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Copper (II) addition to accelerate lactic acid production from co-fermentation of food waste and waste activated sludge: Understanding of the corresponding metabolisms, microbial community and predictive functional profiling

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ABSTRACT

Bio-refinery of food waste and waste activated sludge to high value-added chemicals, such as lactic acid, has attracted particular interest in recent years. In this paper, the effect of copper (II) dosing to the organic waste fermentation system on lactic acid production was evaluated, which proved to be a promising method to stimulate high yield of lactic acid (77.0% higher than blank) at dosage of 15 μM-Cu^{2+}/g VSS. As mechanism study suggested, copper addition enhanced the activity of α-glycosidase and glycolysis, which increased the substrate for subsequent acidification; whereas, the high dosage (70 μM-Cu^{2+}/g VSS) inhibited the conversion of lactic acid to VFA, thus stabilized lactic acid concentration. Microbial community study revealed that small amount of copper (II) at 15 μM/g VSS resulted in the proliferation of *Lactobacillus* to 82.6%, which mainly produced lactic acid. Finally, the variation of functional capabilities implied that the proposed homeostatic system II was activated at relatively low concentration of copper. Meanwhile, membrane transport function and carbohydrate metabolism were also strengthened. This study provides insights into the effect of copper (II) on the enhancement of lactic acid production from co-fermentation of food waste and waste activated sludge.

1. Introduction

Food waste (FW) and waste activated sludge (WAS) are two main types of municipal organic wastes with unprecedented increasing production rate (Dai et al., 2013; Menon et al., 2017; Zhang et al., 2011). Imperative and properly disposal was imminently needed to alleviate the environmental burden from organic waste pollution. Anaerobic fermentation is one of the promising methods to convert carbon from organic wastes such as food waste, to produce high value-added chemicals (Li et al., 2016; Girotto et al., 2015; Rafieenia et al., 2017). Meanwhile, waste activated sludge is rich in facultative microorganisms that can be applied in fermentation (Xue et al., 2018; Ekstrand et al., 2016). To take the advantage of FW and WAS, co-fermentation has been widely studied for the platform molecules production, such as lactic acid (Li et al., 2017, 2018), VFA (Sawatdeenarunat et al., 2017; Zhao et al., 2015), methane (Menon et al., 2017; Mustapha et al., 2016) and hydrogen (Rafieenia et al., 2017; Tawfik et al., 2015).

Lactic acid is one of the most valuable chemicals employed in a wide variety of industrial applications including food, chemicals, pharmaceutical industries, and its demand has reached 15% annual growth rate globally (Gavilà et al., 2015; Tang et al., 2016). At present, inoculating the specific pure strain of lactic acid bacteria (LAB) is adopted mostly as the common strategy to obtain lactic acid (Mufidah and Wakayama, 2016). However, the deficiencies including the cost of cultivating pure strain, the challenge to metabolize complex substrates and the incomplete sterilization of substrate made it uneconomic (Tamis et al., 2015; Li et al., 2017; Zhang et al., 2008). Alternatively, anaerobic fermentation of organic wastes using mixed microbial consortium is an efficient approach to obtain the desired product if the key microbial com-

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munities were proliferated and the relevant metabolic pathway was effectively regulated (Tamis et al., 2015; Liang et al., 2014).

It is well known that metals play an important role in the biological processes, like activating key enzymes in metabolisms, promoting the growth of microbial cells (Facchin et al., 2013; Glass and Orphan, 2012). Copper (II) by far has attracted wide attention as a cofactor within many copper-dependent enzymes (Palumaa, 2013), like Cu-containing laccase, Cu amine oxidase, Cu-containing nitrite reductases, which mainly functioned as electron transfer or oxidation of some small molecules (Solioz et al., 2011). Particularly, copper (II) had positive impacts on butyric acid and hydrogen production in the anaerobic fermentation system (Lin, 2008; Liu et al., 2015a). Besides, the microbial communities shifted in the presence of copper (II), influencing the production of methane in the anaerobic digestion (Ke et al., 2014). For lactic acid production, it was found that copper (II) impeded the conversion of D-lactic acid to pyruvate via inhibiting the activity of NAD-independent D-lactate dehydrogenas (id-LDH) in the pure culture (Tsuneo Hino and Kurod, 1993). In addition, *Lactococcus lactis* subsp. produced more biomass with the addition of copper (Solioz et al., 2011). The evidence above provided the possibility of achieving higher yield of lactic acid from pure culture via copper addition. As yet, no reference is available on the community shift of LAB from the mixed-culture fermentation of organic wastes with the addition of copper. This led us to raise several questions: Does copper have positive effect on lactic acid production from organic wastes using undefined mixed cultures? If it does, how does copper influence the corresponding metabolism during the fermentation? How does it impact the microbial community and corresponding functional capabilities?

Therefore, this study aims to use copper (II) to regulate the indigenous microorganism in the co-fermentation of FW and WAS to enhance lactic acid production. Firstly, the effects of four metal ions including copper on lactic acid production were determined. Then, copper dosage and the variation of metabolic intermediates involved in the pathways were investigated accordingly. Furthermore, the microbial community analysis was performed to disclose the correlation between community structure and copper dosage. Predictive functional profiles of the microbial communities were finally explored. This paper reveals the effects of copper (II) on lactic acid production from co-fermentation of food waste and waste activated sludge, and may benefit the research on the application of copper in anaerobic fermentation.

2. Materials and methods

2.1. Substrate and batch fermentation

Food waste containing mainly rice, tofu and meat was collected from a canteen of Donghua University in Shanghai and then milled to slurry state by the food grinder, followed by a dilution by tap water. The waste activated sludge was collected from the sludge bed of a municipal waste water treatment plant in Shanghai, China. Prior to inoculating, the waste activated sludge was settled for 24 h and decanted the supernatant to obtain the concentrated sludge. Then, the food waste and waste activated sludge were mixed at volatile suspended solid mass ratio of 6:1 (VSS_{FW}/VSS_{WAS}) according to our previous study (Li et al., 2015). Tap water was added to make the TCOD of mixture to be 39,076.0 ± 1022.0 mg/L. The main characteristics of FW, WAS and final fermentative substrate were summarized in Table 1 (averaged data plus standard deviation of triplicate tests). Then, the batch fermentation was conducted in the fermentation reactors with working volumes of 1 L at 35 °C and mechanically stirred at 150 rpm. Sodium hydroxide (5M) and hydrochloric acid (5M) were used to adjust the pH value to be 7 every 6 h.

Table 1
Initial characteristics of food waste, waste activated sludge and the mixed fermentative substrate.

Parameter	Food waste	WAS	Mixture
pH	6.6 ± 0.2	6.6 ± 0.2	7.0 ± 0.2
Total suspended solid (mg/L)	201,540 ± 1286	12,550 ± 288	39,450 ± 1243
Volatile suspended solid (mg/L)	200,760 ± 1322	8950 ± 99	36,332 ± 998
Total COD (mg/L)	201,353 ± 1532	13,558 ± 1054	39,076 ± 1022

2.2. Suitable metal determination for lactic acid fermentation

Metallic nutrition played vital roles in activating certain metabolic enzymes during the fermentation processes (Demirel and Scherer, 2011; Voelklein et al., 2017), like ferrum (Fe^{3+}) (Zhang et al., 2015), manganese (Mn^{2+}) (Liu et al., 2015a) and magnesium (Mg^{2+}) (Yuan et al., 2014), which had positive effects on the generation of fermentative products. Copper (Cu^{2+}) was also reported to inhibit the consumption of D-lactic acid to pyruvate, which could potentially promote the accumulation of lactic acid (Hino and Kurod, 1993). Thus, to examine the impacts of those abovementioned metals on lactic acid production from co-fermentation of FW and WAS, five identical fermentation reactors were conducted as mentioned above with addition of Fe^{3+}, Mn^{2+}, Cu^{2+} and Mg^{2+} in the forms of $Fe_2(SO_4)_3{\cdot}5H_2O$, $MnSO_4{\cdot}4H_2O$, $CuSO_4{\cdot}5H_2O$ and $MgSO_4{\cdot}7H_2O$, respectively (dosage detailed in Table 2). The reactor without any metal addition was set as Blank. Samples were fetched every day and filtered through membranes with pore sizes of 0.45 μm for L- and D-lactic acid.

2.3. Effect of copper (II) dosage on lactic acid fermentation

Initial copper (II) concentration in WAS was determined using inductively coupled plasma mass spectrometry (ICP) to avoid the possible effects on this study (Table S1). To maximize the lactic acid production, four identical reactors, namely Blank, Cu-15, Cu-30 and Cu-70, were conducted as mentioned above with different copper dosage (μM-Cu^{2+}/g VSS) of 0, 15, 30 and 70, respectively. Samples were taken each day for L-lactic acid, D-lactic acid, soluble carbohydrate, soluble protein, NH_4^+-N, VFA assay. FT-IR spectrum of solid samples from Blank, Cu-15 and Cu-70 on 4 d was detected to investigate the variation of chemical groups in the presence of copper. Enzymatic activity of α-glucosidase and protease were determined on the initial three days. Samples in Blank and Cu-15 were also taken for Excitation-emission matrix (EEM) fluorescence spectroscopy analysis.

2.4. Microbial community and predictive functional profiles analysis

Since microbial community determines the performance of lactic acid fermentation, samples were fetched out from Blank, Cu-15 and Cu-70 when lactic acid production was stabilized for analysis (Personal Biotechnology, Shanghai). For PCR amplification of the16S ribosomal RNA (rRNA) gene, the primer 338F (5′-ACTCC TACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTC TAAT-3′) in V3-V4 were used. The bacteria community structure was analyzed through 16s-rDNA gene cloning and sequencing performed on Illumine Miseq system (detailed in Supplementary Information). The closest matching sequences were compared with reference sequences in the GenBank database of BLAST

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