Applied Energy 129 (2014) 89–93

Contents lists available at ScienceDirect

Applied Energy

journal homepage: www.elsevier.com/locate/apenergy

Feasibility of converting lactic acid to ethanol in food waste fermentation by immobilized lactate oxidase

Hong-zhi Ma *, Yi Xing, Miao Yu, Qunhui Wang

Key Laboratory of Educational Ministry for High Efficient Mining and Safety in Metal Mine, University of Science and Technology Beijing, Beijing 100083, China
Department of Environmental Engineering, University of Science and Technology, Beijing 100083, China

HIGHLIGHTS

- Residue lactic acid in food waste could be converted to pyruvic acid.
- Calcium alginate immobilized the lactate oxidase with high pH and thermal stability.
- Immobilized enzyme could convert 70% lactic acid to pyruvic acid.
- Ethanol yield could be increased by 20% with lactate oxidase added.

ARTICLE INFO

Article history:
Received 17 February 2014
Received in revised form 24 April 2014
Accepted 28 April 2014
Available online 22 May 2014

Keywords:
Lactate oxidase
Lactic acid
Food waste
Pyruvate
Ethanol fermentation

ABSTRACT

Adoption of lactic acid bacteria (LAB) into ethanol fermentation from food waste can replace the sterilization process. However, LAB inoculation will convert part of the substrate into lactic acid (LA), not ethanol. This study adopted lactate oxidase to convert the produced LA to pyruvate, and then ethanol fermentation was carried out. The immobilization enzyme was utilized, and corresponding optimum conditions were determined. Results showed that calcium alginate could successfully immobilize the enzyme and improve pH and thermal stability. The optimum pH and temperature were 6.2 and 55 °C, respectively. The utilization of immobilized enzyme with catalytic time of 5 h could convert 70% LA to pyruvate, and the addition of enzyme increased the ethanol yield by 20% more than that of the control. The process could be applied in food waste storage and can help in reducing carbon source consumption.

1. Introduction

The universal concern on energy and environmental preservation has generated increasing research focus on biomass energy worldwide. Finding proper raw materials for biomass energy production is important. Food waste is a type of biomass waste with high organic quantity and moisture content; it is easily perishable and harmful to the environment if not treated properly [1]. High carbon source and abundance of nutrients make food waste an ideal raw material for value-added products, such as methane, lactic acid (LA) fermentation and hydrogen production [2–4]. Ethanol production from food waste can reduce pollution and serve as a resource alternative [5,6]. Fuel ethanol is an important biomass energy because it is simple and has the ability to be mixed with gasoline. Ethanol production usually undergoes cooking process, saccharification, fermentation, and distillation. The traditional cooking process consumes 30–40% of the total energy investment and a large quantity of cooling water, which both increase the cost of ethanol production [7–9]. Production cost is one of the barriers for ethanol production application. Uncooked fermentation cancels the cooking and cooling processes, decreases steam and water use, and simplifies the technological route and equipment. The successful utilization of this process for food waste ethanol fermentation will benefit the environment and reduce the ethanol fermentation cost [9].

Another disadvantage of ethanol from food waste is storage for the raw materials. Preventing the occurrence of corruption during storage and deterioration caused by microorganisms is important in this process [10,11]. High organic content in food waste is useful for microorganism growth. LA is accumulated under methophilic condition without lactic acid bacteria (LAB) inoculation [1,12,13]. LAB is the dominant microorganism. No pathogenic microorganisms are observed in this process. The accumulated LA is used to prevent corruption in ethanol production from food waste. A few studies on this utilization are available, and the mutual effect

* Corresponding author at: Department of Environmental Engineering, University of Science and Technology, Beijing 100083, China. Tel.: +86 13426135537; fax: +86 1062332778.
E-mail addresses: MHZ527@sina.com (H.-z. Ma), xingyiustb@163.com (Y. Xing).

http://dx.doi.org/10.1016/j.apenergy.2014.04.098
0306-2619/

during this process has been studied [14]. Previous studies have focused on removing the corruption effect during the process. No investigation on the effect caused by LAB on the carbon source is available. Ethanol and LA production share the same metabolic pathway, which begin from pyruvic acid. LAB utilization will compete with ethanol production microorganisms for carbon resource. Adopting a proper biochemical method to reduce carbon consumption and prevent corruption results in a double-win effect, which is important for ethanol fermentation from food waste. A possible approach for this goal is to utilize the produced LA during the anti-corruption process to produce pyruvic acid and then yeast in citric acid cycle metabolism for ethanol. The utilization of lactate oxidase can change LA into pyruvic acid [15,16], which is a possible approach to enhance ethanol yield. In fact the pyruvic acid could be produced by fermentation and enzyme-catalyzed reactions [17]. Industrial pyruvate is produced by the dehydration and decarboxylation of tartaric acid [18]. This process has the merit of simple process, while had the disadvantages of expensive cost and low yield. When it came to fermentation method, complex technology route as well as the difficulty in separation had made it hard for industrial application. Enzyme conversion thus became the proper choice [19], there existed several kinds of enzymes for pyruvic production. Lactic dehydrogenase could be used for pyruvic production from lactic acid, since the reaction was a balanced one and corresponding conversion yield was low, and the cost was high [20]. There existed research on pyruvate from lactate by using whole-cell gylcolate oxidase as the enzyme [21]. While among them lactate oxidase was often adopted due to its high yield and low cost [22].

But free enzyme is easily to lose its ability and difficult to be recycled. This would cause difficulty for its separation. If not recycled, the cost for the enzyme would be great. Thus the immobilized enzyme would be easily for recovery of enzyme and could reuse [23]. Take the research for lactate oxidase for example, the utilization for the immobilized enzyme had the following merits, the procedure for cell broken and enzyme extraction could be omit, the stability for the enzyme would be increased, the cost for immobilization had reduced to a large extent, the product could reused for a long time, and it would be easy for separation from product. All these advantages had showed that immobilized enzyme would be a suitable technology. Furthermore, our study utilized food waste as the substrate, which caused a high concentration of suspended solid, which would cause bad effect for the free enzyme, the immobilized enzyme had high tolerance for the impurities inside the food waste ethanol fermentation broth. Which would in turn maintained a high and stable conversion efficiency.

Thus the immobilized lactic oxidase should be utilized in this process. Currently more researches were carried out in immobilized enzyme in the field of enzyme electrodes and biosensors [24]. Few research was carried out for its utilization of converting lactic acid inside food waste to ethanol, in this study, sodium alginate will be used for enzyme immobilization, the characteristic for the immobilized enzyme as well as its utilization in food waste were studied in this process, also the relationship between lactic acid and ethanol were studied as well.

2. Materials and methods

2.1. Materials

Food waste was collected from the dining room of the University of Science and Technology Beijing, China. LAB, designated as TD175, was obtained from our lab. *Escherichia coli* [BL21 (DE3)/pET-LOD] was also obtained from the University of Science and Technology Beijing.

2.1.1. Culture media

Luria broth (LB): Peptone (1 g/100 mL), yeast powder (0.5 g/100 mL), NaCl (1.0 g/100 mL), and kanamycin (50 μg/L) were dissolved in water, and pH was adjusted to 7. The solution was sterilized at 121 °C for 15 min. SB3 media: Peptone (44 g/L), yeast powder (30 g/L), NaCl (10 g/L), and kanamycin (50 μg/L) were dissolved in water. The solution was adjusted to pH 7 and sterilized at 121 °C for 15 min.

2.2. Methods

2.2.1. Preparation of lactic oxidase and detection of enzyme ability

E. coli was initially cultured in LB media for 12 h and then transferred to SB3 media for 48 h. The fermentation broth was centrifuged at 12,000 r/min for 3 min. Then, the supernatant was discarded and the broth was washed twice with phosphate buffer. The broth was dissolved in 20 mL of PBS, and ultrasonic treatment was carried out for 5 min (minimum power, 200 W; probe amplitude, 40%). After a 20 min centrifugation (n = 10,000 r/min, 4 °C), the supernatant was poured into the tube to collect the lactic oxidase crude enzyme solution.

The enzyme was determined based on Ref. [16]. One unit of enzyme was determined as the amount of enzyme that catalyzed LA to produce 1 nmol pyruvate in 1 min. The relative activity was determined as the ratio of the enzyme detected and the maximum enzyme under the condition.

$$\text{Relative enzyme activity} = \text{enzyme activity detected}/\text{maximum enzyme activity}$$

2.2.2. Lactate oxidase immobilization

Lactic oxidase enzyme solution (20 mL) was mixed with 20 mL of 4% sodium alginate solution. This solution was slowly added into 400 mL of 0.2 mol/L $CaCl_2$ solution with a syringe. The solution was then filtered, washed, dried to obtain the particulate immobilized lactate oxidase, and solidified at 25 °C for 2 h.

2.2.3. Measurement methods for LA, pyruvic acid, ethanol, and reducing sugar content

Lactic acid, ethanol concentration was measured by SBA 40 C biosensor [14]. Pyruvate acid was determined as followed, 1 ml of fermentation supernatant was put into a test tube, added 2 mL 8% trichloroacetic acid plus 1.0 mL of 0.1% 2,4-dinitrophenylhydrazine solution. Shaked properly then added 5.0 mL 1.5 mol/L, NaOH solution, detect the absorbance at wavelength of 520 nm, then referred to the standard solution. The reducing sugar was determined as DNS method [25]. All the detection was performed for three times and the average was used for discussion.

2.2.4. Optimization of catalytic conditions for immobilized lactate oxidase

2.2.4.1. Optimum temperature. Phosphate buffer (0.5 mL) was added to each test tube with 0.2 mL of 20 mmol/L LA and 0.1 g of immobilized enzyme. Each tube was placed at 25, 37, 45, 55, 65, and 75 °C, respectively. The water bath was maintained for 10 min, and then 1 mL of 1 mol/L 2,4-dinitrophenylhydrazine was added. The reaction was performed at 55 °C for 20 min, and then 5 mL of 0.04 mol/L NaOH was added to the solution. Finally, the absorbance values were determined at 520 nm wavelength. For comparison, the free enzyme was used as control.

2.2.4.2. Optimum pH. Phosphate buffer with different pH levels (pH 5.8, 6.2, 6.6, 7.0, 7.4, 7.8, and 8.0) was prepared in advance. Then, 0.5 mL of phosphate buffer, 0.2 mL of 20 mmol/L LA, and 0.1 g of immobilized enzyme were placed into each tube. The solution