

The aeration rate in the aerobic degradation of the organic fraction of municipal solid waste

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Abstract

Microbial and enzymatic kinetics are important factors during the aerobic degradation of the organic fraction of municipal solid waste, these depend mainly on the incubation temperature and aeration rates. The objective of this research was to evaluate the process of aerobic degradation, by multiple variables and their combination to understand the interactions between aeration rates in aerobic degradation and their responses. Aeration rates were set at 0.032, 0.064, 0.125, 0.251 and 0.392 L of moist air kg⁻¹ min⁻¹ at 35 °C with inoculum. Microbial activity was evaluated indirectly by means of respirometry, that is, CO₂ production and O₂ consumption. Extracellular enzymatic activities (ie., pectinases, cellulases, xylanases and proteases) were quantified by releasing the reducing sugars. The different tests were carried out at the Metropolitan Autonomous University, Iztapalapa Unit in September 2019. Finding a strong positive relationship between xylanase and pectinase enzymatic activity and dry weight loss, along with increased cellulase and xylanase activities at higher aeration rates.

Keywords: enzymatic activities, respirometry, aeration rate.

Reception date: June 2021

Acceptance date: August 2021

Introduction

Oxygen is essential for microbial activity during aerobic degradation (Guo *et al.*, 2012). A minimum oxygen concentration of 5% (v/v) in the center of the compost pile is necessary to ensure aerobic conditions (Rasapoor *et al.*, 2009). The optimal aeration rate depends on the composition of the raw materials and ventilation methods (Komilis *et al.*, 2011). If aeration is expressed as flow intensity, that is, air flow per unit mass of a substrate, a variable independent of the scale is obtained (Rodríguez-Fernández *et al.*, 2012). This, in fermentation in solid medium, is generally expressed as the average airflow rate (L) per kilogram of a substrate per time (min) ($v \text{ kg}^{-1} \text{ m}^{-1}$ or vkgm, as used throughout the writing).

The temperature and moisture gradients of the solid mass must be controlled to achieve a satisfactory process (Martins *et al.*, 2011). The temperature range between 35-40 °C favors the development of a diverse microbial consortium (Saludes *et al.*, 2007). The increase in temperature in the compost pile is related to the increase in microbial activity (Raut *et al.*, 2008; Li *et al.*, 2013). The quality (stability and maturity) of the compost is determined by multiple variables or their combination (Bernal *et al.*, 2009).

Several authors have attempted to develop mathematical, stochastic and statistical models to understand the multiple relationships and interactions between operational conditions and composting responses (Li *et al.*, 2013). Multivariate statistical methods have been used to process data from the anaerobic digestion process, in which attempts are made to correlate biochemical composition with methane yields (Bayard *et al.*, 2015; Liu *et al.*, 2015; El Achkar *et al.*, 2017). The combined use of principal component analysis (PCA), cluster analysis, and biodegradability testing can help correlate nutrient levels and microbial activity in anaerobic digestion (Gil *et al.*, 2018).

During composting processes, PCA has been widely applied to classify the generation of bad odors and their correlation with different organic waste and their composition (Toledo *et al.*, 2018). PCA algorithms employ physicochemical parameters in both aerobic and anaerobic processes. The composting process can be characterized well by extracellular enzymatic activity (Tiquia *et al.*, 2002). Based on the above, the objective of this work was to evaluate the process of aerobic degradation based on various variables to model the relationships and interactions between the aeration rates during composting and the response variables such as: oxygen (O₂) consumption, carbon dioxide (CO₂) production, the consumption of reducing sugars and the xylanases, cellulases, proteases and pectinases enzymatic activities.

Materials and methods

Substrates and inoculum

The organic fraction of municipal solid waste (OFMSW) was obtained from food processing establishments in Iztapalapa, in Mexico City (Mexico). The stable compost of the mixed municipal solid waste composting plant of the Bordo Poniente of Mexico City (CPBP) was used as a source of microbial consortia (Mc) as an inoculum. The taking of the compost of the CPBP was carried out in accordance with the standard ASTM D-34 (2016).

Experimental strategy

The degradation of OFMSW was carried out in four tubular bioreactors (TB) with capacities of 0.98, 1.91 and 3.84 L. The TBs were packed with a wet-based mixture of: OFMSW 83%, microbial consortium 8%, sawdust 4%, paper 3% and pruning waste 2% to achieve an initial C/N ratio of 30 (Martínez-Valdez *et al.*, 2015). The initial moisture was 70% (Liang *et al.*, 2003). During aerobic degradation, aeration rates increased by a proportion of 0.5 in the different experiments (0.032, 0.064, 0.125, 0.251 and 0.392 vkgm). The respirometry system was operated according to Saucedo-Castañeda *et al.* (2013); Torres-Mancera *et al.* (2018). The calibration of the respirometry equipment was done with standard mixtures of CO₂ y O₂.

Identification of microorganisms

All TBs were inoculated (CPBP compost) and tests were performed at 35 °C due to the increased microbial activity (shown by respirometry) at that temperature (Martínez-Valdez *et al.*, 2015). Once the process was completed, the TBs were unpacked, the material was weighed on an analytical balance (Ohaus Galaxy[®] 200) and the moisture, pH and enzymatic activity were determined. All samples were obtained at the time of maximum CO₂ generation rate, as this indicated the highest microbial activity.

The analysis of the microbial population was performed using the technique of denaturing gradient gel electrophoresis (DGGE): samples were taken under all conditions and centrifuged at 8 049.6 g. The precipitate (1 g) was taken, and the DNA extraction and purification was carried out using the PowerSoil[®] DNA isolation kit (MoBio). Once the extraction and purification were done a 1% agarose gel was run to corroborate the success of the DNA extraction. Subsequently, a polymerase chain reaction (PCR) was performed to obtain *in vitro* copies of a fragment of the sequence of the 16S rRNA gene from each of the samples, which was carried out in a thermocycler (Multigene, Labnet International, Inc.).

The reaction mixture, with a final volume of 50 µl, contains 10 X of buffer, 1.5 mmol of MgCl₂, 200 µm of each dNTP (Promega Corporation, USA), 10 pM of each primer, 1.5 U of GoTaq[®] Flexi DNA Polymerase (Promega Corporation, USA) and 1-20 ng µl⁻¹ of DNA template. The amplification conditions were as follows: 2 min at 95 °C of initial denaturation, 25 cycles consisting of 30 s at 95 °C, 30 seconds at 50 °C and 1 min at 72 °C, followed by a final extension of 5 min at 72 °C. The amplicons obtained were stored at 4 °C after running by electrophoresis (100 V, 30 min) in a 1% agarose gel. After amplification of the 16S rRNA gene sequence, a new nested amplification was made using the previously obtained amplicon as a DNA template.

The primers 1070F (5' ATG GCT GTC GTC AGC T 3') and 1392R+GC (5' CGC CCG CCG CGC CGC CCC GCC GCC GCC GCC CCG CCC CCC ACG GGC GGR GRG TAC 3') were used using the same amplification conditions by changing the alignment temperature to 55°C. The samples were purified with a commercial kit (Wizard[®] SV Gel and PCR Clean-up System, Promega Corporation) following the protocol described by the supplier. The quantification of DNA nucleic acids was measured with the NanoDrop ND1000 spectrophotometer (Thermo Scientific). There was 1 µl with 500 ng of DNA from each sample added to 5 µl of loading buffer (0.25% bromophenol blue; 0.25% xylene cyanol; 30% glycerol, in water).

Amplicon profiles were analyzed with the universal mutation detection system DCode (Bio-Rad Laboratories, Hercules, CA) using acrylamide gels of 16 cm x 16 cm, using a 7% acrylamide gel with a denaturation gradient of 30-60%, with a ratio of (37.5:1), which were filtered at 0.45 μm . To do this, two solutions were prepared for the elaboration of the degradation gel from 30 to 60%. The 30% solution was prepared using 12.5 ml of 40% acrylamide/Bis, 2 ml of TAE 50X, 12 ml of formamide and 12.6 g of urea Molecular Biology grade (Sigma-Aldrich) and 60.9 ml of milli Q water per 100 ml. The 60% solution contained 12.5 ml 40% acrylamide/Bis, 2 ml of TAE 50X, 24 ml of formamide, 25.2 g of urea and 36.3 ml of milli Q water per 100 ml.

Both solutions were filtered through a 0.45 μm filter and degassed. The formation of the gel was carried out by the BioRad 475 gradient former, which was loaded with 20 ml of denaturing solution, 70 μl of 10% persulfate and 10 μl of TEMED. Gel electrophoresis was performed for 16 hours in TAE buffer (0.5 X, pH 8) at 60 °C using a PowerPac universal power supply (Bio-Rad), with an initial voltage of 200 V for 10 min, then the voltage was reduced at 85 V for 16 h (Neilson *et al.*, 2013). The gel was stained according to the technique described by Radojkovic and Kušic (2000). The staining procedure consisted of an initial pre-stain fixation for 3 min in 10% ethanol, 0.5% acetic acid.

Staining for 5 min in fixation solution plus 0.2% silver nitrate, the washing of the gel in water for 2 min, and development for about 5 min in 30% NaOH and 0.1% formaldehyde in Milli-Q water. Once the gel was run, the identified bands were cut, and a 5 min extraction was performed. With water and stir. A 30% agarose gel (80 V, 45 min) was applied to corroborate the presence of DNA and then sequencing was performed by Macrogen.

Evolutionary history was deduced using the maximum likelihood method based on the Kimura 2-parameters model (Kimura, 1980), previously selected 8 by a Bayesian information criterion. The tree with the highest probability of trunk (-1499) is shown. The percentage of trees in which the associated taxa were grouped is shown next to the branches.

The initial trees for the heuristic search were obtained automatically by applying the Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach and then selecting the topology with the superior log likelihood value. The tree is drawn to scale, with the lengths of the branches measured in the number of substitutions per site. The analysis used 32 nucleotide sequences. All positions containing gaps and missing data were removed. There were 239 positions in the final dataset. Evolutionary analyses were performed in MEGA7 (Kumar *et al.*, 2016).

Measurement of enzymatic activity

The activities of the extracellular enzymes, pectinase, cellulase and xylanase, were quantified by the release of reducing sugars. Reactions were carried out in a citrate buffer (50 mmol, pH 5.5). Pectinase activity was measured using 1% birch pectin incubated at 37 °C for 30 minutes (Zhang *et al.*, 2000). Cellulase activity was quantified using 2% carboxymethylcellulose incubated at 50°C for 30 minutes (Ghose, 1987).

Xylanase activity was measured using 1% xylan from birch wood incubated at 50 °C for 15 minutes (Loera and Córdoba, 2003). All reaction volumes were: 750 ml of substrate with 250 ml of enzymatic extract, mixed in an orbital stirring of 250 rpm. The solutions were measured at a wavelength of 540 nm with a Beckman® DU 640 spectrophotometers.

Protease activity was determined using a modified method from Alef and Nannipieri (1995). An aliquot of 1 ml of enzymatic extract was added to 5 ml of 2% casein solution and incubated at 50 °C under stirring for 2 h. Enzymatic activities were expressed as U per gram of dry matter, where 1 unit (U) is the amount of enzyme that catalyzes the generation of 1 μ mol of product per minute under enzymatic reaction conditions.

Statistical analysis

All measurements and tests were performed in triplicate. A multi-variable analysis ($\alpha= 0.05$) and the principal components analysis were performed using Statgraphics® Centurion XVII.

Results and discussion

Effect of aeration during composting

To analyze the effect of the aeration rate (vkgm), the CO₂ content (v/v) produced under all conditions was evaluated (Figure 1). CO₂ production was 31, 20, 14, 9, and 2% at 0.032, 0.064, 0.125, 0.251, and 0.392 vkgm, respectively. A principal component analysis (PCA) is shown in Figure 2a. The PCA showed that a main group containing cumulative CR, instantaneous CR, pectinase and CO₂ as grouped variables, which are associated with each other, and another main group contained reducing sugars, xylanase and cellulase (Figure 2a). In addition, it was observed that vkgm and protease are negatively correlated with pectinase, CO₂ and CR.

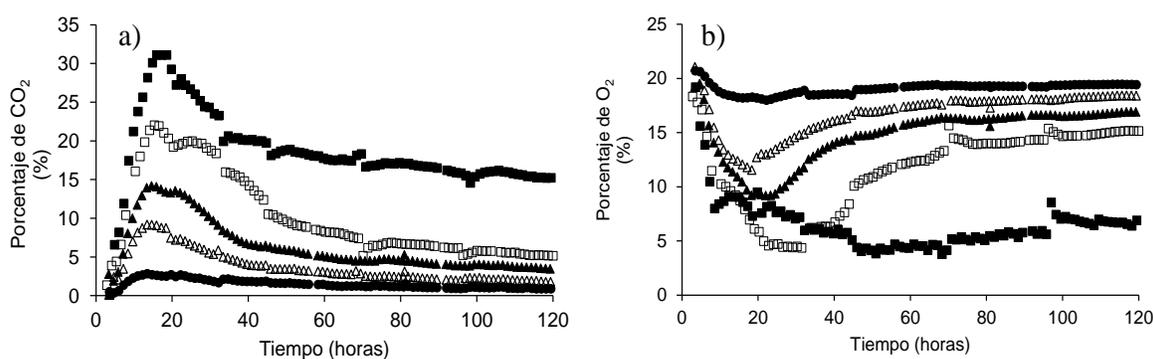


Figure 1. Concentration of a) CO₂; and b) O₂ at the gaseous outlet during the aerobic degradation of OFMSW at different aeration rates (vkgm) ■ 0.032, □ 0.064, ▲ 0.125, △ 0.201 and ● 0.392).

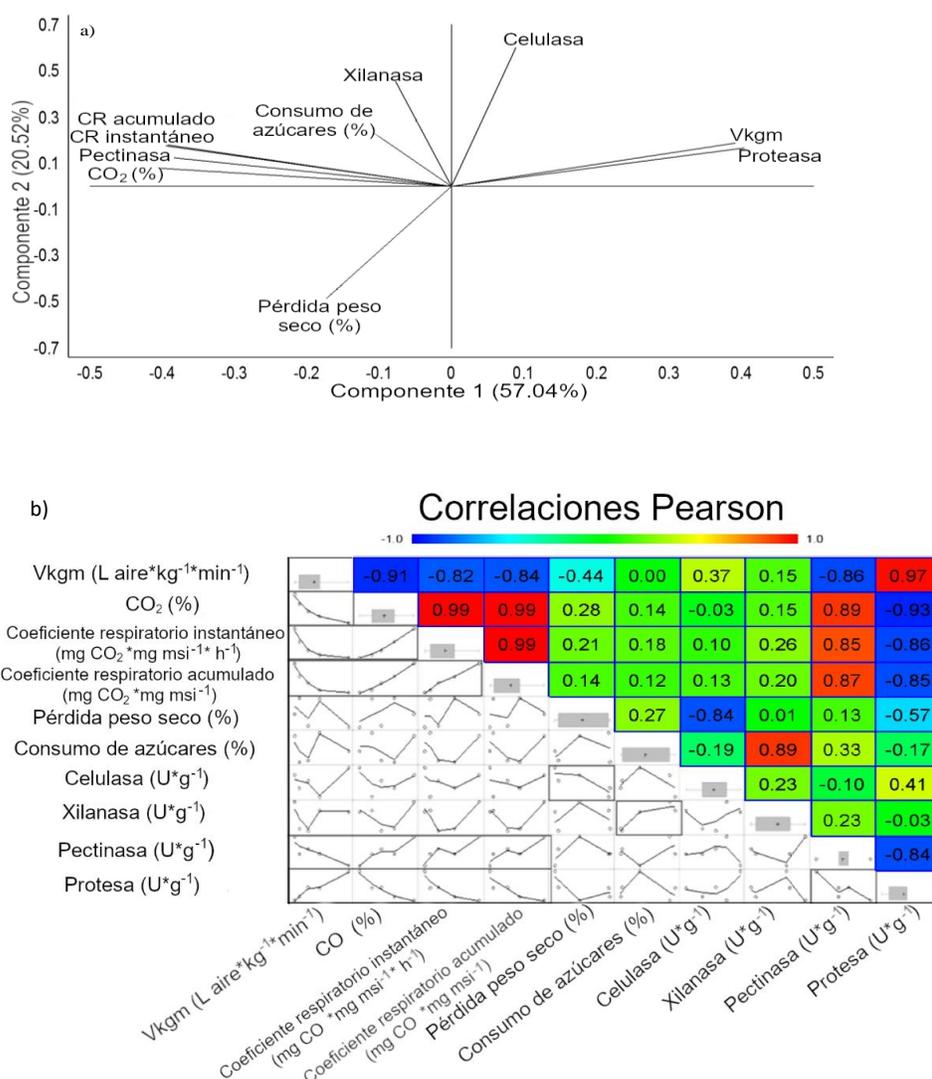


Figure 2. a) principal component analysis (PCA); and b) correlation matrix and Pearson’s correlation coefficients between aeration rate, physicochemical, respirometric and enzymatic responses determined.

The analysis showed that a 3-component model explains 96.34% of the variability and a 2-component model explains 76%. A dispersion matrix and Pearson’s correlation coefficients between each pair of variables are included in Figure 2b. It was observed that the aeration rate (vkgm) has a significant negative correlation with respect to pectinase activity (-0.86) and with respirometric activity responses such as CO₂ content (-0.91), instantaneous CR (-0.82) and cumulative CR (-0.84). on the other hand, vkgm has a significant positive correlation with protease activity (0.97).

In addition, the high activity of proteases indicates that soluble proteins are degraded by proteases, and it could be a consequence of pectinase activity being reduced as a result. Protease activity has a significant positive correlation with cellulase activity (0.41). These observations

were also reported by Goyal *et al.* (2005); He *et al.*, (2013), who observed an increase in protease and cellulase activity during the active phase of composting. Kayikçioğlu *et al.* (2011) reported that the maximum protease activity coincided with the maximum microbial activity.

The PCA revealed that pectinase has a significant positive correlation with the respirometric indices, namely CO₂ (0.89), instantaneous CR (0.85) and cumulative CR (0.87) and a negative correlation with vkgm (-0.86). Meanwhile, proteases have a significant negative correlation with respirometric indices such as CO₂ (-0.93), instantaneous CR (-0.86) and cumulative CR (-0.85) and a significant positive correlation with vkgm (0.97).

The above confirms that protease is correlated with aerobic conditions, while pectinase is only present in anaerobic conditions. Puyuelo *et al.* (2011); Xu *et al.* (2012) have pointed out that the increase in enzymatic activity depends on the microbial consortia present and the concentration of microorganisms. Therefore, the change in protease and pectinase activity could indicate changes in microbial consortia during the process.

Microorganisms present in different aeration conditions

The identification of bacteria (Figure 3) *Citrobacter freundii*, *Klebsiella oxytoca* can be found. *Klebsiella oxytoca* has been reported as a high producer of cellulose enzymes (Golias *et al.*, 2002). In low aeration rates (0.032, 0.064 and 0.125 vkgm), the presence of *Enterobacteria* has been revealed, which is related to low protease and high pectinase activities (i.e., the prevalence of semi-anaerobic environments). These bacteria are chemoorganotrophic with both respiratory and fermentative metabolism. Most are negative oxidases, except for *Plesiomonas*. Most reduce nitrate to nitrite, except for fermentations of *Saccharobacter* and some strains of *Erwinia* and *Yersinia* (Brenner and Farmer, 2015).

Some strains, such as *E. cloacae*, *E. hafniae* or *E. aerogenes* are producers of lipase. On the other hand, *Buttiauxella* sp. is known as a phytase producer and a facultative anaerobe of the *Enterobacteriaceae* family (Mitra *et al.*, 2010). In the condition with the highest rate of aeration tested (0.392 and 0.251 vkgm), *Acinetobacter* bacteria were the most representative, being strictly aerobic with positive catalase and negative oxidase activity. Most strains can grow in a simple minimum medium with a single carbon source and an incubation temperature between 20-30 °C.

Lewin *et al.* (2016); Wang *et al.* (2016) mentioned that *Actinobacter* contributes to the global carbon cycle through the decomposition of plant biomass. Its enzymes may contribute to the industrial-scale decomposition of cellulosic plant biomass into simple sugars that could be converted into biofuels. *Actinobacter* hosts a substantially broad catalog of cellobiohydrolase, β -glucosidase, acetyl xylan esterase, arabinofuranosidase, pectin lyase, and ligninase genes.

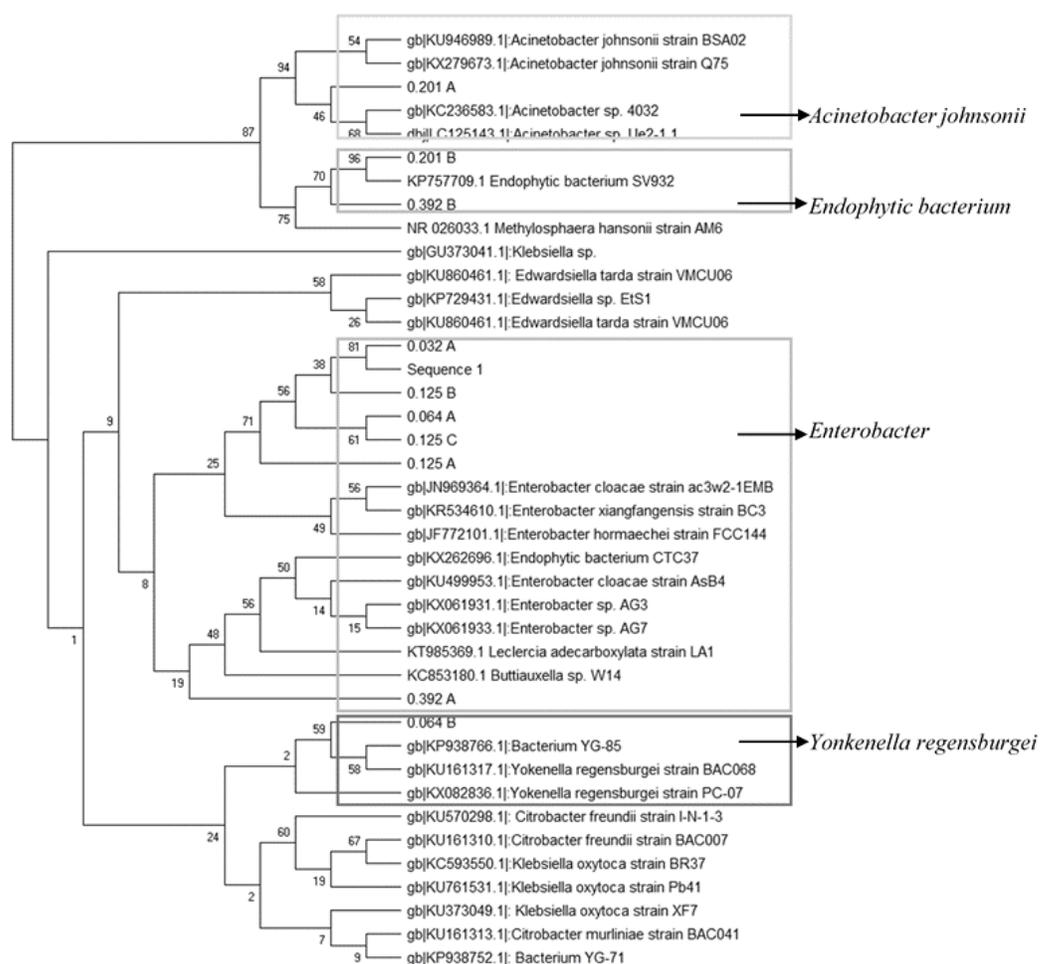


Figure 3. Molecular phylogenetic analysis by the maximum probability method. The experimental conditions used were: Inoculation by composting of CPBP, temperature set at 35 °C, initial moisture 70% (wb), pH 6, various aeration rates, namely 0.032, 0.064, 0.125, 0.251 and 0.392 vkgm. All samples were obtained at the time of maximum CO₂ generation rate.

Conclusions

The results revealed a strong positive correlation between xylanase, pectinase and dry weight loss, while all enzymatic activities increased at higher aeration rates. In this sense, the high consumption of reducing sugars was correlated only with the activity of xylanase and pectinase, since cellulase and protease had a lower activity. DGGE revealed that at the highest aeration rates (0.392 and 0.251 vkgm), the presence of *Actinobacter* was dominant. Therefore, it can be inferred that under a system in aerobic conditions, the presence of *Actinobacter* favors the degradation of organic matter.

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