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Mesophilic bio-liquefaction of lincomycin manufacturing biowaste: The influence of total solid content and inoculum to substrate ratio

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ABSTRACT

As the potential source of environmental antibiotic pollution, large amount of biowaste generated from antibiotic fermentation manufacture highlights its beneficial utilization for resource and nutrients recovery; which is suitable for anaerobic bio-liquefaction (hydrolysis and acidification). However, its high solid content and residual antibiotics are a cause for concern. In this study, batch anaerobic experiments were conducted to evaluate the bio-liquefaction performance of lincomycin manufacturing biowaste at different total solid content (TS) and the ratios of seeding granular sludge (inoculum) to substrate (ISR). The results showed that lincomycin manufacturing biowaste had high bio-liquefaction efficiency, with the highest 10d volatile solid (VS) degradation rate being approximately 38%. The bio-liquefaction efficiency of protein could reach 100%. Predicted by response surface methodology, 1580 mg/L gVS volatile fatty acids (VFAs) and 497 mg/L gVS ammonium were obtained at a TS of 10%, ISR of 3 and solid retention time of 9.5 d, which is the optimum scenario.

1. Introduction

Environmental antibiotics have been of increased concern in recent years (Kummerer, 2009a,b). Solid waste and wastewater generated from antibiotic manufacturing processes are among the major sources of antibiotic pollutants in the environment (Phillips et al., 2010). In 2008, over 70 categories of antibiotics were manufactured in China, which comprised 20–30% of the world yield. Starch, maize slurry, soybean powder, glucose, peptone, beef extract, yeast extract and nutrient salts are often applied as the substrate in antibiotic manufacturing processes due to their easy utilization by microorganisms (Elander, 2003). However, only a portion of the substrates are converted into antibiotics; therefore, a great deal of manufacturing wastes is produced. As a centralized source, these wastes are primarily composed of the original substrates and the cell filaments. As a result, these wastes have a high biomass organic content, especially proteins, which is favorable for resource and nutrients recovery. On the other hand, this waste contains un-extracted antibiotics, which led to the recent prohibition of the use of antibiotic biowastes as animal feedstock in China. Incineration is the main disposal method for this category of antibiotic biowaste. Although this technique is expensive, only a few alternatives have been considered in lab or pilot trials, including mixing and co-treatment with the wastewater or chemical inactivation (Budinova et al., 2008; McKinney, 1962).

Anaerobic digestion is a potential alternative for recovery of the biomass resource in antibiotic wastes. Some studies have evaluated the degradation of antibiotics from wastewater during anaerobic digestion (Gartiser et al., 2007; Mohring et al., 2009), as well as the influence of wastewater antibiotics on anaerobic digestion (Amin et al., 2006). However, to the best of our knowledge, few studies have focused on the anaerobic treatment of antibiotic manufacturing biowaste. In addition to the effect of antibiotics on the anaerobic process, the accumulation of volatile fatty acids (VFAs) and ammonia need to be considered due to the high total solid content of antibiotic manufacturing biowaste.

Anaerobic bio-liquefaction is the anaerobic process in which large amounts of solid organic waste are hydrolyzed into soluble metabolic products in the liquid phase. Correspondingly, bio-liquefaction product is a type of liquid mixture composed of highly concentrated VFAs produced by the degradation of organics, ammonia from protein degradation, and other undegraded soluble polymers. In addition to utilization by methanogens, bio-liquefaction products have many other potential uses, such as the carbon source of wastewater facilities (Soares et al., 2010), the substrate of bio-fuel cells (Chang et al., 2010), materials for polyhydroxyalkanoates (PHAs) (Castilho et al., 2009) production. These downstream source recovery processes require increased and more highly concentrated VFAs and ammonia production from the bio-liquefaction process, which needs to be optimized.

Total solid content (TS) and inoculum to substrate ratio (ISR) are two important parameters involved in anaerobic treatment. High TS can enhance the organic loading rate (OLR) of the anaerobic

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Nomenclature

TS	total solid content	RSM	response surface methodology
ISR	inoculum to solid ratio	SRT	solid retention time
VS	volatile solid		
VFAs	volatile fatty acids		

process by increasing the concentration of VFAs and ammonia, and consequently reducing the reactor volume. Unfortunately, when large amounts of VFAs and ammonia are released into liquors, the anaerobic bio-liquefaction process becomes unstable due to the decrease in pH and accumulation of the liquefaction products (Veeken et al., 2000). The potential inhibitory effect of accumulated liquefaction products on anaerobic processes will be aggravated as a result of mass transfer limitation caused by high TS (Liu and Ghosh, 1997). Inoculum is the biomass that added into the reactor in order to stimulate the anaerobic bio-liquefaction process. The ratio of inoculum to substrate represents the ratio of added biomass amount to substrate amount, calculating by volatile solid (VS). A higher ISR can shorten the acclimation period and avoid organics overload. Conversely, adding larger amounts of inoculum will decrease the effective space of the reactor, resulting in a corresponding decrease in the OLR of the reactor. Although the effects of TS and ISR on methane production have been broadly evaluated, few studies have been conducted to investigate their impact on the bio-liquefaction process.

In this study, batch bio-liquefactions were conducted at different TS and ISR under mesophilic conditions in order to obtain the maximum VFAs and ammonia production, in attempt to reduce and utilize lincomycin manufacturing biowaste. RSM methodology was used to optimize bio-liquefaction process.

2. Methods

2.1. Materials

Lincomycin manufacturing biowaste, with a diameter lower than 2 mm, was obtained from a pharmaceutical company in Henan Province, China. The characteristics of lincomycin manufacturing biowaste are shown in Table 1. The seeding granular sludge was obtained from a UASB wastewater facility with liquid internal recirculation located in a paper mill. The microorganisms in sludge are usually acclimated in an environment of high lignin and alkaline concentration (Kortekaas et al., 1998), which mainly consist of *Clostridium* (Suihko et al., 2005) After collected from the UASB reactor, the granular sludge was incubated with an organic loading of 2 g-COD L^{-1} d^{-1} of glucose and sodium acetate mixture (the COD ratio of glucose to sodium acetate was 2:1) in a semi-continuous stirred reactor for more than 10 d. The TS of the anaerobic granular sludge is 12.8%, with 89.6% VS in TS.

2.2. Batch bio-liquefaction experiments

Bio-liquefaction tests were conducted in 500 mL serum bottles sealed with butyrate rubber septa and an aluminum screw cover. After mixing the anaerobic granular sludge, buffer solution and lincomycin biowaste, deionized water was added to adjust the TS to 3%, 5%, 8%, and 10% and the ISR to 0.5, 1, 2, and 3 (on the VS basis of the inoculum to the substrate). The detailed quantities of the aforementioned mixtures in each scenario are shown in Supplementary Table 1. The composition of the buffer solution is presented in Each vessel was purged with 1 mL/s N_2 to ensure an anaerobic environment and then incubated in 35 ± 2 °C. Liquid samples were taken every 2 d by syringe to measure the VFAs and ammonia concentrations, as well as the total organic carbon (TOC) and pH. Gas samples were collected daily using a gas bag to determine the methane concentration and biogas volume. At the end of the 10 d incubation period, solid samples were collected to determine the TS, VS, and organic element composition. Each data point was tested in duplicate.

2.3. Bio-degradability test of lincomycin manufacturing biowaste

The scenario of a TS of 3% and an ISR of 3 was evaluated to estimate the methane yield and bio-degradability of lincomycin manufacturing biowaste (Supplementary Table 2). Duplicate aliquots containing a corresponding mixture were incubated using the same method as Section 2.2, but with an incubation period of 45 d. The VS content of the mixture at 45 d was measured to calculate the bio-degradability of the biowaste.

2.4. Test methods

Methane concentration was measured by gas chromatography (GC112A, Shanghai Jingke Co. Ltd., China). Gas volume was measured by the water displacement method. pH was determined using a pH meter (PS2-F, Shanghai Leici Co. Ltd., China). TOC was measured using a TN_b/TC multi N/C 3000 analyzer (JenaAG Co. Ltd., Germany). VFAs and lactate concentration were measured using a LC-20AD HPLC (Shimadzu Co. Ltd., Japan). Elemental constitutions of the solids were determined before and after anaerobic incubation with an elemental analyzer (VarioEL Co. Ltd., Germany). Ammonium concentration, TS and VS were determined by the corresponding standard methods (American Public Health Association, 1998).

Lincomycin was extracted as USA method 1694 (USEPA, 2007), and determined by HPLC (HPLC1200, Agilent Co. Ltd., USA). Using C18 reverse phase column, the mobile phase was a mixture solution of phosphate buffer (dilute 13.5 mL phosphate acid to 1000 mL, and adjust the pH to 6.0 using $NH_3{\cdot}H_2O$), ethanol and acetonitrile at the ratio of 700:150:150. The flow rate was 1 mL/min, lasting for 25 min. The samples were detected in UV detector at the wavelength of 210 nm.

Table 1
Physiochemical characteristic of lincomycin manufacturing biowaste.

Component	Proportion (%)	Component	Proportion (%)	Component	Concentration (g/kg)
TS	18.1 ± 0.94	Protein	26.7	Mg	2.1
VS (by TS)	86.1 ± 0.10	Cellulose	15.4	Ca	37.3
C (in VS)	43.1 ± 0.31	Carbohydrate	12.1	Na	5.1
N (in VS)	5.0 ± 0.05	Lipid	10.3	K	2.5
H (in VS)	7.3 ± 0.07	Nitrogen-free extract	35.5	P	10.0