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# Efficient production of optically pure L-lactic acid from food waste at ambient temperature by regulating key enzyme activity



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#### ABSTRACT

Bio-production of optically pure L-lactic acid from food waste has attracted much interest as it can treat organic wastes with simultaneous recovery of valuable by-products. However, the yield of L-lactic acid was very low and no optically pure L-lactic acid was produced in the literature due to (1) the lower activity of enzymes involved in hydrolysis and L-lactic acid generation, and (2) the participation of other enzymes related to D-lactic acid and acetic and propionic acids production. In this paper, a new strategy was reported for effective production of optically pure L-lactic acid from food waste at ambient temperature, i.e. via regulating key enzyme activity by sewage sludge supplement and intermittent alkaline fermentation. It was found that not only optically pure L-lactic acid was produced, but the yield was enhanced by 2.89-fold. The mechanism study showed that the activities of enzymes relevant to food waste hydrolysis and lactic acid production were enhanced, and the key enzymes related to volatile fatty acids and D-lactic acid generations were severally decreased or inhibited. Also, the microbes responsible for L-lactic acid production were selectively proliferated. Finally, the pilot-scale continuous experiment was conducted to testify the feasibility of this new technique.

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## 1. Introduction

Lactic acid has been widely used in medical, food, and general chemical industries. It has two optical isomers, i.e., L- and D-lactic acid. L-lactic acid is the precursor of poly-L-lactate (PLLA), a promising biodegradable plastic (Hofvendahl and Hahn-Hägerdal, 2000). The physical property and

biodegradability of PLLA are highly depended on the L-isomer purity of lactic acid (Lunt, 1998). Nevertheless, chemical synthesis of lactic acid only generates the racemic mixture, whereas microbial fermentation is an alternative approach to produce L-lactic acid (Ilmen et al., 2007).

With the rapid growth of human population in the world, food waste and its environmental impact have become a

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major issue. Anaerobic treatment of food waste has become an interest as it can recover energy and valuable by-products (Carballa et al., 2011; Levis and Barlaz, 2011). Although lactic acid could be biologically produced from organic waste by anaerobic fermentation, the lactic acid was the mixture of Land D-isomers (Gao et al., 2011). Also, the documented methods were usually complicated as they were conducted under sterile and thermophilic or mesophilic conditions and the lactic acid producing bacterial strains need be inoculated (Nakasaki et al., 1999). In addition, the yield was only 0.227 g per initial total chemical oxygen demand of the substrate (g/g TCOD) (Akao et al., 2007).

During anaerobic fermentation the organic compounds are firstly hydrolyzed by enzymes (such as protease and α-glucosidase, Fig. 1). It is well known that food waste is usually rich in carbohydrate. When a carbohydrate-enriched matter was anaerobically fermented to produce volatile fatty acids (VFA), the activity of hydrolysis enzyme and the yield of VFA were observed to be significantly improved by the addition of a certain amount of waste activated sludge (Feng et al., 2009). Thus, it can be speculated that the production of lactic acid from food waste could be enhanced by the addition of waste activated sludge. Also, as shown in Fig. 1, the hydrolyzed products can be bio-converted to L-lactic acid, D-lactic acid, acetic acid or propionic acid. The enzymes responsible for Land p-lactic acid production are respectively NAD-dependent L-lactate dehydrogenase (L-LDH) and NAD-dependent Dlactate dehydrogenases (D-LDH) (Garvie, 1980). Obviously, Optically pure 1-lactic acid could be produced by improving the activity of L-LDH and simultaneously inhibiting that of D-LDH. In addition, if the activities of enzymes (acetate kinase (AK), phosphotransacetylase (PTA), succinyl CoA transferase (CoAT) and oxaloacetate transcarboxylase (OAATC)) relevant to the generations of acetic and propionic acids were decreased, more substrate could be used to produce 1-lactic acid. Also, supposing that NAD-independent lactate dehydrogenase (iLDH) attributed to the consumption of L-lactic acid declined in activity, the yield of L-lactic acid would be further enhanced. Until now, however, no any reference is available regarding the efficient production of optically pure L-lactic acid from wastes at ambient temperature by regulating the activities of these enzymes.

In this paper a new method, i.e., by sewage sludge supplement and intermittent alkaline fermentation to regulate the activity of key enzymes, for effectively producing optically pure L-lactic acid from food waste at ambient temperature was reported. Firstly, the effect of sludge supplemented to food waste fermentation system on L-lactic acid production was studied. In order to increase the optical purity of L-lactic acid the intermittent alkaline fermentation strategy was developed. Then, the mechanisms for remarkably high optically pure L-lactic acid being produced by sewage sludge supplement and intermittent alkaline fermentation were explored. Finally, the feasibility of continuously and effectively producing optically pure L-lactic acid from food waste by sewage sludge supplement and intermittent alkaline fermentation at ambient temperature was testified in a pilotscale reactor.

### 2. Materials and methods

#### 2.1. Food waste and waste activated sludge

After the removal of facial tissue, chopsticks, bones, and inorganic particles, the food waste, which was collected from a dinning restaurant in Shanghai, was milled to slurry state and diluted with tap water. The final characteristics of the food waste were as follows (average data plus standard deviation of triplicate measurements): total suspended solids (TSS) 97.42  $\pm$  3.62 g/L, volatile suspended solids (VSS) 94.68  $\pm$  3.28 g/L, total chemical oxygen demand (TCOD) 137.62  $\pm$  5.76 g/L, soluble chemical oxygen demand (SCOD) 26.67  $\pm$  3.92 g/L, total carbohydrate 68.31  $\pm$  5.97 g COD/L, and total protein 14.31  $\pm$  0.74 g COD/L. The waste activated sludge (WAS) was withdrawn from a municipal wastewater



Fig. 1 – Proposed metabolic pathway for lactic acid production from organic wastes (Garvie, 1980; Feng et al., 2009).

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