



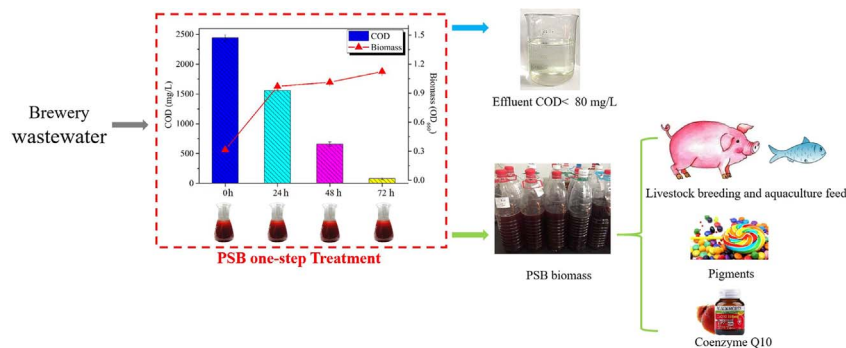
# One-step treatment and resource recovery of high-concentration non-toxic organic wastewater by photosynthetic bacteria

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## GRAPHICAL ABSTRACT



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## ABSTRACT

In order to achieve simple pollutant removal and simultaneous resource recovery in high-COD-non-toxic wastewater treatment, a one-step photosynthetic bacteria (PSB) method was established using batch study experiment. The effluent COD met the national discharge standard, and biomass with rich protein and high-value substances was efficiently produced. It eliminated the demand of post-treatment for conventional PSB treatment. Results showed that *Rhodospseudomonas* effectively treated brewery wastewater and achieved biomass proliferation. Yeast extract was the best additive for PSB growth and the effluent COD was below 80 mg/L with 400 mg/L yeast extract, meeting the national discharge standard. In addition, the PSB biomass increased by 2.6 times, and the cells were rich in protein, polysaccharide, carotenoids, bacteriochlorophyll and coenzyme Q10, reaching 420.9, 177.6, 2.53, 10.75 and 38.6 mg/g respectively. This work demonstrated the great potential of PSB for high-COD non-toxic wastewater treatment in one-step process.

## 1. Introduction

In wastewater treatment, biological treatment method was the most widely applied method (Kovalova et al., 2012; Henze and Harremoës, 1983). The conventional biological wastewater treatment is the process

of biodegradation, which is a process that microorganism decompose macromolecule organics, nitrogen and phosphor to simplest substance such as CO<sub>2</sub>, N<sub>2</sub> and orthophosphate. Typical conventional biological wastewater processes include activated sludge, membrane biological reactor (MBR), sequencing batch reactor (SBR), aerobic-anaerobic

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method (A/O), up-flow anaerobic sludge blanket (UASB), etc (Hinken et al., 2014; Juteau et al., 2004). These processes can effectively remove the organic pollutants and nutrients in the wastewater. However, there is a very big problem, namely the excess sludge. For example, in a typical activated sludge process, roughly 50% organics in the wastewater (in terms of COD) was transformed into the microbial biomass via microorganism metabolism. The so generated excess biomass is called excess sludge. The excess sludge contains ~99% water and high organic and nutrients, and needs careful treatment and disposal. The cost of excess sludge treatment and disposal sometimes counts for 50% of the total cost of the wastewater treatment plants. Further, the release of CO<sub>2</sub> and N<sub>2</sub>O during the biological degradation process contributes to the global warming and has caused great concerns as well (Appels et al., 2008).

Therefore, a new and very different solution has been proposed, namely the biological conversion. This method is to select some special microorganisms that are of economic value and use the nutrients-rich wastewater as a free culturing medium. In this process, macromolecule carbohydrate, nitrogen and phosphorus in the wastewater are transformed into valuable microorganism biomass via microbial metabolism. At the same time, these substances are removed from the wastewater and the wastewater gets purification. Therefore, the problem of excess sludge no longer exists and the cost of excess sludge treatment and handling becomes unnecessary. Please note that to utilize the bio-conversion method, the wastewater must be non-toxic and have abundant organics and nutrients.

The representative bio-conversion method is algae treatment of wastewater that converts pollutants in wastewater into oil-rich algae, which is then used for bio-fuel production. For the past decades, many researchers have devoted to this method and achieved satisfactory results (Judd et al., 2015; Mennaa et al., 2015; Tsiptsias et al., 2016).

Another option is to utilize photosynthetic bacteria (PSB). PSB are a group of bacteria which can utilize light energy and organic to realize autotrophic growth and heterotrophic growth, respectively. PSB have been demonstrated to have great potential in treating wastewater effectively and realizing resource recovery simultaneously. PSB can effectively treat fishing wastewater (Azad et al., 2004), starch wastewater (Getha et al., 1998; Prachanurak et al., 2014), dairy wastewater (Kaewsuk et al., 2010), rubber wastewater (Anwar et al., 2013), livestock wastewater (Ponsano et al., 2008), and domestic wastewater (Nagadomi et al., 2000; Hülsen et al., 2016). The COD removal and ammonia nitrogen removal could reach 85–93% and 99% (Saejung and Thammaratana, 2016; Yang et al., 2017). PSB biomass is rich in single-cell protein, and can be used as fish or livestock feed. They are high-grade feed or feed additive to promote the growth of animals' body, enhance the disease resistance, improve water quality, and reduce production costs and management fees. Furthermore, the PSB cells contain many high-value substances including carotenoids and coenzyme Q10 (CoQ10) (Jeong et al., 2008; Hao et al., 2017). Carotenoids are wide-use food and fat colorant, are added in medicines to enhance the immune system, and are cosmetic additives as well. CoQ10 is an important hydrogen cell in the respiratory chain and is a popular antioxidant with a high market price. Therefore, it is highly attractive to use PSB to treat high concentrated non-toxic wastewater to realize pollutant control and resource recovery in one process. In comparison, the algae technology can handle wastewater with very low N and P but is improper for high COD wastewater, while PSB technology is more suitable for high COD wastewater.

However, one problem hinders the application of PSB wastewater treatment in large scale. In order to achieve economic benefit, concentrated wastewater is needed in order to produce large amount of PSB biomass. For example, starch wastewater with ~10,000 mg/L COD was effectively treated by PSB, and the biomass yield reached 0.51 mg-biomass/mg-BOD-removal (Prachanurak et al., 2014). However, the initial COD was so high that even though the COD removal reached 93%, the effluent COD was 182 mg/L. The COD of seafood wastewater

was rapidly reduced by about 80% and the effluent COD was 400 mg/L. The soybean wastewater was removed COD of 95% and the effluent COD was still 490 mg/L (Lu et al., 2011). Similar phenomenon for other concentrated wastewater as well (Saejung and Thammaratana, 2016). This level of effluent COD obviously cannot meet the environmental standards. For example, the discharge standard of industrial wastewater in China is usually 100 mg/L. In order to achieve the effluent standard, pre-treatment and post-treatment are needed. The post-treatment is usually traditional activated sludge method (Takeno et al., 2005). The inclusion of post-treatment brings three problems: complex operation and higher capital cost; loss of 100–500 mg/L COD which otherwise can be used to culture PSB cells; and generation of excess sludge which needs extra treatment and disposal. The benefits of bio-conversion are then greatly weakened. Therefore, researchers tried to improve the treatment efficiency of PSB process to realize one-step wastewater treatment and resource recovery.

The simplest method to improve biological wastewater treatment is to add some external materials, such as trace elements or organic molecules. Wu confirmed that adding Fe<sup>2+</sup> and Mg<sup>2+</sup> can significantly improve wastewater COD removal and shorten the treatment time (Wu et al., 2012, 2015). However, the effluent still could not be discharged directly. Selection of high efficiency strains was studied (Nagadomi et al., 2000) but the effluent was still high. Another additive, yeast extract, a common nutritional substance used in microorganism-culturing mediums, is rich in essential amino-acids, vitamins b and trace elements. It states that yeast extract is necessary for heterotrophic photosynthetic bacteria culturing (Buchanan and Gibbons, 1984). Therefore, we attempted to add trace elements and yeast extract in the process of PSB wastewater treatment to achieve one-step clarification. Brewery wastewater was used in this study, which is a representative non-toxic high-COD organic wastewater with large production worldwide.

This study aimed to find a simple one-step process for the application of PSB in concentrated non-toxic organic wastewater treatment and resource recovery. Different PSB strains and additives were examined. The biomass growth and value-added substances were evaluated.

## 2. Material and methods

### 2.1. Materials

Four PSB strains were used in this study. PSB 1 was isolated from a local pond, which was identified as a *Rhodospseudomonas* strain, a well recorded strain with a Gene Bank accession number of CP001151.1. PSB 2 was a strain isolated from a polluted local river. PSB 3 (commercial name Zhongguang and identified as *Ectothiorhodospira*) and PSB 4 (commercial name Strain 99) were purchased from Jinlong Bio-product Company, China. All strains were cultured in an improved RCVBN medium in a thermostat shaker (120 r/min, 26–30 °C) (Lu et al., 2013).

Artificial brewery wastewater prepared with a diluting brewery was used in this experiment. The artificial wastewater could imitate practical brewery wastewater with similar chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP) concentrations. The wastewater COD, TN and TP were 2200–2600, 20–22, and 5–6 mg/L, respectively.

### 2.2. Experimental methods

Reactors were sterilized at 121 °C for 30 min before use. The experiments were conducted in batch photobioreactors at the laboratory scale using 1000 mL borosilicate Pyrex bottles. Wastewater and PSB (90/10, v/v) were added to the reactors. According to previous work (Lu et al., 2011), natural light and micro-aerobic conditions were set for PSB, which was anaerobic in daytime (without aeration) and aerobic (with artificial aeration) to keep the dissolved oxygen in the reactor 2–4 mg/L at night. These conditions utilized sunlight and saved the

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