NUTRIENT REMOVAL BY PHOTOSYNTHETIC MICROORGANISMS FROM SYNTHETIC WASTEWATER

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Abstract

The Cyanobactrium *Phormidium fragile* and the green alga *Scenedesmus armatus* are chosen in this study for treatment of synthetic wastewater. The growth rate and nutrient removal rates were investigated under different irradiance condition. The growth rate increased with increasing irradiance, reaching a saturation value at 2000 and 1500 Lux for P. *fragile* and *S. armatus* respectively. The organic acids removal rates by *S. armatus* were higher than those removed by *P. fragile* reaching the maximum values at 1500 and 2000 Lux. On the other hand phosphate, nitrate and ammonia removal rates by *P. fragile* were higher than those by *S. armatus.* A mixed culture of both *P. fragile* and *S. armatus* was therefore used for simultaneous removal of organic acids, nitrate, ammonia and phosphate. Both acetate and propionate removal rates increased with the increase in the concentration of *S. armatus* while removal rates of nitrate and phosphate increased with the increasing in the concentrations of *P. fragile.*

Keywords: wastewater treatment, photosynthetic cells, *Phprmidium fragile, Scenedesmus armatus.*

Introduction

Although industrial and agricultural wastewaters are usually subjected to primary, and secondary treatment, the final effluents typically contain high concentrations of organic acids, inorganic phosphorus and nitrogen that can have eutrophication effects on lakes, rivers and estuaries. Microalgae have a high capacity for inorganic nutrient uptake (Talbot & de la Noüe, 1993; Blier *et al*., 1995), and they can be grown in mass culture in outdoor solar bioreactors (de la Noüe *et al*., 1992). Unicellular green algae such as *Chlorella* and *Scenedesmus* have been widely used in wastewater treatment because they often colonize the ponds naturally, and they have fast growth rates and high nutrient uptake capabilities. However one of the major drawbacks of using microalgae in wastewater purification is the harvesting of biomass (Laliberté *et al.*, 1997). A major advantage of using photosynthetic microalgae for wastewater treatment is the possibility of combining wastewater treatment with production of useful metabolities. Algal biomass can be excellent sources of many useful metabolites

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(Glombitza & Koh, 1989), energy sources such as oil (Boussiba *et al*., 1987; Kishimato *et al*., 1994) and can also be used to remove toxic gases such as NO_x and SO_x from flue gases (Negoro et al., 1991; Yoshihara et al., 1996).

Cyanobacteria have a variety of characteristics that make them well-suited to wastewater treatment. First, they have a high nutrient removal capacity as they can accumulate inorganic phosphorus and nitrogen and store them as polyphosphates and cyanophycin (Fay, 1983), but this feature has been little explored in the contex of wastewater treatment. Second, they are likely to tolerate the highly variable conditions that characterize polluted effluents. Finally, many of the mat-forming strains self-aggregate when in culture and therefore readily sediment in the absence of stirring, which allows the resultant biomass to be easily, harvested Mespoulède, 1997). Potential end use of the harvested biomass includes the extraction of commercially valuable pigments (Mumford and Miura, 1988; Glazer, 1994).

Most of the studies on the use of microalgae for wastewater treatment have been based on the use of a monoculture to a specific nutrient (mainly nitrogen or phosphate) and only few studies have been reported on the use of mixed algal cultures for wastewater treatment (Gantar *et al*., 1991). Most wastewater contains a variety of nutrients and it is difficult to get a single strain that can simultaneously remove all nutrients from the wastewater.

The aim of the present work was to select a good combination of photosynthetic microorganisms whose biomass can be harvested for various applications. In this paper we present the growth and nutrient removal capabilities of the Cyanobacterium (*Phormidium fragile*) and the green alga (*Scenedesmus. Armatus*) under different irradiance conditions, together with the feasibility of using a mixed culture of these phototrophs for simultaneous removal of various nutrients from wastewater.

Materials and Methods

The prokaryotic alga (*Phormidium fragile*) and the chlorophyte alga (*Scenedesmus armatus*) were isolated from the Nile water at Giza district, purified and enriched in Blod's Basal medium (BBM) Bischoff & Bold, (1963). Seven days old cultures were homogenized and diluted under aseptic conditions to give stock cultures.

A synthetic wastewater composed of (per liter of distilled water) sodium acetate, 5.0 gm; sodium propionate, 1.0 g; KNO_3, 20 mg; NH_4NO_3, 100 mg; $K_2H PO_4$, 60 mg; $CaCl_2$ $2HO_2$, 14 mg; $MgSO_47H_2O$, 100 mg; KCl, 30 mg; Fe citrate, 0.1 mg; yeast extract, 1.0 mg and SL7 solution, 0.3 ml (pH, 7) was used in this study. The SL 7 solution was composed of mgL^{-1}) $ZnCl_2$, 70; $MnCl_2$. $4H_2O$, 100; H_3BO_4, 60; $CoCl_2$. $6H_2O$, 200; $CuCl_2$. $2H_2O$, 20; $NiCl_2$. $6H_2O$, 20; NaMo O_4. $2H_2O$, 40 and HCl (25%), 1.0 ml, (Ogbonna *et al*., 1995). This basic synthetic medium was used for studies of nutrient removal by *P. fragile* and *S. armatus*. Ten mls of the dilute stock of each culture and mixed cultures (OD_{750}=0.4) were transferred to 50 ml sterile synthetic wastewater in 250 ml Erlemyer flasks. 6 replicates were exposed to irradiance of 0.0, 500, 1000, 1500, 2000, 2500 Lux and incubated at 26ºC $\pm$ 1.0.

Samples were taken at 24 hours-intervals one flask per strain for each irradiance.

Cyanobacterium (*P. fragile*) flocs were mixed and homogenized in the auto mix (4000 r.p.m.) before OD_{750} measurements. The natural logarithm of OD_{750} was plotted as a function of time and growth rate was estimated from the straight portion of the curve. An aliquot of culture, previously used for biomass determination was filtered through a whatman 934-AH glass fibre filter. For measurement of propionate and acctate concentration 100 μg of the filtrate was applied as a spot using a capillary pipette to silica gel TLC plates (20 $\times$ 20 cm). Perchloric acid was used for elution at a flow rate of 1.0 ml min^{-1}. After development of the chromatograms, the concentration was measured by spectrophotometer. (Cs- 9000 – Shimadzu – Japan). PO_4^{3-} P was analysed follwing the ascorbic acid method outline in APHA (1989) and NO_3^-N by the sulfanilamide method described in MENVIQ (1992). The procedures mentioned by Fawcett and Scott (1960) and Chany and Marbach (1962), were used to determine NH_4^+ - N.

Results and Discussion

Growth (OD) was followed as a function of time and the representative growth rate for each culture was shown in Figure 1. Maximal phototrophic growth rate increased with increasing irradiance, reaching a saturation value at 2000 and 1500 Lux for *P. fragile* and *S. armatus* respectively. A marked decrease in the growth rate of *P. fragile* was observed at the highest irradiance suggesting photo inhibition. This observation was consistent with the finding of Hu et al (2000), who reported that low irradiance favours the growth of the cyanobacteria due to their superior light capturing abilities and their low light compensation point. On the other hand the green alga *S. armatus* grew well at the highest irradiance tested, an important aspect for wastewater treatment performed in outdoor aerated pounds.

Figure 2 shows that the nitrate removal rate for the cyanophyte increased sharply between 500 and 2000 Lux, then it leveled off or decreased at higher irradiances, this suggests that the bright light causes an excess of excitation energy and, as a results, photoinhibition and photobleaching of the cells, as observed by Roos & Vincent (1998). On the other hand the green alga *S. armatus* showed an increased removal rate (3.78 mg L^{-1} d^{-1}) at 2500 Lux.

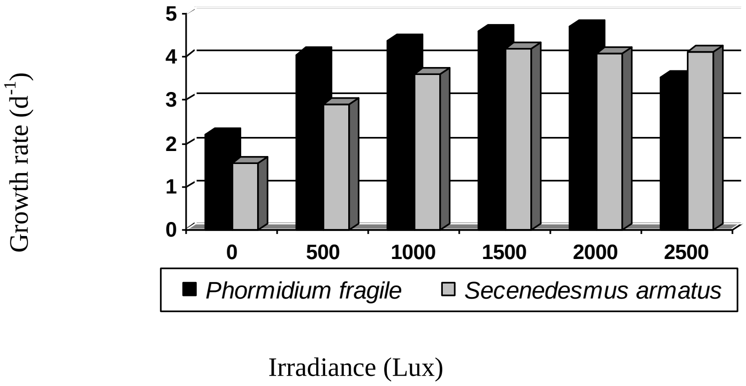

Figure 1: Growth rate of *P. fragile* and *S. armatus* in synthetic wastewater under different irradiance conditions.

The relationship between phosphate removal rate and irradiances was shown in Figure 3, the best removal rate was observed with *P. fragile* at 2000 Lux, (1.08 mg P L^{-1} d^{-1}). However, above 1000 Lux, *S. armatus* showed similar P-removal rates, around 0.7 mg P L^{-1} d^{-1}. It is clear that the two tested organisms were less effective at removing phosphate relative to nitrogen. In this connection Chevalier et al. (2000) reported that nitrogen became limiting before the complete removal of phosphate, or alternatively it may simply reflect the lesser cellular demand for P relative to N.

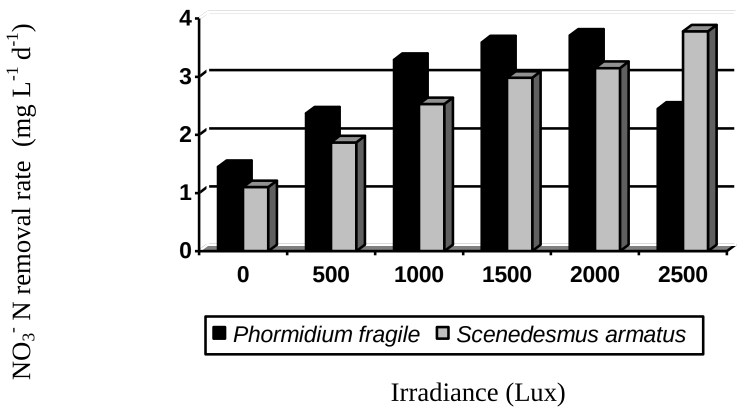

Figure 2: Nitrate (NO_3^--N) removal rate in the synthetic wastewater by *P. fragile* and *S. armatus* under different irradiance conditions.

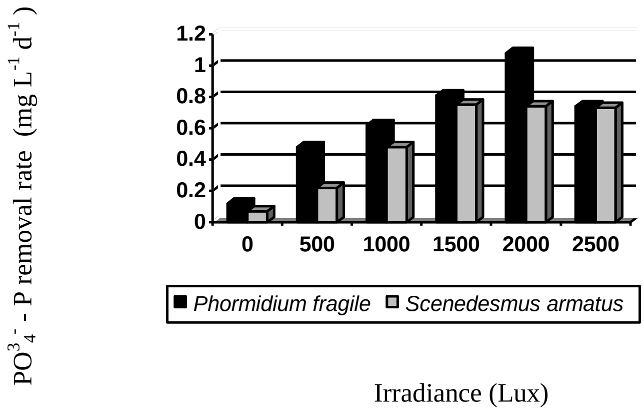

Figure 3: Phosphate (PO_4^{3-} - P) removal rate in synthetic wastewater by *P. fragile* and *armatus* under different irradiance conditions.

Variation in ammonia removal rate by both photosynthetic cells with different light intensities followed the same trend as that of cell growth (Figure. 4). Although the ammonia removal rate by *P. fragile* sharply decreased at the highest irradiances, it was almost two times higher than the values obtained with *S. armatus* at 1500 & 2000 Lux. Generally, the removal rate of ammonia by both tested organisms was very high if compared with that of nitrate. In accordance with this Dortch (1990) reported that in the presence of both NH_4^+ and NO_3^-, most algal cells use the former for growth preferentially even though complete NH_4^+ repression is rare.

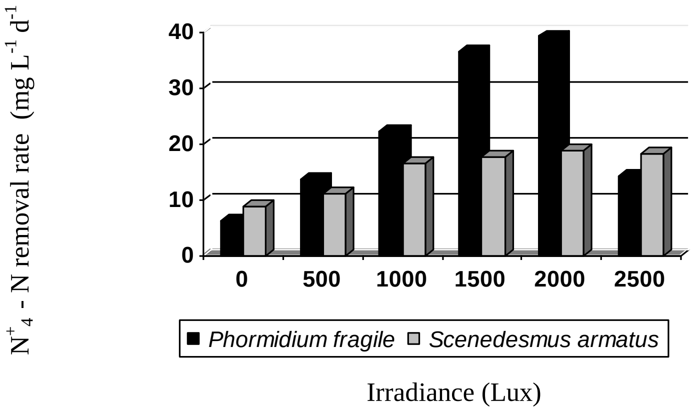

Figure 4: Ammonia (NH_4^+ -N) removal rate in synthetic wastewater by *P. fragile* and *S. armatus* under different irradiance conditions.

Although the growth rate of *P. fragile* was higher than that of *S. armatus* under dark condition the acetate removal rate by *S. armatus* was about 3 times higher than the value obtained with *P. fragile* (1.52 g L^{-1} d^{-1}). This high value increased with increasing the irradiances reaching a maximum value. (2.61 g L^{-1} d^{-1}) at the highest irradiance conditions (Figure 5).

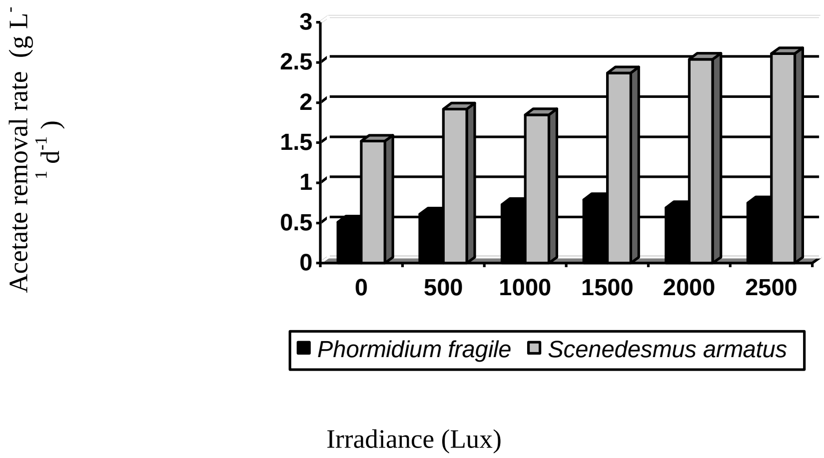

Figure 5: Acetate removal rate in the synthetic wastewater by *P. fragile* and *S. armatus* under different irradiance conditions.

Figure 6 shows that propionate removal rate of both photosynthetic cells was very low although it slightly increased with increasing irradiances. *S. armatus* recorded higher propionate removal rates than *P. fragile* at all irradiance conditions and the highest propionate removal rate was 37 mg L^{-1} d^{-1} at 2500 Lux.

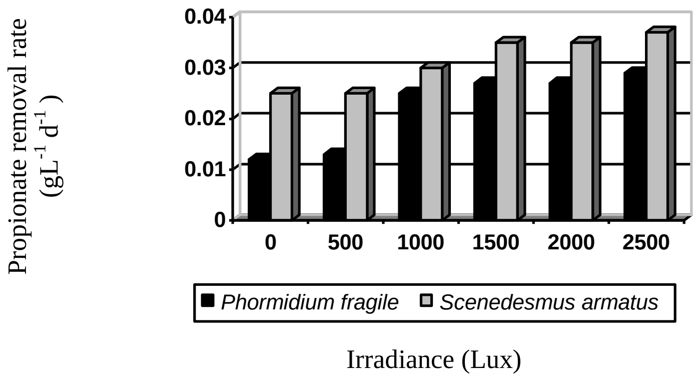

Figure 6: Propionate removal rate in the synthetic wastewater by *P. fragile* and *S. armatus* under different irradiance conditions.

The above results show that although both the Cyanobacterium *P. fragile* and the green alga *S. armatus* can be used for wastewater treatment under different irradiance conditions and moderate temperature, complete removal of the organic acids was not achieved. This is because under these conditions, the mixotrophy can occure, so heterotrophic and photoautrophic metabolisms can take place simultaneously. As a result, nitrogen and phosphate were exhausted within 40 h. treatment. In the absence of nitrogen and phosphate, cell growth and thus organic acids removal stopped.

In this connection Ogbonna *et al*., (2000) reported that, the growth rate of *Chlorella sorokiniana* was more than twice as high as those of *Spirulina platenis* neither *C. Sorkiniana* nor *S. Platenis* could remove propionate from the wastewater.

The effects of variation in the ratio of *P. fragile* to *S. armatus* on nutrient removal rates are shown in Figure 7. The O.D of the initial mixed culture was (0.02). Both acetate and propionate removal rates increased with the increase in the concentration of *S. armatus* while the removal rates of nitrate and phosphate increased with increase in the concentration of *P. fragile.*

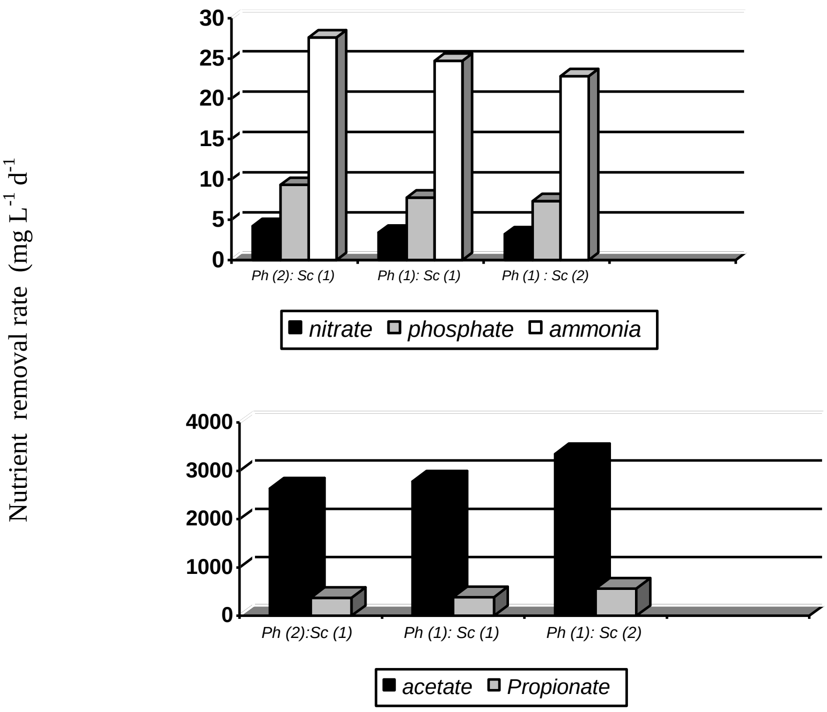

Figure 7: Effect of initial *P. fragile* to *S. armatus* ratio on wastewater treatment by a Mixed culture of the two strains at irradiance of 2000 Lux.

The ammonia removal rate did not vary much when the ratios of the strains were varied and the highest value obtained with increase in the concentration of *P. fragile.* On the whole the organic acids removal rates by *S. armatus* were higher than those of *P. fragile* on the other hand phosphate, nitrate and ammonia removal rates of *P. fragile* were higher than those of *S. armatus.* Thus none of these strains can be used for simultaneous removal of all the nutrients from wastewater. However by using a mixed culture of these strains, all the nutrients could be simultaneously removed from the wastewater.

In conclusion it appears that mixed culture of cyanobacteria and green algae are potential candidates for wastewater treatment at moderate temperature and over irradiance range from 500 to 2000 Lux.

Also for removal of inorganic nutrient from wastewater, cyanobacteria are more appropriate than chlorophytes, because of their natural flocculation properties.

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إزالة التغذية من نفايات المياه المخلقة بواسطة الكائنات الدقيقة الضوئية التغذية

فاطمة الزهراء توفيق زكى
كلية العلوم – قسم النبات
جامعة القاهرة

تم اختيار الطحلب الأخضر المزرق فورميديم فراجيل والطحلب الأخضر سينيدزمص ارماتص لهذه الدراسة لمعالجة نفايات المياه المخلفة . تم دراسة كل من معدل النمو ومعدل ازالة التغذية تحت ظروف إضاءة مختلفة . وقد وجد أن :

1- معدل النمو زاد بزيادة الإضاءة وقد أعطى أعلى قيمة للنمو عند 2000 و 1500 لاكس بالنسبة للطحلب الأخضر المزرق والأخضر بالترتيب .
2- معدل إزالة الأحماض العضوية بواسطة الطحلب الأخضر سينيدزمص ارماتص كانت اعلى من إزالتها بواسطة الطحلب الأخضر المزرق وكانت أعلى قيمة لازالة الاحماض العضوية عند قوة إضاءة 1500 و 2000 لاكس .
3- من ناحية أخرى كان معدل إزالة الفوسفات والنيترات والامونيا بواسطة الطحلب الأخضر المزرق كانت أعلى من مثيلتها بواسطة الطحلب الأخضر.
4- وقد تم استخدام مزرعة من كلا الطحلبين لإزالة كل من الأحماض العضوية والفوسفات والنيترات والامونيا معاً . وكانت النتيجة أن معدل إزالة املاح الاسيتات والبروبيونات زادت بزيادة تركيز الطحلب الأخضر سينيدزمص ارماتص اما معدل إزالة النيترات والفوسفات فقد زادت بزيادة تركيز الطحلب الأخضر المزرق فورميديم فراجيل .