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# Nutrient Removal and Biokinetic Study of Freshwater Microalgae in Palm Oil Mill Effluent (POME)

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## Abstract

**Objectives**: Palm Oil Mill Effluent (POME) is an untreated wastewater that causes water pollution when discharge directly. Therefore, the objective of this research lies on POME treatment using microalgae. **Method**: Batch cultivation of *Chlorella sorokiniana* was conducted with different volumes of POME to distilled water in order to determine it phycoremediation ability. Michealis-Menten equation was used as a model to study the biokinetic. The kinetic coefficients for substrate removal by this algae. **Findings**: This strain showed high efficiency of removing 31-62.2 % nitrate, 30.6-39.5 % phosphate, 54.1-95.1 % ammonium and 11-56.1 COD over 15 days culture. The nutrients biokinetic removals were determined as follows; k = 9.2*10$^{-3}$ mg $NO_3^-$ mg$^{-1}$ DCW d$^{-1}$, $K_m$ = 68.7 mg/L, $Y_N$ = 0.1 g DCW g$^{-1}$ $NO_3^-$ for nitrate, k = 8*10$^{-3}$ mg $PO_4^{3-}$ mg$^{-1}$ DCW d$^{-1}$, $K_m$ = 144.6 mg/L, $Y_P$ = 0.12 g DCW g$^{-1}$ $PO_4^{3-}$ for phosphate, k = 2.3*10$^{-2}$ mg $NH_4$ mg$^{-1}$ DCW d$^{-1}$, $K_m$ = 113 mg/L, $Y_N$ = 0.08 g DCW g$^{-1}$ $NH_4^+$ for ammonium and k = 0.15 mg COD mg$^{-1}$ DCW d$^{-1}$, $K_m$ = 1662 mg/L, $Y_{COD}$ = 0.02 g DCW g$^{-1}$ COD for Chemical Oxygen Demand (COD). **Application/Improvement**: This study concluded that *C. sorokiniana* was successfully cultivated in POME and concomitantly produces important biomass which could be used for sustainable bioenergy production.

**Keywords:** Biokinetic, Freshwater Microalgae, Nutrient Removal, Palm Oil Mill Effluent, Phycoremediation

## 1. Introduction

Palm oil production in Malaysia has grown tremendously in recent years accounting for about 10.3% of world oil production[1]. The increase in palm oil production has resulted in the generation of a large amount of wastewater known as Palm Oil Mill Effluent (POME)[2]. POME is a thick brownish, acidic and colloidal suspension that is non-toxic in its natural form, but when its discharge into the environment it can pollute the water by affecting human health and aquatic ecosystem[3]. For example, high amount of nitrate ($NO_3^-$) are contained in POME and are reported to affect the developmental stages of human offsprings by causing methemoglobinemia[4]. In the aquatic environment, a high concentration of phosphate ($PO_4^{3-}$) accounts for the mass loss of aquatic biota due to eutrophication[1]. Discharge of untreated wastewater such as POME into the environments has long been linked to many environmental problems.

Culturing microalgae in wastewater is a promising biological wastewater treatment method because microalgae assimilate high amount of nitrate and phosphate and use it for growth[5,6]. This process helps in nitrogen, phosphate and ammonium detoxification. Wastewater treatment by microalgae offers several advantages such as generation of non-secondary pollution, affordable, efficient nutrient recycling and production of valuable biomass. Study from[7] have reported efficient nitrate uptake from wastewater by *Chlorella* spp. Also, according to[8], they reported efficient nitrate and phosphate removal in piggery wastewater at a range of 78.71-91.28 % and 53.05-88.72 % by *Schenedesmus* sp. respectively. Nitrogen, phosphorus and COD were reportedly removed of about 59, 81 and 88 % by algae when it grew in municipal wastewater[9].

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The above mention studies mainly discussed the nutrient removing efficiency of microalgae and very few reported the application of algae for the treatment of high nutrient load effluent like POME, no did investigate the biokinetic coefficients governing this nutrient removal. *C. sorokiniana* is a robust freshwater microalga capable of growing faster and has strong nutrient removing ability. Study of the nutrient removal of these nutrients by *C. sorokiniana* will be helpful in finding an efficient method of treating POME and these coefficients will be adequately required in providing information's that are essential to the understanding of algae biological process in wastewater treatment plant. This study thus investigates the biokinetic coefficients and nutrients removal ability of *C. sorokiniana* in POME.

# 2. Materials and Methods

## 2.1 Sample Collection

POME was collected at the Facultative Anaerobic Pond (FAP) from local palm oil mill industry (Kilang Sawit Bukit Besar, Johor Bahru Malaysia). FAP is a conventional wastewater treatment system that consists of four ponds arranged in parallel. POME from the industry is discharged directly into the three ponds where acidification and digestion take place. POME coming out from the final (4th) pond contains a high amount of nutrients Table 1. Due to its turbid characteristic, it was left to settle for four hours before being filtered using Whatman filter paper. POME was sterilized by autoclaving at 121°C for 20 minutes and later placed in a laminar flow under the effect of UV sterilization for 15 minutes in order to obtained sterile filtrate. For the purpose of preservation, POME was refrigerated at 4°C to prevent biodegradation.

## 2.2 Algae and Culture Condition

A pure strain of *C. sorokiniana* was obtained from algae culture collection center at University of Texas, Austin, Texas, USA. The culture was maintained on Proteose media at 29°C. The composition of Proteose media consist of $NaNO_3$ (25 g/L), $CaCl_2.2H_2O$ (2.5 g/L), $MgSO_4.7H_2O$ (7.5 g/L), $K_2HPO_4$ (7.5 g/L), $KH_2PO_4$ (17.5 g/L), NaCl (2.5 g/L), Urea (1.5 g/L). pH of the media was kept at 7. The experiments were conducted in a batch system. 1 L flasks were used for the algae cultivation. Before the commencement of the experiments, about 20% volume of algae was inoculated in 250 mL Proteose media (composition as shown above) for 21 days. This importance of this step is to ensure that, *C. sorokiniana* acclimatize well before inoculation in POME. The justification of using *C. sorokiniana* for these experiments was due to its robust nature and ability to grow under photoheterotrophic mode making it one of the most tolerant microalgae to grow in a strange environment.

## 2.3 Characterization of POME

The characteristics of POME sample are shown in Table **1**. Nitrate, phosphate and ammonium were determined following the Hatch DR 6000 Spectrophotometric Manual (DR/6000, Hatch Co. Ltd. Tokyo 2008).

## 2.4 Determination of Nitrogen, Phosphorus, and Ammonium

Nitrate and phosphate were specifically determined using cadmium reduction method and an acid hydrolysable method respectively while ammonium was determined using Nessler method. Suitable dilutions were made for the high concentration and the final results were computed by multiplying the dilution factor. 5 mL microalgae suspension was taken from each Erlenmeyer flask to measure the nutrient removal throughout the experiments. The samples were centrifuged at 4,000 rpm for 15 min and afterward, the supernatants were analyzed for nitrate, phosphate, and ammonium based on the HACH DR 5000 spectrophotometer manual.

## 2.5 Determination of pH

pH was determined using the portable pH meter according to the standard methods[10].

## 2.6 Determination of COD

About 10 mL of POME was taken and then centrifuged at 4000 rpm for 15 minutes. The supernatant was mixed thoroughly using vortex and was placed in a preheated digester block at 1,500°C. Following the next 2 h, the sample was withdrawn and measured using spectrophotometer.

## 2.7 Determination of Heavy Metals Ions

To determine the concentration of heavy metals in POME, 10 mL of POME was acid digested using 2.5 mL concentrated nitric acid (65% $HNO_3$) and 0.8 mL of

Hydrochloric acid (HCL) at 180°C for 20 min in a microwave oven (model; Berghof *Speed wave 4*). Afterward, the digested samples were taken out, allowed to cool, filtered and diluted with distilled water. The sample was then analyzed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) according to the method portrayed by[11]. All the data were analyzed using Microsoft excel 2010. The percentage removals of nitrate, phosphate, ammonium and COD were computed using the formula *(Ci-Cf)/Ci* ×100, where *Ci* = Initial concentration and *Cf* = is the final concentration.

## 2.8 Experimental Setup

The experiments were conducted in a batch system using 1 L Erlenmeyer flask. At the inception of the experiment, 20% (v/v) of pre-cultured microalgae cell was cultivated in sterilized POME initially diluted to 80, 60, 40 and 20% (v/v) with distilled water. Higher dilution of wastewater was suggested to provide better growth of algae. This initial inoculum concentration was kept constant throughout the experiment. Since POME contains nitrate ($NO_3^-$), phosphate ($PO_4^{3-}$), ammonium ($NH_4^+$) and COD, their initial concentrations after the dilution were measured and the removal of these nutrients was monitored throughout the experiment. The experiment was repeated three times and the average values of the replicate results were used for analysis. The removal rate constant (k) and saturating constant (Km) for $NO_3^-$, $PO_4^{3-}$, $NH_4^+$ and COD by *C. sorokiniana* was determined *in situ*. The cultivation condition was maintained by a continuous supply of Carbon Dioxide ($CO_2$) for mixing purpose using an air pump. The pH was maintained at 7 by the addition of 1 M sodium hydroxide (NaOH) and 2 M HCL. Fluorescence lamps (3000 lux) were used as a source of illumination for 12 h (Day:Night) interval. The experiment was conducted at temperature 25-30°C for 15 days. The reason of using *C. sorokiniana* for the biokinetic study is due to the following: 1, To determine the tolerance level of the *C. sorokiniana* for nutrient removal in POME as industrial wastewater contained a high amount of nutrient and 2. To study the biological processes taking place during the treatment.

## 2.9 Determination of Biomass Concentration

Algal biomass was determined using Cell Dry Weight (CDW) based on the method described by[12]. 20 mL of algal culture was centrifuged at 4000 rpm for 15 min. The supernatant was discarded and the pellet was washed three times with distilled water. The pellets were dried at 70°C in a hot air oven until constant weight was achieved. Biomass was determined in terms of cell dry weight per volume of culture (g/L).

## 2.10 Determination of Specific Growth Rate

The specific growth rate was calculated by the equation;

$\mu = \frac{1}{t} In\left(\frac{Xm}{Xo}\right)$ Where $X_o$ and $X_m$ are the initial and final concentration of biomass respectively and t is the duration of the batch run.

## 2.11 Determination of Nutrient Removal Kinetics

Michaelis-Menten kinetic equations were employed for the determination of two kinetic coefficients; 1. Saturation constant ($K_m$) and 2. Reaction rate constant (k). This equation is suitable for the data because the rate of substrate utilization gets higher with high organic content and vice versa. The kinetic coefficients $K_m$ and k were derived based on Michaelis-Menten kinetic relationship as seen in Equation **2**. The equation was adopted from[13] and was derived as follows;

$$R = \frac{R\max S}{Km + S}$$

$R = \frac{RmaxS}{Km + S}$ Where $R_{max}$ stands for maximum substrate removal rate and S is the substrate concentration. Maximum removal rate and substrate concentration at initial batch system modify the equation as follows;

$$Rso = \frac{R\,\mathrm{m}\,oSo}{Kmo + So} \quad (3)$$

Hence, $R_{mo}$ is proportional to $X_o$ which is equal to $Rmo = k.Xo$ (Equation 2). The equation can be written as;

$$WRso = \frac{kXoSo}{Kmo + So} \quad (4)$$

Where "k" is the reaction rate constant ($time^{-1}$), $X_o$ is the initial biomass concentration for *C. sorokiniana*. The specific rate of substrates removal ($R_{xi}$) can be computed

by dividing both sides of Equation (3) by $\frac{Ro}{Xo}$ to get;

$$Rxi = \frac{RSo}{Xo}\frac{RSo}{Kmo + So} \quad (5)$$

To obtain straight line graph, let linearized Equation (4) by doubling the reciprocal form of the equation.

$$\frac{1}{Rxi} = \frac{1}{k} + \frac{km}{k}.\frac{1}{So} \quad (6)$$

The plot of $1/R_{xi}$ against 1/So gives linear line consisting of slope = $K_m/k$ and intercept at y-axis = 1/k.

## 3. Results and Discussion

### 3.1 Nitrogen and Phosphorus Removal

Microalgae growth in wastewater has long been linked to its nutrient assimilation. However, this study gave more priority to nitrate, phosphate, ammonium and COD removal because they formed the major component of nutrient for algae growth and is significantly required for determining kinetic coefficients. The kinetic coefficient of these nutrients was determined *in situ* in order to provide information on biological process governing wastewater treatment and inhibition model. Table 1 showed the general characteristics of anaerobic digested POME and the amount of $NO_3^-$—N, $PO_4^{3-}$—P, $NH_4^+$—N and COD obtained indicated the need for treating POME before discharge out. The nutrient removal ability of *C. sorokiniana* in POME was carried out for a period of 15 days.

Nitrogen constitutes important nutrients necessary for the growth of microalgae. The sole inorganic source of nitrogen that can be assimilated directly by most microalgae is in form of nitrate ($NO_3^-$), nitrite ($NO_2^-$) and ammonium ($NH_4^+$)[14]. In POME, the available nitrogen sources for assimilation by algae are in the form of nitrate and ammonium.

Nitrate was greatly removed in all the dilutions at a range of 31-62.2 % Table 2. The nitrate reduction curve in Figure 1 follows a similar pattern as ammonium removal does except for 80% and 60% dilutions which indicated that these are the two forms of nitrogen readily available for utilization by algae in POME. Results in Table 2 showed that *C. sorokiniana* removed 54.1- 95.1 % of ammonium over 15 days culture. It can be seen that the percentage removal of ammonium was higher than nitrate, the preference of ammonium to nitrate as a nitrogen source in other microalgae was also documented in the literature[7]. The $NH_4^+$—N removal curve can be seen in Figure 2. Even though Ding and his coworkers reported 100% $NH_4^+$—N removal by *Chlamydomonas* sp, in this study, the total $NH_4^+$—N removal was found to be 54-95 %[15]. This could be the due difference in microalgae species used for the treatment because microalgae species respond to the different level of ammonium toxicity. This showed the possibility of *C. sorokiniana* becoming more saturated with ammonia in POME than *Chlamydomonas* spp.

**Table 1.** General characteristic of POME from anaerobic pond

| General Characteristics | POME[a] | Malaysian discharge limits[b] |
|---|---|---|
| pH | 6.5 | 5-9 |
| COD | 2100 | - |
| TSS | 3200 | 400 |
| Nitrate | 181 | - |
| Phosphate | 131 | - |
| Ammonium | 245 | - |
| Turbidity | 420 | |
| Heavy metals | | |
| $Zn^{2+}$ | 0.79 | 1 |
| $Fe^{2+}$ | 118.1 | 5 |
| $Mn^{2+}$ | 9.2 | 1 |

[a] All parameters unit is in mg/L except pH and turbidity (NTU).

[b] Reference,[1]

Microalgae obtained energy via metabolism of phosphorus, which means that phosphorus is another necessary nutrient for the growth of algae. Results in Table 2 showed that phosphorus removal in all the dilutions was found to be 30.6-39.5 %. Phosphate removal curves in Figure 3 showed the growth of *C. sorokiniana* in 20% (v/v) dilution as it almost immediately reached stationary phase between days 9 to 15. In this dilution, insufficient phosphate was the limiting nutrient for growth. Study have reported that high amount of phosphate affect algae growth[16] in this study, the high concentration of nutrients in 20 and 40% of POME medium might be one of the priority factor responsible for the low nutrient removal of *C. sorokiniana* in POME.

**Table 2.** Nutrient removal efficiency of *C. sorokiniana* in various POME dilutions

| Nutrient parameters | Removal efficiency | 80% | 60% | 40% | 20% |
|---|---|---|---|---|---|
| $NO_3^-$ | Initial value (mg/L) | 45.0 | 81.0 | 112.0 | 145.0 |
| | Final value (mg/L) | 17.0 | 41.0 | 67.0 | 100.0 |
| | Removal (%) | 62.2 | 49.4 | 40.2 | 31.0 |
| $PO_4^{3-}$ | Initial value (mg/L) | 81.0 | 93.0 | 111.0 | 121.0 |
| | Final value (mg/L) | 49.0 | 58.0 | 72.0 | 84.0 |
| | Removal (%) | 39.5 | 37.6 | 35.1 | 30.6 |
| $NH_4^+$ | Initial value (mg/L) | 37.2 | 57.7 | 115.5 | 231.0 |
| | Final value (mg/L) | 1.82 | 6.4 | 45.0 | 106.0 |
| | Removal (%) | 95.1 | 88.9 | 61.0 | 54.1 |
| COD | Initial value (mg/L) | 314.0 | 421.0 | 921.0 | 1921.0 |
| | Final value (mg/L) | 138.0 | 240.0 | 681.0 | 1710.0 |
| | Removal (%) | 56.1 | 42.9 | 26.1 | 11.0 |

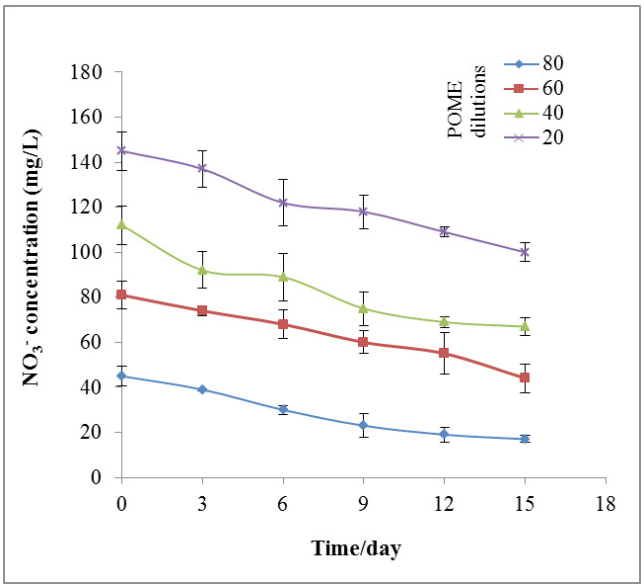


**Figure 1.** $NO_3^-$ consumption in the medium of different dilutions of POME with C. *sorokiniana* growth.

The COD content of POME has become one of the major environmental threats, especially when it's discharged to a bare water-course. The anaerobic digested POME contains inorganic carbon in form of COD that was not consumed by bacteria but are available for assimilation by microalgae. Based on the results in Table 2**,** *C. sorokiniana* removed COD of 11-56.1 %. Results of this study demonstrated that *C. sorokiniana* is capable of utilizing small amount COD content of POME as a carbon source. In anaerobic digested POME acetogenic bacteria convert volatile fatty acid to acetate, which is then converted to methane and carbon dioxide by methanogens via methanogenesis[17]. Thus the occurrence of acetate as a source of carbon for microalgae growth in anaerobic POME was due to partial breaking down of acetate into methane. COD removal from POME ratio of 80% and 60% coincide at uniform based line in day 9, causing *C. sorokiniana* to reach stationary phase between day 9 to 15 due to deficient nutrients source Figure 4.

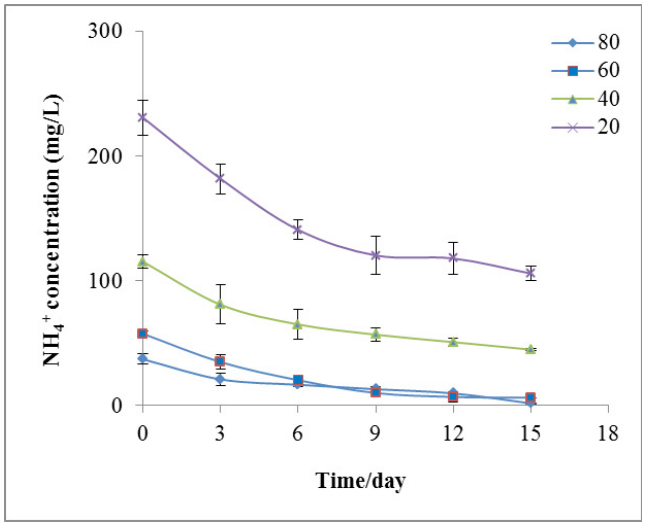


**Figure 2.** $NH_4^+$ consumption in the medium of different dilutions of POME with C. *sorokiniana* growth.

Therefore in all the experiments conducted, 80% dilution of POME was identified as the best condition for the cultivation of *C. sorokiniana* in POME because it accounts for better nutrient removal. Therefore it is sub

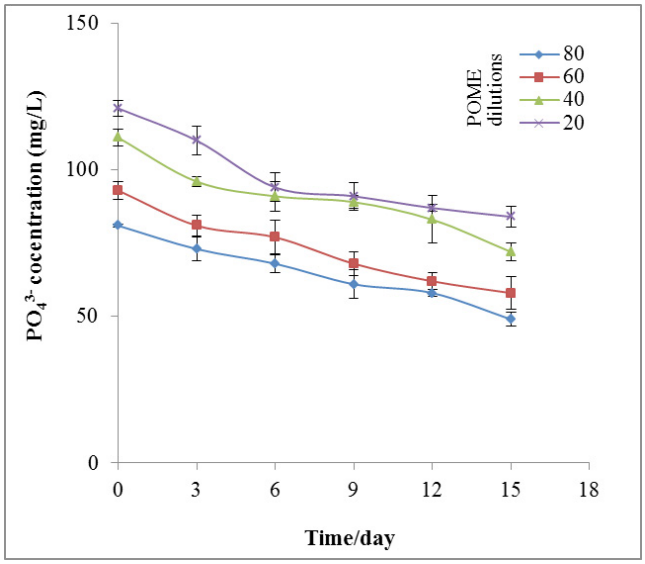


**Figure 3.** $PO_4^{3-}$ consumption in the medium of different dilutions of POME with C. *sorokiniana* growth.

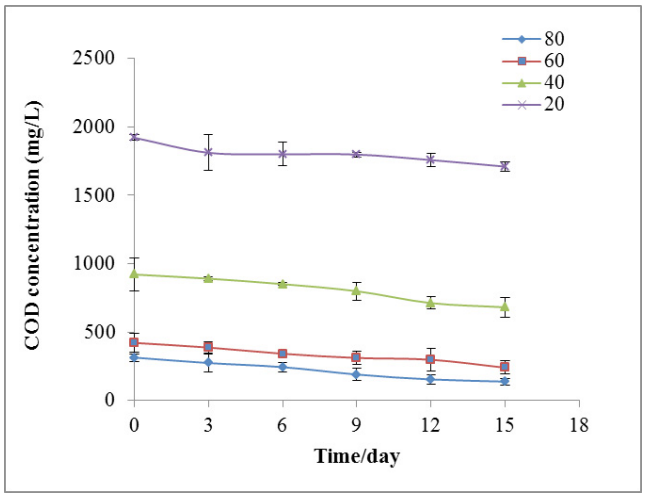


**Figure 4.** COD consumption in the medium of different dilutions of POME with C. *sorokiniana* growth.

jected to further experiments to investigate the biokinetic coefficients such as saturation constant ($K_m$) and removal rate constant (k) for the removal of nitrate, phosphate, ammonium and COD in 80% dilution by *C. sorokiniana*. Study of these coefficients is helpful in understanding the biological wastewater treatment and the designing of a bioreactor for enhancing wastewater treatment. It is also important in proposing a model for studying the presence of an inhibitory substance in POME. Although[16] has used *Chlorella* sp. species of microalgae to treatment digested dairy manure effluent, but could provide the biokinetic data governing the biological treatment as this research does. The overall nutrient removal efficiency reported in this study was not 100%, this concluded there is a portion of organic nutrients which cannot be assimilated by *C. sorokiniana* possibility due to the presence of some inhibitory substances such as tannic acid in POME.

## 3.2 Nutrients Removal Yield and Kinetic Coefficients Study

The Michaelis-Menten relationship in Equation **6** was used to determine the kinetic coefficients by plotting $1/R_{xi}$ against $1/N_o$ giving a straight line with slope $K_m/k$ and y-axis intercept of 1/k. Thus, experimental data in Table **3** was plotted based on $1/R_x$ versus $1/ NO_3^-$ for nitrate, $1/R_x$ versus $1/ PO_4^{3-}$ for phosphate, $1/R_x$ versus $1/ NH_4^+$ for ammonium and $1/R_x$ versus 1/ COD for COD respectively as shown in Figure 5-8.

Results from Figures 5-8 showed that the specific rate ($1/R_x$) of nitrate, phosphate, ammonium and COD removal increases with the increase in nutrient removals. Based on the slope and the intercept of the plot, the kinetic coefficients for nitrates and phosphate removal by *C. sorokiniana* are determined as; $k = 9.2*10^{-3}$ mg $NO_3^-$ $mg^{-1}$ DCW $d^{-1}$, $K_m$ = 68.7 mg/L ($R^2$ = 0.94) and $k = 8*10^{-3}$ mg $PO_4^{3-}$ $mg^{-1}$ DCW $d^{-1}$, $K_m$ = 144.6 mg/L ($R^2$ = 0.89) respectively. Similarly, the coefficients for $NH_4^+$—N and COD removal by *C. sorokiniana* were reported as $k= 2.3*10^{-2}$ mg $NH_4^+$ $mg^{-1}$ DCW $d^{-1}$, $K_m$ = 113 mg/L ($R^2$ = 0.96) and k = 0.15 mg COD $mg^{-1}$ DCW $d^{-1}$, $K_m$ = 1662 mg/L ($R^2$ = 0.95). The implication of these results is that the $K_m$ values represent the maximum amount of the nutrients that *C. sorokiniana* can assimilate for growth if the system were to be in continuous mode. This is significant in avoiding the use of excess nutrients which might be toxic to the growth of microalgae. This implies that the *C. sorokiniana* possessed an excellent nutrients removal ability giving it the potential to be cultivated in other wastewater of high nutrient load. The low rate of nutrients removal (*k*) values obtained from this study further confirmed the reason why *C. sorokiniana* could not provide 100% removal of these nutrients which could be due to the presence of inhibitory substances such as tannic acid and high COD content in POME. The R values for the determination of these coefficients are above 80; this indicates that the kinetic coefficients computed from this experiment are accurate.

The initial concentrations of $NO_3^-$—N, $PO_4^{3-}$—P, $NH_4^+$—N and COD used for the cultivation of *C. sorokiniana* were 45, 81, 37.2, and 314 mg/L respectively, whereas

**Table 3.** Increase in biomass and nutrients removal of *C. sorokiniana* in 80% dilution (v/v) POME

| Time (days) | Biomass (g/L) | $NO_3^-$ (mg/L) | $PO_4^{3-}$ (mg/L) | $NH_4^+$ (mg/L) | COD (mg/L) |
|---|---|---|---|---|---|
| 0 | 0.44 | 45.0 | 81.00 | 37.2 | 314.00 |
| 3 | 0.95 | 39.0 | 76.20 | 27.3 | 275.60 |
| 6 | 1.91 | 30.0 | 68.10 | 18.6 | 221.60 |
| 9 | 2.71 | 23.0 | 62.10 | 10.8 | 179.60 |
| 12 | 3.26 | 19.0 | 58.26 | 8.2 | 156.20 |
| 15 | 3.59 | 17.0 | 56.13 | 6.9 | 143.15 |

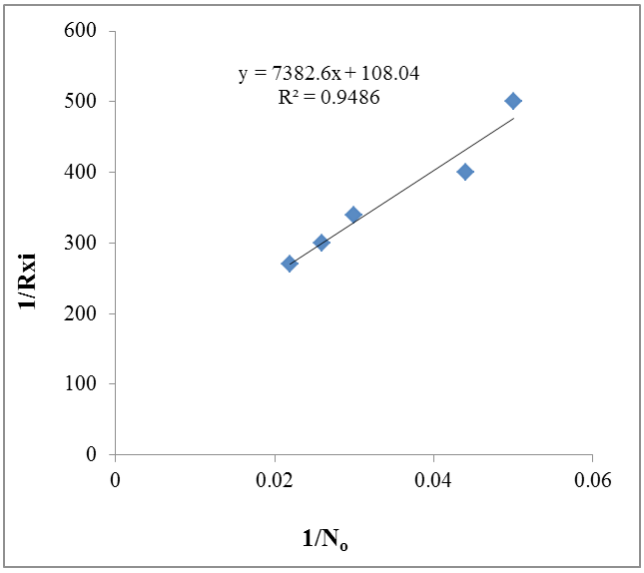


**Figure 5.** Determination of kinetic coefficients, $K_m$ and k for specific $NO_3^-$ removal.

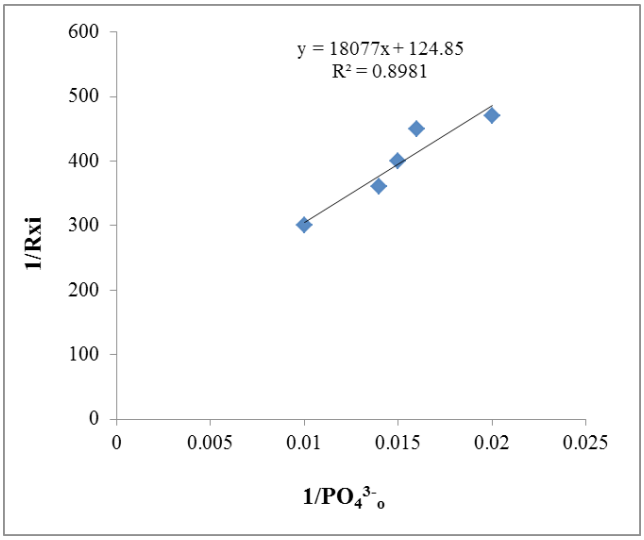


**Figure 6.** Determination of kinetic coefficients, $K_m$, and k for specific $PO_4^{3-}$ removal.

according to Michaelis-Menten equation, *C. sorokiniana* can assimilate individual nutrients concentrations up to 69, 144.6, 113 and 1662 mg/L of $NO_3^-$ —N, $PO_4^{3-}$ —P, $NH_4^+$ —N and COD respectively. This means that, *C. sorokiniana* can grow in wastewater of high nutrient load.

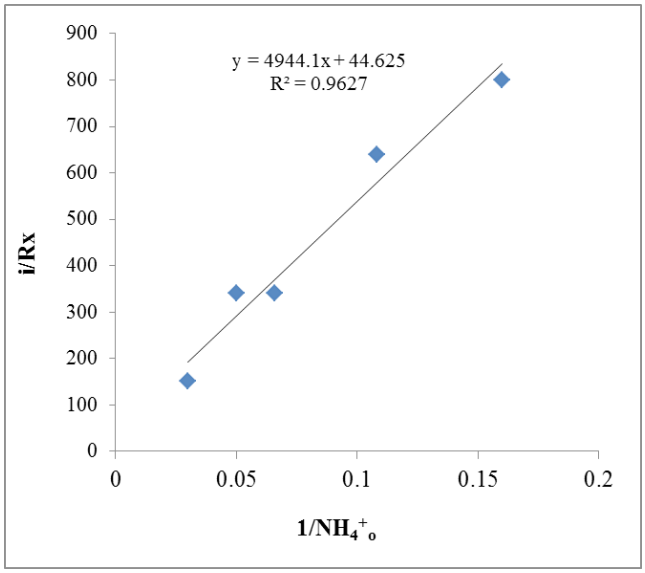


**Figure 7.** Determination of kinetic coefficients, $K_m$ and k for specific $NH_4^+$ removal.

Moreover, different microalgae species tolerate different level of nutriets load. This is because, the optimal amount of the nutrients in a medium induces the growth of microalgae and enzymes activities such as nitrate reductase and phosphatase. But at a high amount, it causes a repressive effect on these enzymes leading to genetic modification which makes the enzymes unable to convert the nutrients. The inability for enzymes to function can thus halt the nutrient assimilation process. Microalgae consist of the cell membrane that is permeable for nutrients translocation, thus there will be rapid diffusion and subsequent nutrient accumulation pool in

the cell and that will further affect the algal cell leading to death. This implies that, different microalgae species response to different toxicity level due to high nutrients load. For example, study by[18] reported that the ammonium tolerant level of different algae species varies from 25 µmol $NH_4^+$—N $L^{-1}$ to 1000 µmol $NH_4^+$—N $L^{-1}$.

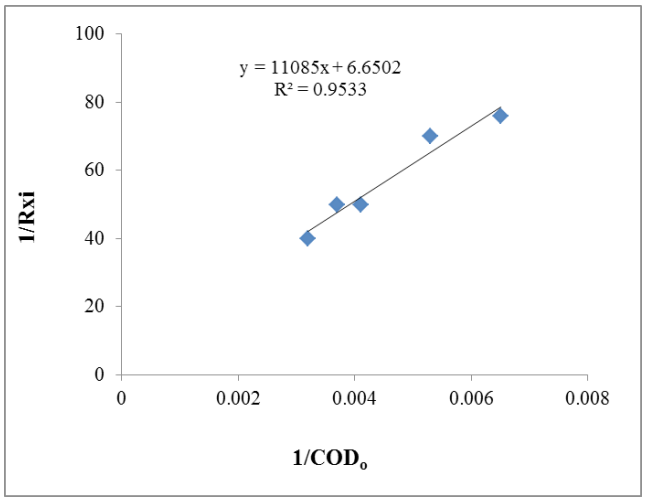


**Figure 8.** Determination of kinetic coefficients, $K_m$ and k for specific COD removal.

In case of Yield coefficient (Y) for $NO_3^-$—N, $PO_4^{3-}$—P, $NH_4^+$—N and COD, they were computed according to the following Equations (9) to (12) as follows;

$$(Biomass)_f - (Biomass)_o = Y_N (NO_3^- - N)_o - (NO_3^- - N)_f \quad (9)$$

$$(Biomass)_f - (Biomass)_o = Y_P (PO_4^{3-} - P)_o - (PO_4^{3-} - P)_f \quad (10)$$

$$(Biomass)_f - (Biomass)_o = Y_N (NH_4^+ - N)_o - (NH_4^+ - N)_f \quad (11)$$

$$(Biomass)_f - (Biomass)_o = Y_{COD} (COD)_o - (COD)_f \quad (12)$$

$(Biomass)_f$ stands for a final concentration of biomass (mg/L) synthesized, $(Biomass)_o$ is the initial biomass concentration (mg/L) synthesized and $(NO_3^- —N)_o$ and $(NO_3^- —N)_f$ are the initial and final concentration of nitrate (mg/L) respectively. Y can be obtained by plotting a graph of $(Biomass)_f$ - $(Biomass)_o$ against $(NO_3^- —N_o$ - $NO_3^- —N_f)$, a linear graph was obtained and the slope of the graph gives the yield coefficients. Similar calculations were applied for determining Y of $PO_4^{3-}$—P, $NH_4^+$—N and COD. Therefore, the coefficient yield (Y) for nutrients removal was computed as shown in Figures 9-12. Both the four graphs showed that increase in biomass concentration increases the nutrient removal. The slope of $((Biomass)_f - (Biomass_i))$ versus $((NO_3^- —N)_o$ and $(NO_3^- —N)_f)$ plot gives yield coefficient $(Y_N)$ values of $NO_3^-$ N as $Y_N$ = 0.1 g DCW $g^{-1}$ $NO_3^-$ ($R^2$ = 0.87). Similar computation was applied for $PO_4^{3-}$—P, $NH_4^+$—N and COD obtained as $Y_P$ = 0.12 g DCW $g^{-1}$ $PO_4^{3-}$ ($R^2$ = 0.89), $Y_N$ = 0.08 g DCW $g^{-1}$ $NH_4$ ($R^2$ = 0.85) and $Y_{COD}$ = 0.02 g DCW $g^{-1}$ COD ($R^2$ = 0.87) respectively. This concluded that nutrient removal by microalgae is dependent on the component of the substrate and the types of the species used.

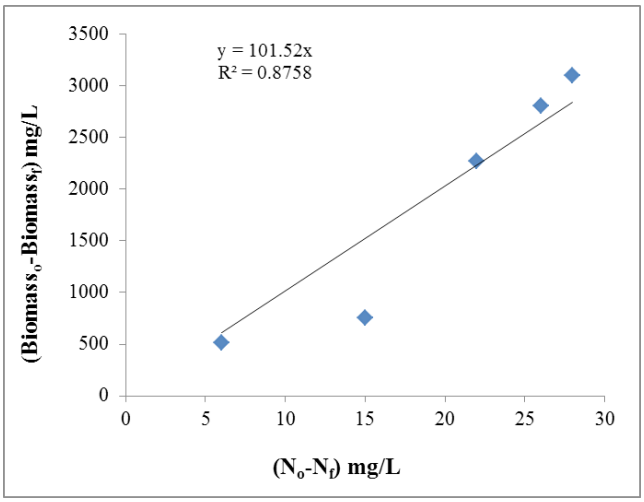


**Figure 9.** Determination of yield coefficient for $NO_3^-$ removal by C. *sorokiniana*.

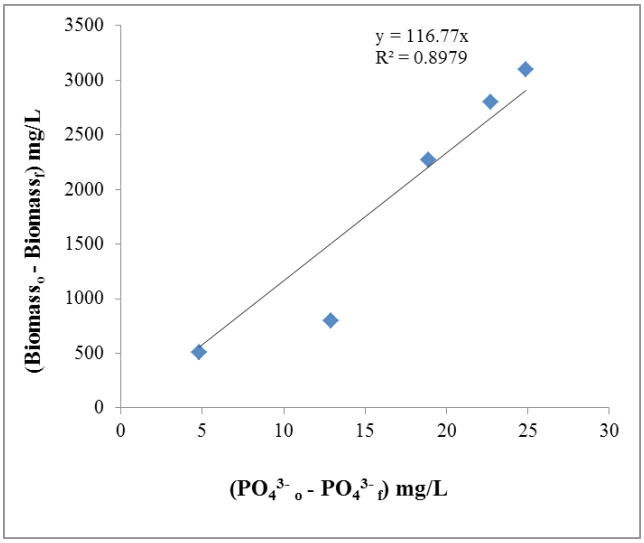


**Figure 10.** Determination of yield coefficient for $PO_4^{3-}$ removal by C. *sorokiniana*.

There is a slight difference of kinetic coefficients of nitrogen, phosphate and ammonium removal reported in the literature. For example, a study by[13] cultivated

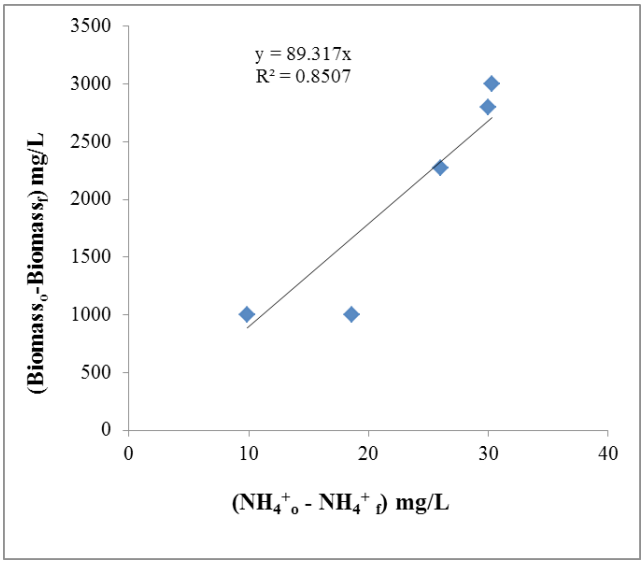


**Figure 11.** Determination of yield coefficient for $NH_4^+$ removal by C. *sorokiniana*.

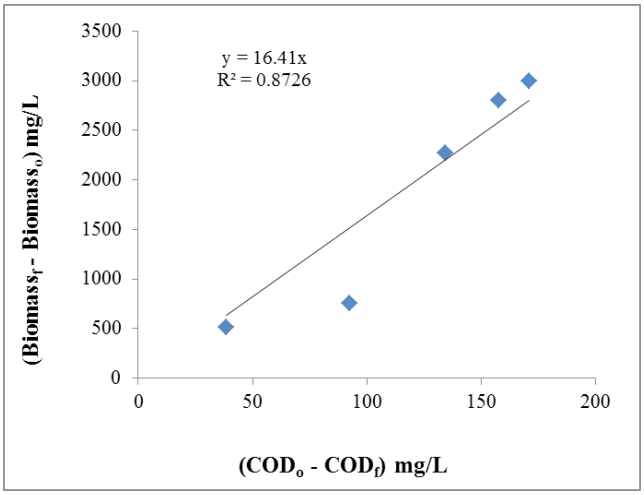


**Figure 12.** Determination of yield coefficient for COD removal by C. *sorokiniana*.

*Chlorella vulgaris* in synthetic wastewater and reported the rate of nitrogen and phosphate removal (k) as 1.5 mg $NH_4^+$—N $mg^{-1}$ chl a $d^{-1}$ and 0.5 mg $PO_4$ —P $mg^{-1}$ chl a $d^{-1}$. High k value, usually favors high biodegradation reaction of an organic substrate. Other factors that might cause variation of k values are differences of experimental set-up, a variation of aeration time, pH, types of wastewater used as nutrient feed for algae, types of microalgae species, among others[19–21] Considering the fact that these factors have an influence on the values of the kinetic coefficient, they are kept constant in this study. This is because the study aimed at investigating the possible inhibitory model that affect *C. sorokiniana* for removal of toxic nitrate, phosphate, ammonium, and COD from POME than optimizing the operating condition and nutrients. In addition, the kinetic coefficients data presented in this study are relevant understanding the biological wastewater treatment and designing of a bioreactor for pilot-scale wastewater treatment. This is the first report of kinetic coefficients of nutrients removal in POME using *C. sorokiniana*.

## 4. Conclusion

Biokinetic coefficients of nitrate, phosphate, ammonium and COD removal by *C. sorokiniana* in POME were investigated. Results from this study showed that *C. sorokiniana* is tolerant to nutrients presence in POME. *C. sorokiniana* was capable of removing 31-62.2 % nitrate, 30.6-39.5 % phosphate, 54.1-95.1 % ammonium and 11-56.1 COD in 15 days. Based on the results of this study, the biokinetic coefficients of nitrate removal by *C. sorokiniana* was determined as; $k = 9.2*10^{-3}$ mg $NO_3^-$ $mg^{-1}$ DCW $d^{-1}$, $K_m$ = 68.7 mg/L. Similarly, the biokinetic coefficients of phosphate, ammonium and COD removal was determined as $k = 8*10^{-3}$ mg $PO_4^{3-}$ $mg^{-1}$ DCW $d^{-1}$ and $K_m$ = 144.6 mg/L, for ammonium, $k = 2.3*10^{-2}$ mg $NH_4$ $mg^{-1}$ DCW $d^{-1}$, $K_m$ = 113 mg/L and for COD, k = 0.15 mg COD $mg^{-1}$ DCW $d^{-1}$ and $K_m$ = 1662 mg/L. It can be seen clearly that the nitrogen removal rate is higher than phosphorus. The coefficient yield (Y) for nitrate, phosphate, ammonium and COD was computed to be 0.1 g DCW $g^{-1}$ $NO_3^-$, 0.12 g DCW $g^{-1}$ $PO_4^{3-}$, 0.08 g DCW $g^{-1}$ $NH_4$ and 0.02 g DCW $g^{-1}$ COD respectively. The result of this study concluded that *C. sorokiniana* is tolerant to high nutrient load in POME and therefore it can be applied for the treatment of other wastewater of high toxic level.

## 5. Acknowledgement

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