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EFFICIENCY OF AMMONIA AND PHOSPHORUS REMOVAL FROM A COLOMBIAN AGROINDUSTRIAL WASTEWATER BY THE MICROALGAE *CHLORELLA VULGARIS* AND *SCENEDESMUS DIMORPHUS*

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Abstract
The ammonia and phosphorus removal efficiencies of the microalgae Chlorella vulgaris *and* Scenedesmus dimorphus, *during biotreatment of secondary effluent from an agroindustrial wastewater of a dairy industry and pig farming, were evaluated. The microalgae were isolated from a wastewater stabilization pond near Santafé de Bogota, Colombia. Batch cultures were made using both species in 4-l cylindrical glass bio-reactors each containing 2 1 of culture.* Chlorella vulgaris *was also cultivated on wastewater in a tri-angular bioreactor Three 216-h experimental cycles were run for each microalga and in each bioreactor In the cylindrical bioreactor,* S. dimorphus *was more effi-cient in removing ammonia than* C. vulgaris. *However, the final efficiency of both microalgae at the end of each cycle was similar. Both microalgae removed phos-phorus from the wastewater to the same extent in a cylindrical bioreactor Using* C. vulgaris, *the triangular bioreactor was superior for removing ammonia and the cylindrical bioreactor was superior for removing phos-phorus. This study shows the potential of using these microalgae to reduce the environmental pollution of heavily contaminated agroindustrial waters currently disposed of untreated into the waterways and streams of tropical Colombia. © 1997 Elsevier Science Ltd.*

Key words: Microalgae, *Chlorella vulgaris, Scenedes-mus dimorphus,* agroindustrial wastewater, nutrient removal, bioreactors.

INTRODUCTION

Microalgae culture systems show a versatility that allows them to participate in different processes, such as wastewater treatment, production of animal

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food, production of fertilizers, and production of common and fine chemicals (De la Noüe and De Pauw, 1988). The wastewater treatment by micro-algae cultures has a major advantage. It does not generate additional pollution when the biomass is harvested and it allows efficient recycling of nutrients (De la Noüe *et al.,* 1992). The use of several microalgae and cyanobacteria in wastewater treatment is known (Calzada *et al.,* 1991; Cañizares *et al.,* 1994; De la Noüe *et al.,* 1993; De la Noüe and Proulx, 1988; Serodes *et al.,* 1986; Oswald, 1988).

Our aims were: to find microalgal species with a high efficiency of ammonia and phosphorus removal, and to compare the efficiency of the removal in two bioreactor types, cylindrical and triangular. We studied the potential of two Chlorophyceae: *Chlor-ella vulgaris* and *Scenedesmus dimorphus*. The importance of Chlorophyceae and the above micro-algae for nutrient removal is known (Cañizares and Casas, 1991; Chevalier and De la Noüe, 1985; Lav-oie and De la Noüe, 1985; Serodes *et al.,* 1991), but not for the type of wastewater used in this study.

METHODS

Microalgae

Wild strains of *Chlorella vulgaris* Bejerinck and *Sce-nedesmus dimorphus* (Turp.) Kütz were isolated from a secondary effluent of a wastewater treatment stabilization pond. They were identified by micro-scopic observation according to the descriptions of Chlorophyceae in the literature. The characteristics of the wastewater in the pond are given in Table 1.

Culture media

Two sterile synthetic media were used to mantain the cultures. The medium for *Chlorella vulgaris* con-tained (g/1): KNO_3, 25; $MgSO_4.7H_2O$, 10; KH_2PO_4,

Table 1. Characteristics of the agroindustrial wastewater in a secondary treatment aerobic stabilization pond

Parameter	Mean value
pH	7.4
Temperature	19.5°C
Conductivity	1.6 mS/cm
Total dissolved solids	819.5 mg/l
Nitrates	1.9 mg/l
Nitrites	0
Ammonia	36.3 mg/l
Total phosphorus	111.8 mg/l
Chlorides	589.9 mg/l .
Alkalinity	477.2 mg/l
Chemical oxygen demand (COD)	282.9 mg/l
Total suspended solids (TSS)	111.2 mg/l
Fecal coliforms	2465 cfu/ml
Total coliforms	2815 cfu/ml

Note: The solid residues came mainly from a dairy industry, with a small quantity from pig farming. The water had high levels of fats and proteins (quantities not determined in this study). No heavy metals or industrial toxic materials were present in the wastewater (Valder-rama, 1995).

4; K_2HPO_4, 1; $FeSO_4.7H_2O$, 1; Micronutrients (mg/1) H_3BO_3, 2.86; $MnCl2.4H_2O$, 1.81; $ZnSO_4.7H_2O$, 0.11; $CuSO_4.5H_2O$, 0.09; $NaMoO_4$, 0.021. Bold's Basal medium used for *Scenedesmus dimorphus* contained (g/1): $NaNO_3$, 10; $CaCl_2.2H_2O$, 1; $MgSO_4.7H_2O$, 3; K_2HPO_4, 3; KH_2PO_4, 7; NaCl, 1;(pg/l) Na_2EDTA, 50; $FeSO_4.7H_2O$, 4.98; H_3BO_3, 11.42; $ZnSO_4.7H_2O$, 8.82; $MnC1_2.4H_2O$, 1.44; $MoNO_3$, 0.71; $CuSO_4.5H_2O$, 1.57; $Ca(NO_3)_2.6H_2O$, 0.49 (Bischoff and Bold, 1963). To eliminate bacteria and protozoa, the wastewater was filtered using a S and S 595 paper filter (0.45 mm), 185 mm in diameter (Schleicher and Schuell, Germany) and sterilized by autoclaving for 20 min. A 1:1 dilution of the waste-water with fresh water was necessary to reduce turbidity. The diluted wastewater was used throughout this study. The percentages of ammonia and phosphorus removal were calculated from the diluted cultures.

Incubation

To determine the nitrogen and phosphorus removal from wastewater by the microalgae, batch cultures were carried out in two types of bioreactors: a 4-1 cylindrical glass (working volume of 21) and a 50-1 triangular acrylic (30 1 working volume). The triangular bioreactor had dimensions of 60x70x33 cm (W/L/H). At the bottom tip, it had an aeration system composed of a silicone tubing with holes connected to ambient air supply. The microalgae inoculation volume (suspended in microalgae growth media) in each bioreactor was 10% (v/v), with an initial concentration of approx. $2x10^6$ cells/ml. All the cultures were incubated at ambient temperature (20±2°C) under continuous fluorescent

illumination (60 mmole/m^2/s) and bubble-aerated by a commercial aquarium pump.

Analytical determinations

Ammonia and phosphorus removal were followed by analyzing 45-ml samples taken every 24 h. The first determination was made 144 h after beginning the culture. Each sample was centrifuged for 15 min at 1800 x g to eliminate the algae. Ammonia nitrogen, nitrates and total phosphorus were determined according to standard methods (AWWA-APHA-WPCF, 1990).

Experimental design and statistical analysis

Every experiment was composed of three independent but otherwise similar cycles. Each cycle lasted 216 h for each microalga in each bioreactor type. Samples for analysis were taken in triplicate for every determination. Results of the three cycles were combined and used for statistical analysis. Statistical analysis was done by One-way Analysis of Variance (ANOVA) at $P \leq 0.05$. Data were converted to arcsin before analysis.

RESULTS AND DISCUSSION

As a result of the nature of the wastewater used, variations in nutrient removal probably can be attri-buted to the impossibility of having similar initial nutrient concentrations for each cycle. Nevertheless, as noted by Cañizares *et al.* (1994), this variety of concentrations permitted the establishment of removal efficiency percentages.

Scenedesmus dimorphus always removed signify-cantly more NH_3 from the wastewater than *C. vulgaris* in the 41 cylindrical bioreactor. However, at the end of each cycle (after 216 h) the percentage removed was the same (Fig. 1). In *C. vulgaris cul*tures, the nitrate levels in the wastewater increased from 5 to 45 mg/1 after 144 h and stayed at this level thereafter. This phenomenon did not occur with *S. dimorphus* (data not presented). Phosphorus removal efficiencies obtained by the two microalgae in a 41 bioreactor were statistically similar. The removal ranged from 20% after 144 h to 55% after 216 h (data not shown). Our results showed lower efficiencies of phosphorus removal by *C. vulgaris* and *S. dimorphus* than those reported by Lavoie and De la Noüe (1985) for other microalgae, such as the 75% for *S. obliquus* and 71-90% for *Phormidium* (De la Noüe and Proulx, 1988).

The efficiencies of *C. vulgaris* removal of ammonia and phosphorus were compared in cylin-drical vs triangular bioreactors. In the triangular bioreactor, *C. vulgaris* removed more NH_3 than in the cylindrical bioreactor. After 216 h of incubation, the efficiency removal was equal [Fig. 2(a)]. How-
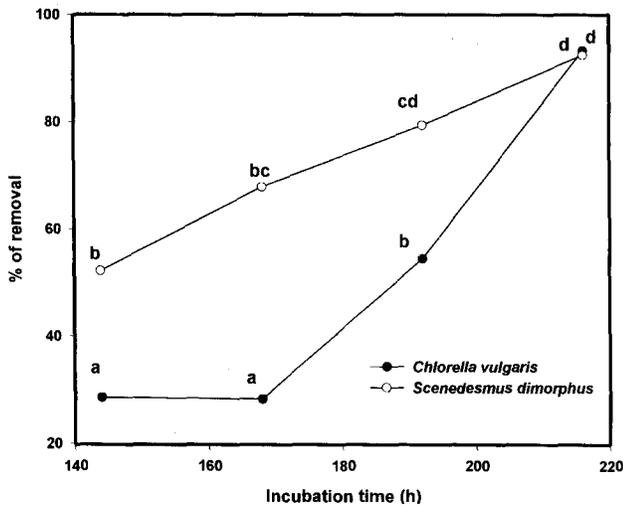
Fig. 1. Percentage of removal of NH_3 from agroindustrial wastewater by *C. vulgaris* and *S. dimorphus*. Points denoted by a different letter differ significantly at $P \le 0.05$ in one-way ANOVA.

ever, the cylindrical bioreactor was more efficient in removing PO_4^{3-} than the triangular bioreactor. There, no removal of PO_4^{3-} occurred [Fig. 2(b)].

The depletion of ammonia and increase in nitrates in the microalgae wastewater culture cannot be exclusively attributed to nitrogen conversion. Other mechanisms capable of eliminating ammonia are non-biological, such as air-stripping, absorption and sedimentation. In intensively-aerated microalgal syste-ms, like ours, the air-stripping mechanism seems to be the most important. In addition, microalgal photosynthetic activity in all culture systems 16 raised the temperature to 27-28°C and the pH to 9, thus improving the air-stripping phenomena (Talbot and De la Noüe, 1993).

The removal efficiency of ammonia by *S. dimorphus* in our study was higher than that docum-ented for *S. quadricauda* by Chevalier and De la Noüe (1985), who reported a removal efficiency of only 23.31%. However, the results we obtained with *C. vulg-aris* and *S. dimorphus* are similar to *Phormidium bonh-eri* efficiency (95%) [De la Noüe and Proulx, 1988].

There are two major ways to remove phosphorus from wastewater: (i) direct cellular absorption under ae-robic conditions, and (ii) sedimentation to anoxic cond-itions. Phosphorus removal in our, study under aerated conditions can be explained by its interaction with the nitrogen in the water. Because nitrogen is the limiting nutrient factor in the medium, the phosphorus concen-tration will still be high even after ammonia exhaustion. This leads to a saturation in the phosphorus cellular absorption mechanisms (Chevalier and De la Noüe, 1985). In our study, in spite of the high oxygenation rate, the absence of a sedimentation zone in the bioreac-tors prevented phosphorus chelate precipitation. In con-

trast, Proulx *et al.* (1994) determined higher phosphorus removal efficiencies (57%) than ours, in a triangular bioreactor during *Phormidium bonheri* cultivation.

In tropical countries like Colombia (having an excess of freshwater caused by heavy rainfall, over 10,000 mm annually, in some regions), agricultural reuses of the treated wastewaters are unlikely to occur as there is no shortage of irrigation water in the country. However, disposal of less contaminated wastewater will have a major benefit for environmental pollution control. The current disposal method nationwide is a direct disposal of untreated wastewater into the waterways and streams.

In conclusion, the two microalgae wastewater treatment systems present a good option of biological tertiary treatment of agroindustrial wastewater. Both species showed a fairly high ammonia-removal efficiency, comparable to those obtained in other studies with cyanobacteria like *Phormidium* and *Spir-ulina,* and a moderate phosphate removal.
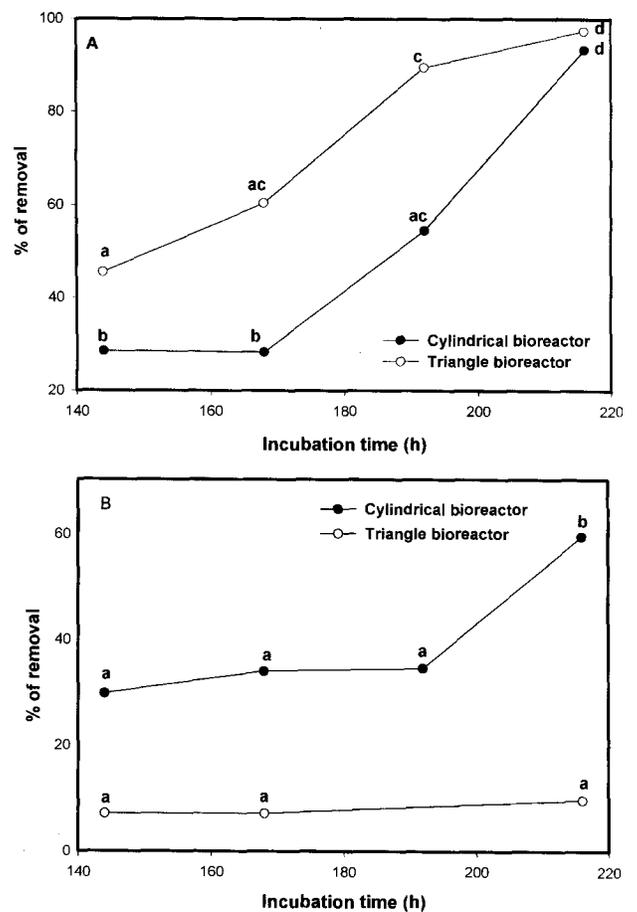

Fig. 2. Comparison of removal of ammonia (A) and phos-phorus (B) by *C. vulgaris* growing in agroindustrial wastewater in cylindrical and triangular bioreactor. Points denoted by a different letter, in each sub-figure, differ significantly at $P \le 0.05$ in one-way ANOVA.

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