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GROWTH AND NUTRIENT REMOVAL EFFICIENCY OF *SCENEDESMUS OBLIQUUS* IN SETTLED AND ACTIVATED SEWAGES

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Abstract

Algal cultures of *Scenedesmus obliquus* were grown in two different sewages (settled and activated). Both types of wastewater supported good algal production, which was comparable and even better than that found in the commercial Bristol medium. Much higher biomass production was recorded in settled sewage than activated sewage under semi-continuous culture condition. High correlation was recorded between algal growth and nutrient removal in both settled and activated sewages. The $N_4^+ - N$ dropped faster in the settled sewage from its initial 30.5 mg/l to 3.2 and 4.6 mg/l in the batch and semi-continuous cultures, respectively, after 8 days. Some fluctuations appeared in nitrate concentrations in both sewages. The rate of removal was significantly higher in the activated sewage in all treatments, and reached about 91%, removal. The changes in phosphate concentrations were similar in both sewages. There were increases in the first two days, then decreases, especially in the semi-continuous cultures. The maximum P removal (90%) was recorded in semi-continuous cultures in settled sewage. The results of this study suggest that the semi-continuous algal culture is more suitable and efficient in wastewater treatment than the batch cultures.

Key words: microalgae, wastewater, treatment, nutrient, removal.

Introduction:

Many studies have demonstrated that algal cultivation is a mean of eliminating residual inorganic nutrients from secondarily treated wastewater, and represents a potential food source, or a source of a variety of chemicals and pigments (Matusiak *et al.*, 1976; Oswald *et al.*, 1978; Shelef and Soeder, 1980; Przytocka-Jusiak *et al.*, 1984; Rodrigues and Oliveira, 1987). However, the technology of algal cultivation on a large scale has not yet been highly developed (de la Noue and Ni Eidhin, 1988). One major problem associated with algal treatment is the substantial requirement of land-space, and it is important to keep the space requirement to a minimum. The semi-continuous culture system is one possible way to maximize plant-space. de la Noue and Ni Eidhin (1988) reported that algal culture on secondarily treated wastewater can be operated satisfactorily by intensive semi-continuous cultures of *Scenedesmus obliquus*. They found that the culture system could be operated stably at dilution rates of up to 0.8 day^{-1}, with successful algal production and nutrient depletion from wastewater. Another possible solution for space problem is to use the algal system as the secondary treatment step, which can reduce the cost involved in the conventional secondary activated sludge process. Although relatively few studies had been carried out using algal culture as the secondary treatment process, Tam and Wong (1989) found that a microscopic green alga, *Chlorella pyrenoidosa* cultivated in settled sewage (primary treated effluent) had better growth and was more effective in reducing wastewater N and P than activated sewage (secondary

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treated effluent) in a bacteria-free batch culture system.

The present investigation attempts to compare the growth and nutrient removal efficiency of the microscopic green alga *Scenedesmus obliquus* in settled and activated sewages. The experiment also aims to evaluate the feasibility and potential of employing a semi-continuous culture system.

Materials and Methods:

Two kinds of sewage samples (settled and activated sewage) were collected from the wastewater treatment station at Beni-Suef governorate, mainly from domestic source. The settled sewage (SS) was the supernatant collected after the primary screening and primary sedimentation processes. The average constituents of such wastewater is 300 mg/l COD, 90 mg/l BOD, 30.5 mg/l $N_4^+ - N$, 3.9 mg/l $N_3^- - N$, 11 mg/l organic N, 9.5 mg/l $PO_4^{-3} - P$ and the sewage was near neutral with a pH value of 7.1. Minute amounts of heavy metals were monitored in the sewage. The activated sewage was the effluent after treated by primary and secondary treatment processes. Activated sewage consisted of 120 mg/l COD, 26 mg/l BOD, 14 mg/l $N_4^+ - N$, 16.4 mg/l $N_3^- - N$, 12 mg/l organic N, 10.2 mg/l of $PO_4^{-3} - P$ and the pH was 6.9.

Stock cultures of *Scenedesmus obliquus* (obtained from Culture Collection of Algae & Protozoa, Cambridge, UK) were cultured in commercial Bristol medium (pH 6.8-7.2) under aseptic conditions (Nichols and Bold 1965). Algal suspensions in the logarithmic growth phase were centrifuged at 3000 rpm for 10 minutes and the cell pellets were washed twice with 0.1 M phosphate buffer (pH 6.5) and then resuspended in 4 liter plastic tank containing 2 liter of either settled or activated sewage. The initial algal concentration in each tank was 8.1×10^6 units/ml. Nine tanks were set up for each sewage sample, three for "batch" culture, three for "semi-continuous" culture and three acted as the "control". The batch culture meant that the algae were kept in the wastewater for a period of 8 days without any renewal of fresh sewage samples, while the semi-continuous culture was maintained by replacing half volume of the sample with fresh sewage at every two days intervals (0.25/day). This was carried out by removing one liter algal suspension from each tank, then centrifuged at 4000 rpm for 10 minutes, the algal cells were resuspended to the original volume with one liter fresh sewage sample and returned to the plastic tank. The procedure was repeated four times during the 8-days experimental period and resulted in a semi-continuous culture with algal biomass recycled at the dilution rate of 0.75/day. Besides the control tanks (without any algal inoculation) *Scenedesmus* cells were also grown in batch in commercial Bristol medium (Nichols and Bold, 1965) and used for comparing algal growth in wastewater. The tanks were prepared and each tank was aerated with air, and illuminated with white fluorescent tubes ($300\mu E\ m^{-2}s^{-1}$) at 16 and 8 hours light-dark cycle. Temperature was maintained at 22°C.

During the experimental period 50 ml cell suspension were harvested daily. Algal growth was monitored by air-dry weight and chlorophyll content (acetone extraction, Yung and Mudd 1966). The algal cells were then separated from the culture medium by centrifugation (as above). The supernatant was analyzed for pH, $NH_4^+ - N$, $NO_3^- - N$ and $NP_4^{-3} - P$, following the methods described by APHA (1985).

Results and discussion:

Growth of Algal cells:

The growth curves of *Scenedesmus obliquus* cultured in different kinds of media for 8-days period are shown in figure 1. The growth rate was indicated by air-dry weight of the algal cells. It is clear that the two sewage samples supported significantly higher amounts of algal growth than the Bristol medium.

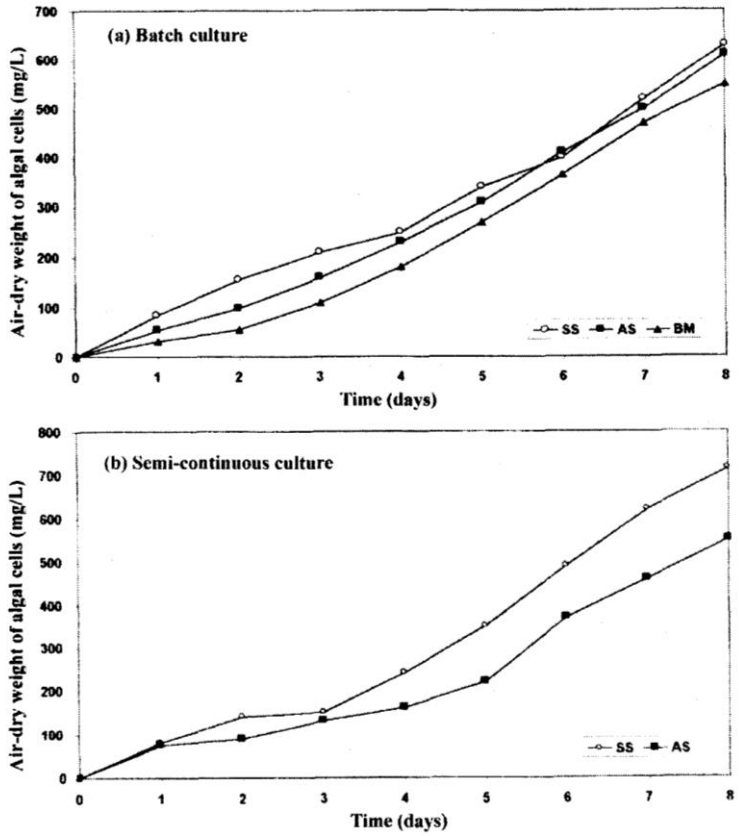

Fig. 1: Growth curve, in terms of air dry weight of *Scenedesmus* cells, under different consitions. (BM: Bristol medium; SS: Settled sewage; AS: Activated sewage).

Many previous studies have concluded the suitability of using sewage as growth medium for algal cultures and high rate algal ponds have been put into practice successfully (Oswald *et al.*, 1978; Shelef *et al.*, 1978; Kawia *et al.*, 1984; Tam arid Wong 1989; Hammouda *et al.*, 1995; Abdel Hamed 1997). Fig. 1,a reveals that the dry weight of algal cells in batch settled sewage insignificantly differs from that found in activated

sewage. However, much higher biomass production was recorded in settled sewage than activated sewage under semi-continuous culture condition (Fig. 1,b). Recent studies have shown that microscopic green algae appeared to grow better in primarily treated sewage than secondarily treated effluent (Przytocka-Jusiak *et al.*, 1984; Tam and Wong 1989). The lesser algal production found in activated sewage might be due to the fact that this sewage contained lower amount of $NH_4^+ - N$ than settled sewage, and the difference in $NH_4^+ - N$ content between two sewages was even more obvious in semi-continuous cultures. There was higher algal growth observed in settled than activated sewage in a bacteria free batch culture condition (Tam and Wong 1989). Przytocka-Jusiak *et al.* (1984) concluded that algae utilize $NH_4^+ - N$ preferentially in stationary culture and exclusively in continuous cultures, as they do not produce active nitrate reductase.

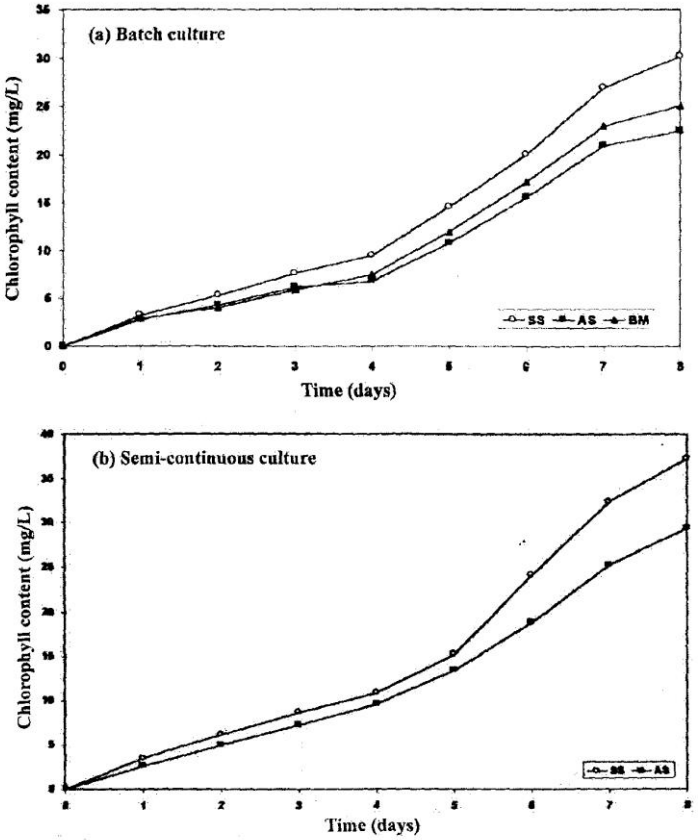

Fig. 2: Growth curve, in terms of chlorophyll content of *Scenedesmus* cells, under different consitions. (Same symbols as Fig.1).

Figure 2 revealed that algal growth in batch in Bristol medium had similar amounts of chlorophyll content as that found in activated sewage, and the highest amounts were recorded in settled sewage. The relatively low chlorophyll content of algal cells in activated sewage might be due to the lack of other compounds such as Mg and B as the activated sludge process could have removed these trace but essential elements. These findings suggest that settled sewage was a more favourable algal culture medium than activated sewage. Thus, the high rate algal ponds might be more effective and economical if it was installed as a secondary rather than a tertiary treatment process.

Wastewater nitrogen removal

Figure 3 shows the changes in $NH_4^+ - N$ content in wastewater in the algal cultures and the control. Significantly more $NH_4^+ - N$ was removed in wastewater containing *Scenedesmus obliquus* cells then the control. Many studies have been focused on the mechanisms of removal ammonia from wastewater and stressed the role of ammonia stripping, nitrification, denitrifaction and assimilation by algae (Reeves 1972; Matusiak 1976; Przytocka-Jusiak *et al.*,1984). Ammonia stripping (volatilization) is significant only under alkaline, high temperature conditions (Reeves 1972) and the presence of a large amount of urea in the wastewater (Matusiak *et al.*, 1976). In this study the pH was nearly neutral and urea concentration was not high, so $NH_4^+ - N$ removal due to stripping should not be significant. On the other side, a significant correlation was found between $NH_4^+ - N$ removal and algal production in settled sewage ($r=0.88$) indicating that the direct utilization of $NH_4^+ - N$ by algae was an important process (Hammouda *et al.*, 1995). By comparing the two kinds of sewage more rapid decrease in NHJ-N was found in the settled sewage. The $NH_4^+ - N$ concentration of the settled sewage containing algal cultures dropped from 30.5 mg l^{-1} to 3.2 mg l^{-1} after 8 days of treatment in the batch and semi-continuous cultures, respectively. The removal efficiency was 89.5 %. The activated sewage starts with a much lower initial $NH_4^+ - N$ (14 mg l^{-1}). This low initial concentration dropped rapidly to 1.2 mg l^{-1} in the hatch culture after 3 days of treatment, and to 1.4 mg l^{-1} after 4 days in the semi-continuous culture. However, the $NH_4^+ - N$ concentrations fluctuated at the latter part of the experiment. A small initial drop followed by a rise in $NH_4^+ - N$ was observed in the control tanks. This may be due to the activities of some endogenous bacteria presented in wastewater. The uptake and immobilization of N by these bacteria may explain the initial drop while mineralization of organic N from aged dead bacteria cells may result in the latter rise in $NH_4^+ - N$. The treatment efficiency reached 92 and 85% in the semi-continuous and batch systems, respectively (Fig.3). Previous results by Matusiak (1976) and Przytocka-Jusiak *et al.* (1984) showed that dense cultures of *Chlorella vulgaris* were adapted to high $NH_4^+ - N$ concentration and were capable of N removal from highly loaded nitrogenous industrial wastes.

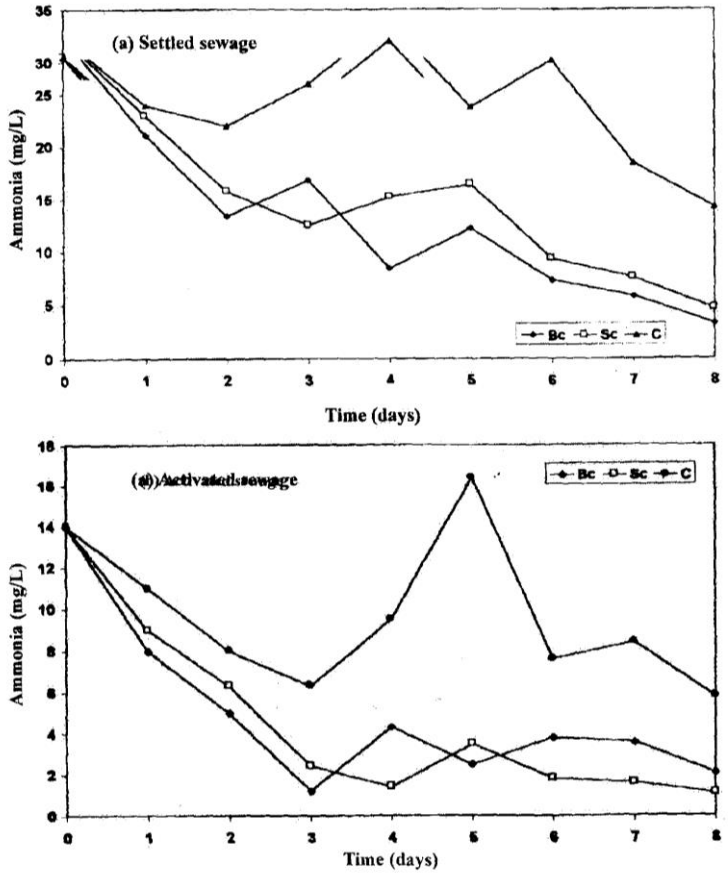

Fig. 3: Changes in wastewater $NH_4^+ - N$ content during algal cultivation. (Bc: Batch culture; Sc: Semi-continuous culture; C: control).

In case of the semi-continuous culture, addition of $NH_4^+ - N$ did not result in its accumulation in wastewater. The concomitant increase of algal production in the semi-continuous culture indicates that the added $NH_4^+ - N$ was rapidly assimilated by the growth of algae. This result indicates that $NH_4^+ - N$ removal by algal cultures would be more efficient in semi-continuous than batch cultures. Abdel Raouf (1994) reported similar findings.

Figure 4,a shows that $NO_3^- - N$ concentration in the settled sewage was relatively low and constant throughout the study, at the level of 2.5-4 mg/l. As its value

was low, there were no significant differences between the removal of NO_3^- – N in settled sewage in all the treatments. The initial NO_3^- – N concentration of activated sewage was high (16.4 mg/l). In the first 4 days of the experiment, there was little change in NO_3^- – N content. Then, the concentration dropped rapidly, with the final concentration around 1.5 mg/l. The treatment efficiency was about 91% in both batch and semi-continuous cultures. It is clear that NO_3^- – N decreased when the ammonia concentration falls below certain limits or depletes from the medium (Przytocka-Jusiak *et al.*, 1984; Maestrini *et al.*, 1986; Tam and Wong 1989). The removal of NO_3^- – N was mainly due to direct utilization by algae and loss due denitrification process was not important, because denitrification can only take place under anoxic or anaerobic condition, which was not available in this study. Moreover, a significant correlation was found between NO_3^- – N removal and algal production as dry weight (r=0.98) and as Chlorophyll content (r= 0.97).

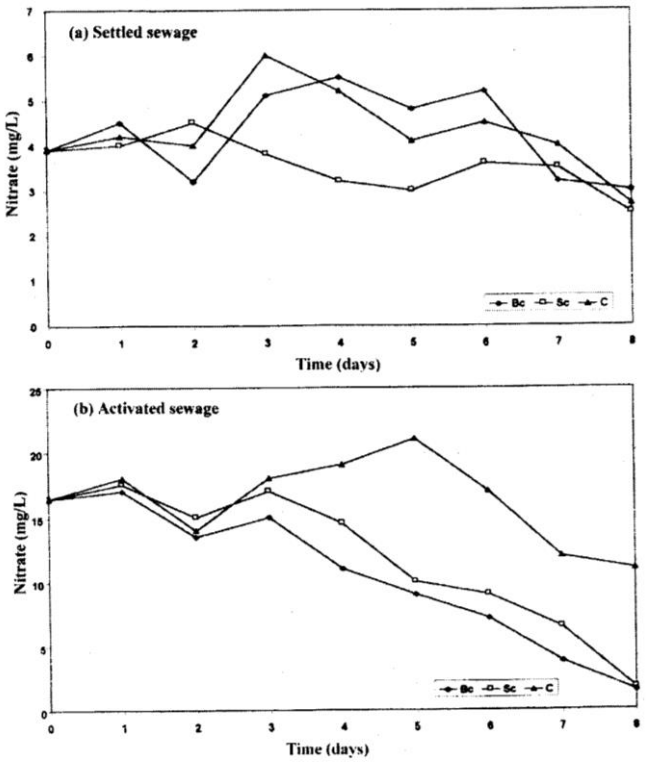

Fig. 4: Changes in wastewater NO_3^- – N content during algal cultivation. (Bc: Batch culture; Sc: Semi-continuous culture; C: control).

Wastewater phosphorus removal:

Figure (5) reveals that there was no obvious change in orthophosphate concentrations of both sewages during the experimental period in the control tanks. The P content fluctuated randomly and maintained around 9 and 10 mg/i in the settled and activated sewages respectively The P concentration in the settled sewage increased in both algal tanks in the first 2 days then decreased. The drop of wastewater P concentration was faster in the semi-continuous culture than the batch culture. The removal efficiency in the batch culture was about 73%, and the final P concentration was 2.5 mg/l. Kobbai *el al.* (2000) reported 86% P removal from wastewater in 7 days in a batch culture. In contrast the final P concentration in the semi-continuous culture was less than 1 mg/l and the treatment efficiency reached 90%. Similar patterns of P removal were seen in the activated sewage. The P content fluctuated in the semi continuous culture till the fourth day then there was steady decrease to reach a final concentration of 1.5 mg/l. The treatment efficiency reached 85%. P removal in the batch culture was significantly lower than the semi-continuous culture, with a final concentration of 4.2 mg/l. The efficiency of P removal was significantly less and accounted for only 59%.

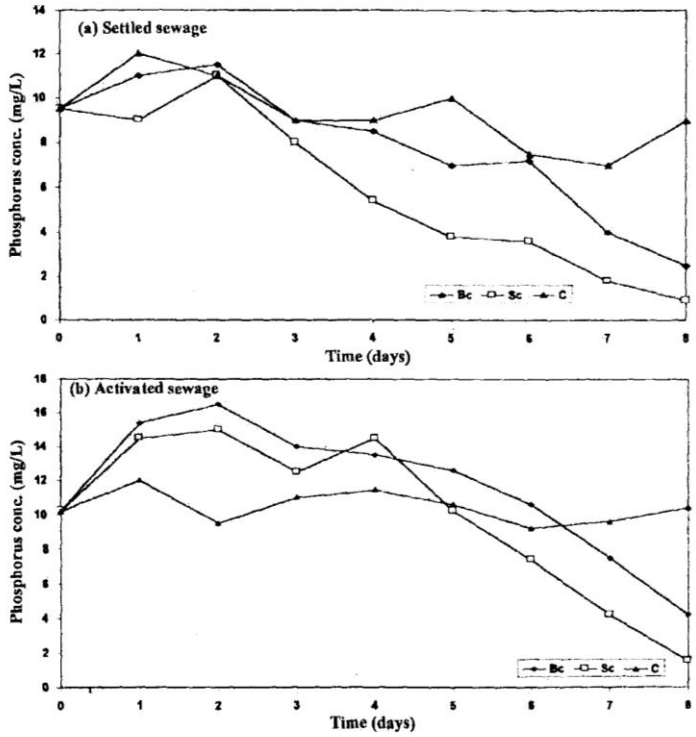

Fig. 5: Changes in wastewater PO_4^{-3} – P content during algal cultivation. (Bc: Batch culture; Sc: Semi-continuous culture; C: control).

Conclusion:

This experiment has shown that both settled and activated sewages were able to support more growth of *Scenedesmus obliquus* than the commercial Bristol medium. Settled sewage seemed to be the best culture medium. Significant correlation was found between algal production and nutrient removal from wastewater in both sewages. This indicated that the purification mechanism for both wastewaters was due to direct uptake and assimilation of inorganic nutrients by algal cells. In general, the % nutrient reduction from settled sewage was not significantly different from that found in activated sewage except for phosphorus removal. These results indicated that it is possible to use the algal system as the secondary treatment process for the removal of inorganic nutrients from wastewater. In both settled and activated sewages, the semi-continuous culture seemed to have a higher potential for the subsequent nutrient removal.

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نمو طحلب *Scenedesmus obliquus* وكفاءته فى التخلص من المغذيات من مياه الصرف الصحى بعد المعالجة الأولية والثانوية

محمد السيد عبد الحميد

كلية العلوم – جامعة القاهرة (فرع بنى سويف)- قسم النبات

تم تنمية *Scenedesmus obliquus* على نوعين من مياه الصرف الصحى (المعالجة أوليا والمعالجة ثانويا)، كلا النوعين من المياه ساعد على نمو وإنتاج كميات كبيرة من الطحلب مقارنة بالوسط الغذائى الصناعى. سجلت الدراسة نمو أكثر للطحلب فى المياه المعالجة أوليا تحت ظروف النمو النصف-مستمر. كان هناك ارتباطا وثيقا بين نمو الطحلب ومعدل التخلص من المغذيات فى كلا النوعين من المياه. انخفض تركيز الأمونيا أسرع فى المياه المعالجة أوليا من ٣٠,٥ ملج/ل إلى ٣,٢ و ٤,٦ ملج/ل فى كلا من الزراعة الثابتة والنصف مستمرة على الترتيب خلال ٨ أيام. ظهرت بعض الارتفاعات والانخفاضات فى تركيز النترات فى كلا النوعين من المياه. كان معدل التخلص من النترات أعلى (معنويا) فى كل المعلجات فى المياه المعالجة ثانويا، وبلغت نسبة المعالجة ٩١٪. كان التغير فى عنصر الفسفور متشابها فى كلا النوعين من المياه. حدثت زيادة فى أول يومين، ثم تلاها إنخفاضا سريعا خاصة فى المياه المعالجة أوليا تحت ظروف النمو النصف-مستمر. بلغت أقصى نسبة تخلص من الفوسفور ٩٠ ٪ وسجلت فى المياه المعالجة أوليا تحت ظروف النمو النصف-مستمر.

تقترح النتائج المسجلة فى هذه الدراسة أن تنمية الطحالب تحت ظروف النمو النصف-مستمر أكثر كفاءة ومناسبة لمعالجة مياه الصرف الصحى عن ظروف النمو المثبتة.