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Klebsiella pneumoniae sp. LZU10 degrades oil in food waste and enhances methane production from co-digestion of food waste and straw

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ABSTRACT

High content of lipid in food waste (FW) often causes inhibition on anaerobic digestion (AD) and methane production. To facilitate the transformation of oily FW to biogas, a bacterial strain *Klebsiella pneumoniae* sp. LZU10 (hereafter LZU10) which could degrade waste cooking oil to soluble chemical oxygen demand (sCOD) was isolated from restaurant FW-contaminated soil. The strain also demonstrated high resistance to heavy metals, whose level were found elevated in FW. Thus LZU10 was used to pretreat FW prior to anaerobic co-digestion (coAD) with straw. Results showed that after pretreatment by LZU10 the lipid content decreased from 59.6% to 39.5%, and sCOD increased by 25.03%. The methane production and methane recovery rate were increased by 41% and 58%, respectively, after pretreatment with LZU10, compared with those of untreated FW. The present study suggests that pretreatment of lipid-rich FW with lipolytic bacteria is a promising solution to reduce lipid inhibition and enhance methane production.

1. Introduction

Food waste (FW) is a major form of municipal solid waste. In China, FW is generated at a rate of 92.4 million tons per year, which accounts for about 35% of global FW (Girotto et al., 2015). FW is rich in carbohydrates, proteins, celluloses, and lipids (Gustavsson et al., 2011). Thus, untreated FW can generate putridity and toxic gases that could cause environmental pollution and endanger human health (Chen et al., 2015). Traditional treatment methods of FW encompass landfilling, incineration, and feeding to animals. However, landfilling has been banned because of greenhouse gas emissions and landfill site limitations (Zhang et al., 2014a). Incineration is constrained because of the high water content (>85%) in FW and the possibility of dioxin generation (Shibamoto et al., 2007). FW used directly as animal feed would increase the risk of disease propagation due to the shorter food chain (Zhang et al., 2014a). Hence, efficient and ecofriendly methods to treat FW need to be explored.

Anaerobic Digestion (AD) has proved to be an effective technology in converting biodegradable wastes, such as agricultural wastes, yard wastes, and livestock manure, to clean energy (Li et al., 2014). FW, with its 80–97% volatile solids to total solids ratio (VS/TS) and high moisture content, is an attractive substrate for AD (Pham et al., 2015). Chinese FW often contains high lipid content (>20% dry basis) because of traditional cooking methods (Liu et al., 2012). Lipid has a higher biochemical methane production potential (1.425 L/g) than carbohydrates (0.830 L/g) and proteins (0.921 L/g), however, due to the low solubility and poor degradation of lipid, a high proportion of lipid in FW would cause inhibition on the biogas production with lag phase occurrence (Rasit et al., 2015). Researchers also found that accumulated long-chain fatty acids (LCFAs), the main intermediate of lipid degradation, were the primary inhibitor because they could be adsorbed onto the surface of anaerobic microorganisms and block mass transfer (Pereira et al.,

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2005). To deal with the above problems caused by high lipid concentration, oil removal and means to increase the solubility of oil, including physical, chemical, biological and combinative approaches, have been applied pre- or during AD. For example, ultrasound, ozonation and thermal treatment of FW were proved to enhance AD and biomethane production (Ariunbaatar et al., 2014; Naran et al., 2016). High voltage pulse discharge pretreatment increased the cumulative methane yield by 134% (Zou et al., 2016).These procedures demonstrated utilities in increasing the solubilisation of FW, but required either complicated manipulation or extra energy input. Utilization of lipases to hydrolyze lipids was currently restricted at lab-scale owing to its limited oil-degrading ability and high cost (Meng et al., 2015). In addition, reducing the LCFA concentration by addition of LCFA adsorbents such as calcium salts was proved effective, however, extra chemicals such as chloroform and methanol were required and too much precipitate would conglomerate and inhibit methane production (Dereli et al., 2014). More importantly, removal of LCFA from FW would cause reduction of the total methane yield due to the loss of lipid.

Pretreatment of FW with oil-degrading bacteria, which could degrade lipids into glycerol and acetic acids with the benefits of simplicity and low capital investment, provided an alternative way to pretreat lipid-rich wastes. Gumisiriza et al. (2009) reported that pretreatment of fish processing wastewater using two lipolytic bacterial strains enhanced the AD process and hence methane production. Peng et al. (2014) utilized a *Bacillus* strain to pre-treat oily wastewater and the methane yield was significantly augmented. Some bacteria, such as *Pseudomonas*, *Bacillus*, *Klebsiella*, and *Acinetobacter*, can degrade lipids, suggesting the application of these bacteria in the pretreatment of lipid-rich wastes (Kis et al., 2015; Mongkolthanaruk and Dharmsthiti, 2002; Odeyemi et al., 2013). Nonetheless, bacterial pretreatment thus far has seldom been applied in the AD of FW, partially because the waste cooking oil (WCO) in the FW is more difficult to be degraded by bacteria than refined oils (data not shown). On the other hand, the organics-degrading bacteria are normally sensitive to heavy metals whose concentrations were often found elevated in foods, and thus might not perform very well during pretreatment and AD process (Huang et al., 2016). Heavy metal pollution of soils is a serious concern worldwide. Previous reports found that in some contaminated areas, increased concentrations of heavy metals, e.g. Cd, Pb, Cu, Zn, and Ni, are often detected in various foodstuffs, which might lead to higher levels of residual heavy metals in FW (Zheng et al., 2013). Hence, in order to perform bacterial pretreatment of FW, it is of importance to screen bacterial strains that could not only degrade WCO, but also tolerate heavy metals in FW.

The objectives of this study were: (1) to screen strains from FW-polluted soil that could efficiently degrade various lipids, including edible oils and WCO; (2) to evaluate the heavy metal resistance of the screened strains and compare with existing heavy metal-resistant strains; (3) to pretreat FW with the oil-degrading strains and monitor the subsequent methane yield on a batch model. The present study could provide a potentially economic and simple way to reduce lipid inhibition on AD of FW and obtain an augmented methane production by pretreating oil-rich FW with oil-degrading bacteria.

2. Materials and methods

2.1. Enrichment and isolation of microorganisms that degrade WCO

FW was collected from the canteen of Lanzhou University, which represented typical FW generated in northwest China. FW was homogenized with a fruit mixer and stored at −20 °C. WCO was separated from FW in a separatory funnel. Soil samples were collected from a restaurant FW-contaminated site in Lanzhou, China. Modified M9 medium at pH 7.0 used for screening was composed of 10 mL WCO, 17.09 g $Na_2HPO_4{\cdot}12H_2O$, 3 g KH_2PO_4, 0.5 g NaCl, 1 g NH_4Cl, and 0.49 g $MgSO_4{\cdot}7H_2O$ per liter. WCO degraders were enriched using the following procedures. To 9 mL 0.85 mM NaCl solution, 1 g of soil was added, and the mixture was cultivated at 25 °C for 4 h. Then, 100 μL of the suspension was transferred into 5 mL modified M9 medium and incubated at 180 rpm and 37 °C (Crystal Incubator Shaker, IS-RSV3, USA). 50 μL of this enrichment culture was transferred to 5 mL fresh modified M9 medium, and incubated at 180 rpm and 37 °C. Liquid culture was then spread onto a solid LB medium. Single colonies were collected and reselected for in modified M9 medium containing WCO.

2.2. Microbial identification and phylogenetic analysis

Gram staining was performed using the standard method. Genomic DNA was extracted using MiniBEST Bacterial Genomic DNA Extraction Kit (Takara, Dalian, China). The 16S rRNA gene was amplified using PrimeSTAR HS DNA polymerase (Takara, Dalian, China) and the universal primers 27 F (5′GGTTACCTT GTTACGACTT3′) and 1492 R (5′AGAGTTTGATCMTGGCTCAG3′). PCR was conducted with pre-denaturation at 98 °C for 5 min, and thermal cycling parameters were 98 °C for 30 s, 55 °C for 45 s, and 72 °C for 90 s, followed by 72 °C for 10 min. PCR products were purified using a Gel Extraction Kit (OMEGA, China) and sequenced at ShangHai Majorbio Bio-pharm Technology Co. Ltd (Shanghai, China). Subsequently, the sequences were compared to those in the NCBI database (http://www.ncbi.nlm.nih.gov/). A phylogenetic tree was generated using the maximum likelihood method by MEGA5.

2.3. Lipid degradation experiment

LCFAs (linolenic acid, stearic acid, oleic acid, palmitic acid, linodeic acid) were purchased from Shyuanye Reagent (Shanghai, China). Edible oils (linseed oil, colza oil, sesame oil, blend oil, and lard) were purchased from local grocery store. The blend oil was a mixture of soybean oil, canola oil, sunflower oil, corn oil, peanut oil etc. WCOs were collected from Szechuan restaurant, hot pot restaurant, barbecue restaurant, and university cafeteria in Lanzhou China. Oils were added into flasks containing 100 mL of modified M9 medium to a final concentration of 1% (v/v %), and bacteria were inoculated followed by incubation at 30 °C and 180 rpm for 48 h. After incubation, the lipid content was analyzed using chloroform-methanol extraction method according to Sugimori and Utsue (2012). The degradation rates of different oils were calculated by comparing the content of residual oils in inoculated flasks to that in uninoculated flasks. All experiments were carried out in triplicate.

2.4. Gas chromatogram-mass spectrometry (GC-MS) analysis and thin-layer chromatography (TLC) analysis

1 mL of 12-h culture of LZU10 was added to 100 mL modified M9 medium with 1% WCO, and the mixture was cultivated at 30 °C, 180 rpm for 48 h. The culture was centrifuged and the supernatant(2 mL) was transferred to a 16 × 125 mm screw-cap Pyrex tube. 2 mL of BF_3-MeOH (14%, wt/vol) was added and the tubes were incubated in a 55 °C water bath for 1.5 h with vigorous hand-shaking for 5 s every 20 min. 2 mL of a saturated solution of $NaHCO_3$ and 3 mL of hexane were added then vortex-mixed (O'Fallon et al., 2007). After centrifugation the hexane layer (1 μL) containing the fatty acid methyl esters was injected in a Thermo Trace DSQ II GC-MS with a split ratio of 20:1. Meanwhile, modified