Journal of Bioscience and Bioengineering
VOL. xx No. xx, 1–9, 2017

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# Conversion of vegetable wastes to organic acids in leaching bed reactor: Performance and bacterial community analysis

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Received 26 July 2016; accepted 24 February 2017
Available online xxx

**Vegetable wastes (VWs), which contained abundant solid content, were digested in a leaching bed reactor (LBR). The tested VWs for acidification in the LBR included cabbage, potato, and tomato vine. The digestion in the LBR was carried out at the same organic loading rate of 25 g volatile solid (VS) per liter. It was found that cabbage and potato produced much more volatile fatty acids (VFAs) than tomato vine, probably because readily degradable components were more abundant in cabbage and potato. The retention time to digest each feedstock was different. It took 4, 5, and 8 days for cabbage, potato, and tomato vine to completely disappear in the reactor, respectively. The profiles of the VFAs generated from the three VWs were examined. In all cases, the predominant VFAs were acetic acid and butyric acid, although the relative abundance of individual VFA varied across the tested leachate samples. The bacterial community compositions of the leachates were analyzed by high throughput sequencing, and it was found that the feedstock strongly affected the bacterial community structure in the acidogenic process. All leachates had distinct bacterial community structure, although they did share a common set of core communities that included Proteobacteria, Firmicutes, and Bacteroidetes.**



[**Key words:** Vegetable wastes; Acidification; Volatile fatty acid; Leaching bed reactor; Bacterial community]

Vegetable wastes (VWs) are generated in large quantities in markets and crop lands, and they may cause considerable environmental pollution if not properly treated. Anaerobic digestion is an excellent means to dispose the VWs in large amount because they contain abundant volatile solids and are easily degradable. However, the rapid hydrolyzation of VWs can result in acid accumulation, which will inhibit methanogenesis if the reactor is overloaded. Ganesh et al. (1) summarized that the maximum organic loading rate (OLR) for the single-phase anaerobic digestion of fruit and vegetable wastes was within 3.6 kg VS/($m^3$·d). Shen et al. (2) found that volatile fatty acids (VFAs) would accumulate to 2.3 g/L if the OLR increased to 3.5 kg VS/($m^3$·d). Mata-Alvarez et al. (3) investigated the single-stage digestion of vegetable and fruit wastes and found that the OLR was limited to less than 3 kg VS/($m^3$·d). Since the rapid production and accumulation of VFAs can limit the OLR, co-digestion of VWs with other organic wastes has been introduced as a remedy to improve buffering capacity (4). However, co-digestion depends on feedstock availability and transportation efficiency (5). Alternatively, two-stage anaerobic digestion has been proposed as a viable solution that outperforms the traditional single-stage process in both process efficiency and energy recovery (6,7). The two-stage anaerobic digestion process involves an acidogenesis unit and a methanogenesis unit connected in series (8). The acidogenesis unit has strong buffering capacity of the OLR, and guarantees a constant feeding rate to the methanogenisis unit. Consequently, this approach allows a more stable operation and higher OLR capacity than the traditional single stage anaerobic digestion (2,7,8).

The continuous stirred-tank reactor (CSTR) is usually used as the acidogenic reactor to treat materials with high solid content, and diluting the materials to an appropriate total solid concentration can generate large amounts of effluent and thus require large storage capacity. Fortunately, leaching bed reactor (LBR) has been developed for use as the acidogenesis unit in the two-stage anaerobic digestion (9). The LBR has distinct advantages over the most often applied CSTR. The LBR is especially suitable for treating materials with high solid content, because the leachate can be circulated to increase the accessibility of substrate and microbes. As a result, the volume of the effluent is much lower, and the energy consumption can be reduced as well. The LBR leachate contains high concentrations of dissolved organic matters, mainly in the forms of VFAs, and they can be further treated in the methanogenic reactor (10).

The microbial community composition is a key factor in the anaerobic digestion of VWs. High throughput sequencing is widely used to analyze the microbial community compositions (11–14), because it can generate one million bp reads with an average length of over 400 bp and can provide much better information of the microbial communities than traditional methods such as DGGE and PLFA (15). The microbial community diversity can help regulate the digestion process. However, little information is available regarding

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http://dx.doi.org/10.1016/j.jbiosc.2017.02.020

**TABLE 1.** Characteristics of cabbage, potato and tomato vine.

| | Cabbage | Potato | Tomato vine |
|---|---|---|---|
| Total Solid (TS, %) | 6.7 | 18.2 | 32.7 |
| Volatile Solid (VS, %-TS) | 92.0 | 94.6 | 92.3 |
| Carbon (%-TS) | 38.0 | 40.6 | 40.7 |
| Nitrogen (%-TS) | 2.9 | 1.9 | 4.4 |
| C/N | 13.1 | 21.4 | 9.3 |

how the microbial community affects the acidification of specific materials in the LBR.

This study examines the production of VFAs in the anaerobic digestion of VWs in an LBR. Three different kinds of feedstock (cabbage, potato, and tomato vine) were tested and their fermentation behaviors during the leaching process were analyzed. The key indicators included the yield and relative abundance of VFAs, the pH value, and the chemical oxygen demand (COD). In addition, the structure and diversity of the bacterial communities were also evaluated by high throughput sequencing.

## MATERIALS AND METHODS

**Substrate and inoculum** The VWs used in this study were obtained from a market in Jinan, China. The VWs were shredded to 3–5 cm before feeding. The general properties of the VWs are summarized in Table 1. The sludge used as the inoculum for the reactors was collected from Jinan Wastewater Treatment Plant, Jinan, China. The inoculum was firstly incubated at 36°C for 7 days to deplete the existing nutrients in the inoculum and hence decrease the endogenous biogas production. The total solids (TS) content and the volatile solids (VS) content of the inoculum were $7.4 \pm 0.4\%$ and $21.3 \pm 0.4\%$-TS, respectively.

**Design of the leaching bed reactor** The acidification process was carried out in leaching bed reactors (12 cm in diameter, 50 cm high), which were double-walled glass reactors of 4 L effective volume, maintained at 35°C by a regulated water bath. The leaching bed was mounted vertically, and the perforated plate that supported the substrate was fitted at the bottom. The top plate had a port to allow the gas to vent into a gas collection bag. The leaching bed was operated in down-flow mode, and this was achieved by pumping the leachate to the top of the reactor and letting it flow through the substrate bed. Each reactor was equipped with a leachate reservoir tank with a holding capacity of 5 L.

To start the anaerobic digestion, different kinds of VWs (cabbage, potato, and tomato vine) were added into the reactors, and a mixture of the sludge and tap water (total volume 2 L) was added as the initial leachate. The system was initially operated with 100 g VS for the acidification. The anaerobic sludge was supplemented to maintain an inoculum-to-substrate ratio of 1:10 on VS/VS basis. The recirculation of

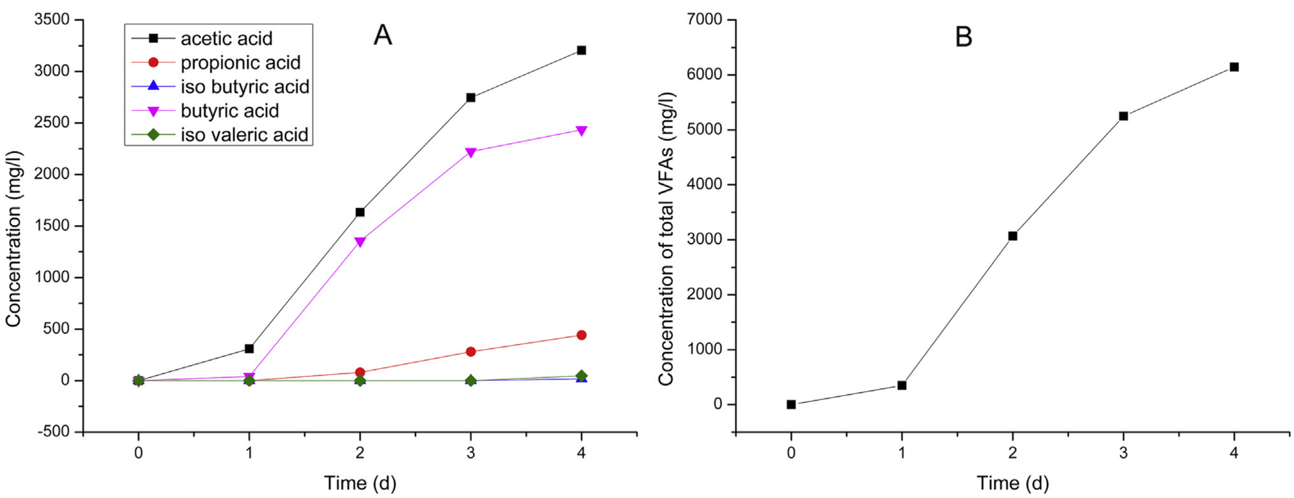


FIG. 1. Distribution and concentration of volatile fatty acids in the leachate of cabbage.

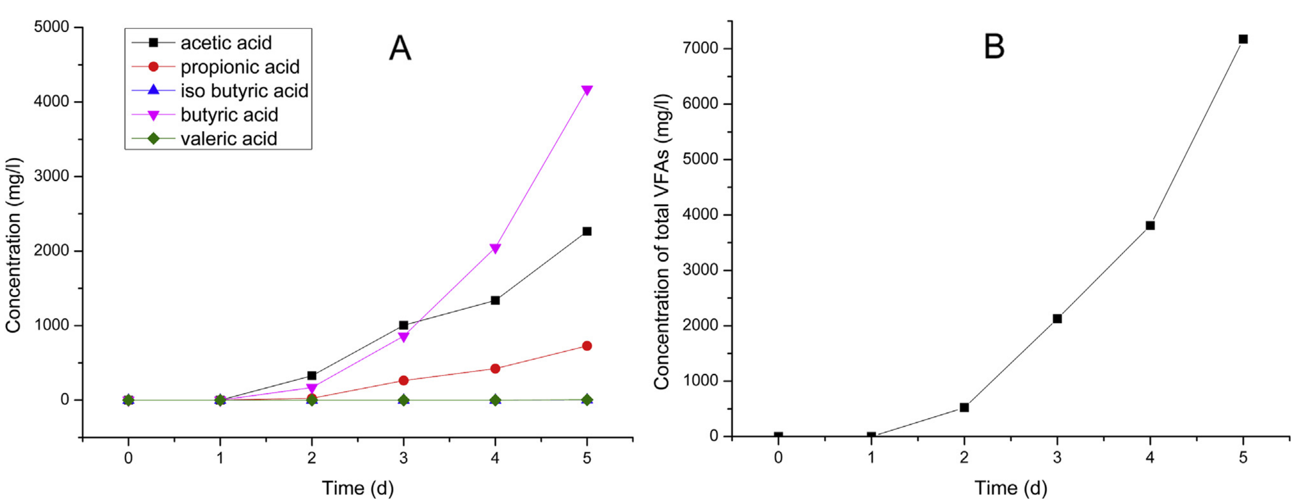


FIG. 2. Distribution and concentration of volatile fatty acids in the leachate of potato.

Please cite this article in press as: Li, Y., et al., Conversion of vegetable wastes to organic acids in leaching bed reactor: Performance and bacterial community analysis, J. Biosci. Bioeng., (2017), http://dx.doi.org/10.1016/j.jbiosc.2017.02.020