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ORIGINAL PAPER

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Nitrate and phosphate removal by *Spirulina platensis*

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Abstract The cyanobacterium *Spirulina platensis* was used to verify the possibility of employing microalgal biomass to reduce the contents of nitrate and phosphate in wastewaters. Batch tests were carried out in 0.5 dm^3 Erlenmeyer flasks under conditions of light limitation (40 μmol quanta m^{-2} s^{-1}) at a starting biomass level of 0.50 g/dm^3 and varying temperature in the range 23–40°C. In this way, the best temperature for the growth of this microalga (30°C) was determined and the related thermodynamic parameters were estimated. All removed nitrate was used for biomass growth (biotic removal), whereas phosphate appeared to be removed mainly by chemical precipitation (abiotic removal). The best results in terms of specific and volumetric growth rates ($\mu = 0.044$ day^{-1}, $Q_x = 33.2$ mg dm^{-3} day^{-1}) as well as volumetric rate and final yield of nitrogen removal ($Q_{N-NO_3^-} = 3.26$ mg dm^{-3} day^{-1}, $Y_{N-NO_3^-} = 0.739$) were obtained at 30°C, whereas phosphorus was more effectively removed at a lower temperature. In order to simulate full-scale studies, batch tests of nitrate and phosphate removal were also performed in 5.0 dm^3 vessels (mini-ponds) at the optimum temperature (30°C) but increasing the photon fluence rate to 80 μmol quanta m^{-2} s^{-1} and varying the initial biomass concentration from 0.25 to 0.86 g/dm^3. These additional tests demonstrated that an increase in the inoculum level up to 0.75 g/dm^3 enhanced both NO_3^- and PO_4^{3-} removal, confirming a strict dependence of these processes on biomass activity. In addition, the larger surface area of the ponds and the higher light intensity improved removal yields and kinetics compared to the flasks, particularly concerning phosphorus removal ($\mu = 0.032$–0.050 day^{-1}, $Q_x = 34.7$–42.4 mg dm^{-3} day^{-1}, $Q_{N-NO_3^-} = 3.24$–4.06 mg dm^{-3} day^{-1}, $Y_{N-NO_3^-} = 0.750$–0.879, $Q_{P-PO_4^{3-}} = 0.312$–0.623 mg dm^{-3} day^{-1}, and $Y_{P-PO_4^{3-}} = 0.224$–0.440).

Keywords Biological nitrogen removal · Abiotic phosphorus precipitation · *Spirulina platensis* · Thermodynamics

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Introduction

The effluents generated by industrial and civil activities need pre-treatment before their disposal into rivers, lakes and oceans in order to reduce contaminants to environmentally safe levels. Special attention is required for inorganic substances such as ammonium, nitrates and phosphates, which encourage growth and contribute to the eutrophication of water bodies.

Microalgal mass culture appears to be a feasible way to remove inorganic nutrients and, in some instances, to convert them into useful biomass. Various systems and species have been investigated in past years to improve the effectiveness and economic feasibility of such biotreatment systems [14, 15]. When nutrients and agitation are not limiting, the growth efficiency of algal systems is strictly dependent on light penetration and temperature [11, 21].

Among microalgae, the blue-green algae (cyanobacteria) appear to be particularly attractive for the production of high-quality biomass. In order to improve their cell productivity, certain factors must be controlled during cultivation, including cell density, depth, and turbulence of suspension [17]. The use of microalgae has been proposed to reduce the content of nitrogen, phosphorus and residual organic carbon in wastewater [9].

Spirulina platensis biomass was used in the present work to remove nitrates and phosphates from a standard growth medium. *S. platensis* is a blue-green photoautotrophic, unicellular, filamentous microalga, which grows abundantly in aqueous and saline habitats [20]. Because of its high content of amino acids and proteins

it has long been used as human food in Mexico and Africa. When used as a biological agent for treatment, it could be employed as food for animals, as starting material for energy production processes (anaerobic digestion), as fertiliser or to produce fine chemicals such as pigments, polysaccharides, carotenoids, steroids, vitamins, polyunsaturated fatty acids, and lipids [2, 3, 10, 13].

The purposes of this study were to (1) determine the optimum temperature for *S. platensis* growth, (2) remove nitrate and phosphate, (3) investigate the mechanisms of nutrient removal by material balances, and (4) estimate the thermodynamic parameters of both cell growth and thermal inactivation. To evaluate the possibility of employing such a microalga in a full-scale treatment, tests of nitrogen and phosphorus removal were also carried out in 5.0 dm^3 vessels at different initial biomass concentrations, under conditions simulating those of actual outdoor systems.

Materials and methods

Microorganism and culture medium

The microalgal strain *S. platensis* UTEX 1926, obtained from the University of Texas Culture Collection (Austin, Tex.), was cultured in the medium of Schlösser [19], supplemented with potassium nitrate and phosphate up to the desired levels of N (0.097–0.11 g/dm^3) and P (0.085–0.088 g/dm^3).

Experimental set-up and cultivation conditions

Batch tests were performed, under light-limited conditions, in 0.5 dm^3 Erlenmeyer flasks covered with cotton caps, agitated at 50 rpm on a reciprocal shaker placed inside a chamber thermostatted at the selected temperature. Following the suggestions of Vonshak [22], the temperature was varied between 23°C and 40°C and the starting biomass concentration (X_o) was set at 0.50 g/dm^3. Two fluorescent lamps (40 W) were used to ensure a photon fluence rate (PFR) of about 40 μmol quanta m^{-2} s^{-1}. The pH increased from 8.7 to about 10 at the end of cultivation. Four lengthened open mini-ponds, similar to those described by Craggs et al. [9], each having a 7.0 dm^3 total volume and 0.15 m^2 surface area, were also used to simulate the real-scale removal of nitrogen and phosphorus. They were agitated at 18 rpm by linear paddle wheels ensuring gentle axial thrust and inoculated with increasing biomass concentrations (0.25, 0.42, 0.60, 0.75 and 0.86 g/dm^3). Temperature was maintained at the optimum value suggested by preliminary batch tests (30°C) in the same way as for Erlenmeyer flasks; illumination was provided by four fluorescent lamps (40 W), ensuring a PFR of about 80 μmol quanta m^{-2} s^{-1}. The fermentation volume (5.0 dm^3) was kept constant by daily replacement of water lost by evaporation. Samples were taken periodically for determination of nitrogen, phosphorus and biomass concentrations. Data were collected in triplicate; standard error never exceeded 8%.

Yields and kinetic parameter estimation

Experimental nitrogen and phosphorus removal yields (Y_{N-NO3-} and $Y_{P-PO43-}$) were determined as the ratios of the amounts removed to the starting levels of these elements. The corresponding theoretical yields ascribed to biomass uptake were calculated from the data of cell mass production, assuming an average biomass composition for *S. platensis* of $CH_{1.650}O_{0.531}N_{0.170}S_{0.007}P_{0.006}$ as reported by Cornet et al. [8]. The removal yields ascribed to abiotic chemical precipitation of phosphate were calculated as the difference of the experimental and theoretical yields. For both types of calculation, total times of 22 and 25 days were considered for tests performed in Erlenmeyer flasks and in open ponds, respectively.

The volumetric rates of nitrogen and phosphorus consumption $Q_{N-NO_3^-}$ and $Q_{P-PO_4^{3-}}$ as well as the volumetric cell mass productivity (Q_x) were calculated at the end of each test as the ratios of their respective concentration variations to the total time.

Analytical procedures

Cell concentration was determined by dry weight after filtration through Millipore filters (0.45-μm pore diameter) after pH adjustment to 8.0 with 3.0 *N* acetic acid to eliminate precipitates. Nitrogen and phosphorus concentrations were determined by standard methods [1].

Results

Batch cultivations in Erlenmeyer flasks

Figures 1 and 2 describe the trends of nitrate and phosphate consumption at 25, 30, 35 and 40°C. The data points indicate the experimental values obtained at variable temperature, while the dotted lines show the theoretical removal trends, calculated according to their respective biomass production.

Table 1 lists the average values of specific growth rate, biomass productivity, rates and yields of nitrogen and phosphorus removal, calculated after triplicate experiments. Kinetic parameters, growth yield, and nitrogen removal exhibited maximum values at 30°C under the operating conditions of stirring and illumination used, in agreement with the value reported by Vonshak [22] for the growth of *S. platensis* DA, while significantly higher optima were reported for other isolates and strains (35–38°C). In addition, as highlighted by Torzillo and Vonshak [21], the optimum growth temperature is lower than those of both photosynthesis (35°C) and respiration (45°C).

A completely different behaviour can be seen for phosphorus removal: both removal rate and experimental yield progressively decreased from 0.463 to 0.254 mg P dm^{-3} day^{-1} and from 0.124 to 0.068 with increasing temperature from 25 to 40°C.

Batch cultivations in vessels

One of the most difficult tasks in outdoor mass culture is scale-up, which is the stage when contamination by other algae and bacteria may occur because of dilution of the initial inoculum. According to Richmond [17], there is a direct relationship between density of *S. platensis* in the culture and density of contaminants.

The possibility of realising a treatment system using this microalga was investigated using 5.0 dm^3 vessels at the optimum temperature selected by means of previous

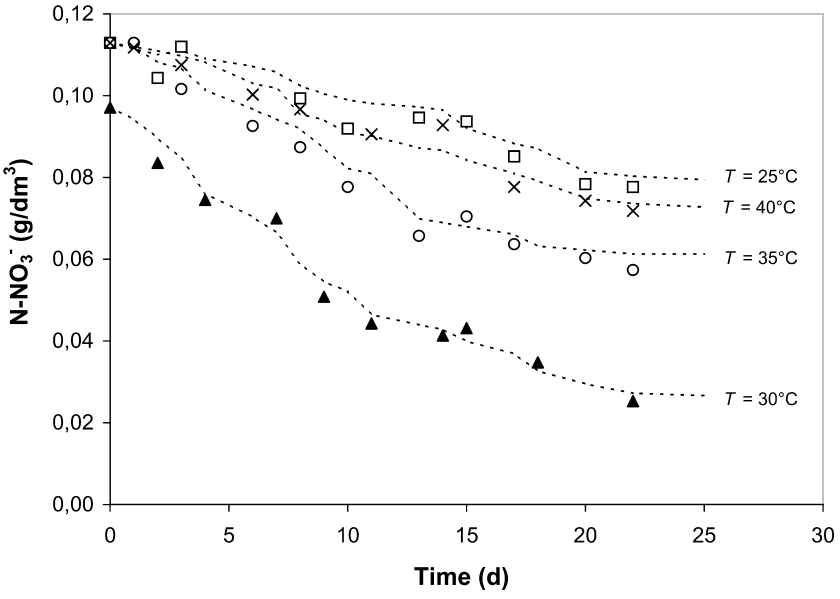

Fig. 1 Changes in nitrogen concentration during batch cultivations of *Spirulina platensis* carried out in 0.5 dm^3 Erlenmeyer flasks at different temperatures. T (°C): □ 25, ▲ 30, ○ 35, × 40. *Dotted lines* Theoretical consumptions estimated by material balances

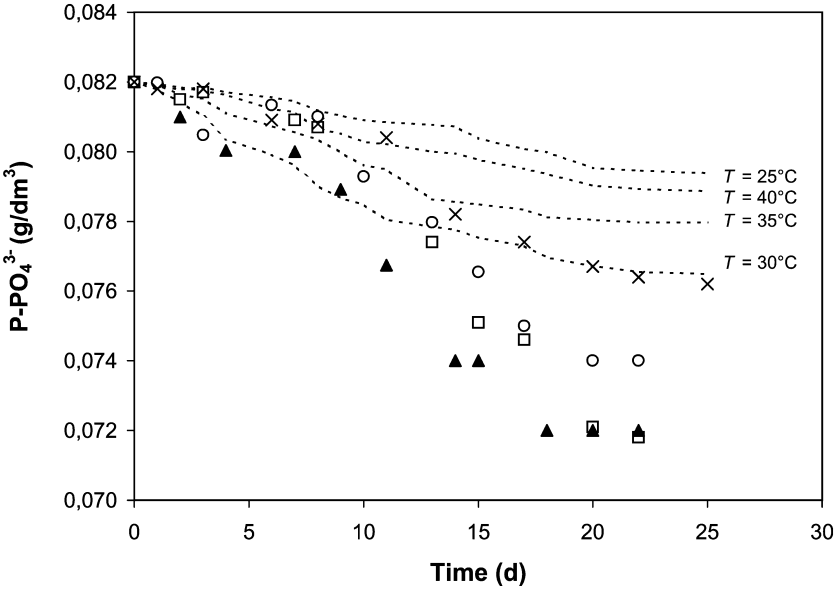

Fig. 2 Changes in phosphorus concentration during batch cultivations of *S. platensis* carried out in 0.5 dm^3 Erlenmeyer flasks at different temperatures. T (°C): □ 25, ▲ 30, ○ 35, × 40. *Dotted lines* Theoretical consumptions estimated by material balances

flasks tests (30°C) and increasing the PFR to a value (80 μmol quanta m^{-2} s^{-1}) comparable to that of full-scale outdoor cultivation systems [12]. The results, in terms of both removal yields and kinetic parameters of tests performed by increasing the inoculum level from 0.25 to 0.86 g/dm^3, are listed in Table 2. This study may be considered a bench-scale attempt to simulate and model the full-scale removal of nitrogen and phosphorus from water.

The increase in starting biomass concentration up to 0.75 g/dm^3 enhanced both nitrogen and phosphorus removal, confirming the strict dependence of these processes on biomass activity. At 30°C and 80 μmol quanta m^{-2} s^{-1}, biomass growth depended on the starting amount of inoculum, even if the amount of phosphorus removed during the first 10 days due to biotic activity was hardly affected. After this period, the increase in pH associated with biomass growth favoured its chemical precipitation in the same way in all cases.

A further increase in the inoculum level did not improve either nitrogen or phosphorus removal, whereas a clear inhibition of these activities took place for X_o > 0.86 g/dm^3 (results not shown). Such behaviour can be ascribed to a limitation of biotic activity caused by excess biomass with respect to light penetration (shading effect) [11].

Discussion

Effect of temperature

The experimental data for nitrogen concentration (Fig. 1) were in excellent agreement (errors ranging from

Table 1 Influence of temperature on the average yields and kinetic parameters of *Spirulina platensis* batch cultivations in 0.5 dm^3 Erlenmeyer flasks. μ Specific growth rate, Q volumetric growth rate or removal rate, Y removal yield

	T (°C)			
	25	30	35	40
Growth				
Q_X (mg dm^{-3} day^{-1})	15.9	33.2	24.5	18.7
μ (day^{-1})	0.028	0.044	0.035	0.029
$N{-}NO_3^-$ removal				
$Q_{N-NO_3^-}$ (mg dm^{-3} day^{-1})	1.60	3.26	2.52	1.87
$Y_{N-NO_3^-}$ (−)[a]	0.312	0.739	0.492	0.364
$Y_{N-NO_3^-}$ (−)[b]	0.289	0.720	0.457	0.364
$P-PO_4^{3-}$ removal				
$Q_{P-PO_4^{3-}}$ (mg dm^{-3} day^{-1})	0.463	0.454	0.362	0.254
$Y_{P-PO_4^{3-}}$ (−)[a]	0.124	0.122	0.098	0.068
$Y_{P-PO_4^{3-}}$ (−)[b]	0.031	0.067	0.049	0.037
$Y_{P-PO_4^{3-}}$ (−)[c]	0.093	0.055	0.048	0.031

[a]Experimental removals
[b]Theoretical biotic removals estimated assuming the *S. platensis* composition reported by Cornet et al. [8]
[c]Abiotic removals calculated as the difference between experimental and theoretical biotic removals

Table 2 Influence of inoculum level on the average yields and kinetic parameters of *S. platensis* batch cultivations in 5.0 dm^3 vessels

	X_o (g/dm^3)				
	0.25	0.42	0.60	0.75	0.86
Growth					
Q_X (mg dm^{-3} day^{-1})	34.7	36.0	36.8	41.2	42.4
μ (day^{-1})	0.050	0.046	0.037	0.035	0.032
$N-NO_3^-$ removal					
$Q_{N-NO_3^-}$ (mg dm^{-3} day^{-1})	3.24	3.50	3.44	3.96	4.06
$Y_{N-NO_3^-}$ (−)[a]	0.750	0.776	0.762	0.878	0.879
$Y_{N-NO_3^-}$ (−)[b]	0.745	0.762	0.779	0.872	0.897
$P-PO_4^{3-}$ removal					
$Q_{P-PO_4^{3-}}$ (mg dm^{-3} day^{-1})	0.312	0.568	0.581	0.599	0.623
$Y_{P-PO_4^{3-}}$ (−)[a]	0.224	0.330	0.335	0.440	0.437
$Y_{P-PO_4^{3-}}$ (−)[b]	0.053	0.064	0.064	0.084	0.084
$Y_{P-PO_4^{3-}}$ (−)[c]	0.171	0.266	0.271	0.356	0.353

[a]Experimental removals
[b]Theoretical biotic removals estimated assuming the *S. platensis* composition reported by Cornet et al. [8]
[c]Abiotic removals calculated as the difference between experimental and theoretical biotic removals

−7.8 to +7.4%) with the theoretical values estimated assuming the dry biomass elemental composition experimentally determined by Cornet et al. [8], which suggests that all removed nitrate was used for biomass growth. Conversely, the experimental data for phosphorus consumption (Fig. 2) were much lower than the theoretical values estimated according to the above assumption, which means that only a portion of the phosphate taken up was used for biomass growth under conditions of light limitation.

The phosphorus concentration in solution fell at pH values above 9.5 at every temperature tested, confirming that its removal depended on pH more than on temperature. The progressive alkalinisation of the medium due to OH^- release and CO_2 uptake during photoautotrophic growth, was responsible for pH increases up to pH 10.0. This caused insoluble phosphates to precipitate, thus considerably increasing the fraction of phosphorus removed. These results are in agreement with those of Laliberté et al. [12], who reported that, in an aerobic treatment system operating with *S. platensis*, phosphorus could be removed by two different mechanisms. Biological assimilation (biotic process) preferentially took place during the biomass growth phase, while chemical precipitation (abiotic process) predominated when biomass concentration reached a threshold value. Under the light-limited conditions investigated in this study, growth slowed down due to light shading [22].

The optimum temperature observed in this work for growth and nitrate removal confirms the biological nature of such an activity. Therefore, nitrate removal yield is expected to depend on nitrate level in the medium, i.e. the lower the nitrate concentration, the higher the removal. Thus, *S. platensis* would be effective for nitrate removal only at relatively low concentrations and when growth could be fast and uninterrupted, as in continuous culture.

Since phosphorus is removed mainly as phosphate by chemical precipitation (Table 1), its removal is expected to increase with phosphate concentration. A hypothetical treatment system based on the microbial activity of *S. platensis* should be particularly suited for the removal of phosphate, the compound responsible for most eutrophic phenomena in final receiving water bodies. In fact, although the proposed system did not ensure yields of phosphate biotic removal comparable with those of common activated sludge plants ($Y_{P-PO_4^{3-}} \sim 0.20$), it is expected, from simple material balances, to become more effective under conditions of growth limitation by phosphorus, such as those occurring in domestic wastewater (up to 6.5 mg P dm^{-3}) [6]. Taking into account the additional abiotic phosphate removal promoted by alkalinisation of the medium, one should expect performances comparable with those of typical plants for biological phosphate removal [7].

The specific growth rate values from 23 to 40°C have been plotted according to Arrhenius (Fig. 3) to estimate the thermodynamic parameters of both growth and its inactivation, assuming that the latter phenomenon is the result of an equilibrium favoured by a temperature increase. As expected, the enthalpy variation of thermal inactivation equilibrium (90.5 kJ/mol) was higher than the activation enthalpy of growth (64.7 kJ/mol) and both values compare with those estimated for other bioprocesses and enzymatic systems [5, 16, 18]. These results are consistent with the peculiar sensitivity of *S. platensis* growth to temperature, and with the hypothesis that the growth rate could be limited, at high tempera-

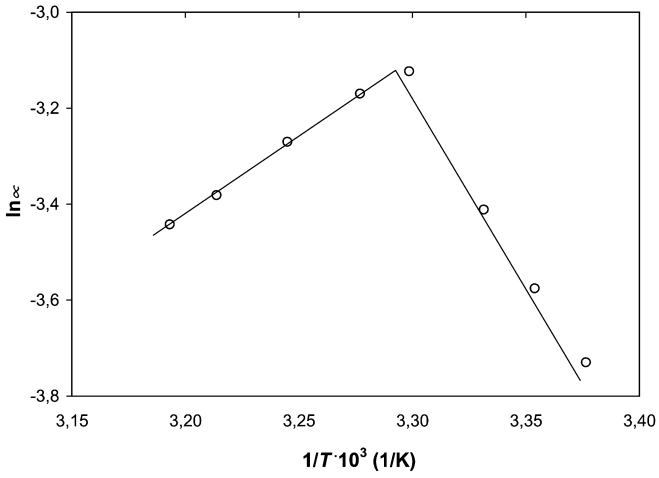

Fig. 3 Arrhenius plots for the estimation of thermodynamic parameters of either *S. platensis* biomass growth or its thermal inactivation

ture, by reversible inactivation of a growth-controlling enzyme [16].

Effect of the inoculum level

At 30°C *S. platensis* was able to effectively remove nitrogen and phosphorus under conditions of variable biomass concentration (0.25–0.86 g/dm^3), such as those naturally present in uncontrolled actual plants. Comparison of these data with those obtained at the same temperature in Erlenmeyer flasks (Table 1) suggests that, under the light-limited conditions investigated in this study, the larger surface area of the ponds exposed to the light, and, mainly, the higher light intensity, favoured algal activity, enhancing nearly all the kinetic parameters and yields.

These data will be used in future work using the stage approach [4] for scaling-up reactors with partial similarity, in order to evaluate the possibility of exploiting such a technology in a full-scale system.

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