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High-strength fermentable wastewater reclamation through a sequential process of anaerobic fermentation followed by microalgae cultivation

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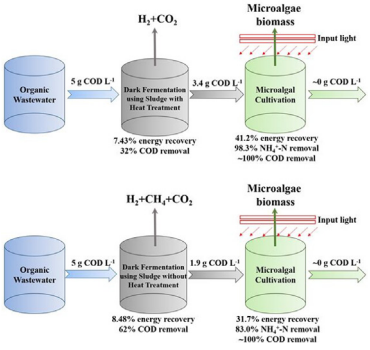
HIGHLIGHTS

- Anaerobic fermentation and microalgae cultivation was used to treat wastewater.
- H_2 fermentation produced lots of organic residuals that could be used by algae.
- H_2 fermentation broth supported better algal growth than methane fermentation.
- The nutrient and energy recoveries were evaluated for the sequential processes.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, the sequential process of anaerobic fermentation followed by microalgae cultivation was evaluated from both nutrient and energy recovery standpoints. The effects of different fermentation type on the biogas generation, broth metabolites' composition, algal growth and nutrients' utilization, and energy conversion efficiencies for the whole processes were discussed. When the fermentation was designed to produce hydrogen-dominating biogas, the total energy conversion efficiency (TECE) of the sequential process was higher than that of the methane fermentation one. With the production of hydrogen in anaerobic fermentation, more organic carbon metabolites were left in the broth to support better algal growth with more efficient incorporation of ammonia nitrogen. By applying the sequential process, the heat value conversion efficiency (HVCE) for the wastewater could reach 41.2%, if methane was avoided in the fermentation biogas. The removal efficiencies of organic metabolites and NH_4^+-N in the better case were 100% and 98.3%, respectively.

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1. Introduction

High-strength sewage is usually treated via anaerobic fermentation, an important approach that can convert the stored energy in the wastewater to methane, hydrogen or other combustible gases. Anaerobic fermentation via methane generation has been widely applied in the treatment of high-strength fermentable wastewaters generated in agricultural, food and winery sectors (Lee et al., 2013; Riaño et al., 2011; Siles et al., 2007). However, more and more studies have switched the focus to fermentation for the production of hydrogen, since hydrogen is deemed as the

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most ideal substitute for fossil fuels because of its high energy density, recyclability and non-polluting nature (Batista et al., 2015).

The difference between hydrogen and methane fermentation also relies on the fact that most carbonaceous materials originated from the substrate still remains in the fermentation broth instead of being converted from liquid to gaseous phase in hydrogen fermentation processes, thus resulting in higher usable organic residuals, such as VFAs and alcohols, that can be further used by heterotrophic or mixotrophic microorganisms (Oh et al., 2003; Wang et al., 2008). The fermentation broths from both processes still contain significant amount of VFAs, nitrogen and phosphorus, which would cause potential threat to the natural environment if not properly managed (Yang et al., 2010).

Bacteria-based wastewater treatment processes possess the advantage of being highly efficient in COD removal, but they are less competent in nitrogen and phosphorus reduction, which would often require complicated design in the flow chart in order to achieve effective operation (Hao et al., 2001; Wang et al., 2016). The history of applying microalgae in wastewater nutrient remediation using various strains like *Chlorella* has a long time span of about 75 years (Abdel-Raouf et al., 2012). Bio-treatment with microalgae is appealing due to the photosynthetic capabilities that are lacking for bacteria, which could convert solar energy into useful biomass and incorporate nutrients such as nitrogen and phosphorus (Noue and Pauw, 1988). In previous studies, the ability of *Chlorella* sp. in the removal of nutrients has been widely witnessed. For instance, the elimination of nitrogen and phosphorus by *Chlorella* sp. reached 80.9% and 99.2%, respectively, in a piggery wastewater (Lee and Chen, 2016). In another study done on a primary settled wastewater, nutrient removal efficiencies achieved 86% and 70%, respectively, for inorganic N and P by culturing *Chlorella* sp. (Lau et al., 1996). And for a diary manure wastewater, microalgae removed ammonia, total nitrogen and total phosphorus by 100%, 75.7–82.5%, 62.5–74.7%, respectively, in differently diluted broths after the anaerobic fermentation (Wang et al., 2010a).

Above all, mixotrophic microalgae cultivation in dark fermentation effluents should be a promising alternative for the wastewater nutrients' reclamation. Meanwhile, mixotrophic microalgae can utilize a variety of organic compounds, as both carbon and energy sources, to accumulate biomass. The major soluble metabolites from dark fermentation, such as ethanol, acetic and butyric acid, could be readily assimilated by microalgae according to previous studies (Liu et al., 2012). *Chlorella vulgaris* consumed almost all of the soluble metabolites in the fermentation broth and the highest biomass production rate was 0.22 g/L/d (Liu et al., 2013b).

From both energy and nutrient recovery points of view, the sequential process of dark fermentation followed by microalgae cultivation for treating high-strength fermentable wastewaters holds great promise. However, the information on the whole process's evaluation is limited, let alone taking the different fermentation types' effects into account. Therefore, this paper designed and performed two experimental routes, i.e., fermentation using the anaerobic sludge (AS) with heat treatment for producing hydrogen-dominating biogas, and fermentation using the AS without heat treatment for producing methane-containing biogas, followed by microalgae cultivation in the fermentation broths to remediate the metabolites, nitrogen and phosphorus. The two technical routes were compared with regard to the biogas generation, broth metabolites' composition, algal growth with simultaneous nutrients' reduction, and energy conversion efficiencies for the whole processes.

2. Materials and methods

2.1. Seed anaerobic sludge and algal inoculum

The AS used in the anaerobic fermentation process was collected from a starch wastewater treatment plant located in Wuxi, Jiangsu Province, China. The AS was used as the inoculum for dark anaerobic fermentation process with and without heat treatment. The heat treatment was designed in order to suppress the activity of hydrogen-consuming bacteria, leading the anaerobic fermentation to generate hydrogen instead of methane. The heat treatment was performed at 121 °C for 10 min in an autoclave to kill the non-spore-forming hydrogen-consuming bacteria while retain the spore-forming hydrogen-generating bacteria. The freshwater microalgae strain used in this study was identified as *Chlorella* sp. and named L3. It was isolated in a local ditch with longitude and latitude of N: 31°43′40″ and E: 121°30′8″ and retained on solid Tris-Acetate-Phosphate (TAP) plate in our lab. The seed was grown on liquid TAP medium and illuminated under cool white fluorescent lamps with around 150 μmol photons/m^2/s at room temperature (26 ± 2 °C).

2.2. Batch dark anaerobic fermentation in 1 L glass bottles

1 L glass bottles with the working volume of 900 mL were used as the batch fermentation reactors. The aluminum foil composite film sampling bags were connected with glass bottles to collect biogas during the fermentation process. Glucose (5 g/L) was used as the only substrate in dark anaerobic fermentation processes in an artificial wastewater containing (per liter of deionized water) the following: 500 mg NH_4Cl, 250 mg KH_2PO_4, 250 mg K_2HPO_4·$3H_2O$, 300 mg $MgCl_2{\cdot}6H_2O$, 25 mg $FeCl_3$, 16 mg $NiSO_4$, 25 mg $CaCl_2$, 11.5 mg $ZnCl_2$, 10.5 mg $CoCl{\cdot}6H_2O$, 5 mg $CuCl_2{\cdot}2H_2O$, and 15 mg $MnCl_2{\cdot}4H_2O$. The reactors were inoculated with AS at a solid concentration of 4 g/L. The initial pH was adjusted to 6.50 as suggested in a previous study using 1 M NaOH solution (Abdallah et al., 2016). The reactors were placed on magnetic stirrers (MYP11-2, China) with stirring speeds at 500 r/min and temperature controlled at 37 °C ± 1 °C. Oxygen in headspace was replaced with nitrogen gas (purged for 5 min) to provide anaerobic conditions. The two experimental treatments for dark anaerobic fermentation process were: (1) Biogas generation using the AS with heat treatment; (2) Biogas generation using the AS without heat treatment. Other conditions were the same in the two sets of experiments.

2.3. Cultivation of Chlorella sp. L3 in the fermentation broths

The two kinds of fermentation broth from the dark anaerobic fermentation systems using (1) the AS with heat treatment and (2) the AS without heat treatment were collected after the same hydraulic retention times (HRTs) of 43 h, and subjected to centrifugation at 8000 rpm for 10 min to remove the suspended solids. Inoculants of *Chlorella* sp. L3 harvested in the exponential growth phase were inoculated into the fermentation broths with initial biomass densities of around 0.10 g/L and the initial pHs were adjusted to 6.00 according to previous studies (Cho et al., 2014; Liu et al., 2013a; Ren et al., 2014b). The algal growth batch experiments were performed in 100 mL erlenmeyer flasks with working volumes of 50 mL. All groups were cultured at 26 ± 2 °C. The effective area of light intercepted in each reactor was around 1.60×10^{-3} m^2. Each experiment was carried out in triplicate.

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