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ScienceDirect
Bioresource Technology 99 (2008) 4980–4989
BIORESOURCE TECHNOLOGY

Chlorella sorokiniana UTEX 2805, a heat and intense, sunlight-tolerant microalga with potential for removing ammonium from wastewater

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Received 2 April 2007; received in revised form 31 August 2007; accepted 9 September 2007
Available online 19 November 2007

Abstract

In the summer of 2003, a microalga strain was isolated from a massive green microalgae bloom in wastewater stabilization ponds at the treatment facility of La Paz, B.C.S., Mexico. Prevailing environmental conditions were air temperatures over 40 °C, water temperature of 37 °C, and insolation of up to 2400 μmol m^2 s^{-1} at midday for several hours at the water surface for four months. The microalga was identified as *Chlorella sorokiniana* Shih. et Krauss, based on sequencing its entire 18S rRNA gene. In a controlled photo-bioreactor, this strain can grow to high population densities in synthetic wastewater at temperatures of 40–42 °C and light intensity of 2500 μmol m^2 s^{-1} for 5 h daily and efficiently remove ammonium from the wastewater under these conditions better than under normal lower temperature (28 °C) and lower light intensity (60 μmol m^2 s^{-1}). When co-immobilized with the bacterium *Azospirillum brasilense* that promotes growth of microalgae, the population of microalga grew faster and removed even more ammonium. Under exposure to extreme growth conditions, the quantity of four photosynthetic pigments increased in the co-immobilized cultures. This strain of microalga has potential as a wastewater treatment agent under extreme conditions of temperature and light intensity.

Keywords: *Azospirillum*; Biological wastewater treatment; *Chlorella sorokiniana*; Microalgae

1. Introduction

Wastewater treatment in all urban areas, including those located in extreme cold and hot environments is a necessity of any human community. Biological wastewater treatment in these areas depends on whether the microorganism of choice grows and functions efficiently under the prevailing extreme conditions. Microalgal species suitable for tertiary wastewater treatment are commonly studied (de-Bashan and Bashan, 2004; Oswald, 1988; Talbot et al., 1991; Valderrama et al., 2002), but under extreme conditions are scarcely investigated (Tang et al., 1997). This situation occurs despite the ability of many microalgae to grow over a wide temperature range (de la Noüe and de Pauw, 1988; Walker et al., 2005). This is particularly true for *Chlorella* sp. that can adapt to 5–42 °C (Kessler, 1985; Maxwell et al., 1994).

Of all *Chlorella* species, *Chlorella sorokiniana* Shih. et Krauss is most resistant to heat and high light intensity and has been studied mainly for biomass production and photosynthesis research (Morita et al., 2000a,b, 2001, 2002). However, when used in wastewater treatment, stud-

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0960-8524/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2007.09.065

ies have been done under moderate heat and light conditions (Muñoz et al., 2004, 2005; Yoshida et al., 2006). Immobilization of microalgae in polymers to assist recovery of the biomass produced from wastewater treatment has been studied for two decades (Chevalier and de la Noüe, 1985; Lau et al., 1997; Tam and Wong, 2000). In recent years, an upgrade of this system has been pursued, where *Chlorella* sp. and a microalgae growth-promoting *Azospirillum* bacterium (MGPB) have been immobilized in alginate beads (de-Bashan et al., 2004; Gonzalez and Bashan, 2000).

Several interactions of microalgae with bacteria are documented. *Pseudomonas diminuta* and *Pseudomonas vesicularis*, two obligate aerobes isolated from laboratory algal cultures, stimulated the growth of the microalgae *Scenedesmus bicellularis* and *Chlorella* sp. (Mouget et al., 1995). Some positive effects of marine bacteria on marine diatoms have been known for decades (Suminto and Hirayama, 1996; Ukeles and Bishop, 1975). Inoculation of the marine diatom *Chaetoceros gracilis* is used as feed in pearl oyster hatcheries with the MGPB *Flavobacterium* sp. in mass culture production of the diatom, resulting in significantly higher specific growth rate than the control cultures (Suminto and Hirayama, 1997). Inoculation of freshwater fish aquaculture ponds with *Azospirillum* sp. and *Azotobacter* sp. significantly increased the phytoplankton population and, consequently, the yield of carp (Garg and Bhatnagar, 1999). Co-culturing of *C. sorokiniana* and *Ralstonia basilensis* degraded sodium salicylate and acetonitrile (Muñoz et al., 2004, 2005). Co-immobilization of *Chlorella vulgaris* with *Azospirillum brasilense* (more commonly known as an agricultural plant growth-promoting bacterium (Bashan et al., 2004), significantly enhanced the growth of the microalga (Gonzalez and Bashan, 2000), but similar immobilization procedures with the naturally occurring, nitrogen-fixing association of *C. vulgaris* and *Phyllobacterium myrsinacearum* (Gonzalez-Bashan et al., 2000) led to senescence of the microalga (Lebsky et al., 2001).

This study attempted to isolate and characterize a microalgal strain capable of growing under extremely hot desert conditions and evaluate its potential as an agent for wastewater treatment under these extreme conditions. This was done by exposing the strain, under completely controlled conditions in a photo-bioreactor, to the maximum heat and light exposure prevailing in the area, combined with possible synergistic help of a MGPB.

2. Methods

2.1. Isolation of the microalgae

During routine collection of samples in August 2003 from the wastewater treatment facility of the city of La Paz, Baja California Sur, Mexico, a large green microalgal bloom was observed on abandoned stabilization ponds. During sampling at midday, the air temperature was over 40 °C and insolation approached 2500 μmol m^2 s^{-1}. Water samples were collected from the surface of the ponds and brought to the laboratory. The microalga was isolated, cultivated, and purified on C-30 medium (Gonzalez and Bashan, 2000) using standard microbiological techniques. The microalgae were routinely grown and preserved in slants of C-30 medium under constant white light of 25–60 μmol m^2 s^{-1}. The strain was deposited in the University of Texas Algae Collection in Austin, Texas, USA (UTEX 2805).

2.2. Identification of the microalgae

The 18S rDNA gene sequence was determined as follows: the microalgae were cultivated in C-30 medium for 10 days, harvested by centrifugation, washed twice with saline, and lyophilized. A 0.5-g sample of lyophilized *Chlorella* cells was incubated for 30 min in 30 ml TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). After centrifugation, the cells were extracted with some drops of TE buffer and ground in a mortar with liquid nitrogen. DNA isolation and purification was performed according to standard methods. PCR and internal sequencing primers are listed in Huss et al. (1999). Sequencing was done by cycle sequencing with an ABI Prism 310 Genetic Analyzer (Perkin–Elmer/Cetus, Boston, MA, USA). The 18S rRNA sequence was manually aligned with several reference green microalgae for comparison and identification.

2.3. Growth of microalgae under extreme temperature and insolation

This microalga was grown in carbon-free synthetic wastewater containing the following (in mg l^{-1}): NaCl, 7; $CaCl_2$, 4; $MgSO_4 \cdot 7H_2O$, 2; K_2HPO_4, 21.7; KH_2PO_4, 8.5; Na_2HPO_4, 33.4; and NH_4Cl, 10 (Gonzalez et al., 1997), either in suspension culture or immobilized with bacteria in alginate beads (explained below) in a specially designed environmental chamber constructed with the following (Fig. 1): a 1-l closed fermentor (Omni-Culture, Virtis, NY, USA 400 ml liquid) with controlled bottom aeration and mixing connected to an external cooling system (Model 1160S, VWR, Niles, IL, USA) at 11–13 °C water temperature. This was placed in a standard growth chamber for plants (Biotronette Mark III, Melrose Park, IL, USA) covered with aluminum foil on the inside of the chamber to enhance light reflection at 28–40 ± 2 °C under photon flux density of 30–2500 ± 3 μmol m^2 s^{-1}. Since the growth chamber provides full-spectrum light up to 250 μmol m^2 s^{-1}, additional light was provided by four incandescent bulbs (200 W) and four additional bulbs (100 W) mounted on a pole on two sides of the fermentor and by two, side-mounted 400-W halogen lamps whose distance from the bioreactors can be changed to provide different light intensities. The setup can provide and control light intensity up to 2500 μmol m^2 s^{-1} with air and water temperatures at 40–42 °C. Light intensity was measured with a photometer (Model 840010, Sper Scientific, Scotts-

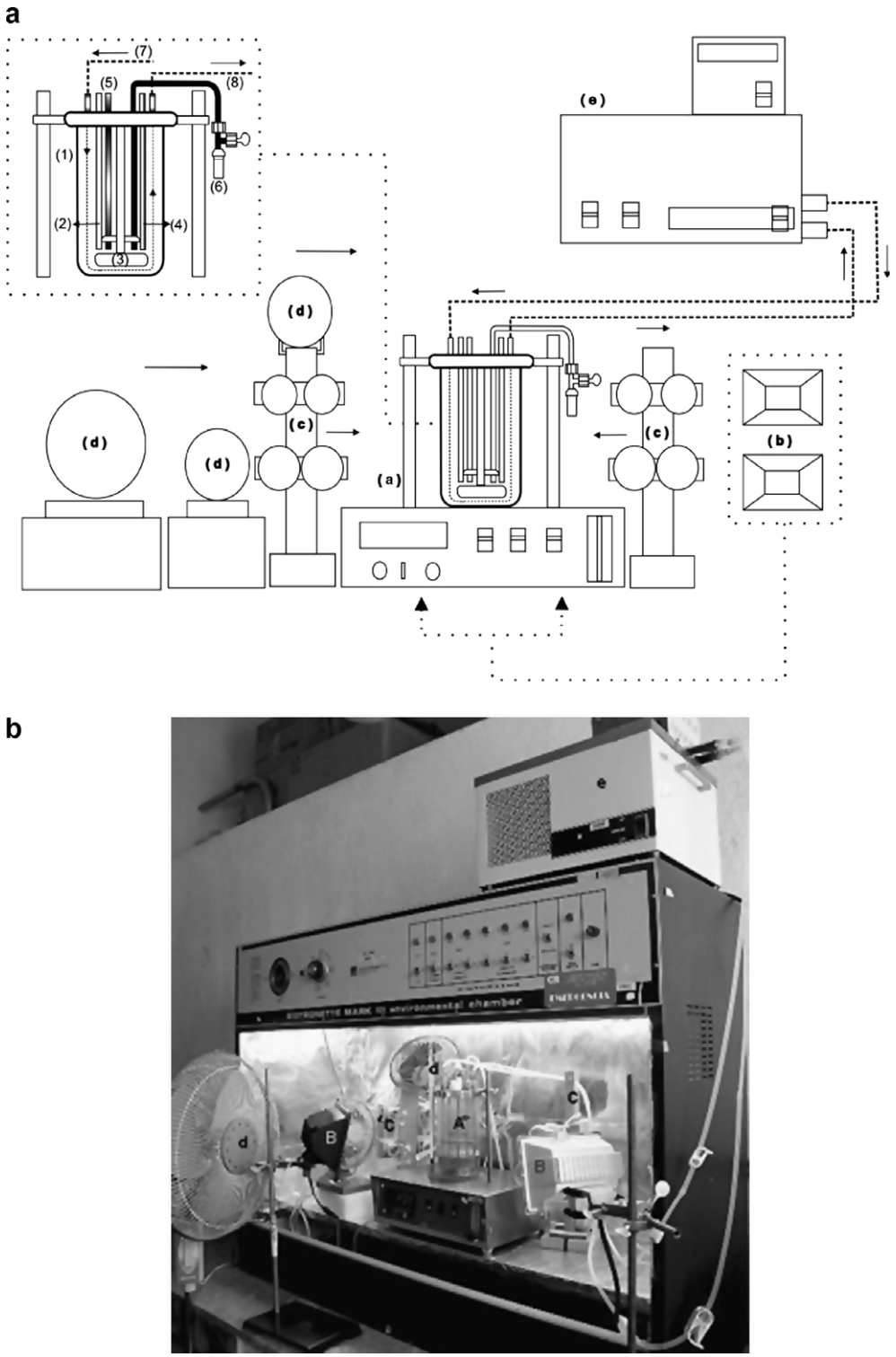

Fig. 1. Schematic representation (A) and photograph (B) of the environmental chamber, with its accessories, used for high temperature and high light intensity experiments. (a) Fermentor composed of (1) vessel, (2) thermometer, (3) agitator, (4) aeration, (5) heater, (6) entry for extracting samples, (7) cooling water inlet, (8) cooling water outlet; (b) two halogen lamps (400 W each) located in front of the vessel; (c) two columns with four incandescent lamps each (600 W per column) located on both sides of the vessel; (d) three fans (one large and two small); (e) water-circulation cooling unit; aluminum foil covers all walls of the environmental chamber. (Schematic is not to scale.)

dale, AZ, USA). Temperature within the growth chamber and within the fermentor was controlled by the cooling system and a commercial air conditioning unit. Additionally, three commercial, variable-speed ventilators (two with 15 cm diameter and one with 40 cm diameter) mounted on both sides of the environmental chamber eliminated

excessive heat at high light intensities from around the fermentor. The variation of temperature measured within the environmental chamber were <1 °C. At low light intensities, the temperature was controlled by the heating and cooling system of the fermentor itself with variation of ±1 °C.

2.4. Immobilization with A. brasilense in alginate beads, nitrogen removal, and pigment analysis

Immobilization of microalgae with bacteria was done according to de-Bashan et al. (2004). Briefly, axenic cultures, either *C. sorokiniana* (at a level of 0.5×10^6 cfu ml^{-1}) or the microalgae growth-promoting bacterium *A. brasilense* (DSM 1843, Braunschweig, Germany; at a level of $1.3 \pm 0.2 \times 10^6$ cfu ml^{-1}), were mixed with 2% alginate solution. The solution was dripped from a sterile syringe into 2% $CaCl_2$ solution, mixing the solution periodically. To immobilize the two microorganisms in the same bead, after washing the cultures, each of them was re-suspended in 10-ml 0.85% saline solution and then mixed together in the alginate before forming the beads. Because immobilization and co-immobilization procedures normally reduce the number of *Azospirillum* cells in the beads, a second overnight incubation in diluted nutrient broth was necessary. For cell counting, the beads were solubilized by immersing beads in 4% $NaHCO_3$ solution for 30 min, and had no negative effects on either microorganism. *A. brasilense* was counted by plating a series of dilutions (in 0.85% saline) on nutrient agar plates (Sigma) and *Chlorella* was counted under a light microscope with a Neubauer hemocytometer.

Ammonium was measured using standard water analysis techniques (APHA, AWWA, WPCF, 1992) using kits (Hach, Loveland, CO, USA) and a spectrophotometer (Hach DR/2000, Loveland, CO, USA) according to the manufacturer. Pigments (chlorophyll *a* and *b*, violaxanthin, and zeaxanthin) were determined after dissolving the beads and releasing *C. sorokinina* cells and measuring by HPLC, as described by de-Bashan et al. (2002a). In this method, pigments were immediately extracted by HPLC-grade acetone overnight at −40 °C and immediately thereafter, analyzed without storage. Pigments were detected with the diode array absorbance signal at 440 nm. Identification was made by comparing retention time and spectral characteristics with commercial pigment standards supplied by DHI (International Agency for ^{14}C Determinations, Denmark, http://www.c14.dhi.dk/index.htm). Quantification of results used the pigment response factor (HPLC peak area/pigment mass) obtained from the commercial pigment standards, as described by Mantoura and Repeta (1997). Results are expressed as ng pigment $cell^{-1}$.

2.5. Adaptation of the microalga to high temperature and intense light

Because abrupt exposure to high temperatures or intense light or both result in death of *C. sorokiniana* (S. Fendrich, 2005, Diploma thesis, Technische Universität, Hamburg–Harburg, Germany), adaptation periods were necessary. Because there is little knowledge on temperature and light regimes needed for acclimation of this microalgal species, the following three adaptation schemes, each covering five days, were tested. (i) The temperature of the culture was raised to 40 °C in two steps (30–35 °C for 30 min and 35–40 °C for 30 min) at a light intensity of 45 μmol m^2 s^{-1}. Once reaching 40 °C, the temperature was maintained for 5 h and returned to 30 °C. This scheme was repeated over the next four days. (ii) Intensity of light was raised to 1032 μmol m^2 s^{-1} in three steps, all at 30 °C (115 μmol m^2 s^{-1} for 25 min, 115–500 μmol m^2 s^{-1} for 20 min, and 500–1032 μmol m^2 s^{-1} for 15 min). Once reaching the high light intensity, conditions were maintained for 5 h and then reduced to the original 60 μmol m^2 s^{-1}. This process was repeated as described for five days. (iii) Raising the temperature to 40 °C and light intensity to 2500 μmol m^2 s^{-1} at the same time was done, as described for the first two schemes, with an additional acclimation period of 1032–2500 μmol m^2 s^{-1} for 30 min.

2.6. Experimental design

Each run in the fermentor used 17 g of beads suspended in 400 ml synthetic wastewater for five days. Each exposure to extreme conditions (light, temperature, or both) lasted 5 h of each day. The remaining 19 h of each run was maintained at 30 °C and 60 μmol m^2 s^{-1}. Initial ammonium chloride (NH_4Cl) concentrations used in the synthetic wastewater were 25 and 50 mg l^{-1}. Data is presented as amount of removal of the NH_4^+ ion. Because the fermentor was closed, ammonia could not volatilize and escape. Various controls (beads without microorganisms, wastewater alone, and microalgae and bacteria immobilized separately (de-Bashan et al., 2004) were routinely used. Data concerning microalgae and bacteria immobilized separately is presented.

2.7. Statistical analysis and calculations of growth rate and doubling time

Each experiment was performed in triplicate, where one run of the fermentor served as a replicate. Three 20-ml samples of each run were taken for each water analysis at 24-h intervals. Pigments were analyzed in six replicates. Each experiment, including adaptation to extreme conditions schemes, was repeated twice. Results were analyzed by one-way ANOVA coupled with Tukey's post-hoc analysis and Student's *t*-test analysis, both with the significance level at $P \leqslant 0.05$, with the Statistica, version 6 software (StatSoft, Tulsa, OK, USA). Growth rate (K) of the microalgae was calculated as $K = \mathrm{Ln}(N_1/N_0)/t_1 - t_0$, where N_1 and N_0 are cells at time 1 (t_1) and time 0 (t_0). Generation time (gt) was calculated as $\mathrm{gt} = \mathrm{Ln}\,2/K$ (Levasseur et al., 1993) to validate calculated data on growth rates.

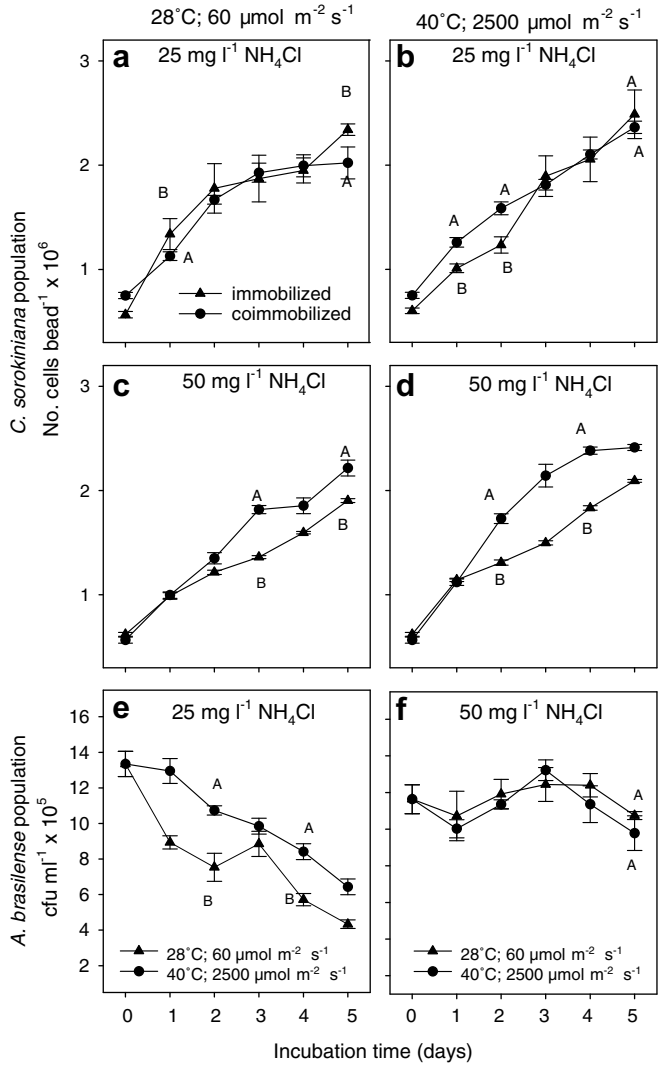

Fig. 2. Growth of *Chlorella sorokiniana* UTEX 2805 in synthetic wastewater, immobilized alone or with *Azospirillum brasilense* Cd in alginate beads, cultured at 28 and 40 °C under two light intensities (60 and 2500 µmol m^2 s^{-1}) and two initial ammonium chloride concentrations (25 and 50 mg l^{-1}) (a–d). Populations of *A. brasilense* Cd under these growth conditions (e,f). Points for each incubation time, in each separate subfigure is denoted by a different capital letter and differ significantly by Student's *t*-test at $P \leqslant 0.05$. Bars represent SE. Data presented refer to full cycles of 24 h each.

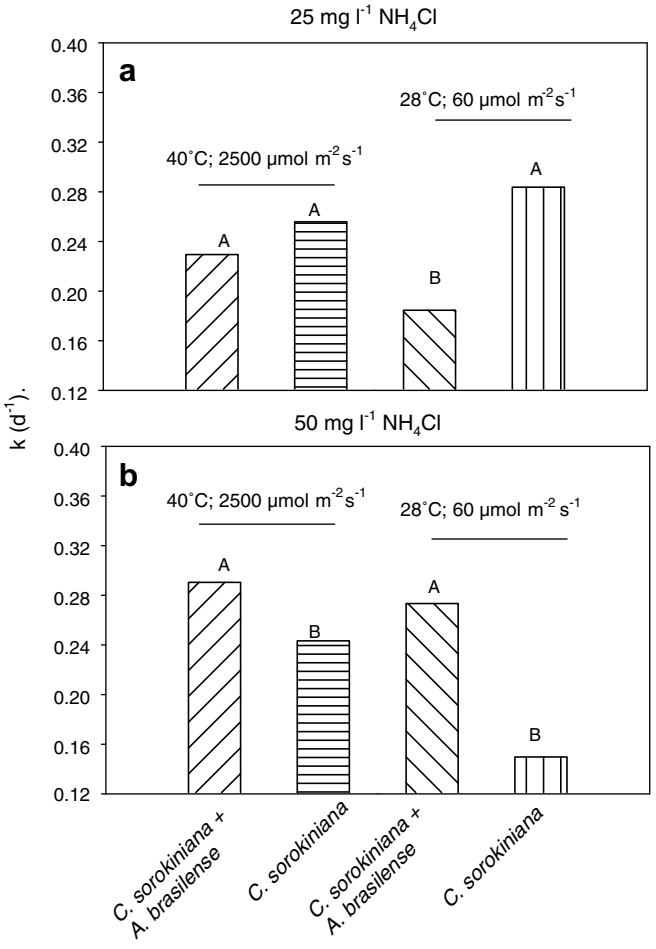

Fig. 3. Growth rate (*K*) (a, b) of *Chlorella sorokiniana* UTEX 2805 immobilized alone or with *Azospirillum brasilense* Cd in alginate beads and cultured in synthetic wastewater at 28 and 40 °C under two light intensities (60 and 2500 µmol m^2 s^{-1}) and two initial ammonium chloride concentrations (25 and 50 mg l^{-1}). Columns in each sub-figure, denoted by a different capital letter, differ significantly by one-way ANOVA and Tukey's post-hoc analysis at $P \leqslant 0.05$. $K = \mathrm{Ln}(N_1/N_0)/t_1 - t_0$.

3. Results

3.1. Temperature and insolation prevailing in the wastewater ponds

The temperature and insolation in this region were measured for three consecutive years (2003–2005) from June to September. Although the values varied from year to year and the days when measurements were taken, the general characteristics are similar. Midday air temperatures were (low to high): June (39.2–40.6 °C), July (40–41.4 °C), August (38.9–39.2 °C), and September (37.2–39.5 °C). Midday insolation (in µmol m^2 s^{-1}) was (low to high): June (1969–2176), July (2048–2137), August (2002–2434) and September (1878–2040). The surface water temperature, where the microalgae produced the bloom, was 36.6 °C when the air temperature was 41 °C (5 July 2005 at midday).

3.2. Identification of the microalga

The complete 18S rDNA sequence of strain UTEX 2805 is available from GenBank (accession number AM423162). With two nucleotide exchanges, which are in the most variable regions of the gene (Huss et al., 1986), it has 99.9% similarity with the type strain SAG 211-8k (Sammlung für Algenkulturen in Göttingen, Germany) of *C. sorokiniana* (X62441). Similarity with other *C. sorokiniana* strains (X73993, X74001, AB080307) ranges from 99.6% to 99.9%, corresponding to seven, three, and one nucleotide exchanges, respectively. Similarity to type strains of other closely related *Chlorella* species, *C. vulgaris* SAG 211-11b

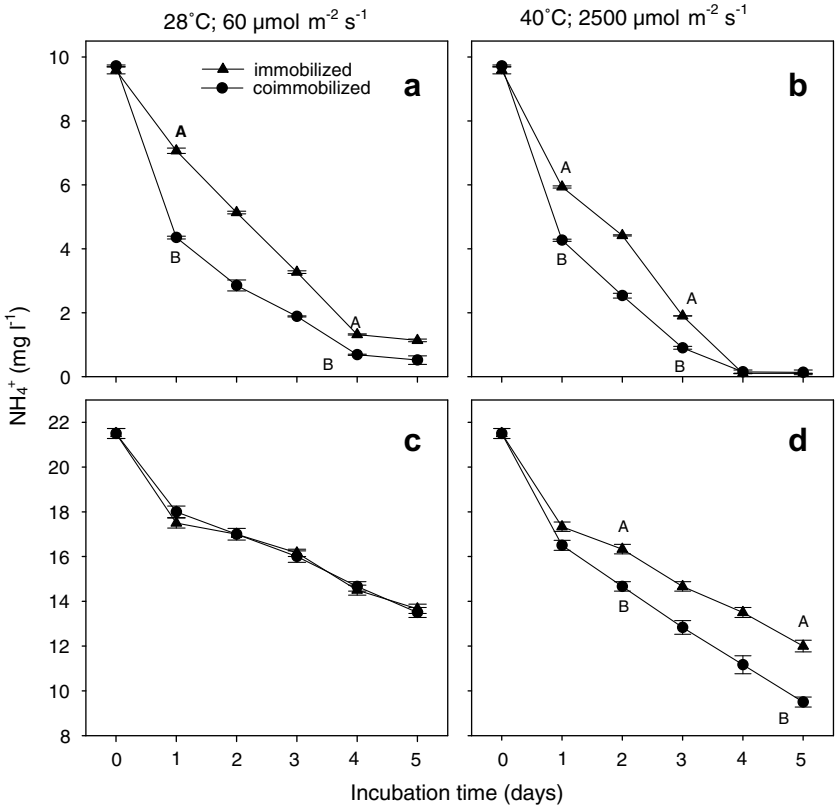

Fig. 4. Removal of ammonium by *Chlorella sorokiniana* UTEX 2805 from synthetic wastewater, immobilized alone or with *Azospirillum brasilense* Cd in alginate beads, at 28 and 40 °C under two light intensities (60 and 2500 µmol m^2 s^{-1}) and two initial ammonium chloride concentrations of 25 (a, b) and 50 (c, d) mg l^{-1}. Points for each incubation time, in each separate subfigure, denoted by a different capital letter, differ significantly by Student's *t*-test at $P \leqslant 0.05$. Bars represent SE. Data presented refer to full cycles of 24 h each.

(X13688) and *Chlorella lobophora* Andreyeva 750-I (X63504), is 99.5% (9 and 10 differences, respectively). Based on these data and on its distinct thermo-tolerance, which is a key characteristic for *C. sorokiniana* (Kessler, 1985), strain UTEX 2805 can unambiguously be assigned to *C. sorokiniana*.

3.3. Growth of *C. sorokiniana* at 40 °C in suspension culture under high light intensity

In suspension, *C. sorokiniana* UTEX 2805 alone could not grow at continuous exposure to 40 °C at any light intensity and died (S. Fendrich, 2005 Diploma thesis, Technische Universität, Hamburg–Harburg, Germany). Exposure for 5 h at 40 °C, out of every 24 h (after an adaptation period), was tolerated by the microalga and they grew successfully at all light intensities. At 45 µmol m^2 s^{-1}, the calculated, specific growth rate was 0.22 (doubling time 3.27 days). At 1032 µmol m^2 s^{-1}, the calculated, specific growth rate was 0.28 (doubling time 2.47 days), demonstrating high survival and growth. At 2500 µmol m^2 s^{-1}, the calculated specific growth rate was 0.06 (doubling time 11.43 days), a very significant indicator of stress. The growth limit of this microalga was 42 °C for the tested period.

3.4. Growth of *C. sorokiniana* immobilized with *A. brasilense* in alginate beads under increased temperature and intensity of light

Under the influence of different ammonium concentrations and inoculation with the MGPB *A. brasilense*, higher temperatures (40 °C) and intensity of light (2500 µmol m^2 s^{-1}) than under common growth conditions (28 °C and 60 µmol m^2 s^{-1}) significantly improved growth of *C. sorokiniana* (Fig. 2). Growth promotion by of *A. brasilense* on *C. sorokiniana* was more marked at higher concentrations of ammonium chloride (50 mg l^{-1}) (Fig. 2c and d). Similar populations of microalgae were observed at the extreme temperature and light conditions and under common growth conditions (Fig. 2b and d). Growth rate was greatest when *A. brasilense* was co-immobilized with *C. sorokiniana* when exposed to extreme culture conditions (Fig. 3b) under high ammonium concentrations. The effect was not observed under low ammonium concentrations (Fig. 3a). At the same time, populations of *A. brasilense* decreased at low ammonium levels, but still being significantly higher under extreme conditions (Fig. 2e), while maintaining similar populations under high ammonium concentration regardless of growth conditions (Fig. 2f).

3.5. Removal of ammonium from synthetic wastewater by C. sorokiniana immobilized with A. brasilense under increased temperature and intensity of light

At lower initial ammonium level, co-cultures of *C. sorokiniana* and *A. brasilense* removed ammonium better than cultures containing only *C. sorokiniana* (Fig. 4a and b), where 100% removal was achieved by the co-cultures after 96 h under extreme incubation conditions (Fig. 4b). Under regular growth conditions, co-cultures did not reach this level of removal of ammonium, but were close (compare Fig. 4a and b after 96 h of incubation). At higher initial ammonium levels under regular growth conditions, co-cultures removed ammonium similar to cultures of the microalga alone (Fig. 4c). However, under extreme conditions, co-cultures always removed more ammonium (Fig. 4d).

3.6. Change in photosynthetic pigment content of C. sorokiniana immobilized with A. brasilense following exposure to extreme temperature and intensity of light

With the bacterium *A. brasilense* present under extreme ambient conditions, increased pigment production occurred in *C. sorokiniana* (Fig. 5). These pigments were chlorophylls *a* and *b* and two auxiliary photo-protective pigments, violaxanthin and zeaxanthin. Under extreme conditions, co-immobilization always yielded higher content of photosynthetic pigments (Fig. 5).

4. Discussion

Devising new biological wastewater treatments depends more on the necessities of the human community than on the availability of organisms and microorganisms where the demand occurs. The usefulness of any strain of microalgae for wastewater treatment depends on two major parameters: (i) the capacity of the strain to grow under the environmental conditions prevailing in the area and (ii) its capacity to remove the pollutants efficiently. Therefore, when wastewater treatment plants are located in areas with extreme environmental conditions, either very cold with low insolation (Talbot et al., 1991) or very hot with very high insolation (de-Bashan et al., 2004), the choice of microorganisms that can proliferate under harsh environmental conditions is paramount. Searching for local microorganism capable of growing in the local wastewater treatment facility requires an isolation strategy for candidate microorganisms. What must be determined is whether they pass the scrutiny of adaptability to the local wastewater scheme.

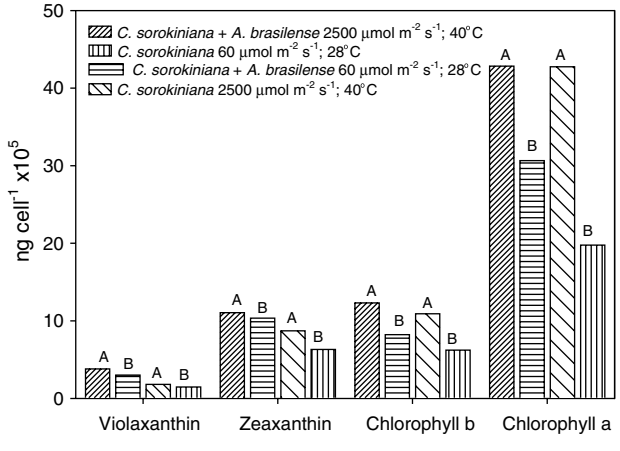

Fig. 5. Photosynthetic pigment contents of *Chlorella sorokiniana* UTEX 2805 immobilized alone or with *Azospirillum brasilense* Cd in alginate beads, cultured in synthetic wastewater at 28 and 40 °C under two light intensities (60 and 2500 µmol m^2 s^{-1}) and initial ammonium chloride concentration of 50 mg l^{-1}. Columns for each pigment, denoted by a different capital letter, differ significantly by one-way ANOVA and Tukey's post-hoc analysis at $P \leqslant 0.05$.

Following this concept, we isolated a microalgal strain capable of forming massive algal blooms in wastewater stabilization ponds. This microalga was identified as *C. sorokiniana*, a species known to grow at relatively high temperatures of 35–40 °C (Morita et al., 2000a,b, 2002; Yamamoto et al., 2003), but not as high as occurs in the deserts of the Baja California Peninsula, where midday temperatures are 38–44 °C in the shade for several months and over 50 °C in direct sunlight, reaching extremes of up to 2500 µmol photon m^2 s^{-1}.

This particular strain does not require high temperature and intense insolation for growth, and can normally grow at 28 °C and 30–60 µmol m^2 s^{-1}. Adaptation of microalgae to different growth regimes often requires acclimation periods (Claustre and Gostan, 1987; Maxwell et al., 1994; Vonshak et al., 1996). Hence, after a period of scaling-up acclimation, our strain could successfully grow at 40 °C and 2500 µmol m^2 s^{-1} for 5 h each day. With this priming, *C. sorokiniana* UTEX 2805 is suitable for growth under conditions prevailing in the desert areas of the Baja California Peninsula of Mexico during the summer, as well as at locations exhibiting similar extreme conditions.

A microbial candidate for wastewater treatment has to show a significant capacity to remove pollutants under the intended environmental conditions. Ammonium, used as a model, common pollutant of domestic wastewater (de-Bashan et al., 2004), was completely removed from wastewater at the most extreme conditions tested. The mechanism for ammonium removal in this experimental wastewater treatment system is by absorption within the cells, which was later removed, together with the beads where they were immobilized (de-Bashan et al., 2002b). At the lowest temperatures, removal of ammonium was less efficient. When the microalga was immobilized in alginate beads with *A. brasilense*, a technology currently under development for wastewater treatment (de-Bashan et al., 2004; Yabur et al., 2007), growth of *C. sorokiniana* was stimulated by ammonium and removal of ammonium increased. A plausible explanation for the increased effect of the MGPB on growth of microalgae under high ammonium level may be related to the pH of the culture. Normally, during the process of growth, the pH of the

medium increases slightly, up to 0.5 pH units (de-Bashan et al., 2002b; Gonzalez and Bashan, 2000). This study shows that this level of ammonium supported a larger population of the MGPB. This MGPB was shown to alleviate pH stress on growth of *C. vulgaris*, also used for wastewater treatment, but under lower temperatures and light intensities (de-Bashan et al., 2005). Hence a similar effect of larger populations of *A. brasilense* is also possible under extreme conditions. These characteristics are similar to other *C. vulgaris* and *C. sorokiniana* strains that had been tested under more regular, less extreme conditions of low light intensity and temperatures (Gonzalez and Bashan, 2000; Gonzalez-Bashan et al., 2000; de-Bashan et al., 2002b, 2004), but were unable to grow under the extreme environmental condition tested here.

Microalgae in nature or in culture are always associated with bacteria (Litchfield et al., 1969). These associations may exert positive or negative influence on growth of microalgae. As demonstrated in this study, under certain conditions, the growth of and removal of ammonium by *C. sorokiniana* UTEX 2805, when immobilized with a MGPB, improved positive interaction patterns, similar to other *Chlorella* species used to treat wastewater (de-Bashan et al., 2002b, 2004; Hernandez et al., 2006).

The capacity to remove pollutants quickly and efficiently from wastewater dictates the nature of the microbial agent to be used (de-Bashan and Bashan, 2004). Good biological agents should remove pollutants in short retention times of 1–4 days (de-Bashan et al., 2002b, 2004). *C. sorokiniana* UTEX 2805 fulfills this basic requirement. It is also known that culturing conditions, including the level of nitrogen controls, affects the removal of ammonium from wastewater by *C. vulgaris* (de-Bashan et al., 2005). This study showed that removal of ammonium by *C. sorokiniana* UTEX 2805 is more efficient when concentrations of ammonium are higher, conditions of temperature and light intensity are extreme, and helper MGPB are present. Although this strain of microalga can remove ammonium under moderate environmental conditions, removal is less efficient. Similar, but preliminary, results were obtained for phosphorus removal by this species (S. Fendrich, 2005, Diploma thesis, Technische Universität, Hamburg–Harburg, Germany).

C. sorokiniana can grow under strong intensity of light and temperature (46.5 °C; 1737 μmol m^2 s^{-1}) in bioreactors (Morita et al., 2000a,b) or under field conditions for biomass production (Morita et al., 2002). However, for practical treatment of wastewater, phyto-remediation, or other types of mass production, a lower intensity of light (250–300 μmol m^2 s^{-1}) and temperatures (30–35 °C) had been tested (Lee et al., 1996; Muñoz et al., 2004, 2005; Vona et al., 2004; Yoshida et al., 2006). Our strain, because it is intended for use under extreme field conditions (>2000 μmol m^2 s^{-1} for several hours daily) far exceeded the conditions reported elsewhere for this species.

When the intensity of light increased, photosynthetic pigments in microalgae (*Scenedesmus quadricauda* and *C. sorokiniana*) increased. This happens, as in higher plants, in the xanthophyll cycle pigments that participate in dissipating the excess absorbed light (Masojídek et al., 1999). It is also known that inoculation with the MGPB *A. brasilense* enhances production of auxiliary photosynthetic pigments in wheat plants (Bashan et al., 2006). Similarly, after exposure of common strains of *C. vulgaris* and *C. sorokiniana* cultivated under "regular conditions" with the added influence of *A. brasilense*, the level of some photosynthetic pigments increased (de-Bashan et al., 2002a). The same phenomenon occurs in *C. sorokiniana* UTEX 2805.

5. Conclusions

A strain of *C. sorokiniana* was isolated from wastewater stabilization ponds located in an area with extremely hot desert conditions (40–42 °C and insolation of up to 2500 μmol m^2 s^{-1}). The identified strain is capable of growing at high or ambient temperature at very intense levels of insolation, similar to the conditions in the desert. The strain responds positively, that is, increased in population, to inoculation with bacteria that promote growth of microalgae. The strain can efficiently reduce ammonium from wastewater (from 10 mg l^{-1} NH_4^+ to undetectable levels in four days).

Acknowledgements

Yoav Bashan participated in this study in memory of the late Avner Bashan and Uzi Bashan. We thank Lina-Maria Gonzalez at the Universidad Nacional de Colombia, Bogota, and Silke Fendrich at the Technische Universität Hamburg–Harburg, Germany for technical assistance during the initial stages of the study and Francisco Hernandez at CIBNOR in Mexico for pigment analysis. This study was mainly supported by Consejo Nacional de Ciencia y Tecnología (CONACYT Investigacion Cientifica Basica 2005 contract 50560-Z), Secretaria de Medio Ambiente y Recursos Naturales of Mexico (SEMARNAT contract 23510) and partly funded by The Bashan Foundation, USA.

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