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# Nutrient Recycling by *Chlorella vulgaris* from Septage Effluent of the Bangkok City, Thailand

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**ABSTRACT:** Only a small amount of septage (1,200 $m^3$/day) from the Bangkok Metropolitan Region (BMR), Thailand, undergoes secondary treatment at a central wastewater treatment plant. The Nongkhaem Nightsoil Treatment Plant, however, has its treating capacity only 600 $m^3$/day. More often, the effluent from the plant fails to meet with the industrial effluent standards as well as building effluent standards set by the government, especially for total kjeldahl nitrogen and total phosphorus. Thus, recycling nutrients back from this effluent before discharging to the public drains by waste reclamation via algal culture is of interest to improve water resource assimilation capacity. This study aimed to investigate bio-engineering factors such as light intensity and hydraulic retention time which influence nutrient uptake and growth development of *Chlorella vulgaris* in treated septage from the Nongkhaem plant. In a series of batch cultures of *Chlorella vulgaris* starting from 100 mg/L of biomass density, there was a significant increase in algal growth ($p<0.05$) with increasing light intensity (3,000, 5,000, and 8,000 lux). The culture conducted under natural sunlight (2,500-9,000 lux), however, gave the maximum algal and chlorophyll production within four days (390 mg/L and 5.8 mg/L, respectively), whereas the removal percentages of total Kjeldahl nitrogen (TKN) and total phosphorus (TP) were approximately 62% and 55%, respectively. Conversion of TKN into algal cell protein was equivalent to 24% and the water remaining after algal harvested after 4 days met Thai government effluent quality standards. It could be concluded that *Chlorella vulgaris* has the potential to be incorporated into a program of septage wastewater treatment for nutrients (N and P) polishing since only a short period (4-7 days) is required for the algal development to reach its full growth. However, harvesting of algae is necessary and should be done before it starts to decompose and releases those nutrients as well as organic substances to the effluent again.

**KEYWORDS:** *Chlorella vulgaris*, Light Intensity, Retention Time, Treated Septage, Nutrient Recycling.

## INTRODUCTION

More than 80% of wastewater directly discharged into the rivers of the Bangkok Metropolitan Region (BMR) consists of domestic wastewater, including that from toilets. Most of the wastewater has received primary treatment in septic tanks, which results in low treatment efficiency, and further treatment is needed at central wastewater treatment plants. Studies from the few secondary treatment plants that currently exist have shown that their effluents contain high levels of pollutants, such as organic matter and inorganic nutrients. These excess nutrients can cause serious environmental problems in natural receiving waters and additional tertiary treatment is needed for their removal before the effluents are discharged.

Methods for removal of organic substances and nutrients include extended aeration, aerated lagoon treatment, etc. Another alternative is treatment in an algal pond or high rate oxidation pond that can better remove macronutrients such as nitrogen and phosphorus, into aquatic phytoplankton biomass. Algae have been used to remove organic matter and nutrients from wastewater for the past 20 years. It reduces pollution and simultaneously produces an algal protein by-product that can be further used for various purposes.[1] These include protein for animal feed, soil conditioner, and raw materials for energy and chemical production.[2]

The use of the green algae *Chlorella vulgaris* as a tertiary treatment for the septage effluent from secondary treatment plants provides high treatment efficiency. Aziz and Ng [2] studied the feasibility of using an activated-algal process to treat wastewater and found that it was able to remove 80-88% of BOD, 70-82% of COD, 60-70% of nitrogen and 50-60% of phosphorus, with a retention period of 15 days. A study by Narkthon [3] on the efficiency of nitrogen and phosphorus removal from swine wastewater by *Chlorella vulgaris* showed that 77-86% of nitrogen and 53-75% of phosphorus was removed with a retention period of 8 days.

This research paper is focused on nutrient removal and growth of *Chlorella vulgaris* in treated septage. Of particular interest was the influence of light intensity and retention time on growth and nutrient removal efficiency.

## Materials and Methods

### Characterization of Treated Septage

Grab samples of treated septage were collected from the Nongkhaem Nightsoil Treatment plant, Bangkok. They were either characterized immediately or fixed with sulfuric acid or hydrochloric acid for preservation until they were characterized by the standard methods described by APHA, AWWA and WEF. [4] All samples were kept in the refrigerator at 4°C. Properties measured were pH (electrometric method), Chemical Oxygen Demand (COD) (closed reflux method), Suspended Solids (SS), Total Kjeldahl Nitrogen (TKN) (total Kjeldahl method) and Total Phosphorus (TP) (ascorbic acid method).

### Stock Culture of *Chlorella vulgaris*

A stock culture of *Chlorella vulgaris* was obtained from the Institute of Food Research and Product Development (IFRPD), Kasetsart University, Thailand.

### Conditions of Culture for *Chlorella vulgaris* Inoculum

*Chlorella vulgaris* stock was cultured in treated septage at a light intensity of 10,000 lux. The lighting was provided by a constant artificial source for 3 days (16 hours/day). The dry weight concentration (mg/L) of the *Chlorella vulgaris* obtained was determined by filtration of a culture liquor sample through GF/C paper and oven drying at 103 – 105°C for 1 hour. Based on the dry weight result, *Chlorella vulgaris* inocula for succeeding tests were adjusted to 100 mg/L by the addition of an appropriate amount of septage treatment plant wastewater.

### Effect of Light Intensity on Growth of *Chlorella vulgaris* in Treated Septage

The glass reactor (five liters volume) containing raw treated septage wastewater (3 L) was inoculated with *Chlorella vulgaris* to 100 mg/L. It was batch cultured in the laboratory room under a fluorescence lamp (36 W) for 12 hours/day. There were three treatments with different levels of light intensity : 3,000, 5,000 and 8,000 lux, equivalent to 37.5, 62.5, and 100 mE/m$^2$/s, respectively. There was another batch cultured under natural sunlight (without artificial light provision in the laboratory room condition) where the light intensities measured from 8.00 a.m.-6.00 p.m. ranged between 2,500-9,000 lux (31.25-112.5 mE/m$^2$/s). All treatments were done with three replications. They were aerated with a siphon air pump at a rate of 1 liter of air per minute and 24 hours/day. Culture samples were spectrophotometrically analyzed daily to determine the total chlorophyll (a and b) and the amount of algal biomass calculated.[4] Culture sample water was also analyzed every 2 days for total Kjeldahl nitrogen (TKN), total phosphorus (TP), suspended solids (SS), and chemical oxygen demand (COD), after removal of algal material by filtration.

### Statistical Analysis

A Kruskal Wallis test was applied to compare the differences in growth of *Chlorella vulgaris* and TKN and TP removal efficiencies. Differences were considered significant when $p<0.05$.

## Results and Discussion

### Characteristics of Septage Treatment Plant Wastewater

The treated septage effluent was brown to pale yellow and quite turbid. Analysis (Table 1) showed that it still contained high levels of organic and inorganic

**Table 1.** Characteristics of treated septage effluent compared with Thailand effluent standards.

| Parameter | Value | Effluent Standard* |
|---|---|---|
| pH | 7.5-7.7 | 5.5 – 9 |
| Total Kjeldahl Nitrogen (TKN), mg/L | 128-240 | 100 |
| Total Phosphorus (TP), mg/L | 8.9-28.3 | - |
| Suspended Solids (SS), mg/L | 46-3,300 | 150 |
| Chemical Oxygen Demand (COD), mg/L | 262-1,260 | 120 |
| Chromium (Cr), mg/L | 0.029 | ≤ 0.5 |
| Total Organic Carbon (TOC), mg/L | 87.6-45.7 | - |
| Cadmium (Cd), mg/L | BDL | ≤ 0.03 |
| Lead (Pb), mg/L | 0.001 | ≤ 0.2 |
| Nickel (Ni), mg/L | 0.006 | ≤ 0.2 |
| Copper (Cu), mg/L | 0.092 | ≤ 1.0 |
| Zinc (Zn), mg/L | 0.322 | ≤ 5.0 |
| Mercury (Hg), mg/L | 0.002 | ≤ 0.005 |

*Source: Ministry of Industry (1996).
BDL = Below Detectable Limit.

pollutants, most of which came from the septage and remained even after the treatment processes. The high COD and SS and TKN concentrations exceeded the Thai Industrial Effluent Standard. [5] It was clear that additional tertiary treatment was required to improve the quality of the effluent before it was discharged into natural receiving waters.

## Growth of *Chlorella vulgaris*

Culture of *Chlorella vulgaris* at light intensities of 3,000, 5,000 and 8,000 lux (Figs. 1, Tables 2 and 3) showed that culture at 8,000 lux gave significantly higher amounts ($p<0.001$) of both algal biomass and chlorophyll production than 3,000 and 5,000 lux. However, it did not give statistically different algal growth when compared with a batch of natural culture. The maximum biomass concentration (390 mg/L) was attained on the fourth day for natural culture whereas light provision batches at 3,000, 5,000 and 8,000 lux had slightly lower biomass values of 277, 305, and 356 mg/L, respectively. Thus, algal growth yielded 2.8-3.9 times the biomass of the inoculum in 4 days. After this, the concentrations of algal biomass and chlorophyll decreased continuously to the starting concentration by the sixth day and most of the *Chlorella vulgaris* died by the eighth day. Sreesai et al [6] also reported that the maximum growth of *Chlorella vulgaris* culture in swine wastewater reached its peak within only four days. Olguin [7] explained that high rate algal pond can operate at short hydraulic retention time in the range of 4 to 10 days, depending on climatic conditions, thereby reducing the required surface area. However, note should be made here that variation of light intensity during the day and culturing algae in a natural open system could have some favorable conditions for algal growth.

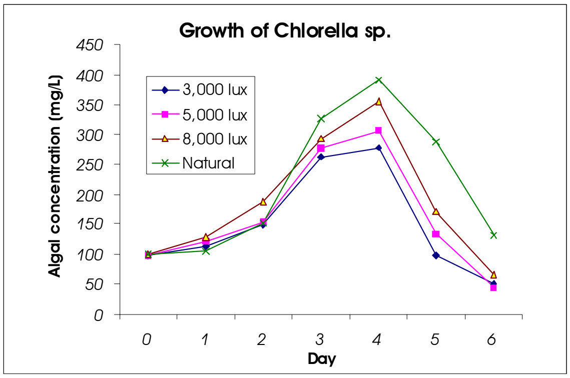


**Fig 1.** Amount of algal production under different conditions of light intensity.

Light intensity is an important factor for photosynthesis, a process that provides energy to convert $CO_2$ into organic substances. The process is driven by the light absorbent pigments chlorophyll a and chlorophyll b. Thus, it is expected that light would influence chlorophyll concentration and biomass in *Chlorella vulgaris* cultures. The lighting used in this study did not significantly raise the system temperature. Olguin et al [8] concluded that light intensity seemed to exert a stronger effect over productivity than temperature when cultured *Spirulina* under tropical conditions. In addition to $CO_2$ uptake for carbon skeletons, increase in biomass also requires uptake of nitrogen and phosphorus for proteins and energy transfer reactions.

Our results were similar to those of Assavaree and Pechmanee [9] who showed that *Chlorella vulgaris* gave higher growth at 8,000 lux than 3,000 lux ($p<0.05$). In our study, the lack of a significant difference in algal growth between 3,000 and 5,000 lux was probably due

**Table 2.** Amount of algal production (mg/L) under different conditions of light intensity at the fourth day of cultivation.

| Light Intensity (lux) | Range of Algal Production (mg/L) | Mean Algal Production (mg/L) | Standard Deviation (n = 9) |
|---|---|---|---|
| 2,500-9,000 (natural) | 340.12-451.32 | 390 | 40 |
| 3,000 | 269.92-291.14 | 277.1 | 8.8 |
| 5,000 | 291.90-321.06 | 305 | 10 |
| 8,000 | 339.43-375.59 | 356 | 12 |

**Table 3.** Amount of chlorophyll production (mg/L) under different conditions of light intensity at the fourth day of cultivation.

| Light Intensity (lux) | Range of Chlorophyll Production (mg/L) | Mean Chlorophyll Production (mg/L) | Standard Deviation (n = 9) |
|---|---|---|---|
| 2,500-9,000 (natural) | 5.10-6.76 | 5.86 | 0.61 |
| 3,000 | 3.98-4.37 | 4.16 | 0.13 |
| 5,000 | 4.38-4.82 | 4.59 | 0.15 |
| 8,000 | 5.09-5.63 | 5.34 | 0.19 |

to the high turbidity including colored water of the treated septage arising from suspended solids. This would limit light penetration into the cultivation medium and thus decrease the photosynthesis rate. This was somewhat overcame by increasing the intensity to 8,000 lux. However, there is a limit to this benefit due mainly to the fact that plants eventually become light saturated so that further increases in illumination produce no further increase in photosynthesis. This may explain the finding that the natural culture, which had exposure to more light intensity (up to 9,000 lux) did not have more significantly increased algal growth. In addition, the increasing concentration of algal cell biomass itself can decrease light penetration by self-shading. This was one reason for rapid algal die off after attainment of the maximum growth. The remaining algal cells on sixth day looked unhealthy because there were no additional nutrients and they could not re-grow the culture successfully like the inoculum culture.

This study indicates that recycling nutrients from septage wastewater via algal culture in natural conditions, where sunlight is sufficiently available, is technically and economically feasible. The amount of returned algal products was similar to a typical algal pond. [10] There was some accumulation of nickel (0.002 mg/kg) and chromium (0.006 mg/kg) in the algal cells (Table 4). Most of the analyzed heavy metal concentrations were quite low and less than the commercial algal cells sold in Taiwan. [11] However, quality of the algal biomass still needs a thorough study in terms of pathogens and toxic substance contamination, if the product is to be used as a feed.

On the other hand, in the case of an in-house treatment plant or other sysrem where light provision is required, the energy cost of increased illumination perhaps will outweigh the benefits of increased algal production. Thus, a practical design for algal culture based on costs of energy consumption versus algal production indicates that a light intensity of 3,000 lux would give an adequate yield (277 mg/L). For example, a study of Sreesai et al [6] found that culturing a small herbivorous aquatic arthropod (*Moina macrocopa*) which typically occurs in the *Chlorella* pond presents a challenge. It offers high biomass and Biochemical Oxygen Demand (BOD) removal efficiency, as well as requiring no further investment cost. A starting algal concentration at 260 mg/L will give a *Moina macrocopa* production of 7,500-8,400 organisms/L or 0.5-0.6 gram wet weight/L. In the present study, if *Moina macrocopa* will be used as an algal harvester and its production is estimated at only 60% of the previous study, around 180-216 kg of *Moina macrocopa* could be produced (equal to 180-216 U.S. dollar) in a one time treatment of 600 $m^3$ septage wastewater. At the same time, the biogas generated from a biological treatment of sludge could also be harvested and used as the energy source for light provision in algal culture. Successful algal production would then depend on a sufficient light provision by management practices such as occasional algal harvesting to prevent self-shading and maintain a suitable algal concentration. The other factors that could be manipulated for maximum productivity are mixing or turbulence, cultivation procedure, biomass concentration, and area density. [12]

## Nutrients Removal Efficiency

Nutrient removal as indicated by measurement of TKN and TP from the wastewater during algal culture was high at all light intensities studied. Maximum removal occurred on the $8^{th}$ day under natural culture (88% TKN and 68% TP) (Table 5) but it did not statistical differ from that of the 8,000 lux provided (85% TKN and 71% TP) (Table 5). On the $4^{th}$ day of culture, which corresponded with maximum algal growth, removal efficiencies of both TKN and TP at 8,000 lux were 54% and 41%, respectively, while at natural lighting, they were 62% and 55%, respectively (Table 6).

These results agreed with other studies, such as that by Gonzales [13] who found that the microalgae *Chlorella vulgaris* and *Scenedesmus* Vulgaris removed 95% of ammonium-nitrogen and 50% of phosphorus in wastewater. Likewise, Weerawattanaphong [14] who cultured *Chlorella vulgaris* in poultry wastewater at 10,000 lux with a retention time of eight days, found that TKN and TP removal were 72–85% and 57–77%, respectively. Similarly, Narkthon [3] reported that culture

**Table 4.** Heavy metal concentrations in cultured *Chlorella vulgaris* and commercial *Chlorella vulgaris*.

| Parameter | Cultured *Chlorella vulgaris* | Commercial *Chlorella vulgaris** |
|---|---|---|
| Chromium (Cr), mg/L | 0.006 | d" 0.5 |
| Cadmium (Cd), mg/L | BDL | 0.22 |
| Lead (Pb), mg/L | BDL | 0.67 |
| Nickel (Ni), mg/L | 0.002 | 0.68 |
| Copper (Cu), mg/L | 0.058 | 0.081 |
| Zinc (Zn), mg/L | 0.217 | 2.82 |
| Mercury (Hg), mg/L | BDL | BDL |

*Source: http:// www.taiwanchlorella.com

**Table 5.** Water quality after harvesting algal biomass from light provision at 8,000 Lux and natural algal culture batches at the eighth day.

| Parameter | Light Provision | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Natural | | | | 8,000 Lux | | | |
| | Range | Mean | S.D.(n=9) | % removal | Range | Mean | S.D.(n=9) | % removal |
| pH | 6.71-6.92 | 6.82 | 0.10 | | 6.54-6.98 | 6.78 | 0.19 | |
| TKN (mg/L) | 15.51-16.23 | 15.96 | 0.38 | 88 | 29.6-44.1 | 33.3 | 4.8 | 85 |
| TP (mg/L) | 4.55-4.90 | 4.71 | 0.13 | 68 | 3.35-5.67 | 4.26 | 0.7 | 71 |
| SS (mg/L) | 43.1-65.3 | 54.4 | 7.7 | 80 | 46-80 | 59 | 11 | 78 |
| COD (mg/L) | 55.0-68.4 | 63.5 | 5.9 | 83 | 33-62 | 45 | 11 | 88 |

**Table 6.** Water quality after harvesting algal biomass from light provision at 8,000 Lux and natural algal culture batches at the fourth day.

| Parameter | Light Provision | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Natural | | | | 8,000 Lux | | | |
| | Range | Mean | S.D.(n=9) | % removal | Range | Mean | S.D.(n=9) | % removal |
| pH | 6.32-6.98 | 6.77 | 0.25 | - | 6.45-7.23 | 6.85 | 0.24 | |
| TKN (mg/L) | 41.11-64.52 | 50.54 | 9.4 | 62 | 45.32-79.26 | 61 | 15 | 54 |
| TP (mg/L) | 5.0-8.0 | 6.7 | 1.3 | 55 | 6.5-11.4 | 8.7 | 1.5 | 41 |
| SS (mg/L) | 129-170 | 143 | 12 | 47 | 123-223 | 167 | 28 | 38 |
| COD (mg/L) | 69-141 | 93 | 24 | 75 | 56-99 | 78 | 15 | 79 |

of *Chlorella vulgaris* in piggery wastewater at 8,000 lux with a retention time of eight days gave TKN and TP removal at 85% and 75%, respectively.

Nutrient removal is dependent upon a direct mechanism involving algae uptake and harvesting of the produced biomass and upon two indirect mechanisms, ammonia-nitrogen volatilization and orthophosphate precipitation. [15] The removal efficiencies were as a result of growth of both *Chlorella vulgaris* and other microorganisms found in the treatment effluent, as well as effluent characteristics. However, the effluent without algae (control) always gave lower removal values of TKN and TP than the inoculated treatments throughout the period of the test. High light intensity would promote pollutant removal by stimulating more growth of *Chlorella vulgaris*. Both TKN and TP removal, however, continued to increase even after *Chlorella vulgaris* began to die in the batch culture. In this period, perhaps other microorganisms, such bacteria and protozoa, played a major role in waste removal as they also need nitrogen and phosphorus for their growth. [16] It is possible that they were able to remove further nitrogen and phosphorus in the absence of *Chlorella vulgaris*. This is because additional photosynthetic organic carbon from the dying algae was made available to their nutrient supply. This circumstance, known as a self purification process, could have occurred quite well for a short period after algal harvest and led to better effluent qualities. The other indirect nutrient removal mechanisms, volatilization of ammonia nitrogen and precipitation of phosphate, are the result of active photosynthesis and high pH in algal cultivation. Garcia et al [17] reported that there are some of the nitrogen is recovered in the form of algae biomass, but a large percentage of it is lost to the atmosphere from volatilization process.

## Growth and Nitrogen Uptake by *Chlorella vulgaris*

Since there were substantial concentrations of nitrogen and phosphorus (128-240 mg/L TKN and 8.9-28.3 mg/L TP) present in the treated septage, one could expect a total algal biomass of 1,600-3,000 mg/L, if *Chlorella vulgaris* could utilize all of the available nitrogen to produce plant protein. This estimation was based on the conversion equation: Algal biomass (mg/L) = TKN (mg/L) x 6.25 (nitrogen to protein conversion factor) x 2 (50% of protein contained in algal biomass) [14, 16]. However, the actual production of algae from this present study ranged from 17-24% with 21% on average or equivalent to 277-390 mg/L of algal biomass.

Lower than expected algal yield might be attributed to several growth-limiting factors. Among those are population density, lighting, temperature, toxic elements, pH, etc. A very crucial factor is the potential growth rate under the environmental conditions prevailing outdoors. Other limiting factors include the concentration or form of carbon source available in the culture medium and lack of vitamins or other organic growth promoters.

In our experiments, there were no additions of any carbon source, phosphorus or trace elements to the treated septage to enhance or optimize *Chlorella vulgaris* growth. It is possible that algal biomass might have increased if mineral nutrients and some trace elements (Cu, Fe, Bo, Zn, etc.) which were found in low concentrations had been added. the presence of any toxic elements or substances in the septage needs to be characterized as well. Further study is also required to optimize the process and properly evaluate the economics of septage waste management in combination with algal production as a sustainable environmental management practice.

Even with the limitations mentioned, the overall efficiency of nutrients (TKN and TP) removal by combined algal growth, bacterial activity and nitrification in the natural condition study reached 62% and 55%, respectively (Table 6), in a short period (4 days). It appeared that algal biomass accounted for removal of approximately 24% of the nitrogen (8 mg TKN removed/L/day). Thus, other microorganisms must have removed the rest of nitrogen, which is equivalent to 38% (13 mg TKN removed/L/day). If considered from only the treatment point of view and there is no limitation of space and facilities, this batch culture bio-reactor should have a retention time longer than four days (after algal harvest) in order to have the purification process well perform. Then, this algal culture system might be feasible as a tertiary treatment process for a septage treatment plant whereas yielding effluent with water qualities (Table 5 and 6) that meet the Thai government effluent standard. [5]

## Conclusion

Growth of *Chlorella vulgaris* was influenced by light intensity and its maximum biomass was obtained on the fourth day of treatment. A batch of natural culture gave the maximum concentrations of chlorophyll and algal biomass (5.8 mg/L and 390 mg/L, respectively), but these were not significantly different from the values from a batch provided with light at 8,000 lux (5.3 mg/L and 356 mg/L, respectively). The algal biomass under natural conditions was increased over the inoculum by 2.8-3.9 times.

There was no significant difference in algal production at 3,000 and 5,000 lux. Thus, for a treatment plant where natural light is scarce, the provision of light at 3,000 lux would best serve the combined purposes of algal production and energy conservation. Besides yielding a better effluent quality, recycling of valuable nutrients via algal culture could give some economical return from culturing algal harvester such *Moina macrocopa*. To reduce the electricity expense, using the biogas generating from a sludge treatment process is recommended.

At the peak of *Chlorella vulgaris* growth on the fourth day of natural batch culture, TKN and TP removal efficiencies were 62% and 55%, respectively, and *Chlorella vulgaris* biomass accounted for 24% of the TKN removal. The effluent water qualities met a Thai standard requirement.

Both TKN and TP removal in treated septage was governed by the growth of *Chlorella vulgaris* in combination with other microorganisms. Therefore, extending a retention time after algal harvesting is recommended to give better effluent qualities.

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