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ANAEROBIC CO-CULTIVATION OF MULTI-ALGAL SPECIES WITH OIL PALM EMPTY FRUIT BUNCHES FOR MILL EFFLUENT TREATMENT AND BIOMETHANE PRODUCTION

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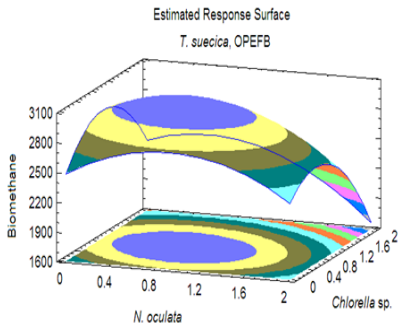
Graphical abstract



Abstract

This study investigated the optimization of anaerobic co-cultivation of multi-algal species with Oil Palm Empty Fruit Bunches (OPEFB) for Palm Oil Mill Effluent (POME) treatment and biomethane production. The highest removal of COD (95-98%), BOD (90-98%), TOC (81-86%) and TN (78-80%) were achieved after 7 days anaerobic treatment with the presence of microalgae. The highest biomethane (4,651.9 mL CH_4/L POME/day) and the specific biogas production rate (0.124 m^3/kg COD/day) with CO_2 (2,265.9mL CO_2/L POME/day) were achieved by co-cultivating N. oculata and Chlorella sp. (each at 1 mL/mL POME) with OPEFB (0.12 g/mL POME). The combination of N. oculata (2 mL/mL POME) with T. suecica or Chlorella sp. (each at 1 mL/mL POME), and OPEFB (0.12 g/mL POME) obtained high biomethane (4,018.9 mL CH_4/L POME /day) but lower biogas (0.097 m^3/kg COD/day) and CO_2 (2,079.5mL CO_2/L POME/day). Generally, low OPEFB and having all the three strains or increasing the level of any (2 mL/mL POME) especially T. suecica, could lower biomethane (870-953 mL CH_4/L POME/day) and CO_2 (803-854mL CO_2/L POME/day), with the biogas around 0.08-0.09 m^3/kg COD/day. The optimum conditions were predicted by Response Surface Methodology and the multiple coefficients of determination, r^2, of 86% suggests good agreement between experimental and predicted values.

Keywords: Anaerobic digestion, biomethane, bioremediation, microalgae, mill effluent, oil palm empty fruit bunch

1.0 INTRODUCTION

The use of fossil fuels as energy sources is unsustainable due to limited resources and accumulation of greenhouse gases (GHGs) in the environment. The combustion of petrol, natural gas or coal has been identified as the major contributors of CO_2 release which eventually causes global warming (Brennan & Owende 2010). Compounding the problem is the depletion of the fossil fuel reserve more than it can meet the demand, a direct consequence of population growth and rapid industrialization (IPCC2007). Algal biofuel has been suggested to be the only renewable energy source that could meet the worldwide demand (Schenk *et al*. 2008). Microalgae could produce biofuel, and biogas

through anaerobic digestion (Abdullah *et al*. 2015). It is cost-effective for CO_2 sequestration and wastewater treatment where algae assimilate nutrients and through photosynthesis, produce dissolved oxygen that is immediately available to bacteria for the oxidization of wastes (Shilton *et al*. 2008).

Co-utilization of microalgae and oil palm wastes such as OPEFBs and POME could resolve both the issues of hazardous wastewater being discharged without treatment into rivers or lakes, and capturing value-added products such as methane as renewable energy and biomass utilization. In Malaysia, the annual production of OPEFB is 19.8 million tonnes on a wet basis which provides huge resources for conversion of biomass solid wastes into value-added products for varied applications (Nazir *et al*. 2013). POME is produced from sterilization of fresh oil palm fruit bunches, clarification of palm oil and effluent from hydrocyclone operations. The production of POME is nearly three times higher than crude palm oil (Wu *et al*. 2009) and is considered as one of the most polluting agro-industrial effluent due to its high COD and BOD. It is however a rich source of organic compounds such as proteins, carbohydrates and lipids along with nitrogenous compounds and minerals (Wu *et al*. 2007; Chan *et al*. 2011). POME and OPEFB therefore can be vital substrates for bioprocessing which may result in a net positive energy or economic balance.

At present, 85% of POME treatment is based on anaerobic and facultative pond system, followed by aerobic treatment in an open tank digester with extended aeration to meet the required discharge standards (Wu *et al*. 2010). Other recent methods such as coagulation (Teh *et al*. 2014), vermitechnology (Lim *et al*. 2014), and adsorption (Mohammed & Chong 2014) have been proposed but their efficiencies in large scale POME treatment require more in-depth investigations. Microalgal anaerobic treatment of POME is an economical route for alternative energy production whilst remediating the environment and reutilizing the wastes (Ahmad *et al*. 2014a, b, c). Use of filtered POME for microalgal cultivation could even enhance the lipid content in microalgae (Shah *et al*. 2014a, b).

The aim of this study was to investigate the effects of anaerobic multi-algal co-cultivation with OPEFB and pond sludge for biomethane production and POME treatment. The biomass production, lipid content and fatty acid profile of Chlorella sp., Nannochloropsis oculata, and Tetraselmis suecica were first evaluated, followed by determining the efficiency of each species on POME treatment. Finally, the effects of combination of different microalgal strains and OPEFB addition on biomethane production at fixed sludge inocula were optimized by Response Surface Methodology.

2.0 EXPERIMENTAL

2.1 Sample Preparation

POME and OPEFB were collected from FELCRA Nasaruddin Oil Palm Mill in Bota Kanan, Perak, Malaysia. The POME was stored in the chilled room at 4°C to avoid microbial degradation activity and composition change. OPEFB was dehydrated in an oven at 105°C for about 6 h and then crushed by using electric blender to form practical sizes of less than 4 mm, and stored in an airtight plastic bottle at room temperature.

2.2 Microalgal Strain and Culture Conditions

Fresh water strain Chlorella sp. and marine strains Nannochloropsis oculata, and Tetraselmis suecica were kindly provided by Dr. Mohd Fariduddin Othman from the Fisheries Research Institute (FRI), Pulau Sayak, Kedah, Malaysia. N. oculata and T. suecica cells were cultured in sterilized sea water and Chlorella sp. were cultured in sterilized freshwater, enriched with Conway medium (MacLachlan 1979). Media in culture flasks were autoclaved at 121°C, for 15 min and all transfer of media and culture took place in aseptic environment in a laminar flow cabinet.

Cultures were sub-cultured on eighteen days basis and placed on an orbital shaker at 80 rpm and 28±2°C. The standard conditions for algal culture were 100 ml culture in 250 ml Erlenmayer flask, with a salinity of 30 ppt and an initial pH 8, under 24 h illumination from fluorescence white light (Phillips) of 90 µmol photons $m^{-2}s^{-1}$ intensity. For cell growth kinetics study, cells were inoculated into 1 L flask at 10% (v/v) inoculum density.

2.3 Batch Anaerobic Experiment

The CHALLENGE AER-200 Aerobic and Anaerobic Respirometer system was used for anaerobic digestion experiment. The system consists of eight 500 ml serum bottles (biological reaction vessels), a stirring base for sample mixing, a water bath for controlling the temperature, a cell base containing eight flow measuring cells, an interface module, and a computer.

For anaerobic experiment, the reaction vessels and related parts were cleaned using deionized water and rinsed thoroughly before autoclaving at 121°C for 15 min. The following procedures were carried out under non-sterile environment to establish the results as it would be applied in the field. Bottles were filled with 50 mL POME, 3 mL/mL POME sludge, OPEFB (0, 0.06, 0.12 g/mL POME), and N. oculata, Chlorella sp. and T. suecica (0, 1, 2 mL/mL) as multi-cultures inoculated at initial density of 60.9 x 10^6 cells/mL, 35.9 x 10^6 cells/mL, 13 x 10^6 cells/mL, respectively. pH of the sample was adjusted to 7.5 by using NaOH or HCL. Each serum bottle was purged by using nitrogen gas to remove oxygen, and then the screw cap with butyl rubber septum was quickly put on to ensure anaerobic

environment. The reaction vessels were then placed on MS8-300 magnetic stirring base water bath with the stirring rate at 300 rpm, and the temperature set at 48°C (Ahmad *et al*. 2014a, b, c). The experiments were run for hydraulic retention time (HRT) of 3 and 7 days.

For total biogas and biomethane collection, the test bottles were vented by briefly inserting a clean 20-gage needle through the septum. The venting prevents gas buildup in the bottle. Reaction vessels were attached to the tubing connected to a flow measuring cell for analysis of total gas production and its production rate. For biogas composition analysis, plastic gas bags (SKC, Japan) were connected to each test bottle. The Challenge Environmental System (CES) program was started when the temperature of water bath was stable and no bubble was detected in the flow measuring cell. The cell counters and timer from the control system of the computer program were reset and the data acquisition was initiated.

2.4 Analytical Methods

2.4.1 Chemical Analyses of POME and OPEFB

Biological oxygen demand (BOD_5) was analyzed using Standard Methods by HACH (HACH, USA). COD measurement was carried out using spectrophotometer DR 5000, according to 8000-Reactor Digestion Methods (HACH). Total Organic Carbon (TOC) and Total Nitrogen (TN) were analyzed by using TOC Analyzer (TOC-$V_{CSH\ SHIMADZU}$, Japan). pH of POME was measured by using Mettler Toledo-320 pH probe. The elemental analysis of OPEFB was performed by using CHNS-932 analyser (APHA 2005).

2.4.2 Cell Density and Dry Weight

Cell density was monitored by using haemocytometer (Hirschmann) and a microscope (Meiji-Techno). For fresh and dry weight determination, 100 mL sample was harvested and filtered through pre-weighed GF/F filters (934-AH, Whatman). The filtered cells were washed with distilled water and dried at 80°C in an oven until constant weight and cooled in a desiccator before weighing. The equation used is as follows:

$$\text{Dry weight} = (DW_A - DW_C) / V \quad (1)$$

where, DW_A is the average dry weight of filtered algal cells (g), DW_C is average dry weight of filter (g) and V is culture volume (L).

2.4.3 Lipid Extraction

Lipid content analysis was conducted based on Bligh and Dyer (1959).

$$\text{Lipid Content Analysis (\%)} = [(W_2-W_1)/W_d] \times 100 \quad (2)$$

where, W_1 is previously weighed glass vial, W_2 is weight of vial along with lipid content and W_d is the dry weight of algae.

2.4.4 Biogas Composition

Biogas level was determined using Gas Chromatography (Shimadzu, GC- 2010): - Column GS-Q (J&W Scientific), to analyze the main composition of biogas- CH_4, H_2 and CO_2.

2.4.5 Experimental Design and Statistical Analyses

For Response surface methodology (RSM), the Box–Behnken design was used for the optimization of factors and the second order model that incorporates curvature was developed to approximate the responses. The responses include specific biogas production rate (m^3/kg COD/day) (y_1) and biomethane rate (mL CH_4/L POME/day) (y_2). Three levels were evaluated: - minimum (x_1=0, x_2=0), central point (x_1= 1, x_2= 0.06) and maximum (x_1= 2, x_2= 0.12) values, for microalgae (Chlorella sp., N. oculata, T. suecica, mL/mL POME) and OPEFB (g/mL POME), respectively. The specific biogas production rate was calculated as follows (Saleh *et al*. 2012):

$$\text{Specific biogas production rate } (m^3/\text{kg COD/day}) = \frac{\text{Total volume of biogas produced } (m^3)}{\text{COD load (kg)} \times \text{Time (day)}} \quad (3)$$

3.0 RESULTS AND DISCUSSION

3.1 Cell Growth Kinetics

The highest cell density and dry weight were achieved with N. oculata at 62.2×10^6 cells/mL and 0.65 g/L, respectively, with maximum biomass formation rate (Table 1) of 0.113±0.002 g/L/d, t_d of 4.98±0.21 day and μ_{max} of 0.14±0.02/d. Chlorella sp. and T. suecica obtained cell density 1.5-5-fold lower although the dry weight was comparable at 0.53-0.69 g/L. The maximum biomass formation rate of N. oculata and T. suecica in this study were comparable to P. lutheri culture at 5–300 L scale with biomass reported at 0.45 g/L (in 250 mL), μ_{max} at 0.14/day (in 30 L) and t_d at 4.95 days (in 30 L tank) (Shah *et al*. 2014b).

The reported maximum cell concentration of 65×10^6cell/mL has been reported for Nannochloropsis, but with 2-fold higher μ_{max} of 0.339/d (Wahidin *et al*. 2013). The lower cell density of T. suecica could possibly be due to its bigger cell size (5-10µm length × 14µm width) (Hansen *et al*. 1996) as compared to N. oculata and Chlorella sp. (2-4 µm in diameter) (Toepel *et al*. 2005). As cells are autocatalytic, the difference is also due to initial cell density where N. oculata and Chlorella sp. recorded initial cell density of 1.3-4.2 × 10^6 cell/mL as compared to 1.03 × 10^6 cell/mL for T. suecica.

3.2 Lipid Contents and Fatty Acids Analyses

The microalgal cells from logarithmic, early stationary and stationary phase were extracted for lipid content. Fresh water Chlorella sp. recorded lipid content of

14.7±0.4, 22.7±0.6, and 30.4±1.1% for respective phases, but had reduced total lipid content of 27.8% on day eighteenth. Both marine N. oculata and T. suecica showed lipid contents of 27.5±1.1% and 23.7±2.2%, respectively. These are comparable to those reported for C. pyrenoidosa with 26% lipid at 0.05 g/L KNO_3 (Nigam *et al*. 2011). Our previous studies suggest that both μ_{max} (0.21/d and 0.20/d) and lipid contents (39.1 ± 0.73% and 27.0 ± 0.61%), respectively, of N. oculata and T. suecica are much enhanced when cultivated in 10% POME in sea water (Shah *et al*. 2014a). Other reported lipid content of N. oculata include 14.9% when grown at room temperature under continuous photon flux density of 70.0 μE/m^2/s (Attilio *et al*. 2009) and T. suecica at 19-32% of total dry weight in photo-bag bioreactors (Navid 2013).

For N. oculata (results not shown), the total saturated fatty acids (TSFA) (53.8%), monounsaturated fatty acids (MUFA) (15.1%), and polyunsaturated fatty acids (PUFA) (12.7%) showed major components comprising of pentadecanoic acid, C15:0 (5.3±0.47%); palmitic acid, C16:0 (36.2±1.89%); palmitoleic acid, C16:1 (9.96±0.46%); oleic acid, C18:1 (5.1±0.32%); and eicosanoic acid, C20:0 (4.9±0.77%). Chlorella sp. showed lower TSFA (45.2%) but higher MUFA (26.9%) and PUFA (28.9%) than N. oculata. The major components identified from the total lipids of Chlorella sp. were palmitic acid, C16:0 (31.3±1.22%); palmitoleic acid, C16:1 (23.4±0.69%); oleic acid, C18:1 (15.2±1.2%); and docosahexaenoic acid (DHA), C22:6 (4.99±0.56%). For T. suecica, TSFA (47.9%) were comparable to Chlorella sp. but with substantially lower MUFA (7.3%) and PUFA (9.1%). The fatty acid compositions of T. suecica were pentadecanoic acid, C15:0 (4.70±0.24%); palmitic acid, C16:0 (20.15±1.39%); pentadecanoic acid, C17:0 (10.7±1.4%), linoleic acid, C18:2 (5.5±0.12%) and DHA, C22:6 (3.80±0.65%).

The main fatty acids present in the lipids of the three microalgal species sp. studied are normally short-chain fatty acids (C14–C18) and this lipid profile is comparable to that reported earlier (Huang *et al*. 2013). The variations of relative fatty acid profile and other species of the genus Nannochloropsis can be attributed to the natural diversity in biological samples and on the growth conditions and the original state in which the samples are obtained (Pal *et al*. 2011; Khozin-Goldberg & Boussiba 2011). The difference in fatty acids composition may have direct bearing on the intended use of microalgae for biodiesel or biogas production.

3.3 Anaerobic Treatment of POME with and without Microalgae

The characteristics of raw POME (results not shown) suggest that the pH was 3.5-5 with COD of 65,772 mg/L, BOD of 24,117 mg/L, TOC of 4,746 mg/L, TN of 385 mg/L, TSS of 6,8367 mg/L and Oil and grease of 3,546 mg/L, indicating high amount of organic matter. These are comparable to previously reported values (Anon 2010; Norhayati *et al*. 2011; Chan *et al*. 2012). The elemental composition of carbon, hydrogen, nitrogen and sulphur of OPEFB (CHNS) were 40.1±0.708, 5.3±0.489, 1.4±0.047 and 0.29±0.028%, respectively. These were comparable to previously reported values of 45.5, 6.1, 1.7 and 0.14%, respectively (Saleh *et al*. 2012). The C:N ratio of 28.6:1 was within the 20-30:1 ratio suggested for the presence of nutrients and minerals required for bacterial growth and good for anaerobic digestion for biogas production (Parkin and Owen 1986).

As shown in Table 2, the final pH of anaerobically treated sample after 3 and 7 days HRT were reduced to 5.6-5.7 from the earlier pH 7.5 adjustment before treatment. The pH drop can be attributed to the accumulation of high volatile fatty acid (VFA) concentration and ammonia. This could influence anaerobic digestion by affecting acetate-utilizing methanogenic archaea, hydrogen-utilizing methanogens, and syntrophic bacteria, which subsequently may inhibit anaerobic bacteria and reduce methanogenesis (Torres & Loréns 2008). The highest removal of COD (95-98%), BOD (90-98%), TOC (80-86%) and TN (80%) were achieved after 7 days anaerobic treatment in the presence of microalgae. On day 3, except for TOC and TN, and BOD with T. suecica treatment, the BOD and COD removal efficiency were already generally higher than without microalgae. Addition of microalgae therefore significantly improved the POME treatment and this can be further optimized by improving organic loading rate, reactor design and conditions.

Our earlier results with filtered POME composition in sea water at different levels (1, 5, 10, 15 and 20%) used as an alternative medium, obtain enhanced cell growth and lipid accumulation. At 10% POME, N. oculata and T. suecica had maximum specific growth rate (0.21/d and 0.20/d) and lipid content (39% and 27%), respectively, after 16 days of flask cultivation. The algal treatment of POME/Seawater media also achieved high removal of COD (93.6-95%), BOD (96-97%), TOC (71-75%), TN (78.8-90.8%) and oil and grease (92-94.9%) (Shah *et al*. 2012a).

Table 1 Kinetics of cell growth and lipid production of N. oculata, Chlorella sp. and T. suecica in basic growth conditions

Microalgal strains	Maximum biomass formation rate, X'_{max} (g/L.d)	Maximum specific growth rate, μ_{max} (/d)	Doubling time, t_d (day)	Maximum Lipid Content (%)
N. oculata	0.113±0.002	0.14±0.02	4.98±0.21	27.5±1.1
Chlorella sp.	0.110±0.001	0.13±0.01	5.05±0.12	30.4±1.1
T. suecica	0.111±0.002	0.14±0.01	4.85±0.04	23.7±2.2

Table 2 Anaerobic treatment of POME with and without microalgae

Parameters	Removal efficiency (%)							
	Day 3				Day 7			
	Without algae	*N. oculata*	*Chlorella* sp.	*T. suecica*	Without algae	*N. oculata*	*Chlorella* sp.	*T. suecica*
pH	6±0.81	6.3±0.12	7.2±0.16	7.2±0.16	5.7±0.08	5.6±0.08	6.8±0.08	7.1±0.08
BOD	78±0.81	90±1.25	86±0.81	67±1.63	87±0.81	98±0.47	95±0.81	90±0.81
COD	73±1.63	83±0.81	86±0.81	87±1.24	87±2.44	97±1.24	98±0.82	95±1.63
TOC	62±1.63	63±1.25	68±1.24	67±0.81	70±2.45	80±1.63	86±0.47	80±1.25
TN	69±0.47	73±1.25	59±1.24	73±2.45	70±0.47	80±1.24	78±2.05	80±1.25

3.4 Specific Biogas and Biomethane Production Rate

Table 3 has been rearranged according to groups for ease of comparison based on OPEFB composition. The actual experimental runs were designed in random to reduce statistical biasness. The highest biomethane rate (4,651.9 mL CH_4/L POME/day) and the specific biogas production rate (0.124 m^3/kg COD/day) were achieved with co-cultivation of N. oculata and Chlorella sp. (each at 1 mL/mL POME) and OPEFB (0.12 g/mL POME) as shown in Run 6. With increasing N. oculata (2 mL/mL POME) but maintaining Chlorella sp. and T. suecica (each at 1 mL/mL POME) at high OPEFB (0.12 g/mL POME) (Run 18), the biomethane rate remained high (4,018.9 mL CH_4/L POME/day) although the specific biogas production rate was slightly lower (0.097 m^3/kg COD/day). Reasonably high biomethane rate (3,500-3,600 mL CH_4/L POME/day) can be achieved even without OPEFB by doubling the N. oculata level but maintaining the 1:1 ratio of Chlorella sp. to T. suecica (Run 4) and also by having high OPEFB (0.12 g/mL POME) even in the total absence of microalgae (Run 28) or just the absence of N. oculata but at 1:1 ratio of Chlorella sp. to T. suecica (Run 22). This suggests that it may not be necessary to increase the level of microalgae other than N. oculata, and if all the three strains present, doubling the level of either Chlorella or T. suecica may have deleterious effects. We have reported high biomethane rate achieved by co-cultivating OPEEFB (0.12 g/mL POME) with 2 mL/mL POME mono-algal culture of Chlorella (biomethane of 5276 mL/L POME/d, specific biogas of 0.129 m^3/kg COD/d); N. oculata (biomethane of 4812 mL/L POME/d, specific biogas of 0.126 m^3/kg COD/d) and T. suecica (biomethane of 3900.8 mL/L POME/d, specific biogas of 0.116 m^3/kg COD/d) (Ahmad *et al*. 2014a, b, c). Reducing the amount of OPEFB (0.06 g/mL POME) but maintaining high mono-algal culture either N. oculata or Chlorella sp. (at 2 mL/mL POME), the biomethane rate (4,443-4,524 mL CH_4/L POME/d) and the specific biogas rate (0.120-0.122 m^3/kg COD/d) remained high (Ahmad *et al*. 2014a, b, c). In general, reducing OPEFB and in the presence of specifically T. suecica in the multi-algal culture, could lower the biomethane rate to around 3000 mL CH_4/L POME/d or below.

The effects of multi-algal species and OPEFB showed positive influence on specific biogas production and biomethane rate (Figure 1). OPEFB ($p< 0.002$-0.005) and the combined multi-algal species had the most significant positive effects on specific biogas production rate, while N. oculata, Chlorella sp. and T. suecica ($p< 0.0001$-0.003) were most significant on biomethane production. Although the models showed r^2 of 80-82 % suggesting good prediction, the effects of all other combined factors showed non-significant effects. Based on ANOVA, the model represents the experimental values well within the

defined experimental range. The multiple coefficients of determination, r^2 for multialgal species co-cultivation were found to be 86%, suggesting good agreement between experimental and predicted values. The optimum value calculated for specific biogas production rate was 0.121 m^3/kg COD/day and the maximum biomethane rate of 4,423.3 CH_4/L POME/day can be obtained at optimum co-cultivation of multi-algal N. oculata and Chlorella sp. (each at 1 mL/mL POME) and OPEFB (0.12 g/mL POME).

Table 3 Box-Behnken design and responses of multi-algal species and OPEFB

Run	Independent Variables				Specific biogas production rate (m^3/kg COD/day)	Biomethane (mL CH_4 L/POME/day)	CO_2 (mL CO_2 L/POME/day)
	N. oculata (mL mL^{-1} POME)	*Chlorella* sp. (mL mL^{-1} POME)	*T. suecica* (mL/mL POME)	OPEFB (g mL^{-1} POME)	Experimental Value	Experimental Value	Experimental Value
Group A							
28	0	0	0	0.12	0.125	3,539.0	3,534.0
6	1	1	0	0.12	0.124	4,651.9	2,265.9
3	1	0	1	0.12	0.101	2,765.2	2,036.6
22	0	1	1	0.12	0.104	3,541.6	1,556.5
Group B							
17	1	0	0	0.06	0.095	3,030.6	1,730.0
10	0	1	0	0.06	0.121	3,165.0	1,883.4
8	0	0	1	0.06	0.099	2,853.6	1,550.6
23	1	1	1	0.06	0.108	3,132.2	1,853.6
9	1	1	1	0.06	0.111	3,072.2	2,272.0
Group C							
27	0	0	0	0	0.104	2,540.0	2,532.0
7	1	1	0	0	0.099	2,579.8	2,301.5
13	1	0	1	0	0.099	2,778.9	1,475.1
19	0	1	1	0	0.099	2,353.0	1,939.8
Group D							
18	2	1	1	0.12	0.097	4,018.9	2,079.5
2	1	2	1	0.12	0.099	2,787.5	2,272.4
26	1	1	2	0.12	0.108	1,064.8	914.38
Group E							
24	2	1	0	0.06	0.124	1,224.8	873.7
14	2	0	1	0.06	0.121	2,123.6	1,643.4
1	0	2	1	0.06	0.104	2,224.8	1,654.3
15	1	2	0	0.06	0.107	2,787.5	1,753.9
5	1	0	2	0.06	0.106	1,229.4	1,153.9
16	0	1	2	0.06	0.089	1,026.7	934.6
Group F							
12	2	2	1	0.06	0.077	952.74	864.8
11	2	1	2	0.06	0.081	943.74	853.8
21	1	2	2	0.06	0.082	952.74	863.8
Group G							
4	2	1	1	0	0.108	3,601.3	1,543.3
20	1	2	1	0	0.096	2,424.0	1,984.9
25	1	1	2	0	0.088	870.5	803.4

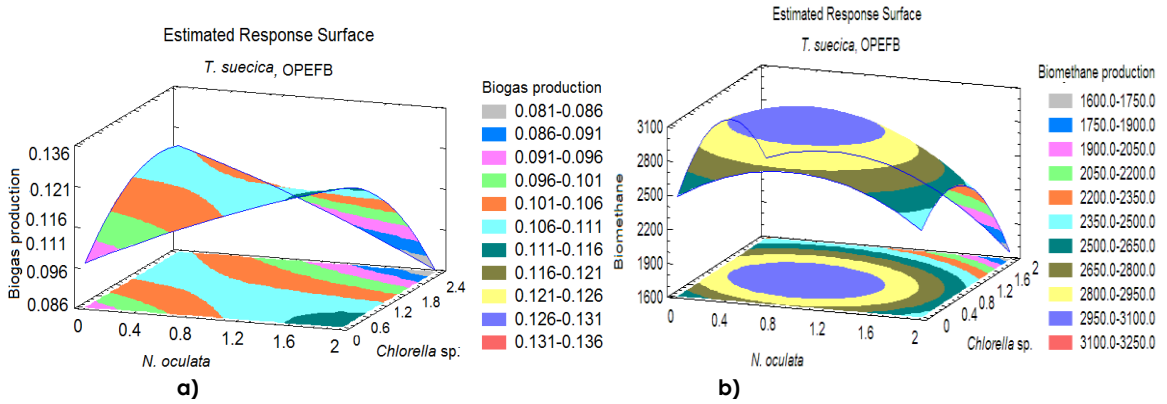


Figure 1 Estimated response surface plot of a) Specific biogas production rate, b) Biomethane production for anaerobic co-digestion of multi-algal species and OPEFB (at constant T. suecica and OPEFB)

3.5 Important Factors

Specific strain of microalgae and OPEFB co-cultivation with POME, at the correct ratio of POME and sludge inocula will maximize biomethane production. Low concentration or absence of microalgae and OPEFB reduced the specific biogas production rate and biomethane production. The addition of microalgae and OPEFB therefore not only create a balance of nutrients for facultative anaerobic bacteria, but also enhances the buffering capacity of the digester. The higher the lipid content of the cell, the higher will be the potential for biomethane yield, as these can serve as nutrients for bacteria, and microalgae may work in tandem with bacteria to breakdown the OPEFB and POME. The lipid content of N. oculata, Chlorella and T. suecica in this study was found to be 27.5, 30.4 and 23.7%, respectively. These may explain the high biomethane yield with the combination of N. oculata and Chlorella, but it does not explain as to why an increased amount of single species or any two species in the cultivation reduced the biomethane yield. The only plausible explanation is the crowding or antagonistic effect which may affect the micro-environment within the digester, and defeats its intended purpose of supporting bacterial growth for substrate conversion.

Our study deals with slightly above mesophilic conditions (48°C) resulting in higher biogas production (0.124-0.125 m^3/kg COD/d) after 3 days HRT. Algal biomass containing lipid between 2 to 22 % produces methane yield ranging from 0.47 to 0.80 m^3 CH_4 VS/kg in anaerobic digestion (Li *et al*. 2011). Several studies have looked at co-digestion of microalgae with sludge under thermophilic and mesophilic conditions (Sreekrishnan *et al*. 2004). The digestion of algal biomass under thermophilic conditions has reportedly resulted in higher gas production than mesophilic conditions, whereas the variations in solids retention times (SRTs) between 11 and 30 days do not affect gas production (Lau *et al*. 2009). The major drawback is the energy to maintain thermophilic condition. The biogas productivity can be increased by mixing the proteinaceous algal biomass with carbon rich waste such as primary sewage sludge which increases the C/N ratio of digester feeding (Chua *et al*. 2010). Co-cultivation is also beneficial because potential toxic NH_4 is diluted which allows improved loading rate and enhanced biogas yield (Sosnowski *et al*. 2013). With excess VFAs, the acidogens grow rapidly and produce more volatile acids to further reduce the pH. In such conditions, methanogenesis cannot occur as it requires pH around 6.5-7.5. The methanogens may not be able to keep up with this change and degrade acids as fast as they are generated, and these may lead to low methane production (Poh & Chong 2009). Optimization needs to be carried out to look at the possible effects of the conditions and reactor configurations in combination with multi-algal species and the substrates.

4.0 CONCLUSION

The highest removal of COD (95-98%), BOD (90-98%), (81-86%) and TN (78-80%) were achieved for 7 days anaerobic POME treatment with the presence of microalgae. The highest biomethane rate (4,651.9 mL CH_4/L POME/day) and the specific biogas production rate (0.124 m^3/kg COD/day) were achieved by co-cultivating N. oculata and Chlorella sp. (each at 1 mL/mL POME) with OPEFB (0.12 g/mL POME). Increased amount of microalgae with OPEFB addition reduced the biomethane and the specific biogas production rate. Without microalgae even at high OPEFB, the biomethane level was lowered although the specific biogas production rate may remain constant. Co-cultivation of multi-algal species therefore enhanced POME treatment and increased the biomethane production depending on species and the amount introduced.

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