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Nitrogen and phosphorus removal by high latitude mat-forming cyanobacteria for potential use in tertiary wastewater treatment

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Abstract

As part of a program to develop biological wastewater treatment systems for cold climate areas four strains of filamentous, mat-forming cyanobacteria isolated from Arctic and Antarctic environments were evaluated for their nutrient stripping and growth capabilities. A tropical strain, *Phormidium bohneri*, known for its excellent performance in wastewater treatment, was used as a comparison. Experiments were done in artificial media under controlled batch culture conditions to avoid interactions with indigenous microorganisms such as bacteria and protozoa. The culture medium simulated real effluents containing high concentrations of nitrate and phosphate. Temperatures (5, 15 and 25°C) and irradiances (80, 210, 350, 640 and 1470 μmol photon $m^{-2}s^{-1}$) were selected according to situations encountered in a variety of field conditions. For all irradiance levels, growth was satisfactory at 15 and 25 °C, but limited at 5 °C. At 25 °C a satisfactory nitrogen removal rate (3.5 and 4.0 mg N $L^{-1}d^{-1}$) was obtained for one polar strain (*Phormidium tenue*) and the control *P. bohneri*. At 15 °C, the best nitrogen removal rate (3.5 mg N $L^{-1}d^{-1}$) was measured with *P. bohneri* while the best rate for the polar strains was around 2.3 mg N $L^{-1}d^{-1}$. At 15 °C, a phosphorus removal rate of 0.6 mg P $L^{-1}d^{-1}$ was obtained with *P. bohneri* and polar strains *P. tenue* and *Oscillatoria* O-210. Nitrogen (NO_3^-) and phosphorus (PO_4^{3-}) uptake rates increased as a function of irradiance over the range 80 to 350 μmol photon $m^{-2}s^{-1}$. Our results indicate that tertiary biological wastewater treatment at low temperatures (5 °C) cannot be anticipated with the polar strains tested, because they are psychrotrophic rather than psychrophilic and thus grow too slowly under conditions of extreme cold. However, it appears that these cyanobacteria would be useful for wastewater treatment at moderately cool temperatures (c. 15 °C), which are common during spring and fall in northern climates.

Introduction

Domestic and agriculture wastewaters retain high concentrations of inorganic phosphorus and nitrogen that may lead to eutrophication of the water bodies that they discharge into. Over the last few decades, efforts have been made to apply intensive microalgal cultures to perform the biological tertiary treatment of secondary effluents (Oswald & Gotaas, 1957; de la Noüe et al., 1992). Unicellular green algae such as *Chlorella* sp. and *Scenedesmus* sp. have been widely used in wastewater treatment as they often colonize the ponds naturally and have fast growth rates and high nutrient removal capabilities. However, one of the major drawbacks of using micro-algae in wastewater purification is the harvesting of biomass (Laliberté et al.,

1997). Species with a natural tendency to aggregate offer an attractive option, and in this respect, epilithic or benthic filamentous cyanobacteria (blue-green algae) are excellent candidates for intensive mass cultures intended for wastewater treatment (de la Noüe & Proulx, 1988).

Phormidium bohneri Schmidle, a tropical cyanobacterium, shows self-aggregation, capacity to sediment (Talbot & de la Noüe, 1988) and growth features that lend themselves to a biotreatment system for wastewater in temperate and warm climates (Blier et al., 1996; Sylvestre et al., 1996). Proulx et al. (1994) performed some preliminary testing under field conditions to assess its capability to remove nutrient, and promising results have been obtained with fish farm effluent (Dumas et al., 1998). However, the nutrient removal rate was negligible at 10 °C, thus limiting the utilization of this species in cold climates.

A potentially alternative approach for northern regions such as Canada is the use of cyanobacteria strains that occur naturally in cold climates. In the Arctic and Antarctica, mat-forming cyanobacteria are often the major component of autotrophic communities in polar lakes and streams, particularly shallow water ecosystems (Vincent, 2000). The polar strains experience bright light throughout summer, prolonged cool temperatures and freezing during the long winters, conditions that are characteristic of wastewaters in cold climates. These cyanobacteria are psychrotrophic rather than psychrophilic, as defined by Morita (1975) because they are tolerant to the cold water conditions, but have a temperature optimum for growth higher than 15 °C (Tang et al., 1997a). These eurythermal characteristics could be exploited for extending wastewater treatment into early spring and late fall seasons, which is not feasible with *P. bohneri.*

In general, 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 context 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).

The aim of the present work was to test some strains of polar cyanobacteria for their ability to remove nitrate and phosphate from wastewaters under cold climate conditions. In this paper we present the growth and nutrient removal capabilities of four strains of polar cyanobacteria, compared to the control strain *P. bohneri*, at three temperatures (5, 15, 25 °C) over a gradient of irradiances (80 to 1470 μmol photon $m^{-2}s^{-1}$).

Materials and methods

Organisms

Four isolates of polar cyanobacteria from glacier-fed meltwater streams or the nearshore region of high latitude lakes were tested. Strain O-025 was isolated from a meltwater pond on the McMurdo Ice Shelf (Antarctica, 78 °S), strains O-104 and O-120 were sampled from the Toolik Lake region in Arctic Alaska (68°N) while strain O-210 was isolated from a lake in subarctic Québec (56°N). Three of these strains have subsequently been identified by Professor Robert Sheath, College of Biological Sciences, University of Guelph, as *Schizothrix calcicola* (O-104), *Phormidium subfuscum* (O-025) and *Phormidium tenue* (O-120). The fourth strain (O-210) was an unidentified *Oscillatoria* with a trichome diameter of 10 μm, and was designated strain *Oscillatoria* O-210. Upon collection, the samples were transfered to agar plates containing BG-11 mineral medium (Rippka et al., 1979). The non-axenic, but monospecific cultures, were maintained in sterilized BG-11 medium at 13.0 °C under a continuous illumination of 45 μmol photon $m^{-2}s^{-1}$.

The control strain of *P. bohneri*, Schmidle was isolated in our laboratory from samples collected in Kenya's Rift Valley lakes and identified by the late Professor P. Bourrelly. Monospecific, but not axenic, stock cultures were grown at room temperature in the liquid medium BG-11 at 45 μmol photon $m^{-2}s^{-1}$.

Experimental conditions

In order to evaluate the effect of light and temperature, strains were grown in culture tubes containing 40 mL BG-11 artificial medium bubbled with atmospheric air at a rate of 0.1 vvm (vol vol.$^{-1}$min.$^{-1}$). Prior to the experiments, cultures were acclimated to their respective experimental conditions for 2 days.

The experiments were conducted in culture chambers in which temperature was controlled to $\pm$ 1.0 °C; experiments were done at 5, 15 and 25 °C. The temperature of the medium was continuously monitored

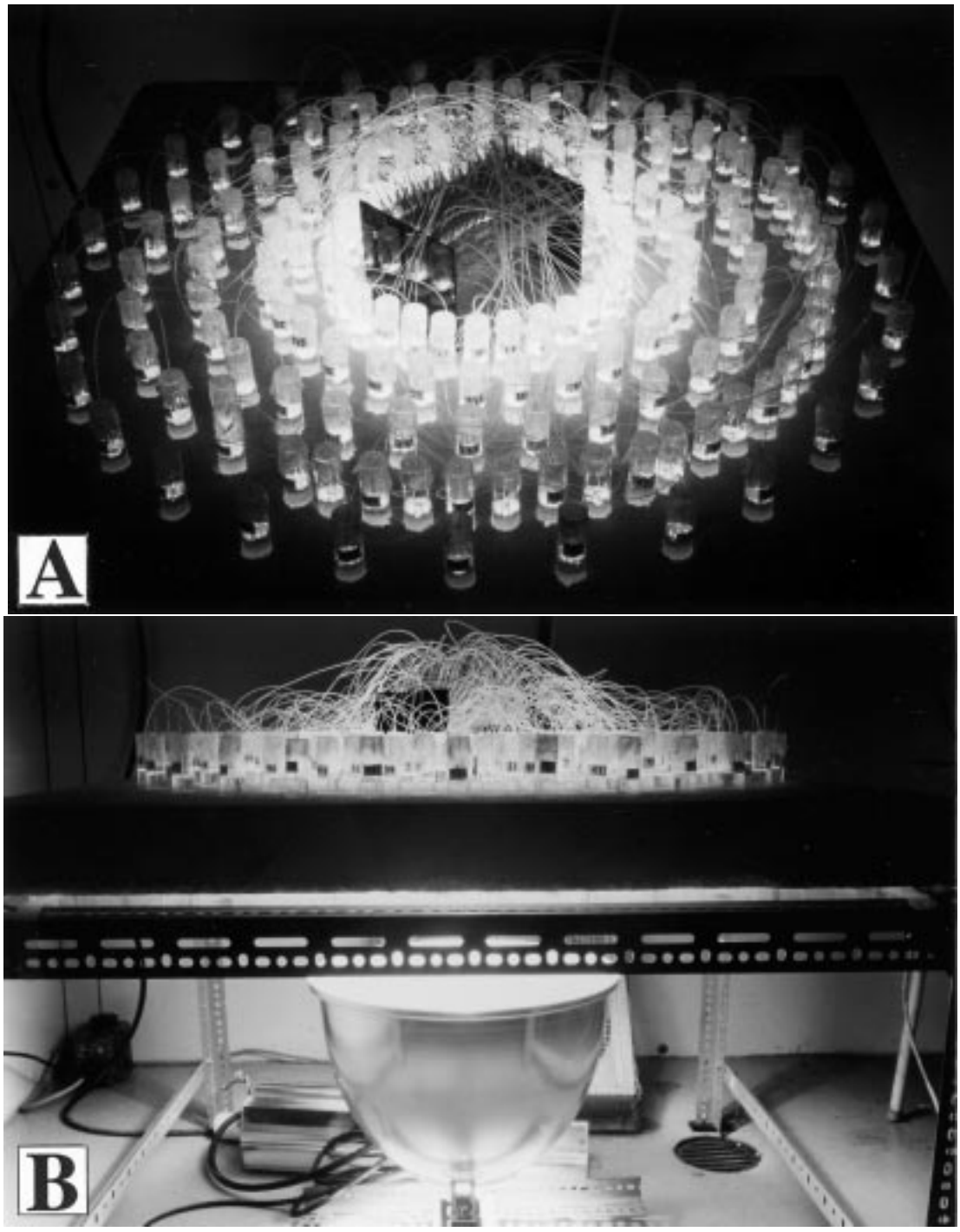

Figure 1. View from above (A) and the side (B) of the experimental setup with the lamp, the five irradiance conditions and the air bubbling system.

with a thermocouple (type T-740 connected to 100-mm Strip Chart Recorder). The cultures were exposed to irradiances of 80, 210, 350, 640 and 1470 μmol photon $m^{-2}s^{-1}$ (as measured at the bottom of each tube with a Quantum Photometer LI-COR 185B fitted with LI-190S Quantum Sensor) in a light gradient incubator. This consisted of an opaque plate with five sets of 30 orifices arranged in concentric circles, giving a total of 150 orifices (Figure 1 A). Each orifice supported one of the 150 culture tubes, allowing illumination only through the bottom of the tube. Each of the cultures of the five cyanobacterial strains was transferred to 30 tubes and these were then positioned on the plate, with 6 replicates placed randomly in each of the circles corresponding to the five irradiance conditions. Below the plate, a 400-W metal-halide circular lamp (Dura-Test, Optimarc) was enclosed in a cupola reflector in order to provide a concentric gradient of irradiances (Figure 1B). By varying the height of the growing plate and the position of the lamp in the reflector, it was possible to finely adjust the desired irradiance gradient. The photoperiod was 14 h light –10 h dark. Ventilation was used under the table to eliminate excess heat, and the entire system was installed in a temperature-controlled room.

Sampling and analyses

Samples were randomly taken at the end of each 14-h light period, one tube per strain for each irradiance. The pH was measured with an Orion pH meter. Cyanobacterial flocs were broken up with a tissue grinder (Tekmar, TP18/10S1) before OD_{750} measurements (Ultrospec II, LKB spectrophotometer). 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 using linear regression analysis. An aliquot of culture, previously used for biomass determination, was filtered through a Whatman 934-AH glass fibre filter (porosity 1.5 μm). The filtrate was analysed for orthophosphate and nitrate using a Technicon Autoanalyser II. Orthophosphate was analysed by the ascorbic acid method as outlined in APHA (1989) and nitrate by the sulfanilamide method described in MENVIQ (1992).

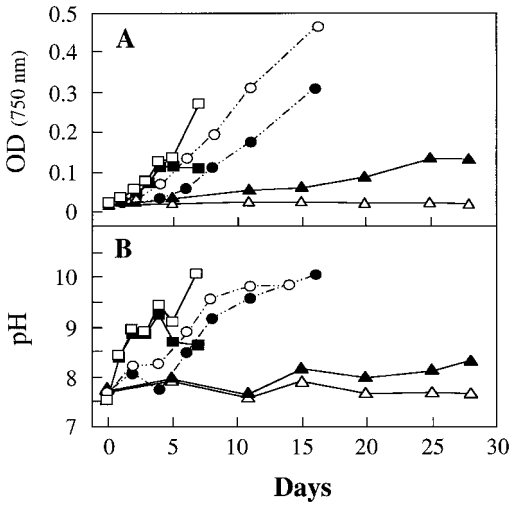

Figure 2. Growth (A) of an Arctic cyanobacterium (strain O-104, *S. calcicola*) and the control *Phormidium bohneri*, and the change in pH (B) of BG-11 culture medium over time at three temperatures. OD = optical density. *P. bohneri:* △ 5 °C; ○ 15 °C; □ 25 °C. Strain O-104: ▲ 5 °C; ● 15 °C; ■ 25 °C.

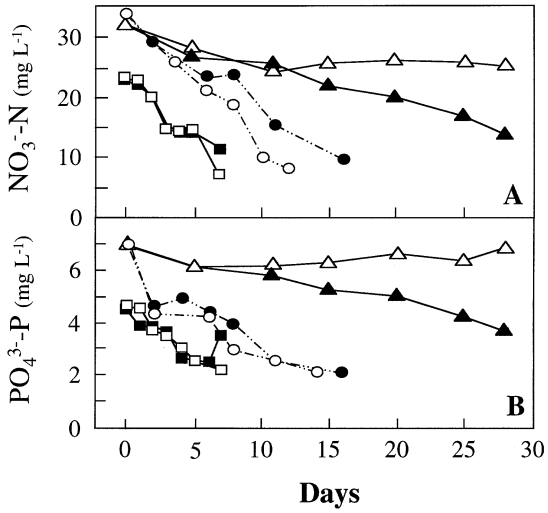

Figure 3. Nitrate (NO_3^--N) (A) and phosphate (P-PO_4^{3-}) (B) concentrations in BG-11 medium in the presence of *P. bohneri* and polar strain O-104 (*S. calcicola*), at three temperatures. *P. bohneri:* △ 5 °C; ○ 15 °C; □ 25 °C. Strain O-104: ▲ 5 °C; ● 15 °C; ■ 25 °C.

Results

Temporal changes in cultures

Growth (OD), pH, nitrogen and phosphorus were followed as a function of time for each culture and representative curves are given in Figures 2 and 3 for two of the strains tested. Figure 2A shows the growth

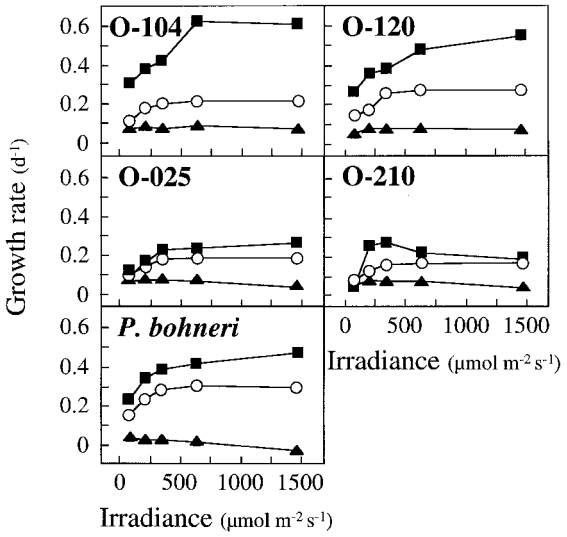

Figure 4. Growth rate of four polar cyanobacterial strains and *P. bohneri* as a function of irradiance at temperatures from 5 °C to 25 °C. ▲ 5 °C; ○ 15 °C; ■ 25 °C.

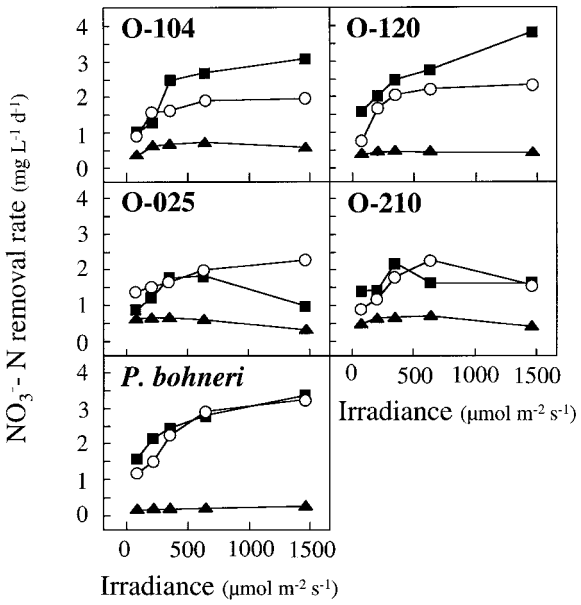

Figure 5. Nitrate (NO_3^--N) removal rate in BG-11 medium using four polar strains and *P. bohneri* as a function of irradiance at temperatures from 5 °C to 25 °C. ▲ 5 °C; ○ 15 °C; ■ 25 °C.

curves for *P. bohneri* and *S. calcicola* (O-104) at the three temperatures tested. At 15 °C and 25 °C, the biomass increased rapidly, as did pH (Figure 2B). At 5 °C, no growth was observed for *P. bohneri* while O-104 grew, but at a relatively slow rate (0.004 OD_{750} per day).

Figures 3A and 3B show the nitrate and phosphate removal from the culture medium over time by strain O-104 *(S. calcicola)* and *P. bohneri.* The concentration of both nutrients declined at a constant rate through time, and the nutrient stripping rates increased with increasing temperature. At 5 °C, the nutrient removal was better with strain *S. calcicola* than with *P. bohneri.* At 25 °C there was no difference in nutrient removal rates between the two strains.

Effects of irradiance and temperature

Maximal phototrophic growth rate increased with increasing irradiance, reaching a saturation value at 350 or 610 μmol photon $m^{-2}s^{-1}$ (Figure 4). A slight decrease at the highest irradiances was observed in some cultures, suggesting photoinhibition. At 5 °C, the optimal irradiance was about 80 μmol photon $m^{-2}s^{-1}$ for *P. bohneri* and 210 μmol photon $m^{-2}s^{-1}$ for polar strains. At 15 and 25 °C, the optimal irradiance was between 350 and 640 μmol photon $m^{-2}s^{-1}$ for all strains tested.

Nitrogen removal

The nitrate removal rate for all strains increased sharply between 5 °C and 15 °C, and to a lesser extent from 15 °C to 25 °C (Figure 5). The best removal rate at 25 °C was observed with *Phormidium tenue* (O-120) (4 mg NO_3^- – N $L^{-1}d^{-1}$) followed by *P. bohneri* (3.5 mg N $L^{-1}d^{-1}$) and *S. calcicola* (3.2 mg N $L^{-1}d^{-1}$). At 15 °C, the most effective isolate was *P. bohneri* (3.5 mg N $L^{-1}d^{-1}$). At 5 °C the N-removal rate was significantly reduced for all strains and particularly for *P. bohneri* which showed near-zero uptake rates at this temperature. As observed with growth rate, there was increasing nitrate removal up to an irradiance saturation level.

Phosphorus removal

The relationship between phosphate-removal rates and temperature (Figure 6) was more complex than for nitrate and, in many cases, the best removal rate was observed at 15 °C rather than 25 °C. For strains O-104 and O-210 this was true for all irradiances while for *P. bohneri*, strains O-025 and O-120 this was observed at lower irradiances. Overall, the best removal rate was observed with *P. bohneri* at 25 °C (1 mg P $L^{-1}d^{-1}$) while at 15 °C, *P. bohneri* and strains O-120 and O-210 showed similar P-removal rates, around 0.6 mg

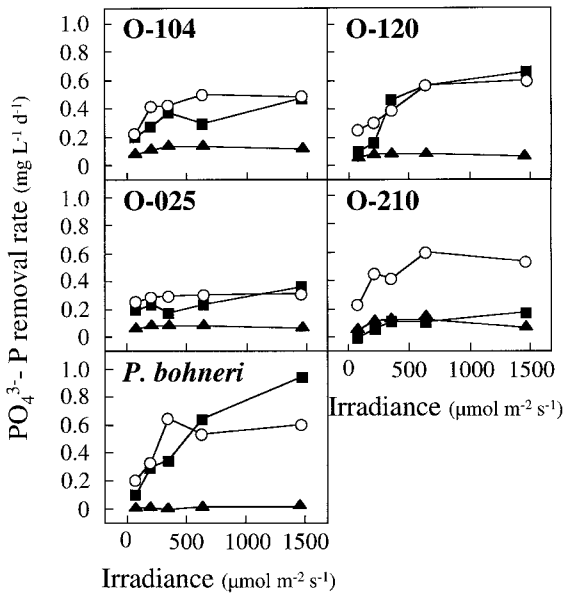

Figure 6. Phosphate (PO_4^{3-}-P) removal rate in BG-11 medium using four polar strains and *P. bohneri* as a function of irradiance at temperatures from 5 °C to 25 °C. ▲ 5 °C; ○ 15 °C; ■ 25 °C.

P $L^{-1}d^{-1}$. At 5 °C the phosphorus removal rate was significantly reduced for all strains. The polar strains removed phosphorus at rates between 0.07 and 0.15 P $L^{-1}d^{-1}$ while the P-removal rate for *P. bohneri* was only slightly above zero.

Discussion

Growth versus temperature and irradiance

Tang et al. (1997a) observed that high latitude cyanobacteria generally have an optimal temperature for growth of ≥ 15 °C, indicating that polar cyanobacteria are psychrotrophic (cold-tolerant) rather than psychrophilic (cold-adapted). According to their observations, cyanobacterial growth can generally be observed over a 20 °C range (minimum 10 °C, maximum 30 °C). Tang & Vincent (1999) similarly showed that high latitude cyanobacteria have high temperature optima for growth, although there is considerable variation between strains in their thermal tolerances and growth range. Optimal temperature for the growth of strains O-025 and O-210 was respectively 16.1 °C and 17 °C at an irradiance of 225 μmol photon $m^{-2}s^{-1}$ (Tang et al., 1997a); our data show that an increase of temperature to 25 °C did not stimulate the growth of these strains beyond the growth at 15 °C (Figure 4). The optimal temperature for growth of strains O-104 and O-120, at 225 μmol photon m^{-2} s^{-1} light irradiance, was respectively 20.7 °C and 35 °C, and again, this was consistent with our experiments showing a better growth at 25 °C (Figure 4).

Light limitation may be a potential problem when using solar biotechnology to treat water heavily loaded with particulate matter, or in the presence of a high biomass concentration which reduces light penetration. However, in general, low irradiance favours the growth of cyanobacteria due to their superior light capturing abilities and their low light compensation point (Hu et al., 2000; Tilzer, 1987). Notwithstanding their ability to tolerate low irradiances, strains *P. bohneri* and O-120 also grew well at the highest irradiance tested. Figure 4 shows that very high irradiances (e.g., full summer radiation of 1600 μmol photon $m^{-2}s^{-1}$ at noon without clouds) can be tolerated by some of the tested strains, an important aspect for wastewater treatment performed in outdoor aerated ponds.

Nutrient removal versus temperature, irradiance and pH

The nitrate removal rate was characterized by very low values at 5 °C, especially for *P. bohneri* (Figure 5). At this temperature, the uptake rate first increased with irradiance, then it leveled off or decreased at higher irradiances. This suggests a bright light effect at a low temperature, probably due to an excess of excitation energy and, as a result, photoinhibition (Roos & Vincent, 1998) and possibly photobleaching of the cells, as observed by Hu et al. (2000). The sensitivity of photosynthetic organisms to bright light at low temperatures may therefore pose an operational constraint on outdoor wastewater treatment in cold climates. Strains O-104 and O-120, which have higher temperatures saturation level, showed an increased removal rate at higher irradiances, unlike the two other polar strains (O-025 and O-210). For this reason, cyanobacterial strains with a higher optimal growth temperature for wastewater treatment are more appropriate because they seem to be more adaptable to different temperature and irradiance conditions. The performance of polar strains evaluated in the present study was better than strain E-18 (another strain of *S. calcicola*) which was previously used by Tang et al. (1997b) under continuous irradiance conditions (0.87 mg N L^{-1} d^{-1} at 15 °C and 1.3 mg N L^{-1} d^{-1} at 25 °C).

P. bohneri and polar strains cultures were less effective at removing phosphate relative to nitrogen, the

best removal rate being 1 mg P $L^{-1}d^{-1}$ (Figure 6), although this is better than the results obtained with strain E-18 (Tang et al., 1997b). This suggests 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.

The pH increased substantially in all of the growing cultures; this increase was as to be expected for photoautotrophic growth in the unbuffered culture conditions that would also be encountered in aerated ponds or sequential batch reactors used for tertiary treatment of wastewaters. It is well known that high pH values are likely to induce phosphorus precipitation by the formation of hydroxyapatite (Diamadopoulos & Benedek, 1984). Laliberté et al., (1997) have shown that phosphorus is removed more rapidly in culture media at pH values greater than 10.5 indicating that the phosphorus removal can be enhanced by this chemical stripping mechanism.

At 5 °C, the removal rate of phosphorus was reduced to less than 20% of maximal values (Figure 5), indicating that the polar strains at these lowermost temperatures have only a poor nutrient-removal capacity and are unlikely to provide a satisfactory performance under conditions of extreme cold. A temperature of 15 °C and irradiances from 640 to 1470 μmol photon $m^{-2}s^{-1}$ were the best for phosphorus removal by *P. bohneri* and O-120. The tropical strain was thus able to remove phosphorus as efficiently as polar strains at this intermediate temperature.

In conclusion, it appears that polar cyanobacteria are potential candidates for wastewater treatment at temperatures ranging from 15 °C to 25 °C and over the irradiance range 640 to 1470 μmol photon $m^{-2}s^{-1}$. Cyanobacteria are more appropriate than chlorophytes, because of their natural flocculation properties. As expected, the tropical strain used here was inactive at temperatures lower than 10 °C. However, our results also indicate that tertiary biological wastewater treatment at 5 °C cannot be achieved with polar cyanobacteria given their psychrotrophic rather than psychrophilic characteristics. These organisms appear to be adapted for nutrient removal at moderately cool water temperatures, but finding appropriate photosynthetic micro-organisms for the efficient tertiary treatment of effluents at less than 10 °C remains an important challenge for applied phycologists.

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