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Seawater toilet flushing sewage treatment and nutrients recovery by marine bacterial-algal mutualistic system

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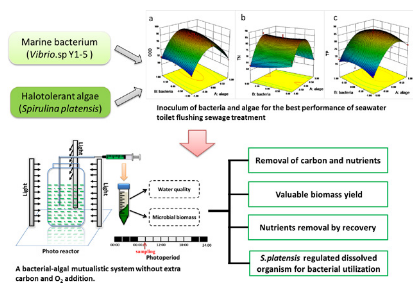
HIGHLIGHTS

- A marine bacterial–microalgal mutualist was used for seawater blackwater treatment.
- Carbon and nutrients simultaneous removal without aeration and extra carbon.
- Nitrogen and phosphorous removal was mainly through assimilation/accumulation.
- Facultative heterotrophic *S.platensis* regulated dissolved organism for the bacteria.
- The biomass obtained from the energy-saving process is a potential biofuel feedstock.

GRAPHICAL ABSTRACT



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ABSTRACT

Seawater toilet flushing sewage with excess eutrophic and high salinity brought a great barrier on the municipal wastewater treatment plants. Nutrients recovery and biomass production as potential biofuel feedstock with less energy consumption will be a key challenge in wastewater treatment. In the optimal inoculation of algae and bacteria, a marine bacterial-algal mutualistic system was established to treat synthetic seawater toilet flushing sewage without extra carbon and O_2 addition. It was showed that 85.5% of total nitrogen (TN) (from 200 mg/L), 91.0% of total phosphorus (TP) (from 40 mg/L) and 98.7% of chemical oxygen demand (COD) (from 1600 mg/L) were removed with 4.28 g/L of biomass yield (biomass productivity 159.3 mg/L/d) containing 16.3% lipid and 62.6% protein, which performance mainly achieved by bacteria during first six days and algae functioned subsequently. Both nitrogen and phosphorus removal of the system were mainly assimilation/accumulation. Algal facultative heterotrophia ensured dissolved organic carbon for bacterial utilization and avoiding excessive organic matter produced. The established algal-bacterial system provided a potential energy-efficient and eco-friendly approach for seawater blackwater treatment and nutrients recovery simultaneously.

1. Introduction

Seawater used for toilet flushing is regarded as an important alternative to alleviate the pressure of fresh water in coastal areas. It has been more than 50 years since the project was successfully operated in Hong Kong (Li et al., 2005), and subsequently

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established in Qingdao since 2004 (Sun et al., 2009). However, high salinity with excess eutrophication of seawater blackwater (BW defined as toilet flushing sewage) have brought a great challenge on the wastewater treatment: (1) Toxicity of high ammonium results in general nitrogen biological communities' collapse and nitrogen removal inefficiently(Huang et al., 2014); (2) High salinity leads to poor performance of denitrifier (Intrasungkha et al., 1999) and PAOs (phosphorous accumulating organisms)(Welles et al., 2014) by causing cell plasmolysis(Vyrides and Stuckey, 2009) and inhibiting the enzyme activity of microorganism; (3) low carbon/nitrogen of blackwater (about 5–7) (Van et al., 2008) may cause inefficient nutrients removal in conventional biological process due to denitrifying OHOs (ordinary heterotrophic organisms) would use organic carbon preferentially over PAOs, which leads to insufficient carbon sources for phosphorus removal (Wang et al., 2015) or additional cost by dosing carbon sources. To address these challenges, establishing a bio-system of halophilic microorganisms with ability of high nutrients removal without external carbon source requirement might be a preferable choice as for time and financial.

Wastewater treatment mainly focused on removing the elements and meeting the discharge standard currently, while nutrients recovery and biomass yield as potential biofuel feedstock with less energy consumption will be a resource-oriented wastewater treatment scheme. In activated sludge or granular sludge treatment, the maximum assimilation of nitrogen was only 15–20% (Fux and Siegrist, 2004) and 20–30% of phosphorous (Jaffer et al., 2002) indicating that substantially all N lost (mainly as N_2 and some greenhouse gas NxO) through denitrification and P deficiency have greatly affected and restricted crop yields worldwide(Cordell et al., 2009). As photosynthetic organism, microalgae-systems have been gradually used in wastewater treatment due to its economical value for microalgae are considered as promising feedstocks for biofuels and provide about 25% of global energy requirements(Rawat et al., 2011), and its potential to assimilate nutrients without additional carbon sources for nitrogen and phosphorous removal(Noüe et al., 1992; Franchino et al., 2013) (e.g. high rate algal ponds (HRAP)). Nevertheless, single algae systems would release excessive alkali and organic carbon via overgrowth result in the deterioration of effluent water qualities(Wang et al., 2010).

In algal-bacterial systems, oxygen and organic carbon produced by microalgal photosynthesis could be used by heterotrophic bacteria while CO_2 released by chemoorganotrophic bacterial respiration could be in turn fixed by algae (Ramanan et al., 2015). A novel shortcut nitrogen removal process of algal-bacterial system achieved 90% nitrogen removal from anaerobically digested swine manure centrate with only 10% by biomass uptaking and the rest through nitration/denitritation(Meng et al., 2015). Effects of photoperiod on nutrient removal and biomass production of a algal-bacterial system was studied in lab-scale photobiorectors treating 10 times diluted municipal wastewater(Chang et al., 2015). *B. licheniformis – C. vulgaris and P. putida - C. vulgaris* systems were selected to treat synthetic municipal wastewater (Liang et al., 2013; Shen et al., 2017), and real municipal wastewater was treated by different inoculum ratios of activated sludge - *C. vulgaris* with microalgae harvesting(Mujtaba and Lee, 2017). However, reported algal-bacterial systems have focused on low-nutrients wastewater treatment of fresh water (Muñoz and Guieysse, 2006) or rich-nitrogen water owing to its high nitrogen assimilation to valuable protein of microalgae, wastewater with high phosphorus and salinity treated by algal-bacterial system have no reported to our knowledge.

Marine *Vibrio* sp.Y1-5 with efficient ability of carbon and nutrients removal from salinity wastewater with high ratio of C/N under aerobic condition, and the special nitrogen metabolic mechanism that nitrate reduction to ammonium for assimilation and high tolerance of ammonium was studied previously (Li et al., 2017). Compare to other microalgae, *Spirulina platensis* was selected in this study due to its high salt-tolerance (Zeng and Vonshak, 1998), facultative heterotrophic that use organic carbon (e.g. glucose, sodium acetate) preferentially with producing more oxygen (Rym et al., 2010) and its superior performance in environmental potential that high ability of nitrogen recovery(Chang et al., 2013) and CO_2 removal(Skjånes et al., 2007) associated with high contents of proteins (70%), polysaccharides, lipid and vitamins(Pulz and Gross, 2004; Madkour et al., 2012).

In this study, a novel marine bacterial-algal system consist of *Vibrio* sp.Y1-5 and *S. platensis* was established to treat synthetic seawater toilet flushing with valuable biomass yield. In this regard, the microorganism's inoculum and removal performance of carbon and nutrients were studied to reveal the interactions and respective function of algae and bacteria in the system. The mechanisms of nutrients and carbon removal, along with algal-bacterial population dynamics were investigated by statistic analysis and facultative heterotrophic test.

2. Material and methods

2.1. Microorganism and experimental wastewater

Studied bacterium *Vibrio*.sp Y1-5 was belongs to the genus of according to NCBI's Bacterial Blast algorithm and phylogenetic relationships(Li et al., 2017). For the followed batch experiments, the bacteria was firstly activated in shake flasks containing experimental wastewater for 36 h on a shaker (200 rpm as DO of 7.8 mg/L) at 25 °C. *S. platensis* was obtained from the Freshwater Algae Bank of the Chinese Academy of Sciences (CAS), inoculum was cultivated in Zarrouk Medium (ZM) (Gòdia et al., 2002) at room temperature under continuous irradiation. To maintain the purity of algae strain, the collected algal suspension was filtered through 0.45 μm cellulose acetate membrane every week and resuspended in fresh ZM for four cycles. The bacterial contamination was examined by microscopic analysis for judging whether the purified process as above is needed again.

Synthetic seawater blackwater was used as experimental wastewater in this study, which parameters referred to toilet flushing sewage reported by Todt(Todt et al., 2015): PO_4-P 40 mg/L (K_2HPO_4 as P source), NH+ 4-N 200 mg/L (NH_4Cl as N source), COD 1600 mg/L(glucose and sodium acetate as C source) and 1 mL/L of trace element solution. The trace elements solution: Na_2EDTA 63.7 g/L, $CaCl_2$ 5.5 g/L, $ZnSO_4 \cdot 7H_2O$ 3.9 g/L, $FeSO_4 \cdot 7H_2O$ 5.00 g/L, $NaMoO_4 \cdot 2H_2O$ 1.00 g/L, $CuSO_4$ 1.01 g/L, $CoCl_2 \cdot 6H_2O$ 1.61 g/L. The pH was adjusted with HCl and NaOH to be 7.5. Salinity of the synthetic wastewater was 3%.

2.2. Experiment set up

A cylindrical glass bottle (1.2 L) with gas-permeable sealing film (to ensure gaseous exchange spontaneously) as the photobioreactors (PBRs) was set up in illumination incubator, which filled with 1 L synthetic wastewater (Fig. 1). To verify and provide a potential approach for energy-saving treatment of seawater application wastewater, all batch experiments were operated in a static status without providing O_2 and CO_2 in light incubator at 28 °C; Light and dark cycles were controlled to 14 h: 10 h(Chang et al., 2013); Bioreactors were ringed on three sides by illumination at the intensity of 90 μmol $m^{-2} s^{-1}$. After washed with deionized water twice, algal cells were incubated into bioreactor systems. Optimal activated bacterial liquid was then inoculated in the bioreactor systems. Initial inoculum concentration of algae and