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(RESEARCH ARTICLE)

Determination of uptake rate of phosphorus and changes in COD and BOD during photoautotrophic cultivation of microalgae in sewage effluent

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Abstract

Photosynthetic organisms like microalgae possess useful potentials such as nutrient uptake, biological oxygen demand (BOD) and chemical oxygen demand (COD) removal from wastewater which are crucial for wastewater treatment. This study was conducted to determine the rate of phosphorus uptake and changes in COD and BOD during microalgae cultivation in sewage effluent. Standard Methods for the Examination of Water and Wastewater were used to analyse the characteristics of the effluent samples (test), BG-11 medium (control) and also determine algal growth rates under continuous light illumination and constant aeration at varying temperatures. Results obtained showed that; removal efficiencies of *Chlorella* sp were higher in the test samples than control. Removal efficiencies for BOD, COD and $PO4^{3-}$ were 92.8%, 59.6% and 61.8% respectively for the test samples and 39.8%, 41.7% and 28.2% for the control. Also, *Chlorella* sp demonstrated better removal efficiencies at higher growth rates, exponential growth phase, constant aeration and temperature range between 25-30 ^{o}C. Hence, microalgae, under controlled and optimal conditions can be efficient in removal of pollutants in wastewater (sewage effluent).

Keywords: BOD; *Chlorella sp*; COD; Phosphorus; Removal efficiency; Sewage effluent

1. Introduction

Wastewater contains quite a number of pollutants that have to be removed before its discharge into surface waters as the discharge of wastewater that is rich in nutrients can cause eutrophication which is a serious problem that occurs particularly in enclosed water bodies [1]. Eutrophication occurs when a dense bloom of algae grow in water due to presence of high concentrations of nitrogen and phosphorus, the main nutrients of concern[1]. This depletes the level of oxygen in the aquatic system leading to loss of aquatic lives. While conventional biological treatment such as activated sludge removes organic matter from wastewater, many other constituents such as pathogens, nutrients and heavy metals are not eliminated to a satisfactory level. Over the years, many modifications have been introduced to traditional treatment processes to improve performance. However, majority of such modifications come with some limitations such as high cost and increased complexity in its operation and maintenance [2, 3]. Use of microalgae in wastewater treatment has been gaining attention due to their effective roles in the uptake of pollutants from the environmental wastes as well as their ability to produce valuable biomass that can be used in different industrial applications such as food processing, pharmaceuticals, organic fertilizer, animal feed, bio-fuel and biogas [4]. In wastewater treatment applications, for instance, microalgae can assimilate nutrients, uptake CO_2, provide O_2 for aerobic bacteria, and produce biomass that can be used to boost biogas generation from anaerobic digesters [5]. Microalgae require nutrients basically nitrogen and phosphorus which are found in wastewater to grow. Nitrogen is widely available in wastewater in many forms. However, microalgae can assimilate only the inorganic nitrogen. Phosphorus is the second essential nutrient

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which promotes algal growth in wastewater. The most important form of phosphorus for microalgae is orthophosphate PO_4^{3-} that is utilized directly by microalgae. Microalgae cannot take other forms of phosphorus until they are hydrolyzed by extracellular enzymes to PO_4^{3-}[6, 7], microalgae also require inorganic carbon sources such as CO_2 while growing under phototrophic conditions, alternatively, under heterotrophic conditions, they use dissolved organic carbon such as acetate, sugar, and organic acids as a carbon source causing the concentration of chemical oxygen demand (COD) to decrease. BOD is determined by the ability of microorganisms to convert organic material to CO_2 and water using the oxidizing power of molecular oxygen. Therefore, BOD can result in asphyxiation or death of aquatic life since it depletes the dissolved oxygen of receiving water, hence, its removal in wastewater treatment is paramount [7]. Different kinds of microalgae have been used in wastewater treatment but Chlorella and Scenedesmus are most commonly used since they have been reported to be more tolerant and adaptive and can survive under different wastewater physical and biochemical conditions [8]. Microalgae are being used in waste stabilization ponds (WSPs) and high rate algae ponds (HRAPs) for wastewater treatment and its overall performance in waste removal is satisfactory. Nevertheless, microalgae treatment presents certain limitations because it requires large surface area for treatment and long hydraulic retention times. Global warming and shortage in fossil fuels have been the driving forces for humans to investigate sources of renewable energy that could provide a better alternative to fossil fuels [9, 10]. According to [11], microalgae biomass is an attractive option not just for waste water treatment, but also bio-fuel production since it uses fewer chemicals and emits less CO_2 due to its high oil content. Some researchers [3, 12, 13] have studied algae as a phyco-remediating agent for wastewater, however, not much attention has been paid to the impacts of temperature and cultivation time on the efficiencies of COD, BOD and orthophosphate removal. Hence, the overall objective of this research was to determine the uptake rate of phosphorus and to evaluate the changes in COD and BOD of photoautotropic cultivation of Microalgae in sewage effluent at different temperature and cultivation periods.

2. Material and methods

2.1. Study Area

The samples (sewage effluents) for this research were obtained from the sewage treatment plant, University of Nigeria, Nsukka. Nsukka lies on latitude 6.86°N and longitude 7.39°E longitude and covers a total land mass of 1,810km^2.

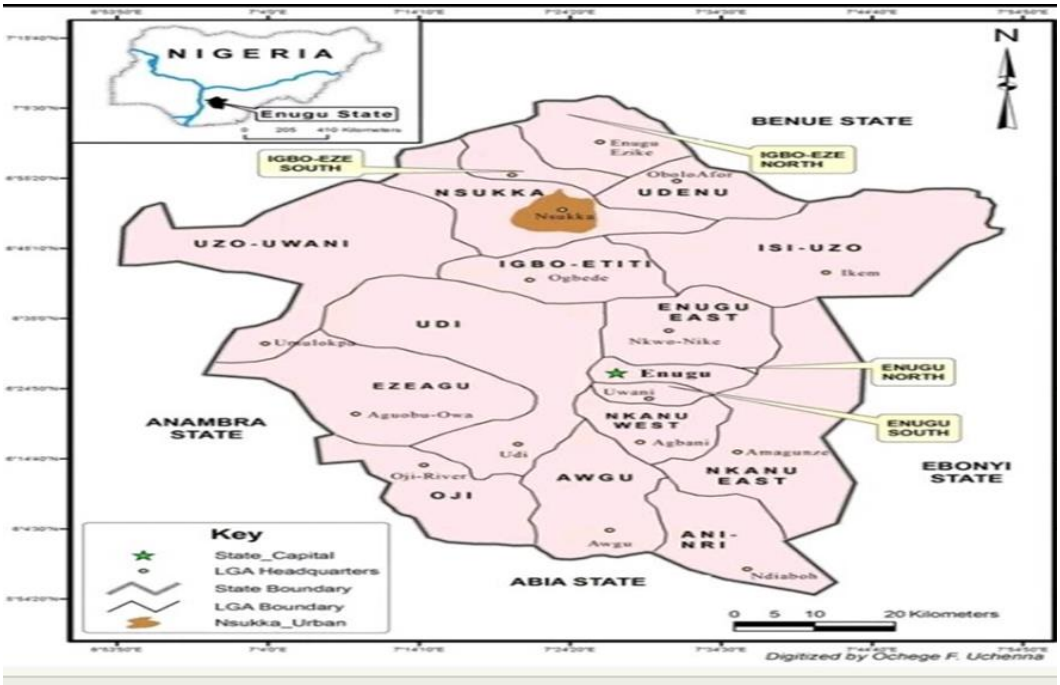

Figure 1 Map of Nsukka town (Adapted from Department of Geography Archives, UNN).

2.2. Sample Collection

Pretreated sewage sample was obtained at the terminal point of primary sewage treatment i.e. after sedimentation from University of Nigeria, Nsukka treatment plant. The sample was taken in 20 liter gallon and immediately transferred to

the Water and Public Health Research Laboratory (WPHRL), Department of Microbiology, University of Nigeria, Nsukka where it was immediately subjected to sterilization to kill any faecal coliform present.

2.3. Cultivation of Microalgae Species

Sterilized sample (sewage effluent) was used as the broth medium for the cultivation and enrichment of the Microalgae. The broth culture was incubated for 48 hrs at 25 ^{o}C and under a fluorescent light source. After incubation, sterile petri dishes containing different concentrations of agar-agar medium (2.4 g/200ml and 2.0 g/200ml of sterilized sewage effluent) were inoculated with the broth culture via a sterile wire loop, labeled as TP 2.4 g and TP 2.0 g respectively and incubated for 14 days under 25 ^{o}C in the presence of light source. Cultivation of alga in BG-11 medium ($NaNO_3$) was used as control.

2.3.1. Pure Culture Isolation

Three colonies were obtained after incubation in the plates having TP 2.4g concentration while there was no growth in TP 2.0g plates. The colonies were subcultured into 3 different agar-agar media, labeled TP-1, TP-2 and TP-3 and incubated under a light source for 4 days, the pure isolates were picked and inoculated into 8ml sterilized sewage effluent broth in 5 bijou bottles for enrichment.

2.3.2. Identification of the Isolate

A drop of the TP-1, TP-2 and TP-3isolates was placed on different glass slides, covered with cover slip and examined microscopically under X40 objective. The morphology of the algae seen was compared to the algae found in the Atlas of Microalgae.

2.4. Batch reactor experimental set up

Batch reactor experiments were conducted for 15 days at varying temperatures (15-35 ^{o}C). Four Bama bottles with 3 litres capacity were used for the set up. Aeration and agitation pump for O_2 distribution and proper mixing were also supplied. Sewage effluent (1 litre) was poured into the 1st bottle and inoculated with *Chlorella sp*., the 2nd bottle contained 1L of un-inoculated sewage effluent (TWR) while the 3rd had 1Lof BG-11 medium inoculated with *Chlorella sp*. (positive control).They were incubated at 30 ± 2 ^{o}C with continuous light intensity(1.42 mWcm^{-2}) and constant agitation. Samples were taken on 3 days intervals for analyses of soluble or dissolved concentrations of COD, BOD, and Phosphorus (PO_4^{3-}).

2.5. Wastewater Characterization

2.5.1. Wastewater Characterization

Orthophosphate content: this was done as described by Standard Methods for the Examination of Water and Wastewater [14]. An aliquot of the solution (50 ml) was dropped into a flask, followed by the addition of few drops of phenolphthalein indicator. Upon pink colour development, strong acid solution was added drop wise. An aliquot of ammonium molybdate (4 ml) was gently added into the mixture followed by subsequent addition of 4-5 drops of stannous chloride with through mixing. Samples were left unshaken for 10 minutes at room temperature for colour development. The absorbance was measured at 640nm.

2.5.2. Biochemical Oxygen Demand

HACH Method 8000 method was used [15] and BOD was measured as oxygen difference between the initial sample and after incubation for 5 days at 20 °C.

2.5.3. Chemical Oxygen Demand (COD)

COD was also analyzed according to HACH Method 8000 [15]. The samples were heated for two hours with sulfuric acid and a strong oxidizing agent, potassium dichromate. All oxidizable organic compounds reacted and reduced the dichromate ion (Cr2O72-) to green ion (Cr3+). COD was measured at wavelength of 620nm.

2.6. Determination of microalgal growth rate

Using a spectrophotometer, the optical density of serially diluted sample (0.1 ml) from each test tube was determined at OD640nm. Growth rate per day was calculated by fitting the OD for each day into the exponential function:

$GR = (\ln ODt - \ln OD_0)/t$

where $\ln OD_0$ is the natural log of optical density at the initial day; lnODt is the natural log of optical density measured on day t.

3. Results and discussion

3.1. Morphological/ microscopic characterization and Identification of Isolate

After cultural examination and microscopy, the isolated algae were characteristic of *Chlorella* sp. as shown in Table 1.

Table 1 Morphological/ microscopic characteristics of isolates

Colony code	Colour	Edge	Elevation	Surface	shape	Probable organism
TP-1	Greenish	Smooth	Raised	Rough	globular	Chlorella sp
TP-2	Greenish	Smooth	Raised	Rough	Spherical	Chlorella sp
TP-3	Greenish	Smooth	Raised	Rough	spherical	Chlorella sp

3.2. Microalgal growth rate

The growth pattern (Fig.2 & Fig. 3) obtained for Chlorella sp in sewage effluent showed that; 0 to 4th day of cultivation marked the lag phase of the organism while exponential growth was observed from day 5-10. The stationary phase set in after the 10th day due to reduced nutrient availability followed by decline on day 14 and 15. Similar pattern was observed in Bg-11 (control) except that the onset of exponential phase was a little bit prolonged due to lesser nutrient supply, followed by a rapid onset of stationary and decline phases.

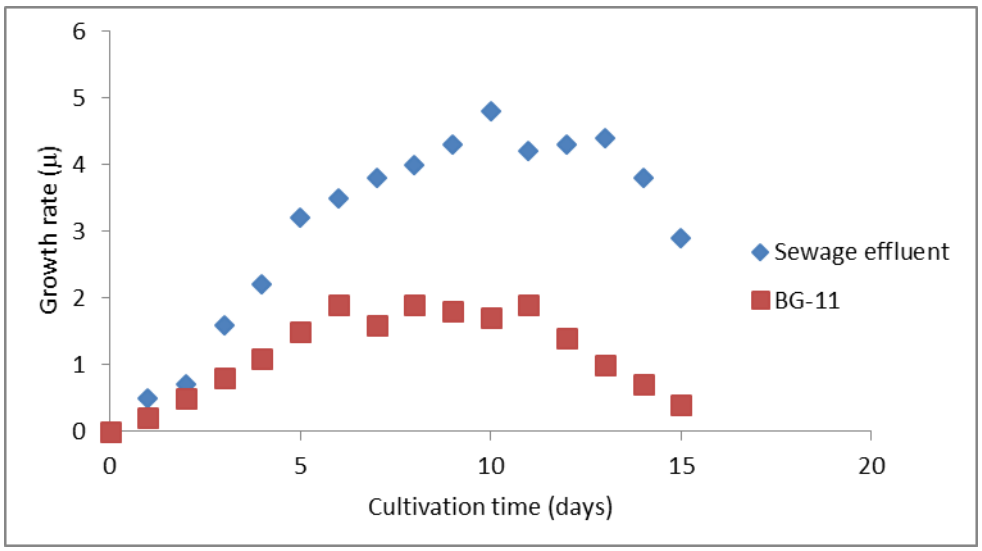

Figure 2 Growth curve of *Chlorella* sp. in sewage effluent and BG-11 (control)

Figure 3 Showing from up-left; days 2, 5, 10 and 15 of microalgae cultivation. At day 15, microalgae have taken up all the nutrients especially the Total phosphorus content and soluble solids present in the sewage effluent broth.

3.3. Estimation phosphorus uptake by Microalgae

Chlorella sp. showed maximum reduction in phosphorus concentration (61.8% removal) in day 12 of cultivation. This is attributable to the fact that the organism exhibited exponential growth at this period and hence phosphorus requirement for growth (with subsequent removal from sewage effluent) was at its peak.

Table 2 Uptake of phosphorus by *Chlorella* sp.

Time (days)	TWR(control)		Chlorella spp	
	Initial conc(mg/L)	% removal	Initial conc(mg/L)	% removal
0	11.0±0.2	0	11.0±0.2	0
3	9.5±0.2	13.6	9.6±0.05	12.7
6	8.0±0.1	27.3	6.0±0.1	45.5
9	7.90±05	28.2	4.2±0.01	61.8
12	8.3±0.4	24.5	6.5±0.3	40.9
15	9.1±0.05	17.3	7.9±0.4	28.2

Results are expressed as mean± SD

3.4. Removal of Biochemical Oxygen Demand

As shown in Table 3, Chlorella sp was able to reduce BOD up to 92.8% of its initial concentration in the sewage effluent while only 39.8% removal was recorded for the control. This connotes that biodegradable organic matter in the medium were utilized by the organism during growth/metabolism.

Table 3 Removal of BOD by *Chlorella* sp

Cultivation time (days)	TWR (control)		Chlorella sp	
	Initial Conc. (mg/L)	% removal	Initial Conc. (mg/L)	% removal
0	119.2±0.8	0	119.2±0.8	0
3	113.2±0.5	5.0	98.1±0.02	17.7
6	78.3±0.09	34.3	29.0±0.09	75.7
9	71.7±0.09	39.8	8.6±0.1	92.8
12	92.4±1.4	22.5	42.6±0.6	64.3
15	101.9±0.1	14.5	60.1±0.1	59.1

Results are expressed as mean± SD

3.5. Estimation of COD removal

COD was more efficiently removed with increasing time. Percentage removal of 59.6 and 41.7 were obtained for *Chlorella* sp and control respectively (Table 4).

Table 4 Estimation of COD removal

Time (days)	TWR(control)		Chlorella spp	
	Initial Conc. (mg/L)	% removal	Initial Conc. (mg/L)	% removal
0	240	0	240	0
3	223	7	188	21.7
6	218	9.2	165	31.3
9	192	20	133	44.6
12	179	25.4	109	54.6
15	140	41.7	97	59.6

Data are expressed as mean of three replicate ± SD (standard deviation).

3.6. Effects of temperature on BOD and COD removal

It was observed that temperature had significant impact on the elimination of BOD and COD by *Chlorella sp.* The temperature of the experimental set up varied between 15°C to 35°C showed that the best removal efficiency ranged between 25-30°C for all the chemical parameters examined (Fig.4).

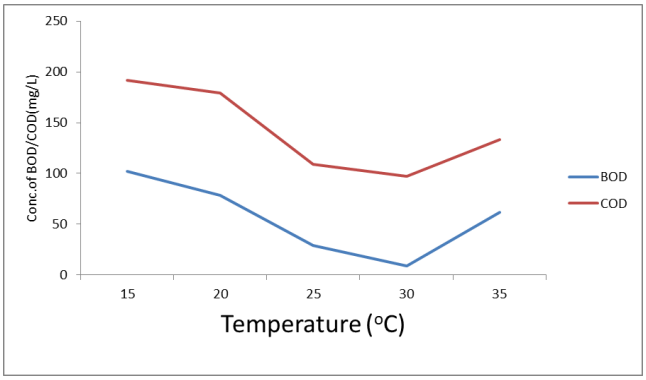

Figure 4 Changes in BOD/COD concentration with temperature

3.7. Effects of temperature phosphorus removal

As seen for BOD and COD removal, highest phosphorus removal by *Chlorella* sp. was recorded at 30°C (Fig. 5).

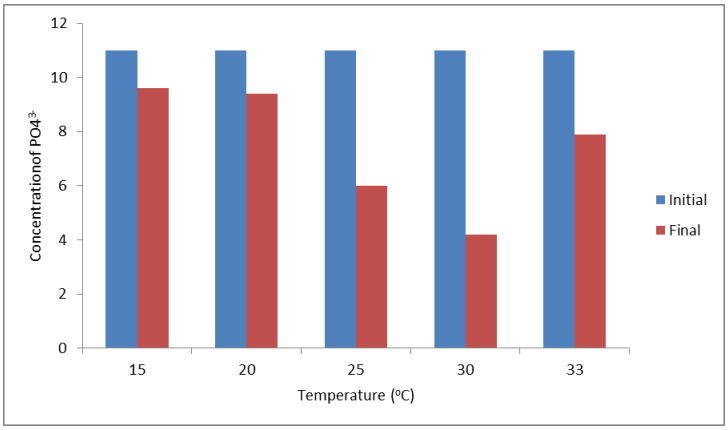

Figure 5 Concentration of $PO4^{3-}$ at varying temperatures

The removal of Phosphorus, BOD and COD from the effluent in this study showed strong dependence on factors including temperature, aeration, cultivation time and photoperiods. It was recorded that temperatures between 25-30°C were optimum for the reduction/ elimination of these chemical parameters from wastewater. This shows there is a relationship between temperature and growth rate whereby growth rate tend to increase with increasing temperature of growth until it reaches a maximum value of (30°C in this case) and then begin to decline. This implies that, beyond 30°C, the activity of microorganism (*Chlorella* sp) begins to decrease hence less BOD, COD and $PO4^{3-}$uptake. This corroborates a study by [16] where maximum removal of COD, BOD, nitrogen and phosphorus from industrial wastewater was obtained at 30°C

Similarly, cultivation period (days) had significant effect on the removal efficiency of *Chlorella sp*. Peak activities were observed at the exponential phase in both sewage effluent and control. At this time, *Chlorella* sp had fully adapted to medium and rapidly utilizing its constituent for its growth and other metabolic activity, hence, the efficiency of nutrient removal is increased. This means phosphorus and some other oxidizable organic matter present in the effluent serve as nutrient for *Chlorella* sp growth. [17, 18] had previously reported optimum removal of chemical contaminants from wastewater at exponential phase of growth of Algal species. At the elapse of the experimental period, 61.8% and 28.2% phosphorus removal was recorded in test and control medium respectively. *Chlorella* sp is capable of taking up phosphorus as inorganic orthophosphate ($PO4^{3-}$) because it serves as a micro-nutrient essential for growth. Though satisfactory, this finding compared to studies by [4, 19, 20] which reported up to 80.9%, 87% and 90% phosphorus removal by *Chlorella* sp is relatively low. The average percentage BOD reduction calculated for the present study is 92.8 % and 39.8% respectively for the test and control samples while COD gave 59.6% and 41.7% respectively. This implies Chlorella is more efficient in BOD removal compared to COD. This is probably due to high concentration of xenobiotic compounds elevating the COD of the wastewater which remains unaffected by microflora [21] or a pointer that sufficient oxygen needed to oxidize all organic material in the sewage effluent was not adequately supplied. From a study conducted by [22], they observed that aeration facilitated algal biomass due to enhanced photosynthetic activity of the algal, thereby releasing more oxygen for COD removal. Likewise, [4] recorded maximum reduction efficiencies of 90.8% and 80.1% by *Chlorella* sp after 20 days of incubation period.

4. Conclusion

This study showed that microalga (Chlorella sp.) can be cultivated in sewage effluents with appreciable removal of phosphorus, COD and BOD. To improve the efficiencies of this removal, physical factors such as temperature; cultivation periods and light intensity must be optimized. Hence, at optimal conditions, Chlorella sp. offers an excellent, cost effective and eco- friendly technology for the treatment of municipal and industrial wastewater.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interests.

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