Available online at http://www.rsu.ac.th/rjas

Rangsit Journal of Arts and Sciences, January-June 2017 RJAS Vol. 7 No. 1, pp. 49-57

ISSN 2229-063X (Print)/ISSN 2392-554X (Online)

Harvesting of microalgae oil from brackish water in Thailand

Sasirindara Labua[1*] and Thanawat Karkew[2]

Faculty of Biotechnology, College of Agricultural Innovation, Biotechnology and Food,
Rangsit University, Patumthani 12000, Thailand

*Corresponding author; E-mail: sasirin.l@rsu.ac.th

Submitted 9 August 2016; accepted in final form 24 February 2017
Available online 29 June 2017

Abstract

The aim of this study was to assess 7 methods used for harvesting and concentrating microalgae from brackish water in Thailand and to perform a comparative analysis to determine the most efficient and economical dewatering methods for large scale processing of microalgae biomass. The harvesting techniques investigated included sedimentation, vacuum filtration, centrifugation, organic flocculation, inorganic flocculation, auto flocculation, and bio-flocculation. Five criteria were used for evaluating microalgae harvesting technique, namely: a. dewatering efficiency, b. cost, c. suitability for industrial scale, d. time, and e. reusability of media. The results showed that harvesting microalgae oil by flocculation with 1.2 g/l $Al_2(SO_4)_3 \cdot 18H_2O$ was the most efficient and economically viable dewatering methods for large scale processing of microalgae biomass.

Keywords**:** *biomass, brackish water, efficient and economically, flocculation, harvesting and concentrating, microalgae*

1. Introduction

Microalgae are regarded as the best candidate for the production of biodiesel as they do not compete with edible crops (Wahlen, Willis, & Seefeldt, 2011) and can produce between 20,000 to 80,000 L of oil per acre per year which is 7-31 times greater than that produced by the best terrestrial crop (palm tree) (Demirbas, 2010). Microalgae biomass can be used to produced numerous value added products such as biofuels (biodiesel, bioethanol, biogas and biohydrogen) (Slade & Bauen, 2013), fish feed, animal feed, human food supplements such as vitamin A, B1, B2, B12, C, E, nicotinate, biotin, folic acid and pantothenic acid, Omega 3 fatty acid (Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA), and chlorophyll and skin care products such as anti-aging creams, anti-irritant creams and skin regenerate creams (Chen et al., 2009; Harun, Singh, Gareth, & Micheal, 2010). However, processing microalgae into biodiesel and other value added products requires culturing of the microalgae, recovery of the microalgae biomass and the extraction and downstream processing of the oil and other value added products (Pulz, 2001). Microalgae range in size from 2 to 30 µm. The major obstacle for using microalgae bio mass on an industrial-scale for production of value added products is the dewatering step which accounts for 20-30% of the total costs associated with microalgae production and processing (Al hattab, Ghaly, & Hammoud, 2015; Zitelli, Rodolfi, Biondi, & Tredici, 2006). Microalgae cultures need to be concentrated because they exist as a dilute suspension containing 0.1-2.0 g of dried biomass per liter (Danquah, Gladman, Moheimani, & Forde, 2009).

The method used to harvest microalgae cells is dependent on the characteristics of the microalgae, such as size and density (Olaizola, 2003). Harvesting also usually requires a separate step after the cell cultivation. All of the available harvesting approaches are only economically feasible for production of high-value products and thus have limitations for effective, cost-efficient production of biofuels (Gultom & Hu, 2013; Shelef, Sukenik, & Green, 1984).

Microalgae can be separated from aqueous solution by settling after treatment with flocculants, coagulants and polymers or a combination of these inorganic additives. At the pilot or laboratory scale, this can be performed using a graduated cylinder and measuring the settling speed and final clarification of the aqueous medium. Inorganic coagulants were tested using Jar tests to evaluate coagulation on algae removal. By disrupting the stability of the system, successful microalgae harvesting can be obtained (Uduman, Qi, Danquah, Forde, & Hoadley, 2010).

The aim of study was to assess 7 methods used for harvesting and concentrating microalgae from brackish water in Thailand and to perform a

comparative analysis in order to determine the most efficient and economically viable dewatering method for large scale processing of microalgae biomass. The harvesting techniques investigated included sedimentation, vacuum filtration, centrifugation, organic flocculation, inorganic flocculation, auto flocculation and bio flocculation. Selection of the most suitable harvesting methods was based on the dewatering efficiency, cost, suitability for industrial scale, time, and reusability of media for operating on a large scale.

2. Materials and methods

2.1 Microalgal strain and culture condition:

The microalgal strain was obtained from brackish water in Thailand and grown in Watanabe's medium. Algae samples were cultivated in Watanabe's media containings 1.5 gL^{-1} KNO_3, 1.25 gL^{-1} KH_2PO_4 , 1.25 gL^{-1} $MgSO_4.7H_2O$, 20 mgL^{-1} $FeSO_4.7H_2O$ and 1 ml A_5 solution. The pH was adjusted to 6.5 with distilled water before autoclaving. Growth was in a 70 L photobioreactor containing 50 L of medium. Samples were cultured at room temperature with sun light and bubble aeration for 15 days. After 15 days, the culture was then used for testing the harvesting method of microalgae.

2.2 Harvesting methods of microalgae

2.2.1 Inorganic flocculation:

The microalgal suspension (1 L) was placed in a 1.5 L PET bottle. $FeCl_3.6H_2O$ (0.2 mg/l) Low dose, (70 mg/l) High dose, pH 8.0, $Fe_2(SO_4)_3$ (0.7 g/l, 0.8 g/l), pH 8.0, $Al_2(SO_4)_3.18H_2O$ (1 g/l, 1.2 g/l), pH 7.0 were added, respectively. Each concentration was performed in triplicate.

2.2.2 Organic flocculation:

The microalgal suspension (1 L) was placed in a 1.5 L PET bottle. Chitosan (4 mg/ml, 5 mg/ml), pH 8.0 were added respectively. Each concentration was performed in triplicate.

2.2.3 Auto flocculation

Adding NaOH or KOH 0.1 mg/l, pH 10.0 into the microalgae mediums (1 L) in a 1.5 L PET bottle. Comparisons of the efficiency of auto flocculants were assessed.

2.2.4 Cylindrical sedimentation tank

The pH of microalgae media was adjusted to pH 10.0 by NaOH. Then, the algal suspension was placed in a cylindrical sedimentation tank.

2.2.5 Bio flocculation

Applying bacterial bio flocculants produced by Bacillus subtilis (10%, 20%, 30% (v/v)) into the microalgal suspension. Comparative studies of the efficiency of bio flocculation were performed

2.2.6 Centrifugation

The microalgal suspension was centrifugation at 8000 rpm, 15 min and 13000 rpm,3 min. Each force was performed in triplicate.

2.2.7 Vacuum filtration

The microalgal suspension was filtered through 0.45µm (microfiltration) and 11 µm (macrofiltration) (What man, Sigma-Aldrich, UK).

3. Results and discussion

3.1 Inorganic flocculation:

Harvesting microalgae by inorganic flocculations are shown in Table 1 and Figure 1. The results showed that the most effective method was the addition of $Al_2(SO4)_3.18H_2O$ (Alum) 1.2 g/l, pH 7.0 which gave sludge height of 2 cm. in 24 h and 0.54±0.05 g/l dried weight. Milledge and Heaven (2013) noted that Alum was a superior flocculating agent compared to ferric sulfate in terms of pH, amount of flocculent and the quality of the final water slurry. Microalgae cells are negatively charged, as a result of adsorption of ions originating from organic matter and dissociation of ionization of surface functional groups (Uduman et al., 2010). Addition of an iron-based or aluminium-based coagulant will neutralize and reduce the surface charge (Grima, Belarbi, Fernandez, Medina, & Chitsi, 2003). Microalgae can also be flocculated by inorganic flocculants at sufficiently low pH (Sayyed, Sayyed, & Mazahar, 2010). However, despite its advantages coagulation using inorganic coagulants suffers from several drawbacks. A) A large concentration of inorganic flocculent is needed to cause solid-liquid separation of the microalgae, thereby producing a large quantity of sludge. B) The process is highly sensitive to pH. C) Although some coagulants may work for some microalgae species, they do not work for others. D) The end product is contaminated by the added aluminum or iron salts (Guzine et al., 2011).

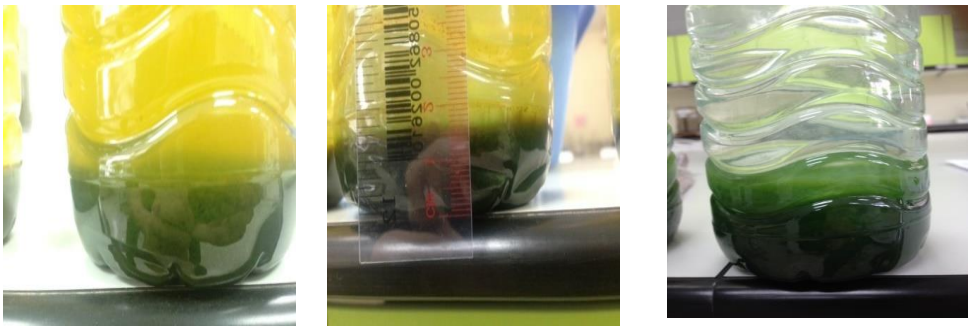
RJAS Vol. 7 No. 1 Jan.-Jun. 2017, pp. 49-57
ISSN 2229-063X (Print)/ISSN 2392-554X (Online)

Table 1 Harvesting microalgae with inorganic flocculation

Chemicals	Concentration (mg/l)	pH	Sludge Height (cm)		Dried Weight (g/l)
			1 h	24 h	
$FeCl_3.6H_2O$	0.2	8.0	0.56±0.06[d]	0.56±0.06[d]	0.25±0.06[d]
	70.0	8.0	1.03±0.06[c]	1.03±0.06[c]	0.36±0.02[c]
$Fe_2(SO_4)_3$	0.7	8.0	1.90±0.01[b]	1.50±0.10[b]	0.45±0.02[b]
	0.8	8.0	1.96±0.06[b]	1.57±0.06[b]	0.42±0.02[bc]
$Al_2(SO_4)_3.18H_2O$	1.0	7.0	2.97±0.55[a]	2.07±0.11[a]	0.53±0.03[a]
	1.2	7.0	3.03±0.15[a]	2.00±0.01[a]	0.55±0.05[a]

[a]*Each measurement is the mean of three replications ± one standard deviation. Means within a column with different letters (a,b,c,d) are significantly different at P < 0.05.*

[a,b,c,d]*Dependent variables:lipid; different letters refer to significantly different lipid contents at 95% confidence intervals; identical letters refer to insignificantly different lipid contents at 95% confidence intervals.*



(a) $FeCl_3.6H_2O$ (b) $Fe_2(SO_4)_3$ (c) $Al_2(SO_4)_3.18H_2O$

Figure 1 Harvesting microalgae with inorganic flocculation

3.2 Organic flocculation:

Chitosan not only has been proven highly effective for water treatment and environmental protection, but also has shown interesting properties in harvesting both freshwater algae and marine algae. Harvesting microalgae with chitosan concentrations of 4 mg/l and 5 mg/l had no difference in cell dried weight and sludge height (Table 2 and Figure 2). As the overall charge of microalgae cells is negative, the positively charged chitosan is strongly adsorbed on microalgae cells to the same amount, which results in most of the charged groups being close to the surface of the cells and effectively destabilizes the microalgae (Guanyi, Liu, Yun, & Yuan, 2014; Wu , Zhu , Huang , Zhang, & Li , 2012). The ability of chitosan to harvest microalgae effectively at low dosages is partly caused by its'- properties. Chitosan not only acts as an adsorbent, but also spontaneously coagulates to agglomerate the microalgae cells. However, when using high dosages of chitosan, the percentage of microalgae cells harvested declined sharply, which may be caused by charge neutralization and bridging phenomena. During flocculation, the cationic charge of chitosan attracts the negatively charged microalgae, reducing the electrostatic repulsion among microalgae cells and then forms the flocs. Excess amino groups led to restabilization of the microalgae and a decrease of separation efficiency (Rashid, Rehman, & Han, 2013).

Table 2 Harvesting microalgae with organic flocculation

Chemicals	pH	Sludge Height(cm)		Dried Weight (g/l)
		1 h	24 h	
Chitosan (4 mg/l)	8.0	1.20±0.10[a]	1.30±0.15[a]	0.46±0.01[a]
Chiosan (5 mg/l)	8.0	1.20±0.01[a]	1.36±0.06[a]	0.46±0.01[a]

[a]*Each measurement is the mean of three replications ± one standard deviation. Means within a column with different letters (a,b,c) are significantly different at P < 0.05.*

[a,b,c]*Dependent variables:lipid; different letters refer to significantly different lipid contents at 95 %confidence intervals; identical letters refer to insignificantly different lipid contents at 95 %confidence intervals.*

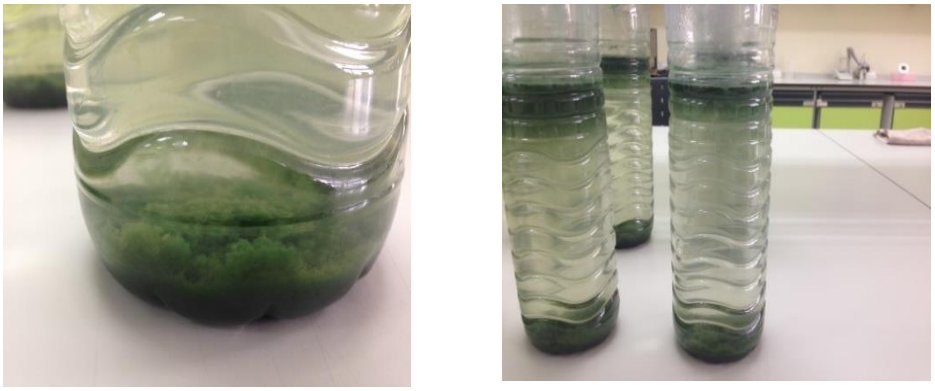


Figure 2 Harvesting of microalgae with organic flocculation

3.3 Autoflocculation

Some microalgae species can flocculate spontaneously in response to certain environmental stresses. This phenomenon is known as autoflocculation. There are several factors that affect the efficiency of autoflocculation, which include the following: pH, dissolved oxygen content, nitrogen concentration and the amount of calcium and magnesium ions in solution. When the pH of the medium is increased, the cells come together and settle by gravitational force. The addition of more bases into the medium increased the formation of dense flocs which resulted in less settling time (Wu et al., 2012). A comparision was performed by adding NaOH or KOH at the same pH and concentration to test the efficiency of autoflocculation (Table 3 and Figure 3). The results showed that auto flocculation of microalgae by NaOH increased the flocculation efficiency more than KOH. Harith et al. (2009) noted that increasing the pH from 8.0 to 10.0 using NaOH or KOH increased the flocculation efficiency from 13 to 82% and from 35 to 78% in 4 h, respectively (Harith, Yusoff, Mohammed, Shariff, & Din, 2009).

3.4 Cylindrical sedimentation tank

Sedimentation tanks are cylindrical with a funnel shaped bottom so that the settled microalgae are concentrated near the outlet (Figure 4). The outlet is placed at the bottom of the tank so that the collection of the settled microalgae can more easily be recovered. These tanks work by allowing the denser solids to settle on the bottom of the tank, leaving the clear water at the surface. Once the settling process is complete, the microalgae can be retrieved from the tank through the outlet. The dried weight of microalgae harvested by cylindrical sedimentation tank (0.79 g/l) was higher than autoflocculation under the same conditions. The factors influencing the settlement rates of microalgae include density and particle size, temperature, aging of the cells, light intensity and time (Harith et al., 2009). Cole and Wells (2003) indicated that the rate of settlement is dependent on the type of microalgae present and found the green microalgae to have an average settling rate of 0.1 m/d.

Table 3 Autoflocculation of microalgae

Chemicals	pH	Sludge Height (cm)		Dried Weight (g/l)
		1 h	24 h	
NaOH (0.1 mg/l)	10.0	0.21±0.07[b]	1.00±0.10[a]	0.40±0.00[a]
KOH (0.1 mg/l)	10.0	0.50±0.05[a]	1.10±0.05[a]	0.25±0.01[b]

[a]*Each measurement is the mean of three replications ± one standard deviation. Means within a column with different letters (a,b,c) are significantly different at P < 0.05.*

[a,b,c]*Dependent variables:lipid; different letters refer to significantly different lipid contents at 95 %confidence intervals; identical letters refer to insignificantly different lipid contents at 95 %confidence intervals.*

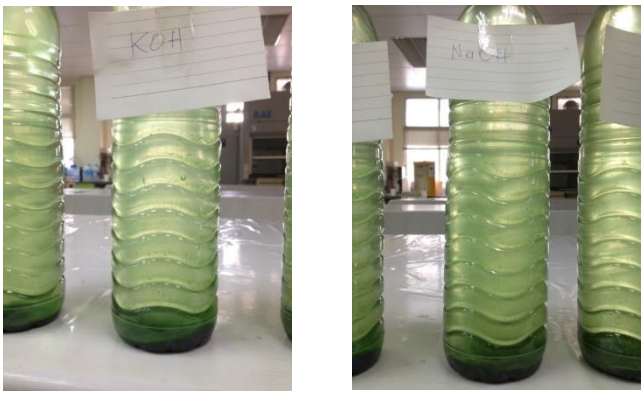


Figure 3 Autoflocculation of microalgae

Figure 4 Harvesting microalgae with cylindrical sedimentation tank

3.5 Bio flocculation

The use of microorganisms for the recovery of microalgal biomass has been investigated (Salim, Bosma, Vermue, & Wijffels, 2011; Xuan, 2009). The factors affecting bio flocculation include: concentration of the bio flocculant, pH and the selectivity of the microorganism. This methods works by the addition of *Bacillus subtilis* (10%, 20%, 30% (v/v)) into the microalgal suspension, which adheres to the microalgae cells causing the weight to increase and resulting in settlement of the cells to the bottom of the vessel. The supernatant containing the culture medium is decanted and washed with water in order to reduce the salinity. The results showed that adding 30% (v/v) *Bacillus subtilis* gave the highest flocculating microalgae (0.90 g/l). Vandamme et al. (2013) indicated that the use of fungi or bacteria as flocculating agents results in microbiological contamination of the microalgae biomass, which needs to be assessed before use in feed or food products.

3.6 Centrifugation.

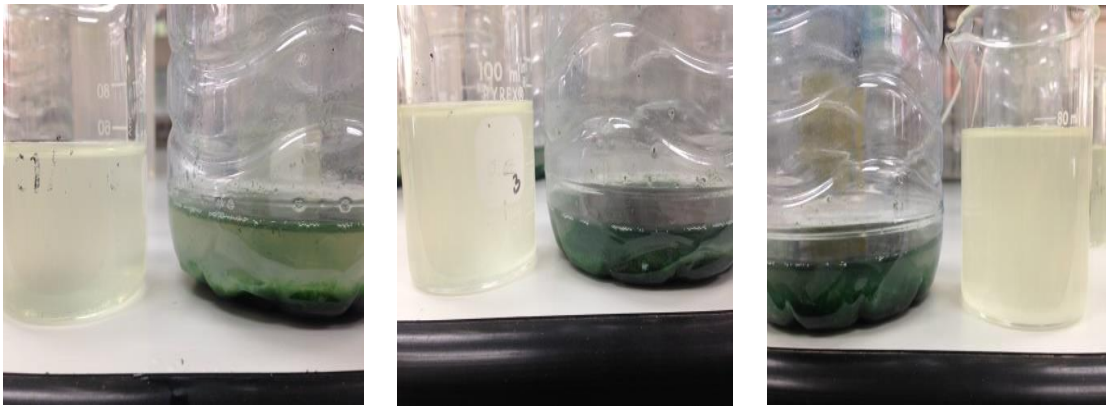
Centrifugation is a process in which a centrifugal force is used to enhance the separation of solids. Spinning the suspension creates the pressure differential necessary for particle separation from the liquid suspension. Thus, the efficiency of the recovery process is dependent on the centrifugal force (Grima et al., 2003). In this study harvesting microalgae at 8000 rpm for 15min gave no difference in microalgae dried weight as compared with 13000 rpm for 3min (Table 5). This means that the centrifugal force at 8000 rpm for 15min was suitable for harvesting microalgae. Al Hattab et al. (2015) noted a recovery efficiency of microalgae greater than 95% at a force of 13000 rpm. However, the energy consumption is higher than low centrifugal force.

Table 4 Bio flocculation of microalgae

Chemicals	Concentration (%v/v)	pH	Sludge Height (cm)		Dried Weight (g/l)
			1 h	24 h	
B .subtilis	10	8.1	1.80±0.20[a]	0.90±0.10[b]	0.84±0.08[c]
	20	7.7	1.50±0.05[b]	1.20±0.05[a]	0.86±0.04[b]
	30	7.0	1.45±0.05[c]	1.20±0.05[a]	0.90±0.05[a]

[a]*Each measurement is the mean of three replications ± one standard deviation. Means within a column with different letters (a,b,c) are significantly different at P < 0.05.*

[a,b,c]*Dependent variables:lipid; different letters refer to significantly different lipid contents at 95 %confidence intervals; identical letters refer to insignificantly different lipid contents at 95 %confidence intervals.*



(a) 10% (v/v) *B. subtilis* (b) 20% (v/v) *B. subtilis* (c) 30% (v/v) *B. subtilis*

Figure 5 Harvesting microalgae with bio flocculation

Table 5 Centrifugation of microalgae

Force (rpm)	pH	Time (min)	Dried Weight (g/l)
8000	8.0	15	0.25±0.02[a]
13000	8.0	3	0.25±0.02[a]

[a]*Each measurement is the mean of three replications ± one standard deviation. Means within a column with different letters (a,b,c) are significantly different at P < 0.05.*

[a,b,c] *Dependent variables:lipid; different letters refer to significantly different lipid contents at 95% confidence intervals; identical letters refer to insignificantly different lipid contents at 95% confidence intervals.*

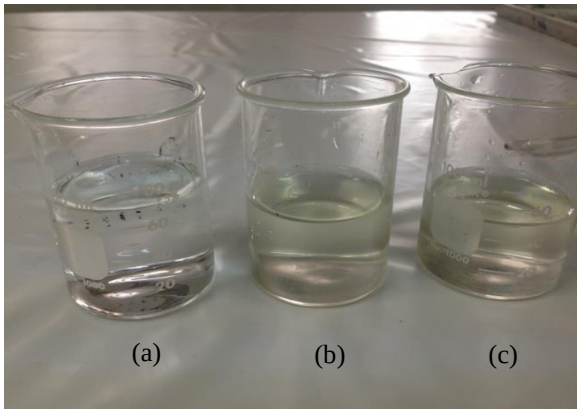
3.7 Vacuum filtration

Vacuum filtration separates solids from liquid media by capturing the solid particles onto a filter while pulling the liquid through by suction from the filter (Science, 2014). Microalgae range in size from 2 to 30 µm indicating that a microfiltration membrane is suitable for vacuum filtration (Brennan & Owende, 2010). The microalgae from brackish water was filtered through 0.45 µm (microfiltration) and 11 µm (macrofiltration) (Table 6). The results showed that the microalgae suspension could not be filtered through a microfiltration membrane (0.45µm) but could be filtered through a macrofiltration membrane (11 µm). This indicates that the microalgae cells from brackish water were greater than 11 µm (Figure 6). Milledge and Heaven (2013) stated that the macrofiltration membrane can be used for large microalgae cells. Uduman et al. (2010) reported that the vacuum filtration harvesting technique is the most suited for large microalgae cells (greater than 10 µm).

Comparison of seven methods deemed suitable for harvesting microalgae on an industrial scale and 5 evaluation criteria are shown in Table 7

Table 6 Vacuum filtration of microalgae

Filter Pore sized	Dried Weight (g/l)
0.45 µm (microfiltration)	ND
11 µm (macrofiltration)	0.42±0.03



(a) Distill water (b), (c) Supernatant after vacuum filtration

Figure 6 Supernatant of microalgae after vacuum filtration

Table 7 Seven methods used for harvesting microalgae

Method	Chemical	Sludge Height (cm, 1 h)	Sludge Height (cm, 24 h)	Dried Weight (g/l)	Chemical (Baht/g)
Inorganic flocculation	$FeCl_3.6H_2O$ 0.2 ppm	0.56 ± 0.06^{e}	0.56 ± 0.06^{i}	0.25 ± 0.06^{f}	0.60
	$FeCl_3.6H_2O$ 70 ppm	1.03 ± 0.06^{d}	1.03 ± 0.06^{gh}	0.36 ± 0.02^{e}	0.60
Inorganic flocculation	$Fe_2(SO_4)_3$ 0.7 g/l	1.90 ± 0.01^{b}	1.50 ± 0.10^{b}	0.45 ± 0.02^{d}	3.20
	$Fe_2(SO_4)_3$ 0.8 g/l	1.96 ± 0.06^{b}	1.57 ± 0.06^{b}	0.42 ± 0.02^{de}	3.20
	$Al_2(SO_4)_3.18H_2O$ 1 g/l	2.97 ± 0.55^{a}	2.07 ± 0.11^{a}	0.53 ± 0.03^{c}	0.26
	$Al_2(SO_4)_3.18H_2O$ 1.2 g/l	3.03 ± 0.15^{a}	2.00 ± 0.01^{a}	0.55 ± 0.05^{c}	0.26
Auto flocculation	NaOH	0.21 ± 0.07^{f}	1.00 ± 0.10^{gh}	0.40 ± 0.00^{de}	0.31
	KOH	0.50 ± 0.05^{ef}	1.10 ± 0.05^{g}	0.25 ± 0.01^{f}	0.43
Centrifugation	8000 rpm 15 min	-	-	0.25 ± 0.02^{f}	-
	13000 rpm 3 min	-	-	0.25 ± 0.02^{f}	-
Organic locculation	Chitosan 4 g/l	1.20 ± 0.1^{cd}	1.30 ± 0.15^{de}	0.47 ± 0.01^{d}	0.70
	Chitosan 5 g/l	1.20 ± 0.01^{cd}	1.36 ± 0.06^{d}	0.46 ± 0.01^{d}	0.70
Cylindrical tank	NaOH	-	-	0.79 ± 0.01^{b}	0.31
Bio flocculation	*B .subtilis* 10% (v/v)	1.80 ± 0.20^{cd}	0.90 ± 0.10^{h}	0.84 ± 0.08^{ab}	-
	B .subtilis 20% (v/v)	1.50 ± 0.05^{c}	1.20 ± 0.05^{ef}	0.86 ± 0.05^{a}	-
	B .subtilis 30% (v/v)	1.45 ± 0.05^{b}	1.20 ± 0.05^{ef}	0.90 ± 0.05^{a}	-
Vacuum filtration	0.45 µm (micro filtration)	-	-	ND	290
	11 µm (macro filtration)	-	-	0.42 ± 0.03^{de}	1000

Criteria	Description
Dewatering efficiency	The system should be able to effectively concentrate and remove a high percentage of the cells from their surrounding liquid media.
Cost	The operational costs of the process should be low in order to reduce the total processing costs associated with microalgae recovery.
Suitability for large scale use	The method should be effective in handing large volume for industrial production.
Time	The rate of harvest should be quick to ensure the sustainability purposes.
Reusability of media	The media should be recycled for reuse in order to minimize costs.

4. Conclusion

Selection of the most suitable harvesting methods was based on dewatering efficiency, cost, suitability for industrial scale, time and reusability of media for operating on large scale. The results showed that harvesting microalgaeoil with 1.2 g/l $Al_2(SO_4)_3.18H_2O$ gave the most efficient and economically viable dewatering methods for large scale processing of microalgae biomass .

Although each of the optimal techniques was deemed suitable for harvesting of microalgae from brackish water in Thailand on their merits, a combination of methods can also be used to enhance the recovery efficiency and improve the economics. The use of inorganic flocculation as an initial harvesting step to concentrate the algae suspension and the centrifugation as a secondary dewatering step will reduce the time and costs associated with dewatering. Flocculation allows for effective removal of algae from large amounts of liquid media and as such the costs associated with energy intensive centrifugation can be reduced by using them as secondary techniques since less volumes of microalgae suspension will undergo the secondary treatment. The cost of the purification and extraction processes decreases with increased biomass concentration.

5. Acknowledgements

The authors express their gratitude to the Research Institute of Rangsit University for providing research fund.

6. References

Al Hattab, M., Ghaly, A., & Hammoud, A. (2015). Microalgae harvesting methods for industrial production of biodiesel: Critical review and comparative analysis. *Journal Fundamentals of Renewable Energy and Applications, 5*(2), 1-26. DOI: http://dx.doi.org/10.4172/2090-4541.1000154

Brennan, L., & Owende, P. (2010). Biofuels from microalgae - A review of technologies for production, processing and extractions of biofuels and co products. *Renewable and Sustainable Energy Reviews*, *14*, 557-577. DOI:http://dx.doi.org/10.1016/j.rser.2009.10.009

Chen, P., Min, M., Chen, Y., Wang, L., Li, Y., Chen, Q., . . . Ruan, R. (2009). Review of the biological and engineering aspects of algae to fuels approach. *International Journal of Agricultural and Biological Engineering, 2*(4), 1-30. DOI: http://dx.doi.org/10.3965/j.issn.1934-6344.2009.04.001-030

Cole, T. M., & Wells, S.A. (2003). A two-dimensional, laterally averaged, hydrodynamic and water quality model, version 3.1. Instruction Report EL-03-1, US Army Engineering and Research Development Center, Vicksburg, MS, USA.

Danquah, M. K., Gladman, B., Moheimani, N., & Forde, G. M. (2009). Microalgal growth characteristics and subsequent influence on dewatering efficiency. *Chemical Engineering Journal*, *51*(1-3), 73-78. DOI:http://dx.doi.org/10.1016/j.cej.2009.01.047

Demirbas, A. (2010). Use of algae as biofuel sources. *Energy Conversion and Management*, *51*(12), 2738-2749. DOI: http://dx.doi.org/10.1016/j.enconman.2010.06.010

Grima, E. M., Belarbi, F. G., Fernandez, A., Medina, A. R., & Chitsi, Y. (2003). Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnology Advances*, *20*(7-8), 491-515. DOI: http://dx.doi.org/10.1016/S0734-9750(02)00050-2

Guanyi, C., Liu, Z., Yun, Q., & Yuan, L. C. (2014). Chitosan and its derivatives applied in harvesting microalgae for biodiesel production: An outlook. *Journal of Nanomaterials*, *4*(1-9). DOI: http://dx.doi.org/10.1155/2014/217537

Gultom, S. O., & Hu, B. (2013). Review of microalgae harvesting via co-pelletization with filamentous fungus. *Energies*, *6*(6), 5921-5939. DOI: http://dx.doi.org/10.3390/en6115921

Guzine, I., Diwanni. E. I., Shadia, A., Rafei, E. I., Hawash, S. I., & Enin, S. A. (2011). Optimized flocculation of microalgae for fuel oil and antioxidant production. *Der Chemica Sinica*, *4*(12-25).

Harith, Z. T., Yusoff, F. M., Mohammed, M. S., Shariff, M., & Din, M. (2009). Effect of different flocculants on the flocculation performance of microalgae, Chaetoceros

calcitrans. *African Journal of Biotechnology*, *8*(21), 5971-5978.

Harun, R., Singh, M., Gareth, M. F., & Micheal, K. D. (2010). Bioprocess engineering of microalgae to produce a variety of consumer products. *Renewable and Sustainable Energy Reviews*, *14*, 1037-1047. DOI: http://dx.doi.org/10.1016/j.rser.2009.11.004

Milledge, J. J., & Heaven, S. (2013). A review of the harvesting of micro-algae for biofuel production. *Reviews in Environmental Science and Biotechnology*, *12*(2), 165-178. DOI: http://dx.doi.org/10.1007/s11157-012-9301-z

Olaizola, M. (2003). Commercial development of microalgal biotechnology: From the test tube to the marketplace. *Biomolecular Engineering*, *20*, 459-466. DOI: http://dx.doi.org/10.1016/S1389-0344(03)00076-5

Pulz, O. (2001). Photobioreactors: production systems for phototrophic microorganisms. *Applied Microbiology and Biotechnology*, *57*(3), 287-293.

Rashid, N., Rehman, M. S., & Han, J. I. (2013). Use of chitosan acid solution to improve separation efficiency for harvesting of the microalga Chorella vulgaris. *Chemical Engineering Journal*, *226*, 238-242.

Salim, S., Bosma, R., Vermue, M. H., & Wijffels, R. H. (2011). Harvesting of microalgae by bio-flocculation. *Journal of Applied Phycology*, *23*(5), 849-855. DOI: http://dx.doi.org/10.1007/s10811-010-9591-x

Sayyed, H., Sayyed, A., & Mazahar, F. (2010). Adsorption studies of Fe. (II) on low cost biomaterial. *Advances in Applied Science Research*, *1*(3), 147-152.

Science, U. A. (2014). Filtration. Department of Chemistry and Biochemistry. University of Arizona, Tucson, Arizona. Accessed on October 22, 2014 from http://www.chem.arizona.edu/

Shelef, G., Sukenik, A., & Green, M. (1984). Microalgae harvesting and processing: A literature review. National Technical Information Service. U.S. Department of Commerce.

Slade, R., & Bauen, A. (2013). Microalgae cultivation for biofuels: Cost, energy balance, environmental impacts and future prospects. *Biomass and Bioenergy*, *53*, 29-38. DOI: http://dx.doi.org/10.1016/j.biombioe.2012.12.019

Uduman, N., Qi, Y., Danquah, M. K., Forde, G. M., & Hoadley, A. (2010). Dewatering of microalgal cultures: a major bottleneck to algae-based fuels. *Journal of Renewable and Sustainable Energy*, *2*, 012701-012715. DOI: http://dx.doi.org/10.1063/1.3294480

Vandamme, D., Foubert, I., & Muylaert, K. (2013). Flocculation as a low-cost method for harvesting microalgae for bulk biomass production. *Trends in Biotechnology*, *31*(4), 233-239. DOI: http://dx.doi.org/10.1016/j.tibtech.2012.12.005

Wahlen, B. D., Willis, R. M., & Seefeldt, L. C. (2011). Biodiesel production by simultaneous extraction and conversion of total lipids from microalgae, cyanobacteria, and wild mixed-cultures. *Bioresource Technology*, *102*, 2727-2730. DOI: http://dx.doi.org/10.1016/j.biortech.2010.11.026

Wu, Z., Zhu, Y., Huang, W., Zhang, C., & Li, T. (2012). Evaluation of flocculation induced by pH increase for harvesting microalgae and reuse of flocculated medium. *Bioresource Technoogyl*, *110*, 496-502. DOI: https://dx.doi.org/10.1016/j.biortech.2012.01.101

Xuan, D. T. T. (2009). *Harvesting marine algae for biodiesel feedstock*. Department of Environmental Engineering, National University of Singapore. Accessed on November 16, 2014 from www.nus.edu.sg/nurop/2009/FoE/U067436X.PDF

Zitelli, G. C., Rodolfi, L., Biondi, N., & Tredici, M. R. (2006). Productivity and photosynthetic efficiency of outdoor cultures of *Tetraselmis suecica* in annular columns. *Aquaculture*, *261*, 932-943. DOI:http://dx.doi.org/10.1016/j.aquaculture.2006.08.011