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Short Communication

An oleaginous filamentous microalgae *Tribonema minus* exhibits high removing potential of industrial phenol contaminants

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HIGHLIGHTS

- One oleaginous filamentous microalgae exhibited strong phenol removing ability.
- *Tribonma minus* uptakes maximum 449.46 mg g^{-1} of phenol.
- *T. minus* could degrade phenol efficiently up to phenol 700 mg L^{-1}.
- Residual phenol after *T. minus* cultivation was around range 0.1–0.5 mg L^{-1}.

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ABSTRACT

Discharge of industrial phenol contaminants could cause great harm on natural environment. Through oleaginous microalgae cultivation in phenolic wastewater, pollutants can be phototrophically biofixed into biomass as feedstock for bioenergy production. It was firstly reported in this study that, an oleaginous filamentous microalgae *Tribonema minus* exhibited strong environmental phenol removal ability. *T. minus* filaments showed 449.46 mg g^{-1} of phenol-uptake capacity, obviously higher than those strains with low phenol absorption such as *Scenedesmus dimorphus*. And phenols could be removed efficiently at the initial phenol concentration up to 700 mg L^{-1}. Simultaneously, through *T. minus* growth, phenol concentration could be decreased from 100 mg L^{-1} to the range of 0.1–0.5 mg L^{-1}, which meet industrial discharge need of phenol contaminants in most countries. So *Tribonema minus* is a potential algal specie to help the construction of integrated process for both oleaginous biomass production and bioremediation of phenol contaminants.

1. Introduction

Phenol and its derivatives often occur as hazardous pollutants in industrial effluents (Mohammadi et al., 2015), which have strong corrosive effect and can lead to intensively environmental problems. And a large number of microorganisms including bacteria, fungi, and microalgae have been proved with high degrading capacity of phenolics (Al-Khalid and El-Naas, 2012). Some oleaginous microalgae such as *Scenedesmus* (Lutzu et al., 2016) and *Chlorella* (Cheng et al., 2016) have shown high potentials as bioremediation agents to treat different wastewater. By the photosynthetic growth, industrial pollutants could be fixed and transformed into oleaginous biomass for biofuel or biochemical production (Chisti, 2007). Previous researches also reported that there are some microalgae strains which could degrade phenolic compounds. Klekner and Kosaric (1992a) firstly reported three strains *Scenedesmus obliquus*, *Chlorella* sp. and *Spirulina maxima* could degrade phenols in the culture. Semple and Cain (1996) found that, *Ochromonas danica* could grow hetertrophically with phenol and p-cresol as its sole carbon substrate. It was also proved that, some strains in *Chlorella* or *Scenedesmus* could degrade different phenolic derivatives, such as 2,4-dichlorophenol, 2,4-dimethylphenol (Klekner and Kosaric, 1992b; Pinto et al., 2002), penta-chlorophenol (Tikoo et al., 1997), nonylphenol (Gao et al., 2011), 2,4-dinitrophenol (Klekner and Kosaric, 1992a). And Di Caprio et al. (2015) investigated phenol degradation and biomass production of *Scenedesmus* sp. during cultivation with olive mill wastewater. All these work provided a potential way of cheap microalgae biomass production combined with phenol degradation via microalgal cultivation.

Tribonema minus was a filamentous yellow-green algae, which has been newly reported as an excellent feedstock for biodiesel

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production due to its distinctive properties of high lipid oil content, robustness to protozoa and harvesting cost-effective (Wang et al., 2013). In this study, *T. minus* was firstly reported to have the capacity of phenol contaminants efficient removal from the culture medium. It means that, *T. minus* could be used as the potential feed stock for biofuel production which is integrated with the biodegradation of phenolic effluents via mass autotrophy.

2. Methods

2.1. Strains and media

Tribonema minus SAG 880-3 was purchased from the Culture Collection of Algae of Gottingen University. The strain *Scenedesmus dimorphus* EA801 was kindly provided by Prof. Hu Qiang from the Arizona State University. *Chlorella* sp. FACHB 31 and *Chlorella pyrenoidosa* FACHB 29 were purchased from Freshwater Algae Culture Collection at the Institute of Hydrobiology. *Chlorella zofingiensis* NIES 2175 was purchased from Microbial Culture Collection at National Institute for environmental studies. All strains were sustained and cultivated in regular BG11 medium.

2.2. Cultivations

For inoculum preparation, all of the strains were cultivated in bubbled column photobioreactors (40 cm height, 2 cm diameter) with 200 mL working volume. 1% CO_2 was continuously aerated into the culture. After 4–5 days cultivation, filamentous *T. minus* were harvested by filter fabric with 300 mesh and concentrated. The cells of those unicellular strains, *S. dimorphus*, *Chlorella* sp., *C. pyrenoidosa* and *C. zofingiensis* NIES 2175, were harvested by centrifugation. All the harvested cells were re-suspended into 150 mL BG11 medium with required biomass density in 250 mL flask. A mount of phenols were added with designed concentration. All the flasks were incubated at 25 °C, shaken 3–5 times per day and irradiance at 50 μmol photons m^{-2} s^{-1} was provided continuously.

2.3. Biomass density determination

Biomass density was measured according to Wang et al. (2013). A certain volume (v) of algal culture was filtered on pre-weighted membrane (DW_0), oven dried at 105 °C overnight and then weighed as DW_1. The biomass density was calculated as $(DW_1 - DW_0)/v$.

2.4. Phenol quantification by UV spectrophotometry

The total phenol content in the culture was determined by ultraviolet spectrophotometry (Noorashikin et al., 2016) on a Cary 50 Ultraviolet–visible Spectrophotometer (Varian, America). 3 mL culture was sampled per day for the phenol measurement. The sample was filtrated by filter fabric (300 mesh) and then membrane (0.45 μm) in sequence, the supernatant was used to measure the optical density at 269 nm (OD269).

2.5. 4-APP determination of phenol residual

A modified 4-aminoantipyrine spectrometric method (4-APP) was utilized for quantification of phenol. 0.1 mL NH_4Cl buffer (pH 10.7, 20 g NH_4Cl dissolved in 100 mL ammonia), 0.2 mL 2% 4-aminoantipyrine solution, and 0.2 mL 8% potassium ferricyanide solution were added into 10 mL filtrated sample in turn. And then the solution was incubated at room temperature for 10 min. The optical density at 510 nm (OD510) was measured on a Cary 50 Ultraviolet–visible Spectrophotometer (Varian, America).

2.6. HPLC determination of phenol residual

High performance liquid chromatography (HPLC) was utilized to quantify phenol residue in the culture. Waters 1525 HPLC system was utilized with Waters 2998, Photodiode Array Detector. Agilent Extend-C18 column (5 μm, 4.6 × 250 mm) was utilized with the solution of methanol and water (3:1) as mobile phase. The flow rate and column temperature were controlled at 0.8 mL/min and 35 °C. 10 μL sample filtrated with 0.22 μm membrane was loaded.

2.7. Statistical analysis

All experiments were done with triplicate samples and repeated at least once. All the data shown in figures, tables, and text are the mean values. The correlation coefficient (R value) was calculated using CORREL function in Excel. The value <0.3 was thought to be uncorrelated and >0.3 was thought to be correlated. Higher value indicates higher correlation.

3. Results and discussion

3.1. Prominent phenol-removal performance of different microalgae

Five strains were compared about their phenol uptake, which were *Tribonema minus*, *Scenedesmus dimorphus*, *Chlorella* sp., *Chlorella zofingiensis* and *Chlorella pyrenoidosa*. The initial phenol concentration and biomass density were respectively controlled at about 250 mg L^{-1} and 3 g L^{-1}. The phenol concentration after 0, 5 and 10 days cultivation were determined by UV absorption method. As shown in Fig. 1, *T. minus* exhibited superior phenol removing efficiency than other algal species. After 5 days incubation, filamentous *T. minus* had absorbed >95% phenol (243.09 mg L^{-1}) from the culture. While only less than 11% (16.97–27.53 mg L^{-1}) phenol was removed by other four strains,. After 10 days incubation, 88.24% (217.72 mg L^{-1}) phenol was absorbed by *Chlorella* sp., however none further increased absorptions by *S. dimorphus*, *C. zofingiensis* and *C. pyrenoidosa* were found. Compared with unicellular microalgae, filamentous strains are more suitable for large-scale cultivation due to their high resistance to grazer-predation and low-cost for harvesting recovery (Hillebrand et al., 2002). A filamentous blue-green algae *Spirulina maxima* was also reported to be capable of removing over 95% phenol from the culture with up to 400 mg L^{-1} initial phenol concentration (Lee et al., 2015). *S. maxima* belongs to prokaryotic blue-green algae, which accumulates little lipids. While *T. minus* showed great advantage for biodiesel production, which could accumulate over 50% lipids in its biomass (Wang et al. in 2013).

3.2. Phenol-absorbing capacity of Tribonema minus

To investigate phenol-absorbing capacity of *T. minus* biomass, its filaments were inoculated into the cultures with different biomass density. Fig. 2 respectively showed their change of phenol concentration in 6 days cultivation. It could be found that 2–4 days acclimation period was necessary for subsequent phenol degradation. Similar phenomena were also reported by Klerkner and Kosaric (1992a), who also found the acclimation period length differed among different species. The results in Fig. 2A also showed that, increased inoculum concentration shorten acclimation duration of *T. minus*. While 0.5 g L^{-1} biomass density was enough for removing of 96.77% (225 mg L^{-1}) phenol from the culture in