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The combined effect of bacteria and *Chlorella vulgaris* on the treatment of municipal wastewaters

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HIGHLIGHTS

- *C. vulgaris* contributes to the removal of nitrogen and phosphorus in wastewater.
- Microorganisms eliminate most organic matter in wastewater.
- Nitrogen removal rate by algae is faster than that by algae-microorganism consortium.
- More than 44.1–64.0% of total nitrogen is recycled into the biomass.

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ABSTRACT

Impacts of *Chlorella vulgaris* with or without co-existing bacteria on the removal of nitrogen, phosphorus and organic matter from wastewaters were studied by comparing the wastewater treatment effects between an algae–bacteria consortium and a stand-alone algae system. In the algae–bacteria system, *C.vulgaris* played a dominant role in the removal of nitrogen and phosphorus, while bacteria removed most of the organic matter from the wastewater. When treating unsterilized wastewater, bacteria were found to inhibit the growth of algae at >231 mg/L dissolved organic carbon (DOC). Using the algae–bacteria consortium resulted in the removal of 97% NH_4^+, 98% phosphorus and 26% DOC at a total nitrogen (TN) level of 29–174 mg/L. The reaction rate constant (k) values in sterilized and unsterilized wastewaters were 2.17 and 1.92 mg NH_4^+–N/(mg algal cell ·d), respectively.

1. Introduction

Using microalgae to improve water quality is an environment-friendly wastewater treatment method (Wang et al., 2010). Microalgae have been successfully used for the treatment of fecal wastewater (An et al., 2003), domestic wastewater (de-Bashan et al., 2004), and industrial wastewater (Lim et al., 2010). Simple and low-cost technology is needed when utilizing microalgae to treat wastewater, in a system that recycles nutrients, such as nitrogen and phosphorus (Pittman et al., 2011).

Chlorella sp., a microalgal species that has a fast growth rate and short generation time (Canovas et al., 1996), exhibits great potential in wastewater treatment and biodiesel production. *Chlorella* can tolerate the rigorous environmental conditions found in wastewaters and efficiently assimilate nitrogen and phosphorus from the wastewater (Pittman et al., 2011). *Chlorella* sp. has high removal efficiency (more than 80%) of nutrients in primary and second treatment effluents and, under certain conditions, can completely remove ammonia nitrogen, nitrate nitrogen and total phosphorous (TP) (Lau et al., 1995; Wang et al., 2010). The adaption of *Chlorella* sp. to high nutrient concentrations leads to its wide application in the treatment of agricultural, and fecal wastewater, and wastewaters (Borowitzka, 1999).

Bacteria are inevitably present in the wastewaters; hence, considerable research has been focused on the interaction between

Abbreviations: DOC, dissolved organic carbon; MPGB, microalgae and a bacterium; PBRs, photobioreactors; TN, total nitrogen; TP, total phosphorous.

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microalgae and bacteria, for the purpose of wastewater treatment. Co-immobilization of microalgae and a bacterium (MGPB) was found to be superior to a stand-alone algae system, reaching removal rates of up to 100% ammonium, 15% nitrate, and 36% phosphorus (de-Bashan et al., 2002). Su et al. (2012) found that a 5:1 algae/sludge culture had 5–40% higher removal efficiencies of nitrogen and phosphorus and achieved a 2–4 day faster operation than systems with other inoculation ratios. Peterson et al. (2011) hypothesize that, under un-enriched conditions, changes in algal species assemblages due to succession, would influence bacterial denitrifiers, due to the dependence of the latter on algal exudates. Vasseur et al. (2012) found that the presence of bacteria in the algae–bacteria system increased the maximum carbon conversion efficiency up to 6.3%. The focus of such research has been on the treatment efficiency of the combined systems. However, there has been very little focus on the separate contributions made by *C.vulgaris* and bacteria, respectively, to the removal of nitrogen, phosphorus and organic matter.

In this study sterilized and unsterilized experiments were set up to assess the individual contributions of *C.vulgaris* and bacteria to the removal of pollutants such as N, P and organic matter. Removal of NH_4–N was studied using kinetic modeling and nitrogen recovery was evaluated by a nitrogen balance calculation.

2. Methods

2.1. Microalgal strain and culture

The *C.vulgaris* strain ESP-6 used for this study was obtained from the Department of Chemical Engineering, National Cheng Kung University, Taiwan. Liquid Bold's Basal Medium (BBM) (Nichols, 1973) was used to cultivate *C.vulgaris* which was inoculated into 1.5 L of BBM medium in tubular photobioreactors (PBRs) consisting of vertical columns (each of diameter 14 cm, height 24 cm and a working volume of 2 L) and incubated at 27 ± 1 °C. The PBRs were continuously illuminated by 8000–10,000 lux LED lights (WD-TM-D35 W, Widen Photodiode Technology Co., China) and aerated with 5% CO_2 at a volumetric flow rate of 0.1 vvm (i.e., 10% of PBR working volume per min). Magnetic stirrers, at 50 rpm, were used to agitate the media.

2.2. Experiment design

Samples of the *C. vulgaris* cultures were transferred to PBRs with mixed medium – composed of effluents prepared with different ratios of wastewaters – from a septic tank and secondary sedimentation tank. As the solids in the effluents may reduce the light intensity which impacts the photosynthesis process, the effluents were filtered through 0.22-μm membrane filters prior to mixing to exclude most of solids. Therefore, the inoculum size of algae cultures was set at 5 mg dry weight/L of medium to match the bacteria inoculum size. Initial concentrations of total nitrogen (TN) in the wastewaters were 29, 56, 72, 86, 99, 174 and 246 mg/L, and the corresponding wastewaters were designated as RN30, RN55, RN70, RN85, RN100, RN175 and RN245. The characteristics of the filtered wastewaters are listed in Table 1.

To compare the function of *C.vulgaris* and bacteria during wastewater treatment, sterilized experiments were prepared by autoclaving the wastewaters at 121 °C for 1 h. The wastewaters were filtered through 0.22-μm membrane filters and repeatedly autoclaved at 121 °C for 20 min. According to Craggs (2005), algal biomass typically has a composition of $C_{106}H_{181}O_{45}N_{16}P$. Thus, after wastewater sterilization, the sterilized KH_2PO_4 was utilized to adjust the TN/TP ratio to between 4.0 and 6.0, to ensure that the TP concentration would not limit the absorbance of TN. The concentrations of TN in wastewaters were 30, 66, 81, 134, 168 and 208 mg/L, and the corresponding wastewaters were designated as SN30, SN65, SN80, SN135, SN170 and SN210, respectively. The characteristics of the wastewaters are listed in Table 2. All experiments were conducted in duplicate and mean values are reported.

2.3. Analytical methods

A 50-mL sample was centrifuged at 3600×*g* for 15 min, after which the supernatant was filtered through a 0.45-μm membrane. The filtrate was used for the determination of physical–chemical parameters; COD was determined by the HACH method (DR890 colorimeter, Hach Company, USA); Nash reagent photometry was used for the NH_4^+–N determination; ultraviolet spectrophotometry method for the NO_3^-–N determination; the molybdenum-antimony anti-spectrophotometric method for the TP determination, and a TOC-TN detector (TOC-VCAP, SHIMADZU Co., Japan) was used for TN and DOC determinations. BOD_5 was detected using the BOD detector (OxiTopIS12, WTW Company, Germany) and a LIDA-PHS-25ws meter (Shanghai LiDa instrument factory, China) was used to measure pH.

The sediment from previous centrifugation was lyophilized for 48 h at −50 °C before analysis. Elemental analysis of nitrogen in the solid biomass samples was carried out using a C/H/N analyzer (Vario EL III, Elementar Corporation, Germany).

2.4. Kinetic modeling

Eq. (1), below, was used to model batch dynamics, where kinetic parameters were obtained from the initial substrate utilization rate (R_i, mg/(L d)):

$$R_t = -\frac{S_0 - S_t}{t_0 - t_t} \tag{1}$$

where S_0 (mg/L) denotes the initial substrate concentration at initial time (t_0, d) and S_t (mg/L) is the substrate concentration at t_t (d)

Table 1
The characteristics of wastewater in all tests in the unsterilized experiment*.

Components	RN30	RN55	RN70	RN85	RN100	RN175	RN245
pH	6.47	6.96	7.12	7.10	6.82	7.42	7.19
TN (mg/L)	29	56	72	86	99	174	246
NO_3^-–N (mg/L)	6.3	10.6	10.4	10.9	8.4	10.6	11.6
NH_4^+–N (mg/L)	17	39	52	65	77	143	207
COD (mg/L)	99	170	225	279	391	602	1032
BOD (mg/L)	23	84	118	151	200	350	596
DOC (mg/L)	32	61	82	104	125	231	340
TP (mg/L)	1.4	3.9	5.0	6.2	7.1	13.1	19.6

* Initial concentrations of total nitrogen (TN) in the unsterilized wastewaters were 29, 56, 72, 86, 99, 174 and 246 mg/L, termed as RN30, RN55, RN70, RN85, RN100, RN175 and RN245 tests, respectively. (The values were the average of duplicate experiment and the error bars represented the upper and lower value).