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Characterization of microalgae-bacteria consortium cultured in landfill leachate for carbon fixation and lipid production

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HIGHLIGHTS

- Microalgae-bacteria consortium was cultivated in the landfill leachate.
- The TN was removed by 90% in medium with 10% leachate spike ratio.
- Maximum lipid productivity and carbon fixation was 24.1 and 65.8 mg L^{-1} d^{-1}.
- Humic substances in the landfill leachate were decomposed.

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ABSTRACT

The characteristics of cultivating high-density microalgae-bacteria consortium with landfill leachate was tested in this study. Landfill leachate was collected from Laogang landfill operated for over 10 years in Shanghai, China. The maximum biomass concentration of 1.58 g L^{-1} and chlorophyll *a* level of 22 mg L^{-1} were obtained in 10% leachate spike ratio. Meanwhile, up to 90% of the total nitrogen in landfill leachate was removed in culture with 10% leachate spike ratio with a total nitrogen concentration of 221.6 mg L^{-1}. The fluorescence peak of humic-like organic matters red shifted to longer wavelengths by the end of culture, indicating that microalgae-bacteria consortium was effective for treating landfill leachate contaminants. Furthermore, with the leachate spike ratio of 10%, the maximum lipid productivity and carbon fixation were 24.1 and 65.8 mg L^{-1} d^{-1}, respectively. Results of this research provide valuable information for optimizing microalgae culture in landfill leachate.

1. Introduction

Renewable biofuels have attracted much attention due to the soaring demand of fossil fuel and associated concerns about global greenhouse effect. Microalgae is widely considered as one of the most promising feedstock for renewable and carbon neutral biofuel production (Chisti, 2007). Microalgae as phototrophic microorganism has a high carbon fixation ability capable of converting solar energy, inorganic carbon and nutrients into chemical energy stored in their biomass (Mata et al., 2010). Microalgal biomass is also promising feedstock for lipid and high-value chemicals such as pharmaceutical or cosmetic products production, while the residual biomass could be used for bioenergy production through various means including anaerobic digestion (Sostaric et al., 2012) or hydrothermal liquefaction to bio-crude oil (Zhou et al., 2013). In addition, the incineration, pyrolysis, and gasification are also optional strategies for bioenergy production from microalgal biomass. However, cultivating algae requires significant water and nutrient inputs. For instance, it has been estimated that the production of one billion gallons of biodiesel from algal feedstock requires 1238 billion gallons of water and 564 million kg of nitrogen per year (Yang et al., 2011). Therefore, it is highly advantageous to develop alternatives that provide nutrients and water for algae cultivation from current waste.

Integrating wastewater treatment with microalgae cultivation is promising for microalgae-based biofuel production (Mata et al., 2010). Studies have been reported on the microalgal culture with municipal, agricultural, and industrial wastewater (Cai et al., 2013). Landfill leachate, a by-product from biodegradation process in landfills, contains a high concentration of nitrogen (2000–5000 mg L^{-1}), other nutrients including phosphorous and metals, which could be utilized for microalgae cultivation (Lin et al., 2007; Mustafa et al., 2012). In this regard, 29.7 million tons of leachate produced by the landfills in China per year (Lou, 2006) could provide enough nitrogen to produce 0.15 million dry tons

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of algae. This estimate is based on a typical algal cell composition of $C_{106}H_{263}O_{110}N_{16}P$ (Stumm et al., 1981), and the microalgal biomass could subsequently produce 44 million liters of oil assuming 30% oil content by weight (Chisti, 2007).

Previous research focused on finding the tolerant microalgae species and toxicity testing related to deleterious contaminants in landfill leachate (Tsarpali et al., 2012). It is reported that the toxicity of landfill leachate, as measured by seed germination rates, was significantly reduced, by culturing *Chlorella pyrenoidosa* and *Chlamydomonas snowiae* in leachate (Lin et al., 2007). Nevertheless, few researches have been conducted on cultivating high-density microalgae in landfill leachate for inorganic carbon fixation and biofuel production. Therefore, investigating landfill leachate as the main nutrient source for microalgae cultivation will be valuable.

The objectives of this study are as follows: (1) to study the feasibility of cultivating high-density microalgae using landfill leachate; (2) to investigate the removal of the nutrients and organic substances during cultivation of microalgae-bacteria consortium; (3) to investigate the effect of leachate spike ratio on carbon fixation and the microalgal lipid production.

2. Methods

2.1. Landfill leachate and municipal wastewater

A mixture of municipal wastewater and landfill leachate was used as the medium for the microalgae culture. The landfill leachate was obtained from Laogang Landfill in Shanghai, China. The municipal wastewater used was collected from a grit chamber at Quyang Wastewater Plant (Shanghai, China). Both the municipal wastewater and landfill leachate samples were filtered with 1–3 μm filters before inoculation. The parameters of the municipal wastewater and landfill leachate are provided in Table 1.

2.2. Microalgae cultivation

Chlorella pyrenoidosa (FACHB-9) was provided by the Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China. The Chlorella pyrenoidosa was cultured in flasks with sterile BG11 medium containing 1500 mg L^{-1} $NaNO_3$, 40 mg L^{-1} $K_2HPO_4.3H_2O$, 75 mg L^{-1} $MgSO_4.7H_2O$, 36 mg L^{-1} $CaCl_2.2H_2O$, 6 mg L^{-1} citric acid, 6 mg L^{-1} Ferric ammonium citrate, 1 mg L^{-1} EDTA, 20 mg L^{-1} Na_2CO_3 1 mL L^{-1} A_5 + Co solution. The A_5 + Co solution (per 1000 mL) was composed of 286 mg H_3BO_3, 181 mg $MnCl_2.4H_2O$, 22 mg $ZnSO_4.7H_2O$, 39 mg $Na_2MoO_4.2H_2O$, 7.9 mg $CuSO_4.5H_2O$ and 4.9 mg $Co(NO_3)_2.6H_2O$. The microalgae culture was pre-cultivated in a light incubator before inoculation with light intensity of 3000 Lux, light cycles of 12:12 (light:dark), and temperature of 25 °C.

All the cultivation were conducted in 500 mL flasks with 400 mL municipal wastewater spiked with 0%, 5%, 10%, 15%, 20% leachate spike ratio, respectively. Sintered stones were used for air supply into the culture. The mixing of the algal solution can be conducted by the aeration of gas. Chlorella pyrenoidosa were inoculated in the unsterilized medium. The microalgae solution was centrifuged and washed for three times by distilled water, then re-suspended in distilled water before inoculation. The initial concentration of biomass was 0.05 g L^{-1}. All the inoculations were transferred in a light incubator under temperature of 25 °C, light intensity of 8000 Lux, light cycles of 20:4, and aeration rate of 100 mL min^{-1}.

Table 1
Characteristic of landfill leachate and municipal wastewater.

Parameters	Wastewater	Landfill leachate
pH	7.0–7.6	8.3–8.5
Conductivity (μs cm^{-1})	761	14390
Chemical oxygen demand (mg L^{-1})	174.6	900
Total organic carbon (TOC) (mg L^{-1})	32	293.7
Inorganic carbon (IC) (mg L^{-1})	63.1	1196
Total nitrogen (TN) (mg L^{-1})	61.4	1786
Ammonia nitrogen (mg L^{-1})	33.7	1381
Total phosphorous (TP) (mg L^{-1})	3.1	4.0
Ortho-phosphate (Ortho-P) (mg L^{-1})	2.5	3.2
Cl^- (mg L^{-1})	69.1	1764.0
SO_4^{2-} (mg L^{-1})	33.1	210.7
K (mg L^{-1})	11.8	454.6
Na (mg L^{-1})	31.3	171.1
Mg (mg L^{-1})	11.5	56.3
Fe (mg L^{-1})	2.9	3.7
Cr (mg L^{-1})	0.59	0.47
Ni (mg L^{-1})	0.23	0.54
Zn (mg L^{-1})	0.43	0.87

2.3. Analytical procedures

2.3.1. Biomass quantification

The dry biomass was measured by filtering microalgal liquid sample with 0.7 μm nominal pore size glass microfiber filter (Whatman, GF/F) and subsequently dried at 105 °C for 24 h. Chlorophyll a was measured by the following procedure: 3 mL algal liquid was centrifuged at 5000 rpm for 5 min before supernatant was removed. Same volume of methanol was added for chlorophyll extraction. The chlorophyll was measured with a spectrophotometer (UV–Vis 2550, Shimadzu, Japan) at 652 and 665 nm. The chlorophyll concentration was measured with the following formula (Zhou et al., 2013).

$$\text{chlorophyll } a = 16.29 \times A_{665} - 8.54 \times A_{652} \quad (1)$$

2.3.2. Water quality analysis

pH of the culture medium was measured with a pH electrode (PHB-4, Shanghai) during the cultivation. All the liquid samples for water analysis have been filtered by cellulose acetate membrane filter (0.45 μm). The dissolved organic carbon (DOC) in the medium in form of total organic carbon (TOC), inorganic carbon (IC) and total nitrogen (TN) in the culture medium were measured by a TOC/TN analyzer (TOC-VCPN, Shimadzu, Japan). Ammonium nitrogen and phosphorous were measured according to the standard methods (State Environmental Protection administration of China, 2002). The three dimensional excitation–emission matrix (EEM) spectra of the liquid was measured using a fluorescence spectroscopy (FluoroMax-4, HORIBA JobinYvon Co., France). The fluorescence spectra of Milli-Q water were subtracted from all of the spectra to eliminate water Raman scattering and to reduce other background noise. The scanning emission (Em) wavelength and excitation (Ex) wavelength are 210–550 nm at 5 nm increments and 300–550 nm at 5 nm increments, respectively. The contour line is given to express fluorescence intensity.

2.3.3. Biomass composition analysis

At the end of culture, the cells were removed by centrifugation (5000 rpm, 15 min), washed with distilled water and centrifuged twice. After being stored at −20 °C, the pellets were dried in a vacuum freezing dryer for 24 h. The crude lipid was determined according to the modified Bligh and Dyer method (Bligh and Dyer, 1959). The composition of carbon and nitrogen in the freeze dried cells were analyzed using an elemental analyzer (Vario EL III, German).

2.3.4. Specific growth rate

Specific growth rate (μ, d^{-1}) was calculated by:

$$\mu = \frac{\ln X_2 - \ln X_1}{t_2 - t_1} \quad (2)$$