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Microbial lipid production and organic matters removal from cellulosic ethanol wastewater through coupling oleaginous yeasts and activated sludge biological method

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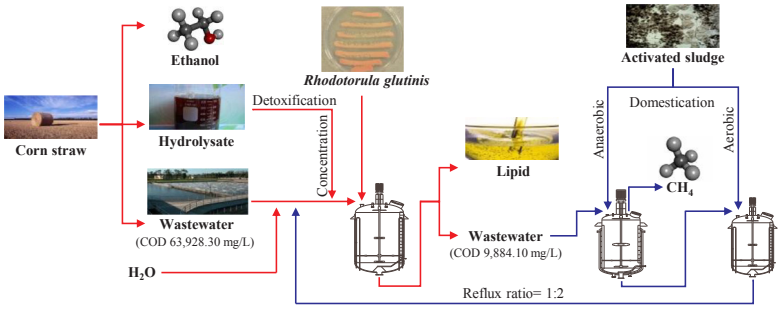
GRAPHICAL ABSTRACT



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ABSTRACT

In this paper, a novel strategy for lipid production through coupling oleaginous yeasts and activated sludge biological methods by cultivation of *Rhodotorula glutinis* in cellulosic ethanol wastewater was studied. Under optimal conditions in wastewater medium (dilution ratio of 1:2 and glucose supplement of 40 g/L), the maximum biomass and lipid content as well as the lipid yield reached 11.31 g/L, 18.35% and 2.08 g/L, with the associated removal rates of COD, TOC, NH_4^+-N, TN and TP reaching 83.15%, 81.81%, 85.49%, 70.52% and 67.46%, respectively. Cellulosic ethanol wastewater treated by the anaerobic-aerobic biological process resulted in removal of COD, NH_4^+-N, TP and TN reaching 67.55%, 94.17%, 90.16% and 48.89%, respectively. The reused water was used to dilute medium of *R. glutinis* for microbial lipid production reaching 2.38 g/L and caused positive effects on the accumulation of biomass and lipid.

1. Introduction

Development of a partial replacement of fossil fuels is critical to resolve environment and population issues (Ajanovic and Haas, 2010; Nigam and Singh, 2011). Recently, biodiesel has attracted increasing attention due to its green and renewable properties (Ambat et al., 2018; Oliveira et al., 2017; Kumar et al., 2016).

Microbial lipid is regarded as potential renewable feedstock for producing biodiesel due to its short production cycle, convenience in scale-up and resistance to seasons and sites (Zhou et al., 2013; Tinoi and Rakariyatham, 2016). Oleaginous yeasts, especially *Rhodotorula glutinis*, possessing obvious advantages over bacteria, moulds and algae because of higher growth rate and lipid accumulation, are regarded as promising feedstock for biofuel production (Liu et al., 2016; Yu et al.,

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2011). However, using glucose as substrate and nutrient source, accounting for more than 80%, could cause high costs and restrain application and extension of microbial lipid (Xue et al., 2010). Previous research gradually transferred attention to cheap and abundant carbon resources and focused on various low-cost substrates, such as glycerol, organic wastewater, lignocelluloses and hydrolysates.

Cellulosic ethanol is widely recognized as another clean energy source. Lignocellulose, the raw material in cellulosic ethanol production, is a renewable resource that is abundant in nature (Baeyens et al., 2015). Cellulosic ethanol production process consumes a large amount of industrial water. According to statistics, the production of 1 L cellulosic ethanol produces ~20 L cellulosic ethanol wastewater (Wilkie et al., 2000; Hu et al., 2017). Low pH values, dark color, high chemical oxygen demand (COD) combined with dissolved organic and inorganic components and inhibitors from the pretreatment of lignocellulose induce negative effects on microbial growth and result in necessary wastewater treatment (Shan et al., 2017).

Oleaginous yeasts, especially *R. glutinis,* grow well with high carbon and nitrogen ratios, and require a lot of water and nutrients to accumulate biomass and lipid content in fermentation process (Amirsadeghi et al., 2015). Therefore, high COD of wastewater is suitable to microbial lipid production by oleaginous yeasts to enhance added value. Pulp and paper wastewater could be utilized as fermentation medium for lipid production by *R. glutinis*, and lipid yield can reach 1.3–2.9 g/L (Amirsadeghi et al., 2015). *R. toruloides* Y2 used organic substances from bioethanol wastewater to produce lipid of 1.3 g/L, with removal of COD was 72.3%. (Zhou et al., 2013). But oleaginous yeasts treating wastewater result in a relatively lower COD removal of < 80% (Huang et al., 2017). After treatment by *R. glutinis*, COD in corn cob bagasse wastewater decreased by 33–42% (Cai et al., 2016), and nitrogen and phosphorus were relatively sufficient, which was not able to be discharged directly and so required a further processing.

To reduce pollutant concentration of wastewater, biological treatment is considered as a promising approach. (Huang et al., 2017; Chan et al., 2009). Generally, when biodegradable COD is < 1000 mg/L, an aerobic system is more suitable to treating wastewater than anaerobic system, which works better in refractory wastewater with biodegradable COD in excess of 4000 mg/L (Chan et al., 2009). However, when treating industrial wastewaters containing high organic and inorganic components, the aerobic and anaerobic treatment effluent is not capable to meet the discharge standard of COD < 120 mg/L (Ji et al., 2002) due to the high concentration of organic wastewater exceeding the load of activated sludge. Neither anaerobic nor aerobic approach was effective in treating green olive debittering wastewater with COD of 25,000 to 100,000 mg/L (Aggelis et al., 2001). According to the respective advantages of anaerobic and aerobic approach, the combined process was proposed to solve the problem of lower COD removal rate (Ros and Zupančič, 2004). Over the recent decades, anaerobic-aerobic treatments received great attention because of low energy requirement and chemical input and simple operation with recycled resource (Chan et al., 2009). COD removal of anaerobic- aerobic process was employed to treat beet molasses alcoholic fermentation wastewater and the COD removal ratio could reach 96.5% (Jiménez et al., 2003). In previous studies, oleaginous yeasts could grow well in high-concentration organic wastewater with high lipid accumulation, and the digestion of wastewater was significant (Matsakas et al., 2014; Ruan et al., 2015; Gong et al., 2016). Coupling oleaginous yeasts and activated sludge, as a biological method, treating high-strength cellulosic wastewater is considered operationally and economically advantageous.

Besides, wastewater treatment and reuse is critical to deal with water shortage and water pollution (Sharma and Kennedy, 2017; Šrámková et al., 2018). After a biological treatment, high-concentration organic wastewater effluent is not able to meet the discharge standard, and the treatment of wastewater needs further process through in-depth physicochemical pathways (Ranade and Bhandari, 2014). Hence, in order to reduce industrial water consumption, simplify subsequent wastewater treatment and achieve the purpose of comprehensive utilization of wastewater, biological treatment of cellulosic ethanol wastewater is expected to act as a potential approach. It has been reported that the EGSB (Expanded Granular Sludge Bed), SBR (Sequencing Batch Reactor) effluent re-used in the cellulosic ethanol washing and fermentation section exhibited a significant effect on ethanol fermentation (Yu, 2010).

Thus, utilization of *Rhodotorula glutinis* cultivation in cellulosic ethanol wastewater for producing microbial lipid can not only reduce the cost of producing microbial lipid but also utilize concentrated organic wastewater.

In this work, cellulosic ethanol wastewater was used as raw material for microbial lipid production by *R. glutinis*. Meanwhile, coupling *R. glutinis* and activated sludge biological method was used for degradation of organic compounds in cellulosic ethanol wastewater. In addition, the feasibility of using treated cellulosic ethanol wastewater as reused water to dilute the medium for producing microbial lipid was explored.

2. Material and methods

2.1. Microorganism, seed sludge, and wastewater

Rhodotorula glutinis CGMCC No. 2258 was obtained from the China National Research Institute of Food and Fermentation Industries (CNRIFFI), stored at 4 °C on agar slant medium with yeast extract, peptone and dextrose.

Seed sludge including anaerobic sludge and aerobic sludge was obtained from a wastewater treatment plant located in Harbin. Before dealing with the target cellulosic ethanol wastewater, seed sludge was domesticated using different concentrations of wastewater to adapt to the growth environment.

Cellulosic ethanol wastewater was obtained from the COFCO of Zhao Dong, China. The main components of wastewater are measured as shown in the Table 1. In order not to affect subsequent culturing and analysis, the samples were stored at −20 °C.

2.2. Medium and culture conditions

The seed/basic medium of *R. glutinis* contained (g/L) Yeast extract 1.5, Na_2SO_4 2, $(NH_4)_2SO_4$ 2, $MgSO_4$ 1.5, KH_2PO_4 7 and glucose 40. The cellulosic ethanol wastewater medium contained cellulosic ethanol wastewater/pure water of 1:2 (v/v), glucose 40 g/L, pH 5.5. The reused wastewater medium consisted of cellulosic ethanol wastewater/reused water = 1:2 (v/v), glucose 40 g/L, pH 5.5. The glucose from detoxified and concentrated cellulosic hydrolysate served as supplementary carbon source. The *R. glutinis*, kept on the YPD agar slant, was precultured for 24 h at 30 °C and then inoculated in a 250 mL flask containing 50 mL seed medium with the conditions of 30 °C, 220 rpm, 24 h. 4 L fermentation medium in a 7 L reactor with inoculating seed of 10% (v/v) was cultured at 30 °C, 180 rpm. The initial pH of all medium was

Table 1
Characteristic of cellulosic ethanol wastewater sample.

Parameter	Concentration (mg/L)	Parameter	Concentration (mg/L)
COD	63,928.30	Lactic acid	22.62
TOC	30,290.00	Acetic acid	7.55
pH	3.78	Citric acid	1.27
SS	136,700.00	Succinic acid	1.30
TP	1735.00	Xylose	10.58
TN	2025.00	Glycerin	3.22
NH_4^+-N	1523.00	Furfural	0.90
Furfur alcohol	0.22	HMF	0.80

COD: Chemical Oxygen Demand; TOC: Total Organic Carbon; SS: Suspended Solids; TP: Total Phosphorus; TN: Total Nitrogen; HMF: Hydroxymethylfurfural.