**Bioprocess Engineering**
2017; 1(3): 69-76
http://www.sciencepublishinggroup.com/j/be
doi: 10.11648/j.be.20170103.12

# The Wastewater Nutrient Removal Efficiences of *Chlorella sorokiniana* and *Scenedesmus obtusiusculus*

**Bryant Isaac Mbir, Appah John Kwame Mensah**

Department of Environmental Science, University of Cape Coast, Cape Coast, Ghana

**Email address:**
ibryant@ucc.edu.gh (B. I. Mbir)

**To cite this article:**
Bryant Isaac Mbir, Appah John Kwame Mensah. The Wastewater Nutrient Removal Efficiences of *Chlorella sorokiniana* and *Scenedesmus obtusiusculus*. *Bioprocess Engineering*. Vol. 1, No. 3, 2017, pp. 69-76. doi: 10.11648/j.be.20170103.12

**Received**: April 21, 2017; **Accepted**: May 27, 2017; **Published**: July 3, 2017

**Abstract:** Urine treatment and nutrient removal was studied on a pilot scale in the DESAH building for a period of 3 months. The essence of the study was to evaluate the practical nutrient removal efficiencies of *Chlorella sorokiniana* and *Scenedesmus obtusiusculus*. The microalgae were grown on 3 different media— namely; mixture (mixed treated and untreated urine), untreated urine and control, and their nutrient removal efficiencies were investigated. Urine that has passed through the OLAND RBC system served as treated urine, and Bold's basal medium served as the control. The OLAND RBC system was able to remove 95.7% of total chemical oxygen demand (COD), 27.1% total nitrogen, 99.7% ammonium, 88.6% total phosphorus and 89.3% ortho-phosphate from the influent urine. Low nutrient removal performance at a very high N: P molar ratios were observed in microalgae in the untreated urine. However, the nutrient removal capacities of microalgae were very high at reduced N: P molar ratios in the mixed medium. *Chlorella sorokiniana* was able to remove 63.2% TN and 55.8% TP at a low N: P molar ratio of 8.5:1, while *Scenedesmus obtusiusculus* removed 45.9% TN and 76.3% TP at an N: P molar ratio of 6.9:1. The results indicate that nutrient removal by microalgae is most efficient in mixed OLAND RBC treated and untreated urine culture. Therefore, the integration of the OLAND RBC system when designing microalgae induced wastewater treatment technologies for sanitation purposes is advocated.

**Keywords:** Urine Treatment, Nutrient Removal, Microalgae Cultivation, Domestic Wastewater

## 1. Introduction

The recycling and reuse concept of sanitation has created the paradigm that nothing is waste. As such, we must not perceive domestic wastewater which contains valuable resources in terms of nutrients and energy as waste. The biomass of microalgae cultivated on wastewater practically offers an alternative to a sustainable renewable biodiesel production in the future. Seventy% of potential energy in the form of chemical oxygen demand (COD) and 80 - 95% of nutrients can be removed from domestic wastewater and reused [1]. Domestic wastewater streams can be characterised into: black water—faeces and urine; grey water—shower, kitchen, laundry water, and organic kitchen waste. The highly concentrated black water can be diverted at source into its component faeces (brown water) and urine (yellow water). Quantitatively, in a day, a healthy adult human produces 1.5 L of urine, 0.17 L of faeces and 0.2 L of kitchen waste [2]. From these streams 90% of nitrogen, 80% each of phosphate and potassium and 70% of COD can be removed. Urine has highest nutrient content, including, pathogens, pharmaceuticals and hormone residues as well as high salt concentrations [2]. The nutrient composition of urine, including 50 – 70% phosphorus, 80 – 90% nitrogen, 60% potassium and 7% ammonia are all soluble in water [3, 4]. Any state-of-the-art wastewater treatment technology that integrates the decentralized sanitation and reuse (DESAR) concept as a target for practical wastewater nutrients removal avails itself to more sustainable and economical sanitation practices [5]. Furthermore, source-separation treatment techniques concentrate the wastewater to maximise energy recovery and higher nutrient removal or nutrient incorporation into the cells of microalgae, in physical chemical treatments coupled with anaerobic treatment techniques. Oxygen-limited autotrophic nitrification/denitrification (OLAND) rotating biological contactor (RBC) urine collection and treatment system is designed to effectively and efficiently remove substantial amounts of nutrients from wastewater at a fully functional state, leading to a discharge of effluents that complies with and

meet environmental standards. Besides, the treated effluents can be used to irrigate food crops in agricultural farms.

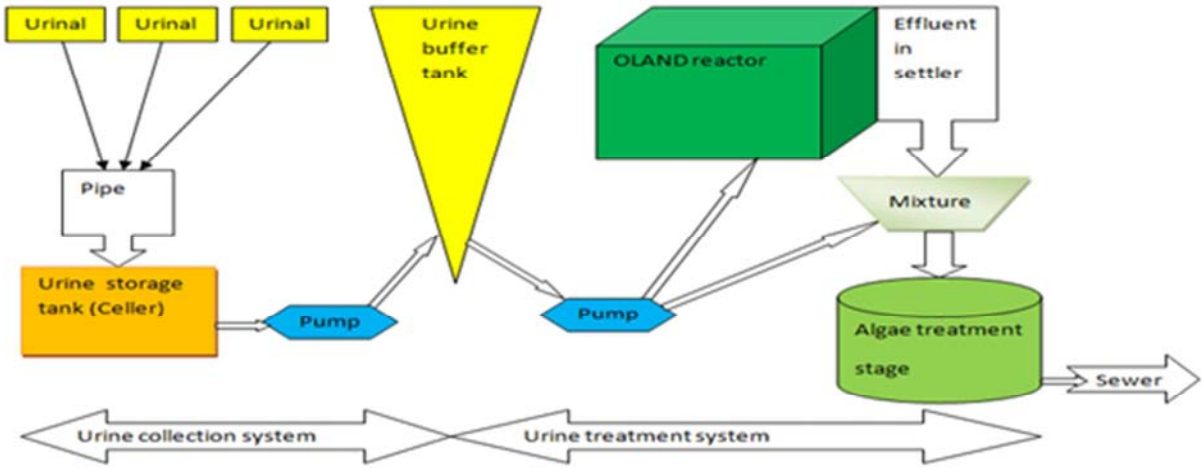


***Figure 1.*** *A pilot scale OLAND RBC urine collection and treatment system.*

The DESAR concept focuses on source-separation-based approach of treating domestic wastewater with resource recovery [6, 7]. The DESAR concept is applicable to urine treatment technologies which have been developed for use. This is because the separation and management of urine from domestic waste streams depends on source-separation. *Chlorella sorokiniana* and *Scenedesmus obtusiusculus* have been effectively and successfully used to remove COD, nitrogen and phosphorus in piggery and municipal wastewaters [8]. However, wastewater nutrient removal efficiency of microalgae is affected by light, temperature, pH and molar concentrations of nitrogen and phosphorus [9, 10, 11]. Hitherto, few known studies have reported on the exact N: P ratio for algae cultivation and phosphorus removal. As such, this study aims to investigate the possibility of removing phosphorus using microalgae at a defined N: P ratio, thereby making the nutrients available to microalgae for energy recovery and nutrient reuse.

## 2. Materials and Methods

The pilot scale OLAND RBC (Figure 1) has length of 1.77 m, width 0.84 m and height 0.42 m with a semi-cylindrical basal perimeter of 1.19 m. It has four rotating basket discs with a space of 0.11 m each in between them. Each rotating disc has a diameter of 0.68 m and width 0.26 m, and filled with carrying materials which occupy 19.23% of the total volume of the disc. The RBC has total volume of 0.95 $m^3$ while its operating volume is 0.39 $m^3$. Undiluted urine was collected via urimat waterless urinals in the offices of Landustrie and stored in a cellar of the building daily. The stored urine was pumped using Homa PS H11-W/K30/60R pump to a urine buffer tank of capacity 0.86 $m^3$ and stored for treatment. The urine stored in the buffer tank was pumped to an OLAND RBC which operated at an average hydraulic retention time (HRT) of 14.9 days with loading rates of 0.34 Kg TN $m^{-3}$ $d^{-1}$ and 0.02 Kg TP $m^{-3}$ $d^{-1}$. The effluents from the OLAND RBC are post-treated with microalgae for further nutrients removal.

***Table 1.*** *Concentrations of nutrients present in influent urine and OLAND effluent.*

| Parameter (mg/l) | Influent Urine±s.d | OLAND RBC effluent±s.d | % removal |
|---|---|---|---|
| COD | 5560.7±549.28 | 239.7±108.48 | 95.7 |
| Total Nitrogen | 4518.7±329.27 | 3293.0±821.28 | 27.1 |
| Ammonium | 4110.0±540.58 | 11.4±20.30 | 99.7 |
| Nitrate | 6.29±1.54 | 3075.5±386.10 | n.d |
| Nitrite | 0.16±0.10 | 0.26±0.52 | n.d |
| Total Phosporus | 241.0±24.04 | 27.4±17.14 | 88.6 |
| Ortho-phosphate | 225.1±30.71 | 24.0±16.53 | 89.3 |
| Dissolved Oxygen | n.d | 2.62±1.91 | n.d |

s.d= standard deviation, n.d= not detected

### *2.1. Analyses of Influent and Effluent Urine Samples*

Filtered (0.45 µm PTFE filter) and unfiltered samples of both influent and effluent urine were measured. Hach Lange kits, from Germany were used to measure ortho-phosphate, nitrate, nitrite, ammonia-nitrogen, and total phosphorus, COD and total nitrogen. The pH of influent and effluent samples with a pH 211 microprocessor pH meter were also measured. All the mentioned parameters were measured every week.

### *2.2. Chlorella sorokiniana and Scenedesmus obtusiusculus Culture Conditions*

Microalage were obtained from the culture collection of algae and protozoa, Oban, UK. A single colony of *Chlorella sorokiniana* from an agar plate was grown on 0.0065 M Bold's Basal medium. A 250 ml Erlenmeyer flask containing the culture was placed on a magnetic stirrer at 350 rpm under two white Sylvania Grolux fluorescent lights (54 W) throughout the study. We used a digital pH controller (pH-201 with HI/LO Action Optional), which supplied the culture with $CO_2$, to buffer the pH. The initial pH of the photobioreactor containing *Chlorella sorokiniana* was adjusted with 2 M HCl and measured to be 6.8. The same procedure was repeated for *Scenedesmus obtusiusculus* with

an initial pH of 6.8. We prepared both cultures on the same day and determined the optical densities (OD) from 5 ml of the start cultures at 450 nm, 680 nm and 750 nm to observe the growth of the microalgae. The ODs for the two microalgae were determined every day. Five ml each of the filtered algal culture was wet-mounted on a slide and observed under a microscope.

*Preparation of Mixed Treated (OLAND RBC) and Untreated Urine at Defined N: P Ratio*

Microalgae thrive on wastewater with N: P molar ratio of 16:1 [12]. A mixture of treated urine from OLAND, and untreated urine with N: P ratio of 16:1 was prepared according to the formula:

$$V_2 = \frac{R_3V_1 - R_1V_1}{R_2 - R_3}$$

where $V_2$ is volume of untreated urine to be added to the treated urine to attain N: P ratio of 16:1; $V_1$ the volume of treated urine; $R_3$ the desired ratio of nitrogen to be achieved; $R_1$ the ratio of nitrogen in the treated urine from OLAND RBC, and $R_2$ the ratio of nitrogen in the untreated urine.

***Table 2.*** *Concentration of nitrogen and phosphorus in the 3 media used for culturing Scenedesmus obtusiusculus before and after dilution.*

| Nutrient concentrations (mg/l) | | Culture | | | | | |
|---|---|---|---|---|---|---|---|
| | | Before dilution | | | After dilution | | |
| | | control | untreated | mixed | control | untreated | mixed |
| Nitrogen | Initial | 132 | 425 | 362.5 | 49.7 | 197.6 | 119.3 |
| | Final | 108 | 411 | 232 | 3.96 | 190.0 | 64.5 |
| Phosphorus | Initial | 55.4 | 23.9 | 24.9 | 23.7 | 12.8 | 17.3 |
| | Final | 32.9 | 21.5 | 23.8 | 21.2 | 8.97 | 4.1 |

### *2.3. Nutrient Removal by Microalgae*

#### *2.3.1. Nutrients Removal with Scenedesmus obtusiusculus*

*Scenedesmus obtusiusculus* was grown on: a) a mixed untreated and treated urine culture, b) an untreated urine and c) a control, to assess nutrients removal efficiencies and the N: P ratios at which maximum nutrients removal was achieved. The nutrients composition of the mixed treated and untreated urine was measured after the solution had been diluted 10 times. We determined the initial nutrient concentrations before inoculation and subsequently inoculated the solution with *Scenedesmus obtusiusculus* at 0.1 cells at $OD_{750}$. The procedures were repeated for the control and untreated urine samples and their nutrient compositions were measured before inoculation with *Scenedesmus obtusiusculus*. However, before the untreated urine was inoculated, 1 ml each of trace metals in the form of $ZnSO_4.7H_2O$, $MnCl_2.4H_2O$, $CuSO_4.5H_2O$, $Co(NO_3)_2.6H_2O$ and $Na_2MoO_4.2H_2O$ were added. After the sixth day, we measured the nutrients composition of the filtrates from the mixed culture, untreated urine and control, because light had become limiting at this time. We twice diluted the cultures continuously for 12 days for the control and the untreated urine, and 25 days for the mixed culture. The microalgae were observed under a high powered microscope (Axio Zeiss Lab A.1) at the end of the study.

#### *2.3.2. Nutrients Removal with Chlorella sorokiniana*

The procedures for urine treatment and nutrient removal with *Chlorella sorokiniana* was done as for *Scenedesmus obtusiusculus.* But in the case of *Chlorella sorokiniana,* light became limiting after 7 days. And the cultures were diluted 5 times, continuously for 6 days. Moreover, no trace metal was added to the untreated urine before inoculation with *Chlorella sorokiniana.* The nutrients compositions of the mixed culture, untreated urine and control cultures were analyzed at the end of the study.

#### *2.3.3. Nutrients Removal Efficiencies of Microalgae*

To compare the nutrients removal efficiencies of the microalgae, two media of similar nutrient concentrations which served as control for the microalgae were prepared. Similarly, mixtures of untreated and treated urine at N: P molar ratios of 11:1 to measure the nutrient removal capacities of *Chlorella sorokiniana* and *Scenedesmus obtusiusculus* were prepared. The pH, light and $CO_2$ were kept constant for the microalgae. The initial concentrations of both the control and mixed sample before inoculation were measured. For *Chlorella sorokiniana,* the media was inoculated at 0.044 cells for the control and 0.050 cells for the mixed culture at $OD_{750}$. The media of *Scenedesmus obtusiusculus* were inoculated at 0.066 cells for the control and 0.065 cells for the mixed culture at $OD_{750}$. At the end of the exponential growth phases of the microalgae, the filtrates of all the cultures were analysed. The nutrients removal efficiencies were estimated from:

$$\%\ N_r = \frac{N_1 - N_2}{N_1}(100)$$

where $N_r$ is the nutrient removal; $N_2$ the final nutrient concentration and $N_1$ is the initial nutrient concentration.

## 3. Results

### *3.1. Treatment of Urine Using OLAND RBC*

Table 1 show that 95.7% of the organic matter present in the urine in the form of Chemical Oxygen Demand (COD) was removed in the OLAND RBC. Ninety-one% of the total nitrogen in the influent urine was available as ammonium ($NH_4^+$), 0.14% as nitrate ($NO_3^{2-}$) and 0.004% as nitrite ($NO_2^-$), respectively (Table 1). Ortho-phosphate constituted 93% of the phosphorus present (Table 1). It was noticed that phosphorus removal was more than nitrogen removal in the

OLAND RBC (Table 1). The concentrations of nitrogen and phosphorus in the undiluted human urine used in this study were similar to what is reported in literature [13, 14].

### *3.2. Nutrients Removal by Microalgae*

Different microalgae species have different preferences for nutrients uptake and this affects their nutrients removal efficiencies. Initial concentrations of nitrogen and phosphorus in the 3 media used for culturing *Scenedesmus obtusiusculus* varied from the final concentrations after the growth experiments, suggesting there have been nutrients removal. Removal of nitrogen by *Scenedesmus obtusiusculus* in the diluted media was highest in the control medium and lowest in the untreated urine (Table 2). Phosphorus removal was in the order: mixture > untreated > control (Table 2). *Scenedesmus obtusiusculus* preferred more phosphorus than nitrogen in the untreated urine and mixed culture (Table 2). Ammonium was the dominant nitrogen source in the mixed treated and untreated urine.

***Table 3.*** *Performance of Chlorella sorokiniana and Scenedesmus obtusiusculus for nutrient removal on 3 different diluted media.*

| Culture | *Chlorella sorokiniana* | | | | *Scenedesmus obtusiusculus* | | | |
|---|---|---|---|---|---|---|---|---|
| | N: P ratio | | % removal | | N: P ratio | | % removal | |
| | Initial | Final | N | P | Initial | Final | N | P |
| control | 1.7:1 | 0.02:1 | 100 | 63.8 | 2.1:1 | 0.19:1 | 92 | 10.5 |
| untreated | 22.7:1 | 17:01 | 21.3 | 48.6 | 15.4:1 | 21.2:1 | 3.8 | 29.9 |
| mixed | 8.5:1 | 7.1:1 | 63.2 | 55.8 | 6.9:1 | 15.7:1 | 45.9 | 76.3 |

Table 3 shows that for *Scenedesmus obtusiusculus,* the N: P ratio increased from 6.9:1 to 15.7:1 in the mixed untreated and treated urine culture, and 15.4:1 to 21.2:1 in the untreated urine. However, for *Chlorella sorokiniana*, the N: P ratio dropped from 8.5:1 to 7.1:1 in the mixed treated and untreated urine culture, and 22.1:1 to 17.0:1 in the untreated urine culture (Table 3). Phosphorus uptake by *Senedesmus obtusiusculus* was higher than nitrogen uptake, but

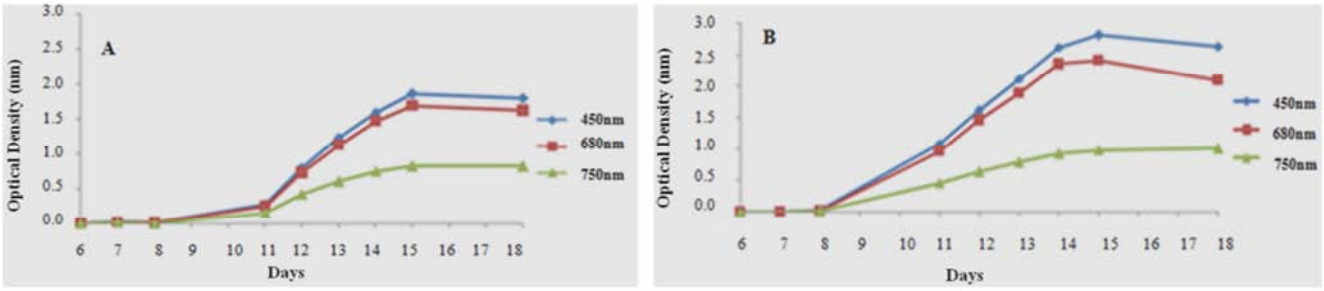


***Figure 2.*** *Growth rates of microalgae on Bold's Basal medium during a period of 12 days A) Chlorella sorokiniana on B) Scenedesmus obtusiusculus.*

nitrogen removal was more compared to phosphorus removal for the mixed culture in *Chlorella sorokiniana* (Table 3). Growth of *Scenedesmus obtusiusculus* on mixed treated and untreated urine was steep. Different N: P ratios influenced different nutrient uptake by *Chlorella sorokiniana* and *Scenedesmus obtusiusculus*. Nutrients removal by microalgae in the control medium was more than in the mixed culture (Table 3). *Chlorella sorokiniana* removed more nitrogen at N: P ratio of 7.2:1 for the mixed culture, while *Scenedesmus obtusiusculus* removed more phosphorus at N: P ratio of 7.6:1 in the mixed culture (Table 3). Nutrients removal by *Chlorella sorokiniana* within all cultures was in the order: control > mixed culture > untreated culture (Table 4). *Chlorella sorokiniana* preferred more phosphorus than nitrogen in the untreated urine compared to the control and mixed cultures (Table 4). After 5 days of growth at $OD_{750}$, there were only 0.18 cells of *Chlorella sorokiniana*, which increased gradually until the tenth day and stabilized (Figure 2A). But, there were 0.4 cells of *Scenedesmus obtusiusculus* after 5 days at $OD_{750}$, which increased gradually until the tenth day (Figure 2B). *Scenedesmus obtusiusculus* cells increased more rapidly than *Chlorella sorokiniana* cells in the growth medium. The growth curve of Figure 2 reveals that the microalgae levelled-off after 10 days, with *Scenedesmus obtusiusculus* recording 1.0 cells compared to 0.9 cells counted for *Chlorella sorokiniana* at $OD_{750}$.

***Table 4.*** *Concentration of nitrogen and phosphorus in the three media used for culturing Chlorella sorokiniana before and after dilution.*

| Nutrient concentrations (mg/l) | | Culture | | | | | |
|---|---|---|---|---|---|---|---|
| | | Before dilution | | | After dilution | | |
| | | control | untreated | mixed | control | untreated | mixed |
| Nitrogen | Initial | 92 | 487.5 | 286 | 18.4 | 97.5 | 57 |
| | Final | n.d | n.d | n.d | n.d | 76.7 | 21 |
| Phosphorus | Initial | 54.3 | 21.5 | 33.6 | 10.9 | 4.3 | 6.7 |
| | Final | n.d | n.d | n.d | 3.9 | 4.5 | 3 |

n.d= not detected

Figure 3A shows the growth of *Chlorella sorokiniana* on untreated urine. Growth of *Chlorella sorokiniana* was slow and gentle on the untreated urine before it was diluted. The sudden dips in the growth curves for the 3 wavelengths were observed because the culture was diluted 5 times to delay depletion of the nutrients (Figure 3A). *Chlorella sorokiniana* grew gradually after the dilution but stopped growing a few days afterwards. High $NH_3$ concentration in the untreated urine may have inhibited the growth of *Chlorella sorokiniana* [21]. The growth of *Chlorella sorokiniana* was steep and rapid in the mixed culture until diluted, which eventually contributed to the slumps in the growth curves (Figure 3B). The growth of *Chlorella sorokiniana* was steep immediately after dilution but steadied a few days afterwards (Figure 3B). Figure 4A shows the growth rate of *Scenedesmus obtusiusculus* on untreated urine with an increase in N: P ratio from 17.8:1 to 19.1:1. After dilution, the growth slumped and picked up a few days afterwards. Generally, the untreated urine culture did not support the growth of *Scenedesmus obtusiusculus.* The growth slumped the first time because light became limiting. Moreover, insufficient $CO_2$ supply increased the pH to 8.1 and resulted in the drop in growth a second time (Figure 4B). It is possible that the cells lacked a carbon source for their photosynthetic activities. High uptake of nitrogen in the mixed culture occurred in the first 4 days for both *Chlorella sorokiniana* (Figure 5B) and *Scenedesmus obtusiusculus* (Figure 5A) but the nutrients uptake in *Scenedesmus obtusiusculus* did not result in sharp growth. There was a gradual increase in growth of *Scenedesmus obtusiusculus* after addition of magnesium, however. Uptake of phosphorus was gradual in both cultures compared to nitrogen (Figures 5A and B).

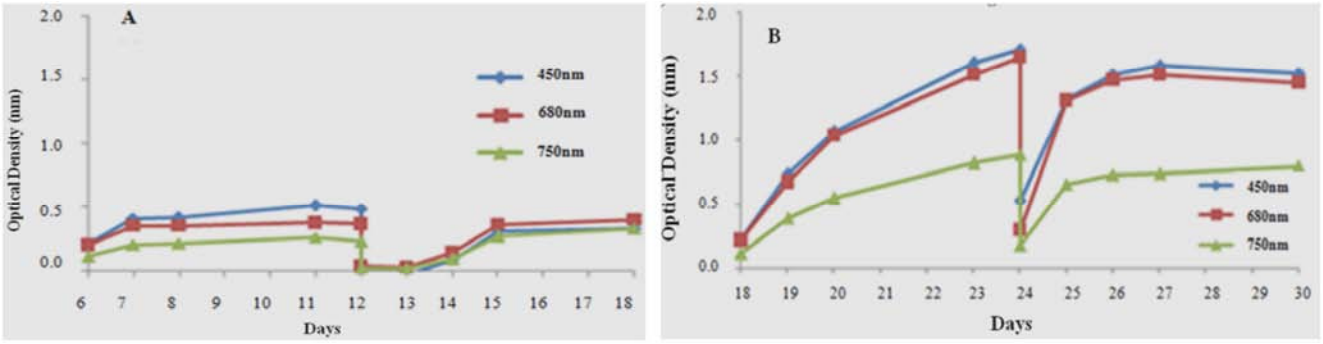


***Figure 3.*** *Growth of Chlorella sorokiniana in a batch culture for 12 days A) untreated urine culture B) mixed treated and untreated urine culture.*

# 4. Discussion

### *4.1. Treatment of Urine with OLAND RBC*

The OLAND RBC is very efficient in removing excess nitrogen. According to [15] and [16], a fully functional OLAND RBC can remove 500 to 2000 mg N $L_{reactor}^{-1}$ $d^{-1}$ of nitrogen, corresponding to 84% of TN in influent urine. However, in this study, 88.9 mg N $L_{reactor}^{-1}$ $d^{-1}$ of total nitrogen was removed by the OLAND RBC, representing 27.1% of total nitrogen in the influent urine. This suggests that the OLAND RBC used in this study is not fully functional yet. Furthermore, stabilized biofilm affected the efficiency of OLAND RBC for phosphorus removal.

### *4.2. Nutrient Removal by Microalgae and Algal Growth*

#### *4.2.1. Nutrient Removal by Chlorella sorokiniana*

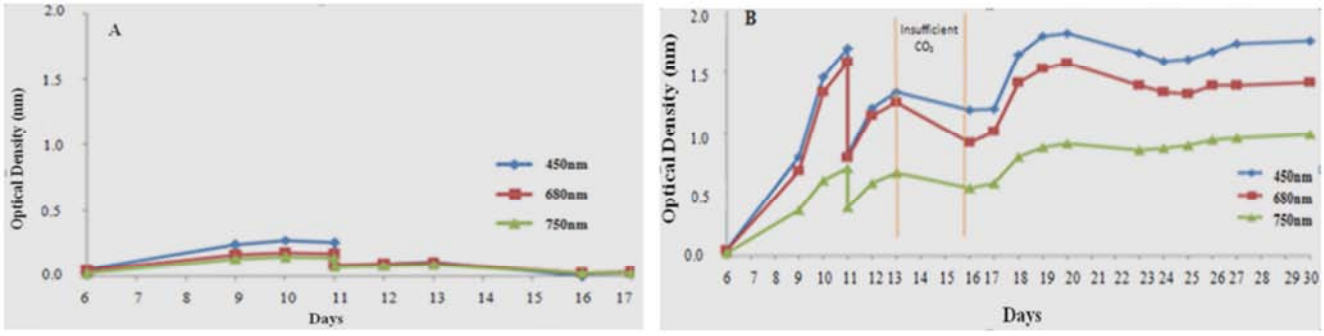


***Figure 4.*** *Growth of Scenedesmus obtusiusculus in a batch culture A) untreated urine culture for 11 days B) mixed untreated and treated urine culture for 23 days.*

The type of nutrient that is taken up more by microalgae is the one required for maintenance and growth [17]. From Figures 2A and B, growth of *Chlorella sorokiniana* was slower because few cells were inoculated. The growth for *Chlorella sorokiniana* was rapid and steep at day 6 but light became limiting at day 9 even though 50% of the nutrients remained after 12 days of growth. Elevated levels of $NH_4^+$ (350 mg/l) in the untreated urine medium raised the pH of the solution. A high $NH_4^+$ concentration suggests that $NH_4^+$ was not the only nitrogen source used by the microalgae, although there was discernibly rapid and steep uptake by *Chlorella sorokiniana* during the first 4 days (Figure 5B). High pH causes the reaction to favour more $NH_3$ production from $NH_4^+$ [14]. As a result, the mediated $NO_2/NO_3$ formation processes may have become affected, and availed more $NO_3$ to the microalgae [18]. Microalgae can also assimilate $NO_3$ as a nitrogen source. Also, we recognize $NH_3$ stripping as one of the processes that may deplete nitrogen in a microalgae wastewater treatment system [19, 20]. In addition, elevated free $NH_3$ affects the photosynthetic capabilities of microalgae [21, 14, 12], and further raises the pH of the solution. However, [18] reported that *Chlorella sp* and *Scenedesmus sp* performed better at $NH_3$ concentrations of 30 – 300 mg/l and at pH of 8, but in a different culture condition. We were unable to quantify the exact amount of phosphorus removed by *Chlorella sorokiniana* at such high pH, because phosphorus precipitation occurs at high pH of 8.9 − 9 [22, 14], and depends as well on the calcium and magnesium levels in the wastewater [23, 13, 14]. Thus the high phosphorus concentrations depleted could be attributed to both microalgae metabolic uptake and chemical phosphorus precipitation. Growth of *Chlorella sorokiniana* on the mixed culture stopped after the 3rd day possibly because of trace nutrients limitation since there were some amounts of both nitrogen and phosphorus. This is inferred from the fact that, addition of magnesium gave a slight increase in the growth of microalgae [10, 13]. Thus an inhibitory factor instead of nutrient [14] could have resulted in the decline of the growth of *Chlorella sorokiniana* on the mixed culture (Figure 3A). *Chlorella sorokiniana* removed more nitrogen in the mixed culture but less nitrogen in the untreated medium, indicating that *Chlorella sorokiniana* thrives in nitrogen rich environment as the mixed culture contained high concentrations of nitrogen. The microalgae tested on the mixed culture of treated and untreated urine exhibited disproportionate nutrients removal at different molar N: P ratios. The highly unbalanced molar N: P ratios and the culture conditions could account for the nutrients depletion patterns observed in the microalgae on the different media. [24] revealed that the minimum nitrogen and phosphorus concentrations that support microalgae growth are 14 mg/l and 1.55 mg/l, respectively. *Chlorella sorokiniana* was able to remove more total nitrogen (63.2%) and phosphorus (55.8%) at a low N: P molar ratio of 8.5:1. However, we observed a drop in the nutrient removal activity of *Chlorella sorokiniana* at a very high N: P ratio in the untreated urine. A high N: P ratio is evidence that phosphorus is limiting and may affect algal growth and nutrient removal [13]. *Chlorella sorokiniana* removed 21% and 49% of total nitrogen and total phosphorus, respectively, at a high N: P ratio of 22.5: 1. [13] reports a peak algal biomass and nutrient removal at N: P ratio of 23:1 and magnesium concentrations of 1.5 – 1.8 mg $Mg^{2+}$ g $biomass^{-1}$. Nutrients removal efficiency of microalgae in diluted and undiluted human urine has been investigated by several researchers [14], 60.0 - 80.4% N and 60.9 - 96.6% P; [13], 36 – 87% N and 57 – 100% P; [25], 75 – 85% N. In *Chlorella sorokiniana,* N: P molar ratio was not observed to influence the direction of nutrient removal in the control as similar N: P ratio was used before and after dilution.

#### *4.2.2. Nutrient Removal by Scenedesmus obtusiusculus*

Many *Scenedesmus obtusiusculus* cells were inoculated, giving rise to high pigment detection and more cells. The microalgae exhibited rapid growth on the third day. However, its growth was limited by light on day 9. Increased $NH_4^+$ concentrations of 400 mg/l raised the pH of the culture and consequently, showed similar associated deleterious effects of increased pH and $NH_3$ levels observed in the *C. sorokiana* culture. *Scenedesmus obtusiusculus* removed more phosphorus in the mixed culture compared to *Chlorella sorokiniana*. In the untreated urine, 29.9% phosphorus and 3.8% nitrogen was removed by *S. obtusiusculus* at N: P ratio of 21.2:1. Similarly, higher phosphorus removal was achieved in the mixed culture, 76.3% total phosphorus and 45.9% total nitrogen at N: P molar ratio of 6.9:1. Possibly, more phosphorus was assimilated and incorporated in the cells of the microalgae to maintain optimal growth when light became limiting. But, after the culture was diluted, more nitrogen was taken up than phosphorus. Thus, light limitation favoured the removal of phosphorus by *S. obtusiusculus* in the untreated urine, while abundant light favoured removal of nitrogen by both *Chlorella sorokiniana* and *Scenedesmus obtusiusculus*. There was disproportionate removal of nutrients in the control culture. Nutrients uptake by microalgae depends on the concentrations and availability of a particular nutrient [10] as well as the N: P molar ratio. Comparing the molar N: P ratios between mixed treated and untreated urine culture and the control, we noticed that the nutrient removal efficiency in the control were higher. Other factors such as free $NH_3$ concentration, light, temperature, micronutrients and $CO_2$ concentration could be influencing the nutrients uptake in urine by the microalgae [9, 10, 13].

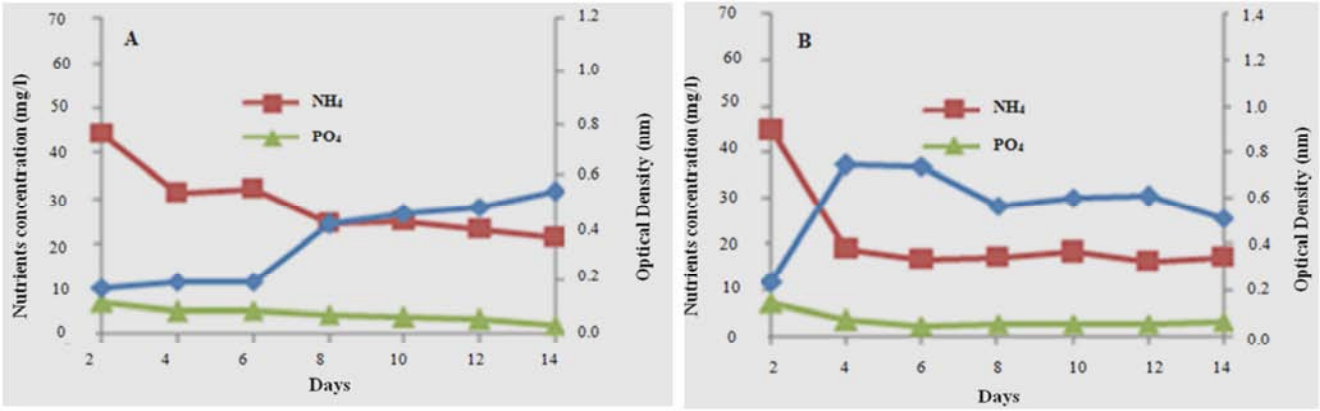


***Figure 5.*** *Relationship of nutrients uptake and growth rate of microalgae in a batch experiment for 14 days A) Scenedesmus obtusiusculus on mixture B) Chlorella sorokiniana.*

### *4.3. Microalgae Growth*

Figures 2, 3 and 4 reveal the general characteristic growth phases of the microalgae: the lag phase, exponential phase, stationary phase and the decline phase. Microalgae cultured in the Bold's basal medium exhibited a lag phase whiles growth in the undiluted and mixed culture revealed no lag phase. A decline phase was not noticed by the end of the study. The dearth of a lag phase is indicative that the microalgae may have already entered exponential phase at the time of inoculation, and also demonstrate their adaptability in the undiluted and mixed culture. Furthermore, the absence of lag phase makes the two test cultures relatively promising, in that, nutrient removal activity becomes rapid and starts immediately after inoculation into those media. In the study, [13] found that the growth of *Chlorella sorokiniana* is enhanced on undiluted human urine, which our findings affirm. It was found that the undiluted and mixed culture supported the growth of *Chlorella sorokiniana* more than *Scenedesmus obtusiusculus* except for the Bold's basal medium. It is noteworthy that the different cultures in this study were not sterilised before inoculation and so microalgae may enter into competition with other already existing microorganisms. Their growth may have been compromised also because of toxic micropollutants and pathogens that exist in yellow water streams [13].

## 5. Conclusion

The findings from the study show that pre-treatment of urine with OLAND RBC and post-treatment with microalgae for excess nitrogen and phosphorus removal is effective and economical. Generally, *Chlorella sorokiniana* removed more nutrients from both the control medium and the untreated urine than *Scenedesmus obtusiusculus*. But, for the mixed treated and untreated urine culture, *Chlorella sorokiniana* removed more nitrogen, while *Scenedesmus obtusiusculus* removed more phosphorus. Nutrients preferences and uptake by the microalgae is influenced by the molar N: P ratio and nutrients removal rate. As such, more studies need to be done to ascertain the exact N: P ratio in urine for optimal microalgae growth and nutrients removal. The combined activity of OLAND RBC and microalgae on practical wastewater nutrient removal will reduce drastically the concentration of excess nitrogen and phosphorus that will be discharged into the various compartments of the environment, and can even find use in agricultural farms as irrigation water.

## Acknowledgement

Sincere thanks goes to Mrss Brendo Meulman, Nico Classens and Ms. Elizabeth Wiersma (all of DeSaH) for their continuous guidance and support during my laboratory set-ups and analyses. Without you, this work would not have been successful. Sincere thanks also go to the Netherlands Government for granting me the Netherlands Fellowship Programme (NFP-NUFFIC) award to pursue M.Sc. in the Netherlands.

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