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Scale-up and cultivation of microalgae from brackish water in Thailand in transparent high density polyethylene bags

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Abstract

The microalgae from brackish water in Thailand e.g. Bang Poo accommodation next to the sea, Bang Poo mangrove forest, watersides of both Asokaram Temple and Srichan waterside were cultivated in Watanabe's medium, pH 6.5 in 5 liters PET bottles and transparent high density polyethylene bags. Cultivation of microalgae in transparent high density polyethylene bags resulted maximum dry weight of 10.92 g l^{-1}, 8.61 g l^{-1}, 6.49 g l^{-1} and 5.47 g l^{-1} respectively. Comparing the addition of glucose 0 g/L, glucose 1 g/L, $FeCl_3$ 0.003 g/L and $FeCl_3$ 1 g/L in Watanabe's medium was investigated. The results showed that adding glucose 1 g/L and $FeCl_3$ 0.003 g/L in Watanabe's medium gave the maximum lipid content of microalgae from Bang Poo mangrove forest 50.72 %(w/w), microalgae from accommodation next to the sea 47.58%(w/w), microalgae from Srichan Temple's waterside 34.36 %(w/w) and microalgae from Asokaram Temple's waterside 32.35%(w/w) respectively. In addition, scale-up and cultivation of microalgae from Bang Poo mangrove forest to 155 liters in transparent high density polyethylene bags, adding glucose 1 g/L ,$FeCl_3$ 0.003 g/L and CO_2 10%(v/v) in Watanabe's medium for 15 days was assessed. Microalgal biomass was recovered by filtration and centrifugation at 8000 rpm for 15 min at 4 °C. Algal cell were dried in an oven at 60°C for 2h and kept in a vacuum desiccator before use. Determination of lipid content by hexane extraction gave 46.53 %(w/w) algae oil.

Keywords: *scale-up, microalgae, transparent high density polyethylene bag, lipid content, brackish water*

บทคัดย่อ

เลี้ยงสาหร่ายน้ำมันขนาดเล็กจากแหล่งน้ำกร่อยบริเวณบ้านพักตากอากาศบางปู บริเวณป่าชายเลนสถานตากอากาศบางปู ท่าน้ำวัดอโศการาม และท่าน้ำวัดศรีจันทร์ ในอาหารสูตร Watanabe's medium ที่ pH 6.5 ในขวด PET และถุงโพลิเอทิลีนใสความหนาแน่นสูง ปริมาตร 5 ลิตร พบว่าน้ำหนักแห้งของสาหร่ายที่เลี้ยงในถุงโพลิเอทิลีนใสความหนาแน่นสูงมีปริมาณมากกว่าที่เลี้ยงในขวด PET โดยน้ำหนักแห้งของสาหร่ายจากแหล่งน้ำบริเวณท่าน้ำวัดอโศการามเท่ากับ 5.47 กรัมต่อลิตร ท่าน้ำวัดศรีจันทร์ เท่ากับ6.49 กรัมต่อลิตร สถานตากอากาศบางปูบริเวณป่าชายเลน 8.61 กรัมต่อลิตร และ บริเวณบ้านพักตากอากาศบางปูเท่ากับ 10.92 กรัมต่อลิตร ตามลำดับ เปรียบเทียบการเลี้ยงสาหร่ายในสูตรอาหาร Watanabe's medium ที่เติมและไม่เติม glucose กับ สูตรอาหารที่เติม $FeCl_3$ 0.003 g/L และ 1 g/L และ ตามลำดับ พบว่าสาหร่ายจากป่าชายเลนสถานตากอากาศบางปู ที่เลี้ยงในสูตรอาหาร Watanabe's medium ที่เติม glucose 1 g/L ,$FeCl_3$ 0.003 g/L ให้ปริมาณน้ำมันสูงสุด เท่ากับ 50.72 %(w/w) ส่วนสาหร่ายจากบริเวณบ้านพักตากอากาศบางปู ท่าน้ำวัดศรีจันทร์ และท่าน้ำวัดอโศการาม มีปริมาณน้ำมันเท่ากับ 47.58% , 34.36 % และ 32.35%(w/w) ตามลำดับ เมื่อขยายกำลังการผลิตของสาหร่ายจากป่าชายเลนสถานตากอากาศบางปู ที่เลี้ยงในสูตรอาหาร Watanabe's medium ที่เติม glucose 1 g/L ,$FeCl_3$ 0.003 g/L และคาร์บอนไดออกไซด์ 10%(v/v)ในถุงโพลิเอทิลีนใสความหนาแน่นสูง ปริมาตร 155 ลิตร นาน 15 วัน เก็บเกี่ยวเซลล์โดยการกรอง และตกตะกอนด้วยการปั่นเหวี่ยงที่ความเร็วรอบ 8000 รอบต่อนาที นาน 15 นาที อบแห้งสาหร่ายที่อุณหภูมิ 60º C นาน 2 ชั่วโมง สกัดน้ำมัน โดยใช้เฮกเซนเป็นตัวทำละลาย พบว่า สาหร่ายมีปริมาณน้ำมัน เท่ากับ 46.53 %(w/w).

คำสำคัญ: *ขยายกำลังการผลิต, สาหร่ายขนาดเล็ก, ถุงโพลิเอทิลีนใสความหนาแน่นสูง, ปริมาณน้ำมัน, น้ำกร่อย*

1. Introduction

Climate change is currently the most pressing global environmental problem. It is widely accepted that using fossil fuels has caused global warming; therefore fossil fuels as a source of energy should be replaced with renewable, clean energy sources to reduce carbon dioxide and greenhouse gas emissions (Amin, 2009). The need for alternative fuels is driven by global warming considerations associated with the consumption of fossil fuels and emission of carbon dioxide. Microalgae can provide several different types of renewable biofuels.

Methane produced by anaerobic digestion of the algal biomass (Spolaore, Joannis-Cassan, Duran, & Isambert, 2006); biodiesel derived from microalgal oil (Banerjee, Sharma, Chisti, & Banerjee, 2002; Gavrilescu & Chisti, 2005); and photologically produced biohydrogen (Ghirardi et al., 2000; Melis, 2002; Fedorov, Kosourov, Ghirardi, & Seibert, 2005; Kapdan & Kardi, 2006); algal biomass for combustion; algal crude oil for direct combustion, or for use in production of other transportation fuels such as diesel, gasoline and jet fuel (kerosene) (Lestari, Maki-Arvela, Beltramini, Lu, G., & Murzin, 2009); biohydrogen; bioethanol via fermentation of carbohydrates derived from algae (Matsumoto et al., 2003; Ho, Huang, Chen, Kondo, & Chang, 2013); and bioethanol produced directly through algal photosynthesis (Ho et al., 2013; Lu, Sheahan, & Fu, 2001). Of the different forms of fuels that algae can provide, algal oil for making liquid transport fuels is the focus of most attention, including commercialization efforts by many companies.

Microalgae contain about 50% carbon in their biomass. In most cases, all of this carbon can be obtained from atmospheric carbon dioxide (Doucha, Straka, & Livansky, 2005). Microalgae are sunlight-driven cell factories that convert carbon dioxide to potential biofuels, foods, feed and high-value bioactives (Metzger & Largeau, 2005; Singh, Kate, & Banerjee, 2005; Spolaore et al., 2006; Walter, Purton, Becker, & Collet, 2005). The normal atmosphere contains around 0.039% of carbon dioxide by volume (Kumar et al., 2010). Algae can capture the atmospheric carbon dioxide with ten-times higher efficiency than terrestrial plants, and store captured carbon dioxide as lipids in their cells. This stored lipid can be utilized to produce the biofuel such as bioethanol, methane and biohydrogen (Nakano et al., 2014). In addition, using carbon dioxide from the atmosphere is likely to greatly reduce the carbon footprint of algal fuels. However, carbon dioxide absorption from the standard atmosphere into the culture medium is never sufficiently fast to rapidly grow a large concentration of algae. Therefore, low-energy physical-chemical strategies are needed to capture and concentrate the carbon dioxide that is already in the atmosphere, for use in algal culture. Insufficiency of concentrated sources of carbon dioxide may suggest the use of organic carbon for algae culture. Growing certain microalgae on fixed organic carbon sources derived from plants is of course possible. Such heterotrophic growth achieves extremely high biomass productivities compared with growth on inorganic carbon and sunlight (Huang, Chen, Wei, Zhang, & Chen, 2010; Bumbak, Cook, Zachleder, Hauser, & Kovar, 2011; Tabernero, Martin del Valle, & Galan, 2012). The available technologies for non-biological capture of carbon dioxide are currently expensive (Metz, Davidson, de Coninck, Loos, & Meyer, 2005; Folger, 2010). Also, in seawater the carbon dioxide solubility is low compared to freshwater. For algae that can be grown in highly alkaline conditions, carbon dioxide may be supplied in the form of bicarbonate (Chi, O'Fallon, & Chen, 2011).

Microalgae are more efficient than terrestrial plants in converting sunlight to biochemical energy (Stephenson, Moore, Terry, Zubkov, & Bibby, 2011). The green microalgae share the same basic photosynthetic machinery as the C3 photosynthetic land plants. The maximum efficiency of photosynthesis in C3 plants growing in the normal atmosphere has been estimated to be 4.6 which means 4.6% of the total solar energy received can be converted to biomass energy (Zhu, Long, & Ort, 2008). In contrast, for algae growing in a carbon dioxide supplemented culture outdoors, the annual average photosynthetic efficiency of >5% has been observed and a possible maximum of 8.3% has been estimated for wild type species (Chisti, 2012). Producing 100 tons of algal biomass fixes roughly 183 tons of carbon dioxide (Sanchez Miron et al., 2003).

Unlike other oil crops, microalgae grow extremely rapidly and many are exceedingly rich in oil. Microalgae commonly double their biomass within 24h. Oil content in microalgae can exceed 80% by weight of dry biomass (Metting,1996; Spolaore et al., 2006). Oil levels of 20-50% are common. Oil productivity, that is the mass of oil produced per unit volume of the microalgal broth per day, depends on the algal growth rate and the oil content of the biomass. Algal oil includes diverse components: in addition to triglycerides, the oil may contain terpenoid hydrocarbons, polar lipids, oxygen containing nontriglyceride carotenoid oils and chlorophylls (Banerjee et al., 2002). All of these compounds are energy rich and carbon rich. Algal crude oil has an energy content of around 35,800 kJ kg^{-1}, or around 80% of the average energy contained in petroleum (Chisti, 2012). Algal crude oil tends to be rich in long chain polyunsaturated fatty acids (Belarbie, Molina, & Chisti,2000; Ward & Singh, 2005, Guschina & Harwood, 2006, Nichols, Petrie,

& Singh, 2010). Algal crude oil is a potential substitute for producing various kinds of transport fuels. Lipids from microalgae are considered as a sustainable source ideally suited for a renewable, biodegradable and environment friendly liquid fuel alternative because microalgae can grow 2-10 times faster than any terrestrial crop, currently being used as a source of biodiesel (Sayre, 2013).

Recovery of microalgal biomass from the broth is necessary for extracting the oil. Biomass is easily recovered from the broth by filtration, centrifugation, and other means (Grima, Fernandez, Acien Fernandez, & Chisti, 2001; Grima, Belarbi, Acien Fernandez, Robles Medina, & Chisti, 2003). In order to develop a feasible commercial production process for biodiesel, microalgal strains that are high in biomass and lipid productivity need to be selected (Griffiths & Harrison, 2009). Therefore, large numbers of isolates have to be screened or genetic modification of already cultivated isolates has to be carried out. In each case, screening large numbers of microalgae using a rapid, accurate and reliable method for detection and quantification of lipids production is required (Elsey, Jameson, Raleigh, & Cooney, 2007). The quantification of microalgal lipids is usually achieved by gravimetric means after solvent extraction (Bligh & Dyer, 1959; Lee et al.,2010) which requires about 3-4 days and needs a minimum of 10-15 mg wet weight of cells (Elsey et al., 2007). Gas chromatography-mass spectrometry (GC-MS) analysis of fatty acid methyl esters (FAME) requires specialized equipment. Therefore, detection of microalgal lipid is most often accomplished by staining techniques employing fluorescence microscopy (Chen, Zhang, Song, Sommerfeld, & Hu, 2009; Cooksey, Guckert, Williams, & Callis, 1987; Elsey et al., 2007).

Nile Red (NR) has been used for staining of lipid droplets in smooth muscle cells, macrophages, yeasts, fungi, single-cell eukaryotes including microalgae and ciliated protozoan (Kimura, 2004; Carman, Thistle, Ertman, & Foy, 1991). The concentration of NR required for staining of different microalgae varies considerably (0.01-100 µg/ml) between the different classes of algae. The major disadvantage of using NR for microalgal staining is that the accuracy and precision of staining differs depending on strain of algae and is greatly affected by uneven dye uptake due to the polarity of solvents used to dissolve the dye (Chen et al., 2009). Uptake of NR is also affected by cell wall structure and composition, growth conditions and environment (Gao, Xiong, Zhang, Yuan, & Wu, 2008; Laurens & Wolfrum, 2010). NR dye is unable to efficiently penetrate the thick, rigid cell walls of microalgae. Thus, an improvement of the NR staining techniques was carried out by Chen, Sommerfeld, & Hu, 2011, who used dimethyl sulfoxide (DMSO) as stain carrier and microwave irradiation to increase staining efficiency. Nevertheless, the major drawbacks of NR are its limited photostability and interference of chlorophyll (Laurens & Wolfrum, 2010). However, it was recognized that various factors including algal species, the polarity of the solvent to dissolve Nile Red and different measure conditions might greatly affect the combination of Nile Red to lipid components in algal cells (Cooksey et al., 1987).

Microalgae cultivation can be done in open-cultures, such as ponds or in highly controlled closed-culture systems called photobioreactors (PBRs). One common PBR requires growing the algae in transparent high density polyethylene bags. Open-culture systems are normally less expensive to build and operate, more durable than large closed reactors. However, ponds use more energy to homogenize nutrients and the water level must be at least 15 cm for the microalgae to receive enough solar energy to grow (Tredici, 2004). Additional drawbacks, which must be weighed against large algal production, are the need for extensive land areas and increased susceptibility to contaminations from other microalgae, fungi or bacteria (Mata, Martins, & Caetano, 2010). PBRs are flexible systems that can be optimized according to the biological and physiological characteristics of the algae species being cultivated. With PBRs, direct exchange of gases and contaminants between the cultivated cells and atmosphere are limited or not allowed by the impermeable reactor walls, but advantages do exist including that PBRs offer better control over culture conditions and growth parameters (pH, temperature, mixing, CO_2 and O_2), prevent water evaporation, reduce CO_2 losses, allow higher microalgae densities or cell concentration, higher volumetric productivities, offer a more safe and protect environment and prevent contamination or minimize invasion by competing microorganisms (Mata et al., 2010).

Large-scale production of microalgal biomass generally uses continuous culture during daylight. A growth mode combining photosynthesis and heterotrophic or mixotrophic production, has limited potential as a large-scale photosynthetic culture because it cannot be operated as a pure

culture and contamination with bacteria growing on organic carbon will inevitably reduce productivity (Chisti, 2013).

Here, for the purpose of scale up and cultivation microalgae in transparent high density polyethylene bags from brackish water in Thailand in displacing fossil fuels, the cultivation will be done using Watanabe's medium. An important criterion when selecting algae is to select algae which give high level of biomass and high lipid content. The optimal parameters were determined by regression analysis.

2. Meterials and methods

2.1 Material and samples

Nile Red (9-diethylamino-5H-benzo[α]phenoxazine-5-one) was purchased from Sigma (CAS number:7385-67-3; Catalogue number: N3013).

Four different sources of brackish water were sampled from Bang Poo accommodation next to the sea, Bang Poo mangrove forest, Asokaram Temple's waterside and Srichan Temple's waterside, Samutprakarn Province, Thailand.

Transparent high density polyethylene bags with 2 m length, 20 cm width and 0.2 mm thickness were kindly provided by PTT Global Chemical Public Company limited, Rayong Province, Thailand.

2.2 Algae cultivation and growth conditions

Algae samples from different sources were cultivated in Watanabe's medium which contains 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. All samples were gathered in duplicate. The addition of ferric chloride (0, 0.003 g/l) and glucose (0, 1 g/l) in Watanabe's medium were investigated. Samples were cultured at room temperature with fluorescence light and bubble aeration for 15 days. Cells were harvested by centrifugation at 8,000 g for 10 min at 4 °C.

2.3 Biomass assay

Algal growth was monitored using the optical density of the culture at 680 nm (OD_{680}) using a Shimadzu UV 2401 PC Spectrophotometer. The pellets were centrifuged at 8000 g for 10 min at 4 °C. Algal cell were dried in oven at 60 °C until weight constant and kept in a vacuum desiccator before weighing with electronic balance (Sartorius Company, Germany).

2.4 Determination of lipid content by spectrofluorometer

Five milliliters of diluted algae suspension were mixed with $5x10^{-2}$ mg/ ml concentration of Nile Red solution in acetone. The suspensions were analyzed on a spectrofluorometer (HITACHI F-4500, Japan) with a 480 nm narrow band pass excitation filter and a 570~590 nm emission filter (Govender et al., 2012). Lipid content of algae from different water sources was determined from the fluorescence intensity of Nile Red stained cells according to solvent extraction.

2.5 Determination of lipid content by gravimetric analysis

Algae powders (approximately 1 g) were mixed with 130 ml of hexane in Soxhlet apparatus (TOPO, Model EAM9202-06, China). The same process were repeated three times and all lipids collected were combined.

2.6 Scale-up and cultivation of algae

After choosing the suitable algae from different sources, algae were cultivated in 2 m length, 20 cm width and 0.2 mm thickness transparent high density polyethylene bags. Each bag was filled with algae, 2.5 L of tap water, and 9 L of Watanabe's medium. Transparent high density polyethylene bags were fixed on a metal stand in 3 m^2 area. Sunlight was used at temperature range 30-35 °C. Carbon dioxide supplemented efficiency of 10% (V/V) has been observed.

2.7 Statistical analysis

Results were presented as mean ± standard deviation (SD) from three replicates. Data obtained were analyzed statistically to determine the degree of significance using one way analysis of variance (ANOVA) at probability level $P < 0.05$.

3. Results and discussion

In order to keep costs down in scale - up cultivation of microalgae, it is important to know the physical properties of brackish water to develop massive cultivation systems characterized by low production costs, high biomass production and ease of handling.

3.1 Physical properties of brackish water

The physical properties of brackish water from each source are shown in Table 1. The most

abundant components were calcium carbonate (8,028 mg/l) and chloride (10,016.00 mg/l). The analysis verifies that water from each source contains different physical properties.

Table 1 Physical properties of brackish water

Water Source	Total soluble solid (mg/l)	pH	$CaCO_3$(mg/l)	Chloride (mg/l)
Asokaram Temple's waterside	12.60 ± 0.12 [a,A]	7.70	3,862.50 ± 3.54 [a,A]	7,020.21 ± 10.61 [a,A]
Bang Poo mangrove forest	30.93 ± 0.73 [b,B]	7.90	5,493.00 ± 5.66 [b,B]	8,471.81 ± 3.54 [b,B]
Bang Poo accommodation next to the sea	35.12 ± 2.90 [c,C]	8.00	8,028.00 ± 2.83 [c,C]	10,016.01 ± 7.08 [c,C]

[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; different letters refer to significantly different lipid contents at 95% confidence intervals; identical letters refer to insignificantly different lipid contents at 95% confidence intervals.

3.2 Comparison of microalgal biomass cultivation

In the present study, comparison of biomass cultivation in PET bottles and transparent high density polyethylene bags was investigated. Two hundred milliliters of microalgae was filled with 5 L of Watanabe's medium in PET bottles and transparent high density polyethylene bags.

Sunlight was used at temperature range of 30-35 °C. Samples were cultured with bubble aeration for 15 days. Each samples have duplicates. The pellets were centrifugation at 8,000 g for 10 min at 4 °C. Algal cells were dried in oven at 60 °C for 2h. Microalgae from Bang Poo accommodation next to the sea cultivated in transparent high density polyethylene bags gave the maximum cell dried weight (Table 2). PET bottles use more energy to homogenize nutrients and the water level cannot be kept much lower than 15 cm for the microalgae to receive enough solar energy to grow. The transparent high density polyethylene bags are thus simple and scalable enough to support growth (Tredici, 2004). Suspension stirring through blowing air in at the bottom of bottle facilitates gas exchange and temperature equalization in the highly turbulent upperzone. Grima et al. (2001) reported that excessive levels of dissolved oxygen released from photosynthesis inhibits photosynthesis and causes photo-oxidation, which leads to damage of algal cells.

Table 2 Microalgal biomass cultivation

Water source	Cell dried weight (g/l)	
	PET bottles	Transparent high density polyethylene bags
Asokaram Temple's waterside	3.41 ± 0.20 [a,A]	5.47 ± 0.71 [a,A]
Srichan Temple's waterside	2.04 ± 0.11 [b,B]	6.49 ± 0.40 [b,B]
Bang Poo mangrove forest	3.41 ± 0.03 [a,NS]	8.61 ± 0.27 [c,C]
Bang Poo accommodation next to the sea	5.19 ± 0.14 [c,C]	10.92 ± 0.58 [d,D]

[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: cell dried weight; different letters refer to significantly different lipid contents at 95% confidence intervals; identical letters refer to insignificantly different lipid contents at 95% confidence intervals.

3.3 Determination of lipid content by spectrofluorometer

Under spectrofluorometer, Nile Red stained microalgae cells were distinguished from unstained by specific emission at 580 nm (Figure1). As shown in Figure 1, Nile Red has rather low background fluorescence. Morphology of lipids excited by blue light (480 nm) is observed under fluorescence microscope. When infused with Nile Red, lipids show characteristic yellow fluorescence. Therefore, it was chosen in the following experiments focusing on optimizing growth medium cultivation for microalgae lipid accumulation.

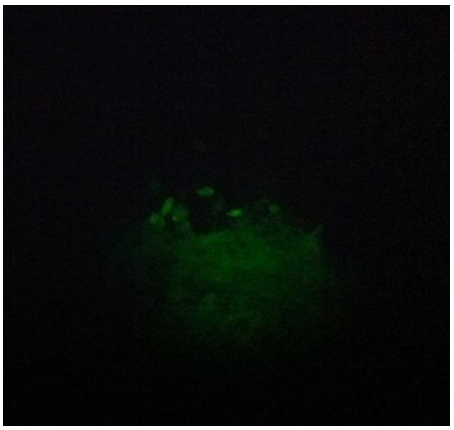

Figure 1 Lipid content observed by spectrofluorometer

3.4 Microalgae biomass cultivated in different growth medium

Microalgal biomass cultivations were distinguished in each growth conditions (Table 3). The critical point for the whole microalgal biofuel production is to select the optimal growth medium and the optimal strain with suitable lipid yield. The relative composition of algal lipids depends greatly on the species used and the medium, environmental and developmental conditions in which the cells are cultured and harvested. In this study, the addition of ferric chloride (0, 0.003 g/l) and glucose (0, 1 g/l) in Watanabe's medium were investigated. The results showed that the microalgae cultivated in Watanabe's medium with the addition of ferric chloride (0, 0.003 g/l) and glucose (0, 1 g/l) gave lipid amounts near the amount of cell dried weight. Our underlying hypothesis is that, 1) positive charged ferric ions (Fe^{3+}) act to coagulate negatively charged microalgae cells (Zhang & Hu, 2012); 2) ferric ions associated with the resulting biomass floc mediate when hydrogen peroxide (H_2O_2) is added (Seo & Han, 2014), and 3) $FeCl_3$, a representative Lewis acid, is again used as an esterification catalyst; by the reaction of its strong acidity, FFAs are preferentially transformed into FAMEs rapidly, leaving a neutral lipid triglyceride (TG) relatively intact (Yoo et al., 2014).

Table 3 Cell dried weight and growth conditions

Water Source	Cell dried weight (g/l)		
	Glucose 1 g/l , $FeCl_3$ 0 g/l	Glucose 0 g/l , $FeCl_3$ 0.003 g/l	Glucose 1 g/l , $FeCl_3$ 0.003 g/l
Asokaram Temple's waterside	$2.92 \pm 0.10^{a,A}$	$1.18 \pm 0.07^{b,B}$	$3.00 \pm 0.70^{a,A}$
Srichan Temple's waterside	$2.81 \pm 0.13^{a,NS}$	$3.26 \pm 0.52^{a,NS}$	$1.50 \pm 1.18^{bc,NS}$
Bang Poo mangrove forest	$0.71 \pm 0.17^{b,B}$	$1.56 \pm 0.13^{b,A}$	$0.65 \pm 0.06^{c,B}$
Bang Poo accommodation next to the sea	$1.88 \pm 0.077^{c,A}$	$1.32 \pm 0.44^{b,AB}$	$0.74 \pm 0.04^{c,B}$

[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.

3.5 Microalgae lipid contents (%W/W) cultivated in different growth medium

Enhancing lipid production relies on modifying the production environment to favor the production or accumulation of lipids. The physiological stresses (e.g. nutrient starvation, salinity increase) redirect metabolic fluxes to accumulation of lipids. Microalgae from Bang Poo mangrove forest gave the maximum lipid contents(50.72 % W/W) when cultivated in 5 L transparent high density polyethylene bags supplemented with the addition of 0.003 g/l ferric chloride and 1 g/l glucose in Watanabe's medium.

Table 4 Lipid contents (%W/W) of microalgae

Water source	Lipid contents (% W/W)		
	Glucose 1 g/l , $FeCl_3$ 0 g/l	Glucose 0 g/l , $FeCl_3$ 0.003 g/l	Glucose 1 g/l , $FeCl_3$ 0.003 g/l
Asokaram Temple's waterside	$20.67 \pm 6.31^{b,A}$	$5.19 \pm 3.59^{b,B}$	$32.35 \pm 0.98^{b,A}$
Srichan Temple's waterside	$17.23 \pm 0.16^{b,C}$	$28.85 \pm 0.17^{a,B}$	$34.36 \pm 4.99^{b,A}$
Bang Poo mangrove forest	$34.58 \pm 6.00^{a,B}$	$29.08 \pm 0.30^{a,B}$	$50.72 \pm 3.28^{a,A}$
Bang Poo accommodation next to the sea	$26.61 \pm 1.22^{ab,B}$	$28.26 \pm 0.08^{a,B}$	$47.58 \pm 0.92^{a,A}$

[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.

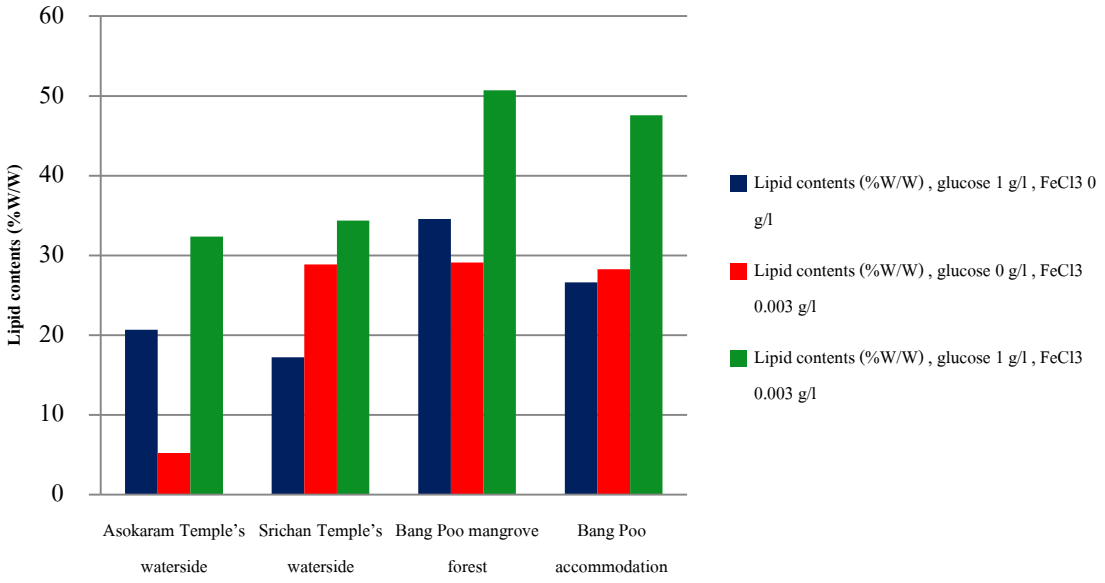

Figure 2 Lipid contents (%W/W) cultivated in different growth media

3.6 Scale-up and cultivation of microalgae

Choosing the microalgae from Bang Poo mangrove forest, scale - up and cultivation microalgae to 155 liters in transparent high density polyethylene bags, adding glucose 1 g/L, $FeCl_3$ 0.003 g/L and CO_2 10% (v/v) in Watanabe's medium for 15 days. Transparent high density polyethylene bags were fixed on a metal stand in 3 m^2 area (Figure 3). Microalgal biomass was recovered by filtration and centrifugation at 8,000 rpm for 15 min at 4 °C. Algal cells were dried in oven at 60 °C for 2h and kept in a vacuum desiccator before use. Determination of lipid content by hexane extraction gave 46.53 % (w/w) algae oil. Estimations suggest that the algal biomass with an oil content of 40% by weight will have to be produced at a cost of no more than $0.25 per kg (Chisti, 2012) , if algal oil is to compete with petroleum at around the current price of $629 per cubic meter ($100/barrel). The actual cost of producing the biomass at present appears to be at least 10-fold greater.

Figure 3 Scale up and cultivation of microalgae

4. Conclusions

The results demonstrate that scale-up and cultivation of microalgae from Bang Poo mangrove forest may be useful for biodiesel production. Maximal lipid contents (46.53 % w/w) were observed at 155 liters in transparent high density polyethylene bags, adding glucose 1 g/L, $FeCl_3$ 0.003 g/L and CO_2 10% (v/v) in Watanabe's medium for 15 days. The simple and efficient process described in this study could benefit the biofuels industries without requiring alteration of existing plant equipment. However, what can be done to consistently maintain high productivity during the entire cultivation period at least in regions where sunlight and temperature are essentially invariant with time is a question yet to be answered for large scale outdoor culture of algae. Inexpensive and low energy processes for recovering the algal biomass from a fairly dilute broth and for extracting the oil from the moist biomass paste, are certainly required. Large-scale solvent extraction of algae oils from the biomass paste should be developed, but biomass recovery and oil extraction are not the major impediments to commercialization of algae oil as is sometimes claimed. The major difficulty is actually in producing a large quantity of the biomass sustainably and at a sufficiently low cost (Cooney, Young, & Nagle, 2009; Uduman, Qi, Danquah, Forde, & Hoadley, 2010). A theoretical maximum productivity of algal crude oil has been estimated to be 354,000 $Lha^{-1}yr^{-1}$ and the best case estimates have ranged from 40,700 to 53,200 $Lha^{-1}yr^{-1}$, depending on geographic location (Weyer, Bush, Darzins, & Wilson, 2010).

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