 μm during the exponential growth phase.

after the sixth day—resulting from the buffering capacity of the seawater and the complete adaptation of the microalgal cells to the culture environment. During the night, the pH value of the culture decreases massively as the microalgal cells do not photosynthesise but actually undergo respiration to release more CO_2 which turns the culture suspension acidic with pH ~ 6.4. This phenomenon is confirmed by early mornings and afternoons quantifications of dissolved CO_2 levels during the cultivation process. As shown in Fig. 5, the afternoon dissolved CO_2 level decreased from 6.16 to 5.56 mmol/L during the first 4 days after inoculation and plateaued afterwards whilst the early morning dissolved CO_2 level maintained a constant value of ~6 mmol/L after inoculation. Dissolved CO_2 quantification was performed by titrating the culture samples with standard HCl to determine the stoichiometric amount of HCO_3^- converted into CO_2 gas. In brief, there is a maximum accumulation of CO_2 during the dark phases of the cultivation cycle where the microalgal cells do not photosynthesise but rather respire. Hence low pH levels are encountered during the night.

3.3. Effect of growth on zeta potential and size of microalgae

The zeta potential profile of the microalgal culture was determined during the cultivation process in order to understand the physical intercellular interactions between the microalgal cells. This study is a useful criterion to determine the optimum harvesting time that would improve dewatering efficiency. The result as in Fig. 6 shows a sharp increase in the electronegative zeta potential from -24.5 ± 0.5 mV to -36.7 ± 0.8 mV for the microalgal cells in the inoculum and in the pre-exponential phase of the cultivation process respectively. The zeta potential decreased further to a minimum of -43.2 ± 0.7 mV during the exponential phase and started increasing as the growth approaches the stationary phase. During the exponential growth phase or HGRP of the microalgal cells, the growth rate of the cells is at its maximum value, hence the intracellular metabolic rate, unicellular mobility and differential growth kinetics of the cells are optimal. Due to the increase in the kinetics of cell growth and unicellular mobility, there is a minimal intercellular interaction between individual cells in the culture and this induces a net electronegative zeta shield around the cells, thus creating a massive repulsion between the cells. This phenomenon explains the high electronegative nature of the microalgal cells during the HGRP. However, on approaching and during the stationary phase or LGRP, the metabolism rate of the microalgal cells is low with reduced unicellular mobility and this generate a less electronegative zeta shield (-34.5 ± 0.4 mV) around the individual cells; resulting in an improved intercellular interactions and cell agglomeration. This is confirmed by the microalgal particle size profile obtained during the cultivation process. The microalgal particle size as shown in Fig. 6 shows a minimum value of ~4.1 μm during the HGRP and an average value of 5.3 μm during the LGRP. The higher microalgal cell size during the LGRP is due to improved intercellular cohesion and agglomeration. Fig. 7 shows a microscopic picture of the nature of the microalgal cells under LGRP and HGRP. Under the same magnification of ×100 the microalgal cells are closely attached to each other during the LGRP than the HGRP.

3.4. Dependence of growth phases and storage conditions on microalgal settling rate

10 L of microalgal culture was harvested during the HGRP and LGRP, placed in a transparent glass container and allowed to settle under daylight and dark conditions. The results as showed in Fig. 8 and tabulated in Table 1 show that the microalgal culture

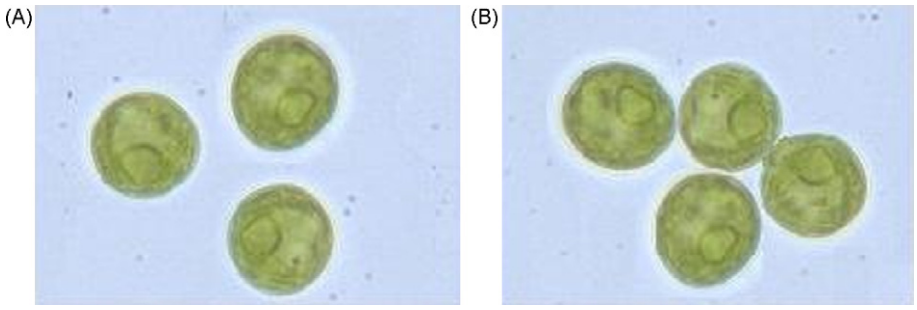

Fig. 7. Microscopic pictures of microalgal cells at (A) HGRP and (B) LGRP during the cultivation process. Result shows the coalescing nature of the LGRP microalgal cells.

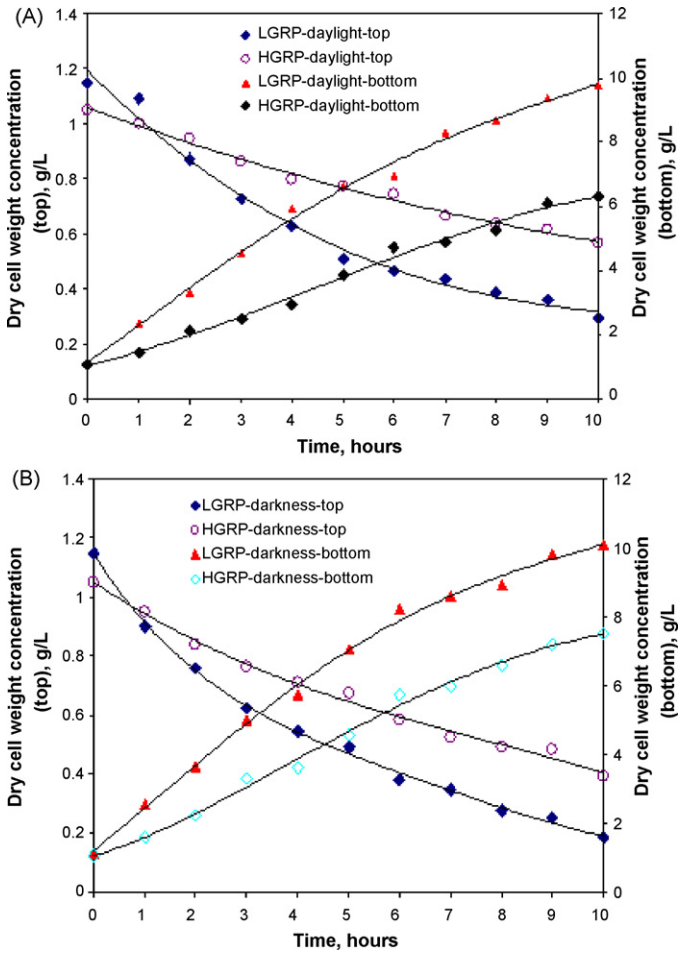

Fig. 8. Gravity settling of microalgal cultures harvested at LGRP and HGRP under (A) daylight condition and (B) dark condition. Result shows the highest settling rate for LGRP under dark conditions. The results represent the average values of 3 replicates.

Table 1
Summary of results from gravity settling of microalgal cultures after 10 h. Cultures were harvested at LGRP and HGRP and kept in daylight and dark conditions at room temperature. Results represent the average values of three replicates.

Conditions	HGRP		LGRP	
	x_f (top) (g/L)	x_f (bottom) (g/L)	x_f (top) (g/L)	x_f (bottom) (g/L)
Daylight	0.57 ± 0.01	6.29 ± 0.12	0.28 ± 0.02	9.76 ± 0.08
Darkness	0.39 ± 0.02	7.50 ± 0.09	0.17 ± 0.01	10.1 ± 0.06

stored under darkness generally settles faster than that stored under daylight conditions and also the LGRP microalgal culture settles faster than that of the HGRP. After 10 h of storage, the LGRP culture recorded a top and bottom dry cell weight concentrations of 0.28 and 9.76 g/L respectively for daylight and 0.17 and 10.1 g/L respectively for darkness whilst the HGRP culture recorded a top and bottom dry cell weight concentrations of 0.57 and 6.29 g/L respectively for daylight conditions and 0.39 and 7.50 g/L respectively for dark conditions. Source of light is an important

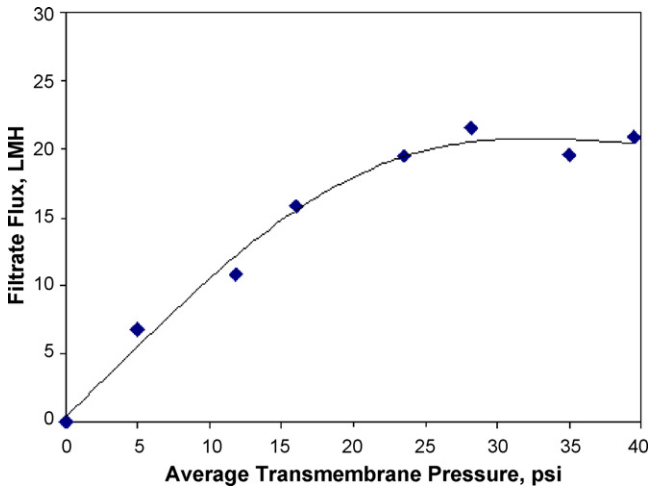

Fig. 9. Pressure excursion data obtained with HGRP microalgal culture. Results show an optimum transmembrane pressure of ~30 psi (207 kPa) corresponding to a filtrate flux of 20 L/m^2 h.

requirement for microalgal growth, so in the presence of daylight and/or HGRP the microalgal cells are actively photosynthesizing with high metabolism rate and unicellular mobility, thus inducing the net electronegative zeta shielding effect. This retards their agglomeration rate and therefore lowers their settling rate. On the other hand, microalgal cells exposed to darkness and/or LGRP do not photosynthesise, hence their metabolism rate is low and this reduces the net electronegative zeta shielding effect. This causes the cells to agglomerate and settle faster.

3.5. Effect of growth phases on tangential flow filtration performance

20 L of microalgal culture harvested from the bag photobioreactor at the HGRP was used to generate a pressure excursion data, where the optimal transmembrane pressure was determined in an experiment that tested the impact of filtrate flux at various transmembrane pressures. As shown in Fig. 9, increasing the transmembrane pressure produces a higher filtrate flux up to approximately 30 psi (207 kPa). At higher transmembrane pressures, no improvement in the filtrate flux is observed whereas losses of microalgae to the filtrate stream were observed visually from the colour change of the filtrate stream. It was therefore concluded that the optimal transmembrane pressures is approximately 30 psi (207 kPa) which corresponds to a filtrate flux of ~20 L/m^2 h. Table 2 shows a summary of the performances of the tangential flow filtration of microalgal culture harvested during the HGRP and LGRP. The results show a two-fold higher degree of dewatering of the LGRP microalgal culture than that of the HGRP, resulting from the coalescing nature of the LGRP microalgal cells. As expected, the wattmeter readings increased during each experiment. This is due to the gradual increase in feed viscosity as more water is removed. The amount of energy consumed by the tangential flow filtration of HGRP and LGRP microalgal cultures is also presented in Table 2. It can be seen that 25 min tangential flow filtration of HGRP microalgal cul-

Table 2
Results from tangential flow filtration of LGRP and HGRP microalgal feedstocks. Runs were performed with a feed volume of 20 L at a pressure of 30 psi (207 kPa). Results represent the average values of three replicates.

Runs	Pressure (psi)	Growth rate (per day)	Feed volume (L)	Feed conc. (g/L)	Final retentate conc. (g/L)	Conc. factor	Energy consumed (kWh/m^3)	Time (min)
HGRP	30	0.11	20	1.05	24.68	23×	0.51	25
LGRP	30	0.03	20	1.15	55.71	48×	0.38	25

ture consumes 0.51 kWh per cubic metre of supernatant removed to concentrate up to 23 times whilst the LGRP culture consumes 0.38 kWh per cubic metre of supernatant removed within the same time duration to concentrate up to 48 times. The energy consumption values show that improved dewatering levels can be achieved more economically by harvesting cultures in the LGRP. Hence the growth phasal point of harvesting microalgal culture is a critical factor that affects the ease of dewatering of microalgae.

4. Conclusion

The economics of dewatering of microalgal cultures is a major bottleneck hampering the realization of algae-based fuels. This body of work suggests an upstream methodology of improving the efficiency of dewatering of microalgae during the cultivation process by investigating the ease of dewatering of microalgal cultures harvested at different growth phases. From the experimental results obtained, microalgal culture harvested under low growth rate conditions presents better dewatering performance than cultures harvested under high growth rate conditions even though the latter presents a higher differential biomass growth. Energy consumption analysis based on tangential flow filtration of the microalgal cultures shows a significant cost benefit and savings by dewatering microalgal cultures harvested during the low growth phase. These results illustrate that the harvesting time strongly affects the ease of dewatering of the microalgal culture and that operating costs and time can be reduced by filtering the microalgae during growth phases which exhibit higher intercellular interaction.

Acknowledgements

This work was supported by an Australian Research Council (ARC) Linkage grant between The Department of Chemical Engineering, Monash University (Victoria, Australia) and Bio-Fuel Pty Ltd., Victoria, Australia.

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