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Microbial community distribution and extracellular enzyme activities in leach bed reactor treating food waste: Effect of different leachate recirculation practices

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HIGHLIGHTS

- Continuous leachate recirculation is ideal for food waste hydrolysis in LBRs.
- L/S ratio of 1:1 and buffer addition selectively enriches hydrolyzing bacteria.
- Key enzymes in LBRs are α-mannosidase, α-fucosidase, lipase and β-galactosidase.
- *Lactobacillus* sp. was found to be predominant in food waste treating LBRs.

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ABSTRACT

This study aimed at understanding the relationship between microbial community and extracellular enzyme activities of leach bed reactor (LBR) treating food waste under different leachate recirculation practices (once per day and continuous) and liquid to solid (L/S) ratios (1:1 and 0.5:1). Microbial community analysis using PCR-DGGE revealed that *Lactobacillus* sp., *Bifidobacter* sp., and *Proteobacteria* were the most abundant species. Number of phylotypes was higher in LBRs with intermittent recirculation; whereas, lower number of phylotypes dominated by the key players of degradation was observed with continuous recirculation. The L/S ratio of 1:1 significantly enhanced the volatile solids removal compared with 0.5:1; however, this effect was insignificant under once a day leachate recirculation. Continuous leachate recirculation with 1:1 L/S ratio significantly improved the organic leaching (240 g COD/kg volatile solid) and showed distinct extracellular enzyme activities suitable for food waste acidogenesis.

1. Introduction

In Hong Kong, food waste (FW) represented about 36% of the municipal solid waste (MSW) stream disposed in landfills during 2012 (HKEPD, 2014). The FWs are generally characterized with high moisture (>70%), volatile solids (85–92% of total solids) contents and low carbon to nitrogen (C/N) ratio of 14.5–20.0% (Browne et al., 2013; Zhang et al., 2007a). Therefore, anaerobic digestion of FW is considered a more feasible option due to its energy recovery and associated greenhouse gas mitigation benefits. Due to technical simplicity and high organic loading/conversion rates, dry anaerobic digestion technology (>20% of total solids) is considered to be more advantageous over the wet technology (<10% of total solids) to treat the highly biodegradable waste components like FW (Karthikeyan and Visvanathan, 2013).

The leach bed reactor (LBR) is conceptualized by Ghosh (1981) for treating high-solid organic substrate and more commonly used for FW treatment under single or two-phase configurations in recent studies (Browne et al., 2013; Selvam et al., 2010; Stabnikova et al., 2008; Wang et al., 2005; Xu et al., 2012; Zhu et al., 2009). Advantages of two-phase anaerobic digestion of FW using LBR and UASB (up-flow anaerobic sludge blanket) reactors were well documented in earlier publications (Browne et al., 2013; Lü et al., 2008; Wang et al., 2005; Xu et al., 2011). However, hydrolysis of FW is critically a rate limiting step depending on the solids retention time (SRT) in LBRs and subsequent biomethanation of organics in UASB which is essential to be well understood to improve the process rate.

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Leachate recirculation is the most commonly used approach (as detailed in Table 1) to improve the rate of hydrolysis/acidogenesis in LBRs that redistributes the available nutrient contents and buffer the system, leading to a more effective microbial activity. In many cases, combination of leachate recirculation with other process controls namely, particle size reduction (Kim et al., 2008), pH adjustment (Selvam et al., 2010; Xu et al., 2011), micro-aeration (Xu et al., 2014; Zhu et al., 2009), enzyme addition (Romano et al., 2009), inoculum addition (Charles et al., 2009) and temperature control (Lee et al., 2008), were also considered. But most of these studies did not have a clear understanding of metabolic complexity during hydrolysis/acidogenesis of FW in LBRs.

Each of the above mentioned process variables are expected to positively influence the distribution of microbial communities and associated extracellular enzyme activities within the LBRs under various stages of operation/waste stabilization (Cirne et al., 2007; Dearman et al., 2006; Lü et al., 2009; Wang et al., 2010). Therefore, a clear understanding is thus required for further control of the reactors positively. Lü et al. (2009) found divergence in microbial community and metabolites in anaerobic batch reactors due to the effect of pH. Dearman et al. (2006) found that the methane production rate is significantly correlated with the bacterial community distribution structure within the old and new LBRs operated in a sequential mode. The development and distribution of microorganisms in LBRs treating grass silage with continuous leachate recirculation revealed that bacteria belonging to Bacteroidetes, Betaproteobacteria, Alphaproteobacteria, and Gammaproteobacteria were the dominant ones (Wang et al., 2010). In addition, they found Archaea (hydrogenotrophic genus *Methanobacterium*) in the 10th and 17th day of leachate samples. Members of the phylum Firmicutes, Actinobacteria, Chloroflexi and *Flavobacterium* were reported from 1st stage LBRs while treating energy crops (Cirne et al., 2007).

Thus, it is very clear that the favorable physical and chemical conditions namely pH, buffering capacity and metabolite re-distribution through leachate recirculation, and feedstock characteristics were probably of equal importance for microbial distribution and effective enzyme production in LBRs. However, the available literature on the distribution of microbial diversity and enzyme activities associated with solid organic substrates under various liquid recirculation regimes are inadequate.

Thus, the main challenge for maturation of two phase technology for FW treatment is the inadequate information on the microbial dynamics under optimized acidogenic LBR. Particularly, the microbial metabolic complexity i.e., microbial composition, enzymes and metabolites distribution pattern in LBRs, as this governs the degradation and gas production rates, needs to be investigated. Therefore, this study aimed to investigate the metabolic complexity of hydrolytic/acidogenic LBRs treating FW under various leachate recirculation conditions (buffering) mainly to establish the correlation between microbial community and enzyme distribution patterns to address the existing knowledge gaps.

2. Methods

2.1. Food waste and inoculum

FW was prepared using bread, boiled rice, cabbage and cooked meat at 35%, 25%, 25% and 15% (on wet weight basis), respectively. Particle size of the FW was reduced to less than 10 mm before feeding into the reactor. The total solids (TS) and volatile solids (VS) contents of the FW were 39.5 ± 1.3% and 97.1 ± 0.8% of TS, respectively; and total organic carbon and total nitrogen contents were 56.4% and 4.5%, respectively. The active inoculum used as the seed in LBRs was collected from the anaerobic sludge digester at Shek Wu Hui wastewater treatment plant, Hong Kong and stored at 4 °C before use.

2.2. Reactor design, loading and operational sequences

Four identical LBRs, as reported previously (Xu et al., 2011), were used in this study. Each reactor was initially loaded with 1 kg of FW and 0.2 kg of anaerobic sludge as inoculum. About 75 g of wood chips, as a bulking agent, was mixed with the FW in all the LBRs to avoid the substrate compaction and channeling of leachate. Bulk density of the substrate mixture was 0.65 kg/L. Organic acids were found to be effectively leached from the FW with wood chips in the LBRs (Demirer and Chen, 2008; Xu et al., 2011).

The LBRs were loaded with food waste at two different liquid to solid (L/S) ratios of 1:1 (LBR-A and LBR-C) and 0.5:1 (LBR-B and LBR-D) and received intermittent (LBR-A and LBR-B) and

Table 1
Comparison of various leachate recirculation practices employed in previous studies using leach bed reactor.

Reactors (capacity)		Substrate	Operational conditions	Leachate recirculation sequences	Monitoring period and operating temperature	Organic removal (%)	Reference
1st stage	2nd stage						
LBR (200 L)	LBR (200 L)	OF-MSW	Batch mode (SEBAC systems)	Interexchange of leachates between the two LBRs (once in a day)	55 days @ 38 °C	NA	Lai et al. (2001)
LBR (3.9 L)	UASB	Food waste	Batch mode (BIOCELL system)	UASB (2nd stage) effluent recirculated into the LBRs (1st stage)	6 days @ 38 °C	70.3–72.5	Han and Shin (2004)
LBR (5.4 L and 80 L)	UASB	Food waste	Batch and semi-continuous modes (HASL system)	UASB (2nd stage) effluent recirculated into the LBRs (1st stage)	10 and 253 days (for batch) & 36 and 286 days (for semi-continuous) @ ambient temperature	77–78	Wang et al. (2005)
LBR (5.4 L)	UASB	Food waste	Batch mode (HASL system)	Recirculation of leachate mix (UASB + LBR) in LBR (1st stage)	14 days @ ambient temperature	NA	Stabnikova et al. (2005)
LBR (1.4 L)	UASB	Vegetable and fruit waste	Batch mode (Two-phase system)	Recirculation of leachate mix (UASB + LBR) in LBR (1st stage)	10 days @ ambient temperature	62–63	Lü et al. (2008)
LBR (7 L)	-	OF-MSW	Batch mode (DiCOM system)	Pre-aeration; methanogenic leachate flooding and recirculation	12 days @ 55–60 °C	41–42	Charles et al. (2009)
LBR (6.4 L)	UASB	Food waste	Batch mode (HASL system)	Leachate recirculation from LBR (1st stage) with 50% replacement of buffer solution	16 days @ 35 °C	69.4 (maximum value)	Present study