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High-efficiency bioconversion of kitchen garbage to biobutanol using an enzymatic cocktail procedure

Hua Chen[a,1], Hong Shen[a,*], HaiFeng Su[b,1], HongZhen Chen[b], FuRong Tan[d,*], JiaFu Lin[c,*]

[a] School of Resource and Environment, Southwest University, Beibei, Chongqing 400714, PR China
[b] Chongqing Institute of Green and Interligent Technology, Chinese Academy of Science, 266, Fangzheng Avenue, Shuitu High-tech Park, Beibei, Chongqing 400714, PR China
[c] Antibiotics Research and Re-evaluation Key Laboratory of Sichuan Province, Sichuan Industrial Institute of Antibiotics, Chengdu University, Chengdu, PR China
[d] Biogas Institute of Ministry of Agriculture, Chengdu 610041, Sichuan, PR China

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ABSTRACT

Research on methods to produce biobutanol production from kitchen garbage (KG) as a potential substrate is thus far lacking. Here, the effect of various enzymatic hydrolysis procedures (EHP) was first tested using different enzyme cocktails, on the decomposition of KG. The efficiency of *Clostridium acetobutylicum*-mediated biobutanol production was then measured using two modes: separate hydrolysis and fermentation (SHF) and simultaneous saccharification fermentation (SSF) in the condition of adjusting pH. The optimal results were obtained using (1) an enzymatic hydrolysis cocktail procedure (EHC5), (2) use of the SSF approach and (3) pH control. This approach results in a biobutanol production of 16.37 g/L and total solvent concentration of 32.96 g/L. Compared to experiments that use pure glucose as a substrate, our results show that KG is a promising feedstock for biobutanol production. The results demonstrate the feasibility of this waste source for an industrial application via the EHP.

1. Introduction

Kitchen garbage (KG) is the most important component of food waste and a significant source of urban pollution (Wang et al., 2002). Recently, there has been a rapid rise in the generation of KG across the globe. Because KG increases environmental pollution, and can spread diseases that are harmful to human health, how to effectively and safely dispose of KG is of great concern to the China government. In recent years, legislative restrictions concerning waste treatment, including KG disposal and environmental protection, have forced KG producers to create new solutions for KG disposal.

In most countries, KG is currently deposited in landfills or incinerated together with other combustible municipal garbage as a supplemental source of electricity (Cheng, 2015). However, these two approaches are facing increasing economic and environmental pressures. KG typically contains high levels of moisture and putrefactive vegetable oil, which can pollute drinking water if it comes into contact with drinking water sources. Incineration produces dioxins during combustion of low-humidity and high-calorie waste products. Another disposal option is converting KG to "pigwash", which can cause disease in pigs and other domestic animals. Improper treatment of KG leads to its putrefaction, which allows toxic, hazardous liquids to leach into the environment (Wang et al., 2015). Therefore, developing a safe and sustainable treatment approach for KG is essential.

Most of the KG generated by the catering industry consists primarily of decomposable lignocellulose, and thus KG can potentially be a substrate for the production of high value-added products. Due to its organic and nutrient-rich composition, KG could theoretically be converted into a useful primary microbial feedstock for biofuels through various fermentation processes. KG has been treated using biological fermentation methods to create biofuels, including methane, hydrogen, ethanol, and swill-cooked dirty oil (Furukawa and Hasegawa, 2006; Uçkun Kiran et al., 2014; Wang et al., 2008). However, these production methods do not fully capitalize on KG's potential economic value. Methanogenic fermentation and extraction of cooking oil result in low-economic-value products. Additionally, ethanol is not a desirable biofuel replacement for fossil fuel energy. Biobutanol is a more advanced energy source, and has greater potential to replace fossil fuels than

Abbreviations: KG, kitchen garbage; EHP, enzymatic hydrolysis procedures; SHF, separate hydrolysis and fermentation; SSF, simultaneous saccharification fermentation; TOC, total organic carbon; TN, total nitrogen

* Corresponding author.
E-mail addresses: shenhong@swu.edu.cn (H. Shen), furong987@126.com (F. Tan), linjiafu@cdu.edu.cn (J. Lin).
[1] Equal contribution.

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ethanol. However, there are limited reports in the literature regarding conversion of KG to biobutanol.

KG is mainly composed of carbohydrate polymers (including starch, cellulose, and hemicelluloses), lignin, proteins, lipids, and organic acids. Therefore KG can be hydrolytically decomposed into simple sugars via a series of reactions catalyzed by cellulolytic enzymes. Using enzymes to break down carbohydrates into economically valuable substances is an earth-friendly and effective pretreatment approach. Conversion of polysaccharides to oligosaccharides or monosaccharides does not produce poisonous compounds in hydrolysate solution; therefore the products of these reactions are usually more amenable to microbial uptake. Thus, the EHP has growing appeal, because it requires less energy and milder environmental conditions and generates fewer fermentation-inhibiting products compared to chemical approaches that utilize sulfuric acid or other acids. However, considering the high levels of moisture and putrefactive vegetable oil and the variety of impurities found in KG, it is not clear whether the enzymatic hydrolysis approach can be used to break down KG.

In this study, using KG as feedstock for *Clostridium acetobutylicum*, the potential for KG to be a biofuel resource for producing high levels of biobutanol were enriched. Specifically, to improve microbial growth and increase sugar yields, various high-efficiency enzymatic cocktail procedures were tested, which combined different enzymes to control the enzymatic hydrolytic process. Finally, the effectiveness of resulting hydrolysates to drive butanol production using two fermentation methods: SHF and SSF were assessed. In biofuel production via fermentation, SHF is the most conventional and widely used method. The SSF was another method used for conversion of sugars to avoid inhibitory effect occurs in SHF method. In addition, to attempted to enhance the fermentation process by using a pH-stat to monitor and adjust pH, and thereby further improve biobutanol production.

2. Materials and methods

2.1. Assessment of KG's physicochemical properties

2.1.1. Determination of total solids and moisture

KG was acquired from a school canteen in China. This canteen can provide food for 4000 people. In the study, small samples were taken after after a lunch on a single day. Total solid and moisture content of samples (400 g) were determined as follows: KG samples (three replicates) were dried in a thermostatic oven with hot air at 60 °C in order to quickly evaporate volatile compounds and water during the first 24 h. Subsequently, we incubated KG samples at a low drying temperature (40 °C) rather than a high drying temperature (70.5 °C) in order to prevent caramelization of sugars or alterations in the KG nutrient content. Samples were dried for 24 h or until no further weight change was detectable. Total solids are reported as the percentage of dry solids within the fresh samples.

2.1.2. Determination of ash content

Ash content of KG samples was determined as follows: After weighing previously dried KG samples, we incinerated samples in a forced-air muffle furnace (Shanghai Shinbae Industrial Co., Ltd; Model: TNX1700-30, Temperature Range: 60 °C–1600 °C) in the presence of excess air at 800 °C for 4 h. Next, the oven temperature was lowered to 60 °C and samples were moved to a desiccator and cooled to room temperature. The resulting ash residue was weighed and calculated as the percentage ash content (dry wt., w/w) in dried samples.

2.1.3. Total organic carbon (TOC)

TOC of KG was measured in accordance with TMECC's standard method 04.01-A (25; Combustion with CO_2 detection) (Council, 2002; Leege, 2001). According to TMECC's definition, total organic carbon does not include inorganic carbonate fractions, such as calcium and magnesium carbonates. A carbon analyzer (Model: vario MAXCN-Elementar Americas) was used to determine the TOC content of the KG samples. Briefly, samples were combusted in an oxygen-rich atmosphere resistance furnace at 1100 °C. The CO_2 produced was then channeled into an oxygen stream through anhydrone tubes to scrub water vapor out of the stream. The dehydrated CO_2 stream was fed into an infrared detector, which generates a signal that is proportional to the amount of CO_2 produced. Milled and oven-dried samples were used for TOC determination and values are reported as the percentage of TOC content (dry wt., w/w) in dried samples.

2.1.4. Estimation of total nitrogen (TN)

Total nitrogen comprises the sum of Kjeldahl nitrogen (organic nitrogen and ammonia nitrogen), nitrate nitrogen, and nitrite nitrogen. Total nitrogen is usually used to estimate the carbon to nitrogen ratio (C:N) of a sample. The total nitrogen content was measured using TMECC's 04.02-D method (oxidation by dry combustion) and an automated Nitrogen analyzer (Model: vario MAX CN-Elementar Americas) according to the manufacturer's instructions. About 150 mg milled and oven-dried samples were used for total nitrogen determination. Values are reported as the percentage of total N_2 content (dry wt., w/w) in dried samples.

2.2. Pretreatment of KG

Because KG usually contains high levels of fatty oil, and it inhibit the *Clostridium acetobutylicum* growth, it is necessary to remove fatty oil from KG. The KG was added to ethyl acetate, then stirred by mixing, then allowed to rest for 20 min until obvious stratification appeared using a separatory funnel, and the supernatant was removed. The solid was collected and moved to constant temperature oven of 40 °C to remove the ethyl acetate. The experiment was performed in triplicate.

2.3. Composition analysis

Starch content of KG was determined according to a simple modification of a method previously described in the literature (Pirt and Whelan, 1951; Rose et al., 1991). First, 1% H_2SO_4 was used to hydrolyze KG and then determined the total sugar content in the hydrolysate. Starch content was calculated based on the total sugar content (starch content = glucose content × 0.91). Using the Kjeldahl method, the crude protein (CP) content of KG were measured based on the following formula: CP = kJ N × 6.25 (kJ indicates use of the Kjeldahl method) (Thiex et al., 2002; Xiao et al., 2013). Cellulose content was determined based on spectrophotometry using anthrone as the reaction reagent. This testing process and handling procedure have been previously described in the literature in detail (Black, 1951; Hansen and Møller, 1975). The cellulose content of samples was then calculated according to a standard curve: cellulose content Y (%) of KG = X (cellulose content of standard sample) × a (diluted multiples) × 100/W (total weight of samples). Lignin content was determined using acetyl bromide according to standard methods (Iiyama and Wallis, 1990; Iiyama and Wallis, 1988). Composition analysis of the KG samples is presented (Table 1).

2.4. Rejuvenation of microorganisms and inoculum cultivation

The experimental *C. acetobutylicum* ATCC 4259 strain were obtained from the American Type Culture Collection (ATCC, Manassas, USA) and stored it in sterilized water with 20% glycerol at 4 °C. The dormant strain was heat shocked at 76 °C for 2.5 min in a water bath, then cooled to room temperature. To rejuvenate the strain, spores were transferred to 100 mL culture including tryptone, glucose, yeast medium (TGYM) with 5 g meat medium (Sigma-Aldrich, Beijing) and 2 g glucose. Next, the heat-shocked spores were incubated in an anaerobic jar at 35 °C for 48 h.

The fermentation inoculum was prepared as follows: Modified