British Biotechnology Journal
4(3): 305-316, 2014

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Use of Palm Oil Mill Effluent as Medium for Cultivation of *Chlorella sorokiniana*

Charles Ogugua Nwuche[1,2*], Doris Chidimma Ekpo[1], Chijioke Nwoye Eze[1], Hideki Aoyagi[2] and James Chukwuma Ogbonna[1,2]

[1]Department of Microbiology, Faculty of Biological Sciences, University of Nigeria, Nsukka 40001, Nigeria.
[2]Department of Bioscience and Bioengineering, Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan.

Authors' contributions

This work was carried out in collaboration between all authors. Author CON was the project leader and was responsible for the project plan, experimental design, data analyses and writing the manuscript. Authors DCE and CNE collected the samples and contributed to the data analyses. Authors HA and JCO are senior specialists who provided resources for the execution of the study and edited the manuscript. All authors read and approved the final manuscript.

Original Research Article

Received 16th September 2013
Accepted 28th October 2013
Published 19th February 2014

ABSTRACT

Aims: Palm oil mill effluent (POME) erodes the principal biophysical characteristic of both soil and water when discharged untreated but could be exploited as medium for microalgae cultivation due to its vast mineral contents.
Place and Duration of study: POME samples were collected from a local palm oil processing mill at Nsukka, Enugu State, Nigeria. A part of the study was done at the Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan while the rest at the University of Nigeria, Nsukka between March and September, 2012.
Methodology: *Chlorella sorokiniana* C212 was grown in several Batches (A-D) of POME supplemented with urea (60 mg/L) before subjecting to different sterilization protocols. Cultivation was conducted in shaker flasks at 150 rpm, 1 vvm, 3000 lux and pH 7.0±0.2.
Results: The filter sterilized Batch (B) promoted the highest (1070±30 mg/L) dry cell weight (DCW), lipid (156±12 mg/g-cell) and chlorophyll (1.59±0.11 mg/g-cell) contents while chemical oxygen demand (COD) decreased by 45±08%. The autoclaved medium

**Corresponding author: Email: charles.nwuche@unn.edu.ng;*

(Batch A) gave the least DCW (310±20 mg/L), lipid production (40±05 mg/g-cell) and chlorophyll content (0.58±0.02 mg/g-cell) while COD reduced by 20±04%. The highest COD decrease (70±05%) was achieved in the unsterilized Batch (D). Batch B was most positively affected by dilution because at 75% concentration, DCW increased to 1360±30 mg/L, lipid contents to 174±10 mg/g-cell, chlorophyll to 1.87±0.14 mg/g-cell the while COD declined by 63±03%.
Conclusions: POME has potential for use in microalgae cultivation with significant saving in treatment costs.

Keywords: Palm oil mill effluent; microalgae; cultivation; chlorella sorokiniana; sterilization; chlorophyll; chemical oxygen demand; dilution.

1. INTRODUCTION

Palm oil mill effluent (POME) has received considerable attention in recent years due to its capacity for considerable environmental damage when discharged untreated into inland waterways and cultivated lawns. POME is produced during palm oil production and is a viscous, acidic brown liquid that is predominantly organic with a highly unpleasant odour. In Nigeria, POME is discharged into the environment in its raw form by small scale operators which constitute the major proportion of palm oil refiners. During palm oil extraction from the fresh fruits, about 50% of the water results in POME. It is estimated that for 1 tonne of crude palm oil that is produced, 5 to7.5 tonnes of water ends up as POME [1]. According to the produce department of the Ministry of Agriculture, Kogi State, Nigeria, approximately 3,600 metric tonnes of palm oil is produced annually [2].

Currently interests are growing in the use of POME as feedstock for the production of valuable products and biochemicals because the presence of high concentrations of carbohydrate, proteins, nitrogenous compounds, lipids and minerals in POME [3] make it an excellent raw material for bioconversion by biotechnological means. Presently, one area of application is the cultivation of microalgae which can be processed into biofuels to support the world's continually growing energy demand and curtail the ecological dangers associated with the use of fossil fuel as well as provide a ready environmentally friendly alternative to fossil based energy in the reality of depleting global reserves.

Microalgae are minute photosynthetic organisms that are cultivated on a large scale because they supply many active biological products which are beneficial to man [4]. Apart from biofuel, microalgae can be processed into dietary proteins [5], vitamins, carotenoids, antioxidants [6], fatty acids, enzymes, polymers, toxins and sterols [7]. They are typically found in freshwater and marine system where they have sufficient access to light, water, carbon dioxide and inorganic salts [8]. The major components of media used for photoautotrophic cultivation of microalage include inorganic nitrogen sources and both macro and micro elements. POME contains vast range of mineral elements and can be exploited for the cultivation of microalgae.

This presents an important way of further treating POME before discharge into the environment. Thus as the biomass increase, the organic load of the effluent decreases due to metabolism and uptake of the POME components by the algae. Although a few authors have reported on the use of POME as medium for the cultivation of microalgae, none hitherto has considered the impact of sterilization patterns on biomass and metabolite

production nor has the influence of the culture on the kinetics of COD of the effluent been adequately highlighted. In this study, the potential of using POME as medium for cultivation of Chlorella sorokiniana was investigated.

2. MATERIALS AND METHODS

2.1 Palm Oil Mill Effluent (POME)

Fresh raw POME used as medium for cultivation of Chlorella sorokiniana C 212 was obtained from a local palm oil processing mill at Nsukka, Enugu State of Nigeria and stored at 4ºC until use. POME for use in experiment was first passed through a double-layered muslin cloth (previously sterilized by autoclaving) to remove oil, plant fibres, broken shells and kernels before filtration through Whatman No.1 filter paper. The filtrate was supplemented with 60 mg/L of urea and then sub-divided into several batches. Batch A was sterilised by autoclaving at 121ºC for 15 min. Batch B was treated by passing through a Millex GV 0.22 µm filters (Millipore SA, France). Batch C contained POME mixed with chloramphenicol at a final concentration of 50µg/ml while Batch D was used without any additional treatment. Batch E was the conventional BG 11 medium and served as control. It was inoculated after sterilizing for 15 min at 121ºC. The treatment which gave the highest cell growth was used to determine the effect of POME concentrations on the kinetic parameters. Identical culture conditions were implemented in a separate batch of POME diluted with distilled water to 25, 50 and 75% concentrations. Except for the BG 11 medium, the optical density of all the POME batches and dilutions were determined against a distilled water blank before inoculation in order to evaluate the degree of light penetration in the media. The media were assessed at 430 and 662 nm which is the absorption maxima for chlorophyll *a* as well as at 453 and 642 for chlorophyll *b.*

2.2 Microalgae and Culture Conditions

Chlorella sorokiniana C212 was initially propagated in a 500 ml Erlenmeyer flask containing BG 11 medium at 29±2ºC. The cultivation condition was maintained at an aeration rate of 1 vvm and 150 rpm for 7 days. Continuous illumination at 3000 lux was provided by two fluorescent lamps arranged in parallel on both sides at an equidistance of 10 cm from the flask. The cells were harvested by centrifugation at 3000 rpm for 10 min and the supernatant discarded. The cells (pellets) were washed twice with distilled water before they were inoculated into the different POME batches at 10% inoculum concentration equivalent to $4.0x10^8$ cells/ml. Approximately 90 ml of each of the different POME treatments were aseptically introduced into 500 ml Erlenmeyer flasks and cultivated as previously described for 21 days. All glass-wares were previously washed, air-dried and sterilized in a hot air-oven at 160ºC for 2 h before use. The chemicals and reagents used were reagent grade and purchased from Wako Pure Chemical Ind., Osaka, Japan.

2.3 Analytical Methods

2.3.1 Cell growth

The growth and concentration of the microalgae during batch cultivation was measured spectrophotometrically at 680 nm and converted to biomass by a calibration curve corresponding to the POME treatment or medium used. Specific growth rate (µ) was calculated using equation from the exponential growth:

$$\text{Specific Growth Rate } (\mu) = \ln (x_2) - \ln (x_1)/t_2 - t_1 \quad (1)$$

Where: x_2 and x_1 are the concentrations of the biomass at the end and beginning of each batch run, while t_2 and t_1 is the duration of the run.

2.3.2 Dry cell weight

At the end of cultivation, biomass was harvested by centrifugation at 4000 rpm for 15 min and the supernatant discarded. The pellets were washed with distilled water and freeze-dried at -52ºC under vacuum. The dry biomass obtained after freeze-drying was stored in airtight containers at 20ºC while cell weight was determined gravimetrically in mg/L.

2.3.3 Chlorophyll content

Chlorophyll *a* content was determined spectrophotometrically at 665 nm wavelength in methanol (90%) extracts of the dried biomass according to a modification of Lee and Shen [9] equation:

$$\text{Chlorophyll } a \text{ (g/g-cell)} = 13.43 \times OD_{665} \quad (2)$$

2.3.4 Lipid content

The lipid content of the microalgal biomass was estimated by a modified method of Bligh and Dyer [10]. The total lipids were extracted by a mixture of chloroform-methanol (2:1 v/v) and the biomass at the ratio of 1:1. The mixture was allowed to stand for 1 h before they were transferred into a separatory funnel, shaken for 5 min and allowed to settle. The lipid fraction was then separated and the solvent evaporated in a rotary evaporator. The weight of the crude lipid was then obtained gravimetrically using a digital balance.

2.3.5 POME characteristics

The pH of the different POME treatment and concentrations were determined according to Standard Methods [11]. Chemical Oxygen Demand (COD), Ammonia-Nitrogen (NH_3-N), Total Nitrogen (N) and Nitrates ($^-NO_3$) were measured by the Hach's Spectrophotometic method (DR/4000, Hach Co. Ltd. Tokyo). Carbohydrate content was analyzed by the phenol–sulphuric acid method [12] while soluble protein was measured by the Bradford method [13].

2.4 Statistical Analysis

The data is presented at the means of triplicate experiments ± standard deviations. Where appropriate, results were statistically analyzed by the completely randomized one-factor analysis of variance (ANOVA) using the statistical software IBM-SPSS (Statistical Product and Service Solutions) 16.0.2 (2008 Version).

3. RESULTS

3.1 Effect of POME Treatment on Biomass Production

Biomass production varied in the different POME batches according to the sterilization protocol applied Table 1. In the batch sterilized by autoclaving (Batch A), the DCW of the algae was 310±20 mg/L while in Batch C (sterilized by chloramphenicol (50µg/ml) treatment), it was 640±15 mg/L. Algal cultivation was conducted in Batch D without initial medium sterilization but the total DCW produced at the end of cultivation was 458±27 mg/L. The Table 1 reveals that the POME treatment most beneficial to biomass production (1070±30 mg/L) was the Batch (B) sterilized by membrane filtration. However, the overall result indicate that biomass obtained from the Batch E (2945±90 mg/L) was about three times the DCW from Batch B and several folds higher than the values obtained from the other Batches. When the algae was cultivated in different concentrations Table 2 of a separate batch of the filter sterilized POME, results show that the DCW of biomass (1360±30 mg/L) harvested from the 75% POME was higher than those obtained from the other concentrations. Statistical analysis showed that the differences were statistically significant (P=0.05). At lower concentrations (50 and 25%), the DCW were 874±40 and 560±25 mg/L respectively. The biomass productivity pattern reflects the trend described for the DCW obtained from the different media. The productivity rate was highest (210.36±6.43 mg/L/d) in cultures cultivated in the conventional medium but among the POME treatments, the rate obtained from the Batch B (76.40±2.15 mg/L/d) was the highest while Batch A (22.14±1.42 mg/L/d), the least. In Batches C and D, biomass grew at the rates of 45.71±1.07 and 32.71±1.93 mg/L/d respectively.

3.2 Effect of POME Treatment on Chlorophyll Production

The chlorophyll content of biomass harvested from the different media shows Table 1 that cultures grown in the control medium (BG 11) had the highest chlorophyll content (2.98±0.12 mg/g-cell). Among the POME batches, the concentration of the pigment was statistically higher (P=0.05) in biomass harvested from Batch B (1.59±0.11 mg/g-cell) than those from batch C (1.07±0.15 mg/g-cell). In the other Batches, chlorophyll extracted from Batch a (0.58±0.02 mg/g-cell) was higher than values (0.41±0.07 mg/g-cell) obtained from those grown in Batch D. The chlorophyll content of biomass from the different POME dilutions Table 2 also showed that pigments from the 75% POME (1.87±0.14 mg/g-cell) was higher than those obtained from the other dilutions. In the 100% POME, for instance, the chlorophyll content was 1.59±0.11 mg/g-cell but at lower concentrations, 0.85±0.18 and 0.48±0.07 mg/g-cell of chlorophyll was obtained from the 50 and 25% POME respectively. In terms of productivity, cultures grown in the BG 11 medium (Batch E) had the highest rate (0.213±0.01 mg/L/d) followed by those from Batches B (0.114±0.01 mg/L/d) and C (0.076±0.01 mg/L/d). Chlorophyll productivity in cultures grown in Batch a (0.041±0.00 mg/L/d) and Batch D (0.029±0.01 mg/L/d) were not different (P>0.05) statistically. At lower POME concentrations, cultures grown in the 75% POME had values (0.134±0.01 mg/L/d) significantly higher than those obtained from other Batches Table 1 and dilutions Table 2. The productivity of cultures from the 50% POME (0.061±0.01 mg/L/d) was also higher (P=0.05) than the rates obtained from the 25% POME (0.034±0.01 mg/L/d).

Table 1. Kinetic parameters of *C. sorokiniana* C212 grown in BG 11 medium and different POME treatments

POME	Cell Dry Weight (mg/L)	Chlorophyll Content (mg/g-cell)	Lipid Content (mg/g-cell)	Biomass Productivity (mg/L/d)	Specific Growth Rate (μ)	Chlorophyll Productivity (mg/L/d)	Lipid Productivity (mg/L/d)	% COD Reduction (mg/L)
Batch A	310±20*	0.58±0.02*	40±05*	22.14±1.42*	0.4098*	0.041±0.00*	2.86±0.36*	20±04
Batch B	1070±0*	1.59±0.11*	156±12*	76.43±2.15*	0.4982*	0.114±0.01*	11.1±0.86*	45±08
Batch C	640±15*	1.07±0.15*	94±25*	45.71±1.07*	0.4615*	0.076±0.01*	6.7±1.79*	58±03
Batch D	458±27*	0.41±0.07*	52±07*	32.71±1.93*	0.4376*	0.029±0.01*	3.71±0.5*	70±05
Batch E∞ (BG 11)	2945±90	2.98±0.12	386±18	210.36±6.43	0.5706	0.213±0.01	27.6±1.29	**

**Asterisk indicates significant difference from the control treatment (N = 5, P = 0.05, mean SE from ANOVA); **COD of the BG 11 medium was not determined; ∞ Batch E is Control Treatment*

Table 2. Kinetic parameters of *C. sorokiniana* C212 grown in different concentrations of the filter-sterilized (Batch B) POME

POME Conc. (%)	Cell Dry Weight (mg/L)	Chlorophyll Content (mg/g-cell)	Lipid Content (mg/g-cell)	Biomass Productivity (mg/L/d)	Specific Growth Rate (μ)	Chlorophyll Productivity (mg/L/d)	Lipid Productivity (mg/L/d)	% COD Reduction (mg/L)
75	1360±30*	1.87±0.14*	174±10*	97.14±2.14*	0.5153*	0.134±0.01*	12.4±0.72*	63 ±03*
50	874±40*	0.85±0.18*	120±10*	55.30±7.12*	0.4838*	0.061±0.01*	8.57±0.72*	45±04
25	560±25*	0.48±0.07*	90±08*	40.00±1.80*	0.4520*	0.034±0.01*	6.42±0.57*	28±04*

** Asterisk indicate significant difference from Batch B values (N = 4, P = 0.05, mean SE from ANOVA)*

3.3 Effect of POME Treatment on Lipid Production

The lipid content of biomass harvested from the control medium was 386±18 mg/g-cell. This value was higher than results obtained from the other POME treatments Table 1. The lipid profile of biomass from Batch B (156±12 mg/g-cell) and Batch C (94±25 mg/g-cell) was different (*P*=0.05) statistically. As earlier indicated, 75% POME was found to be the best medium for lipid accumulation (174±10 mg/g-cell) among cultures grown in the different POME dilutions. Lipid content of the 50% POME was 120±10 mg/g-cell but it reduced to 90±08 mg/g-cell in the 25% POME. Lipid productivity followed the same trend as the earlier results. Among the different batches, Batch B stimulated the highest lipid productivity (11.1±0.86 mg/L/d) while Batch A, the least (2.86±0.36 mg/L/d). At lower POME concentrations, 75% POME promoted the highest lipid productivity (12.4±0.72 mg/L/d) while the 25% had the least (6.42±0.57 mg/L/d). Biomass cultivated in the conventional medium had the highest rate (27.6±1.29 mg/L/d) of lipid accumulation.

3.4 Effect of POME Treatment on COD Changes

COD changes in the different POME batches show Table 1 that the least rate (20±04%) of COD reduction was achieved in the POME treatment sterilized by autoclaving (Batch A) while the highest rate (70±05%) occurred in the unsterilized POME medium. The rate of COD reduction in the batches sterilized by filtration (B) and antibiotic treatment (C) were 45±08 mg/L and 58±03 mg/L respectively. Changes in COD profiles of the different POME concentrations were in line with the trend observed in other results. The rate of COD reduction in the 75% POME (63±03%) was higher than the rates found in the other concentrations. At 50 and 25% POME, COD declined by 45±04% and 28±04% respectively.

4. DISCUSSION

The POME batch treated by membrane filtration (Batch B) promoted the highest biomass (1070±30 mg/L) DCW among the different batches tested. This is thought to be due to the concentration of light available to the microalgae during cultivation. At all the wavelengths and among all the batches tested Table 4, the optical density (OD) of Batch B was found to be the lowest indicating that light penetration was maximum compared to Batch A (autoclaved) which had the least light availability as shown by the high OD values. The OD indicates the degree of clarity of the media. The latter correlate to amount of transmitted light and directly affects biomass production by photosynthesis. At the two maximum absorption regions (430 and 662 nm) for chlorophyll *a* for instance, the OD of Batch A were 1.476 and 0.897 while values for the filter sterilized POME (B) were 0.168 and 0.072. This evidently implies that light was more available to the microalgae in Batch B than in the other treatments. Due principally to this advantage, biomass increased remarkably to a final concentration (1070±30 mg/L) which was significantly (*P*=0.05) higher than values obtained from the other batches.

Microalgae are sunlight-driven organisms capable of converting carbon dioxide to biomass and other high value products [4,14]. One other factor that is noteworthy is the chemistry of the medium following filter-sterilization. Millipore preserves the physical, chemical and biological quality of materials and is known to be effective against both bacterial and fungal contamination [15]. This implies that the various components of the POME were retained after the filtration and available to the cultures in their original and natural forms. The effect of urea was also found to be more prominent in this medium than in the other batches.

Nitrogen has been identified as a primary nutrient for the growth of several microalgae [16]. Urea was selected as a nitrogen supplement in this study based on preliminary evaluation Fig. 1 which ascertained that urea was better (1.47 g/L) than the other inorganic salts in stimulating higher biomass production by the algae. This observation was in agreement with Su et al. [17]. In the present study, the addition of nitrogen salt was justified due to the relatively low content of the element in POME. Table 3 shows that the concentration of nitrate in the raw POME was only 40±05 mg/L while other nitrogen (total and ammoniacal) was 90±04 and 109±07 mg/L respectively. At higher POME dilutions, the concentration of the element further diminished. Urea enhanced the growth of the algae by promoting maximum optical density and shorter lag phases in line with the findings of Hadiyanto and Nur [18]. The modifying effect of urea on pH of the effluent is also worthy of note. The initial pH (4.02±0.07) increased to 7.0±0.2 on addition of urea indicating potential savings in cost as no further chemical was applied for pH adjustment. The final pH was favourable to the growth and metabolism of the algae and meets the discharge criterion for industrial effluents.

Table 3. Characteristics of the different POME concentrations used in the study

Parameters (mg/L)	100%**	75%	50%	25%
pH*	3.97±0.03	3.95±0.05	3.98±0.04	4.04±0.05
Protein	44.2±8.0	35.1±6.5	28.2±4.0	15±5.0
COD***	8200±00	6000±00	3940±00	1790±00
Total Carbohydrate	584±20	490±25	354±15	264±10
Ammonia Nitrogen (NH_3-N)	109±07	75±04	56±03	25±02
Total Nitrogen (N)	90±04	62±02	46±05	21±04
Nitrates ($^-NO_3$)	40±05	27±03	20±04	09±04

** Not measured in mg/L, ** Data in column indicate characteristic ± standard deviation of the different POME batches. *** Values represent the COD of the filter-sterilized (Batch B) POME. COD for batches A, C and D was 9160±120*

Table 4. Optical density of the different POME batches and concentrations before inoculation at the absorption maxima for chlorophylls *a* and *b*

POME	Chlorophyll *a*		Chlorophyll *b*	
	430 (nm)	662 (nm)	453 (nm)	642 (nm)
Batch A	1.476	0.897	1.378	0.930
Batch B	0.168*	0.072*	0.129*	0.075*
75%	0.143*	0.062*	0.113*	0.071*
50%	0.099*	0.051*	0.080*	0.053*
25%	0.074*	0.046*	0.063*	0.047*
Batch C	1.083*	0.550*	0.987*	0.580*
Batch D	1.083*	0.550*	0.987*	0.580*

** Asterisk indicates significant difference from the Batch A values (N = 7, P = 0.05, mean SE from ANOVA)*

Biomass from Batch C was 640±15 mg/L. Although the antibiotic may be effective against susceptible bacterial populations in the medium but the presence of other organisms, including the resistant flora, fungi and their metabolites could be inhibitory to the growth of the algae despite the presence of adequate light and culture conditions. Perhaps, the most likely occurrence of interference by other microbial groups could be seen in cultures grown in the unsterilized POME (458±27 mg/L). The least DCW was found in the POME batch sterilized by autoclaving Table 1. The heat treatment changed the colour of the medium to

blue-black. The exact reason for the change in colour is not clear but it is thought that some chemical reactions involving tannic acid and other components occurred during exposure to the high temperature of steam sterilization. The presence of tannic acid and its involvement in the darkening of POME has been confirmed [19]. The darkening led to shading which limited light penetration in the medium and negatively affected chlorophyll formation.

Chlorophyll traps the radiant energy that powers photosynthesis [20]. Its concentration is also known to correlate with photosynthetic activity and directly influences the production of biomass and the accumulation of target products [21]. This is in line with results obtained in the present study. Findings from Table 1 show that the high chlorophyll content (1.59±0.11 mg/g-cell) of the cells cultivated in Batch B was directly related to the increase in biomass (1070±30 mg/L) and lipid content (156±12 mg/g-cell) of the microalgae. The reverse was equally true in the case of the other batches with lower chlorophyll concentrations. Cultures grown in the conventional (BG11) medium had a chlorophyll content of 2.98±0.12 mg/g-cell which positively affected the overall outcomes of the DCW (2945±90 mg/L) and lipid content (386±09 mg/g-cell). Mineral salt media are usually not as complicated as industrial waste waters. POME contains numerous organic and inorganic compounds [22] as well as toxic components which occur at concentrations that are highly variable and often subject to a wide range of influences [23]. The BG11 is an adequate and nutritionally balanced medium that supplies the right concentrations of essential nutrients and minerals needed for the best possible growth of freshwater algae [24]. This explains the positive impact of the medium on the various kinetic components of the culture as indicated by the high specific growth rate (0.5706).

Evaluation of DCW from the different POME concentrations Table 2 showed that the 75% POME stimulated higher biomass production (1360±30 mg/L) than the 100% POME (1070±30 mg/L) as well as the lower POME concentrations. This contrasts previous study by Hadiyanto et al. [25] which reported higher biomass productivity in the 50% POME. The wide differences in POME composition [23] as well as the concentration of urea added (1 g/L) in the referred article could account for this variation. The lower growth of biomass in the raw POME could be attributed to some toxic components. The presence of phenols and some organic acids is generally believed to be responsible for the phytotoxic and antibacterial activity of POME [26-27]. The growth of *Chlorella* was poor in the 25% POME as shown by the low specific growth rate (0.4520) despite the lowered concentration of the toxic elements and higher light intensity. Such low growth rate could be caused by over dilution of the key nutrients [25]. Evaluation of the rate of COD reduction by the algae under the different culture conditions indicate that *Chlorella* could be exploited as potential candidate for the treatment of POME. The algae grown in Batch A recorded a very low (20±04%) reduction rate due to identified reasons but the COD of the Batch B and C cultures declined by 45±08 and 58±03% respectively. The former represent the metabolic capacity of a homogenous algal monoculture, while the latter suggests the involvement of other microorganisms besides the algae by the higher reduction values. This is confirmed from cultures grown in unsterilized POME (Batch D) in which the highest rate of COD reduction (70±05%) was achieved despite having the least (0.41±0.07 mg/L) chlorophyll content.

In cultures grown in the different concentrations of Batch B POME Table 2, COD reduction was enhanced (63±03%) especially in the 75% POME. This further lends credence to the possibility of interference by some components of the raw POME as indicated by the increase in the rate of COD reduction at a lower (75%) concentration. However, despite the limitations imposed by the inhibitory substances, the organisms present in Batch D (unsterilized) appeared unaffected and even achieved a higher rate of COD reduction

(70±05%) than the other cultures. Monocultures usually metabolize a limited range of substrates but a mixed microbial consortium as may be present in the unsterilized POME can be more efficient in remediation due to their broad enzymatic capacities and higher tolerance to fluctuations of temperature, pH and salinity [28].

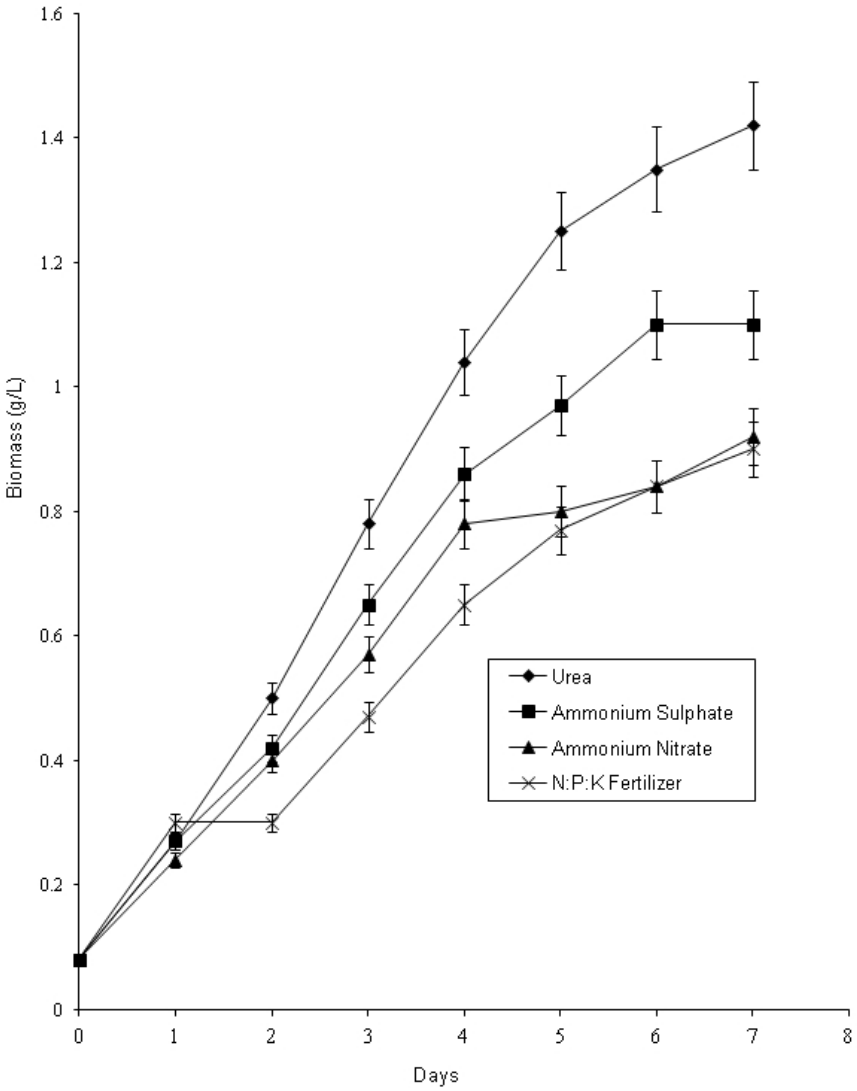

Fig 1. Effect of different nitrogen compounds on the biomass of *C. sorokiniana*

5. CONCLUSION

POME has potential for use as medium for microalgal cultivation with significant saving in treatment costs. However, the low nitrogen content of POME implies that additional supplementation with appropriate compound is needed to improve the nutritional quality of the effluent. Also, due to variation in the composition of POME, the concentration that promotes the best biomass or product yield has to be pre-determined in order to reduce the negative impact of the inhibitory components on the metabolism of cultures.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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