Brazilian Journal
of Chemical
Engineering

ISSN 0104-6632
Printed in Brazil
www.abeq.org.br/bjche

Vol. 32, No. 01, pp. 73 - 86, January - March, 2015
dx.doi.org/10.1590/0104-6632.20150321s00003024

THE “CHEMICAL OXYGEN DEMAND / TOTAL VOLATILE ACIDS” RATIO AS AN ANAEROBIC TREATABILITY INDICATOR FOR LANDFILL LEACHATES

R. C. Contrera[1*], K. C. da Cruz Silva[1], G. H. Ribeiro Silva[2],
D. M. Morita[1], M. Zaiat[3] and V. Schalch[4]

[1]Departamento de Engenharia Hidráulica e Ambiental (PHA), Escola Politécnica (EP), Universidade de São Paulo
(USP), Avenida Prof. Almeida Prado 83, Trav. 2, Cidade Universitária, CEP: 05508-900, São Paulo - SP, Brazil.
Phone: + (55) (11) 3091 1897, + (55) (11) 3091 5396
[*]E-mail: contrera@usp.br or ronancontrera@gmail.com; katita.cris@gmail.com; dmmorita@usp.br
[2]Departamento de Engenharia Civil (DEC), Universidade Estadual Paulista "Julio de Mesquita Filho" (UNESP)
Campus de Bauru, Av. Luiz Edmundo Carrijo Coube 14-01, Vargem Limpa, CEP: 17033-360, Bauru - SP, Brazil.
E-mail: gustavoribeiro@feb.unesp.br
[3]Laboratório de Processos Biológicos (LPB), Departamento de Hidráulica e Saneamento (SHS),
Escola de Engenharia de São Carlos (EESC), Universidade de São Paulo (USP), Engenharia Ambiental,
Bloco 4-F, Av. João Dagnone 1100, Santa Angelina, CEP: 13.563-120, São Carlos - SP, Brazil.
E-mail: zaiat@sc.usp.br
[4]Departamento de Hidráulica e Saneamento (SHS), Escola de Engenharia de São Carlos (EESC), Universidade
de São Paulo (USP), Avenida Trabalhador São-Carlense 400, Centro, CEP: 13566-590, São Carlos - SP, Brazil.
E-mail: vschalch@sc.usp.br

(Submitted: October 10, 2013 ; Revised: April 27, 2014 ; Accepted: May 19, 2014)

Abstract - In some operational circumstances a fast evaluation of landfill leachate anaerobic treatability is necessary, and neither Biochemical Methane Potential nor BOD/COD ratio are fast enough. Looking for a fast indicator, this work evaluated the anaerobic treatability of landfill leachate from São Carlos-SP (Brazil) in a pilot scale Anaerobic Sequence Batch Biofilm Reactor (AnSBBR). The experiment was conducted at ambient temperature in the landfill area. After the acclimation, at a second stage of operation, the AnSBBR presented efficiency above 70%, in terms of COD removal, utilizing landfill leachate without water dilution, with an inlet COD of about 11,000 $mg.L^{-1}$, a TVA/COD ratio of approximately 0.6 and reaction time equal to 7 days. To evaluate the landfill leachate biodegradability variation over time, temporal profiles of concentration were performed in the AnSBBR. The landfill leachate anaerobic biodegradability was verified to have a direct and strong relationship to the TVA/COD ratio. For a TVA/COD_{Total} ratio lower than 0.20, the biodegradability was considered low, for ratios between 0.20 and 0.40 it was considered medium, and above 0.40 it was considered high.

Keywords: Landfill leachate; Total volatile acids; Treatability; Biodegradability; Anaerobic sequencing batch biofilm reactors.

INTRODUCTION

The biodegradability of the leachates varies with time in landfills. Changes in biodegradability of leachates have been evaluated by checking the BOD/COD ratio (Rada *et al.*, 2013). Usually, in young landfills, this ratio is high and falls in mature landfills. Ratios between 0.4 and 0.6 are considered to be an indicator that the organic matter in the leachate is readily biodegradable. In mature landfills,

---

*To whom correspondence should be addressed this ratio is often in the range of 0.05 to 0.2 and this ratio drops because leachate from mature landfills typically contains humic and fulvic acids, besides other recalcitrant organic compounds, which are not readily biodegradable (Tchobanoglous *et al*., 1993).

The BOD/COD and BOD/TOC ratios are widely used as indicators of the biodegradability or treatability of organic matter present in wastewaters, but such ratios must be evaluated with care depending on the type of wastewater and the type of treatment used. For landfill leachates, the BOD test requires acclimated seed to avoid errors and, even so, when toxic substances are present in the leachate, depending on the concentration in the sample, the aerobic seed could be inhibited, leading to BOD values lower than the actual ones (Contrera, 2008).

According to van Haandel and Lettinga (1995), the biodegradability of organic matter can be evaluated by a BOD test in an aerobic environment, but the result cannot be necessarily in an anaerobic environment. In this case, a test of anaerobic biodegradability should be performed considering the particularities of each wastewater and treatment. Thus, in anaerobic environments, the BOD is not an appropriate parameter for assessing the anaerobic biodegradability of landfill leachate and, furthermore, to obtain the value of the BOD at least 5 days is required. The Biochemical Methane Potential (BMP) test is a good alternative to determine the anaerobic biodegradability, but it can take much more time than BOD to give a definitive response, which may not be feasible in some operational situations.

Landfill leachates have some particularities besides the waste types disposed, climate, landfill hydrogeological structure, operational conditions and age of the landfill (Millot and Courant, 1997; Qasim and Chiang, 1994), and its physicochemical characteristics are the result of its formation process. Leachates are composed predominantly by dissolved substances. Most of the solubilization processes, leaching and precipitation occur within the landfill, initially in a short-time aerobic condition, followed by a long-time anaerobic condition. Thus, in general, leachates have a high concentration of dissolved organic and inorganic substances, low concentration of suspended solids and, therefore, little organic matter remaining to be solubilized. In leachates from young landfills, or young cells of landfills, an important fraction of the solubilized organic matter is composed by volatile fatty acids (VFA), usually measured as total volatile acids (TVA).

Scientific and technical literature, in general, does not give much importance to TVA concentration in landfill leachates. In most cases, these acids are considered to be only an indicative of the age of the landfill, emphasizing that, if TVA concentrations are high, the leachate can be treated by biological processes, but never establishing a relative TVA concentration to indicate the level of biodegradability or the kind of biological treatment to be used. In many cases, TVA is the major part of the organic compounds in leachates (Contrera, 2008).

The applicable methods of leachate treatment are biological and physical-chemical, or a combination of these processes (Qasim and Chiang, 1994). Anaerobic and aerobic systems have been extensively researched to remove organic matter and ammonia (Kulikowska, 2012). However, they need an external source of carbon for denitrification when the leachate comes from mature landfill. For this reason, recently, processes such as Annamox and endogenous denitrification has been surveyed and the first results are promising, indicating that anaerobic-aerobic treatment association is possible without an extra carbon source (Kuenen, 2008; van der Star *et al*., 2007; Anfruns *et al*., 2013; Wang *et al*., 2013; Zhu *et al*., 2013).

Anaerobic treatments have been widely used in tropical countries due to their advantages, especially with respect to low energy consumption, low amount of sludge generated and good performance treating high-strength wastewaters. Among the anaerobic systems the Upflow Anaerobic Sludge Blanket (UASB) reactors (Castillo *et al*., 2007; Sun *et al*., 2010), sequencing batch reactors (Timur and Özturk, 1999; K. J. Kennedy and Lentz, 2000; Imen *et al*., 2009) and fluidized bed reactors (Eldyasti *et al*., 2011) can be highlighted.

In order to eliminate the sedimentation step and to minimize the biomass loss in anaerobic sequencing batch reactors (AnSBR), Ratusznei *et al*. (2000) proposed the anaerobic sequencing batch biofilm reactor (AnSBBR), which contains biomass immobilized in an inert support, confined in a basket-like container inside the reactor. As this configuration presented good results treating synthetic wastewaters (removal efficiency up to 86%), the AnSBBR started to be studied and developed (Ratusznei *et al*., 2003; Rodrigues *et al*., 2003; Cubas *et al*., 2004; Pinho *et al*., 2004; Siman *et al*., 2004; Bezerra *et al*., 2010; Costabile *et al*., 2010). The AnSBBR was also tested and optimized with many types of wastewaters, including diluted whey (Damasceno *et al*., 2007), sewage (Sarti *et al*., 2007) wastewater of the personal-care industry (Oliveira *et al*., 2009), biodiesel production effluent (Selma *et al*., 2010; Bezerra *et al*., 2010), sucrose-based wastewater (Manssouri *et al*. 2013) among other studies for optimization and improvement of the AnSBBR (Rodrigues *et al*., 2004; Silva *et al*., 2011; Vich *et al*., 2011).

In the same way, this work evaluated the treatment of landfill leachate under various biodegradability conditions in a pilot-scale AnSBBR. The variations in composition and biodegradability characteristics of the leachate were due to excessive rain and changes in the operational condition of the landfill in São Carlos-SP, Brazil. The variations were noted mainly in the TVA and COD concentrations in the landfill leachate, which resulted in significant variations in the reactor performance explored in this experiment.

Taking advantage of these events, the importance of the TVA/COD ratio was investigated as a fast anaerobic treatability or biodegradability indicator, to help make decisions for managing, designing or planning leachate treatment or pretreatment systems.

MATERIALS AND METHODS

Experimental Set-Up

The experimental pilot plant shown in Figure 1 was installed at the municipal landfill area in São Carlos-SP, Brazil, in a shed constructed near an existing stabilization pond. When the experiment was started, the landfill had about 12 years of operation, generated 100 $m^3.day^{-1}$ of leachate, received about 150 $ton.day^{-1}$ of waste, occupied an area of approximately 0.06 km^2, was 30 m in height and had two extensions (2 and 5 years of operation). Leachate was collected from these extensions and temporarily stored in a 2000 L polyethylene reservoir in front of the shed. Preferably, non-metallic materials were used in the experiment.

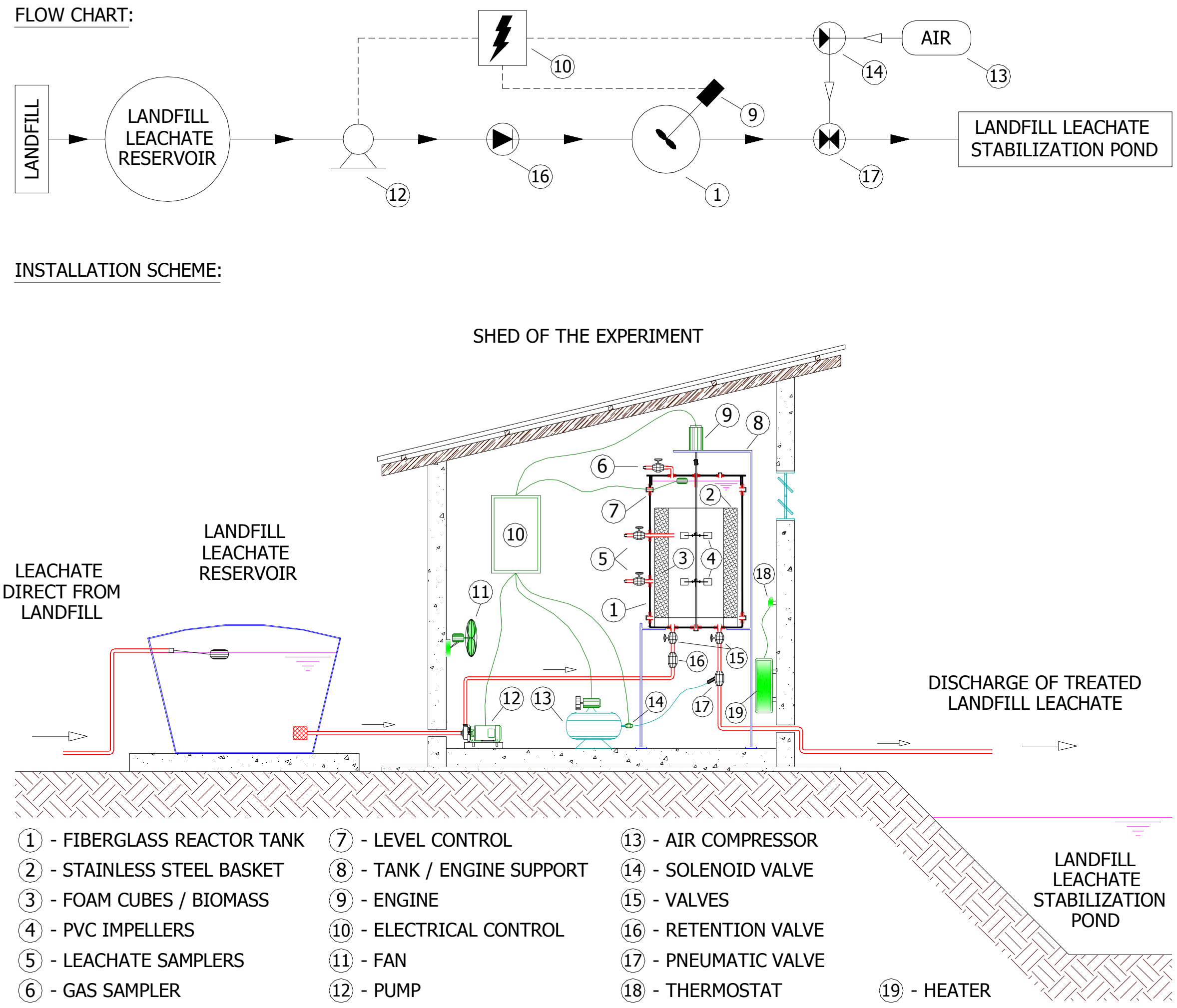


Figure 1: Scheme of the experimental installation at the landfill area in São Carlos-SP, Brazil.

The Anaerobic Sequencing Batch Biofilm Reactor (AnSBBR) treated 746 L of landfill leachate per batch with a total volume of 1300 L (1.65 m high and 1.00 m diameter). The reactor was made of fiberglass and had a stainless steel hollow cylindrical basket (1.20 m high, 0.90 m external diameter and 0.60 m internal diameter) inside it, which was filled with polyurethane foam cubes (4 cm on a side and 23 $g.L^{-1}$ density), as an inert support for biomass (110 L of inoculum) collected from the bottom of an existing stabilization pond. Mechanical stirring was accomplished with two PVC impeller-type turbines, with six flat-blades, operated at 30 rpm, according to Sarti *et al*. (2007), to prevent biomass detachment, producing a velocity gradient ($\overline{G}$) of 118 s^{-1}.

An electrical control triggered the pump, valves and stirring system during the batches guided by level controls and adjusted the reaction times. To operate the pneumatic valve, controlled by a solenoid valve, an air compressor was used. The experiment was conducted at ambient temperature (ranged between 15.0 and 29.2 °C with mean 23.8 ± 2.1 °C) and, during the winter, two oil heaters of 1500 W were used only to reduce the temperature variations and to operate near 25 °C.

The reaction times varied from 5 to 7 days according to the reactor operating stage. The filling time used was 15 minutes and the discharge time was 30 minutes.

Experimental Protocol

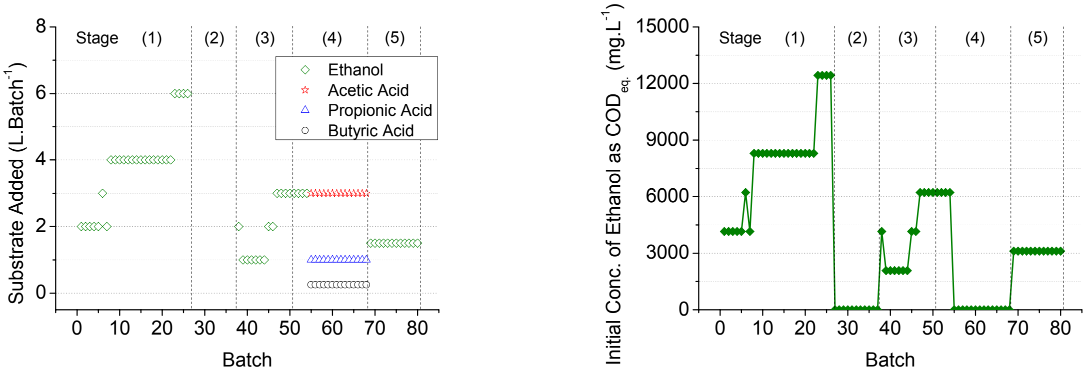
The operation was carried out in five stages on 80 batches lasting 493 days. Stages 1 and 3 were necessary for the acclimation of the reactor for the assays performed at stages 2 and 4. Stage 5 was carried out only to analyze the biomass growth and for a final biomass sampling.

In stage 1, to acclimate the biomass, the leachate was initially diluted in water to 50% and ethanol was added as additional substrate to activate the biomass, according to Figure 2. The dilution was reduced to zero at the end of this stage that lasted 26 batches. Each batch had a reaction time of 7 days. The ethanol used as additional substrate in the AnSBBR was the same commercial hydrous ethanol (92.8% of ethanol and 7.2% of water in mass) used as car fuel in Brazil (Contrera *et al*., 2013). At the end of this stage, the TVA concentrations in the leachate from landfill increased, making it less recalcitrant to anaerobic treatment.

In stage 2, which covers the period of batches 27 to 37, the AnSBBR did not receive any additional substrate or water dilution. From batch 27 to 32, the reaction time was 7 days and four temporal concentration profiles were performed. From batches 33 to 37, based on the previous profiles, the reaction time was reduced to 5 days in order to optimize the operation time, and more four temporal concentration profiles were performed.

In stage 3, which covers the period of batches 38 to 50, ethanol was again added to the leachate at the beginning of each batch in the AnSBBR, according to Figure 2, because the leachate again became extremely recalcitrant to anaerobic treatment, due to the climate and operational changes in the landfill. However, the addition of ethanol at this stage was gradually increased in order to maintain and to acclimate the reactor to high organic loads.

In stage 4, which covers the period from batches 51 to 68, some tests were carried out in the AnSBBR.



(a) (b)

Figure 2: (a) Amount of additional substrate added to each batch; and (b) Initial concentration of ethanol in the batches as COD equivalent.

At the beginning of this stage, with the reactor in steady-state, treating leachate with the addition of ethanol, three temporal concentration profiles were performed to evaluate the reactor performance. After this, trying to reproduce the second stage, as the leachate was extremely recalcitrant, volatile fatty acids were added to the leachate (acetic acid: 3.0 L.batch^{-1}, propionic acid: 1.0 L.batch^{-1} and butyric acid: 0.25 L.batch^{-1}), according to Figure 2, resulting in concentrations near that observed in stage 2. The acetic acid used was glacial 100% and propionic and butyric acids were also 100%. When the system attained a new steady-state, four temporal profiles of COD concentration were performed to evaluate the degradation of the organic matter added to the leachate as VFAs.

In stage 5, which covers the period of batches 69 to 80, the reactor was not monitored, but was kept in operation with a reaction time of 7 days and addition of 1.5 L of ethanol at the beginning of each batch, to provide a final sample of the biomass after batch 80.

Analytical Methods and Microbiological Exams

Influent and effluent samples were collected for physico-chemical analysis in all the first 68 batches performed. When temporal concentration profiles were performed, samples were collected at intermediate times. The chemical oxygen demand (COD) of the total and filtered samples, the pH, total alkalinity (TA), total ammonia nitrogen (TAN), free ammonia nitrogen (FAN), the total solids (TS), total fixed solids (TFS), total volatile solids (TVS), and the metals zinc (Zn), lead (Pb), cadmium (Cd), nickel (Ni), iron (Fe), manganese (Mn), copper (Cu) and chromium (Cr) were determined according to the Standard Methods (APHA, 2005). Gas chromatography was used only for characterizing volatile fatty acids (VFA) in stage 2 of the operation.

In order to accelerate and to simplify the biodegradability evaluation, a direct titration method (Dilallo and Albertson, 1961) for TVA determination was used as it is fast, easy, cheap and does not require sophisticated equipment. Thus, a TVA/COD ratio evaluation can be made in about two hours, which is very fast as compared to the five days necessary for the BOD/COD evaluation which, for an anaerobic treatment, would not be the best indicator. The methodology described by Dilallo and Albertson (1961) was calibrated with standard solutions of volatile fatty acids in the leachate, according to Contrera (2008) using Equation (1):

$$\mathrm{TVA} = \mathrm{fca} \cdot \mathrm{TVAA} - \mathrm{fcb} = 1.854 \cdot \mathrm{TVAA} - 427 \quad (1)$$

Main microbial communities were observed by direct microscopy only at the inoculation and at the end of the experiment after batch 80. An Olympus BX-60 equipped with contrast-phase and fluorescence, measured by ultraviolet light excitation, was used for the observations.

Mathematical Model and Validation

At the beginning of each batch the anaerobic biodegradability (*An.Biod.*$_{COD}$) of leachate was considered to be equal to the efficiency of the treatment to remove its respective COD. At the end of each batch, the anaerobic biodegradability of leachate was considered to be close to zero; the anaerobic biodegradability at any time during the batch could thus be considered to be equal to the fraction of organic matter consumed (%) until that time, referred to the remaining organic matter concentration at the end of each batch, as in Equations (2) and (3):

$$\mathrm{Effic.}_{\mathrm{COD\,Rem.}}\ (\%) = \mathrm{Total\ An.Biod.}_{\mathrm{COD}}\ (\%) = 100 \cdot \left(\frac{C_{S_0} - C_{Sf}}{C_{S_0}} \right) \quad (2)$$

$$\mathrm{Partial\ An.Biod.}_{\mathrm{COD}}\ (\%) = 100 \cdot \left(\frac{C_{S_i} - C_{Sf}}{C_{S_i}} \right) \quad (3)$$

As the organic matter treated by anaerobic processes in leachates is mostly TVA and composed mainly by acetic acid, considering that the theoretical COD of acetic acid is about 1.0 $gO_2.gHAc^{-1}$, the TVA/COD ratio may be a good indicator of the anaerobic biodegradability for landfill leachates.

To validate this assumption, the mathematical correlation between this ratio and the anaerobic biodegradability of the landfill leachate was evaluated, as calculated in Equations (2) and (3). The coefficient of determination (r^2) was used as an indicator of this possible correlation.

RESULTS AND DISCUSSION

Ten months before starting the experiments, the leachate presented concentrations of TVA equal to 2142 mgHAc.L^{-1} and COD$_{\mathrm{Total}}$ equal to 6396 mg.L^{-1}, which could indicate anaerobic treatment as a good option for pretreatment of the leachate due to its high TVA concentration. However, as the reactors were built, the characteristics of the leachate changed, presenting concentrations of TVA equal to 371 mgHAc.L^{-1} and COD$_{\text{Total}}$ equal to 4025 mg.L^{-1}. The experiment was started even so, but as the characteristics of leachate did not change again, reactor failure was observed in a few weeks. This failure was verified by no removal of organic matter and sludge loss without a change in pH, probably due to the absence of biodegradable organic matter in sufficient concentration to maintain the biomass active.

After this, all the foam and the biomass in the reactor were replaced to restart the operation using a new strategy for the biomass acclimation, as previous presented in the methodology. The acclimation was carried out in stage 1, when the leachate was predominantly recalcitrant, presenting an average TVA of 862 mgHAc.L^{-1} and average COD$_{\text{Total}}$ of 4613 mg.L^{-1}.

Excessive rain and changes in landfill operation produced modifications in the leachate characteristics that were explored in this experiment. These changes produced an increasing concentration of biodegradable compounds in the leachate during stage 2, presenting average concentrations of TVA equal to 3658 mgHAc.L^{-1} and COD$_{\text{Total}}$ equal to 8566 mg.L^{-1}, when tests were performed in the pilot-scale reactor. A characterization of VFA by gas chromatography performed on the influent of batch 28 presented: 4130 mg.L^{-1} of acetic acid, 1275 mg.L^{-1} of propionic acid, 232 mg.L^{-1} of butyric acid, 93 mg.L^{-1} of isobutyric acid, 56 mg.L^{-1} of valeric acid, 33 mg.L^{-1} of isovaleric acid and 75 mg.L^{-1} of caproic acid.

At the beginning of stage 3, the leachate became recalcitrant again, presenting average concentrations of TVA equal to 606 mgHAc.L^{-1} and COD$_{\text{Total}}$ equal to 4911 mg.L^{-1}. Thus, ethanol addition was necessary to maintain the biomass active in the reactor for the following tests using volatile fatty acids degradation in stage 4. Thus, stages 1 and 3 were used to prepare the AnSBBR for the tests performed in stages 2 and 4, respectively. Table 1 presents the mean and standard deviation values of the parameters determined in the influent and effluent in stages 1 to 4 of the AnSBBR operation. The parameter values reported for leachate influent are without additions. Stage 5 was performed only to analyze the biomass growth and for a final biomass sampling for a quantitative and qualitative exam.

In batches 1 to 68, the temperature of the liquid in the AnSBBR ranged between 15.0 and 29.2 °C with mean 23.8 ± 2.1 °C. Figure 3 (a) shows the average temperature of the liquid in the AnSBBR and the ranges of variation for each batch.

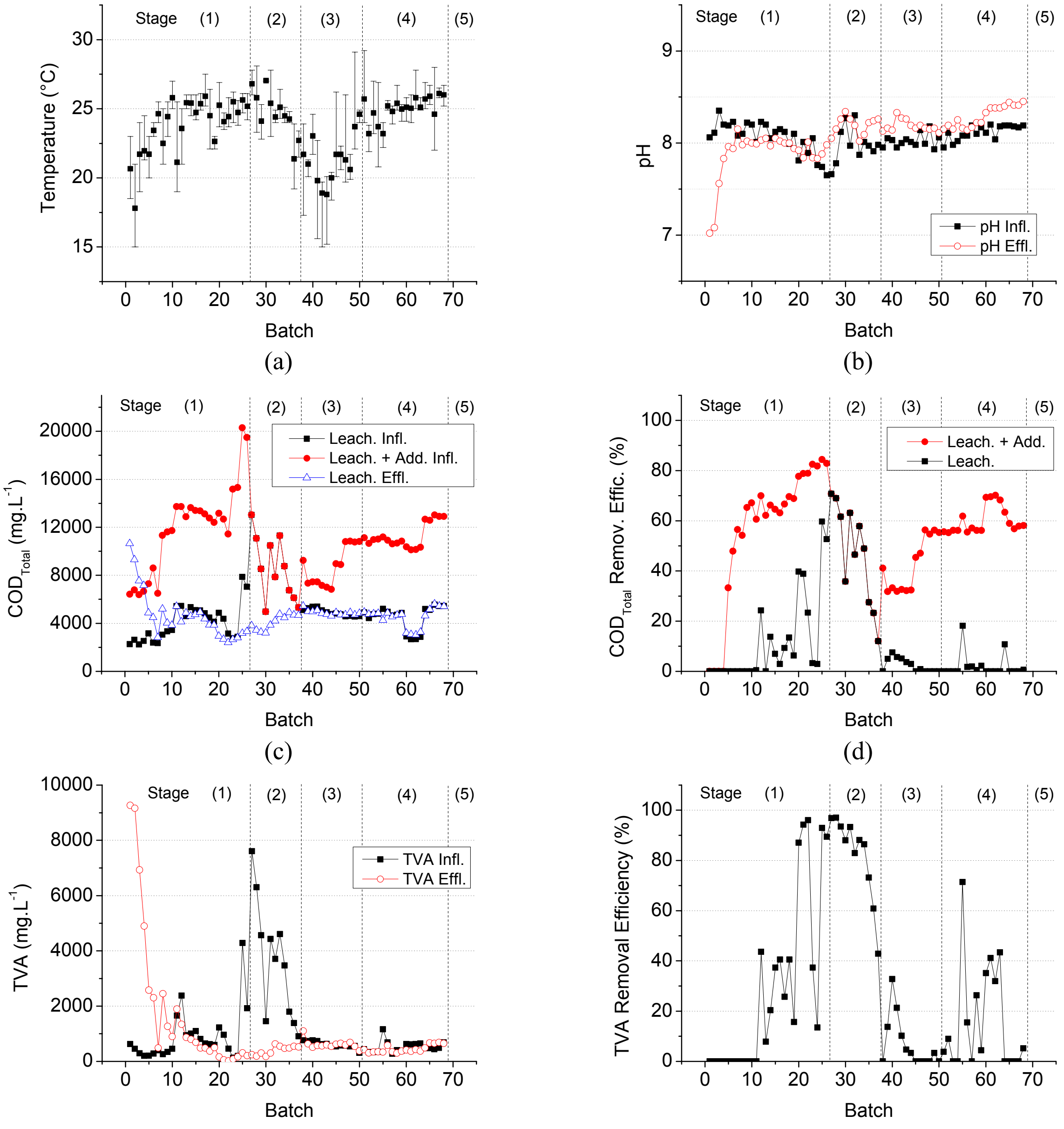
During stages 1, 3 and 4 there was no major removal of organic matter by AnSBBR, with the exception of added substrates. In stage 2, treating pure landfill leachate, the system efficiency reached 71% of COD$_{\text{Total}}$ removal. Considering the added substrate, the major overall removal occurred at the end of stage 1, when 84% of COD$_{\text{Total}}$ was removed. Figure 3 (c) shows the influent leachate with and without additional substrate, and effluent COD$_{\text{Total}}$ concentrations. Figure 3 (d) shows COD$_{\text{Total}}$ removal efficiencies for leachate with and without addition of substrate in the four stages (68 batches) of operation.

Table 1: Mean and standard deviation values of the parameters analyzed in the influent and effluent leachate in stages 1 to 4 of the AnSBBR operation.

Parameter	Unit	Stage 1 (26 Batches)		Stage 2 (11 Batches)		Stage 3 (13 Batches)		Stage 4 (18 Batches)	
		Influent*	Effluent	Influent*	Effluent	Influent*	Effluent	Influent*	Effluent
Temperature	°C	23.8 ± 2.0		24.7 ± 1.7		21.3 ± 1.7		25.0 ± 0.9	
COD$_{\text{Total}}$	mg.L^{-1}	4613 ± 1269	4644 ± 2027	8566 ± 2662	4119 ± 632	4911 ± 304	4885 ± 215	5006 ± 306	4518 ± 826
COD$_{\text{Filtered}}$	mg.L^{-1}	4313 ± 1138	4203 ± 1886	8056 ± 2578	3797 ± 630	4715 ± 376	4487 ± 264	4804 ± 276	4252 ± 850
pH**	-	8.03	7.75	7.95	8.18	8.02	8.18	8.11	8.28
TVA	mgHAc.L^{-1}	862 ± 897	1871 ± 2682	3658 ± 2145	399 ± 163	606 ± 123	613 ± 166	524 ± 206	446 ± 136
TA	mgCaCO$_3$.L^{-1}	8327 ± 1933	7911 ± 2068	10687 ± 1066	10635 ± 1007	12660 ± 790	12345 ± 787	12645 ± 937	14166 ± 2209
NH$_3$-N	mgN.L^{-1}	1762 ± 381	NA	2183 ± 231	NA	2659 ± 209	2620 ± 188	2781 ± 185	3143 ± 715
FAN	mgN.L^{-1}	83 ± 35		166 ± 30		153 ± 21		338 ± 167	
SO$_4^{--}$	mgS.L^{-1}	ND	ND	ND	ND	ND	ND	ND	ND
TS	mg.L^{-1}	11211 ± 2146	10498 ± 2298	15330 ± 2111	12669 ± 1399	13636 ± 1191	13544 ± 887	12803 ± 599	12497 ± 1220
TFS	mg.L^{-1}	7734 ± 1874	7629 ± 1873	9422 ± 663	9085 ± 741	9430 ± 339	9511 ± 261	9078 ± 537	9023 ± 709
TVS	mg.L^{-1}	3477 ± 774	2870 ± 477	5907 ± 1967	3584 ± 922	4206 ± 1063	4033 ± 895	3725 ± 343	3530 ± 747
Zn	mg.L^{-1}	0.46 ± 0.18	0.35 ± 0.16	0.81 ± 0.43	0.64 ± 0.23	0.97 ± 0.62	1.06 ± 0.56	0.67 ± 0.18	0.64 ± 0.20
Pb	mg.L^{-1}	0.12 ± 0.07	0.12 ± 0.05	0.27 ± 0.08	0.23 ± 0.10	0.21 ± 0.15	0.21 ± 0.18	0.20 ± 0.09	0.19 ± 0.09
Cd	mg.L^{-1}	0.03 ± 0.03	0.03 ± 0.02	0.08 ± 0.04	0.06 ± 0.03	0.05 ± 0.02	0.07 ± 0.02	0.04 ± 0.02	0.04 ± 0.03
Ni	mg.L^{-1}	0.43 ± 0.26	0.42 ± 0.22	0.62 ± 0.12	0.62 ± 0.13	0.59 ± 0.07	0.62 ± 0.06	0.47 ± 0.09	0.46 ± 0.10
Fe	mg.L^{-1}	12.82 ± 9.34	13.72 ± 6.79	17.80 ± 6.98	17.48 ± 6.32	9.41 ± 3.85	15.24 ± 3.05	7.22 ± 2.15	15.82 ± 5.03
Mn	mg.L^{-1}	0.47 ± 0.39	0.27 ± 0.11	0.53 ± 0.20	0.22 ± 0.06	0.19 ± 0.03	0.23 ± 0.08	0.19 ± 0.05	0.20 ± 0.05
Cu	mg.L^{-1}	0.04 ± 0.02	0.04 ± 0.02	0.07 ± 0.03	0.08 ± 0.01	0.04 ± 0.02	0.07 ± 0.02	0.05 ± 0.02	0.07 ± 0.02
Cr	mg.L^{-1}	0.07 ± 0.09	0.15 ± 0.15	0.13 ± 0.08	0.19 ± 0.14	0.12 ± 0.13	0.20 ± 0.22	0.31 ± 0.15	0.48 ± 0.18

* Leachate without additions; ** Determined by the mean of H$^+$ concentrations; NA = Not analyzed; ND = Not detected

Due to the addition of ethanol and to the biomass acclimation, at the beginning of stage 1, the AnSBBR produced more TVA than it consumed, as shown in Figure 3 (e), initially reducing the pH of the effluent, as shown in Figure 3 (b). This also explains the higher average concentration and higher standard deviation shown by TVA in the effluent in stage 1 as shown in Table 1. The natural concentration of TVA in the influent leachate ranged from 129 to 7611 mgHAc.L^{-1}. Figure 3 (f) shows the TVA removal efficiency without considering the TVA generated by the ethanol acidification, or VFA added to the leachate. The maximum removal efficiencies reached 96.0% in batch 22 in stage 1, and 97.1% in batch 28 in stage 2. In stage 2 the average removal efficiency was 81.1 ± 16.9%.



(a) (b)

(c) (d)

(e) (f)

Figure 3: (a) Average temperature of the liquid in the AnSBBR and the ranges of variation; (b) Influent and effluent pH variations; (c) Influent and effluent COD$_{Total}$ concentrations; (d) COD$_{Total}$ removal efficiencies for leachate with and without addition of substrate; (e) Influent and effluent TVA concentrations; and (f) TVA removal efficiency without considering the TVA generated or added to the leachate.

The pH of the influent leachate ranged from 7.65 to 8.35 and of the effluent from 7.02 to 8.45. As can be observed in Figure 3 (b), in stage 1, due to the ethanol addition and no consumption of total TVA generated by acidification, the pH of the effluent dropped as compared to the influent leachate pH. In the following stages, the consumption of TVA produced an opposite effect, raising the pH of the effluent in relation to the affluent in most batches.

Most solids removal occurred during stage 2, removing predominantly TVS due to the higher concentration of biodegradable organic matter in the leachate at this stage. During the four stages, the TS concentration ranged from 8401 to 19379 $mg.L^{-1}$, TFS concentration ranged from 4701 to 10718 $mg.L^{-1}$ and TVS concentration ranged from 2330 to 10006 $mg.L^{-1}$ in the natural influent leachate.

N-ammonia ranged from 1264 to 4519 $mgN.L^{-1}$ and FAN ranged from 24 to 634 $mgN.L^{-1}$ without damage to the system performance.

All along the stages, the AnSBBR did not remove metals and, in some cases, an increase in metal concentration occurred in the effluent, possibly due to drag or solubilization of metals from sludge inoculum.

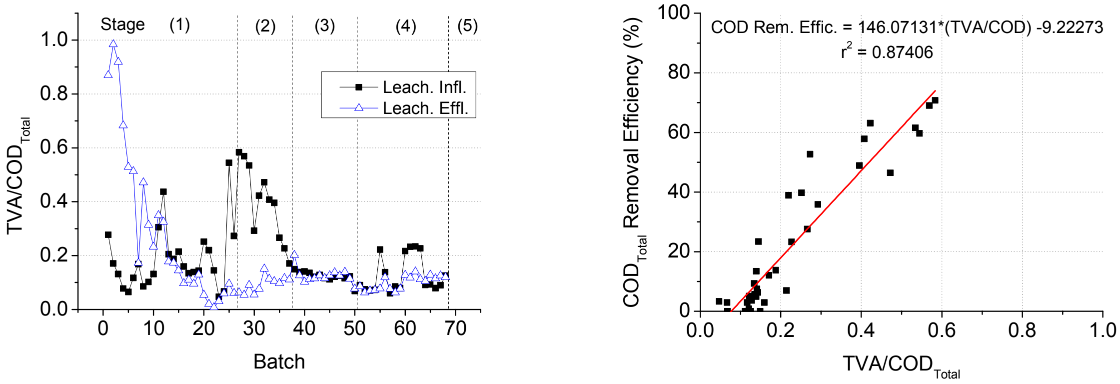
As can be seen in Figure 4 (a), when plotting the TVA/COD_{Total} ratio in the course of the batches, there is a considerable difference in the behavior of this relation for the influent and treated effluent. Note also that, after the acclimation of biomass in the AnSBBR, the TVA/COD_{Total} ratio remained constantly low in the treated effluent. This indicates the importance of this ratio in the anaerobic treatment of landfill leachates.

Plotting the treatment efficiencies, in terms of COD_{Total} removal, in the batch interval in which the biomass was acclimated by receiving leachate without dilution or additions of acids in the AnSBBR (batches 14 to 50) against the TVA/COD_{Total} ratios of the influent gave the graph in Figure 4 (b), where it is possible to note a significant correlation between the efficiencies of the AnSBBR and the TVA/COD_{Total} ratio for the influent leachate. A linear fit to these points gave Equation (4) that correlates the TVA/COD_{Total} ratio and the removal efficiency of COD_{Total}, i.e., the AnSBBR capacity of anaerobically biodegrading leachates. The coefficient of determination (r^2), equal to 0.87406, for Equation (4) indicates a good correlation between the TVA/COD_{Total} ratios and the anaerobic biodegradability of leachate.

$$\text{Effic.}_{\text{COD Rem.}}(\%) = \text{An.Biod.}_{\text{COD}}(\%) = 146.07\cdot\frac{TVA}{COD_{Total}} - 9.22 \qquad (4)$$

In order to further explore the behavior of the TVA/COD_{Total} ratio within the system during a batch, 8 temporal concentration profiles of COD_{Total} and TVA in the AnSBBR were performed in stage 2. Figures 5 (a) to 5 (d) show these profiles performed from batches 28 to 37. The first four temporal concentration profiles (batches 28 to 31) show stabilization in the consumption of biodegradable organic matter after the fifth day. Comparing the COD_{Total} and TVA profiles, they were observed to present the same pattern.

In leachates, the presence of acetic acid as the major component of acids and a relevant part of the organic matter is common. Hence, considering that the theoretical COD of acetic acid is about 1.0 $mgHAc.mgO_2^{-1}$, observing Figures 5 (b) and 5 (d), it is possible to conclude that the anaerobic treatment of leachate removed basically TVA in this experiment.



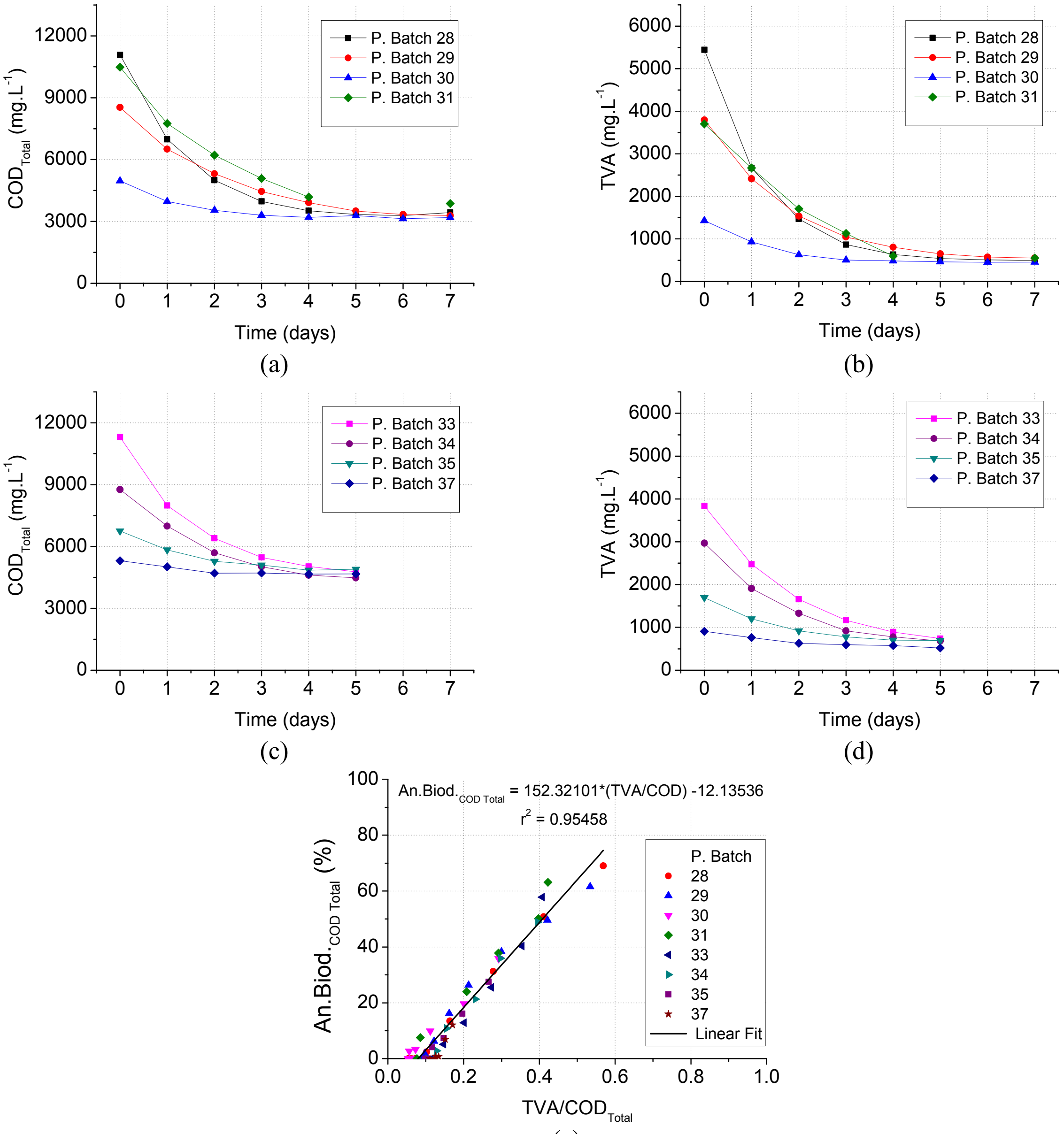
(a) (b)

Figure 4: (a) Variation of the TVA/COD_{Total} ratio in the course of the batches; and (b) Linear relationship between TVA/COD_{Total} ratios and the COD_{Total} removal efficiency for batches 14 to 50.

Grouping these eight concentration profiles, using Equation (3) and plotting the TVA/COD_{Total} ratios against the COD_{Total} anaerobic biodegradation, Figure 5 (e) is produced. This figure shows a strong relationship between these two parameters and, by a linear fit, Equation (5) was obtained, presenting a coefficient of determination (r^2) equal to 0.95458, i.e., very good linear correlation.

$$An.Biod._{COD}\ (\%) = 152.32 \cdot \frac{TVA}{COD_{Total}} - 12.14 \qquad (5)$$

As the leachate became recalcitrant again, due to the operational stabilization and the decrease in rainfall at the landfill, the TVA concentration also decreased. In order to explore this new condition and to verify the potential correlation between the TVA/COD_{Total}



(a) (b)

(c) (d)

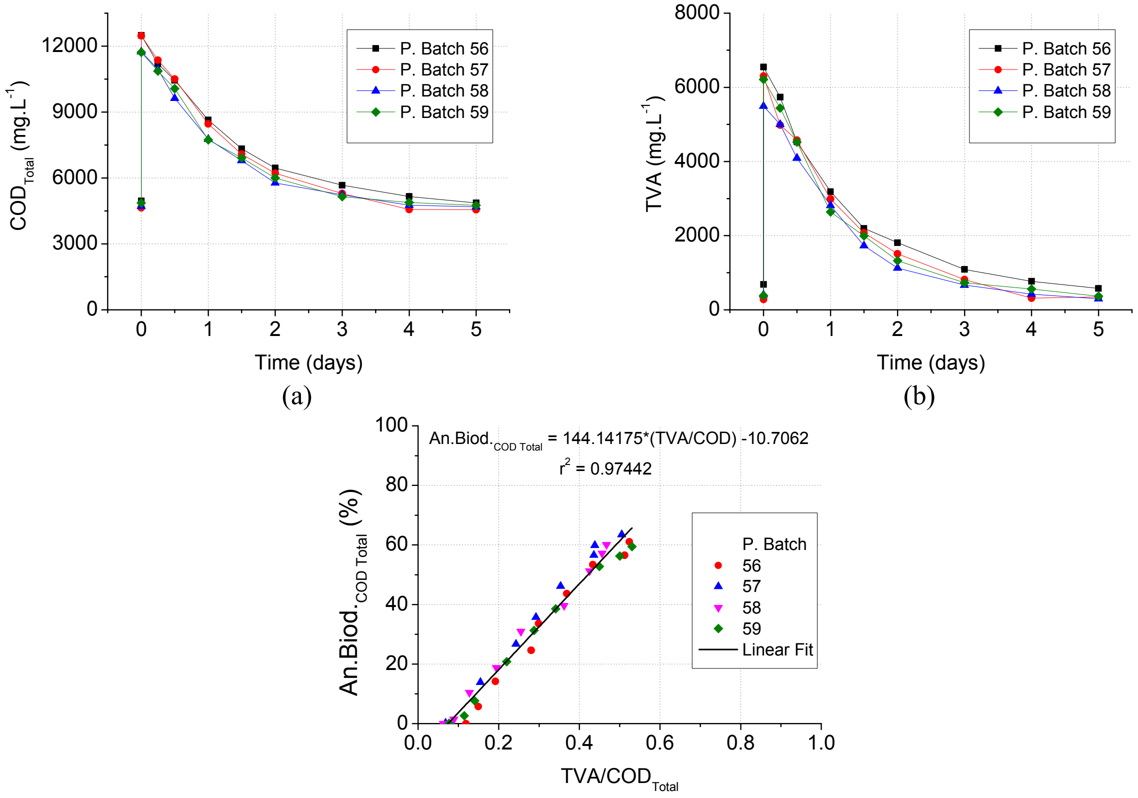
(e)

Figure 5: Stage 2 results of profiles performed on batches 28, 29, 30 and 31 for COD_{Total} (a) and TVA (b); profiles performed on batches 33, 34, 35 and 37 for COD_{Total} (c) and TVA (d); and the linear relationship between TVA/COD_{Total} ratios and the COD_{Total} anaerobic biodegradation for the profiles performed on batches 28, 29, 30, 31, 33, 34, 35 and 37 (e).

ratio and the COD_{Total} anaerobic biodegradation, tests with acetic, propionic and butyric acid were programmed. Before this, in stage 3, a new increase in organic load was performed to stabilize the reactor until the necessary charge for the tests in stage 4. This new stabilization was produced by adding ethanol again to the leachate at the beginning of each batch according to Figure 2. Because ethanol was acidified into acetic acid and the acetic acid was totally consumed, the AnSBBR was ready to receive the volatile fatty acids at the desired concentration at stage 4 in order to verify whether the correlation between TVA/COD_{Total} ratio and the COD_{Total} anaerobic biodegradation was valid. To validate this hypothesis, four temporal profiles of concentration were performed (batches 56, 57, 58 and 59); at the beginning of each batch, 3.0 L of acetic acid ($COD_{Eq.}$ = 4478 mg.L^{-1}), 1.0 L of propionic acid ($COD_{Eq.}$ = 2002 mg.L^{-1}) and 0.25 L of butyric acid ($COD_{Eq.}$ = 568 mg.L^{-1}) were added. Figure 6 (a) shows the COD_{Total} concentration profiles and Figure 6 (b) shows the TVA profiles performed. In Figures 6 (a) and 6 (b) at time zero, the concentration of DQO_{Total} and TVA was plotted before the VFA addition. As the substrate consumption did not go beyond the concentration added, it can be concluded that the leachate was really recalcitrant, as hypothesized.

As in stage 2, taking a similar procedure to investigate the COD_{Total} anaerobic biodegradation dependence of the TVA/COD_{Total} ratio, Figure 6 (c) was produced, where a clear correlation can be noted again. This figure again shows a strong relationship between COD_{Total} anaerobic biodegradation and TVA/COD_{Total} ratios and, by a linear fit, Equation (6) was obtained, which presented a coefficient of determination (r^2) equal to 0.97442, indicating very good linear correlation.

$$\text{An.Biod.}_{COD}\ (\%) = 144.14 \cdot \frac{TVA}{COD_{Total}} - 10.71 \qquad (6)$$



(a) (b)

(c)

Figure 6: Stage 4 results of profiles performed on batches 56, 57, 58 and 59 for COD_{Total} (a) and TVA (b); and the linear relationship between TVA/COD_{Total} ratios and the COD_{Total} anaerobic biodegradation for the profiles performed on batches 56, 57, 58 and 59 (c).

Comparing the results of Equations (4), (5) and (6) in Table 2 and taking the maximum difference between the results, it could be seen that the maximum difference found was less than 4 % in the researched interval and, therefore, a small difference. If anaerobic biodegradation lower than 20 % could be considered low anaerobic biodegradation, between 20 and 50 % medium and above 50 % high, the corresponding TVA/COD_{Total} ratios are lower than 0.20 for low anaerobic biodegradability, between 0.20 and 0.40 for medium and above 0.40 for high.

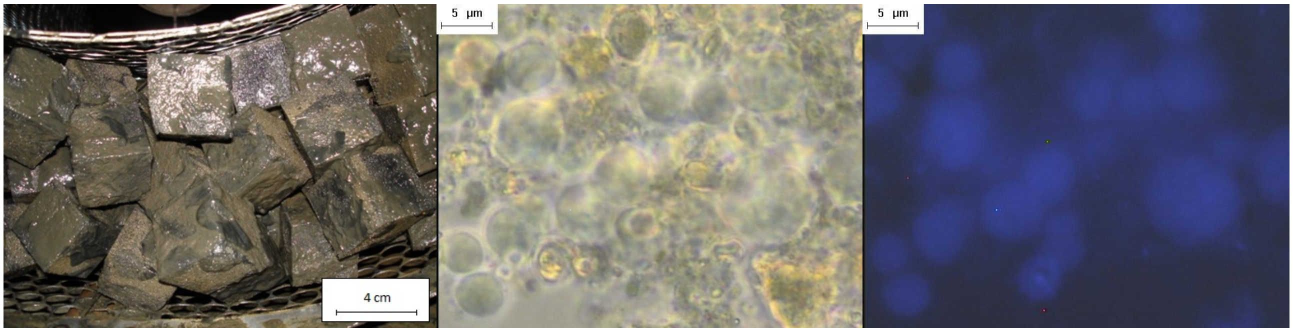
Thus, these results for the leachate from São Carlos-SP, Brazil suggest that TVA/COD_{Total} ratios can indicate the degree of anaerobic biodegradability for landfill leachates as well as the BOD/COD ratio for aerobic biodegradability.

At the end of stage 5, samples of the biomass were taken for examination and no difference was verified regarding the beginning of the experiment. In both cases, the predominant morphology found in the biomass was similar to that of *Methanosarcina sp.* and this is indicative that this morphology can adapt better than others to landfill leachate. The morphology *Methanosaeta sp.* normally found in anaerobic treatment of sewage, was not found by optical examination of biomass. Figure 7 (a) shows the aspect of the biomass in the foam cubes at the end of stage 5, and Figures 7 (b) and 7 (c), respectively, show the images obtained by optical microscopy in phase contrast and fluorescence, which have a high concentration of morphology similar to that of *Methanosarcina sp.* for a sample taken at the end of stage 5 in the AnSBBR.

Unfortunately, gas generation could not be measured, or its composition determined, due to the difficulty in sealing the reactor cover, but the presence of methane was detected by a burning test.

Table 2: Results of Equations (4), (5) and (6), and their maximum differences.

TVA/COD_{Total}	Biod.		An.Biod.$_{COD\ Total}$ (%)			$\Delta_{max.}$ (%)
			Eq. (4)	Eq. (5)	Eq. (6)	
0.10	↑		5.4	3.1	3.7	2.3
0.15	↓	Low	12.7	10.7	10.9	2.0
0.20	--		20.0	18.3	18.1	1.9
0.25	↑		27.3	25.9	25.3	2.0
0.30	|	Medium	34.6	33.6	32.5	2.1
0.35	↓		41.9	41.2	39.7	2.2
0.40	--		49.2	48.8	47.0	2.3
0.45	↑		56.5	56.4	54.2	2.4
0.50	|		63.8	64.0	61.4	2.7
0.55	|	High	71.1	71.6	68.6	3.1
0.60	|		78.4	79.3	75.8	3.5
0.65	↓		85.7	86.9	83.0	3.9



(a) (b) (c)

Figure 7: (a) Aspect of the biomass in the foam cubes within the reactor basket; (b) Image obtained by optical microscopy in phase contrast which has a huge concentration of morphology similar to *Methanosarcina sp.* in a sample taken at the end of stage 5 in the AnSBBR and (c) the same optical microscopy image in fluorescence.

CONCLUSION

The TVA/COD$_{Total}$ ratio presented good linear correlations with the potential of anaerobic biodegradability and the removal efficiency of COD$_{Total}$ for the leachates investigated, presenting coefficients of determination (r^2) above 0.95.

For leachates from the landfill of São Carlos-SP, Brazil, without dilution or additions, TVA/COD$_{Total}$ ratios lower than 0.20 indicated low anaerobic biodegradability; between 0.20 and 0.40 they indicated medium; and above 0.40, they indicated high.

A final test, adding acetic, propionic and butyric acids to a recalcitrant leachate and treating it in the AnSBBR, reproduced the same results presented by treating a non-totally recalcitrant leachate, presenting equivalent TVA/COD$_{Total}$ ratios for each degree of potential anaerobic biodegradability.

Anaerobic treatment of landfill leachate would not be viable with TVA/COD$_{Total}$ ratios lower than 0.20, due to the low efficiencies expected.

ACKNOWLEDGEMENTS

This work was supported by the *Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)*, processes number 03/04812-8 and 03/04602-3, and had the collaboration of *Vega Engenharia Ambiental S.A.*, *Companhia Paulista de Força e Luz (CPFL)* and *Prefeitura Municipal de São Carlos-SP, Brasil*.

NOMENCLATURE

Symbols and Abbreviations

Add.	Addition	
An.Biod.$_{COD}$	Anaerobic Biodegradability in terms of chemical oxygen demand removal	%
AnSBBR	Anaerobic Sequencing Batch Biofilm Reactor	
Biod.	Biodegradability	
BMP	Biochemical Methane Potential	
BOD	Biochemical Oxygen Demand	mg.L^{-1}
COD	Chemical Oxygen Demand	mg.L^{-1}
COD$_{Eq.}$	Chemical Oxygen Demand equivalent	mg.L^{-1}
COD$_{Filtered}$	Chemical Oxygen Demand of filtered samples	mg.L^{-1}
COD$_{Total}$	Chemical Oxygen Demand of non-filtered samples	mg.L^{-1}
Cd	Cadmium	mg.L^{-1}
Cr	Chromium	mg.L^{-1}
C$_{S0}$	Initial Substrate Concentration	mg.L^{-1}
C$_{Sf}$	Final Substrate Concentration	mg.L^{-1}
C$_{Si}$	Intermediate Substrate Concentration	mg.L^{-1}
Cu	Copper	mg.L^{-1}
Effic.	Efficiency	%
Effl.	Effluent	
Eq.	Equation	
FAN	Free Ammonia Nitrogen	mgN.L^{-1}
fca	Correction factor due to recovery and conversion	mgHAc. mgCaCO$_3^{-1}$
fcb	Correction factor due to interfering substances	mgHAc.L^{-1}
Fe	Iron	mg.L^{-1}
HAc	Acetic acid	
Infl.	Influent	
Leach.	Leachate	
Mn	Manganese	mg.L^{-1}
N	Nitrogen	
NH_4^+-N	Ammonium nitrogen	mg**N**.L^{-1}
Ni	Nickel	mg.L^{-1}
P.	Profile	
Pb	Lead	mg.L^{-1}
PVC	Polyvinyl Chloride	
r^2	Coefficient of determination	
Rem.	Removal	
TA	Total Alkalinity	mgCaCO$_3$. L^{-1}
TFS	Total Fixed Solids	mg.L^{-1}
TOC	Total Organic Carbon	mgC.L^{-1}
TS	Total Solids	mg.L^{-1}
TVA	Total Volatile Acids	mg.L^{-1}
TVAA	TVA alkalinity by the DiLallo and Albertson (1961) method	mgCaCO$_3$. L^{-1}
TVS	Total Volatile Solids	mg.L^{-1}
VFA	Volatile fatty acids	
$\Delta_{max.}$	Maximum difference	%

REFERENCES

Anfruns, A., Gabarró, J., Gonzalez-Olmos, R., Puig, S., Balaguer, M. D., Colprim, J., Coupling anammox and advanced oxidation-based technologies for mature landfill leachate treatment. Journal of Hazardous Materials, 258, 27-34 (2013).

Bezerra, R. A., Rodrigues, J. A. D., Ratusznei, S. M., Canto, C. S. A., Zaiat, M., Effect of organic load on the performance and methane production of an AnSBBR treating effluent from biodiesel production. Applied Biochemistry and Biotechnology, 165, 347-368 (2011).

Castillo, E., Vergara, M., Moreno, Y., Landfill leachate treatment using a rotating biological contactor and an upward-flow anaerobic sludge bed reactor. Waste Management, 27, 720-726 (2007).

Contrera, R. C., Tratamento de lixiviados de aterros sanitários em sistema de reatores anaeróbio e aeróbio operados em batelada seqüencial. São Carlos-SP, p. 731, Tese de Doutorado – Escola de Engenharia de São Carlos, Universidade de São Paulo (2008). (In Portuguese).

Contrera, R. C., Sarti, A., Castro, M. C. A., Foresti, E., Zaiat, M., Schalch, V., Ethanol addition as a strategy for start-up and acclimation of an AnSBBR for the treatment of landfill leachate. Process Biochemistry, 48, 1767-1777 (2013).

Costabile, A. L. O., Canto, C. S. A., Ratusznei, S. M., Rodrigues, J. A. D., Zaiat, M., Foresti, E., Temperature and feed strategy effects on sulfate and organic matter removal in an AnSBB. Journal of Environmental Management, 92, 1714-1723 (2011).

Cubas, S. A., Foresti, E., Rodrigues, J. A. D., Ratusznei, S. M., Zaiat, M., Influence of liquid-phase mass transfer on the performance of a stirred anaerobic sequencing batch reactor containing immobilized biomass. Biochemical Engineering Journal, 17, 99-105 (2004).

Dague, R. R., Habben, C. E., Pidaparti, S. R., Initial studies on the anaerobic sequencing batch reactor. Water Sci. Technol., 26, 2429-32 (1992).

Damasceno, L. H. S., Rodrigues, J. A. D., Ratusznei, S. M., Zaiat, M., Foresti, E., Effects of feeding time and organic loading in an anaerobic sequencing batch biofilm reactor (ASBBR) treating diluted whey. Journal of Environmental Management, 85, 927-935 (2007).

Dilallo, R., Albertson, O. E., Volatile acids by direct tritation. J. Water Pollut. Control Fed., 33, 356-365 (1961).

Eldyasti, A., Andalib, M., Hafez, H., Nakhla, G., Zhu, J., Comparative modeling of biological nutrient removal from landfill leachate using a circulating fluidized bed bioreactor (CFBBR). Journal of Hazardous Materials, 187, 140-149 (2011).

Imen, S., Ismail, T., Sami, S., Fathi, A., Khaled, M., Ahmed, G., Latifa, B., Characterization and anaerobic batch reactor treatment of Jebel Chakir Landfill leachate. Desalination, 246, 417-424 (2009).

Kennedy, K. J. and Lentz, E. M., Treatment of landfill leachate using sequence batch and continuous flow upflow anaerobic sludge blanket (UASB) reactors. Wat. Res., 34(14), 3640-3656 (2000).

Kuenen, J. G., Anammox bacteria: from discovery to application. Nature, 06, April, pp. 320-326 (2008).

Kulikowska, D., Nitrogen removal from landfill leachate via the nitrite route. Braz. J. Chem. Eng., 29(2), 211-219 (2012).

Manssouri, M. E., Rodrigues, J. A. D., Ratusznei, S. M., Zaiat, M., Effects of organic loading, influent concentration, and feed time on biohydrogen production in a mechanically stirred AnSBBR treating sucrose-based wastewater. Applied Biochemistry and Biotechnology, 171, 1832-1854 (2013).

Millot, N. and Courant, P., Treatability Characteristics of Landfill Leachate. In Christensen, T. H., Cossu, R., Stegmann, R., Landfilling of Waste: Leachate. Chapman & Hall Ltd., Reprinted (1997).

Oliveira, D. S., Prinholato, A. C., Ratusznei, S. M., Rodrigues, J. A. D., Zaiat, M., Foresti, E., AnSBBR applied to the treatment of wastewater from a personal care industry: Effect of organic load and fill time. Journal of Environmental Management, 90, 3070-3081 (2009).

Pinho, S. C., Ratusznei, S. M., Rodrigues, J. A. D., Foresti, E., Zaiat, M., Influence of the agitation rate on the treatment of partially soluble wastewater in anaerobic sequencing batch biofilm reactor. Water Research, 38, 4117-4124 (2004).

Qasim, S. and Chiang, W., Sanitary Landfill Leachate: Generation, Control and Treatment. Technomic Publishing Company, Inc., Lancaster, Pensylvania, USA (1994).

Rada, E. C., Istrate, I. A., Ragazzi, M., Andreottola, G., Torretta, V., Analysis of electro-oxidation suitability for landfill leachate treatment through an experimental study. Sustainability, 5(9), 3960-3975 (2013).

Ratusznei, S. M., Rodrigues, J. A. D., Camargo, E. F. M., Zaiat, M., Borzani, W., Feasibility of a stirred anaerobic sequencing batch reactor containing immobilized biomass for wastewater treatment. Bioresource Technology, 75, 127-132 (2000).

Ratusznei, S. M., Rodrigues, J. A. D., Camargo, E. F. M., Ribeiro, R., Zaiat, M., Effect of feeding strategy on a stirred anaerobic sequencing fed-batch reactor containing immobilized biomass. Bioresource Technology, 90, 199-205 (2003).

Rodrigues, J. A. D., Ratusznei, S. M., Camargo, E.

F. M., Zaiat, M., Influence of agitation rate on the performance of an anaerobic sequencing batch reactor containing granulated biomass treating low-strength wastewater. Advances in Environmental Research, 7, 405-410 (2003).

Rodrigues, J. A. D., Pinto, A. G., Ratusznei, S. M., Zaiat, M. and Gedraite, R., Enhancement of the performance of an anaerobic sequencing batch reactor treating low-strength wastewater through implementation of a variable stirring rate program. Braz. J. Chem. Eng., 21(3), 423-434 (2004).

Sarti, A., Fernandes, B. S., Zaiat, M., Foresti, E., Anaerobic sequencing batch reactors in pilot-scale for domestic sewage treatment. Desalination, 216, 174-182 (2007).

Selma, V. C., Cotrim, L. H. B., Rodrigues, J. A. D., Ratusznei, S. M., Zaiat, M., Foresti, E., ASBR applied to the treatment of biodiesel production effluent: effect of organic load and fill time on performance and methane production. Appl. Biochem. Biotechnol., 162, 2365-2380 (2010).

Silva, A. J., Domingues, M. R., Hirasawa, J. S., Varesche, M. B., Foresti, E. and Zaiat, M., Kinetic modeling and microbial assessment by fluorescent in situ hybridization in anaerobic sequencing batch biofilm reactors treating sulfate-rich wastewater. Braz. J. Chem. Eng., 28(2), 209-219 (2011).

Siman, R. R., Borges, A. C., Ratusznei, S. M., Rodrigues, J. A. D., Zaiat, M., Foresti, E., Borzani, W., Influence of organic loading on an anaerobic sequencing biofilm batch reactor (ASBBR) as a function of cycle period and wastewater concentration. Journal of Environmental Management, 72, 241-247 (2004).

Standard Methods for the Examination of Water and Wastewater. 21st Ed. American Public Health Association/American Water Works Association/ Water Environment Federation, Washington, DC, USA (2005).

Sun, H., Yang, Q., Peng, Y., Shi, X., Wang, S., Zhang, S., Advanced landfill leachate treatment using a two-stage UASB-SBR system at low temperature. Journal of Environmental Sciences, 22(4), 481-485 (2010).

Sung, S. and Dague, R. R., Laboratory studies on the anaerobic sequencing batch reactor. Water Environ Res., 67, 294-301 (1995).

Tchobanoglous, G., Theisen, H., Vigil, S. A., Integrated Solid Waste Management: Engineering Principles and Management Issues. McGraw-Hill International Editions (1993).

Timur, H. and Özturk, I., Anaerobic sequencing batch reactor treatment of landfill leachate. Wat. Res., 33(15), 3225-3230 (1999).

van der Star, W. R. L., Abma, W. R., Blommers, D., Jan-Willem Mulder, J.-W., Tokutomi, T., Strous, M., Picioreanu, C., van Loosdrecht, M. C. M., Startup of reactors for anoxic ammonium oxidation: Experiences from the first full-scale anammox reactor in Rotterdam. Wat. Res., 41, 4149-4163 (2007).

van Haandel, A. C. and Lettinga, G., Anaerobic Sewage Treatment: A Practical Guide for Regions with a Hot Climate. John Wiley & Sons; 1st Edition (1995).

Vich, D. V., Garcia, M. L. and Varesche, M. B. A., Methanogenic potential and microbial community of anaerobic batch reactors at different ethylamine/sulfate ratios. Braz. J. Chem. Eng., 28(1), 1-8 (2011).

Wang, K., Wang, S., Zhu, R., Miao, L., Peng, Y., Advanced nitrogen removal from landfill leachate without addition of external carbon using a novel system coupling ASBR and modified SBR. Bioresource Technology, 134, 212-218 (2013).

Zhu, R., Wang, S., Li, J., Wang, K., Miao, L., Ma, B., Peng, Y., Biological nitrogen removal from landfill leachate using anaerobic-aerobic process: Denitritation via organics in raw leachate and intracellular storage polymers of microorganisms. Bioresource Technology, 128, 401-408 (2013).