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Enhancing fermentation wastewater treatment by co-culture of microalgae with volatile fatty acid- and alcohol-degrading bacteria

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ABSTRACT

Co-culture of microalgae and bacteria is a promising method for wastewater treatment. The suitable selection of the bacteria in co-cultures with microalgae is critical for wastewater treatment. Three bacteria, *Exiguobacterium aurantiacum*, *Stenotrophomonas acidaminiphila*, and *Chryseobacterium scophthalmus*, were the dominant bacterial species in fermentation wastewater treated by microalgae and activated sludge. Pure cultures and co-cultures of microalgae (*Chlorella sorokiniana* L3) and these bacteria were evaluated for the treatment of fermentation wastewater, which contains high concentrations of acetate acid, butyric acid, ethanol and butanol. The performance of the co-cultures was better than that of pure cultures of microalgae or bacteria for the removal of four organic compounds, and the removal efficiency of volatile fatty acids and alcohols in the best co-culture case was increased by 22.70% compared to that of pure cultures. Butanol and butyric acid were much more difficult for microalgae and bacteria to remove than were acetic acid and ethanol. The co-cultures of *Chlorella sorokiniana* L3 with *Stenotrophomonas acidaminiphila* or *Chryseobacterium scophthalmus* had the highest removal efficiencies for the four organic compounds in all treatments. The co-cultures of *Chlorella sorokiniana* L3 with *Exiguobacterium aurantiacum*, *Stenotrophomonas acidaminiphila* or *Chryseobacterium scophthalmus* promoted not only chlorophyll a + b production but also biomass accumulation of *Chlorella sorokiniana* L3 during the 3 days of cultivation compared with those in pure cultures. More than 77.8% of NH_4^+-N, 45.6% of total PO_4^{3-}-P and 63.7% of COD (chemical oxygen demand) were removed in all co-cultures. These three bacteria are potential candidates for wastewater treatment through co-culture with microalgae.

1. Introduction

High-strength wastewater generated by the agriculture, food and winery sectors is usually treated with anaerobic fermentation [1–3]. Meanwhile, soluble metabolites such as volatile fatty acids (VFAs) and alcohols are usually produced simultaneously during the fermentation process [4]. The wastewater from the fermentation process contains significant amounts of organic compounds, nitrogen and phosphorus, which are potential threats to the water environment in the absence of proper treatment before discharge of fermentation effluent [5].

An important approach that can remove the high contents of organic compounds is the use of photosynthetic bacteria (purple non-sulfur bacteria, PSB) to assimilate those soluble metabolites to convert the stored energy into a high energy density form, H_2, via further photofermentation [6]. However, the lower growth rate of PSB and high sensitivity to environmental conditions (such as the levels of ammonia and oxygen) limit further application [7]. Instead of using bacteria to assimilate the organic compounds during the fermentation process, biological treatment with microalgae shows superior performance in incorporating nutrients such as N and P and converting the energy of wastewater into useful algal biomass [8]. The ability of *Chlorella* sp. to remove N and P during wastewater treatment has been widely observed. For instance, *Chlorella* sp. removed 75.7% to 82.8% and 62.5% to 71.7% of total nitrogen and total phosphorus, respectively, in different dilutions of fermentation wastewater [9].

The co-cultivation of microalgae with bacteria (or cyanobacteria)

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has great promise to replace conventional biological treatment systems that use a single microorganism (microalgae/bacteria) because co-cultivation requires lower inputs of oxygen as the oxidant and chemicals as pH buffers or reducing agents for the denitrification process [10–16]. The combinations of different species of bacteria and *Chlorella* sp. have been used for wastewater treatment. For instance, co-cultures *Chlorella* sp. with *Azospirillum brasilense* in biofilms increased the NH_4^+-N removal ratio to up to 93% when the initial concentration of NH_4^+-N was 3 mg L^{-1} [17]. Only a 53% removal rate was obtained in a single-culture system after 2 days of incubation in synthetic wastewater. In another co-culture system, 80% of COD was removed by a co-culture of *Chlorella* sp. with *Pseudomonas putida* in 3 days, which was significantly higher than the proportion removed by pure cultures [18]. The mechanism underlying the enhanced treatment efficiency of co-cultures can be attributed to the interactions between algae and bacteria, which include nutrient exchange, signal transduction and gene transfer. The interaction between algae and bacteria is closely related to the algae and bacteria species used [19,20]. It should be mentioned that the incorrect choice of bacteria will lead to negative effects on microalgae. It is also important to maintain steady state growth. Algal-bacterial co-cultures may be destroyed if contamination occurs.

According to our previous study [21], mixotrophic cultivation of *C. sorokiniana* L3 in dark fermentation wastewater was proven to be a promising approach for wastewater nutrient and energy reclamation. Microbial community analysis was also carried out on samples from fermentation wastewater after treatment. The scientific hypothesis was that the dominant species in sludge contributed the most to wastewater treatment. Co-cultivation of microalgae and dominant bacteria had similar results to those of a previous study. The wastewater treatment will be enhanced by these co-cultures compared with pure cultures of microalgae.

From the view of nutrient and energy recovery, the co-cultivation of *C. sorokiniana* L3 and bacteria for handling high-strength fermentation wastewater holds great promise. However, information on the co-culture system for high-strength fermentation wastewater treatment is limited. Therefore, seven groups of experiments with *C. sorokiniana* L3 and different species of bacteria were designed in this study. Microalgal growth, nutrient utilization, and energy conversion efficiencies for the entire process were compared. The influence of the bacterial species on the performance of high-strength fermentation wastewater treatment was also explored.

2. Materials and methods

2.1. Experimental microorganisms and cultivation

The *Chlorella sorokiniana* L3 used in this study was isolated from a local ditch with longitude and latitude of N: 31°43′40″ and E: 121°30′8″ and maintained on a solid TAP plate in our lab. The seeding culture was grown in sterilized liquid BG11 medium and illuminated under cool white fluorescent lamps with approximately 150 μmol photons m^{-2} s^{-1} at a temperature of 28 ± 0.05 °C in a constant temperature circulating water bath. A tube photobioreactor was used to achieve a faster growth rate by supplying 0.1 L min^{-1} of CO_2-enriched air before wastewater treatment. The tubes were cylindrical with a height of 40 cm and diameter of 4.5 cm. The working volume was 350 mL.

A microbial community analysis was carried out on samples from fermentation wastewater after treatment in our previous study [21]. This approach allowed for a detailed taxonomic and functional characterization of the ecosystems in the sample system. The dominant bacteria in the treated fermentation wastewater were *Exiguobacterium aurantiacum*, *Stenotrophomonas acidaminiphila*, and *Chryseobacterium scophthalmus*. Thus, it was indicated that several species of bacteria were symbiotic with *C. sorokiniana* L3 during the treatment of high-strength fermentation wastewater. To further study the metabolic regulation mechanisms between different species of bacteria and *C. sorokiniana* L3, the constitution of *C. sorokiniana* L3 + *E. aurantiacum*, *C. sorokiniana* L3 + *S. acidaminiphila*, *C. sorokiniana* L3 + *C. scophthalmus* were analysed to avoid interference from other factors. All bacteria in this study were purchased from the China General Microbiological Culture Collection Center (CGMCC). The aerobic bacteria *Exiguobacterium aurantiacum* CGMCC 1.6137, *Stenotrophomonas acidaminiphila* CGMCC 1.6393, and *Chryseobacterium scophthalmus* CGMCC 1.6281 were used in this study and cultivated in 918 nutrient broth and LB medium, respectively, at 30 °C with shaking at 150 rpm prior to use in wastewater treatment.

Table 1
Characteristics of simulated fermentation wastewater and real fermentation wastewater.

Constituent	Concentration (mg L^{-1})	
	Simulated fermentation wastewater	Real fermentation wastewater
NH_4^+-N	114 ± 0.5	150 ± 2.8
Total PO_4^{3-}-P	282 ± 2.6	426 ± 3.5
Total organic carbon	696 ± 14	/
Inorganic carbon	121 ± 2	/
Ethanol	159 ± 0.6	335 ± 5.1
Butanol	110 ± 0.4	364 ± 3.7
Acetic acid	208 ± 1.7	182 ± 1.9
Butyric acid	931 ± 4.6	920 ± 2.3
pH	3.81 ± 0.02	4.29 ± 0.11

Data shown is the mean ± SD, n = 3.

2.2. Simulated fermentation wastewater and microorganism inoculation

Simulated wastewater was prepared as follows (in mg L^{-1}): 500 NH_4Cl, 250 KH_2PO_4, 250 $K_2HPO_4{\cdot}3H_2O$, 300 $MgCl_2{\cdot}6H_2O$, 25 $FeCl_3$, 16 $NiSO_4$, 25 $CaCl_2$, 11.5 $ZnCl_2$, 10.5 $CoCl_2{\cdot}6H_2O$, 5 $CuCl_2{\cdot}2H_2O$, and 15 $MnCl_2{\cdot}4H_2O$. Ethanol, butanol, acetic acid and butyric acid were added as the four metabolites after dark anaerobic fermentation according to a previous study of the use of real fermentation wastewater [21]. The characteristics of simulated fermentation wastewater and real fermentation wastewater are shown in Table 1. Prepared wastewater was autoclaved at 121 °C for 20 min. The initial pH was adjusted to approximately 7 with 1 M NaOH solution before microorganism inoculation.

After the individual growth of microalgae and bacteria, the cells of *C. sorokiniana* L3 were harvested by centrifugation at 6000 rpm for 5 min, and the cells of bacteria were centrifuged at 8000 rpm for 10 min. Each microorganism pellet was washed three times with sterilized water to remove residual nutrients before inoculation.

2.3. Experimental design

Seven different treatments were set up at laboratory scale for nutrient removal and energy recovery. The initial biomass density of *C. sorokiniana* L3 and bacteria was 0.5 g L^{-1} in both pure and co-culture systems. Three different species of bacteria were added to set up different co-culture systems. The seven batch culture experiments were performed in 100 mL Erlenmeyer flasks with a working volume of 60 mL simulated fermentation wastewater. All experiments were carried out under controlled environmental conditions. The light intensity applied was 150 μmol photons m^{-2} s^{-1}, and all groups were cultured at 26 ± 2 °C with flasks shaken at 150 rpm to provide consistent mixing. Each experiment was carried out in triplicate.

2.4. Analytical methods

The concentrations of the four organic components (ethanol, butanol, acetic acid and butyric acid) were determined by a gas chromatograph (Shimadzu 2010 plus, Japan) equipped with a flame ionization detector (FID) and a molten silica capillary column (DB-FFAP).