Nature Environment and Pollution Technology An International Quarterly Scientific Journal	p-ISSN: 0972-6268 e-ISSN: 2395-3454	Vol. 18	No. 1	pp. 167-173	2019

Original Research Paper | Open Access

Bioflocculation of Two Species of Microalgae by Exopolysaccharide of *Bacillus subtilis*

Musthofa Lutfi*†, Wahyunanto A. Nugroho*, Winny P. Fridayestu*, Bambang Susilo*, Chusak Pulmar and Sandra Sandra***

*Department of Agricultural Engineering, Brawijaya University, Veteran St. 1, Malang, East Java, 65145, Indonesia
**Faculty of Agriculture and Agro-Industry Technology, Rajamangala University of Technology, Suwanabhumi, Ayuthaya 13000 Thailand
†Corresponding author: Musthofa Lutfi

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 30-05-2018
Accepted: 02-08-2018

Key Words:
Microalgae
Bioflocculation
Exopolysaccharide
Bacillus subtilis

ABSTRACT

Harvesting is ultimately an important step on microalgae production since a successful harvesting will increase the performance of the system totally as well as increasing the revenue. Bioflocculation is such type of the harvesting technique that offers high recovery performance, while at the same time it costs less and is more environment friendly. Exopolysaccharide (EPS) is actually the key of the performance. The objective of this research is to observe the performance of EPS produced by the *Bacillus subtilis* for microalgae harvesting. The bacterial growth medium where the EPS has been produced was induced directly to the microalgae. Two different species of microalgae, which were *Nannochloropsis oculata* and *Botryococcus braunii*, were used to compare the influence of microalgae type to the performance. Different concentration of EPS was employed to study how it affects the recovery performance. These various concentrations were 5% v/v, 10% v/v and 15% v/v. The result shows that the highest performance was presented when 5% EPS was induced to both the species, which was about 90%, while the lowest was presented by 15% EPS, which was about 70%. The same trend was applied for initial floc forming time of each treatment.

INTRODUCTION

Microalgae have been proposed as a nature provider for various chemicals ranging from low value to high value chemicals. While in production of some high value chemicals the cost might be an insignificant issue, the production of lower value chemicals such as fuel, the lowest possible cost should be taken into account. Otherwise, it will never be able to compete with conventional source of fuel.

The production of biofuel from microalgae is becoming an attractive option since it offers ease of cultivation (Sing et al. 2013), high oil productivity per dry mass basis (Rodolfi et al. 2009, Griffiths et al. 2009) and sustainable production (Ahmad et al. 2011, Pittman et al. 2011). *Nannochloropsis* sp and *Botryococcus braunii* are among the prospective microalgae due to their high oil content and biomass rate (Moazami et al. 2012, Biondi et al. 2013, Metzger et al. 2005). Another benefit of cultivating *Nannochloropsis* is its robustness, and easy growth in a wide range of environments (Sukenik et al. 2009, Kilian et al. 2011). In regard to *Botryococcus brauni*, it also synthesizes numerous hydrocarbons for wider purposes (Wijffels et al. 2010, Khatri et al. 2013).

The total production cost for biofuel from microalgae is, in fact, a function of several variables such as labour cost, cultivation system, harvesting and refining cost. Such actions have been applied to suppress the constraints: reusing nutrient from wastewater mainly in open pond reactor (Park et al. 2011, Matamoros et al. 2015, Bohutskyi et al. 2015), improving gas transfer to increase the growth rate (Zimmerman et al. 2011, Ying et al. 2013), updating the harvesting technique (Milledge et al. 2013, Barros et al. 2015, Wang et al. 2015), and increasing the efficiency of oil refinery (Halim et al. 2012, Mubarak et al. 2015).

Cell harvesting is a step prior to the refining process of microalgae oil. The uneconomical conventional technique of harvesting could exceed half the cost of total oil production (Richmond et al. 2008). A successful technique for harvesting will not only significantly affect the total performance of the system, but also reduce the total cost. Efficiency of oil recovery in refinery is also affected by the harvesting step (Patil et al. 2015). Innovations have been made in harvesting technique with consideration of high biomass recovery, refining efficiency and low cost.

Chemical flocculation has been applied so far for microalgae harvesting. It is considered as one of the economical techniques since it achieves a good performance

with relatively low flocculant dosage and results in low harvesting cost (Bilanovic et al. 2008, Vandamme et al. 2013, Wan et al. 2015). In general principle, a particle has electrostatical charge that makes them relatively stable solutes in water, besides their small size that makes them suspend easily in the water. Chemical flocculants have opposite charge that attract particles to form a flock. When the bigger flocks are formed, they become unstable. An unstable flock either will settle down or float up, depending on its density and hydrophobicity.

Chemical flocculation has been successful to be applied for water treatment. To apply such technique for microalgae harvesting, some consideration needs to be taken. Besides its several benefits, chemical flocculation has several drawbacks when it is used in full scale microalgae production. The presence of sediment caused by the cationic substance needs more efforts to remove. Chemical flocculation will change the pH medium, temperature, dissolved oxygen as well as nutrient depletion that occur during the process. These adjusted conditions could lead to change of the composition of the harvested cells (Benemann et al. 1996). Hence, more sustainable flocculation method is important to be applied.

Bioflocculation could be an alternative for a cheaper harvesting cost, since it reduces the use of chemical coagulant, hence reducing the following treatment to dispose sediment from the site. Bioflocculation is a term to address any flocculation enhanced by exopolysaccharide (EPS) secreted by microorganisms (Christenson & Sims 2011). It is considered as a biodegradable substance and more environmental friendly than any chemical polymer used in non-biocoagulation process (Kim et al. 2011).

EPS is a major component in microbial biofilm. Several factors affect the composition of EPS as well as its formation. Microbial genetics and the physico-chemical conditions of the growth medium are among the two factors affecting the EPS composition (Castro et al. 2014). Such environmental conditions also affect the character of the EPS in terms of its porosity, density, moisture content, hydrophobicity and mechanical stability (Wingender et al. 2012). According to Pham et al. (2000), EPS itself is produced during the final session of stationary phase of microbial life cycle when it begins to experience a nutrient shortage. However, after any particular time, the number of EPS could decrease, as it will be consumed by microbes itself as a carbon source if more nutrient is not added in the medium. Hence, it is essential to decide the best time to harvest the EPS for further utilization as bioflocculant.

Aggregation of algae occurs as an effect of bridging done by the EPS. As the aggregate becoming larger, its stabilization in the medium becomes weaker and results in settlement or floating of the floc, depending on its density and any other treatment combined with the flocculation process. The recovery performance is affected by several parameters, including EPS dosage, EPS characteristics, morphology of the microalgae and culture density (Manheim & Nelson 2013). Research by Patil et al. (2010) revealed that the flocculation performance increased along with the increasing EPS dosage until it achieved the critical flocculation performance, which was 72% and 500 mg/L for the flocculation performance and dosage of EPS respectively. The study showed that there was no increase in the flocculation performance if the EPS added into the medium exceeded 500 mg/L. The highest performance in the report was 98% recovery when higher concentration of pure γ-PGA from *Bacillus licheniformis* was employed for the harvesting (Ndikubwimana et al. 2016).

Microalgae morphology is linked to the culture density, where the smaller individual microalgae size lead to the denser floc formation resulted in the higher settling performance (Manheim & Nelson 2013). This morphological effect was also applied for the bioflocculant type produced by different species of the bacteria.

This research was to observe the effect of bioflocculants dosage in the recovery performance as well as its settling time. *Nannochloropsis* and *Botryococcus brauni* were the strains used in this experiment to compare their behaviour during floc formation as they are morphologically different. *Bacillus subtilis* was applied as the EPS producer. This type of bacteria has presented to produce biopolymer γ-PGA that was successful in harvesting microalgae by employing the pure polymer in the medium (Zheng et al. 2012). In this study, the *Bacillus subtilis* culture medium with EPS produced in it was directly employed.

MATERIAL AND METHODS

Microorganisms and its Culture

Nannochloropsis oculata and *Botryococcus brauni* were two microalgae strains used in this study. The microalgae were collected from the Institute of Brackish Water Aquaculture (BBAP) Situbondo, East Java, Indonesia. Each strain of microalgae was cultured in a standard Bold's Basal Medium (BBM) in a 5-litre fibreglass tube reactor with conventional agitation to prevent sedimentation. The sparger was placed in the bottom of the reactor to induce carbon dioxide from ambient air. Lighting was provided using fluorescent lamps with intensity of 3000 lux, 24 hours a day for 15 days.

As an EPS producer, *Bacillus subtillis* was employed. The isolate was obtained by the Department of Postharvest

Technology, Brawijaya University, Indonesia. To produce the EPS, 1 dose of isolated bacteria was put into 20 mL of 0.9% NaCl. The inoculate was then cultivated in 980 mL of sterilized nutrient broth. The nutrient broth was prepared by dissolving 8 gram of nutrient broth Merck 105443 into 1 litre of deionized water and stirred at 50°C until perfectly dissolved. The culture was homogenized using a stirrer at 100 rpm for 72 hours at room temperature of 25-30°C. Seventy-two hour was the determined time resulted from the preliminary experiment as the time for the bacteria to achieve late stationary phase in a batch medium.

Exopolysacharide (EPS)

The total EPS was tested gravimetrically by means of putting 25 mL culture of bacterial broth medium into 50 mL centrifuge tubes. It was then centrifuged at a temperature of 4°C, 6000 rpm for 20 minutes. The supernatant was taken, twice as much as cold ethanol (96%) was added and it was allowed to stand for overnight. The second cold centrifugation was carried out at another 4°C, 5000 rpm for 25 minutes. The remaining solid was dried at a 105°C and weighed. The productivity of the bacteria to produce EPS per litre of culture was calculated using the following formula:

$$EPS_{tot} = \frac{W_e}{V_0} \, x \, 100\% \quad ...(1)$$

Where, W_e is the dry weight of the EPS (gram) and V_o is the sample volume (litre).

Microalgae Harvesting

Microalgae was transferred from the glass tube bioreactor into 1 litre of glass beaker with 300 mL of working volume each. The pH of the culture was increased by adding 0.1M NaOH until it reached pH 10. The *Bacillus subtilis* was then added into the beaker. To study the effect of bioflocculant concentration, different volumes of bacterium-EPS inoculum were added ranging from 5% (v/v), 10% (v/v) and 15% (v/v). In this study, undried bacterium-EPS inoculum was added instead of dried mass of EPS since it uses less energy by skipping the separation process for EPS itself from the growth medium. A volume to dry weight conversion was carried out to ease the comparison of the results of this study to others if necessary. As the control of each strain, no bacterial inoculum was added to the beaker. Each treatment was done in triplicate.

The observation of macrofloc formation was carried out using 10x magnifying glass. The time of macrofloc formation was determined when the macrofloc started to be appearing in each beaker. Measurement of the harvesting performance was carried out by calculating the initial cell density of microalgae and the left microalgae suspended in the medium. The cell density was observed with haemocytometer under a microscope. The performance was determined using this formula:

$$Performance = \frac{X_0 - X_1}{X_0} x \, 100\% \quad ...(2)$$

Where, X_0 is the initial cell density (cell/mL) and X_1 is the left microalgae (cell/mL).

RESULTS AND DISCUSSION

EPS Production

Bacillus subtilis is one of the biopolymer producer used for bioflocculation. To produce the EPS, the bacteria needs to be cultured in a growth medium until it reached an optimum production of EPS. As reported by Pham et al. (2000), the optimum EPS production occurs in the late stationary phase just before the death phase. A fall number of EPS will occur in the medium during death phase as it is used by the bacteria for its carbon source. It makes, determining the best harvesting time for the bacterial culture, as essential.

To determine the harvesting time, a preliminary experiment was carried out to observe the growth curve of the bacteria in the prepared growth medium. The growth was observed by counting the number of colonies using a haemocytometer under microscope every 12 hours until it reached the death phase. The time of the bacteria to reach the late stationary phase was then determined as the time when the culture was ready to use for the microalgae harvesting.

Fig. 1 shows the growth curve of the bacterial culture carried out in the preliminary experiment. The first count was done after 12 hours since the culture started. It logarithmically increased for the next 36 hours to reach the point of inflection. From the curve, it can be determined that the death phase occurred after 72 hours of the cultivation. According to these data, the bacterial culture would be best used for microalgae harvesting when its age is 72 hours within the same growth medium type. Different media type, concentration and bacterial species might differ the growth curve. The growth rate could be higher when more nutrients were provided. A higher population (8×10^8 cfu/L) and longer stationary phase (more than 72 hours) has been achieved when the *Bacillus subtilis* was cultured under more optimized conditions (Ghribi et al. 2011).

The EPS production by the *Bacillus subtilis* is presented in Fig. 2. About 50 mL of samples was taken from the culture medium per day in three days of culture. Each sample was dried sequentially to analyse the dried EPS produced. After one day of culture, the bacteria produced only less EPS. The production increased to several folds on the following day. The rate of EPS production was decreased on the third day; while on another side, it reached the highest

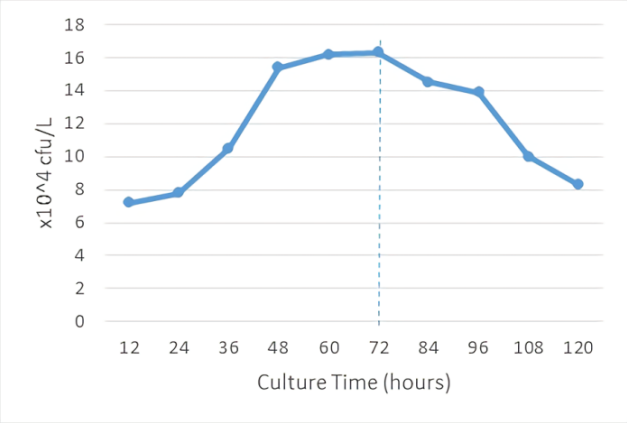

Fig. 1: Kinetics of *Basillus subtilis* in nutrient broth carried out in the preliminary experiment.

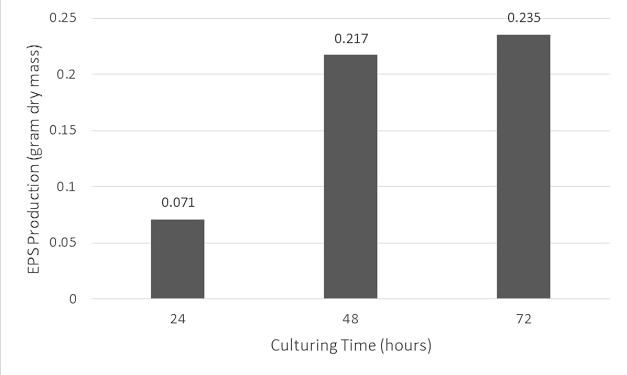

Fig. 2: Dried EPS produced by *Bacillus subtilis* from 50 mL sample.

production number, which was 0.235 gram. Given that the EPS tends to decrease as it passes the stationary phase, no observation of EPS production was carried out.

By converting the result of the last day into gram per litre basis, it can be presented that it was equal to 4.70 gram of dried EPS produced by the bacteria per litre growth medium. Type of medium has revealed to have an effect to the amount of EPS produced by the bacteria, besides the type of bacteria itself. A slightly higher production of EPS by *Bacillus subtilis* has been reported in 48 hours of culture time, which was 4.86 gram/L (Razack et al. 2013). This result was achieved by adding cane molasses into the growth medium, which increased the amount by 2% of the total volume. Molasses does not only provide carbon source for the bacteria, but it also provides some minerals and growth precursors (Liu et al. 2011). The highest reported productivity of microbial exopolysaccharide was probably by the fungus *Phoma herbarum* (13,6 gram/L) with the sorbitol used as the main carbon source, which was 60 g/L.

Microalgae recovery: Given that the harvesting step could enhance the overall performance of microalgae production, it is essential to determine the harvesting method that could recover more microalgae with less cost. Bioflocculation has been proposed as a promising method since such successful method could recover about 90% or more of the total cultured biomass. The less cost when employing this method is attributed by its biodegradability, hence no post treatment is needed to recover the flocculants.

In this study, dried EPS was not employed. Instead, the *Bacillus subtilis* culture medium with the EPS excreted in it was directly used as a flocculant. About 5%, 10% and 15% by volume of EPS was added for microalgae harvesting. By converting this percentage into milligram per litre, these numbers are equal to 220 mg/L, 420 mg/L and 620 mg/L for the 5%, 10% and 15% respectively. It is necessary to highlight that this practice, which was using EPS directly from the bacteria growth medium, is only practical when the EPS is produced in the same site as those of the

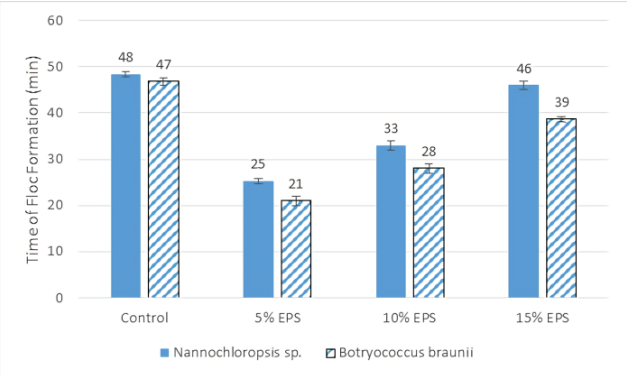

Fig. 3: Time to achieve first floc of EPS-microalgae.

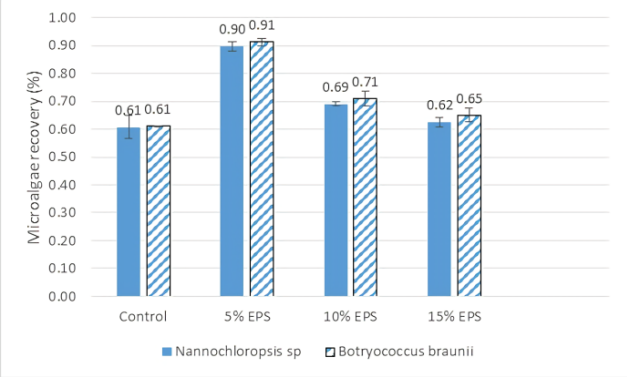

Fig. 4: EPS performance on microalgae recovery.

microalgae cultivation. For any different condition, such as the EPS produced by different producer, the use of commercially dried EPS might be preferable.

The microalgae harvesting started with the addition of the EPS to the microalgae culture. The culture was then homogenized in a magnetic stirrer with faster mixing (100 rpm) for 2 minutes followed by slower mixing (40 rpm) for 8 minutes. This step was followed by letting the samples to settle down for about 2 hours. The time of initial floc formation was observed (Fig. 3). The indicator of macrofloc formation is when the particles began to randomly moving and attach to each other or attach to a closest polymer. In both the microalgal species, the addition of 5% v/v of EPS was the best time to initially forming macrofloc, which was 25 minutes, and 21 minutes for *Nannochloropsis oculata* and *Botryococcus brauni*, respectively. Other than the control, the most unfavourable concentration among all treatment was 15% v/v for both the microalgae species.

To evaluate the recovery performance, the sample of the microalgae growth medium before and after addition of the EPS was taken and was analysed under microscope. It was carried out to see the microalgal cell number initially as well as the left cell suspended in the medium. The same trend also occurred in the EPS performance on harvesting the microalgae with 5% v/v was the most favourable concentration for the treatment with around 90% achievement for both the microalgal species, while the highest concentration shows the lowest performance (Fig. 4). A direct EPS producer bacteria induction to the microalgae medium was also carried to harvest *Clamydomonas* sp. and *Picochlorum* sp. where the recovery performance ranged from 75% to 90% (Díaz et al. 2015). As has been reported by Patil et al. (2010), a concentration did not always linearly affect the harvesting performance. It was suggested that no significant performance increase could happen when the optimum flocculation performance has been achieved. This study was even more interesting when the increase of concentration was inversely proportional to the performance of recovery. An inverse condition was also reported by Ndikubwimana et al. (2016), where the peak performance was achieved with addition of 20% γ-PGA. Beyond this point, the flocculation efficiency has been decreased.

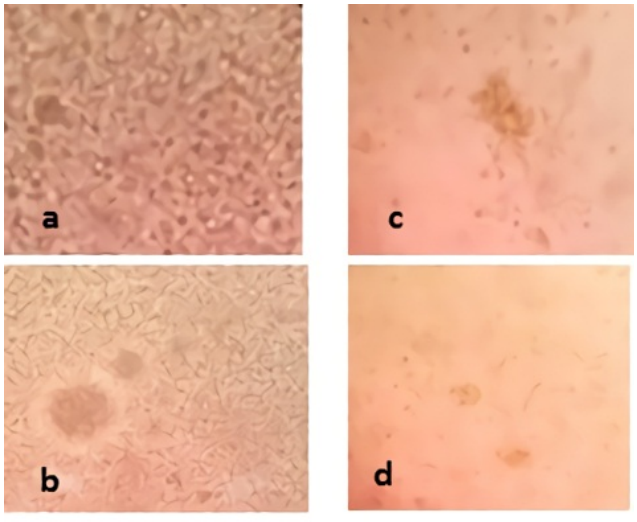

Fig. 5: Microscopic picture of first macrofloc formation of both microalgae in 100x magnitude. *Nannochloropsis oculata* with 5% EPS and 10% EPS (a and b respectively). *Botryococcus brauni* with 5% EPS and 10% EPS (c and d respectively).

However, it cannot be justified that less the concentration of EPS, the better is the performance. It was probably because the optimum concentration has been achieved by the lowest one, or even lower, while increasing the concentration would only distract the performance. It is hypothesized that when the optimum flocculation has happened, an additional flocculant likely will destabilize some macroflocs those have been formed by other biopolymers. A repulsive force between the same charges of the flocculants probably could be the reason of this destabilization. Avoiding more free surface of flocculant probably could be done by optimizing the flocculant-microalgae ratio.

Analysing the form of the floc microscopically is essential to understand the interaction between the microalgae and the EPS. Furthermore, it is necessary to ensure that the formation of the floc was due to the attraction of the EPS to the targeted microalgae, and was not due to any interaction with other substances. According to Salim et al. (2011), there are two ways for the polymer to form the flocs. Bridging occurs when the polymer is partially binding the microalgae on its surface. It normally occurs when the polymer is long enough. On the other side, when the polymer is short, it tends to adsorb the microalgae through its surface. The last sub-mechanism was named as patching. In this study, patching can be concluded as the way of macrofloc formation, as shown in Fig. 5 for all the treatments.

An ANOVA analysis was done using 5% confidential intervals. From the result, it could be concluded that the addition of EPS in the medium has significantly affected the harvesting performance while the type did not affect the performance of those two species employed (Table 1). A significant inverse effect was shown for the EPS concentration employed for the harvesting. While it was hypothesized that a species could affect the performance, this study did not show the effect significantly to such phenomenon. In earlier study, species might affect the harvesting performance mainly due to its density, morphology and microbial

Table 1: Statistical test of the harvesting performance.

Dependent Variable: Recovery

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	0.323[a]	7	0.046	127.235	0.000	0.982
Intercept	12.191	1	12.191	33613.068	0.000	1.000
Microalga species	0.001	1	0.001	3.031	0.101	0.159
EPS Conc.	0.322	3	0.107	295.658	0.000	0.982
Microalga species * EPS Concentration	0.000	3	7.749E-5	0.214	0.885	0.039
Error	0.006	16	0.000			
Total	12.520	24				
Corrected Total	0.329	23				

a. R Squared = .982 (Adjusted R Squared = .975)

community structure (Pham et al. 2000, Díaz-Santos et al. 2015). The two microalgae have clearly different morphology and microbial community structure. While densities of these two algae might not be significantly different, which is close to water density, it probably made those two different microalgae to not show significant recovery performance for the same EPS concentration added.

CONCLUSIONS

In this work, an induction of EPS to the microalgae medium has significantly affected the recovery performance as well as decreasing the harvesting time. Instead of increasing the harvesting performance, an inverse trend was shown when the EPS concentration increased. It is predicted that repulsive force of free surface of the flocculant negatively impacted the recovery performance. To avoid such repulsive force, a study to optimize the EPS-microalgae concentration ratio is suggested for future research.

REFERENCES

Ahmad, A.L., Yasin, N.M., Derek, C.J.C. and Lim, J.K. 2011. Microalgae as a sustainable energy source for biodiesel production: A review. Renewable and Sustainable Energy Reviews, 15(1): 584-593.

Barros, A.I., Gonçalves, A.L., Simões, M. and Pires, J.C. 2015. Harvesting techniques applied to microalgae: A review. Renewable and Sustainable Energy Reviews, 41: 1489-1500.

Benemann, J.R. and Oswald, W.J. 1996. Systems and economic analysis of microalgae ponds for conversion of CO_2 to biomass. Final report. California Univ., Berkeley, CA (United States). Dept. of Civil Engineering, pp. 214.

Bilanovic, D., Shelef, G. and Sukenik, A. 1988. Flocculation of microalgae with cationic polymers - effects of medium salinity. Biomass, 17(1): 65-76.

Biondi, N., Bassi, N., Chini Zittelli, G., De Faveri, D., Giovannini, A., Rodolfi, L., Allevi, C., Macrì, C. and Tredici, M.R. 2013. Nannochloropsis sp. F&M-M24: Oil production, effect of mixing on productivity and growth in an industrial wastewater. Environmental Progress & Sustainable Energy, 32(3): 846-853.

Bohutskyi, P., Liu, K., Nasr, L.K., Byers, N., Rosenberg, J.N., Oyler, G.A., Betenbaugh, M.J. and Bouwer, E.J. 2015. Bioprospecting of microalgae for integrated biomass production and phytoremediation of unsterilized wastewater and anaerobic digestion centrate. Applied Microbiology and Biotechnology, 99(14): 6139-6154.

Castro, L., Zhang, R., Muñoz, J.A., González, F., Blázquez, M.L., Sand, W. and Ballester, A. 2014. Characterization of exopolymeric substances (EPS) produced by *Aeromonas hydrophila* under reducing conditions. Biofouling, 30(4): 501-511.

Christenson, L. and Sims, R. 2011. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnology Advances, 29(6): 686-702.

Díaz-Santos, E., Vila, M., de la Vega, M., León, R. and Vigara, J. 2015. Study of bioflocculation induced by *Saccharomyces bayanus* var. *uvarum* and flocculating protein factors in microalgae. Algal Research, 8: 23-29.

Ghribi, D. and Ellouze-Chaabouni, S. 2011. Enhancement of *Bacillus subtilis* lipopeptide biosurfactants production through optimization of medium composition and adequate control of aeration. Biotechnology Research International, Article ID 653654.

Griffiths, M.J. and Harrison, S.T. 2009. Lipid productivity as a key characteristic for choosing algal species for biodiesel production. Journal of Applied Phycology, 21(5): 493-507.

Halim, R., Danquah, M.K. and Webley, P.A. 2012. Extraction of oil from microalgae for biodiesel production: A review. Biotechnology Advances, 30(3): 709-732.

Khatri, W., Hendrix, R., Niehaus, T., Chappell, J. and Curtis, W.R. 2014. Hydrocarbon production in high density *Botryococcus braunii* race B continuous culture. Biotechnology and Bioengineering, 111(3): 493-503.

Kilian, O., Benemann, C.S., Niyogi, K.K. and Vick, B. 2011. High-efficiency homologous recombination in the oil-producing alga *Nannochloropsis* sp. Proceedings of the National Academy of Sciences, 108(52): 21265-21269.

Kim, D.G., La, H.J., Ahn, C.Y., Park, Y.H. and Oh, H.M. 2011. Harvest of *Scenedesmus* sp. with bioflocculant and reuse of culture medium for subsequent high-density cultures. Bioresource Technology, 102(3): 3163-3168.

Liu, C.T., Chu, F.J., Chou, C.C. and Yu, R.C. 2011. Antiproliferative and anticytotoxic effects of cell fractions and exopolysaccharides from *Lactobacillus casei* 01. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 721(2): 157-162.

Manheim, D. and Nelson, Y. 2013. Settling and bioflocculation of

two species of algae used in wastewater treatment and algae biomass production. Environmental Progress & Sustainable Energy, 32(4): 946-954.

Matamoros, V., Gutiérrez, R., Ferrer, I., García, J. and Bayona, J.M. 2015. Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: A pilot-scale study. Journal of Hazardous Materials, 288: 34-42.

Metzger, P. and Largeau, C. 2005. *Botryococcus braunii*: A rich source for hydrocarbons and related ether lipids. Applied Microbiology and Biotechnology, 66(5): 486-496.

Milledge, J.J. and Heaven, S. 2013. A review of the harvesting of micro-algae for biofuel production. Reviews in Environmental Science and Biotechnology, 12(2): 165-178.

Moazami, N., Ashori, A., Ranjbar, R., Tangestani, M., Eghtesadi, R. and Nejad, A.S. 2012. Large-scale biodiesel production using microalgae biomass of *Nannochloropsis*. Biomass and Bioenergy, 39: 449-453.

Mubarak, M., Shaija, A. and Suchithra, T.V. 2015. A review on the extraction of lipid from microalgae for biodiesel production. Algal Research, 7: 117-123.

Ndikubwimana, T., Zeng, X., Murwanashyaka, T., Manirafasha, E., He, N., Shao, W. and Lu, Y. 2016. Harvesting of freshwater microalgae with microbial bioflocculant: A pilot-scale study. Biotechnology for Biofuels, 9(1): 47.

Park, J.B.K., Craggs, R.J. and Shilton, A.N. 2011. Wastewater treatment high rate algal ponds for biofuel production. Bioresource Technology, 102(1): 35-42.

Park, J.Y., Park, M.S., Lee, Y.C. and Yang, J.W. 2015. Advances in direct transesterification of algal oils from wet biomass. Bioresource Technology, 184: 267-275.

Patil, S.V., Salunkhe, R.B., Patil, C.D., Patil, D.M. and Salunke, B.K. 2010. Bioflocculant exopolysaccharide production by *Azotobacter indicus* using flower extract of *Madhuca latifolia* L. Applied Biochemistry and Biotechnology, 162(4): 1095-1108.

Pham, P.L., Dupont, I., Roy, D., Lapointe, G. and Cerning, J. 2000. Production of exopolysaccharide by *Lactobacillus rhamnosus* R and analysis of its enzymatic degradation during prolonged fermentation. Applied and Environmental Microbiology, 66(6): 2302-2310.

Pittman, J.K., Dean, A.P. and Osundeko, O. 2011. The potential of sustainable algal biofuel production using wastewater resources. Bioresource Technology, 102(1): 17-25.

Razack, S.A., Velayutham, V. and Thangavelu, V. 2013. Medium optimization for the production of exopolysaccharide by *Bacillus subtilis* using synthetic sources and agro wastes. Turkish Journal of Biology, 37(3): 280-288.

Richmond, A. and Q. Hu 2008. Handbook of Microalgal Culture: Biotechnology and Applied Phycology. 1st ed., John Wiley and Sons Ltd: United Kingdom.

Rodolfi, L., Chini Zittelli, G., Bassi, N., Padovani, G., Biondi, N., Bonini, G. and Tredici, M.R. 2009. Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnology and Bioengineering, 102(1): 100-112.

Salim, S., Bosma, R., Vermuë, M.H. and Wijffels, R.H. 2011. Harvesting of microalgae by bio-flocculation. Journal of Applied Phycology, 23(5): 849-855.

Sing, S.F., Isdepsky, A., Borowitzka, M.A. and Moheimani, N.R. 2013. Production of biofuels from microalgae. Mitigation and Adaptation Strategies for Global Change, 18(1): 47-72.

Sukenik, A., Beardall, J., Kromkamp, J.C., Kopecký, J., Masojídek, J., van Bergeijk, S., Gabai, S., Shaham, E. and Yamshon, A. 2009. Photosynthetic performance of outdoor *Nannochloropsis* mass cultures under a wide range of environmental conditions. Aquatic Microbial Ecology, 56(2-3): 297-308.

Vandamme, D., Foubert, I. and Muylaert, K. 2013. Flocculation as a low-cost method for harvesting microalgae for bulk biomass production. Trends in Biotechnology, 31(4): 233-239.

Wan, C., Alam, M.A., Zhao, X.Q., Zhang, X.Y., Guo, S.L., Ho, S.H., Chang, J.S. and Bai, F.W. 2015. Current progress and future prospect of microalgal biomass harvest using various flocculation technologies. Bioresource Technology, 184: 251-257.

Wang, S.K., Stiles, A.R., Guo, C. and Liu, C.Z. 2015. Harvesting microalgae by magnetic separation: A review. Algal Research, 9: 178-185.

Wijffels, R.H., Barbosa, M.J. and Eppink, M.H. 2010. Microalgae for the production of bulk chemicals and biofuels. Biofuels, Bioproducts and Biorefining: Innovation for a Sustainable Economy, 4(3): 287-295.

Wingender, J., Neu, T.R. and Flemming, H.C. 2012. Microbial Extracellular Polymeric Substances: Characterization, Structure and Function. Springer Berlin Heidelberg.

Ying, K., Gilmour, D.J., Shi, Y. and Zimmerman, W.B. 2013. Growth enhancement of *Dunaliella salina* by microbubble induced airlift loop bioreactor (ALB) - the relation between mass transfer and growth rate. Journal of Biomaterials and Nanobiotechnology, 4(2A): 1-9.

Zheng, H., Gao, Z., Yin, J., Tang, X., Ji, X. and Huang, H. 2012. Harvesting of microalgae by flocculation with poly (γ-glutamic acid). Bioresource Technology, 112: 212-220.

Zimmerman, W.B., Zandi, M., Bandulasena, H.H., Tesar, V., Gilmour, D.J. and Ying, K. 2011. Design of an airlift loop bioreactor and pilot scales studies with fluidic oscillator induced microbubbles for growth of a microalgae *Dunaliella salina*. Applied Energy, 88(10): 3357-3369.