RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Factors affecting microalgae harvest efficiencies using electro-coagulation-flotation for lipid extraction

Dawei Zhang[a,c] , Yanling Yu[*a,d] , Chao Li[a,b] ,Chunyan Chai[a,] Lili Liu[a] ,Jia Liu[*a] ,Yujie Feng

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

Microalgae harvest is a relatively costly process in microalgae biodiesel production. In this study, electro-coagulation-flotation (ECF) was employed to harvest microalgae (*Chlorella vulgaris*), Higher current density achieved higher collection efficiency, but also resulted in higher energy consumption and increased levels of dissolved aluminium. At the same ratio of current density to initial cell density, collection efficiency decreased from 99.0% of 0.24 g/L to 30.5% of 1.17 g/L when the electrolysis time was 20 min. For stirring and aeration, the highest collection efficiency of 98.4% with 50 rpm stirring at 20 min was nearly equal to the highest collection efficiency for aeration of 98.3% for 50 mL/min aeration at 30 min. Acidic and neutral culture was beneficial, due to the positively charged aluminium species in the culture; higher collection efficiency at more than 98% occurred with pH levels of 5 to 7 after 20 min. The lowest energy consumption, 0.61 kWh/kg, was achieved at pH5. As part of this research, ECF exhibited higher collection efficiency (99.4%), compared to 93.5% collection efficiency for chemical flocculation ($Al_2(SO_4)_3$).

Introduction

Microalgae biodiesel as a potential bioenergy is currently receiving much attention.[1-4] However, drawbacks hinder large-scale production of microalgae biodiesel, such as lower cell density[5], expensive cultivation, and microalgae harvest costs. Until now, the high cost of harvesting not only limited microalgae bioenergy production, but also hindered microalgae eutrophication removal.

Microalgae cells have been harvested with centrifugation and conventional chemical flocculation. There are disadvantages with these two processes, such as higher costs due to increased energy consumption associated with centrifugation, and contaminated microalgae slurries from chemical flocculation that can make the microalgae product unsuitable for further use as raw material for fuel or food.

As an alternative to chemical flocculation, electro-coagulation-flotation (ECF) is a prospective method that has attracted considerable attention in water and wastewater treatment. Compared with chemical flocculation, ECF has the following advantages: 1) no anions such as chlorides are introduced, which are always a concern with traditional flocculants;[6] 2) no flocculant is required;[7] and 3) pH adjustment is less critical because ECF performs well in a wide pH range.[8] Moreover, the micro-bubbles produced at the anode and cathode can also contribute to the separation of pollutants through flotation.[9]Poelman et al. used ECF for microalgae removal in drinking water treatment; removal efficiencies of 95% or more were easily obtained with different microalgae strains while energy consumption was as low as approximately 0.3 kWh/m^3.[10] Gao et al. reported that 100% of removal was achieved with energy consumption as low as 0.4 kWh/m^3.[11] Vandamme et al. used ECF for microalgae harvest with energy consumption of approximately 2 kWh/kg of microalgae biomass harvested for *Chlorella vulgaris* (freshwater microalgae) and 0.3 kWh/kg for *Phaeodactylum tricornutum* (marine microalgae).[12]

ECF flotation is generally considered more advantageous than sedimentation for microalgae harvest.[13] However, if coagulation is unsuccessful, poor flotation can occur, which results in high coagulant consumption and cell residuals causing downstream filter blockage or breach.[14]

In this work, ECF was employed to harvest microalgae for lipid extraction. The effects of current density, initial microalgae cell density, stirring, aeration, and initial pH on microalgae harvest with ECF were systematically investigated. A comparison of ECF and chemical flocculation for microalgae harvest for lipid extraction was also evaluated.

Materials and Methods

Microalgae strain and culture media

Chlorella vulgaris (Institute of Hydrobiology, Chinese Academy of Sciences Wuhan, China) was preserved in BG11 medium and pre-cultured in light illumination incubator before use.[15] *Chlorella vulgaris* was cultured in 1000 mL Erlenmeyer flasks with 5 g/L glucose as a substrate in BG11 medium under static condition at 30 °C with 3000 lx continuous cool-white fluorescent light illumination. Flasks were hand shaken three to five times daily to avoid wall growth. After cultivation for 5 days, the microalgae culture was diluted to the desired values with

distilled water and then transferred to the ECF reactors. Cultures with mixotrophic and N-deficient medium were both used for ECF.

Table1 Composition of mixotrophic and N-deficient medium

	Mixotrophic medium (g/L)	N-deficient medium (g/L)
$NaNO_3$	1.5	0.1
$K_2HPO_4{\cdot}3H_2O$	0.04	0.04
$MgSO4{\cdot}7H_2O$	0.075	0.075
$CaCl_2{\cdot}2H_2O$	0.036	0.036
Citric acid	0.006	0.006
Ferric ammonium citrate	0.006	0.006
EDTA (dinatrium-salt)	0.001	0.001
$NaCO_3$	0.02	0.02
A_5+Co (mL/L)*	1	1
Glucose	5	5

* A_5+Co solution: consists of H_3BO_3 (2.86 g/L), $MnCl_2{\bullet}H_2O$ (1.81 g/L), $ZnSO_4{\bullet}7H_2O$ (0.222 g/L), $CuSO_4{\bullet}5H_2O$ (0.079 g/L), $Na_2MoO_4{\bullet}2H_2O$ (0.390 g/L) and $Co(NO_3)_2{\bullet}6H_2O$ (0.049 g/L)

ECF reactors - construction and operation

ECF reactors were constructed with 0.65 L effective volume (diameter 9 cm, height 12 cm) using polymethyl methacrylate. A pair of aluminium electrodes (6.0 cm in length × 5.0 cm in width × 0.1 cm in thickness) was employed in each reactor; the electrode gaps were set at 2.0 cm. Before use, alumina (Al_2O_3) film on the surface of the electrodes was removed with emery paper. Current was supplied by a digital DC power source (SK173SL3A9, Nanjing Sunear Electric Appliance Co., Ltd, China). All experiments were carried out under constant-current mode and at room temperature (20 °C). The reactor was stirred by a magnetic stirrer (79-1, Hangzhou Ming far instrument Co., LTD, China) or aerated by an air pump (aco-009D, HAILEA Co., LTD, China). The culture pH was adjusted to 8.0 using 0.1 M NaOH or 0.1 M H_2SO_4, to avoid the effect of Cl^- in electrolysis of the electrodes.[11,16]

When comparing ECF and chemical flocculation, ECF was performed with a stirring speed of 50 rpm, initial cell density of 0.24 g/L, pH of 5.0, and current density of 0.42 mA/cm^2, with electrolysis for 20 min and then standing for 20 min. As the control, the cultures were also centrifuged at 4000 rpm for 10 min. The flocs were dried at 40 °C and then used for lipid extraction. The Al^{3+} in the effluents was also determined.

Chemical flocculation for microalgae collection

The chemical flocculation process was optimized as follows: $Al_2(SO_4)_3$ (0.25 mmol/L) was the flocculant, initial cell density was 0.48 g/L, and pH was 9.0. The stirring rate was 200 rpm for 1 min, 50 rpm for 10 min, and then standing for 15 min.

Analyses

Samples were taken at 5.0 cm below the water surface, followed by standing for 20 min, and then the samples were analyzed. Optical density (OD) of the culture at 658 nm was measured as the cell density indicator using a spectrophotometer (TU-810 UV/Visible-light Spectrophotometer, Purkinje General, China). A linear relationship between OD_{658} and dry weight (DW, g/L) of microalgal biomass was determined previously for this strain:[15]

$$\text{Dry weight (g/L)} = 0.4818*OD_{658}, \quad R^2=0.9962 \quad (1)$$

Collection efficiency (r) was calculated as:

$$r\ (\%) = 1 - C_{end}/C_{initial} \quad (2)$$

where C_{end} is the final cell density (g/L) and Cinitial is the initial cell density (g/L).

Energy consumption (E) of the ECF process was calculated as:

$$E\ (kWh/kg) = U*I*t/(C*V*r) \quad (3)$$

where U is the voltage, I is the applied electrolysis current, t is the electrolysis time during which collection efficiency was more than 95%, C is the initial cell density, and V is the effective reactor volume (0.65 L in this work). The energy consumption of the air pump or magnetic stirrer was not counted here.

The method for determining the total aluminum concentration followed the research of Gao et al.[11] The samples were first digested with 50% HNO_3, and then the aluminum concentrations were measured using inductively coupled plasma-atomic emission spectroscopy (ICP-AES, Optima 5300 DV, PerkinElmer, USA).

The flocs were collected and then dried at 40 °C. Lipids were extracted from the microalgal biomass using a modified method of Bligh and Dyer[17] described in detail by Feng et al.[15]

Results and Discussion

The ratio of current density to initial cell density (CD/ICD) exhibited a significant effect on ECF collection efficiency and energy consumption. This effect was investigated under varied current densities and initial cell densities with stirring at 50 rpm and an initial pH of 8.0.

Current density

ECF processes were carried out with an initial cell density of 0.48 g/L and current densities varying from 0.25 to 2.08 mA/cm^2, which produced CD/ICD results from 0.52 to 4.33 Acm/g. As shown in Fig.1A, collection efficiency increased along with CD/ICD at less than 5 to 40 min of electrolysis time. Collection efficiency increased slightly during 0 to 40 min. When current density was 0.83 to 2.08 mA/cm^2, collection efficiency initially increased rapidly, followed by a steady stage.

Furthermore, it was found that collection efficiency increased with current density. For example, 99.2% collection efficiency was obtained in 20 min with 2.08 mA/cm^2, while only 68.0% efficiency was achieved with 0.83 mA/cm^2. As predicted by Faraday's law, dissolved aluminium from the anode increased with current density and electrolysis time. As the aluminium-ion concentration increased in the reactor, both the coagulant surface area and the number of active sites correspondingly increased, [18]which promoted microalgae aggregation and floc formation. In addition, as reported by Holt et al,[19] micro-bubble density increased and bubble size decreased with increasing current density, leading to a faster upward flow and microalgae flotation.

As shown in Fig.1B, energy consumption of the ECF process increased from 1.277 kWh/kg to 3.198 kWh/kg when current density increased from 0.83 mA/cm2 to 2.08 mA/cm^2. This increase also led to increased levels of dissolved aluminium that were generated rapidly when higher current density was applied.

However, the dissolved aluminium could not disperse in the solution simultaneously and adhered to the flocs. Thus more dissolved aluminium would be needed to achieve the same collection efficiency at a lower current density. Therefore, energy consumption increased with elevated current density.

Once the lowest energy consumption was determined, 0.83 mA/cm^2, this level was used in the later research process.

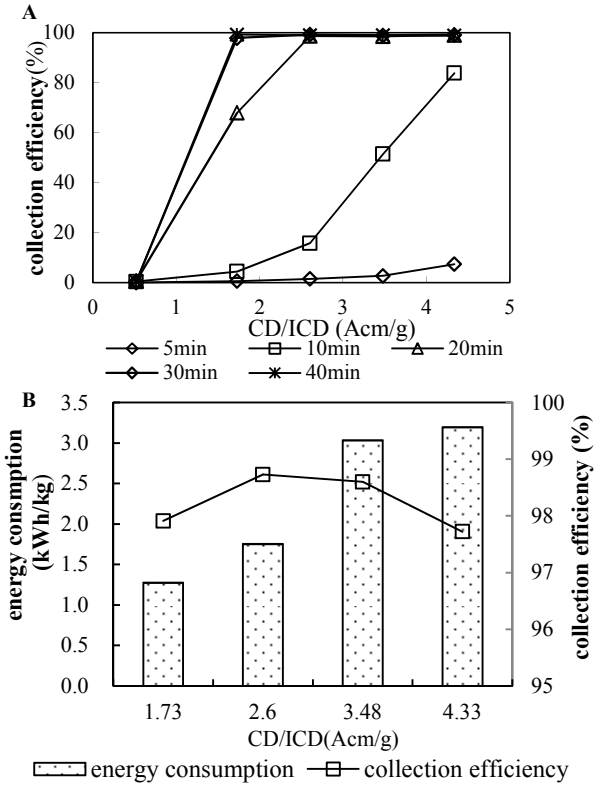

Fig.1 ECF collection efficiency and energy consumption at different current density; A) ECF collection efficiency of different current density at different electrolysis time; B) energy consumption at different current density

Initial cell density

As shown in Fig.2A, collection efficiency decreased significantly from 98.7% to 4.49% in 20 min when the cell density increased from 0.24 g/L to 1.10 g/L. The initial cell densities of 0.96 and 1.10 g/L were not suitable for ECF due to the lower collection efficiencies, 39.1% and 24.2%, respectively, after 40 min, primarily because the higher cell density culture needed additional aluminum for microalgae collection. [20]

Energy consumption was calculated according to Equation 3 with 0.24, 0.48 and 0.72 g/L cell densities (Fig.2B). Energy consumption of 2.26, 1.23, and 1.24 kWh/kg was required for the ECF process with cell densities of 0.24, 0.48, and 0.72 g/L, respectively. In this research, the lower cell density was obtained by diluted original culture with distilled water. Thus the resistance of the microalgae culture was greater with an increased dilution ratio. Therefore, the most energy was consumed due to internal resistance when the microalgae culture of 0.24 g/L cell density was treated.

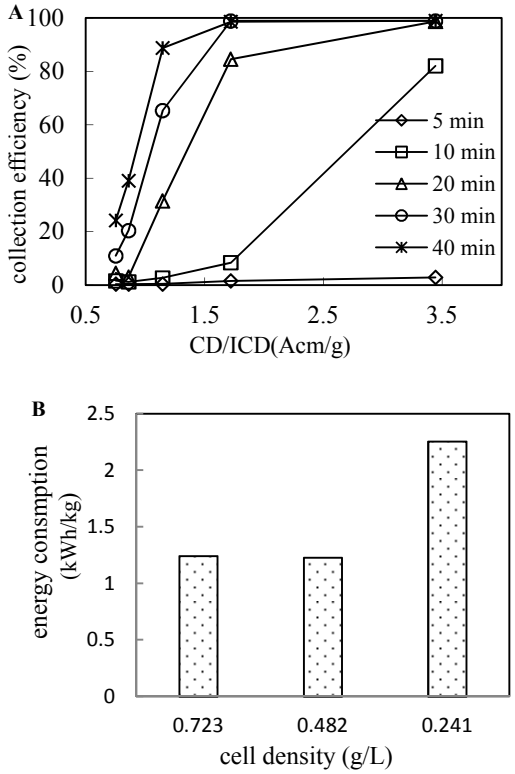

Fig.2 ECF collection efficiency and energy consumption at different initial cell densities; A) ECF collection efficiency of different initial cell density at different electrolysis time; B) energy consumption of different initial cell density

The same CD/ICD

The effect of CD/ICD on microalgae harvest was also investigated. The ECF process was carried out with a range of cell densities (0.24, 0.48, 0.72, 0.96, 1.17 g/L) at a CD/ICD of 1.75 Acm/g, corresponding to the current density of 0.42, 0.84, 1.26, 1.68, 1.92 mA/cm^2. As shown in Fig.3A, collection efficiency decreased from 99.0% of 0.24 g/L to 30.5% of 1.17 g/L in 20 min. As previously discussed, increased dissolved aluminium would be needed when higher current density is applied. However, the amount of dissolved aluminium from the anodes increased linearly with the current density according to Faraday's law. Therefore, insufficient dissolved aluminium was provided when cell density increased. As shown in Fig.3B, energy consumption increased from 1.003 kWh/kg at 0.24 g/L to 1.794 kWh/kg at 0.96 g/L, which was in accordance with decreased collection efficiency.

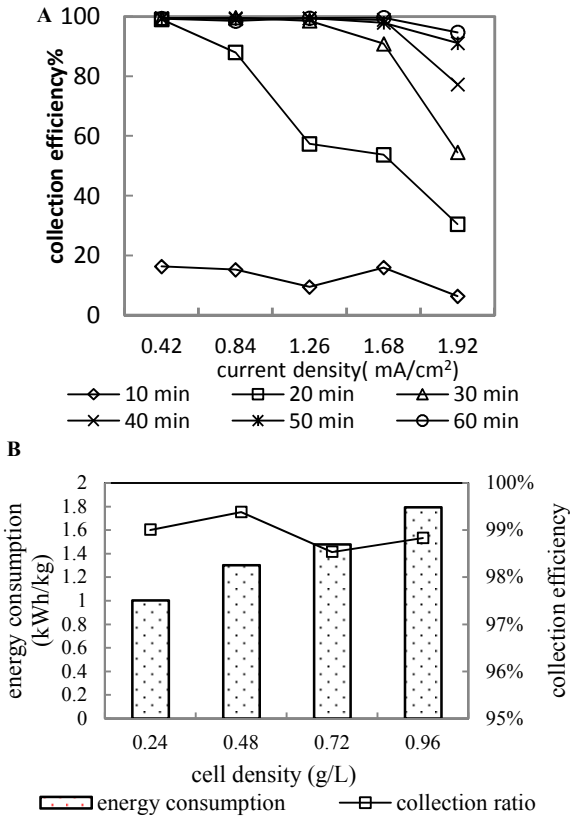

Fig.3 ECF collection efficiency and energy consumption at the same CD/ICD; A) collection efficiency of different current density; B) energy consumption of different cell density

Enhanced ECF collection efficiency with stirring and aeration

Use of both stirring and aeration can enhance ECF collection efficiency by combining the processes of flocculation and flotation. Flocculation of particles in a liquid depends on collisions between particles, caused by their relative motion, which may be caused by Brownian movement, or induced by an external force, e.g. stirring. With stirring at 50 rpm, a layer of algal flocs was observed floating at the water surface, which proved the flocs were not disaggregated. However, stirring at higher speeds resulted in floc disaggregation. For example, ECF collection efficiency with 200 rpm stirring (95.9%) was lower than with 50 rpm (99.4%) after 60 min with an initial 0.48 g/L cell density (shown in Fig.4A). In the first 20 min, the flocs were gradually formed by microalgae cells and aluminium hydroxide, and floated to the water surface. However these flocs were not strong enough to withstand high shear forces such as stirring at 200 rpm speed.

Collection efficiency with aeration was then studied. As shown in Fig.4B, when aeration was supplied at 50 and 100 mL/min, collection efficiency achieved 98.3% and 90.0%, respectively, at 30 min. However, collection efficiency decreased and fluctuated with aeration rates of 150 to 250 mL/min due to shearing, disaggregation of flocs, and poor flotation. As shown in Fig.4B, collection efficiency without aeration was only 74.9% after 50 min; lower efficiency was obtained because the possibility of collisions between algal cells decreased, and most algal cells adhered to electrodes, blocking the diffusion of Al^{3+} and micro-bubbles.

ECF collection efficiency with stirring was then compared to collection efficiency with aeration. While the collection efficiency of 98.4% with 50 rpm stirring at 20 min was almost equal to the collection efficiency for aeration of 98.3% for 50 mL/min at 30 min, the energy consumption for stirring with a shorter electrolysis time was significantly less than for aeration. Therefore, the most energy-efficient stirring rate of 50 rpm, with the highest collection efficiency was used in the following stages of this research.

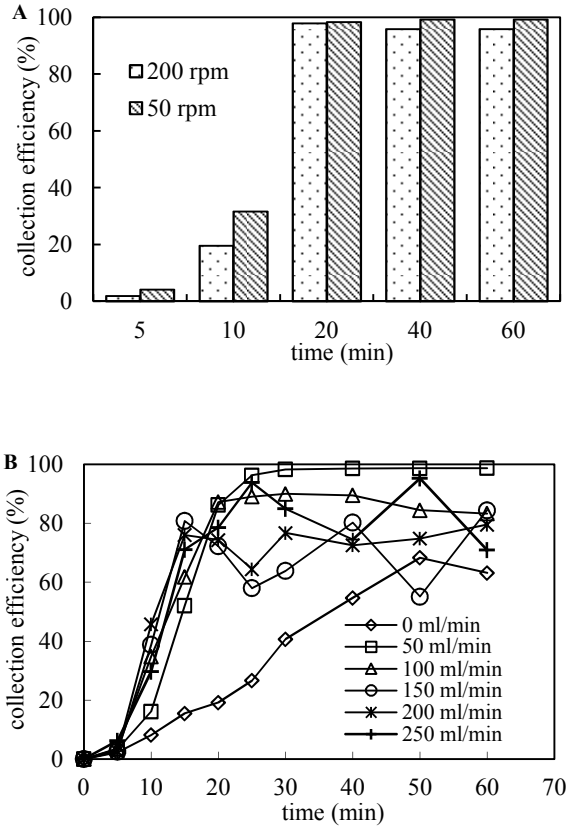

Fig.4 ECF collection efficiency with stirring and aeration with initial cell density 0.48 g/L, pH 8.0, and current density 0.83 mA/cm2; A) Comparison of stirring at 50 rpm and 200 rpm; B) Collection efficiency at aeration rates of 50, 100, 150, 200, and 250 mL/min.

Initial culture pH

The effect of initial culture pH on the ECF process was also investigated, and acidic and neutral pH levels were found to be beneficial (Fig.5A). Collection efficiency was more than 98% with pH of 5 to 7 at 20 min, while only 91.9% collection efficiency was obtained with pH 9. This might be due to the aluminum species in the culture, which relies significantly on the pH and aluminum concentration. [21]At a pH of 5 to 7, $Al(OH)_3$, $Al(OH)^{2+}$ and $Al(OH)^{3+}$, as well as polymeric species such as $Al_{13}O_4(OH)_{24}^{7+}$, are the primary species in the solution.[22] Therefore, the negatively charged microalgal cells would be easily adsorbed onto the positively charged aluminium precipitates. In alkaline culture, $Al(OH)^{4-}$ dominated in solution, which led to negative charges of aluminium hydroxide precipitates, and consequently reduced the adsorption capacity of

the negatively charged cells. Thus, ECF exhibited lower collection efficiency under alkaline conditions than under acid and neutral conditions.

The energy consumption of the ECF process with different initial pH levels is shown in Fig.5B. The lowest energy consumption, 0.61 kWh/kg, was achieved at pH 5. According to 2013 prices of electricity in China of $0.079 for 0.158/kWh, the cost of microalgae harvest was only $0.047 for 0.079/kg with a 0.24 g/L cell density and initial pH of 5.

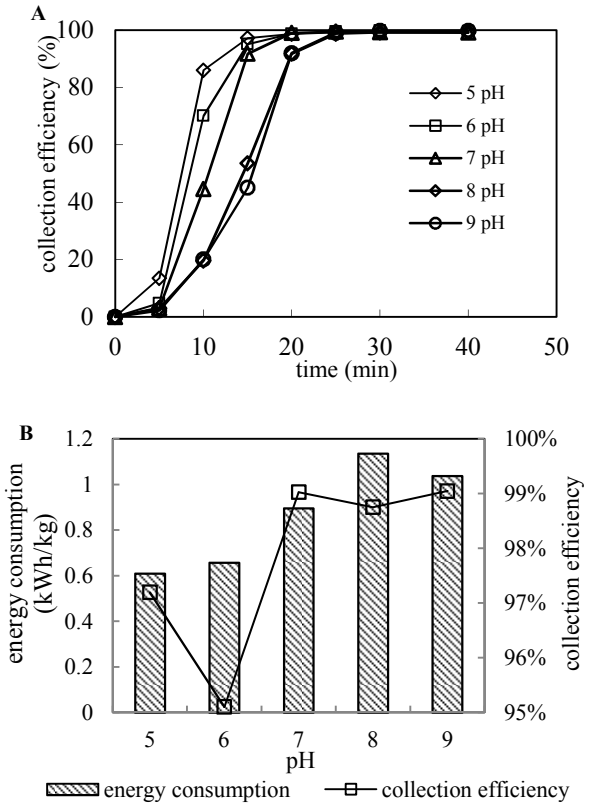

Fig.5 CF collection efficiency and energy consumption at different initial pH levels; A) CF collection efficiency at different initial pH; B) ECF energy consumption at different initial pH

Advantages of ECF over chemical flocculation

As shown in Table 2, ECF exhibited results superior to chemical flocculation for collection efficiency, microalgae content in floc, and lipid recovery efficiency. In N-deficient culture, ECF lipid recovery efficiency was 99.4%, but chemical flocculation achieved only 93.5% efficiency. It is notable that there were no significant differences with the ECF process with the use of mixotrophic culture or N-deficient culture.

Dunahay et al. found that lipid accumulation in algal cell could be stimulated under N-deficiency.[23] According to Converti et al., a threefold increase (from 5.9% to 15.3%) in lipid content was observed in 0.38 g/L $NaNO_3$ culture compared with 1.5 g/L $NaNO_3$ culture.[24] In this research the lipid content in N-deficient culture (0.1 g/L $NaNO_3$) and full quality culture (1.5 g/L $NaNO_3$) was 11.8% and 15.4% respectively. Higher microalgal content in the flocs can reduce the amount of flocs, and thereby decrease the cost of lipid extraction, microalgal residue treatment, and other processes. Furthermore, higher microalgal content corresponded to lower impurity content; impurities in the flocs might absorb microalgal lipids, thus decreasing the lipid recovery efficiency. According to these results, ECF is more suitable for algae harvesting for lipid extraction than chemical flocculation.

The Al^{3+} concentration in the ECF effluent was 1.97 to 2.23 times higher than the chemical flocculation effluent. This result could be attributed to the fact that the ECF electrolysis time was 20 min for reliable collection efficiency, while the ECF collection efficiency was over 97% at 15 min. There were insufficient microalgae cells available for the dissolved aluminium from the anode to form flocs, therefore dissolved aluminium accumulated in the ECF solution.

Table2 Collection results of *Chlorella vulgaris* by ECF and chemical flocculation

	ECF	CF	ECF with N-deficient culture	CF with N-deficient culture
Collection efficiency（%）	100.0	98.7	99.0	98.3
Microalgae content in floc (%)*	73.5	66.0	77.7	65.7
Lipid recovery efficiency (%)**	100.0	92.4	99.4	93.5
Al^{3+} in effluent (mg/L)	6.21	3.15	9.93	4.46

Conclusion

Electro-coagulation-floatation (ECF) was a new technology for algae harvesting with higher collection efficiency and lower energy consumption compare to flocculation. ECF energy consumption was 0.61 kWh/kg under optimal conditions and also achieved reliable collection efficiency (more than 95%) with varying cell densities (0.24 to 0.96 g/L), pH levels (5 to 9), and culture media (mixotrophic and N-deficient).

Acknowledgements

This research was supported by the National Science & Technology Pillar Program during the Twelfth Five-Year Plan Period (2011BAD14B03) and the Scientific Research Foundation for the Returned Overseas Chinese Scholars of the State Education Ministry, China. It was also supported by the State Key Lab of Urban Water Resource and Environment (HIT) (ES200902).The authors also acknowledge funds from the Creative Research Group of China (51121062) and National Science Fund for Distinguished Young Scholars of China (51125033).

Notes and references

a State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China

b Institute of Social Region, Shanxi Academy of Environmental Research,

Taiyuan 030009, China
c Department of Environmental Science & Engineering, Harbin Institute of Technology (Weihai), Weihai 264200, China
d School of Chemical and Engineering & Technology，Harbin Institute of Technology, Harbin 150090, China

Corresponding author email address: yujief@hit.edu.cn; Fax: 86-451-86287017; Tel.: 86-451-86287017.

1 P. K. Campbell, T. Beer and D. Batten, Bioresource Technology, 2011, 102, 50-56.
2 A. Demirbas and M. F. Demirbas, Energy Conversion and Management, 2011, 52, 163-170.
3 B. J. Gallagher, Renewable Energy, 2011, 36, 158-162.
4 S. A. Scott, M. P. Davey, J. S. Dennis, I. Horst, C. J. Howe, D. J. Lea-Smith and A. G. Smith, Current Opinion in Biotechnology, 2010, 21, 277-286.
5 M. A. Borowitzka, Australas Biotechnol, 1994, 4, 212-215.
6 P. Canizares, F. Martinez, C. Jimenez, J. Lobato and M. A. Rodrigo, Environmental Science & Technology, 2006, 40, 6418-6424.
7 F. Ilhan, U. Kurt, O. Apaydin and M. T. Gonullu, Journal of Hazardous Materials, 2008, 154, 381-389.
8 J. Ge, J. Qu, P. Lei and H. Liu, Separation and Purification Technology, 2004, 36, 33-39.
9 A. Y. Hosny, Separations Technology, 1996, 6, 9-17.
10 E. Poelman, N. DePauw and B. Jeurissen, Resources Conservation and Recycling, 1997, 19, 1-10.
11 S. S. Gao, M. A. Du, J. Y. Tian, J. Y. Yang, J. X. Yang, F. Ma and J. Nan, Journal of Hazardous Materials, 2010, 182, 827-834.
12 D. Vandamme, S. C. V. Pontes, K. Goiris, I. Foubert, L. J. J. Pinoy and K. Muylaert, Biotechnology and Bioengineering, 2011, 108, 2320-2329.
13 S. I. Lee, B. Koopman and E. P. Lincoln, Water Science and Technology, 1992, 26, 1769-1778.
14 R. Henderson, M. Chips, N. Cornwell, P. Hitchins, B. Holden, S. Hurley, S. A. Parsons and A. Wetherill, Water and Environment Journal, 2008, 22, 184-192.
15 Y. J. Feng, C. Li, D. W. Zhang, Bioresource Technology,2011, 102, 101-105.
16 C. T. Wang, W. L. Chou, L. S. Chen and S. Y. Chang, Journal of Hazardous Materials, 2009, 161, 344-350.
17 E. G. Bligh and W. J. Dyer, Biochem Physiol, 1959, 37, 911-7.
18 B. T. Zhu, D. A. Clifford and S. Chellam, Water Research, 2005, 39, 3098-3108.
19 P. K. Holt, G. W. Barton, M. Wark and C. A. Mitchell, Colloids and Surfaces a-Physicochemical and Engineering Aspects, 2002, 211, 233-248.
20 S. S. Gao, J. X. Yang, J. Y. Tian, F. Ma, G. Tu and M. A. Du, Journal of Hazardous Materials, 2010b, 177, 336-343.
21 J. M. Duan and J. Gregory, Advances in Colloid and Interface Science, 2003, 100, 475-502.
22 N. Adhoum, L. Monser, N. Bellakhal and J. E. Belgaied, Journal of Hazardous Materials, 2004, 112, 207-213.
23 T. G. Dunahay, E. E. Jarvis, S. S. Dais and P. G. Roessler, Applied Biochemistry and Biotechnology, 1996, 57, 223-231.
24 A. Converti, A. A. Casazza, E. Y. Ortiz, P. Perego and M. Del Borghi, Chemical Engineering and Processing, 2009, 48, 1146-1151.