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Benefits to decomposition rates when using digestate as compost co-feedstock: Part II – Focus on microbial community dynamics

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ABSTRACT

Linkage between composting reactor performance and microbial community dynamics was investigated during co-composting of digestate and fresh feedstock (organic fraction of municipal solid waste) using 25 L reactors. Previously, the relationship between composting performance and various physicochemical parameters were reported in Part I of the study (Arab and McCartney, 2017). Three digestate to fresh feedstock ratios (0, 40, and 100%; wet weight basis) were selected for analysis of microbial community dynamics. The 40% ratio was selected because it was found to perform the best (Arab and McCartney, 2017). Illumina sequencing results revealed that the reactor with a greater composting performance (higher organic matter degradation and higher heat generation; 40% ratio) was associated with higher microbial diversity. Two specific bacterial orders that might result in higher performance were *Thermoactinomycetaceae* and *Actinomycetales* with a higher sequence abundance during thermophilic composting phase and during the maturing composting phase, respectively. *Galactomyces*, *Pichia*, *Chaetomium*, and *Acremonium* were the four fungal genera that are probably also involved in higher organic matter degradation in the reactor with better performance. The redundancy analysis (RDA) biplot indicated that among the studied environmental variables, temperature, total ammonia nitrogen and nitrate concentration accounted for much of the major shifts in microbial sequence abundance during the co-composting process.

1. Introduction

Composting and anaerobic digestion (AD) are the two biological treatment technologies widely used for the stabilization of organic waste (Pognani et al., 2012). Composting technology has higher substrate conversion rates as compared to AD and produce finished compost; however, AD has the benefits of energy recovery and reduction of greenhouse gas emissions. Unlike finished compost, the solid-state by-product (digestate) of AD is not stabilized enough for land application. Aerobic polishing (composting) has been reported as a suitable technology for further stabilization of the digestate (Abdullahi et al., 2008; Bustamante et al., 2013).

The composting process can be enhanced by direct microbial inoculation; however, it can be more economically beneficial to use a by-product such as digestate as an inoculant instead of purchasing or preparing cultivated microbes. The literature review revealed that the co-composting of anaerobic digestate with the organic fraction of municipal solid waste (OFMSW) can bring some advantages to the composting process (Monnet, 2003; De Baere, 2008; Szucs et al., 2012). Since both composting and AD processes are mediated by a wide range of various microorganisms, knowledge on the behaviour, interactions and dynamics of microbial populations is necessary for a better understanding of the co-composting of the OFMSW with digestate.

Both bacterial and fungal communities are present in a typical composting process where the activity of fungi is essential primarily in the maturation phase (Ryckeboer et al., 2003). Microbial populations may be present as active, inactive or spore forms during the composting and their activities are highly dependent on changes in the substrate's properties and physico-chemical conditions. In a study conducted by Partanen et al. (2010), five common bacterial phyla, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Proteobacteria and Deinococcus-Thermus*, were detected in 18 different full and pilot-scale composting facilities. Interestingly, four of these phyla (*Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria)*

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were also present in the AD process (Riviere et al., 2009). The three major classes of the phylum *Firmicutes* present in compost are *Bacillales*, *Clostridia* and *Lactobacillales*. Among them *Lactobacillales*, responsible for the production of lactic acid in the early stages of composting, have also been found in AD processes (Sundberg et al., 2013; Shin et al., 2010; Franke-Whittle et al., 2014). In addition, although composting is an aerobic process, even at the optimum working conditions, the presence of anaerobes is inevitable (Ryckeboer et al., 2003). The facultative anaerobes are possibly responsible for metabolite activities in the composting process. It is reported that anaerobic *Clostridia* and aerobic species of *Bacillus*, both affiliated within the phylum *Firmicutes*, are known to be responsible for metabolizing recalcitrant materials (e.g. cellulose and lignin) in the composting process (Partanen et al., 2010). Fungi also play a very important role, especially in the later stage of the composting process and in the degradation of materials such as lignin (de Bertoldi et al., 1983).

As compared to studies on bacterial communities, limited studies have been reported on the impact of AD fungi populations on the composting processes. The few reported studies however showed that fungi community is highly dependent on the substrate material and composting stages (Neher et al., 2013; Bonito et al., 2010; Ryckeboer et al., 2003).

It is well known that significant changes in microbial communities may occur due to the interactions taking place among the various populations in the composting process (Narihiro et al., 2004). Some of the bacterial communities degrade organic compounds and produce metabolites (e.g. antibiotics and enzymes) that can be detrimental or beneficial to other microorganisms. Aoshima et al. (2001) reported that lactic-acid bacteria secrete metabolites that can be detrimental to other microorganisms in the composting process, while *Acetobacter* sp., affiliated within the phylum *Proteobacteria*, can consume these substances for growth and possibly eliminate the harmful effects on other microbial populations (Partanen et al., 2010). Antagonistic interactions in which one species benefits at the expense of another may also take place during composting and result in changes of microbial populations. With respect to the presence of common microflora in composting and AD processes, using digestate as an inoculant during composting can alter the microbial interactions (e.g. mutualism and antagonism) and possibly enhance the process.

Aside from the benefits that may be obtained from the inoculation, the amount of inoculum is also essential to note. The quantity of inoculum introduced to the compost must be sufficient, otherwise the indigenous microorganisms in the compost do not allow the inoculum microflora to develop and effectively improve the process (Fuchs, 2010). Golueke et al. (1954) reported that composting inoculation has no significant effects on the process because the inoculated microorganisms may have been outcompeted by the indigenous microorganisms. However, Golueke et al. (1954) did not consider the effects of inoculum loading rates and thus different results could have been obtained if various amounts of inoculum had been applied. To better understand the impact of AD digestate on composting processes, information on the impact of AD bacterial and fungal communities and inoculum dosages on composting processes is needed.

The benefits of co-composting the OFMSW and digestate were investigated in the work presented herein. The relationship between composting performance and various physico-chemical parameters including total solids (TS), organic matter (OM), C:N ratio, specific oxygen uptake rate (SOUR), ammonium and nitrate content, pH, and electrical conductivity (EC) were reported in part I of this study (Arab and McCartney, 2017). The results reported in Arab and McCartney (2017) found that the addition of digestate to the OFMSW within the ratio of 20–40% provided the most enhanced composting process by increasing the specific OM removal rate (SOR) and relative heat generation (RHG). In addition, the specific oxygen uptake rate (SOUR) values calculated from respirometry analysis showed that the reactors with 20–40% (%ww) digestate reached the stability point in a significantly shorter period of time (30–36% shorter time) compared to the other reactors. In general, the reactor with 40% digestate (C40) performed best; therefore it was selected for more detailed comparisons to the two controls: zero digestate feedstock (C0) and 100% digestate feedstock (C100) in Part II of the study discussed herein. The objective of Part II was to investigate the digestate benefits in terms of biological parameters, with a focus on microbial community dynamics. The bacterial and fungal diversity at different stages of composting, the correlation between microbial community structure and dynamics, and important environmental parameters were also evaluated.

2. Methodology

2.1. Material used, equipment and operation

Digestate and the organic fraction of municipal solid waste (OFMSW) were the two main feedstocks for the composting reactors. The digestate and OFMSW (fresh feedstock) were mixed in ratios of 0, 40, and 100% (digestate: OFMSW; wet mass). These reactors were coded as C0, C40, and C100. C0 was considered the OFMSW (fresh feedstock) control as no digestate was added to this reactor. C100 was considered the digestate control as no OFMSW was added to this reactor. Full details for the preparation of the reactor materials were described in Arab and McCartney (2017).

The composting experiment was operated and monitored in two stages: aeration and curing. The aeration stage was conducted in an airtight reactor with a working volume of 25 L for 30 days. The second stage (curing/maturation) was conducted for 70 days in 20 L pails with perforated ends on the bottom and top to allow natural ventilation. More details about the instruments used in the setup and operational factors such as aeration, temperature, and compressive loading can be found in Arab and McCartney (2017).

2.2. Microbial community analysis

2.2.1. Sampling and DNA extraction

The representative samples in the reactors were collected at different stages of co-composting, e.g. sampling on day 0 represented the starting or initial phase. Sampling on days, 6, 30, and 100 represented thermophilic phase, post-aeration phase, and the maturing phase, respectively. The DNA was extracted from representative samples collected from all reactors and from the two main feedstocks: OFMSW (C0) and digestate (C100).

The total genomic DNA was extracted from approximately 500 mg of well-homogenized sample using a PowerSoil® DNA isolation kit (MoBio Laboratories, Carlsbad, USA) according to the manufacturer's instructions. For each reactor, DNA was extracted from three replicate samples. A NanoDrop® 2000C spectrophotometer was used to determine the concentrations, quality and integrity of the extracted DNA. Extracted DNA samples were stored at −20 °C until submitted to the microbiology lab for analysis.

2.2.2. Illumina sequencing analysis

The targeted gene sequences were amplified and Illumina Miseq Sequencing was performed at the Research and Testing Laboratory (Lubbock, TX, USA). Samples were amplified for sequencing in a two-step process; forward and reverse fusion primer. The forward primer was constructed with the Illumina i5 sequencing primer (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG) and the 28F primer (GAGTTTGATCNTGGCTCAG) for bacteria and ITS1F primer

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