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Organic carbon recovery and photosynthetic bacteria population in an anaerobic membrane photo-bioreactor treating food processing wastewater

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HIGHLIGHTS

- Photo-bioreactor is used for conversion of organic carbon in wastewater to biomass.
- Photosynthetic bacteria grew in the reactor equipped with infrared transmitting filter.
- Photo-bioreactor was operated with real fluctuating wastewater in long term (>400 d).
- Mixed photosynthetic bacteria culture was characterized by PCR–DGGE method.
- Presence of *Rhodopseudomonas palustris* and diversity of photosynthetic bacteria were revealed.

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ABSTRACT

Purple non-sulfur bacteria (PNSB) were cultivated by food industry wastewater in the anaerobic membrane photo-bioreactor. Organic removal and biomass production and characteristics were accomplished via an explicit examination of the long term performance of the photo-bioreactor fed with real wastewater. With the support of infra-red light transmitting filter, PNSB could survive and maintain in the system even under the continual fluctuations of influent wastewater characteristics. The average BOD and COD removal efficiencies were found at the moderate range of 51% and 58%, respectively. Observed photosynthetic biomass yield was 0.6 g dried solid/g BOD with crude protein content of 0.41 g/g dried solid. Denaturing gradient gel electrophoretic analysis (DGGE) and 16S rDNA sequencing revealed the presence of *Rhodopseudomonas palustris* and significant changes in the photosynthetic bacterial community within the system.

1. Introduction

Purple non-sulfur bacteria (PNSB) are one group of photosynthetic bacteria which widely distributed in the environment and wastewater. Most genera are versatile and subclass to the "*Alphaproteobacteria*" and "Betaproteobacteria" of the proteobacteria. PNSB can participate in the conversion of light energy into chemical energy by a process of anoxygenic photosynthesis (photoheterotrophic). Because they contain photosynthetic pigments; bacteriochlorophylls and carotenoids which can grow in either a phototrophic or a heterotrophic condition depending on the presence or absence of light. PNSB can convert organic wastes to hydrogen gas (Liao et al., 2010) or single cell proteins that reporting rich in proteins and vitamins which can be further utilized (Getha et al., 1998a; Azad et al., 2001; Honda et al., 2006; Chiemchaisri et al., 2007).

Photosynthetic bacteria have been applied to the treatment and utilization of industrial wastewater for their cultivation. Much of the previous researches on photosynthetic biomass production was performed on the cultivation of a single strain or selected species of PNSB, from industrial wastewater using either the test-tube breeding or the batch cultivation method (Prasertsan et al., 1997; Azad et al., 2001; Okubo and Hiraishi, 2007). However, the study on survival and competition of PNSB with other bacteria under natural operating conditions had been still rarely reported. In

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previous studies, it was found that the growth of PNSB cultivation under natural conditions could promoted by installation of an infrared transmitting filter eliminating visible and ultraviolet wave bands thus preventing the algal growth while allowing the near infrared wave band to irradiate into the photo-bioreactors (Honda et al., 2006; Chiemchaisri et al., 2007).

Various types of photosynthetic treatment process for biomass cultivation from industrial wastewater were studied including stabilization ponds (Honda et al., 2006; Chiemchaisri et al., 2007), anaerobic lagoons (Chen et al., 2003), and completely mixed process (Izu et al., 2001) including sequential batch reactor (Chitapornpan et al., 2012). In those treatment processes, poor separation of biomass from water after treatment could lead to the washout of biomass from the system causing instability in their performance or system failure. To overcome this biomass washout problem, membrane technology has been established as a very efficient unit for solid–liquid separation and biomass retention in wastewater treatment process. The use of membrane separation in bioreactor can replace a gravity separation unit and benefit the treatment by completely retaining the biomass within the system while yielding suspended solids free effluent. A higher biomass concentration in membrane incorporated systems thus results in a smaller system footprint and an improvement in treatment efficiencies. With regarding to this improvement, the coupling of the photosynthetic process with membrane separation would enable the reclamation of treated water and energy from recovered biomass.

Although there has been a plethora of previous researches reporting on the application and development of the membrane in the cultivation of PNSB for hydrogen production, few of those previous studies have focused on its application for PNSB biomass cultivation from wastewater (Honda et al., 2006; Chiemchaisri et al., 2007; Kaewsuk et al., 2010; Chitapornpan et al., 2012). The application of a membrane photo-bioreactor to wastewater treatment may lead to a diverse community of naturally growth microorganisms as membrane separation could also retain slow-growing microorganisms within the system. In this respect, a long term investigation into photosynthetic bacterial community is necessary to confirm the microbial population changes within the photo-bioreactor and their effect to the treatment performance. Recent modern molecular techniques developed for identification of predominant microbial species in a mixed culture community can overcome the pure isolation limitations of the traditional approach using liquid enrichment bias. More specifically, polymerase chain reaction (PCR) primers targeting 16S rDNA genes followed by a denaturing gradient gel electrophoresis (DGGE) has been utilized as an efficient tool for the characterization of a microbial community in a mixed culture environment. The effectiveness of this approach has been illustrated in the detection even small percentages of microorganisms within a whole microbial community.

The purpose of this study was to investigate the cultivation and production of photosynthetic biomass in an anaerobic membrane photo-bioreactor (*AnMBR*) treating food processing wastewater. The research of organic removal and biomass production and characteristics was accomplished via an explicit examination of the long term performance of a photo-bioreactor fed with real wastewater, thus ensuring authentic fluctuating characteristic conditions. PCR–DGGE analysis was used for monitoring of the microbial community and diversity of PNSB during the operation of *AnMBR* at six sampling times over the operating period. This enabled comparisons to be made on the microbial community with regards to predominant photosynthetic bacterial species and the diversity profile when the system was operated with and without biomass withdrawal.

2. Methods

2.1. Laboratory scale photo-bioreactor (AnMBR)

The schematic diagram of anaerobic membrane photobioreactor is shown in Fig. 1. A glass vessel with 8 L of working volume was used as a laboratory scale membrane photo-bioreactor. The experiment set-up consisted of a wastewater storage tank, a feed peristatic pump, a photo-bioreactor with gas collection system, an artificial light illumination system, a mixing system and a speed control system. Wastewater from a storage tank was fed once a day using peristatic pump (DELTA model VFD-L, Italy) affixed with pump head (Masterflex Easy load 3 model 77800-50, Japan). An additional peristatic pump was used as for effluent (membrane permeate) extraction from the photo-bioreactor. A completely mixing condition in photo-bioreactor was established and maintained by using a mixing system with a speed control 4 W motor (Oriental Motor Co., Ltd., Japan) and a speed control system. A hollow fiber membrane module made of polyethylene (ECONITY Co., Ltd., Korea) with surface area of 0.04 m^2 and nominal pore size of 0.4 μm was directly immersed in the photo-bioreactor. An infrared IR-2880 transmitting filter, (Tsutsunaka Plastic Industry Co., Ltd., Japan), was arranged over the lighting surface of the photo-bioreactor to completely eliminate visible and ultraviolet wave bands while allowing the near infrared wave band to irradiate through the photo-bioreactors. The observed infrared light intensities at the front surface and within the photo-bioreactors were found to be 270 and 6.18 W/m^2, respectively.

2.2. Food processing wastewater characteristic and analytical methods

The wastewater used in the experiment was the actual wastewater from a food processing factory in Thailand and was collected from the equalization tank using the composite sampling method. Upon the collection, the wastewater samples were refrigerated at 4 °C and stored until they were utilized. The typical characteristics of discharged wastewater varied significantly depending on the time and production schedule of the factory. The influent wastewater characteristics during the study period are shown in Table 1. It was found that pH of fresh wastewater varied from 3.9 to 5.7, while biochemical oxygen demand (BOD) and chemical oxygen demand (COD) ranged from 300 to 5150 mg/l and 700 to 9750 mg/l, respectively. Additionally, total kjeldahl nitrogen (TKN) ranged from 1 to 35 mg/l and sulfate content varied from 10 to 485 mg/l. The average protein content of the food processing wastewater was determined as 78 mg/l.

During the experiment, influent and effluent samples of the photo-bioreactor were collected every 5 days and analyzed for chemical characterization according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1998). The pH and Oxidation Reduction Potential (ORP) of the system were monitored by online pH and ORP controllers. Organic compounds were determined in terms of Biochemical Oxygen Demand (BOD) using a 5-day BOD method, Chemical Oxygen Demand (COD) using closed-reflux method, suspended solids (SS) was determined by gravimetric method. Ammonia nitrogen (NH_3–N) and total kjeldahl nitrogen (TKN) were analyzed by distillation and macro-kjeldahl methods. Total organic carbon (TOC) was quantified using high temperature combustion with TOC analyzer (Shimatzu VCSH-SSM 5000A, Japan).

2.3. Inoculums and reactor operation

The inoculants obtained for this study were samples collecting from the wastewater treatment pond of the sample factory. During