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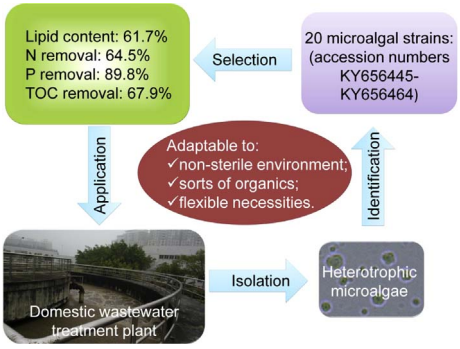
Assessment upon heterotrophic microalgae screened from wastewater microbiota for concurrent pollutants removal and biofuel production

Liang Shen, Jean Damascene Ndayambaje, Theophile Murwanashyaka, Wenqian Cui, Emmanuel Manirafasha, Cuixue Chen, Yuanpeng Wang, Yinghua Lu*

Department of Chemical and Biochemical Engineering, College of Chemistry and Chemical Engineering, The Key Laboratory for Synthetic Biotechnology of Xiamen City, Xiamen University, Xiamen 361005, PR China

GRAPHICAL ABSTRACT



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ABSTRACT

Heterotrophic microalgae, capable of converting organic carbons to biofuel, as well as assimilating nutrients, have a great prospective in wastewater treatment. Meanwhile, the knowledge about heterotrophic microalgae is still far less than the autotrophic conterpart. Hence, in this study, 20 heterotrophic microalgal strains were isolated from a domestic wastewater treatment plant, and identified according to morphology and partial 18S and 23S rRNA gene sequences. Further, their biological traits were assessed in terms of N, P, TOC removal efficiencies, growth parameters, self-settleability and lipids production, expressed through a comprehensive selection index. By such, the optimal strains were chosen and applied back to treat the real wastewater, with or without pretreatment of sterilization. An organic-adaptable strain, i.e., *Botryococcus* sp. NJD-1, was ultimately recommended to achieve the concurrent biofuel production (up to 61.7% lipid content) and pollutants removal (up to 64.5%, 89.8% and 67.9% for N, P and TOC) in pristine wastewater.

1. Introduction

Nowadays, energy and environment are two urgent and closely interrelated issues worldwide. Microalgae appear to be an ideal key for this conundrum as they can uptake pollutants especially nutrients in wastewaters and thereafter reproduce as a feedstock of biofuel, food and chemicals. Meanwhile, autotrophic cultivation, so far the most common way for algae, is only appropriate when CO_2 is available. And the light shading effect will restrict the high-density growth of phototrophic algae as well. So, heterotrophic cultivation of algae suggests a

* Corresponding author.
E-mail address: ylu@xmu.edu.cn (Y. Lu).

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promising way in the circumstance of wastewaters, in which organic carbon can be simultaneously used as energy and carbon source. By such, the cost in the heterotrophic cultivation of algae with wastewater can be greatly lessened due to the elimination of aeration, extra substrate material and sterilization. The operation and daily maintenance also turn to be much simpler than the autotrophic method. With all these merits, heterotrophic cultivation has been reported to enhance the algal biomass yield up to 25-fold of the autotrophic mode (Morales-Sánchez et al., 2017).

However, heterotrophic cultivation of algae with wastewater still faces many obstacles in practice. The primary concern is that there is a limited number of microalgal species that can grow heterotrophically. Indeed, most known microalgae species are obligate autotrophs instead of heterotrophs or mixotrophs (Hamilton et al., 2016). Considering the intrinsic metabolic traits of all microalgae is photosynthetic, the ability to obtain energy through respiration is only shared by unique species of microalgae (Perez-Garcia et al., 2011). The difference among these heterotrophic microalgae responding to environmental factors including nutrient concentrations and C:N:P ratio can be remarkable as well (Wang et al., 2017). As such, screening appropriate microalgal species adaptable to organic substrates is the groundwork of heterotrophic cultivation of algae in wastewater systems. To achieve these objectives, researchers have tried to isolate and cultivate the heterotrophic microalgae in various categories of wastewaters. Zhang et al. (2014) isolated 5 heterotrophic microalgae from a domestic wastewater treatment plant (WWTP) and the heterotrophic metabolism of their isolates was evaluated for biofuel production using the Biolog method. Wang et al. (2010) isolated *Chlorella* sp. from a local wastewater, cultured it in different municipal wastewaters (MWWs) using filtration as pretreatment, and observed 82.4%, 62.5% and 83.0% of NH_4-N, NO_3-N and COD removal respectively. Nevertheless, the heterotrophic microalgae species especially those sourced from wastewaters are still minimal.

Besides, the organic categories that have been documented for heterotrophic cultivation are restricted in a shot list, such as glucose, glycerol and acetate (Girard et al., 2017). Apart from these most commonly used organic substrates, microalgal strains could possibly assimilate a couple of other organics. It was reported that heterotrophic microalgae cultivated could remove several organic acids (e.g., valeric acid, isovaleric acid, isobutyric acid etc) in the filtrated hydrolyzate of waste activated sludge (Wen et al., 2013). Zhang et al. (2014) also evaluated the heterotrophic cultivation of microalgae in 31 substrates but their growth features were not assessed yet. Since it was economically unfeasible to culture microalgae with glucose as that cost can reach up to 80% of the total material (Wang et al., 2016), the wastewater could be used as carbon source, in which the organics would be in a more extensive range for heterotrophic algae growth. Hence, it is necessary to acquire more information about the adaptation of the algae in case of the unusual organics rather than the preliminary differentiation as heterotrophic or autotrophic.

Thus, the combination of heterotrophic algae and wastewater has been attempted by several researchers. Zhou et al. (2012) realized the heterotrophic cultivation of microalgae in concentrated municipal wastewater (CMWW) pretreated by autoclave, in which lipid content reached 33.22% while the nutrient removal efficiencies for total phosphorus, ammonia, nitrogen and COD were 98.48%, 100%, 90.60% and 79.10%, respectively. Ummalyma and Sukumaran (2014) used the heterotrophic microalgae to treat the sterilized dairy effluent for oil production and the organic removal where 39–42% lipid content has obtained. Also, Zhang et al. (2013) treated the autoclaved real wastewater using the newly isolated heterotrophic microalgae where the algal densities of *Scenedesmus* and *Chlorella* species were increased by 203.0% and 60.5%, respectively. And Ramsundar et al. (2017) assessed the potential of *Chlorella sorokiniana* in municipal wastewaters at various stages of treatment as well, using microfiltration to sterilize the wastewater prior to cultivation. In general, sterilization seems an indispensable pretreatment for wastewaters to cultivate algae. Currently, little work on the heterotrophic microalgae cultivation has been carried out using the real wastewater directly.

Additionally, the biomass separation and harvesting has posed another tough problem in the field of the algal cultivation, no matter for microalgae production or wastewater treatment. According to Gutiérrez et al. (2016), the commonly employed biomass harvesting techniques (e.g. flocculation, filtration, centrifugation etc), may account for up to 20–30% of the total cost in microalgae production. Thus, promoting the spontaneous flocculation and gravity sedimentation by way of increasing the dominance of the self-settleable microalgae rose as an appealing strategy for algae harvest in recent years (Park et al., 2013). In this sense, microalgal strains with high self-settleability are of great interest in any cultivation system.

Therefore, the present work attempted to screen and identify a set of heterotrophic algae from a local domestic WWTP, and then perform a thorough assessment on the isolates in terms of growth ability, organics tolerance, nutrient removal potential, lipid recovery and self-settleability. Consequently, the optimized algal strains were applied in the real wastewater to test their potential in practical use. The results of this study are expected to provide instructive information about heterotrophic algal cultivation for the synchronized benefits of wastewater treatment and biofuel recovery.

2. Materials and methods

2.1. Sampling site and wastewaters

The mixed liquor in the aeration tank was sampled in Xiapu Domestic Wastewater Treatment Plant (Xiamen, China). After settling, the pellet was used as the original source for algae screening, and the supernatants with and without autoclaving were used as the autoclaved real domestic wastewater (ARDWW) and the untreated real domestic wastewater (URDWW) in the following experiments, respectively. The characteristics of ARDWW and URDWW were shown in Supplementary data.

2.2. Isolation of microalgal strains

The isolation method was modified from Wang et al. (2013). Briefly, 100 mL of wastewater sample were positioned into sterile 250-mL Erlenmeyer flasks and placed on continuous light bank to encourage algal growth in whole-effluent for 30 h. After the growth was confirmed through microscopic examination (Leica DM500, Switzerland), 4 mL of wastewater sample was transferred to a 50-mL flask containing either modified BG11 (MBG11) (Rippka et al., 1979) or modified Bold-3N media (MBold-3N) (Murwanashyaka et al., 2017), as presented in Supplementary data. The contents of nitrogen (NO_3-N) and phosphorus (PO_4-P) in the media were set as 13.6 mg L^{-1} and 2.22 mg L^{-1}, respectively, according to the content of URDWW. The algae were subjected to purification by serial dilutions followed by inoculation onto agar plates containing flesh medium solidified by 12 g L^{-1} of agar. The citric acid was added to prevent the contamination of bacteria. The single strain culture was ensured by the repeated streaking on the nutrient agar plates and repetitive microscopic examination. Morphologically unique strains were selected and maintained for next experimentation. The preservation conditions used to isolate the microalgal strains were 260 μmol photon m^{-2} s^{-1}, light/dark cycle of 9 h/15 h, initial pH 7 and the temperature range of 25–28 °C for 10 d of incubation. This route was repeated several times to obtain the pure strain. During the growth periods, the cultures were manually shaken 3 times per day to avoid sticking. The overall isolation route of heterotrophic microalgae was shown in Supplementary data and each step was carried out in triplicate.