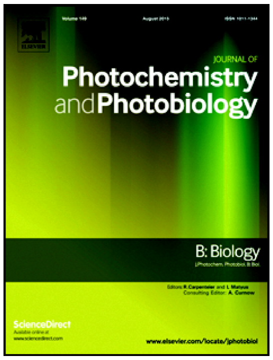

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Mixed microalgae consortia growth under higher concentration of CO_2 from unfiltered coal fired flue gas: Fatty acid profiling and biodiesel production

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Abstract

Biodiesel is produced by transesterification of fatty acid methyl esters (FAME) from oleaginous microalgae feedstock. Biodiesel fuel properties were studied and compared with biodiesel standards. Qualitative analysis of FAME was done while cultivating mixed microalgae consortia under three concentrations of coal fired flue gas (1%, 3.0% and 5.5% CO_2). Under 1% CO_2 concentration (flue gas), the FAME content were 280.3 µg/mL, whereas lipid content was 14.03 µg/mL/D (day). Both FAMEs and lipid contents were low at other CO_2 concentrations (3.0 and 5.5%). However, mixed consortia in the presence of phosphate buffer and flue gas (PB + FG) showed higher saturated fatty acids (SFA) (36.28%) and unsaturated fatty acids (UFA) (63.72%) versus 5.5% CO_2 concentration, which might be responsible for oxidative stability of biodiesel. Subsequently, higher cetane number (52) and low iodine value (136.3 gI_2/100g) biodiesel produced from mixed consortia (PB + FG) under 5.5% CO_2 along with 50 mM phosphate buffer were found in accordance with European (EN 14214) standard. Results revealed that phosphate buffer significantly enhanced the biodiesel quality, but reduce the FAME yield. This study intended to develop an integrated approach for significant improvement in biodiesel quality under surplus phosphorus by utilizing waste flue gas (as CO_2 source) using microalgae. The CO_2 sequestration from industrial flue gas not only reduced greenhouse gases, but may also ensure the sustainable and eco-benign production of biodiesel.

Keywords: FAME yield; fatty acid profile; biodiesel properties; lipid content; mixed microalgae consortia; coal fired flue gas

1. Introduction

World global energy demand has increased due to large industrialization and transportation which led to overdependence of human being on petroleum based fossil fuel [1]. The fossil fuel reserves are decreasing day by day and it is necessary to develop alternate renewable, sustainable and eco-friendly energy sources in nature [2]. Excessive utilization of non-renewable energy sources continuously emit greenhouse gases (GHG) particularly carbon dioxide (CO_2) (reach an alarming level of 400 ppm) into atmosphere [3], which ultimately cause climatic changes and global warming [4]. Clean and renewable energy sources seem as substitute of fossil fuel, as well as solution to the problems related to crude oil exhaustion in near future [5]. Biodiesel is a compatible alternative to fossil fuel and has numerous advantages over diesel fuel including renewable nature and low emission of gases [6]. Biodiesel can be produced from biological derived oil from animals, plants (microalgae) and microbes [7-10].

Microalgae has approximately 40% oil content and yield is many fold higher than other crops [11-14]. Microalgae can also accumulate carbohydrate polymers (starch like compounds) that could contribute to higher biofuel yield [15, 16]. Due to high lipid contents, tolerance to poor quality water, rapid growth rate, utilizing carbon dioxide from flue gas and other favorable qualities attract the interest in developing algal feedstock for biodiesel production [17, 18]. Unicellular photosynthetic microalgae can efficiently fix carbon dioxide as compare to terrestrial plants [19, 20]. Some microalgae strain stored part of this fixed carbon (C) in various lipid forms, including free fatty acids, triacylglycerides (TAGs), wax esters and sterols -up to 60% of dry cell material [21]. Microalgae have ability to utilize CO_2 as carbon source for biomass growth and simultaneously sequester greater amounts of CO_2 [22, 23]. During photosynthesis, microalgae can fix 1.8 kg of CO_2/1kg of biomass [4].

The process of biodiesel production requires initial lipid extraction from microalga biomass followed by conversion to FAMEs. FAME profile is highly dependent on growth conditions and specific producing organism [24]. Biodiesel comprises chain lengths range from C14 to C24 with varying degrees of unsaturation [7]. Biodiesel is generally produced through transesterification of triglycerides with methanol in order to obtain FAMEs. Triglycerides are one of major components of microalgal oil, free fatty acids (FFAs), other lipids with fatty acid ester linkage (saponifiable lipids), and non-saponifiable lipids are also part of the oil composition. Conversely, only free fatty acids (FFAs) and saponifiable lipids produce FAMEs in esterification and catalyzed transesterification, respectively [6, 25]. Biodiesel have comparatively high oxygen content, which ends up in more complete combustion than diesel resulting in lower particulate matter production as well as CO and hydrocarbon emissions [26]. Moreover, this technique can also be used for the sequestration of flue gases to avoid the environmental pollution [27-40]. The present study examined the potential of mixed culture (fresh water) microalgae for biodiesel production. The cultures were subjected to 1% (10% flue gas), 3% (30% flue gas) and 5.5% (50% flue gas) CO_2 from 4 MW (Mega Watt) coal fired boiler at Australian Country Choice. All samples were cultivated in outdoor photobioreactor under abundance of phosphate buffer while treating with 5.5% CO_2 in order to combat with low pH. The effect of flue gas was studied on the basis of growth, lipid content, fatty acid methyl esters (FAME) yield and biodiesel quality.

2. Material and methods

2.1. Culture Conditions

Water samples were collected from two different locations of Brisbane, Queensland, Australian. Composite sampling were done from University of Queensland (UoQ) (27°30'01.98"S 153°00'58.53"E) and Sunstate Cement Factory (27°23'.304"S 153°9'.638"E) as fresh water and storm water algae, respectively. Collected samples were treated with air (as control sample) and carbon dioxide (1% & 3% CO_2) from flue gas. Bold Basal Medium (BBM) was used for inoculation. The two samples (storm water species + UoQ Lake) were mixed together for treating 5.5% CO_2 (flue gas) with addition of 50 mM phosphate buffer in order to combat with low pH. The Mixed consortia with and without phosphate buffer (mixed consortia PB + FG and mixed consortia PB + air) treated with flue gas and air, respectively.

In this study, coal fired boilers smoke were used as flue gas sources from a beef processing industry Australian Country Choice (ACC) located at Cannon Hill, Brisbane, Australia. The fuel gas composed of SO_x (781.8 mg/Nm^3), CO_2 (11.24%), O_2 (8.26%), CO (388.8 mg/Nm^3), NO_x (423.9 mg/Nm^3) and particulates (0.4 mg/Nm^3). The raw flue gas was transferred from smoke stack to algal broth through 2 inch (2-3 m) long pipe. Conical polyethylene bags were used as bubble column photobioreactor for outdoor experiment with 25 L microalgal culture capacity, while 5 L served as the gas space (Figure 1). The bags sleeves were sealed at the bottom in a conical shape in order to prevent cell settling which were hung on steal rod supported by iron frame. This photobioreactor system contained twelve individual connecting bags. The flue gas produced from burning of coal in the flue gas generator is mixed with air to create different v/v mixtures of air-flue gas of desired concentration. The flue gas flow rate was adjustable through gas flow meter. Mixing in all bags was accomplished by sparging air introduced at the bottom through plastic tube at different rate to culture volume. Sunlight was used as natural illumination source corresponding to a photosynthetically active radiation (PAR) of 1650.3 μmol photon·m^{-2}·s^{-1} operated on a 12:12 hours light/dark cycle.

Two stock solutions of KH_2PO_4 and K_2HPO_4 were prepared of pH 7.5. Phosphate buffer found to be more efficient and economically viable (Table 1), which was selected in order to combat the impact of acidic gas component in flue gas. The phosphate concentration in BBM medium was increased by adding 50 mM phosphate buffer in order to avoid significant pH drop by adding CO_2-rich flue-gas.

2.2. Kinetic study

The experiments were carried out in bioreactors operating in intermittent regime, fed with 2.0 L of culture medium in the following experimental conditions: initial cell concentration of 0.1 g/L, isothermal reactor operating at a temperature of 30 ^{o}C, photon flux density of 150 lmol m^2/s^1, and continuous aeration of 1 VVM (Vessel volume per minute) with the injection of air contaminated with 15% carbon dioxide.

2.2.1. Data collection

After inoculation, samples were collected from all bags for the following analysis: pH, OD (optical density) and nutrient (nitrate and phosphate). Sampling was done on 1st, 4th, 8th, 12th and 16th (day) after every inoculation. Samples were collected after 8th day of cultivation (nutrient deficiency) and later samples were collected after 12th day and 16th day of cultivation (after the period of starvation). Samples for lipid analysis were centrifuged (HITACHI, himac CT 6E) immediately at 4000 rpm for 10 min and the pellets were preserved at -80^{o}C. Then all samples were freeze dried and store at -20^{o}C until further analyses.

2.3. FAME analyses

Samples for FAME analyses were collected after one week of starvation at ACC under different treatment (1%, 3% and 5.5% CO_2 (flue gas)). A total of 5 mL of microalgal culture was collected and centrifuged at 8,000 x *g* for 5 min. Biomass was collected and freeze-dried (Alpha 1-2 LD plus) for 30 min. Lyophilized algal biomass was hydrolyzed and methyl-esterified in 300 µL of a 2% (v/v) H_2SO_4 methanol solution for 2 h at 80 ^{o}C with 50 µg C21:0 (Heneicosanoic acid, Sigma-Aldrich) as an internal recovery standard. After the esterification step, 300 µL of hexane and 300 µL of 0.9 % (w/v) NaCl were added in the mixture. The mixture was vortexed for 20 s and centrifuged at 16,000 x *g* for 3 min to facilitate phase separation. Then, 150 µL of the hexane layer was injected into an Agilent 6890 gas chromatograph connected to a 5975 MSD mass spectrometer. The running conditions of Agilent's RTL DBWax method (Application note: 5988-5871EN) was set up [41]. Fatty acids were identified by comparing retention time with pre-run external standards (37 FAME Mix, Supelco, identified through the NIST library). A linear calibration curve based on the internal standard C22:4(n-6) (Adrenic acid) and the internal recovery standard C21:0 were used for fatty acid quantification. TFA content was determined as the sum of all fatty acids. TFA productivity (mg/L/day) was calculated as TFA multiplied by the biomass productivity.

2.4. Quantitative Analysis of Lipid (FAME)

All the experiments were carried out at optimum conditions as precisely reported elsewhere (Reddy et al. 2014). The extraction of crude extract was done by using the formulae shown in Eqs. 1-3. *In situ* transesterification have higher comparative biodiesel yield and is low cost versus conventional biodiesel production route and this method also controls wastes generation in biodiesel production process [42, 43].

$$\text{Crude extract yield} = (\text{Crude extract Wt/dry biomass Wt}) \times 100 \qquad (1)$$

$$\text{FAME in crude extract} = (\text{FAMEs Wt/Crude extract Wt}) \times 100 \qquad (2)$$

$$\text{FAME extracted} = (\text{FAMEs Avg. Wt crude extract/dry biomass Wt}) \times 100 \quad (3)$$

2.4.1. Biodiesel Production from algal oil

Physical characteristics of both triglycerides and fatty acids were determined by number of double bond, chain length and amount of each fatty ester components in both triglycerides and fatty acids [44]. The quality of biodiesel was determine by assessing the saponification value (SV), iodine value (IV), cetane number (CN) and degree of unsaturation (DU), which were determined using relations shown in Eqs. 4-7 [45, 46].

$$SV = \Sigma\ (560 \times F)/MW \quad (4)$$

$$IV = \Sigma\ (254 \times F \times D)/MW \quad (5)$$

$$CN = (46.3 + 5458/SV) - (0.225 \times IV) \quad (6)$$

$$DU = (MUFA;wt\%) + (2 \times PUFA;wt\%) \quad (7)$$

Where, F is the percentage of each fatty acid, MW is the molecular mass of fatty acid, D is the number of double bonds, MUFA is mono-unsaturated fatty acids and PUFA is polyunsaturated fatty acids in percentage weight [47]. Oxidative stress-tolerant microalgae strains are highly efficient for biofuel feedstock production on wastewater.

2.5. Statistics Analysis

All measurement were done in triplicates (n=3) and data was reported as the mean ± standard error using GraphPad Prism 6 statistical software.

3. Results and Discussion

3.1. FAME composition

Fatty acids were primarily produced by esterification and analyzed by gas chromatography (GC). FAME derived from lipids of mix algae subjected to 1%, 3% and 5.5% CO_2

concentrations, which was analyzed in order to assess the quality for biodiesel production. After fatty acid composition analysis, a comparison of algal lipid with respect to saturated, monounsaturated and polyunsaturated compounds was done under different CO_2 concentrations. Fatty acid composition of UoQ Lake, Storm W. and mixed consortia was determined after 15th day of cultivation under different conditions (Air & CO_2). Fatty acid mainly composed of palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) in the absence and presence of CO_2, respectively. The proportions of C16:0 under ambient air, 1%, 3% and 5.5% of CO_2 on 15th day were increased up to 22-31 µg/mg, 23 µg/mg, 27 µg/mg and 32 µg/mg, respectively (Fig. 2). The concentrations of oleic acid were 11-24 µg/mg (1% CO_2), 27 µg/mg (3% CO_2) and 1-12 µg/mg (5.5% CO_2) on 16th day of cultivation. It was found that high level of CO_2 (3%-5.5%) significantly decreased C18:1 concentration from 27 µg/mg (3% CO_2) to 1 µg/mg (5.5% CO_2) on 15th day of cultivation (Figs. 3 and 4). The amount of poly-unsaturated (C18:2 and C18:3) fatty acids were accounted in the range of 26-45 µg/mg, in presence of CO_2. Fatty acid profile of mixed culture cultivated under different CO_2 concentrations showed similar patterns on 15th day of cultivation regardless of the CO_2 concentration. Arachidic acid (20:0) and eicosenoic acid (20:1), C20:5 (n-3) (Eicosapentaenoic acid, EPA) and C22:6 (n-3) (Docosahexaenoic acid, DHA) concentrations were negligible. Moreover, the expected nonanoic (C9:0) and undecanoic (C11:0) were also not detected in any of the microalgal species. It had been reported that more saturated fatty acid could provide biodiesel with a higher CN, decreased NO_x emissions, a shorter ignition delay time and oxidative stability, but poor low-temperature properties (to gel at ambient temperature) [48, 49].

3.2. FAMEs content and productivity

Kao et al. (2014) recorded 98–113 mg/L algal fatty acid methyl ester (FAME) productivity from coke oven and hot-stove exhaust gases under autotrophic conditions. In current study, the main fatty acid components detected were; C16:0, C16:1, C16:2, C16:3, C16:4, C18:0, C18:1, C18:2, C18:3 and C18:4 in microalgal lipid extracted from UoQ-Lake and Storm W. The biodiesel production from microalgal biomass under flue gas (1%, 3% and 5.5% CO_2) was estimated in order to evaluate the effect of CO_2 level. The significant variation in fatty acid profile observed when mix culture cultivated under different CO_2 concentration, particularly C16:0 and C18:1 content. However, regardless of flue gas concentration (air or 1%, 3% and 5.5% CO_2), the content of C16:0+C18:1 (the suitable fatty acids for biodiesel production) of the microalgal biomass was in the range of 30.3-56.3%. The content of fatty acids; C16:0 and C18:0 in the Storm W. species cultivated using fired flue gas in ACC was significantly higher under 1% CO_2 aeration as compared to 5.5% CO_2 concentration (Table 2). Previously, decreasing trend in the lipid content of algal biomass was reported at higher CO_2 concentration [50].

Fatty acid composition generally includes structural unsaturated polar lipids along with neutral lipids (stored), mostly in form of Triacylglycerol (TAG). For biodiesel, the most significant fatty acids include saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFAs) comprising of C14-C18 components [51]. Amount of SFA increased as CO_2 concentration in flue gas increased. Mixed consortia (FG) furnished 36.3% SFA under 5.5% CO_2 (coal derived flue gas) concentration, however the amount of MUFA (30.6%) was high in storm water (FG) under 3% CO_2 (flue gas) concentration (Table 3). High level of saturated fatty acid (SFA) tends to enhance the stability of biodiesel as unsaturated fatty acid have poor oxidative stability [52]. Monounsaturated fatty acid (MUFA) (C16+C18) concentration was slightly higher than reported values, which might be beneficial for excellent oxidative stability and performance at low temperature [53, 54]. The amount of PUFA (62.4%) was higher in UoQ-Lake (FG) under 1% CO_2 (flue gas) concentration from ACC (Table 3). Contrarily, it was observed higher concentration of CO_2 enhanced the PUFA concentration in microalgal cells [55]. Fatty acid composition predicted biodiesel properties, which are in line with standard biodiesel quality produced from microalgae in current investigation [56, 57]. Microalgal lipid composed of saturated and unsaturated fatty acids with C12-C22 components [17]. It is reported that the most suitable feedstock for biodiesel production were enriched with C16-C18 fatty acids, comprising C16:0 (palmitic acid), C18:0 (stearic acid), C18:1 (oleic acid), C18:2 (linoleic acid), and C18:3 (linolenic acid) [54]. Oleic acid, linoleic acid and palmitic acid were considered as the key fatty acids in both heterotrophically and photoautotrophically grown cultures of *Chlorella zofingiensis* [58]. The percentage of C16 and C18 series contents of total FAME are mainly deliberated to evaluate quality of biodiesel [57]. Biodiesel acquired by transesterification of common fatty acid (C16-C18) has number of advantages over petroleum-derived diesel fuel. Biodiesel reduces emissions of CO, hydrocarbons, CO_2 and particle emissions [59].

3.3. Biodiesel properties

Lipid contents improved under stress conditions such as salt addition and nitrogen starvation, but significantly depress the cell growth. Air feeding also enhanced the lipid production by increasing both the intercellular lipid content and biomass productivity. This carries important implication for long term and stable microalgal culture processes, specifically with respect to industrial exhaust flue gases. Mixotrophic culture yield higher biomass over ATP as compare to autotrophic ones. Under optimal mixotrophic conditions, the excess energy in the cells, left out after utilization for cellular maintenance in dark, this could be directed towards energy storage either in form of lipid or starch [60]. Ideal microalgae candidate for biodiesel production need not only high triacylglycerol (TAG) and lipid production, but also appropriate fatty acid composition. Fatty acid composition has profound effect on biodiesel fuel property which was being analyzed in this study for UoQ-Lake & storm water under different CO_2 (flue gas) percentage at ACC. The percentage ratio of saturated fatty acid (SFA) and unsaturated fatty acid (UFA) revealed quality of biodiesel which helps to maintain high oxidative stability and low-temperature property [61]. The most suitable ratio of mixed consortia (FG) was observed in 5.5% CO_2 (flue gas) of 36.28% (SFA) to 63.72% (UFA), with a low percentage of PUFA (48.1%) (Table 3). MUFA were capable of giving the finest compromise between cold flow and oxidative stability [53, 54]. Nayak et al. [22] reported best suitable of SFA to UFA (54.3% to 45.7%) at 2.5% CO_2 concentration and with a low percentage of polyunsaturated fatty acids (16.9%) which may have potential to produce good quality biodiesel.

High degree of unsaturation can reduce the oxidation stability and cetane number, whereas it enhanced cold flow performance [53]. The degree of unsaturation (DU) of oil plays an important role in determination of saponification value, iodine value and cetane number of biodiesel [44]. The saponification value (SV) is inversely proportional to molecular weight of fatty acids. The main purpose of SV is to calculate cetane number of biodiesel. The Serbian and European biodiesel standards does not have saponification value as a restricted property of biodiesel oil [62]. UoQ-Lake (FG) showed higher saponification value (562.1) under 1% CO_2 (flue gas) cconcentration, whereas mixed consortia (PB + FG) had the lowest SV value of 149.2 under 5.5% CO_2 (flue gas) concentration at ACC. The iodine value (IV) calculated to determine the degree of unsaturation (MUFA & PUFA) of biodiesel, more the double bond in the fatty acid chain, higher the IV for that oil [54, 63]. Similarly IV values of UoQ-Lake (FG) were higher (513.59 $gI_2/100g$) when treated with 1% CO_2 (flue gas), whereas mixed consortia (PB + FG) displayed lowest IV (136.3 $gI_2/100g$) under 5.5% CO_2 (flue gas) concentration at ACC (Table 3). Higher IV of the biodiesel may cause polymerization of glycerides and deposition of lubricant in the engine [45].

After evaluating the saponification value and IV, cetane number of biodiesel was calculated. Cetane number (CN) is a dimensionless descriptor linked to fuel ignition quality in the diesel engine [54]. Higher cetane value is indicator of better combustion, less knocking, low nitrous oxide (NO_x) emission and easier startup of engine [49, 62, 63]. Diesel fuel with higher amount of saturated and monounsaturated FAMEs have high value of CN. Cetane number of mixed consortia (PB + FG) was 52 when treated with 5.5% CO_2 (flue gas) concentration, whereas all other samples showed extremely low CN. The CN value increased with increasing FAME saturation. The minimum CN value of standard ASTMD6751, European (EN 14214) and Australian standard and National Petroleum Agency (ANP255) standard are 47, 51 and 45, respectively [45]. The degree of unsaturation (DU) is a crucial parameter to determine IV and CN of the biodiesel [44]. The DU value (139.1) was higher of biodiesel from UoQ-Lake (FG) at 1% CO_2 (flue gas) concentration. The higher PUFA value (more than one double bond) had adverse effect on oxidative stability of biodiesel because carbon reactive sites are susceptible for free radical attack. On the other hand, long chain SFA and MUFA are suitable FAME for biodiesel as they improve oxidative stability without affecting the cold flow properties of biodiesel [49, 52, 64, 65]. According to European standards EN14214, for an ideal biodiesel the percentages of linolenic acid (C18:3) and stearidonic acid (C18:4) must be under 12% and 1%, respectively [66, 67]. The linolenic acid values were agreement with standard values for biodiesel under 1% & 3% CO_2 concentrations, whereas under 5.5% CO_2 concentration this value was beyond and stearidonic acid value was also within the recommended limit at low CO_2 concentration (Table 2).

3.4. Effect of phosphate buffer on FAME yield

With addition of coal fired flue gas under autotrophic batch culture, UoQ-Lake (FG) showed higher FAME content and productivity of 280.3 µg/mL and 14.03 µg/mL/D, respectively, under 1% CO_2 (flue gas) concentration (Table 2). Previously, the algal FAME productivity was reported in the range of 98-113 mg/L from coke-oven and hot-stove exhaust gases under autotrophic conditions [68]. In this study, adsorption of heavy metal by microalgae from flue gas decreased the pH, and inhibits growth which was overwhelmed by addition of 50 mM phosphate buffer. However, the FAME content and yield of mixed consortia (PB + FG) was reduced to 74.4 µg/mL and 4.653 µg/mL/D, respectively, under high 5.5% CO_2 concentration (flue gas) (Table 2). Previous studies also highlighted that the only phosphorous supply was enough for algal growth enhancement [69]. During cultivation stage of microalgae, phosphorous from liquid medium transferred and stored as Poly-P in algal cells [70]. In previous studies, this phenomenon of phosphorous accumulation have also been reported [71, 72]. Subsequently, poly-P is a polymer of orthophosphate linked by phosphoanhydride bonds as it is thermodynamically equal to the ‘‘energy-rich’’ phosphate in adenosine triphosphate (ATP), poly-P can serve as an energy source. Poly-P can be categorized into acid-soluble and acid insoluble poly-P. Acid-insoluble Poly-P is generally deposited in cells and consumed when the amount of extracellular phosphorus found to be insufficient for microalgae growth. Acid-soluble poly-P is involved in protein production and DNA metabolism [73, 74]. Chu et al. [70] found that phosphate assimilated by microalgal cells under nitrogen deficiency to metabolically synthesize compounds consisting of protein (particularly enzymes for lipid production), RNA, DNA, ATP and intermediate metabolic products. So far, these provide adequate material and a source of energy for lipid accumulation in algal cells under nitrogen deficient conditions. Apart from the unfavorable conditions caused by a reduction in a pH in the growth medium, flue gas has been shown to contain a large number of heavy metals [75]. The reduced biomass productivity and FAME yield might be due to heavy metal stress (present in flue gas) which causes damage to chloroplast and disturbance of the endoplasmic reticulum required for CO_2 fixation and lipid biosynthesis [76, 77]. Additionally, the highest FAME productivity were attained under adequate phosphorus conditions [78]. In the present study, as the concentration of total phosphate (TP) increased, the content (%) of polyunsaturated fatty acid was also increased. In addition, with higher concentration of TP, the content of PUFA was also higher, but the content of SFA decreased followed by MUFA. Therefore, the excessive addition of TP decreased the FAME content. While treating algae samples, 5.5% CO_2 found to produce more suitable (saturated) fatty acid according to fuel quality for the production of biodiesel. The production of biodiesel from microalgae is an eco-benign techniques and also biodiesel offers low emission GHG, so far, adaptation of this techniques can reduce the use of conventional fuels as well as combat the issue of environmental pollution [8, 38-40, 79-88].

4. Conclusions

The biomass oil content, productivity and lipid content found to be the adequate criteria for estimating the potential of microalgae for biodiesel production. With addition of coal fired flue gas under autotrophic batch culture, UoQ-Lake (FG) showed FAME content and productivity under 1% CO_2 (flue gas) concentration. However, the FAME yield and content of mixed consortia in the presence of phosphate buffer (PB) and flue gas (FG) was reduced at higher (5.5%) CO_2 concentration (flue gas). Phosphorus addition also reduces the FAME productivity at higher concentration. The linolenic acid and stearidonic acid values were within the recommended limits 1% & 3% CO_2 concentration, whereas at 5.5% (CO_2 concentration) were higher than the recommended values. Regardless of flue gas concentration (air or 1%, 3% and 5.5% CO_2), the content of C16:0+C18:1 (fatty acids) were in the range of 30.3-56.3%. Results revealed that CO_2 sequestration from industrial flue gas not only reduced greenhouse gases, but may also ensure the sustainable and eco-benign production of biodiesel, which need to adopt strategies for higher biomass yield as well as enhancement of free fatty acids, triacylglycerol, and fatty acid ethyl ester (biodiesel) and development of such technology might be a viable technology for the production of biodiesel from microalgae as well as CO_2 sequestration.

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Table 1: Concentration of phosphate buffer vs pH under different concentration (100% CO_2)

Concentration (mM)	**Initial pH**	**pH after 5 mins (100% CO_2)**
10	8.05	5.59
20	8.01	5.80
30	8.01	5.94
40	8.03	6.03
50	8.00	6.10
100	7.94	6.33

Table 2: Total FAME production (µg/mg), lipid content (% DW), biomass productivity (g/L/d) and lipid productivity (µg/mL/d) under different CO_2 concentration

CO_2 (%)	**Samples**	**Total FAME (µg/mg)**	**Lipid Content (% DW)**	**Biomass productivity (g/L/d)**	**Lipid Production (µg/mL/d)**
1% CO_2	UoQ-Lake (A)	165.6	16.56%	0.047458	7.85983
	UoQ-Lake (FG)	280.3	28.03%	0.050042	14.02823
	Storm W. (A)	248.2	24.82%	0.054875	13.61769
	Storm W. (FG)	249.5	24.95%	0.0545	13.5964
3% CO_2	UoQ-Lake (A)	127.7	12.77%	0.045689	5.836428
	UoQ-Lake (FG)	168.6	16.86%	0.042444	7.15753
	Storm W. (A)	146.1	14.61%	0.037733	5.512115
	Storm W. (FG)	210.0	21.00%	0.049867	10.47087
5.5% CO_2	Mixed C. (A)	103.8	10.38%	0.05975	6.204545
	Mixed C. (FG)	100.0	10.00%	0.049542	4.952281
	Mixed C. + PB (A)	70.2	7.02%	0.052583	3.690661
	Mixed C. + PB (FG)	74.4	7.44%	0.046167	3.435559

Key; UoQ-Lake (A) = University of Queensland water, A = air, FG = flue gas, Storm W. = storm water, DW = dry weight, Mixed C. + PB (FG) = Mixed consortia phosphate buffer (flue gas), d = day

Table 3: Percentage of SFA, MUFA, PUFA and values of biodiesel quality parameters

CO_2 (%)	Samples	SFA %	MUFA %	PUFA %	SV	IV (gI2/100g)	CN	DU
1% CO_2	**UoQ-Lake (A)**	25.1640	26.4588	48.3772	332.0952	303.4206	-5.5346	123.214
	UoQ-Lake (FG)	23.2533	14.3598	62.38692	562.1253	513.5887	-59.5479	139.134
	Storm W. (A)	26.2083	28.1375	45.65421	497.6119	454.6458	-45.0269	119.446
	Storm W. (FG)	26.2714	27.6083	46.12031	500.2525	457.058	-45.6276	119.848
3% CO_2	**UoQ-Lake (A)**	28.6273	29.4265	41.94616	256.1524	234.0350	14.94974	113.319
	UoQ-Lake (FG)	30.5780	29.6699	39.75217	338.146	308.9489	-7.07256	109.174
	Storm W. (A)	32.7779	30.0244	37.19767	292.9241	267.6317	4.71569	104.420
	Storm W. (FG)	30.3542	30.643	39.00247	421.0505	384.695	-27.2936	108.648
5.5% CO_2	**Mixed C. (A)**	34.8312	24.1392	41.02961	208.2256	190.2464	29.7065	106.199
	Mixed C. (FG)	36.2825	15.6482	48.06925	200.4457	183.1383	32.3232	111.787
	Mixed C.+PB (A)	31.5301	7.0589	61.41101	140.7402	128.588	56.1483	129.881
	Mixed C.+PB (FG)	33.4486	6.6101	59.9412	149.2213	136.3369	52.2007	126.493

Key; UoQ-Lake (A) = University of Queensland water, A = air, FG = flue gas, Storm W. = storm water, Mixed C = Mixed consortia, PB (FG) = phosphate buffer (flue gas), SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, IV = iodine value, CN = cetane number and DU = degree of unsaturation

Highlights

- Mixed microalgal culture was grown in nutrient rich medium in outdoor photobioreactor
- Photobioreactors connected to unfiltered smoke stack-coal-fired boiler
- FAME was quantified under three concentrations of flue gas
- Phosphate significantly enhance biodiesel quality but reduce FAME productivity
- CO_2 sequestration and biodiesel production seems good option through microalgae growth

Graphics Abstract

Figure 1

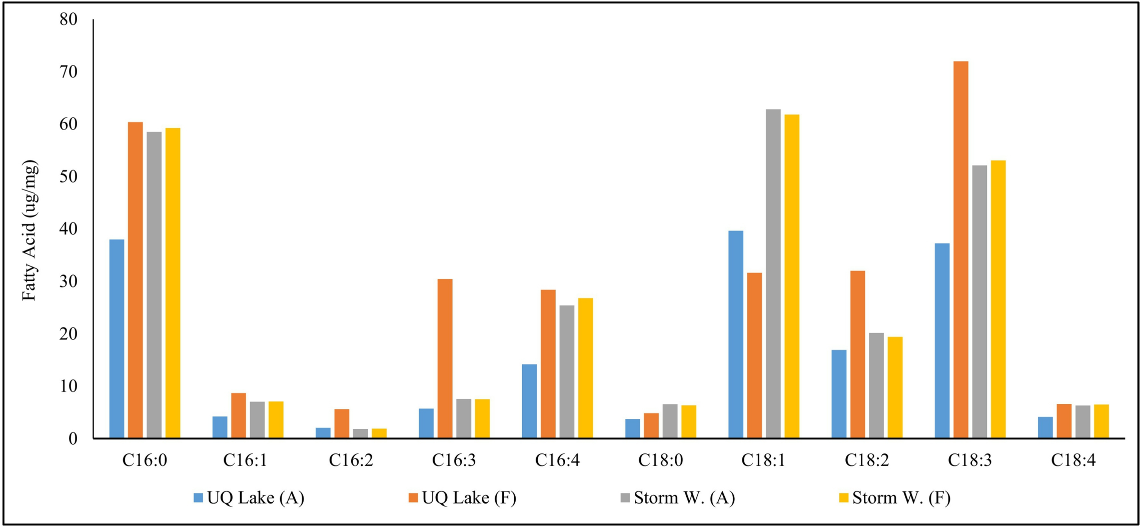
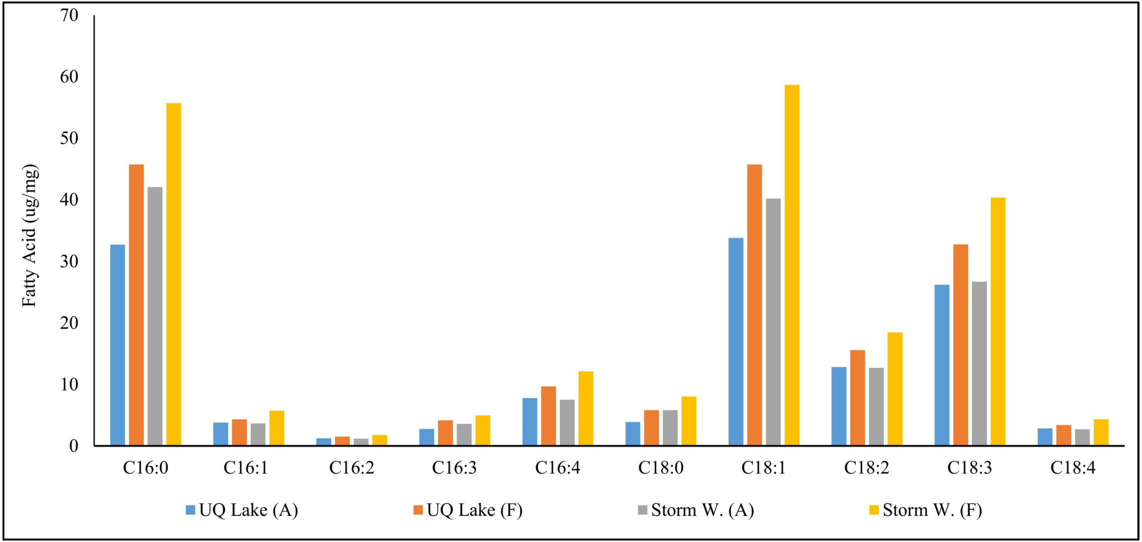

Fatty Acid (ug/mg)
0
10
20
30
40
50
60
70
80
C16:0
C16:1
C16:2
C16:3
C16:4
C18:0
C18:1
C18:2
C18:3
C18:4
UQ Lake (A)
UQ Lake (F)
Storm W. (A)
Storm W. (F)

Figure 2



Fatty Acid (ug/mg)
70
60
50
40
30
20
10
0
C16:0
C16:1
C16:2
C16:3
C16:4
C18:0
C18:1
C18:2
C18:3
C18:4
UQ Lake (A)
UQ Lake (F)
Storm W. (A)
Storm W. (F)

Figure 3

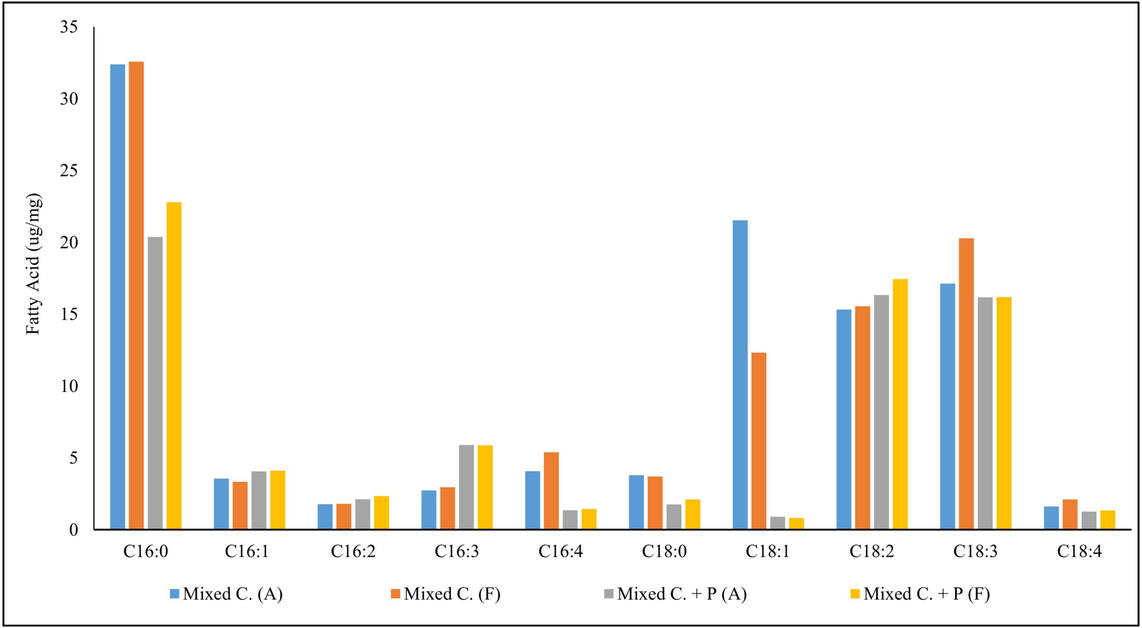

35
30
25
20
15
10
5
0
Fatty Acid (ug/mg)
C16:0
C16:1
C16:2
C16:3
C16:4
C18:0
C18:1
C18:2
C18:3
C18:4
Mixed C. (A)
Mixed C. (F)
Mixed C. + P (A)
Mixed C. + P (F)

Figure 4