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Volatile fatty acid production from spent mushroom compost: Effect of total solid content

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ABSTRACT

To improve volatile fatty acids (VFAs) production from spent mushroom compost (SMC), the effect of total solid (TS) content was studied. The protein and polysaccharide solubilization, ammonium (NH_4^+-N) and phosphate (PO_4^{3-}-P) release, and VFAs production were analyzed. Results showed that the optimal fermentation time was 4 day for VFAs production, longer fermentation time led to VFAs consumption and methane production. Within the TS range from 6% to 18%, a TS of 15% was the optimal for VFAs production, the highest VFAs concentration reached 2781 mg/L, and acetate and propionate acids accounted for about 71% of total VFAs. In addition, the maximum concentration of soluble protein and polysaccharide reached approximately 1648 mg/L and 1394 mg/L, respectively. The NH_4^+-N and PO_4^{3-}-P release was in a range of 2.11–7.59 mg/gVS and 0.26–1.13 mg/gVS, respectively. If the NH_4^+-N and PO_4^{3-}-P can be removed, the application of VFAs utilization might be significantly enhanced.

1. Introduction

Volatile fatty acids (VFAs) are short-chain fatty acids that consist of six or fewer carbon atoms, and they can be distilled at atmospheric pressure. Due to its high added value, it has been proposed a wide range of applications such as biopolymers, the chain elongation and the biological nutrient removal from wastewater (Agler et al., 2011; Kleerebezem and van Loosdrecht, 2007). For example, biological nutrient removal has been widely used in modern municipal wastewater treatment plants. The nutrient removal with biological processes is closely linked to the presence of easily biodegradable carbon source, which is often the limiting factor in application (Tsuneda et al., 2005). It has been reported that the VFAs of 6.0–9.0 mg was required for biological removal of 1.0 mg phosphorus from wastewater (Henze, 1991; Pitman et al., 1992). The fermentation liquid enriching VFAs has been considered as a practical external carbon source for increasing biological nutrient removal, by which both carbon source production and solid waste utilization can be simultaneously accomplished (Zhang et al., 2009). Moreover, VFAs are the substances of great importance and wide use in industry. However, at present commercial VFAs are mostly produced by chemical processes. These processes consume large amounts of fossil carbon resources as raw materials and cause related environmental problem. Therefore, the renewed interest has focused on developing biological routes for VFA production from potential organic residual materials.

Spent mushroom compost (SMC), a waste product of mushroom industry, consists of abundant organic substances and mushroom mycelium. Furthermore, the SMC contains many kinds of residual enzymes, such as protease, cellulase, hemicellulase, LiP, MnP, and laccse (Zhang and Sun, 2014). The production of every kg mushroom corresponds to approximately five kg SMC (Williams et al., 2001). As estimated, there could be more than 8 million tons of SMC produced in China each year (Shi et al., 2014). The high amounts of SMC have not been treated and disposed efficiently. Most SMC is discarded on land, which leads to environmental pollution and public health issues associated with the attraction of flies and other insects (Kapu et al., 2012). To solve SMC treatment and disposal problem and to enhance the waste recycling, the SMC has been explored for potential commercial applications, including soil amendment and bioremediation agent (Chiu et al., 2009; Medina et al., 2009). However, there has been relatively little information in examining the feasibility of SMC as a renewable

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feedstock for VFAs production.

In general, the VFAs production from biowastes is a complex anaerobic process, including hydrolysis and acidification. In the hydrolysis process, complex and higher molecular organic compounds are broken down into simpler organic monomers by the enzymes secreted from the hydrolytic microorganisms (Fang et al., 2014; Mao et al., 2015). Subsequently, acidogens ferment these monomers into VFAs such as acetic, propionic and butyric acid (Lee et al., 2014). During this process, the operational total solids (TS), retention time, as well as additives have great effect on the microbial community and the concentration and composition of VFAs produced (Hu et al., 2006; Kang et al., 2011).

Therefore, this study aimed to investigate the feasibility of VFAs production from SMC, and to examine the effect of TS content on the hydrolysis and VFAs production. The proteins and polysaccharides solubilization, ammonium and phosphate release in anaerobic process were also investigated.

2. Material and methods

2.1. Substrate and inoculum

The SMC used in this study was obtained from a local mushroom grower in Changsha, China. After drying at 60 °C to a moisture content less than 10%, the SMC was processed with a mill (FS1107, Runpu Inc., China) to powders. Then the powders were screened through a 0.71 mm metal sieve and stored at 4 °C for further use. The average characteristics of SMC used in subsequent experiments were as follow: TS of 60.96%, violate solids (VS) of 13.26% and carbon to nitrogen (C/N) ratio of 28.69.

Mesophilic anaerobic digested sludge was taken from an anaerobic digester of a biogas station in Changsha, China, and used as the inocula. The average TS and VS of inocula were 9.93% and 4.73%, respectively.

2.2. Experimental set-up and operation

Batch test experiments were carried out in five identical reactors with a working volume of 250 ml. At the beginning of experiment, the reactors were flushed with nitrogen gas for 5 min to replace the air. The reactors were then put into an orbital shaker at 35 °C and 140 r/min. In every reactor, a certain amount of SMC, 40 ml inoculum and 200 ml distilled water was added, and the TS of the system was controlled as 6%, 9%, 12%, 15% and 18% respectively. The hydrolysis and acidification of SMC lasted for 7 days until the VFAs concentration didn't increase anymore. Samples were taken from reactors every 24 h and analyzed. All the experiments were carried out independently in triplicate.

2.3. Analytical methods

Samples were directly taken from the reactors for VS analysis. The sample was first centrifuged at 5000 r/min for 30 min with a centrifuge (TGL-20B, Anting Inc., China), and then filtered through a cellulose membrane with a pore size of 0.45 μm. The filtrate was used to determine the VFAs, soluble protein, polysaccharide, NH_4^+-N and PO_4^{3-}-P. The TS, VS, NH_4^+-N and PO_4^{3-}-P were measured according to APHA Standard Methods (Eaton et al., 2005). Proteins were measured with Lowry's method (Lowry et al., 1951). Polysaccharide was measured with phenol-sulfuric acid method using glucose as a standard solution (Herbert et al., 1971). To analyze VFAs, the filtrate was collected in a 1.5 ml gas chromatograph (GC) vial, and 3% H_3PO_4 was added to adjust the pH to approximately 4.0. Then the concentration and composition of VFAs (Acetic, propionic, iso-butyric, n-butyric, iso-valeric, n-valeric acid) were determined by GC (Agilent, 3710) equipped with analytical column DB-FFAP (30 m × 0.25 mm × 0.25 mm). Nitrogen was the carrier gas with a flow rate of 2.6 ml/min. The temperature of injector and FID were 250 and 300 °C, respectively. The GC oven was programmed to begin at 70 °C and remain for 3 min, then increase to 180 °C at a rate of 20 °C/min, and hold at 180 °C for 3 min. The sample injection volume was 1.0 μl.

3. Results and discussion

3.1. Changes of proteins and polysaccharides

In the first step of anaerobic digestion-hydrolysis, particulate compounds are firstly converted to soluble forms (proteins, polysaccharides, etc.) and further hydrolyzed to simple monomers (Wahidunnabi and Eskicioglu, 2014). Galí et al. (2009) modified the ADM1 model for agro-waste and stated that TS content obviously influenced the hydrolysis process, which is assumed to be the first limiting step of anaerobic digestion. Fig. 1 presents the effect of TS content on the soluble protein and polysaccharide concentration during SMC fermentation. It can be seen that the concentration of soluble proteins and polysaccharides at different TS content has similar change trend. Large amount of lignocellulose in SMC were decomposed by mycelia during mushroom cultivation, the lignocellulosic structure of SMC became looser and the SMC became

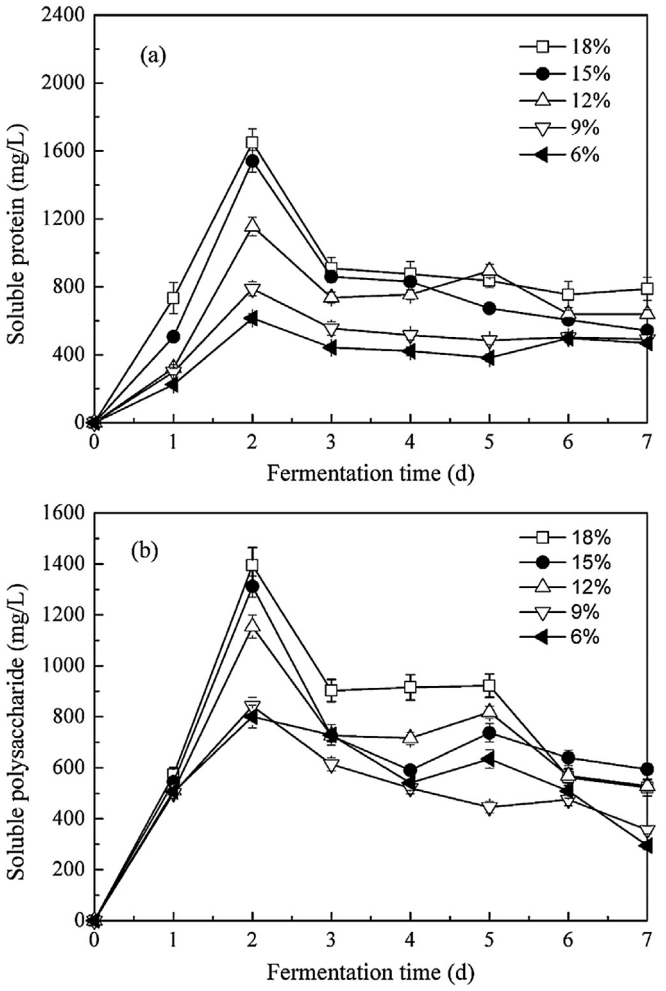

Fig. 1. Effect of TS content on concentration of (a) soluble proteins and (b) soluble polysaccharides during fermentation.

more digestible. Hence, the crude proteins and carbohydrates were quickly hydrolyzed into soluble proteins and polysaccharides during hydrolysis process, which were further consumed by acidogens. In addition, the soluble proteins and polysaccharides depends on the balance between organic release and further degradation (Chen et al., 2007). When the soluble organic matters were quickly consumed by acidogens, their concentrations decreased.

We can also concluded from Fig. 1 that the concentration of proteins and polysaccharides increased as the TS content increased, more organic matters were transformed to soluble matters by hydrolysis bacteria. The maximum concentration of proteins and polysaccharides reached to 1648 mg/L and 1394 mg/L at the 2nd day, respectively. However, Abbassi et al. (2012) investigated the effect of TS content on performance of anaerobic digestion of lignocellulosic biomass, and reported that the hydrolysis rate with a TS content of 10% and 15% showed a similar trend, while the hydrolysis rate linearly decreased with increasing the TS concentration more than 15%. Similarly, Pommier et al. (2007) observed a strong impact of TS content on kinetic rates and maximum methane production in solid waste anaerobic digestion. The reason of the different phenomenon in this study might be contributed to the abundant residual enzymes in SMC, such as protease, cellulase, which were benefit for the SMC degradation.

3.2. VFAs production

As shown in Fig. 2, the soluble small molecule compounds generated during hydrolysis were transformed to VFAs in acidogenesis step. The total VFAs production at various TS contents is shown in Fig. 3. It was observed that the VFAs concentration initially increased and then maintained relatively stable, even slightly decreased. The maximum concentration of VFAs was 2781 mg/L with a TS content of 15%, which was 76.12% higher than that with a TS content of 6%. More organic matters were hydrolyzed and further transformed to VFAs with a higher TS content. Nevertheless, when the TS content further increased to 18%, the VFAs production slightly decreased, compared with the TS content of 15%. Too high TS content might limit the mass transfer between fermentation substrates and microorganisms and hinder the acidification rate (Abbassi et al., 2012).

In this study the maximum VFAs concentration reached at the 4th day of fermentation, which extended two days compared with the time for the maximum soluble protein and polysaccharide production because of the further degradation of these organics. The suitable fermentation time was shorter than that for VFAs production from other lignocellulosic wastes (Li et al., 2013). This might be attributed to amounts of extracellular enzymes released from mushroom crops to substrate during growth and fruiting, which is comparable to a pretreatment of fungal degradation (Chiu et al., 2000). Lin et al. (2015) reported a degradation efficiency of cellulose, hemicellulose and lignin in substrates during 110-day Shiitake cultivation reached approximately 69.08%, 61.56% and 54.30%, respectively. Therefore, compared with other lignocellulosic biomass, a substantial amount of the lignocellulose in the SMC might be decomposed and permeated by mycelia during mushroom cultivation, making the SMC more digestible. Considering the maximal VFAs production and fermentation efficiency, the optimum fermentation condition should be selected as a TS content of 15% and a fermentation time of 4 d.

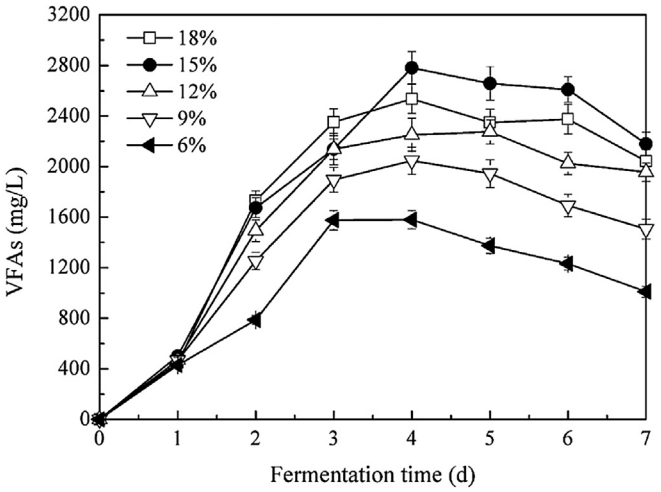

Fig. 3. Effect of TS content on VFAs production.

The VFAs mainly contain acetate, propionate, iso-butyrate, n-butyrate, iso-valerate, and n-valerate acid and the organic acid distribution is very important for the VFAs utilization. Even though there is no strict demand on the type of carbon source for wastewater treatment, the low molecular weight fatty acids, like acetic acid and propionate acid, has been pointed as the best carbon source for biological phosphorous removal because of their faster uptake and assimilation by phosphorus accumulating organisms (Moser et al., 1998; Oehmen et al., 2004). In this study, the acetic and propionate acid were the major compositions in VFAs as shown in Fig. 4, which might be benefit to biological phosphorus removal.

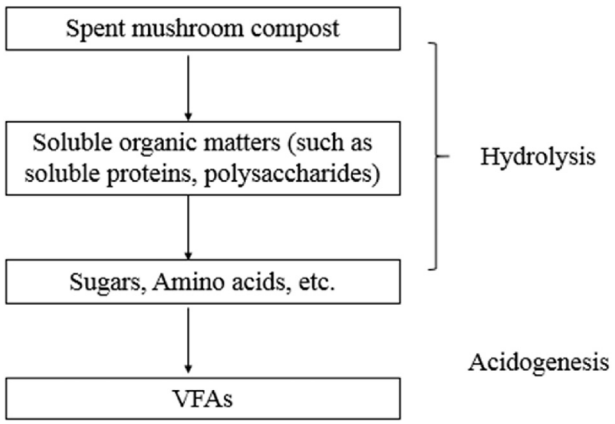

Fig. 2. Pathway of VFA formation from spent mushroom compost.

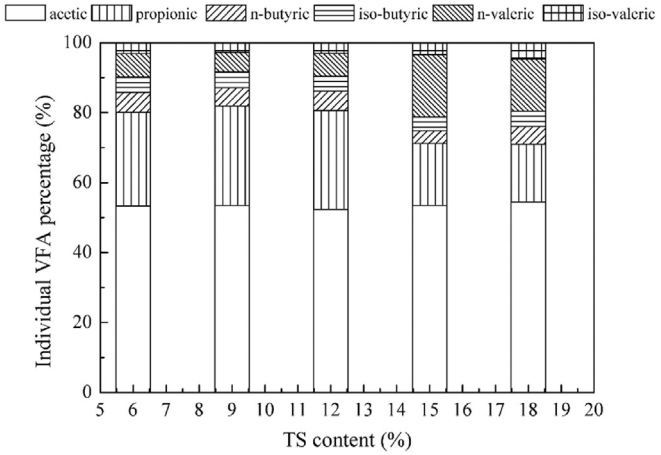

Fig. 4. Effect of TS content on VFAs composition.

In polyhydroxyalkanoates production, acetate and butyrate acids could promote the production of 3-hydroxybutyrate, while propionate and valerate acids favor the synthesis of 3-hydroxyvalerate (Elefsiniotis and Wareham, 2007; Kleerebezem and van Loosdrecht, 2007; Moser et al., 1998). Fig. 4 presented the percentage of individual VFA accounting for the total VFAs at different TS contents. At the TS content from 6% to 18%, the sum of produced acetic and propionate acid accounted for more than 70%, which were the most prevalent in the total VFAs. It was agreement with other publications (Jiang et al., 2013; Zhang et al., 2009). Besides, both the iso-butyrate and n-butyrate acids accounted for less than 10% of total VFAs at all TS contents, and iso-valeric acid was the lowest with no more than 5%. The distribution of VFAs in this study was similar with those reported in the literature (Feng et al., 2009; Zhang et al., 2009). However, it's worth noting that when the TS content increased to 15%, the percentage of propionate acid began to decrease, and the percentage of valerate acid increased at the same time. The bacteria might perform different metabolic pathways to form different fermentation products with different TS contents (Ucisik and Henze, 2008). In addition, the TS content might affect the behavior of the microbial community involved in the anaerobic fermentation (Eastman and Ferguson, 1981). For example, Yi et al. (2014) showed that the initial TS content affected the performance of food waste fermentation and also might change the microbial community in fermentation system.

3.3. Ammonium and phosphate release

It's important to prevent high NH_4^+-N and PO_4^{3-}-P concentration in the fermentation liquid, because excessive amounts of them would influence the conversion performance of VFA into the subsequent steps product and product quality (Dong et al., 2009). In anaerobic fermentation, NH_4^+-N is produced by the biological degradation of nitrogenous compounds, mostly in the form of proteins, which is hydrolyzed to amino acids and further degraded to ammonium (Ucisik and Henze, 2008). As shown in Fig. 5, with the TS content from 6% to 18%, the NH_4^+-N concentration was in a range of 380.1–531.2 mg/L after 7-day fermentation, and the NH_4^+-N yield increased with the increase of TS content. NH_4^+-N release ratio was in a range of 2.11–7.59 mg/g VS, which were much lower than that obtained from food waste fermentation with a range of 43.11–92.75 mg/g VS (Wang et al., 2015). The proteins in food waste were much more than that in SMC.

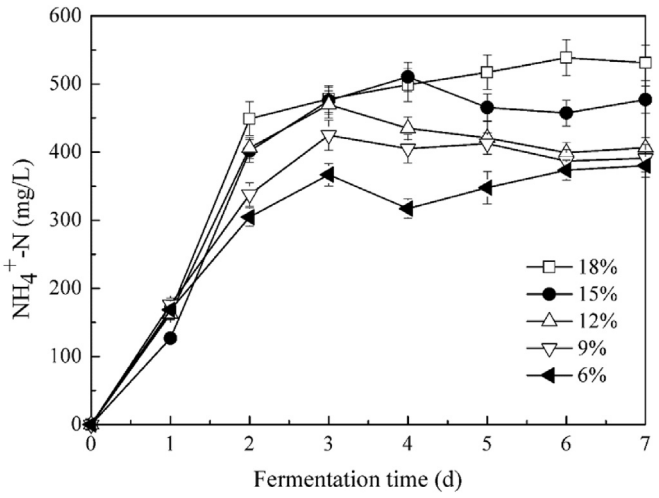

Fig. 5. Effect of TS content on ammonium release.

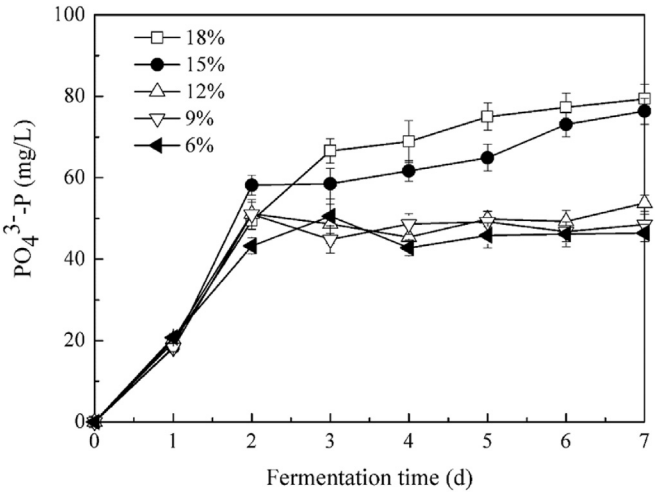

Fig. 6. Effect of TS content on phosphate release.

Similarly, as the degradation products of organic phosphorus in the hydrolysis and acidification process, the PO_4^{3-}-P increased from 46.4 to 79.4 mg/L when the TS content increased from 6% to 18% (Fig. 6). Correspondingly, the PO_4^{3-}-P release ratio was in a range of 0.26–1.13 mg/g VS, which obviously lower than that from sewage sludge fermentation with a range of 9.69–10.8 mg/g VSS (Kang et al., 2011). More PO_4^{3-}-P release might be caused by lipid degradation in sewage sludge (Miron et al., 2000). The NH_4^+-N release was higher than the PO_4^{3-}-P release, which was observed in the fermentation of other solid wastes as well (Banister et al., 1998; Chen et al., 2007).

Even though the NH_4^+-N and PO_4^{3-}-P concentration was lower compared to the fermentation of other solid wastes, the application of VFAs utilization might be significantly enhanced, if the NH_4^+-N and PO_4^{3-}-P was removed and recovered. From another perspective, the recovery of NH_4^+-N and PO_4^{3-}-P from the fermentation liquor can provide a pathway for fertilizer production. The simultaneous recovery of NH_4^+-N and PO_4^{3-}-P can be realized through the formation of struvite as a fertilizer (Sutton et al., 2011).

4. Conclusion

The performance of hydrolysis and acidification of SMC was influenced by the TS content. Considering the maximal VFAs production and fermentation efficiency, the optimal condition was a TS content of 15% and a fermentation time of 4 d. Suitable TS content improved the hydrolysis and VFA yields and released more soluble organics for acidification process by acid-forming bacteria. However, too high TS content limited the mass transfer between fermentation substrates and microorganisms and hindered the acidification rate. Different TS content could influence bacteria community and their metabolic pathways, which resulted in variation of VFAs composition. During fermentation of SMC, ammonium and phosphate were also released from organic substrates, which obviously lower than that in the fermentation of other solid wastes.

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