Bioresource Technology 198 (2015) 828–835

Contents lists available at ScienceDirect

Bioresource Technology

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

Evaluation of various harvesting methods for high-density microalgae, *Aurantiochytrium* sp. KRS101

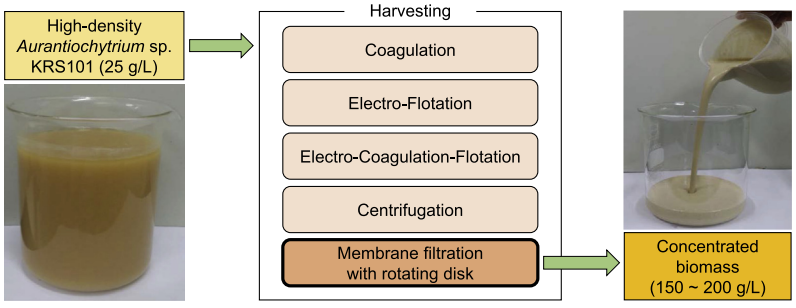
Kyochan Kim [a,1], Heewon Shin [a,1], Myounghoon Moon [a], Byung-Gon Ryu [b], Jong-In Han [c], Ji-Won Yang [a], Yong Keun Chang [a,d,*]

[a] Department of Chemical and Biomolecular Engineering, KAIST, 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea
[b] Decontamination and Decommissioning Research Division, Korea Atomic Energy Research Institute (KAERI), 989-111 Daedukdaero, Yuseong-gu, Daejeon 305-353, Republic of Korea
[c] Department of Civil and Environmental Engineering, KAIST, 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea
[d] Advanced Biomass R&D Center, KAIST, 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea

HIGHLIGHTS

- Harvest of high-density *Aurantiochytrium* sp. was difficult using common methods.
- Dynamic filtration was found to be an ideal alternative for the high-density culture.
- Fast rotation of a perforated disk reduced membrane fouling maximally by 99%.
- Rotating disk helped maintain high permeate flux under above 150 g/L of biomass.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 6 August 2015
Received in revised form 21 September 2015
Accepted 22 September 2015

Keywords:
Aurantiochytrium sp.
Coagulation
Electrochemical process
Centrifugation
Membrane filtration

ABSTRACT

Five technologies, coagulation, electro-flotation (EF), electro-coagulation–flotation (ECF), centrifugation, and membrane filtration, were systematically assessed for their adequacy of harvesting *Aurantiochytrium* sp. KRS101, a heterotrophic microalgal species that has much higher biomass concentration than photoautotrophic species. Coagulation, EF, and ECF were found to have limited efficiency. Centrifugation was overly powerful to susceptible cells like *Aurantiochytrium* sp. KRS101, inducing cell rupture and consequently biomass loss of over 13%. Membrane filtration, in particular equipped with an anti-fouling turbulence generator, turned out to be best suited: nearly 100% of harvesting efficiency and low water content in harvested biomass were achieved. With rotation rate increased, high permeate fluxes could be attained even with extremely concentrated biomass: e.g., 219.0 and 135.0 L/m^2/h at 150.0 and 203.0 g/L, respectively. Dynamic filtration appears to be indeed a suitable means especially to obtain highly concentrated biomass that have no need of dewatering and can be directly processed.

1. Introduction

Microalgae is regarded as an unparalleled and sustainable feedstock for the production of both biofuels (most notably, biodiesel) and high-value added products such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and astaxanthin (Alfafara et al., 2002; Armenta and Valentine, 2013; Cuellar-Bermudeza et al., 2014; Papazi et al., 2010). Among a good many microalgal

* Corresponding author at: Department of Chemical and Biomolecular Engineering, KAIST, 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea. Tel.: +82 42 350 3927; fax: +82 42 350 3910.
E-mail address: ychang@kaist.ac.kr (Y.K. Chang).
[1] Kyochan Kim and Heewon Shin contributed equally to this work.

http://dx.doi.org/10.1016/j.biortech.2015.09.103
0960-8524/

species, *Aurantiochytrium* sp., heterotrophic thraustochytrid, is particularly useful, as it can accumulate up to 80% of lipid of dry biomass and the lipid contains exceptionally high levels of palmitic acid and DHA (Hong et al., 2011; Kim et al., 2013; Vandamme et al., 2011). The high level of palmitic acid renders the microalgal oil suitable for the biodiesel production: the resulting fuel can have high cetane number and oxidation stability, and low iodine content. What is more, the exceedingly high DHA content makes the *Aurantiochytrium* species a competitive candidate for the production of the unsaturated oil (Ryu et al., 2013a; Sijtsma and De Swaaf, 2004).

Recently, various studies based on *Aurantiochytrium* sp. KRS101 have been performed for the purpose of its commercial application. Mass production was attempted by taking advantage of its high biomass productivity (Hong et al., 2013b), cheap substrates including cellulosic biomass (Hong et al., 2012, 2013a) and organic waste (Ryu et al., 2013b) sought for the reduction of cultivation cost, and wet extraction of lipid developed for decreasing the cost of downstream processes (Choi et al., 2014a,b; Yoo et al., 2014). Harvesting, however, is not well studied and a technology with sufficient efficacy has not yet established; and it is more so because the final cell density is much higher than the photoautotrophic cultivation.

The harvesting technologies can be divided as chemical- and physical-based methods: the chemical-based methods include coagulation, electro-flotation (EF), and electro-coagulation–flotation (ECF), and physical-based methods include centrifugation and membrane filtration. The coagulation process is easily operated and inexpensive. However, remaining chemical coagulants in the harvested microalgae may affect the biomass negatively. Although electrochemical approaches like EF and ECF are surely cost- and energy-effective compared to the chemical coagulation, they have substantial downsides: EF has considerably low harvesting efficiency and ECF has the problem of residual chemicals that may degrade the quality of biomass. Centrifugation, a physical method, has merits of efficiency and simplicity and yet its practicality is limited on account of prohibitively high requirement of cost and energy. Cell damage caused by strong centrifugal force is also problematic. Lastly, membrane filtration makes it possible to harvest microalgae in a manner that is highly efficient and biomass quality remains unaltered. This otherwise ideal approach has its own challenging issue, i.e., membrane fouling that greatly degrades filtration performance.

The purpose of this study was therefore to determine a best-suited harvesting means particularly for a heterotrophically grown and hence dense culture of *Aurantiochytrium* sp. KRS101 with biomass concentration of around 25 g/L. Because of its uniquely high density, a level that is far higher than the phototrophic cultivation, it is possible that different methods and/or different condition should be applied. To this end, the aforementioned technologies, namely, coagulation, EF, ECF, centrifugation, and membrane filtration, were examined and compared.

2. Methods

2.1. Preparation of microalgae

Aurantiochytrium sp. KRS101 was obtained from the Korea Research Institute of Bioscience and Biotechnology (KRIBB) and stored in 25% (v/v) glycerol at −80 °C. Seed cultures were grown in 50-mL basal medium, consisting of 60 g/L glucose, 10 g/L yeast extract, 9 g/L KH_2PO_4, 10 g/L sea salt, and 10 mg/L tetracycline, in 250-mL culture flasks on a shaker rotated at 120 rpm for 3 days at 28 °C. The microalgae was cultivated in 1 L of the same medium in a 5-L bioreactor for 3 days at 28 °C with shaking at 120 rpm and 0.5 v/v/min of air. The pH of the culture was 4.6.

2.2. Harvesting experiment

Five harvesting methods, i.e., coagulation, EF, ECF, centrifugation, and membrane filtration, were conducted using the same batch culture of *Aurantiochytrium* sp. KRS101. All experimental materials and operation conditions of each harvesting method are summarized in Table 1.

Coagulation was performed using $FeCl_3$ (Sigma Aldrich, USA) as a coagulant with five different doses: 0, 0.25, 0.5, 0.75, and 1 g/L. After the coagulant was added, the mixture was vigorously stirred to ascertain that microalgae and the coagulant were mixed thoroughly. In each experiment, 3 mL of the sample was obtained to measure harvesting efficiency from the half height of 100 mL of microalgae suspension in a mass cylinder every 15 min for 2 h.

In ECF experiments, a chamber (width, 70 mm; height, 170 mm; thickness, 70 mm) with two perforated electrode plates (width, 57 mm; height, 115 mm; thickness, 2 mm) placed in parallel was installed. An aluminum electrode (Mg, 2.2–2.8%; Cr, 0.15–0.35%) was used as the sacrificing anode electrode and a dimensionally stable anode (DSA®, Ti/IrO_2) as the cathode. The anode was connected to the positive outlet and the cathode to the negative outlet using a DC power supply (S-3005Q, Fine-Power, Korea). Conventional DC was converted to pulsed DC with different duty cycles (0.2–1.0) at 1000 Hz. A voltage meter (GDM-8261, Good Will Instrument, Taiwan) was used to measure voltage to calculate electrical consumption during the experiment. Each of three different current densities (5.7, 11.4, and 17.2 mA cm^{-2}) was supplied to 400 mL of the microalgae suspension. A magnetic stirring bar was placed on the floor of the chamber and rotated at 350 rpm during the experiment. Every 5 min, 3 mL of the sample was taken to measure harvesting efficiency at 90 mm below the water surface using a 5 mL syringe with a 15 cm needle. In the EF mode, a non-sacrificial electrode (DSA®, Ti/IrO_2) was employed as the anode and the placements of Al was used as the cathode; thus on it hydrogen gas was generated, instead of Al released from it. Other than this condition, similar experimental conditions were applied to EF as in ECF.

Centrifugation was performed using a High speed centrifuge Supra 22 K (Hanil Science, Korea) at 1000, 5000, and 9000*g* for 30 min. After centrifugation, a supernatant was discarded and a cell pellet freezer-dried for 3 days in a pre-weighed tube. The tube after freezer-drying was weighed, and the amount of lyophilized biomass was calculated to determine recovered biomass from centrifugation and harvesting efficiency of it.

Membrane filtration was carried out using a dynamic filtration module, a FMX B-class (bench scale) commercial equipment equipped with a perforated disk (BKT Co. Ltd., Korea). Details of the equipment and system were previously reported (Kim et al., 2015).

For the selection of a proper membrane, three commercial membranes were tested: one microfiltration membrane, polyvinylidene fluoride (PVDF) 0.2 μm; and two ultrafiltration membranes, PVDF 150 kDa and polyethersulfone (PES) 150 kDa, from Microdyn-Nadir (Germany). Their membrane permeabilities as measured by pure-water filtration were 2560.2 ± 169.7, 790.9 ± 14.3, and 709.1 ± 11.6 L/m^2/h/bar, respectively. Filtration experiments of microalgal culture were carried out at five rotation speeds of 0, 400, 800, 1200, and 1600 rpm and at a constant transmembrane pressure (TMP) of 100 kPa and feed flow rate of 8 L/min. All the experiments were conducted at temperatures between 25 and 30 °C. The permeate was collected to calculate the permeate mass flow rate using a load cell connected to a computer, then being recirculated back into the feed tank so that initial biomass concentration in the feed tank remained constant.

Once the membrane of PES 150 kDa was selected, dewatering experiments were conducted to reach biomass concentration of

Table 1
Experimental materials and conditions of each harvesting method for *Aurantiochytrium* sp. KRS101.

Harvesting method	Material	Operation condition	Operation time (min)
Coagulation	Coagulant: $FeCl_3$	Coagulant dose: 0, 0.25, 0.5, 0.75, 1.0 g/L	120
Electro-flotation	Anode: DSA Cathode: Al	Current density: 5.7, 11.4, 17.2 mA cm^{-2}	40
Electro-coagulation–flotation	Anode: Al Cathode: DSA	Current density: 5.7, 11.4, 17.2 mA cm^{-2}	40
Centrifugation		Relative centrifugal force: 1000, 5000, 9000*g*	30
Membrane filtration	Membrane: PVDF 0.2 um, PES 150 kDa, PVDF 150 kDa	Rotation speed: 0, 400, 800, 1200, 1600 rpm	180–360

more than 150 g/L using membrane filtration at 1600 and 800 rpm at the constant TMP of 100 kPa and feed flow rate of 8 L/min. In this case, the permeate was not recirculated back into the feed tank; instead, an aliquot was collected from the feed tank for monitoring any change in its biomass concentration.

2.3. Analytical and calculated methods

Biomass concentration was determined by filtering a microalgal suspension through a pre-dried and pre-weighed 47 mm circular glass filter paper (GF/C, Whatman, USA) and weighing the remains after washing by phosphate-buffered saline (PBS, pH 7.4) and drying at 105 °C for 24 h. Optical density (OD) of sample was measured at 600 nm using a UV–Vis spectrophotometer (DU® 700, Beckman Coulter, USA).

Harvesting efficiency (%) of each harvesting method was calculated as

$$\text{Coagulation, EF, and ECF}: \text{ Harvesting efficiency} = 100 \times (1 - \mathrm{OD}_t/\mathrm{OD}_{\text{initial}})$$

$$\text{Centrifugation}: \text{ Harvesting efficiency} = 100 \times (1 - W_{\text{after}}/W_{\text{before}})$$

$$\text{Membrane filtration}: \text{ Harvesting efficiency} = 100 \times (1 - \mathrm{OD}_{\text{permeate}}/\mathrm{OD}_{\text{feed}})$$

where OD_t and $\mathrm{OD}_{\text{initial}}$ are ODs of the suspension at time t and prior to the start of harvesting process, respectively, W_{after} and W_{before} are weights of biomass after and before centrifugation, respectively, and $\mathrm{OD}_{\text{permeate}}$ and $\mathrm{OD}_{\text{feed}}$ are ODs of the permeate and feed, respectively.

In membrane filtration, membrane resistance and fouling resistance were calculated as

$$J_w = \mathrm{TMP}/\mu R_m$$

$$J_{\text{plateau}} = \mathrm{TMP}/\mu(R_{\mathrm{m}} + R_{\mathrm{f}})$$

where J_w is the permeate flux (L/m^2/h) of pure-water filtration, J_{plateau} is the permeate flux at the plateau state (where no large change of permeate flux occurs) during microalgae filtration, μ is the fluid viscosity (Pa s), and R_{f} and R_{m} are the fouling and membrane resistances (1/m), respectively.

3. Results and discussion

3.1. Chemical-based harvesting

Coagulation was found to be effective only when the coagulant $FeCl_3$ dosage was above 0.5 g/L (Fig. 1); the removal rates of microalgae were 81.9%, 90.3% and 98.8% for 0.5, 0.75, and 1 g/L, respectively. This high requirement of coagulant happened likely because of its uniquely high biomass concentration. As expected, the harvesting efficiency was raised with the increase in dosage (Papazi et al., 2010).

As the dosage was increased from 0.5 to 1 g/L, a layer of coagulated biomass was formed, gradually becoming denser and thinner during experiment. When 0.5 or 0.75 g/L was used, the layer was well settled only within 20 min. A dosage of 1 g/L, though nearly 100% of biomass was captured, required 60 min for complete settlement, indicating that large dosages of coagulant are not always beneficial particularly for harvesting *Aurantiochytrium* sp. KRS101. In view of harvesting time, the optimal dose of $FeCl_3$ fell into between 0.75 and 1 g/L, with a limited harvesting efficiency of less than 90%. Considering all this, coagulation seemed not to be an ideal option at least for concentrating *Aurantiochytrium* sp. KRS101.

In the electrochemistry-based methods, namely, ECF and EF, harvesting efficiency was found to be a function of current density supplied (Fig. 2). In the ECF, an increased current density accelerated the release of aluminum ions from the sacrificial anode electrode, thereby causing metal hydroxides to more rapidly be formed; the aluminum hydroxide coagulant then led to faster microalgal floc formation and therefore harvesting. This phenomenon occurred in the EF in a similar way except for floc formation.

The harvesting efficiency of ECF was higher than EF, especially at a current density of 17.2 mA cm^{-2}. At this current density, the maximum efficiency of ECF reached 88.1%, while only 55.5% was reached using EF. This efficiency discrepancy was caused likely by the varied extents of gas bubble entrapment into algae cells: the entrapment was effective with ECF but limited with EF, confirming the flocculation is indeed a necessary step for efficient harvesting of *Aurantiochytrium* sp. KRS101 just like many other microalgal species (Alfafara et al., 2002).

Even though using the larger current density resulted in the higher harvesting efficiency, the decreasing rate of harvesting efficiency also increased with a supply of the larger current density. In ECF experiments, contrary to steadily increasing recovery efficiency with a current density of 5.7 and 11.4 mA cm^{-2}, the recovery efficiency at a supply of 17.2 mA cm^{-2} decreased more than 20% after 40 min. It is speculated that too high current density could release an excessive amount of metal hydroxides and cause the surface of microalgae overly covered by positively charged precipitates. The microalgae surface may eventually become positive, instead of neutralized. As a result, the repulsion among the microalgae cells can occur and each cell stabilized again, resulting in the reduced formation of flocs.

Overall, the electrochemical means appeared to be less effective for harvest of *Aurantiochytrium* sp. KRS101 than other species. For *Chlorella vulgaris* and *Microcystis aeruginosa*, recovery efficiency easily reached more than 95% (Gao et al., 2010; Vandamme et al., 2011), which was substantially higher than 88.1% in this study.

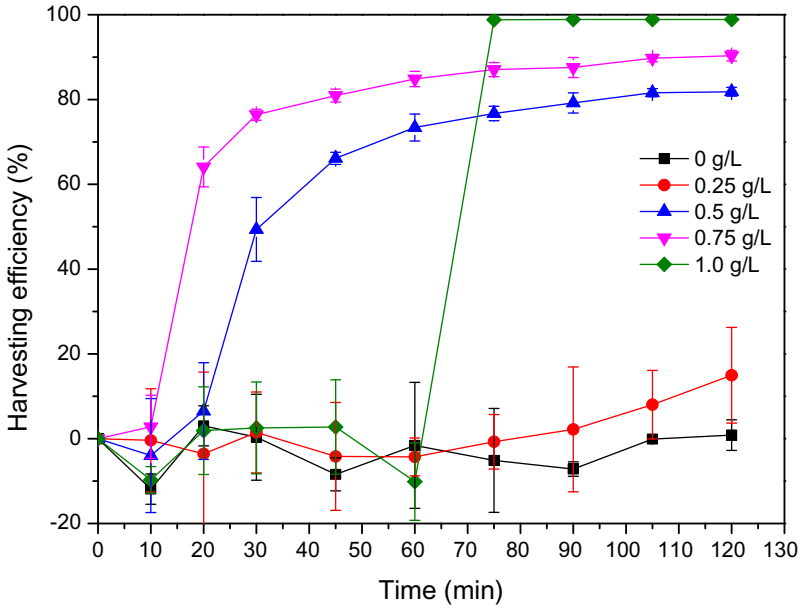


Fig. 1. Effect of $FeCl_3$ dosage on harvesting efficiency of coagulation for *Aurantiochytrium* sp. KRS101.

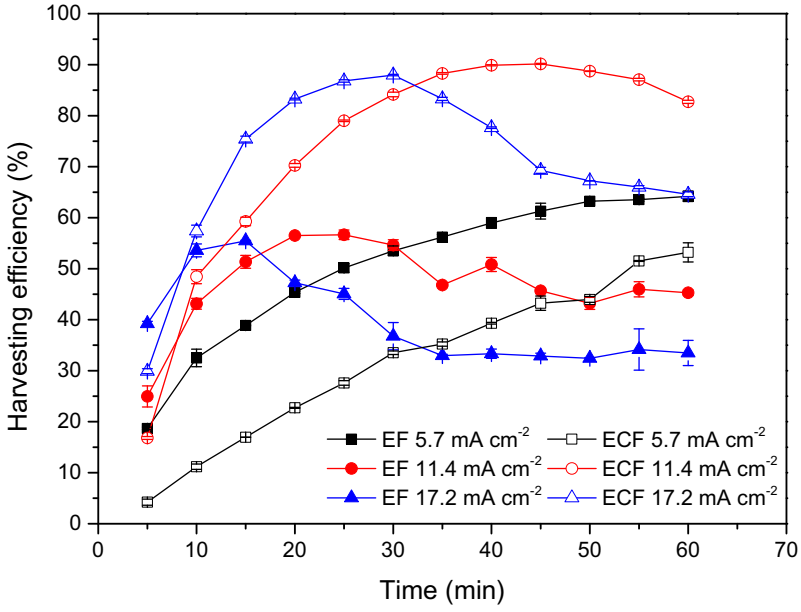


Fig. 2. Effect of current density on harvesting efficiency of EF and ECF for *Aurantiochytrium* sp. KRS101.

One possible cause of this impaired efficiency was the high initial biomass concentration of *Aurantiochytrium* sp. KRS101 which led to poor mixing of suspension; thus, there was less chance for the positive ions to attach to the microalgae surface and cells to be trapped in the gas bubbles.

3.2. Physical-based harvesting

Centrifugation, though effective, lost 12.8–15.4% of biomass at all the tested centrifugal forces (Table 2). This is because a floating layer was formed and not settled down. *Aurantiochytrium* sp. is known as highly sensitive to cell rupture by the external stress, leading to a release of cytoplasm into the medium (Wong et al., 2008). The cell rupture was reported to occur even by osmotic pressure generated through nutrient consumption during cultivation (Kim et al., 2013; Velmurugan et al., 2014). This phenomenon might take place in this study as well, as the cells were grown until glucose was almost consumed. Besides, an increase in relative centrifugal force led to a thicker floating layer, resulting in a great biomass loss: this took place possibly because the increased external force ended up rupturing more cells. Both issues, which are critical, make centrifugation almost impractical to be used for harvesting *Aurantiochytrium* sp. KRS101.

In membrane filtration equipped with the rotational disk, effects of rotation speed and membrane on harvesting performance were firstly investigated in order to find the optimum rotation speed and membrane for harvest of *Aurantiochytrium* sp. KRS101. The plateau permeate fluxes of all the membranes were

Table 2
Summary of harvesting performance of five harvesting methods for *Aurantiochytrium* sp. KRS101.

Harvesting method	Condition and operation time	Harvesting efficiency (%)	Water content in harvested biomass (%)
Coagulation	0 g/L, 120 min	0.9	83.7
	0.25 g/L, 120 min	15.0	85.8
	0.50 g/L, 120 min	81.8	89.9
	0.75 g/L, 120 min	90.3	90.2
	1.00 g/L, 120 min	98.8	91.5
Electro-flotation	5.7 mA cm^{-2}, 40 min	59.0	82.0
	11.4 mA cm^{-2}, 25 min	56.7	86.1
	17.2 mA cm^{-2}, 15 min	55.6	88.0
Electro-coagulation–flotation	5.7 mA cm^{-2}, 40 min	39.3	83.9
	11.4 mA cm^{-2}, 40 min	89.9	85.2
	17.2 mA cm^{-2}, 30 min	88.0	90.2
Centrifugation	1000*g*, 30 min	87.2	72.1
	5000*g*, 30 min	85.9	70.9
	9000*g*, 30 min	84.6	70.2
Membrane filtration	PVDF 0.2 μm, 240 min	97.3	–
	PES 150 kDa, 1600 rpm, 180 min	99.8	79.7
	PES 150 kDa, 800 rpm, 360 min		83.9
	PVDF 150 kDa, 240 min	99.9	–

below 6.0 L/m^2/h without the rotation of the disk, which was similar to the classical cross-flow filtration mode (Fig. 3a). Unlike other photoautotrophic microalgae, high biomass concentration of this heterotrophic microalgae culture rapidly produced harsh fouling layer on the membrane surface during filtration of microalgae, leading to more than 97% of flux decline and very low permeate flux. For other dynamic filtration of *C. vulgaris* using a vibratory membrane, a drastic flux decline was also found to take place when feed concentration increased up to 100 g/L, and permeate flux with feed concentration of 25 g/L was only 16 L/m^2/h at TMP of 276 kPa in the cross-flow filtration mode without the vibration of the membrane (Slater et al., 2015). The application of the rotational disk on the membrane surface to solve this serious fouling problem increased plateau permeate fluxes of all membranes dramatically up to 180.6 L/m^2/h at 800 rpm and 289.5 L/m^2/h at 1600 rpm for PVDF 0.2 μm (Fig. 3a). In addition, an increase in rotation speed of disk decreased fouling resistance notably by more than 97% from 800 rpm (Fig. 3b). These results demonstrated the positive effect of rotational disk generating high shear stress on the prevention of membrane fouling formation and as a result the improvement of filtration performance, as proven in the previous results (Hwang and Lin, 2014; Hwang and Wu, 2015; Kim et al., 2015; Ríos et al., 2012).

PVDF 0.2 μm had higher permeate flux than PVDF 150 kDa obviously due to larger mean pore size, and yet showed similar performance to PES 150 kDa possibly due to smoother surface of PES membrane (Fig. 3a) (Zhang et al., 2008). Fouling resistance of PES 150 kDa was lower than the other membranes with less severe membrane fouling and lower flux decline on account of low surface roughness of PES membrane (Fig. 3b). Harvesting efficiency was quite high: microfiltration and ultrafiltration membranes were 97.3% and 100%, respectively, regardless of rotation speed of the perforated disk (Table 2). Because the ultrafiltration membranes, which displayed high enough efficiency and flux, has an added advantage of being able to capture all types of cells, even the smallest ones that pass through the microfiltration membrane, PES 150 kDa was chosen as the membrane for further dewatering experiments of *Aurantiochytrium* sp. KRS101.

Dewatering experiments were done to increase biomass concentration from 25 g/L and up to 150 g/L. To this end, two rotation speeds, 800 and 1600 rpm, were employed separately. During filtration, permeate flux decreased slowly in the first stage, and then remained relatively constant above 250 and 100 L/m^2/h at 1600 and 800 rpm, respectively, until biomass concentration reached around 100 g/L (Fig. 4). Afterwards flux decline became rapid between 100 and 150 g/L, and far more so between 150 and 200 g/L. This result was in line with a previous study that reported dynamic filtration experienced fast flux decline from 160 to 40 L/m^2/h when biomass concentration of *C. vulgaris* was increased from 0.5 to 100 g/L at TMP of 207 kPa (Slater et al., 2015). To our surprise, high permeate fluxes were maintained even at very high concentration of 150.0 g/L: 219.0 L/m^2/h at 1600 rpm and 75.5 L/m^2/h at 800 rpm. This implied that the rotation of high speed of disk and thus turbulence generated by it effectively alleviated serious fouling on membrane. Even more surprisingly, operation at 1600 rpm showed still a significant permeate flux of 135.0 L/m^2/h with biomass concentration of 203.0 g/L from which dewatering, in a practical sense, was known to be impossible with in the classical cross-flow filtration. The dynamic filtration well compensates for the raised energy consumption by disk rotation by way of its reduction in a required filtration area and membrane cleaning frequency (Ríos et al., 2012).

Besides, operation at 1600 rpm led to reach more than 150 g/L of biomass in 150 min, which was 200 min faster than at 800 rpm; permeate flux at 1600 rpm was 2 to 3-fold higher than 800 rpm throughout dewatering process. This means that dynamic filtration has another option for controlling harvesting time, i.e., rotation speed; in the traditional cross-flow filtration TMP is the only parameter that can be manipulated. All this supported that dynamic filtration equipped with ultrafiltration membrane and rotational disk with perforations was indeed well-suited for dewatering *Aurantiochytrium* sp. KRS101 and particularly so for exceedingly high biomass concentration.

3.3. Comparison of each harvesting method

Table 2 summarizes maximum harvesting efficiency, corresponding operation time, and water content in harvested biomass according to each harvesting method and its operation condition. For coagulation, an increase in coagulant dose improved harvesting efficiency, but also raised water content in the harvested biomass that required further dewatering steps. Similarly, for EF and ECF, an increase in current density enhanced harvesting efficiency or lowered operation time to obtain maximum harvesting efficiency, but also elevated water content in the harvested biomass. Using these chemical-based harvesting methods, it was literally

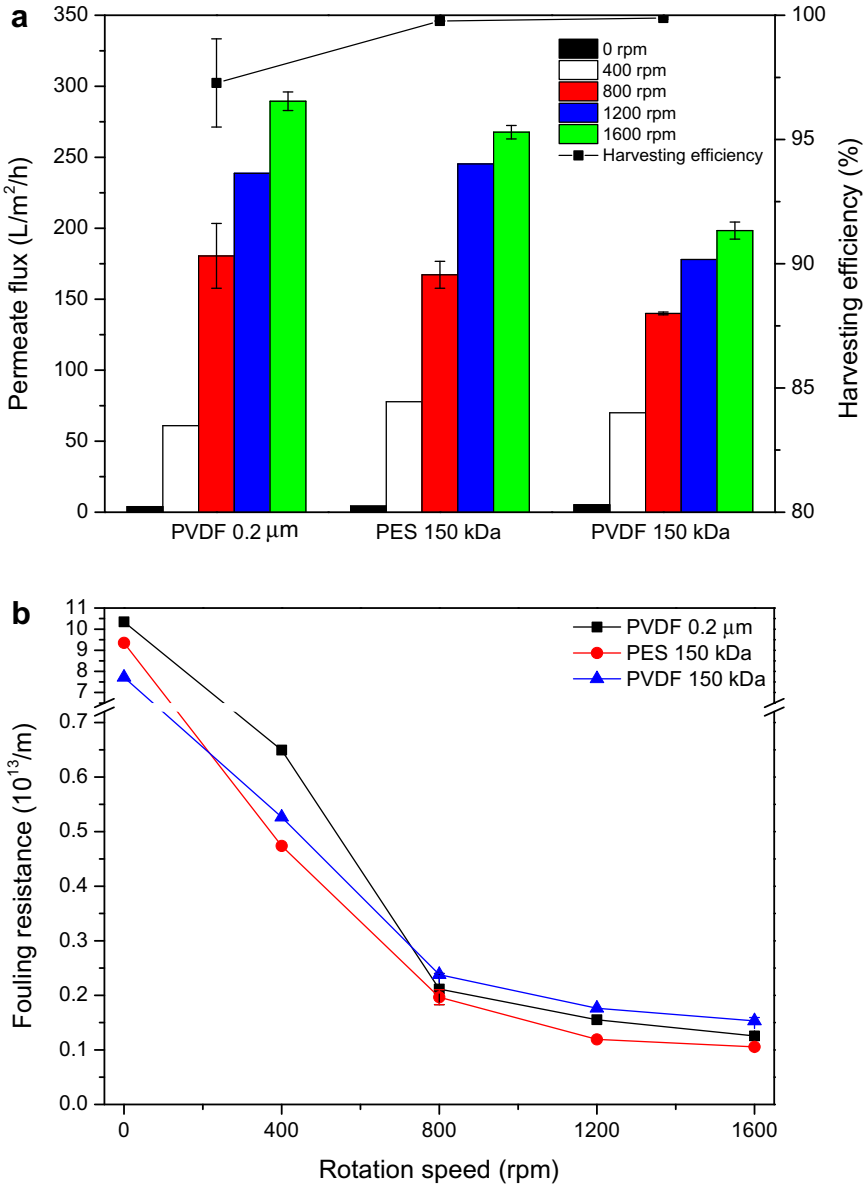


Fig. 3. Effects of membrane and rotation speed on (a) plateau permeate flux, harvesting efficiency, and (b) fouling resistance of membrane filtration for *Aurantiochytrium* sp. KRS101.

impossible to achieve sufficiently high harvesting efficiency and at the same time low enough water content of harvested biomass because the increased coagulant addition and/or bubble generation for the sake of higher harvesting efficiency induced loose layers of harvested biomass. What is worse, their efficiency was not satisfactory: only half was recovered by EF, and only 90% by ECF. Centrifugation, on the other hand, enabled to produce biomass with very low water content, in fact, the lowest among the tested means and in so doing very rapidly. However, more than 10% of biomass failed to be recovered by it. Membrane filtration possesses all the merits that the aforementioned techniques has partly: high harvesting efficiency and low water content in the harvested biomass when operated with ultrafiltration membrane. One more distinctive feature that the other methods cannot offer is its ability to control water content in harvested biomass according to a downstream requirement. This can be done to the point where harvested biomass becomes too viscous to be transported by a circulation pump. In terms of energy consumption, membrane filtration was acceptable: it was much lower than centrifugation and only slightly higher than EF and ECF (Table 3). Considering energy consumption as well as harvesting performance of membrane filtration, operations at two speeds of 1600 and 800 rpm had its own advantage: 1600 rpm for rapid harvesting rate and 800 rpm for efficient harvesting energy consumption.

The dynamic filtration can be better utilized by means of combining another technology in a synergetic manner. For example, based on the results in this study, entire operation time for dewatering of *Aurantiochytrium* sp. KRS101 up to 200 g/L can be reduced from 180 min at 1600 rpm to 90 min by the two-step harvesting using first coagulation at 0.75 g/L of $FeCl_3$ or ECF at 17.2 mA cm^{-2} for 30 min followed by membrane filtration at 1600 rpm for 60 min.

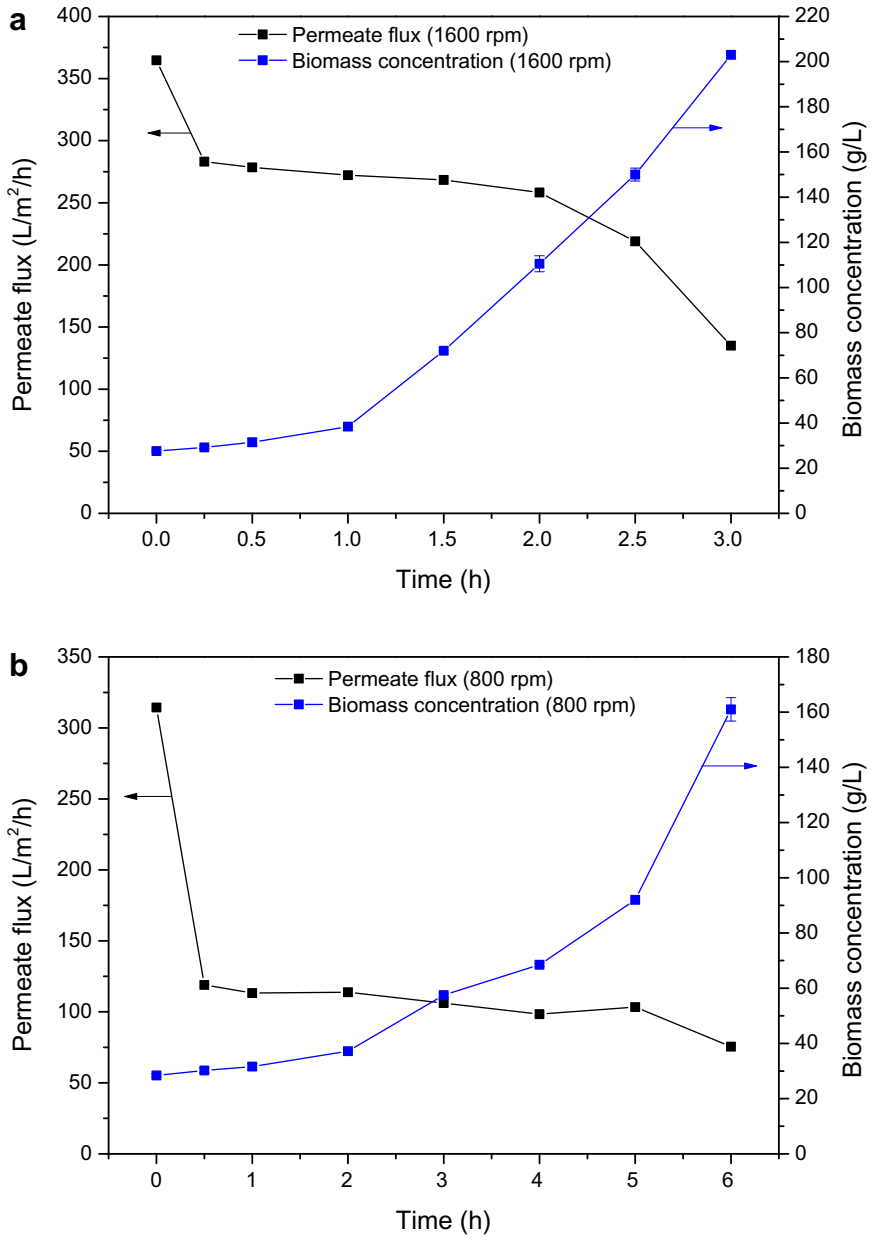


Fig. 4. Changes of permeate flux and biomass concentration during dewatering of *Aurantiochytrium* sp. KRS101 using membrane filtration at (a) 1600 rpm and (b) 800 rpm.

Table 3
Electrical energy consumption of five harvesting methods for *Aurantiochytrium* sp. KRS101.

Harvesting method	Condition and operation time	Electrical energy consumption (Wh/g)
Coagulation	0.75 g/L, 120 min	0.11–0.57
Electro-flotation	17.2 mA cm^{-2}, 15 min	0.125
Electro-coagulation–flotation	17.2 mA cm^{-2}, 30 min	0.077
Centrifugation	1000*g*, 30 min	1.94
Membrane filtration	1600 rpm, 180 min	0.39
	800 rpm, 360 min	0.25

4. Conclusions

Aurantiochytrium sp. KRS101, a potent DHA producer and heterotroph, needs a different way of harvesting, as their final cell density is much higher than photoautotrophic microalgae. Among harvesting means tested, dynamic filtration adopting rotation of a perforated disk was demonstrated to uniquely serve the purpose by way of effectively limiting membrane fouling even under considerably high biomass concentration; it had no biomass loss, low water content in harvested biomass, reasonable energy consumption, and operation manageability.

Acknowledgements

This work was supported by the Advanced Biomass R&D Center (ABC) of Global Frontier Project of Korea funded by the Ministry of Science, ICT and Future Planning (ABC-2010-0029728). We thank Dr. Chul-Ho Kim at Korea Research Institute of Bioscience and Biotechnology (KRIBB) for providing *Aurantiochytrium* sp. KRS101.

References

Alfafara, C.G., Nakano, K., Nomura, N., Igarashi, T., Matsumura, M., 2002. Operating and scale-up factors for the electrolytic removal of algae from eutrophied lakewater. J. Chem. Technol. Biot. 77 (8), 871–876.

Armenta, R.E., Valentine, M.C., 2013. Single-cell oils as a source of omega-3 fatty acids: an overview of recent advances. J. Am. Oil Chem. Soc. 90 (2), 167–182.

Choi, S.A., Jung, J.-Y., Kim, K., Kwon, J.H., Lee, J.-S., Kim, S.W., Park, J.-Y., Yang, J.-W., 2014a. Effects of molten-salt/ionic-liquid mixture on extraction of docosahexaenoic acid (DHA)-rich lipids from *Aurantiochytrium* sp. KRS101. Bioprocess. Biosyst. Eng. 37 (11), 2199–2204.

Choi, S.A., Jung, J.-Y., Kim, K., Lee, J.-S., Kwon, J.-H., Kim, S.W., Yang, J.-W., Park, J.-Y., 2014b. Acid-catalyzed hot-water extraction of docosahexaenoic acid (DHA)-rich lipids from *Aurantiochytrium* sp. KRS101. Bioresour. Technol. 161, 469–472.

Cuellar-Bermudeza, S.P., Romero-Ogawaa, M.A., Rittmannb, B.E., Parra-Saldivara, R., 2014. Algae biofuels production processes, carbon dioxide fixation and biorefinery concept. J. Pet. Environ Biotechnol. 05 (04).

Gao, S.S., Du, M.A., Tian, J.Y., Yang, J.Y., Yang, J.X., Ma, F., Nan, J., 2010. Effects of chloride ions on electro-coagulation–flotation process with aluminum electrodes for algae removal. J. Hazard. Mater. 182 (1–3), 827–834.

Hong, W.-K., Kim, C.H., Rairakhwada, D., Kim, S., Hur, B.-K., Kondo, A., Seo, J.-W., 2012. Growth of the oleaginous microalga *Aurantiochytrium* sp. KRS101 on cellulosic biomass and the production of lipids containing high levels of docosahexaenoic acid. Bioprocess. Biosyst. Eng. 35 (1–2), 129–133.

Hong, W.-K., Rairakhwada, D., Seo, P.-S., Park, S.-Y., Hur, B.-K., Kim, C.H., Seo, J.-W., 2011. Production of lipids containing high levels of docosahexaenoic acid by a newly isolated microalga, *Aurantiochytrium* sp. KRS101. Appl. Biochem. Biotechnol. 164 (8), 1468–1480.

Hong, W.-K., Yu, A., Heo, S.-Y., Oh, B.-R., Kim, C.H., Sohn, J.-H., Yang, J.-W., Kondo, A., Seo, J.-W., 2013a. Production of lipids containing high levels of docosahexaenoic acid from empty palm fruit bunches by *Aurantiochytrium* sp. KRS101. Bioprocess. Biosyst. Eng. 36 (7), 959–963.

Hong, W.-K., Yu, A., Oh, B.-R., Park, J.M., Kim, C.H., Sohn, J.-H., Kondo, A., Seo, J.-W., 2013b. Large-scale production of microalgal lipids containing high levels of docosahexaenoic acid upon fermentation of *Aurantiochytrium* sp. KRS101. Food Nutr. Sci. 04 (09), 1–5.

Hwang, K.-J., Lin, S.-J., 2014. Filtration flux–shear stress–cake mass relationships in microalgae rotating-disk dynamic microfiltration. Chem. Eng. J. 244, 429–437.

Hwang, K.-J., Wu, S.-E., 2015. Disk structure on the performance of a rotating-disk dynamic filter: a case study on microalgae microfiltration. Chem. Eng. Res. Des. 94, 44–51.

Kim, K., Jung, J.-Y., Kwon, J.-H., Yang, J.-W., 2015. Dynamic microfiltration with a perforated disk for effective harvesting of microalgae. J. Membr. Sci. 475, 252–258.

Kim, K., Jung Kim, E., Ryu, B.-G., Park, S., Choi, Y.-E., Yang, J.-W., 2013. A novel fed-batch process based on the biology of *Aurantiochytrium* sp. KRS101 for the production of biodiesel and docosahexaenoic acid. Bioresour. Technol. 135, 269–274.

Papazi, A., Makridis, P., Divanach, P., 2010. Harvesting *Chlorella minutissima* using cell coagulants. J. Appl. Phycol. 22 (3), 349–355.

Ríos, S.D., Salvadó, J., Farriol, X., Torras, C., 2012. Antifouling microfiltration strategies to harvest microalgae for biofuel. Bioresour. Technol. 119, 406–418.

Ryu, B.-G., Kim, J., Kim, K., Choi, Y.-E., Han, J.-I., Yang, J.-W., 2013a. High-cell-density cultivation of oleaginous yeast *Cryptococcus curvatus* for biodiesel production using organic waste from the brewery industry. Bioresour. Technol. 135, 357–364.

Ryu, B.-G., Kim, K., Kim, J., Han, J.-I., Yang, J.-W., 2013b. Use of organic waste from the brewery industry for high-density cultivation of the docosahexaenoic acid-rich microalga, *Aurantiochytrium* sp. KRS101. Bioresour. Technol. 129, 351–359.

Sijtsma, L., De Swaaf, M., 2004. Biotechnological production and applications of the ω-3 polyunsaturated fatty acid docosahexaenoic acid. Appl. Microbiol. Biotechnol. 64 (2), 146–153.

Slater, C.S., Savelski, M.J., Kostetskyy, P., Johnson, M., 2015. Shear-enhanced microfiltration of microalgae in a vibrating membrane module. Clean Technol. Environ. Policy.

Vandamme, D., Pontes, S.C.V., Goiris, K., Foubert, I., Pinoy, L.J.J., Muylaert, K., 2011. Evaluation of electro-coagulation–flocculation for harvesting marine and freshwater microalgae. Biotechnol. Bioeng. 108 (10), 2320–2329.

Velmurugan, N., Sathishkumar, Y., Yim, S.S., Lee, Y.S., Park, M.S., Yang, J.-W., Jeong, K. J., 2014. Study of cellular development and intracellular lipid bodies accumulation in the thraustochytrid *Aurantiochytrium* sp. KRS101. Bioresour. Technol. 161, 149–154.

Wong, M.K.M., Tsui, C.K.M., Au, D.W.T., Vrijmoed, L.L.P., 2008. Docosahexaenoic acid production and ultrastructure of the thraustochytrid *Aurantiochytrium mangrovei* MP2 under high glucose concentrations. Mycoscience 49 (4), 266–270.

Yoo, G., Yoo, Y., Kwon, J.-H., Darpito, C., Mishra, S.K., Pak, K., Park, M.S., Im, S.G., Yang, J.-W., 2014. An effective, cost-efficient extraction method of biomass from wet microalgae with a functional polymeric membrane. Green Chem. 16 (1), 312–319.

Zhang, G., Ji, S., Gao, X., Liu, Z., 2008. Adsorptive fouling of extracellular polymeric substances with polymeric ultrafiltration membranes. J. Membr. Sci. 309 (1), 28–35.