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Recovery of Nitrogen and Phosphorus from Microalgae by Subcritical Water

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Sustainable energy supply and its security are the prime concern for the establishment of the low carbon society. Microalgal biomass produced by mass cultivation has a high potential as a renewable and sustainable energy resource that is a tangible alternative to fossil energy resource. Since a microalga fixes atmospheric carbon dioxide as organic matters such as lipids and carbohydrates as a result of photosynthesis, microalgal lipids are considered to be a carbon-neutral feedstock for fuel production. Number of previous studies have shown that microalgae can be utilized as the source of energy as well as of biologically active substances, supplements, food and feed. However, only a limited number of studies have been conducted on utilization of microalgal biomass itself, after extraction of useful substances, as the nutrients source for its mass cultivation, although it is necessary to establish an economically feasible process to recycle nutrients to the mass cultivation system of the microalga. Cost of nitrogen and phosphorus would be a substantial part of the running cost of such mass cultivation systems without a nutrients recycling process. The results from this experiments of the sub-critical water (SCW) process, which has been carried out at relatively moderate temperature conditions of up to 250 ºC and under the saturation steam pressure, is found as an unique process that can possibly recover both lipids as the feedstock and water soluble nutrients, N and P, necessary for the mass-cultivation of microalga simultaneously in a single process. Further, the experiments were carried out to optimize the SCW operational conditions to maximize lipids and nutrients recovery from microalgal biomass. The preliminary experimental data imply that effective and energy-saving extraction of the both classes of products is possible by applying the SCW technology to the microalgal biomass.

1. Introduction

Some species of aquatic microalgae have high potential to accumulate neutral lipids, which can be produce bio-diesel fuel by transesterification (Wen et al., 2014). Bio-diesel is one of the highly expected alternatives to fossil fuel (Suganya et al., 2016). Microalgae have been studied as material and energy source for many years and microalgal lipid production for more than a decade (Sahay and Braganza, 2016). But sustainable and commercially feasible mass cultivation system for microalgae is yet to be developed due to some limitations of appropriate strains, culturing system, extraction procedure of lipids and utilization of residues. Though a lot of research has been carried out to fill the knowledge gaps of pre-treatment (Yeoh et al., 2015), solvents used in the lipid extractions (Dvoretsky et al., 2016) medium for culturing mass cultivation of microalgae (Cicci and Bravi, 2014) and utilization of microalgae residues (Barontini et al., 2016). However, technical and economical limits have so far prevented the successful implementation of microalgae mass cultivation (Pearce, 2016). It has been pointed out that the following issues need to be addressed urgently in order to reduce the fossil fuel induced carbon impact and production cost of bio fuels; efficient technique to extract lipids in large quantities, utilization of cheap source of nutrients and effective use of solid algal residue after extraction of lipids.

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According to the previous study, nutrient supply has been identified as the most cost-intensive factor in mass cultivation of microalgae (Fushimi et al., 2016). Therefore, in order to minimize the production cost of bio fuel and make it competitive with fossil fuel cost, a practical technique (Misra et al., 2016) to utilize the lipid extracted algal residue (LEAR) as the source of N, P and other minute nutrients needs to be established (Patel et al., 2016). It is reported that microalgal biomass has been used as soil conditioner, which implies that microalgal biomass can be a good source of nutrients (Griffiths et al., 2016). With regards to conventional fertilization two major issues concern: heavy metals contamination and eutrophication, which can be minimized by recycling nutrients from microalgal residues and easy to supply available plant up-taking forms.
In order to establish a practical nutrient recovery system, the present research focuses on subcritical water (SCW) treatment of the lipid extracted algal residue (LEAR). SCW system, which has been applied to treat organic matters such as sewage sludge, is considered to be less energy intensive and more environmentally friendly than other conventional thermal and/or chemical systems. However, behaviors of LEAR in SCW treatment have not been investigated in detail. According to the researcher (Sinag et al., 2004) temperature, pressure, reaction time, heating rate, biomass type have a great effects on product distribution during hydrolysis of biomass is important to optimize as a future SCW treatment process. So the main research objective is optimization of three operational conditions of temperature, retention time and biomass-solvent ratio during SCW treatment for efficient recovery of N and P from LEAR as the nutrient source for mass cultivation of microalgae.

2. Methodology

In this study, *Chlorella sp*. used as the model microalga. Total N and Total P were selected as the indices to evaluate the properties of solid phase and liquid phase of SCW product of *Chlorella* sp.

2.1 Microalgae dry biomass sample preparation

Chlorella sp. provided by a Japanese agency were used to conduct the experiments. It was supplied as a concentrated liquid (140 g/L) which was kept at 4 ºC in a refrigerator until used. *Chlorella* sp. which is spherical, eukaryotic and unicellular alga, is a typical microalga with low lipid and high protein contents.
Solids in the *Chlorella* sp. slurry were collected by filtration by a glass microfiber filter (Whatman, GF/F, pore size 0.7 um) and oven-drying at 60 ºC for 18 h. The filters that had been oven-dried at 60 ºC for 24 h and kept in desiccators were used for filtration. The recovered dry biomass was stored in a freezer at -31 ºC until further process. The characteristics of the biomass are summarised in Table 1.

Table 1: Characterization of Chlorella sp.

Items	% (dry basis)
Carbohydrate	35 %
Protein	45 %
Lipid	20 %
Total Solids	8.9 %
Total Nitrogen	7.2 %
Total P	0.45 %

2.2 Experimental Design

The response curve method was used to design the experiments in the study, because, compared with the “one-factor-at-a-time” approach, the response surface method has an advantage of capable of investigating the interactions of the different operating parameters on the variations of targets and providing a perspective of the entire process. As the key variables affecting nutrients recovery, reaction temperature (180 ºC, 200 ºC, 220 ºC and 240 ºC), retention time (15 min, 30 min, 60 min) and biomass-solvent (water) ratio (2.5g/100mL, 5g/100mL and 7.5g/100mL) were selected as independent factors. To achieve the research objective the path way outlined in the figure 1.

2.3 Experimental Procedure (SCW experiments)

SCW experiments were carried out by a subcritical water reactor system developed and fabricated by OM LABTECH CO., LTD. It consists of a 200 mL stainless steel cylindrical reactor with a thermocouple and pressure gauge to monitor the reaction temperature and the internal pressure. The reactor is designed to operate at the temperature up to 400 ºC. The reactor has an internal agitator paddle at a fixed speed of 300 rpm. The system is fitted with rapid cooling system by water running in the cooling coil around the heater and an electric fan.

2.4 Determination of Weight of Lipid

A quantity of the dried algal biomass was ground using a pestle and mortal. After fine powder of the dried alga was obtained, 1 g of sample was taken to the plastic test tube of 50 mLThe dried algal powder was then mixed with approximately 40 mL mixture of methanol, chloroform and water in pre-determined ratio for lipid extractions. The mixture of the sample and solvent was vortexed for 2 min and kept still for one to several hours for extraction. The centrifuge was used to separate the solids and liquid at 4 ºC at 10,000 rpm for 10 min. The lipid was recovered by removing solvent by using a rotary evaporator with the water bath set at 70 ºC and a constant rotational speed. After complete evaporation weight of the lipid obtained was determined by gravimetric method.

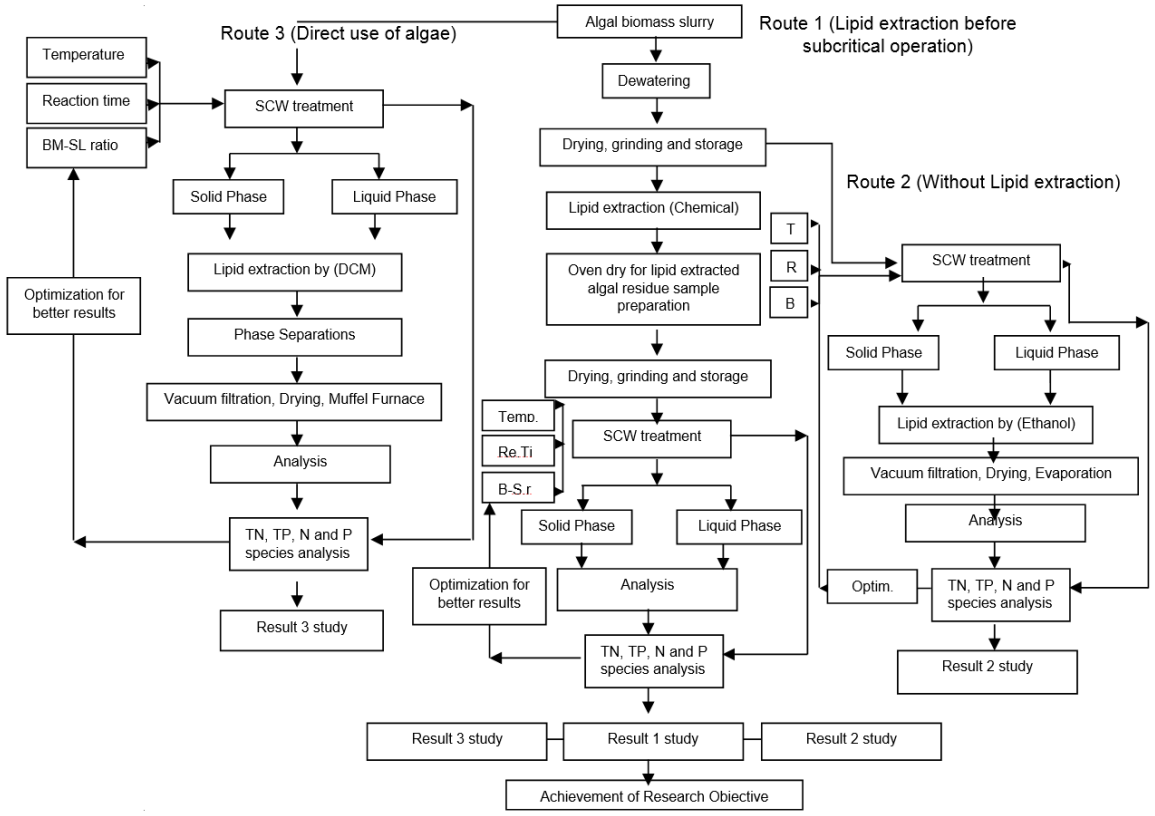

Figure 1: Flow chart of the research

2.5 Analysis of Products

2.5.1 P in the water soluble fraction

The total amount of soluble P in the water fraction was determined by the molybdenum blue method. An aliquot of 50 mL of water fraction was mixed with 10 mL of potassium peroxodisulfate in a Teflon vessel. The solution was heated at 121 ºC for 0.5 hin an autoclave to convert phosphorus species to PO_4^{3-} by hydrolysis. Then, the solution was mixed with reagent A (1.5 g L (+)-ascorbic acid, 1.0 g sodium dodecyl sulfate in 1 L distilled water) and reagent B (67 mL hydro sulfuric acid, 5.48 g ammonium molybdate 4-hydrtae and 0.25 g potassium antimony tartrate, and adding distilled water to the total volume of 1 L). The mixture was analyzed for phosphate concentration using a flow injection analyzer (UV-vis Spectrophotometer, SHIMADZU, Japan). P recovery ratio in the aqueous phase RP was calculated using Eq(1).

$$\mathrm{Rp} = \frac{\text{Weight of total P in water soluable fraction (g)}}{\text{Weight of P in dry algae (g)}} \times 100 \quad (1)$$

2.5.2 N in the water soluble fraction

The total amount of soluble N in the water fraction was determined by an ultraviolet spectrophotometer method. An aliquot of 50 mL of water fraction was mixed with 10 mL each of potassium peroxodisulfate and sodium hydroxide solutions in a Teflon vessel. The solution was heated to 121 ºC for 0.5 h in an autoclave to convert

all the nitrogenous spices to NO_3-. After 5 mL of supernatant was mixed with 1 mL of (1+16) HCl solution, NO_3-. Concentration in the solution was determined by an absorption spectrophotometer (UV-vis Spectrophotometer, SHIMADZU, Japan) at the wavelength of 220 nm. Total N recovery ratio RN in the aqueous phase was calculated using Eq(2)

$$\mathrm{Rn} = \frac{\text{Weight of total N in water soluable fraction (g)}}{\text{Weight of N in dry algae (g)}} \times 100 \quad (2)$$

2.5.3 Liquefaction biomass conversion %

The dry solid in feed stock (LEAR) was taken after oven dried at 105 ºC for 1.0 h. The weight of LEAR was taken and put it in to the reactor. 100 mL of MQ water were added before the process started. After successful SCW operation the reactor was allowed to cool down at room temperature to collect the mixer. After using a separator (Vacuum filter) the solid phase was separated from liquid phase and kept at storage conditions until further use. The solid residue then oven dried at 105 ºC for 24 h to make a complete dry which termed as 'dry solid residue'. Conversion % was calculated using Eq(3).

$$\mathrm{Conversion\%} = \frac{\text{Dry solid in Feed Stock} - \text{Dry Solid Residue}}{\text{Dry Solid in Feed Stock}} \times 100 \quad (3)$$

3. Results and Discussion

The conversion of solid matters by SCW process are summarised in Figures 2, 3 and 4. As shown in the figures, it was demonstrated that the liquefaction efficiency by SCW was in the range between 44 % and 80 % of conversion. The conversion efficiencies were observed to be dependent on the parameter such as temperature, reaction time and biomass-solvent ratio, which are all manipulatable, in SCW process.

Figure 2 shows the effects of temperature on conversion %. It is shown that the solid residue decreases with increasing temperature. The increment is monotonic in the temperature range evaluated. According to the previous study, an organic matter in hot compressed water condition, which is a subcritical water condition, firstly is hydrolysed at lower temperature region where polysaccharides and proteins are degraded to small molecules, and dehydration, deoxygenation and decarboxylation reactions take place. Then, when the temperature increases, the repolymerization occurs and the reactions are accelerated. As the temperature increases, it is speculated that the rate of hydrolysis decreases and the mass of solid residue increases.

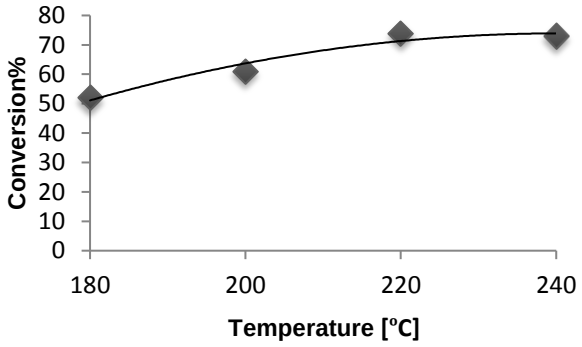

Figure 2: Effects of temperature on conversion %

Reaction time showed substantial effects on conversion % as can be seen in Figure 3. A holding time of 10 min was found insufficient to increase N and P concentrations in water fraction at the lower range of temperature (180 ºC – 200 ºC). Reaction time longer than 25 min and temperature higher than 200 ºC, it was observed that a greater conversion %, which is expected to result in more soluble N and P formation in water fraction, was achieved. But, with the reaction time greater than 35 min, repolymerization to produce solids was observed. Therefore, reaction time of 25 to 35 min is considered to be optimal in case of temperature range between 200 ºC to 260 ºC. Other studies (Garcia et al., 2011) have suggested that the optimal reaction time is in the range of 30 to 60 min at a similar temperature conditions, the difference may be due to different cooling rates after the SCW treatments.

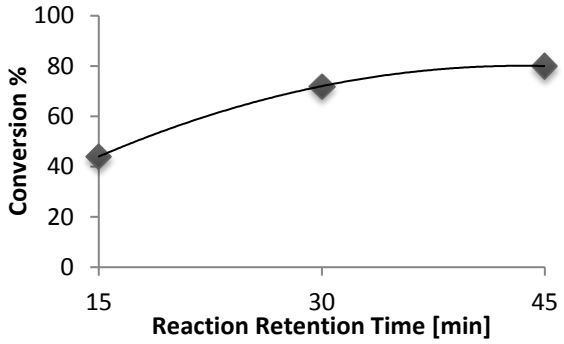

Figure 3: Effects of reaction time on conversion %

As shown in Figure 4, the highest conversion % was observed at the ratio of 5 g/100 mL. It is expected that the soluble N and P concentrations are also the highest at the ratio. Under the SCW treatment conditions used in this experiment, the solid to solvent ratio of 5 g/100 mL is considered to be optimal.

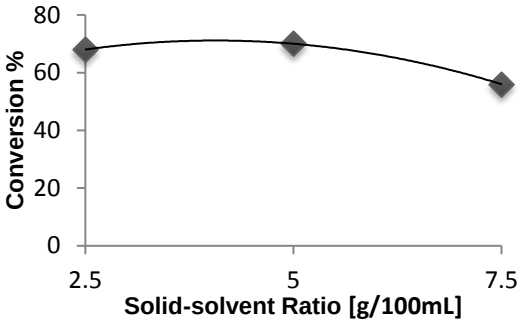

Figure 4: Effects of biomass to solvent ratio on conversion %

Assuming the optimal reaction time of 30 min and solids to solvent ratio of 5 g/100 mL, the effects of temperature on concentrations of total soluble phosphorus and nitrogen are shown in Figures 5 and Figure 6.
As shown in Figure 5, a positive correlation was observed between total soluble phosphorus (Rp) and temperature. However, in case of total soluble N (Rn), a local maximum concentration of Rn was observed at 220 ºC as shown in Figure 6. It is implied that volatile nitrogenous compounds are formed and vaporized at higher temperature, but the mechanism is not clear yet.

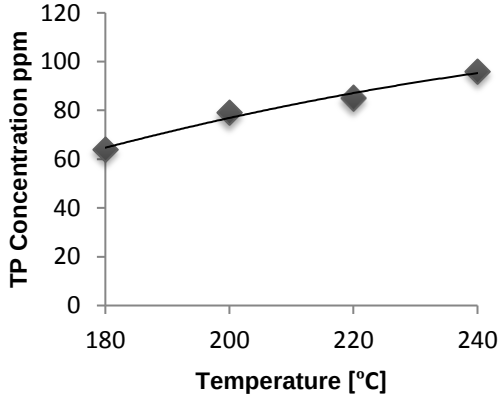

Figure 5: Effects of reaction temperature of total soluble phosphorus concentration

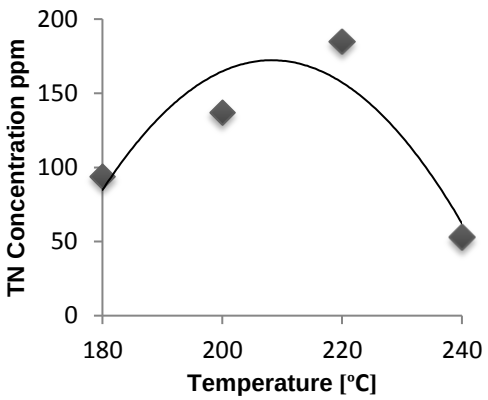

Figure 6: Effects of reaction temperature of total soluble nitrogen concentration

4. Conclusions

Subcritical water treatment of C*hlorella* sp. was conducted at various temperatures, reaction retention time and solid-solvent ratio conditions. From the results it can be concluded that to get the maximum recovery of soluble phosphorus and nitrogen, as the nutrient source for mass cultivation of microalgae, can be accomplished at 220 ^{o}C and 30 min of reaction time under the experimental set-up used in the study. The rates of temperature rising and cooling, internal agitation appeared to have significant effects on the efficacy of SCW treatment, but the effects are not well known yet, therefore need to be further investigated.

On the other hand, the extracted N and P are highly concentrated to direct use as a medium. So, prior to application in the algal farming it must be diluted. So, dilution rate for successful algal growth may be a new research priority from the algal nutrient recovery system.

References

Barontini F., Biagini E., Dragoni F., Corneli E., Ragaglini G., Bonari E., Tognotti L., Nicolella C., 2016, Anaerobic digestion and co-digestion of oleaginous microalgae residues for biogas production, Chemical Engineering Transactions 50, 91-96.

Cicci A., Bravi M., 2014, Production of the freshwater microalgae scenedesmus dimorphus and arthrospira platensis by using cattle digestate, Chemical Engineering Transactions 38, 85-90.

Dvoretsky D., Dvoretsky S., Temnov M., Akulinin E., Peshkova E., 2016, Enhanced lipid extraction from microalgae chlorella vulgaris biomass: experiments, modelling, optimization, Chemical Engineering Transactions 49, 175-180.

Fushimi C., Kakimura M., Tomita R., Umeda A., Tanaka T., 2016, Enhancement of nutrient recovery from microalgae in hydrothermal liquefaction using activated carbon, Fuel Processing Technology 148, 282-288.

Garcia Alba L., Torri C., Samorì C., van der Spek J., Fabbri D., Kersten S.R., Brilman D.W., 2011, Hydrothermal treatment (HTT) of microalgae: evaluation of the process as conversion method in an algae biorefinery concept, Energy and Fuels 26 (1), 642-657.

Griffiths M., Harrison S.T., Smit M., Maharajh D., 2016, Major Commercial Products from Micro-and Macroalgae, Algae Biotechnology, Springer International Publishing, Berlin, Germany, 269-300.

Misra N., Panda P.K., Parida B.K., Mishra B.K., 2016, Way forward to achieve sustainable and cost-effective biofuel production from microalgae: a review, International Journal of Environmental Science and Technology, 13, 2735.

Patel B., Guo M., Chong C., Sarudin S.H.M., Hellgardt K., 2016, Hydrothermal upgrading of algae paste: Inorganics and recycling potential in the aqueous phase, Science of The Total Environment 568, 489-497.

Pearce M.W., 2016, An integrated approach to microalgae biomass generation and processing, PhD Thesis, Cranfield University, Cranfield, England.

Sahay S., Braganza V.J., 2016, Microalgae based biodiesel production–current and future scenario, Journal of Experimental Sciences 7, 31-35.

Sinag A., Kruse A., Rathert J., 2004, Influence of the heating rate and the type of catalyst on the formation of key intermediates and on the generation of gases during hydropyrolysis of glucose in supercritical water in a batch reactor, Industrial and Engineering Chemistry Research 43 (2), 502-508.

Suganya T., Varman M., Masjuki H.H., Renganathan S., 2016, Macroalgae and microalgae as a potential source for commercial applications along with biofuels production: A biorefinery approach, Renewable and Sustainable Energy Reviews 55, 909-941.

Wen X., Geng Y., Li Y., 2014, Enhanced lipid production in Chlorella pyrenoidosa by continuous culture, Bioresource Technology 161, 297-303.

Yeoh K.P., Cheung T.C.K., Pahija E., Hui C.W., 2015, Effects of pretreatment on microalgae drying, Chemical Engineering Transactions 45, 565-570.