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Bioremediation of Eutrophicated Water by *Acinetobacter Calcoaceticus*

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Abstract Removal of phosphorus and nitrogen from eutrophicated water was carried out by in situ bioremediation. With the addition of *Acinetobacter calcoaceticus*, 65.0% ± 4.0% of total phosphorus (TP), 37.0% ± 4.0% of total nitrogen (TN), 75.0% ± 7.0% nitrite (NO_2^-–N), and 87.0% ± 4.0% of ammonia (NH_4^+–N) were removed. Furthermore, chlorophyll *a* removal in the inoculated treatments reached 83.7% ± 1.5%, and algae in the water was basically controlled.

Keywords *Acinetobacter calcoaceticus* · Bioremediation · Biodegradation · Eutrophication

Currently, eutrophication is a major environmental problem worldwide, leading to water quality deterioration and significant losses of biodiversity. Eutrophication is caused by agroindustrial wastewater, detergents, pesticides and animal husbandry. In eutrophicated waters, perennial algal blooms, or their frequent recurrence, and high turbidity are well-known water quality issues. Moreover, toxic cyanobacteria can harm aquatic life and human beings.

Eutrophication control by removal of nutrients, in particular phosphorus (Correll 1998), has been the focus of attempts to find ways to improve water quality in natural waters. Among these methods, UV irradiation (Whangchai et al. 2004), application of copper sulphate, chlorine, potassium permanganate, lime [CaO, $Ca(OH)_2$], alum [$Al_2(SO_4)_3 \cdot 14H_2O$; Lam and Prepas 1997; Boyd and Massaut 1999), and ferric salts (Randall et al. 1999), as well as wetland creation (Tilley et al. 2002), and bioremediation (Ripl 1976) have been widely used. An emerging technology available for natural waters is bioremediation, which is advantageous because of its relatively low environmental impact (Head 1998). A large number of bioremediation experiments have suggested that microbial products can be employed to improve water quality or to clean up contaminated environments (Burford et al. 2003; Queiroz and Boyd 1998; Devaraja et al. 2002; Douillet 2000; Vezzulli et al. 2004). The published literature about bioremediation for controlling eutrophication has emphasized the effects of microbial products on water quality. Due to commercial secrets, the species used in these microbial products are not generally known. However, the metabolic pathways of two or more bacteria are difficult to describe. Thus, it is important to study a single bacterium for remediation of natural waters.

The use of *Acinetobacter calcoaceticus* in wastewater treatment and various bioreactors for eliminating phosphorus from wastewater is well documented (Muyima and Cloete 1995; Mino et al. 1998; Srivastava and Srivastava 2005, 2006). However, no information is currently available on in situ bioremediation in natural waters by this species. Following evidence supporting its active role in biological phosphorus removal, we investigated the effects of *A. calcoaceticus* on in situ remediation of eutrophicated water and provide a theoretical basis to further exploit new microbial products.

Materials and Methods

Eutrophicated water was collected from Water Bird Lake in the Beijing Zoo, China, in plastic buckets. The water was then placed in a laboratory or a greenhouse. The

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properties of wastewater used in the study are shown in Table 1.

A. calcoaceticus was obtained from the Institute of Microbiology, Chinese Academy of Sciences. A medium containing (g L^{-1}) glucose (2.0), $(NH_4)_2HPO_4$ (3.0), K_2HPO_4 (1.2), $MgSO_4$ (0.2) and NaCl (0.6) dissolved in distilled water (pH 7.2) was used for cultures. The medium was solidified with 2% agar and used for plate counts. Cultures were incubated at 37°C in a rotary shaker (150 rpm) for 8 h. Cells were collected by centrifugation at 12,000×*g* at 4°C for 10 min. The cells were washed and suspended in 0.8% NaCl solution. *A. calcoaceticus* suspended liquid was inoculated in an experimental water sample. Simultaneously, 0.1 ml of the suspended liquid was plated on an agar medium plate and the plates were incubated at 37°C overnight for plate counts. The suspended liquid had a bacterial cell density of 6×10^8 CFU mL^{-1}.

In August 2006, 12 3 L triple-necked flasks with 1,000 mL of wastewater were inoculated with different concentrations of *A. calcoaceticus* suspended liquid [three each of 0‰, 0.05‰, 0.1‰, or 0.2‰ (v/v)]. They were incubated in a rotary shaker (150 rpm) for 5 days after inoculation, then water quality analyses were conducted. This experiment determined the ideal quantity of *A. calcoaceticus* for efficient removal of total phosphorus.

All experiments were carried out in a greenhouse at China Agricultural University. Six plastic buckets filled with 20 L of wastewater were prepared. Three buckets were treated with *A. calcoaceticus* suspension liquid and aeration, and three buckets were treated with aeration only (controls). Dissolved oxygen levels in the treated water were maintained above 6.0 mg L^{-1} by air blowers (Model ACO-009, RESUN, China).

Wastewater was inoculated with 0.1‰ (v/v) of *A. calcoaceticus* suspension liquid in each 20 L wastewater sample once every 5 days. Water samples were collected in plastic bottles for water quality analyses both before and after inoculation. All samples were stored at –4°C if not analyzed immediately.

Table 1 Characteristics of wastewater

Parameter	Mean ± standard deviation
COD[a] (mg L^{-1})	88.1 ± 0.09
Total nitrogen (mg L^{-1})	1.95 ± 0.03
NH_4^+–N (mg L^{-1})	0.07 ± 0.004
NO_2^-–N (mg L^{-1})	0.09 ± 0.006
Total phosphorus (mg L^{-1})	0.33 ± 0.006
PH	7.2 ± 0.02
Turbidity (NTU)	53.78 ± 1.83
Chlorophyll *a* (mg m^{-3})	117.31 ± 0.006

[a] Chemical oxygen demand

Water quality analyses were conducted using standard methods (AWWA 1999). Chemical oxygen demand (COD) was measured by the potassium dichromate-boiling method; the colorimetric method was used for total nitrogen (TN), total phosphorus (TP), NH_4^+–N, and NO_2^-–N. Chlorophyll *a* was estimated according to Clesceri et al. (1989). Water temperature, dissolved oxygen, pH, and turbidity were read in situ using handheld meters (model OXi 3152, WTW, Germany; models pH 315i and HI 93703-11, Hanna Instruments, Portugal). For each parameter, duplicate samples were analyzed.

To evaluate the effect of inoculation with *A. calcoaceticus*, we used a one-way ANOVA (SPSS, release 11.5) for each parameter.

Results and Discussion

Addition of *A. calcoaceticus* suspension liquid at ratios of 0‰, 0.05‰, 0.1‰, and 0.2‰ resulted in TP removals of 17.0% ± 1.0%, 28.3% ± 2.1%, 36.3% ± 3.1%, and 26.7% ± 7.5%, respectively. All concentrations of *A. calcoaceticus* removed significantly more TP from the wastewater than the control. The highest removal rate was observed with the addition of 0.1‰ *A. calcoaceticus* suspension liquid, which was significantly higher than the other treatments. Consequently, addition of 0.1‰ *A. calcoaceticus* suspension liquid was used as the ideal amount for the second experiment.

Average TP levels in the treatments decreased sharply in the first 10 days of treatment but did not decrease much in the subsequent 15 days (Fig. 1). TP removal was significantly higher in the inoculated buckets (48.5% ± 2.6%) than in the control buckets (20.8% ± 8.0%) during the first 10 days. This TP removal rate is higher than in aerobic batch tests in sequencing batch reactors (e.g., 42%, Srivastava and Srivastava 2005). TP removal was seen after 5 days and continued for the remainder of the experiment. Removal of 65.0% ± 4.0% of TP was seen after 25 days.

On average, the reduction in TN, NO_2^-–N, and NH_4^+–N was higher in the inoculated treatment than in the control (Fig. 2).

The TN concentration in the treatments changed greatly with time. Removal rates were significantly higher in the inoculated treatment ($p < 0.01$; Fig. 2a) and varied from 37.0% ± 4.0% to 21.7% ± 4.0%. The lack of significant improvement in removal rate over time may be due to fixing of atmospheric nitrogen either by free-living or symbiotic organisms (Häder et al. 1998).

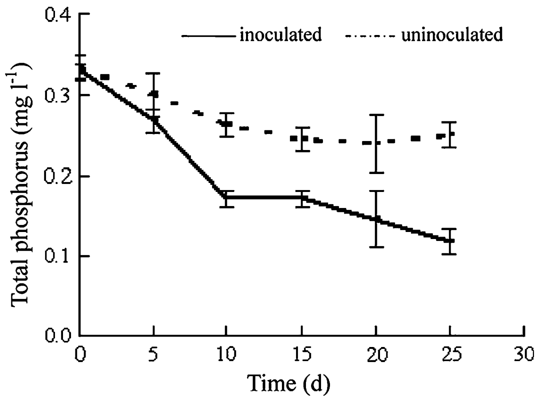

Fig. 1 Change in total phosphorus concentration over time in the two treatments. *Error bars* indicate one standard deviation, based on three replicates

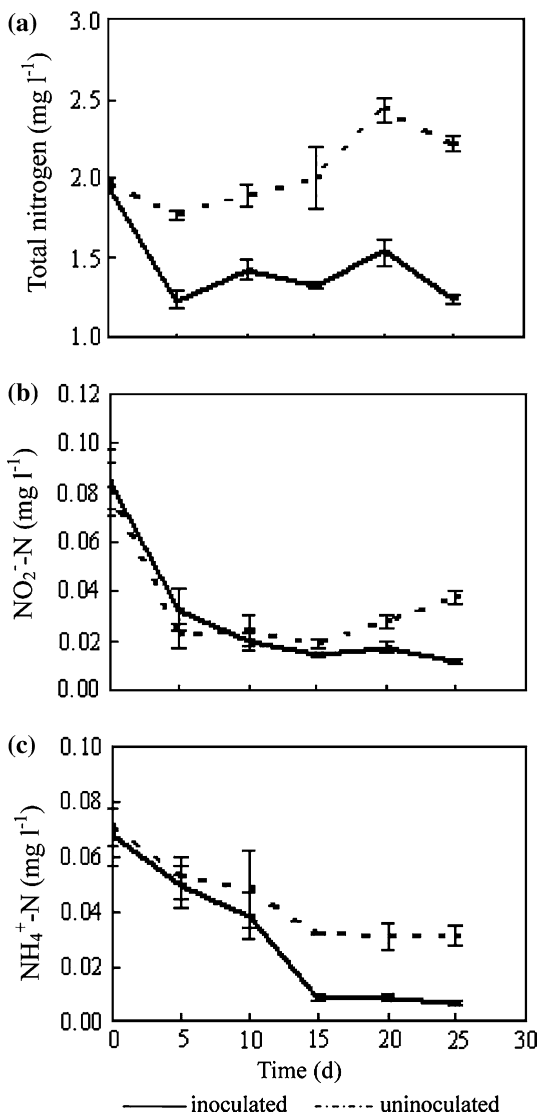

Fig. 2 Change in average **a** total nitrogen, **b** NO_2^-–N, and **c** NH_4^+–N concentrations over time in the two treatments. *Error bars* indicate one standard deviation, based on three replicates

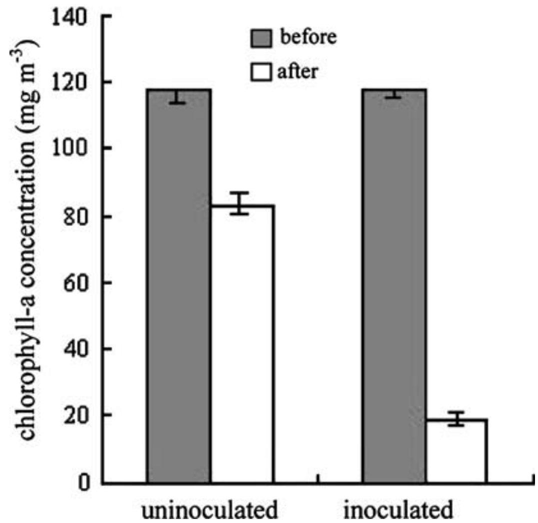

Fig. 3 Chlorophyll *a* concentration before and after the experiment in each treatment. *Error bars* indicate one standard deviation, based on three replicates

The accumulation of NO_2^- (Jensen 2003) and NH_4^+ (Augspurger et al. 2003) can be toxic to fish and other aquatic organisms. NO_2^-–N removal (Fig. 2b) was initiated immediately in both treatments. The highest removal rate in the inoculated treatment (75.0% ± 7.0%) was seen from day 15 onward and was slightly higher than the highest rate in the control (70.0% ± 10.0%). There were no significant differences between the treatments ($p < 0.05$). This phenomenon might be caused by the sufficient oxygen supply provided by the air blowers (Wiesmann 1994). The NO_2^-–N concentration in the control was maintained at 0.01 mg L^{-1} from day 15 onward, far lower than the 0.08–0.35 mg L^{-1} range adequate to protect sensitive aquatic animals (Camargo and Alonso 2006). In addition, NH_4^+ removal (Fig. 2c) was similar to NO_2^- removal. The maximum removal rate (87.0% ± 4.0%) was significantly higher than the control ($p = 0.009$). In the uninoculated treatment, NH_4^+–N concentration was near zero from day 15 onward.

After 25 days, chlorophyll *a* removal in the inoculated treatment reached 83.7% ± 1.5%, and was significantly higher than in the control ($p < 0.01$, Fig. 3). The residual chlorophyll *a* concentration in the treated buckets was 19.1 ± 1.79 mg m^{-3}, far lower the 83.4 ± 3.13 mg m^{-3} in the control. The greater removal ability in the inoculated treatments may be due to the phosphorus removal ability of *A. calcoaceticus*.

The nutrient removal characteristics of bacteria are of particular value for in situ bioremediation. The use of *A. calcoaceticus* in natural waters has not been previously reported. These results warrant further exploration, in terms of new microbial products, microbial ecology and food chain interactions.

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