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Using pig manure to promote fermentation of sugarcane molasses alcohol wastewater and its effects on microbial community structure

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HIGHLIGHTS

- PM is an ideal candidate for co-digestion with MAW.
- The optimal COD ratio of PM:MAW for anaerobic digestion is 1.0:1.5.
- The structure and composition of bacterial communities varied in the early and late stage.
- The type of main OTUs did not differ much among tanks with different ratios of PM to MAW, only the proportion is different.

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ABSTRACT

Molasses alcohol wastewater (MAW) is difficult to be bio-treated and converted into biogas. In this study, MAW mixed with pig manure (PM) in different ratios was co-digested. Biogas production, chemical oxygen demand (COD) removal and the structure of microbial communities were monitored in the process. Our results showed that under the optimal COD ratio of PM:MAW (1.0:1.5), $COD_{removal}$ and biogas yield were the highest. And in fermentation tanks with different PM to MAW ratios, the structure and composition of bacterial communities varied in the early and late stage. Furthermore, the type of main bacterial operational taxonomic units (OTUs) have no differences, yet the relative abundance of OTUs varied. The current research showed that there was a good potential to the use of PM as a co-digested material to anaerobic treatment of MAW and provided references for further improving bio-treatment of MAW.

1. Introduction

During the manufacturing of cane sugar, a large amount of molasses is produced. Sugar refineries typically use molasses to produce alcohol, and for 1 ton of alcohol produced, 13–15 tons of wastewater (i.e., MAW) are typically generated. MAW contains approximately 88% molasses (Jain et al., 2002), and its components vary with different alcohol production techniques and operation methods. MAW typically has relatively high COD and biochemical oxygen demand (BOD), as well as elevated phosphate, sulfate, and organic carbon content. In addition, MAW is highly corrosive, acidic, with a pH value 3.5–5, and contains a relatively high content of solids (Wilkie et al., 2000; Cheng et al., 2000). MAW also contains 2% melanoidin (a dark brown pigment) with complex components, which have not been fully elucidated (Kalavathi et al., 2001; Rivero-Peĭrez et al., 2002). Melanoidin has antioxidant effects, and is toxic to microbes present in MAW treatment (Sirianuntapiboon et al., 2004). Besides melanoidin, there are other colorants in MAW, such as melanin, caramel and phenolics (Satyawali and Balakrishnan, 2008). Direct emissions of MAW can cause serious pollution to the environment, leading to problems such as hypoxia and deterioration of water quality in discharge streams. The melanin contained in MAW can also block sunlight, interfere with photosynthesis of aquatic organisms, and thus affect their activities (Fitzgibbon et al., 1998). Studies on the treatment of MAW have been carried out for many years. A range of biological and physico-chemical methods have been investigated, which collectively suggest microbial fermentation of MAW to biogas as a promising approach to effectively treat this waste stream (Satyawali and

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Balakrishnan, 2008). But a comprehensive treatment scheme for MAW leading to effective removal of both organics and color is not available. Although biological methods especially anaerobic treatment have been employed extensively as the first treatment step on a variety of industrial wastewater, there is no successful researches on anaerobic treatment for MAW. The treatment processes of MAW with microbiorganisms have been rarely reported recently. Jain and his co-workers found that the $COD_{removal}$ of MAW is up to 66–81% with *Xanthomonas fragariae*, *Bacillus megaterium*, *Bacillus cereus* and additional carbon and nitrogen sources (Jain et al., 2002). Sirianuntapiboon and co-workers demonstrated that acetogenic bacteria No.BP103 can reduce COD of MAW to 35.5–71.2% in the medium containing glucose 3.0%, yeast extract 0.5%, KH_2PO_4 0.1% and $MgSO_4{\cdot}7H_2O$ 0.05% (Sirianuntapiboon, 2004). Kumar and Chandra reported that *Bacillus thuringiensis, Bacillus brevis, Bacillus sp*. decrease COD of MAW to 41–63% (Kumar and Chandra, 2006). What is more, the treatment system with multilevel procedures, such as combined anaerobic digestion and ultraviolet photodegradation and consisting of an acidification reactor, an upflow staged sludge bed (USSB) reactor, an upflow anaerobic sludge blanket reactor and an aerobic trickling filter were studied (Apollo et al., 2013; Onodera et al., 2013), which appears a high cost. There is thus an urgent need to develop integrated treatment processes to treat MAW.

The development of industrialization and urbanization, and population growth has simultaneously increased environmental pollution and the demand for energy. Anaerobic fermentation of wastewater and residues to produce methane have been both extensively studied and widely applied. Technologies in anaerobic fermentation of domestic wastewater, anaerobic fermentation of a variety of animal manures, and anaerobic fermentation of industrial wastewater and wastes have achieved satisfactory economic and environmental benefits (Schomaker et al., 2000). However, components of the organic carbon present in MAW are difficult to process and reports of successful fermentation of MAW to methane are lacking. One challenge of MAW fermentation lies in the imbalance of carbon to nitrogen ratios (up to above 120). In addition, MAW is rich in sulfate, sulfate-reducing bacteria compete with methanogens during the anaerobic bio-digestion process (Hilton and Archer, 1988). Anaerobic co-digestion of animal manures and organic wastes or wastewater is currently undergoing new developments and rapid expansion, and there have been many reports on successful application of this technology that produces methane, e.g., that by Neves et al. (Mshandete et al., 2004). In the present study, co-digestion of PM and MAW was performed under laboratory conditions to explore optimal ratio of PM to MAW, which in effect modulates the C/N ratio, for the optimal anaerobic conversion to biogas. In addition, next generation sequencing methods were applied to characterize the structure and composition of bacterial communities in early and late stages of fermentation. A better understanding of microbial activities and community structures should aid in evaluation and control of process operation, and is significant for investigation of degradation mechanism. The results of this study provide unique insight into the interplay between nutrient composition, bacterial community structure, and gas production during the anaerobic treatment of MAW.

2. Methods

2.1. Establishment of methane fermentation device

Brown jars of 5.5 L with a working volume of 4 L were used as fermentation tanks. Each jar was sealed with a rubber stopper. In the rubber stopper, there was a sampling port and an air duct, the air tightness of which was ensured. Starting fermentation liquid was obtained from IC reactor that was in the process of treating bagasse spray wastewater from Guangxi Guitang (Group) Co. Ltd., and MAW was wastewater produced from alcohol production of cane molasses (concentrated 6 times) from the same company. Wastewater was subjected to 10-fold dilution prior to initiation of preliminary fermentation experiments. Based on the preliminary results, COD ratios of PM to MAW were set as H1 (1.0:0.0), H2 (1.0:1.0), H3 (1.0:1.5) and H4 (1.0:2.0), total COD was 5000 mg/mL, respectively. Three replicate reactors were set up for each PM to MAW ratio. The starting fermentation liquid was diluted to 20% before use and fermentation was allowed to proceed at 37 °C for 81 days.

2.2. Sample collection

Samples were collected on the 2nd (early stage) and 81st (late stage) day of fermentation. A, B, C and D were numbers of samples collected from tanks H1, H2, H3 and H4 on the 2nd day, respectively, and SA, SB, SC and SD were numbers of samples collected from tanks H1, H2, H3 and H4 on the 81st day, respectively. Prior to sample collection, fermentation tanks were shaken to homogenous contents and fermentation liquid in the middle of the tank was collected. About 100 g samples were collected. Part of the sample was stored in −80 °C freezer for further analysis.

2.3. Analysis methods

Biogas was collected during the fermentation process using the saturated brine displacement method. The volume of the produced biogas was determined by measuring the volume of displaced saturated brine. Gas chromatography was applied to quantify the methane content in biogas produced during the course of the fermentation. A Shimadzu GC-14C (Shimadzn Co. Ltd., Japan) gas chromatograph was used employing a 0.3 mm × 2 m capillary column and a stationary phase Porapak Q (80–100 mesh) column and FID detector. Inlet temperature was set as 100 °C, column temperature was set to 60 °C, and the detector temperature was set to 100 °C. The hydrogen gas pressure was set as 40 Kpa, the pressure of the nitrogen carrier gas was set to 65 Kpa, and the pressure of oxygen was maintained at 30 Kpa. Manual injection was applied, and the injection volume was 3 μL. The concentration of methane was determined by comparison of peak areas determined from a standard sample (99.9% methane). COD was measured using potassium dichromate method (Andrew et al., 2005).

2.4. DNA extraction and PCR amplification

Extraction of genomic DNA from the samples was performed using the FastDNA® Spin Kit for Soil (Q-BIOgene, Carlsbad, USA) according to the manufacturer's instructions. PCR primers F357 and R518 (Muyzer et al., 1993) were used for amplification of the V3 region of bacterial 16S rRNA genes. PCR conditions were: 10 × PCR buffer, 0.25 mmol/L dNTPs, primers 0.4 μmol/L each, and 1U Ex Taq polymerase (TaKaRa Company, Japan), sample DNA template 10 ng, and molecular grade water added to make a total volume of 25 μL. PCR amplification conditions were: predenaturation 95 °C for 5 min followed by 25 cycles of stepdown PCR at 94 °C for 45 s, 65–55 °C 30 s (annealing temperature dropped from 65 to 56 °C, reduced by 1 °C every two cycles and 5 cycles for 55 °C at the end), 72 °C 1 min, followed by a final extension at 72 °C for 8 min. The size (~200 base pairs) and concentration of the PCR product was verified and quantified agarose gel electrophoresis. In order to remove false positive products, the above PCR product was recovered and purified to serve as template, and reconditioning PCR was performed as the second amplification, in order to obtain PCR product with higher specificity. Reconditioning PCR system contained: 10 × PCR buffer,