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Improving pollutants removal by microalgae *Chlorella PY-ZU1* with 15% CO_2 from undiluted anaerobic digestion effluent of food wastes with ozonation pretreatment

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HIGHLIGHTS

- Microalgae mutant with 15% CO_2 was used to remove various pollutants from the UADEFW.
- Ozonation pretreatment increased biomass yield and pollutants removal efficiency.
- Removal efficiencies of COD, NH_3-N and TP from the UADEFW increased to 68–99%.
- Lipid content of microalgal biomass increased when UADEFW was pretreated with ozone.

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ABSTRACT

In order to purify various pollutants (3108 mg COD/L, 2120 mg NH_3-N/L) in the undiluted anaerobic digestion effluent of food wastes (UADEFW), ozonation pretreatment was employed to improve pollutants removal by microalgae mutant *Chlorella PY-ZU1* with 15% CO_2. Ozonation pretreatment broke C=C bonds and benzene rings of refractory organics such as unsaturated fatty acids and phenols in UADEFW and degraded them into low-molecular-weight organics such as methanoic acid and methanal, but excessive ozone induced the accumulation of toxic by-products. The microalgal growth rate and biomass yield markedly increased to the peaks of 456 mg/L/d and 4.3 g/L, respectively, when the UADEFW was pretreated with 2 mg-O_3/mg-C of ozone. The removal efficiencies of NH_3-N, TP and COD reached 99%, 99% and 68%, respectively. The lipid and carbohydrate contents of microalgal biomass increased because of the relative lack of nitrogen when microalgae was cultured with 15% CO_2 to purify the UADEFW with ozonation pretreatment.

1. Introduction

Chinese cities produce more than 60 million tons of food wastes annually. From 2011 to 2015, China established five batches comprising 100 cities for pilot-scale recycling and harmless treatment of food wastes. Many large and medium-sized cities, such as Beijing, Shanghai, Guangzhou, Hangzhou and Ningbo, are involved in the pilot of clean disposal of food wastes. Biogas production through anaerobic digestion is a clean method for disposal of food wastes because of its harmlessness, economic benefits and high load-carrying capacity for waste minimization. In 2014, the daily production of food wastes in Zhejiang province reached 6500 tons, and the biogas-producing potential of those food wastes was 150 million m^3 yearly. However, the effluent liquid after anaerobic digestion still contains high concentrations of chemical oxygen demand (COD), nitrogen, phosphorus, and other nutrients (Shin et al., 2015). Thus, efficient purification and recycling of biogas effluent is a hot issue.

Microalgae are applied to wastewater purification because of their high photosynthetic efficiency, rapid growth rate, strong adaptability and ability to utilize inorganic nutrients from wastewater (Beevi and Sukumaran, 2014; Kumar et al., 2010; Park et al., 2009; Van Wagenen et al., 2015).

Microalgae can assimilate and utilize nutrients in wastewater during growth, thereby reducing major pollutants, such as nitrogen and phosphorus, in wastewater, deep processing wastewater, and producing high value-added microalgal biomass which can be further used to produce biodiesel (Levine et al., 2011) or animal feed (Singh et al., 2011). Therefore, microalgae are used to deeply purify wastewater for effective utilization of wastewater and low-cost production of microalgal biomass.

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Singh et al. (2011) utilized 6% (v/v) poultry litter anaerobic digester effluent (maximum TN concentration of 152 mg/L) to cultivate three algal strains *Chlorella minutissima*, *Chlorella sorokiniana* and *Scenedesmus bijuga*. The observed maximum growth rate was 76 mg/L/d, and the nitrogen and phosphorus removal efficiencies were 60% and 80%, respectively. Cai et al. (2013) cultivated *Synechocystis* sp. and *Nannochloropsis salina* by using artificial seawater medium supplemented with 3–24% (v/v) anaerobic digestion effluent containing maximum COD and total nitrogen (TN) concentrations of 639 and 640 mg/L, respectively. A maximum biomass yield of 212 mg/L/d was obtained in semi-continuous cultivation. The removal efficiencies of ammonia nitrogen (NH_3-N), TN, and total phosphorus (TP) were 82.5–100%, 71.2–100% and 83.6–100%, respectively, during 10-day batch cultivations. Wang et al. (2010) investigated the effectiveness of using digested dairy manure as nutrient supplement to cultivate *Chlorella* sp. NH_3-N, TN, TP, and COD removal efficiencies of 100%, 75.7–82.5%, 62.5–74.7%, and 27.4–38.4%, respectively, were observed in differently diluted dairy manure with maximum COD and TN concentrations of 2376 and 223.2 mg/L, respectively. Cheng et al. (2015) cultivated *Chlorella* mutants through continuous introduction of 15% CO_2 to purify undiluted anaerobic digestion effluent of swine manure, with 3745 mg COD/L and 1135 mg TN/L content. The biomass yield and average growth rate of *Chlorella PY-ZU1* reached 4.81 g/L and 601.2 mg/L/d, respectively; moreover, the removal efficiencies of TP, COD and ammonia nitrogen increased to 95%, 79% and 73%, respectively. Kim et al. (2014) utilized ozone to pretreat piggery wastewater and cultivated *Scenedesmus quadricauda*; the maximum biomass yield was 3.4 g/L, and the maximum removal efficiencies of TN and TP were 4% and 29.8%, respectively. Obviously the removal efficiencies of total nitrogen and total phosphorus remained low, and the removal of COD was not investigated.

Few studies to date have used microalgae to purify anaerobic digestion effluent of food wastes. Ji et al. (2015) cultivated *Scenedesmus obliquus* in Bold's Basal Medium supplemented with 0.5–10% (v/v) food wastewater. The maximum removal efficiencies of TN and TP were 75% and 12%, respectively, while the biomass yield of microalgae was 0.41 g/L. However, the anaerobic digestion effluent of food solid wastes was not inoculated into microalgae for purification experiment; moreover, 100% (v/v) wastewater was not used as culture medium to cultivate microalgae, and the purification efficiency of COD in food wastewater was not investigated. Heo et al. (2015) cultivated the marine microalgae *Tetraselmis suecica* in the seawater-diluted food-waste recycling wastewater with 246.7 mg NH_3-N/L and 141.8 mg TP/L. The maximum biomass yield was 2 g/L in fivefold diluted medium in 2.5 L fermenters, whereas the removal efficiencies of NH_4^+ and PO_4^{3-} were 99.0% and 52.3%, respectively. However, the anaerobic digestion effluent of food solid wastes was not inoculated into microalgae for purification experiment; moreover, 100% (v/v) wastewater was not used as culture medium to cultivate microalgae, and the purification efficiency of COD in food wastewater was not investigated. Shin et al. (2015) used primary effluent of municipal wastewater to dilute anaerobically digested food wastewater effluent with three different volume ratios of 1/10, 1/20, and 1/30; Primary effluent of municipal wastewater is the effluent of primary treatment aiming at removing solids by settlement and other physical treatment. *S. bijuga* was then cultivated to purify food wastewater and produce biodiesel. The maximum biomass yield was 1.49 g/L, and the removal efficiencies of COD, TN and TP were 66.4%, 90.7% and 90.5%, respectively. However, the anaerobic digestion effluent of food solid wastes was not inoculated into microalgae for purification experiment; moreover, 100% (v/v) wastewater was not used as culture medium to cultivate microalgae.

High concentrations of undiluted anaerobic digestion effluents can inhibit microalgal growth because high turbidity limits photosynthesis and high ammonia content is toxic to microalgae (Wang et al., 2010). Therefore, most studies used diluted anaerobic digestion effluents. However, dilution of effluents are not economical because they require plenty of fresh water. Although using primary effluent of municipal wastewater and seawater as replacement can save some fresh water resources, this strategy is not feasible for engineering applications because it requires high effluent processing capacity and reactor volume.

In this study, the removal efficiencies of COD, NH_3-N, and TP were increased by pretreating the effluent of undiluted anaerobic digestion food wastes through ozonation before cultivating *Chlorella* mutants under 15% CO_2. Limitations with regard to inhibition of Chlorella growth and pollutants purification by high concentrations of COD and NH_3-N were resolved. Moreover, issues on fresh water wasting and increased quantities of wastewater were avoided.

2. Methods

2.1. UADEFW resources and microalgal strain

Anaerobic digestion effluent of food wastes was collected from the export of an anaerobic fermenter (Hangzhou Environmental Group Co., Ltd.). The fermentation substrate was the solid parts of food wastes collected from household kitchen. The volume of the fermenter was 500 m^3 and the retention time was 25 d. The UADEFW was centrifuged to remove suspended solids. The supernatant was sterilized and its pH was adjusted. The supernatant was then inoculated with *Chlorella* for purification experiment.

The microalgal strain *Chlorella PY-ZU1* was obtained through mutagenesis of *Chlorella pyrenoidosa* with 500 Gy ^{60}Co γ-rays radiation, followed by domestication through continuous introduction of 15% CO_2 (Cheng et al., 2015). The cells of *Chlorella PY-ZU1* were maintained in Brostol's solution (also known as soil extract, SE) containing 0.25 g of $NaNO_3$, 0.075 g of $K_2HPO_4{\cdot}3H_2O$, 0.075 g of $MgSO_4{\cdot}7H_2O$, 0.025 g of $CaCl_2{\cdot}2H_2O$, 0.175 g of KH_2PO_4, 0.025 g of NaCl, 40 mL of soil extract, 0.005 g of $FeCl_3{\cdot}6H_2O$, 1 mL of Fe-EDTA, and 1 mL of A5 solution in 958 mL of de-ionized water (Cheng et al., 2013).

2.2. Pretreatment process of UADEFW and conditions in cultivation

During aeration pretreatment, air was introduced to the bottom of Erlenmeyer flasks containing 1 L of UADEFW through a long steel pipe (180 mm × ϕ 3 mm) by an air pump. The experiment set up comprised different aeration times of 0, 24, 48, 60, and 72 h. During ozonation pretreatment, ozone was generated by an air source ozone generator, and then introduced to the bottom of Erlenmeyer flasks containing 1 L of UADEFW through a long steel pipe (180 mm × ϕ 3 mm) at a constant flow rate of 1 L/min. The experiment set up comprised different ozone dosages of 0, 0.3, 1, 2, 6, 12 mg-O_3/mg-C. UADEFW after aeration and ozonation pretreatment were autoclaved at 112 °C for 30 min, and the initial pH was adjusted to 6.0–6.5 by adding 1 mol/L HCl or 1 mol/L NaOH before inoculation. Then UADEFW was used as culture medium for *Chlorella PY-ZU1*, with an inoculum density of 0.1 g/L. The concentrations of COD, NH3-N, and TP shows negligible change after autoclaving. The effect on pollutants removal by autoclaving at high temperature can be ignored. *Chlorella PY-ZU1* was cultivated in 300 mL column bioreactors under continuous illumination of 6000 lux at 27 °C. UADEFW was continuously aerated with 15% CO_2 at a flow rate of 30 mL/min during the growth period. The control condition included aeration time of 0 h and ozone dosage of 0 mg O_3/mg C.