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Biochemical and microbial changes reveal how aerobic pre-treatment impacts anaerobic biodegradability of food waste



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ABSTRACT

Aerobic pre-treatment of food waste (FW) was performed at different oxygen concentrations (0%, 5%, 10% and 21%O₂) and different durations (1, 2, 3 and 4 days) to investigate its impact on biochemical and microbial community characteristics of the waste and its ability to improve anaerobic biodegradability. Whatever the duration, the highest effect of pre-treatment was observed at full aerobic pre-treatment (21%O₂) while 5%O₂ and 10%O₂ showed lower transformation performances. Biochemical variations at 21%O₂ were mainly a decrease of simple carbohydrates, volatile fatty acids (VFA) and low molecular weight water soluble compounds and an increase of high weight water soluble compounds. Microbial community analysis showed a clear modification of populations after 21%O₂ aerobic pre-treatment, changing from an initial dominance of lactic acid bacteria to a final dominance of VFA consumers (like *Acetobacter*) and a higher presence of *Fungi*. Enzymatic tests showed an increase of exoenzymes content and a higher presence of protein and carbohydrates degrading enzymes. Finally, the aerobic pre-treatment did not negatively impact methane potential of FW (496 NLCH₄·kgVS⁻¹) which remained unchanged after two days of pre-treatment at 21%O₂. These latter optimal pre-treatment conditions are proposed to be tested in future investigation of anaerobic digestion (AD) process with low inoculum to substrate ratio in order to assess their ability to avoid acidification risk during AD of FW.

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1. Introduction

The management and treatment of food waste (FW) is a subject of growing interest worldwide. In Europe, about 89 Mt of FW are produced each year, mainly by households and food services (53% and 12% of the total FW respectively) (Monier et al., 2010; Stenmarck et al., 2016). Beyond waste prevention, EU is thus promoting FW valorisation, essentially via composting or anaerobic digestion (article 5 of EU Directive 99/31/EC).

Anaerobic digestion (AD) has proven to be an appropriate technique to treat FW, resulting in the production of an energy-rich biogas and a digestate which can be used as biological fertiliser (Li et al., 2011; Zamanzadeh et al., 2016). However, the stability and efficiency of AD of FW can be problematic on two points. On short-term, a rapid accumulation of volatile fatty acids (VFA), caused by the high concentration of simple carbohydrates in FW, may inhibit methane production (Ren et al., 2018; Scano et al., 2014; Zhang et al., 2012). A total inhibition can be observed at a VFA concentration of $40-50 \text{ gCOD-L}^{-1}$ (Veeken and Hamelers, 2000). On longer term, anaerobic conditions result in slow hydrol-

* Corresponding author. *E-mail address:* anne.tremier@irstea.fr (A. Tremier). ysis kinetics and only partial degradation of the structural carbohydrates of FW: only about 50% of the cellulose is degraded after 30 days of AD (Deublein and Steinhauser, 2011). Moreover, fermentation during storage step between the collection of the FW and its introduction in the AD treatment can produce an additional VFA accumulation contributing to instability (Fisgativa, 2016).

Several studies have been conducted to improve the AD yield and stability, using mechanical, thermal, chemical or enzymatic pre-treatments of the substrates (Brémond et al., 2018; Carrere et al., 2016; Kondusamy and Kalamdhad, 2014). Even though some pre-treatments demonstrated their ability to enhance AD, side effects as the release of recalcitrant and inhibitory molecules or further difficulty for digestate dewatering were also observed. These undesired phenomena, in addition to the difficulty of implementation and high energy requirements of pre-treatments, often counteracted their positive effects (Carlsson et al., 2012; Ma et al., 2011). In comparison, the interest of a biological aerobic pre-treatment of substrates before AD has been revealed more recently with a real potential. Indeed, it has been shown that pre-treating FW enhances the hydrolysis of slowly biodegradable compounds by promoting the microbial excretion of hydrolytic enzymes (Charles et al., 2009; Lim and Wang, 2013; Meng et al., 2016; Sarkar and Venkata Mohan, 2017). Ni et al. (2017) stated



an increase of methane production during municipal solid waste (MSW) dry anaerobic digestion with an 8-day sequencing preaeration because of accelerated degradation of proteins (airflow rate of 0.5 L min⁻¹ kg waste⁻¹ performed by cycle of 10 min aeration followed by a 20 min pause). This positive effect of aeration was also observed by Rafieenia et al. (2017) on methane production with one day pre-aerated $(5 \text{ L} \cdot \text{h}^{-1})$ protein-rich FW. However, the duration and conditions of aerobic pre-treatment should be well managed to not oxidise excessively the organic matter of FW and limiting the methane yield. Brummeler and Koster (1990) observed that 10-days of aerobic pre-treatment, on the organic fraction of municipal solid waste (OFMSW), resulted in lower initial acid formation during AD linked to the degradation of the easily degradable part of the substrate but induced a loss of 40% of methane production. Wu et al. (2018) showed that an aerobic storage of FW during 8 days produced a diminution of 15% of the methane vield comparing to raw FW. Charles et al. (2009) showed that carbon emitted as CO₂ (during six days of pressuring aerobic pre-treatment of OFMSW), accounts for the reduction of 26% of the CH₄ produced during subsequent AD. Finally, Rafieenia et al. (2017) observed that even a short (one day) aerobic pre-treatment causes a decrease of methane potential of a lipidrich FW, compared to a non-pre-aerated sample.

In spite of these studies, there is still a lack of knowledge on the biochemical and microbial community modifications promoted by such aerobic pre-treatments while there is a real challenge at the industrial level in assessing the ability of aerobic pre-treatment to limit the risk of instabilities potentially occurring during AD of FW. The aim of this study was thus (1) to understand how short time aerobic pre-treatments influence further anaerobic biodegradability of the waste and which variations of FW characteristics (physicochemical characteristics, biochemical fractionation, microbial populations and enzymes content), promoted by the pre-aeration explain this influence; and (2) to highlight which pre-treatment condition could be interestingly later tested in AD process conditions. The impacts of the oxygen level and of the pre-treatment duration were specifically assessed. Compared to previously published experiments that were performed at small laboratory scale (1-L bottles) with grounded FW (Meng et al., 2016; Rafieenia et al., 2017; Wu et al., 2018), our study was realised at a 10-L laboratory pilot scale with raw FW.

2. Materials and methods

2.1. Composition and sampling of food waste feedstock

The study used FW (kitchen waste and leftovers of the dishes served) produced by a collective catering establishment, which produces 17 tons of waste per year, corresponding to nearly 700 customers per day. FW was collected over a period of three days (stored at 4 °C) and mixed to obtain a representative sample with the following typological composition: 30% vegetables, 24% fruits, 19% meat, 13% starchy food, 9% bones and shells and 5% paper per wet weight (WW). Initial FW (hereafter referred to as T0 or raw FW) was sub-sampled and stored at -20 °C for later use in the experiments to ensure the same initial feedstock conditions. The characteristics of the raw FW (analysed as described in Sections 2.4 and 2.5 below) are listed in Table 1.

2.2. Experimental pre-treatment set-up

The pre-treatment pilot consisted of four 10-L glass cells equipped with a gas flow inlet and outlet. The cells containing 3 kg of FW were maintained in a water bath heated by an automated thermostat at a temperature of 37 °C. This temperature is

Table 1

Characteristics of the raw food waste.

Parameter	Raw FW (T0)
рН	5.3
Total solids (TS, g·kgWW ⁻¹)	295.3 ± 14.7
Volatile solids (VS, g·kgWW ⁻¹)	256.5 ± 13.6
Volatile solids (VS, g·kgTS ⁻¹)	868.9 ± 20.9
Total chemical oxygen demand (COD, $gO_2 \cdot kgVS^{-1}$)	1714.0 ± 51.4
Total carbon (C, %VS)	56.1 ± 4.0
Total nitrogen (N, %VS)	3.7 ± 0.4
C/N ratio (%)	15.1
Carbohydrate (%VS)	68.8
Protein (%VS)	25.0
Fat (%VS)	6.2
Volatile fatty acids (VFA, g·kgVS $^{-1}$)	9.8
Ammonium content (NH ⁺ ₄ , g·kgVS ⁻¹)	0.3 ± 0.01
Biomethane potential (BMP, NLCH ₄ ·kgVS ^{-1})	496.0 ± 31.9

where WW- wet weight.

close to conditions of mesophilic AD processes. The temperature within the cell contents was recorded using a Platinum resistance thermometer. The pre-treatment tests were carried out by circulating 50 L·h⁻¹ of gas, to optimise the aerobic degradation of FW as described by Tremier et al. (2005), with a recirculation rate of 300 L·h^{-1} to ensure homogeneous conditions. The inlet of gas was located at the bottom of the cell and the gas flowed through the waste to reach the outlet located at the top of cell. The O₂ content in the gas flow entering the experimental cell was set at 0%, 5%, 10% or 21% by mixing compressed air with nitrogen (N₂) to study the effects of anaerobic conditions (0%O₂), limited oxygen conditions (5%O₂ and 10%O₂) and ambient air or full aeration conditions (21%O₂). Incoming and outgoing O₂, CO₂, N₂O and CH₄ concentrations in the air of each cell were measured at 3-min intervals using an ABB gas analyser (model URAS26EL3020, ABB).

Because it is not possible to get a homogeneous FW sample from a cell without disturbing waste characteristics, doing a time course experiment required running as many cells as the number of samples needed. Thus, for each O_2 concentration, four cells were started at the same time and one cell was stopped every day of the 4-day experiment in order to collect and sample the waste and to measure variations in FW characteristics. Along this paper, the durations of 1, 2, 3 and 4 days of pre-treatment, regardless the oxygen conditions, will be also referred as T1, T2, T3 and T4 respectively. The maximal duration of 4 days of pre-treatment was chosen to limit excessive organic matter degradation in aerobic conditions according to previous studies (Charles et al., 2009). The total content of each cell, thereafter called treated FW, was immediately homogenised and frozen at -20 °C prior to analyses.

2.3. Samples preparation

One kilogram of each frozen sample (raw FW and treated FW from each cell) was ground to 2 mm using a Robot Coupe R15 Food Processor for physicochemical and biochemical analyses. About 7 g of these ground FW samples were further ground in liquid nitrogen for 1 min with a ball mill (300 Dangoumill, Prolabo) to obtain a homogeneous powder, which was stored at -80 °C for further molecular microbiology and enzymatic analyses.

2.4. Physicochemical and biochemical analyses

Total solids (TS) were analysed after drying at 80 °C until constant weight. Volatile solids (VS), chemical oxygen demand (COD) total Kjeldahl Nitrogen (TKN) and ammonium (NH_4^+) were measured using standard methods (APHA, 2012).

Aqueous extraction was performed to separate water soluble (SOL_W) and non-water-soluble (NSOL) organic matter. The NSOL

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