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6 **Performance evaluation of micro-aerobic hydrolysis of mixed sludge: Optimum**
7 **aeration and effect on its biochemical methane potential**
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19 **ABSTRACT**
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21 This study evaluated the performance of a micro-aerobic hydrolysis of mixed sludge and its
22 influence as a pretreatment of this waste for its subsequent anaerobic digestion. Three
23 experimental series were carried out to evaluate the optimum micro aeration levels in the
24 range from 0.1 to 0.5 vvm and operation times within the range 24-60 h. The maximum

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methane yield (35 mL CH₄/g VSS added) was obtained for an aeration level of 0.35 vvm.
This methane yield value increased 114% with respect to that obtained with the non-aerated
sludge. In the micro aeration process carried out at an aeration level of 0.35 vvm, increases
in soluble proteins and total sugars concentrations of 185% and 192% with respect of their
initial values were found respectively after 48 h of aeration. At the above micro-aerobic
conditions, soluble COD augmented 150% while volatile suspended solids (VSS) content
decreased until 40% of their initial respective values. Higher COD_s increases and VSS
decreases were found at 60 h of micro-aeration, however, the above parameters did not vary
significantly with respect to the values found at 48 h.

Keywords: Aerobic hydrolysis, anaerobic digestion, methane production, mixed sludge.

INTRODUCTION

The anaerobic process, commonly called anaerobic digestion, is the most used
treatment technology for organic matter removal from primary and waste activated sludge
(WAS).^[1-3] The benefits associated with anaerobic technology include mass reduction, odor
removal,^[4-5] pathogen reduction, and more significantly, energy recovery in the form of
methane.^[6] However, it should be emphasized that sludge contains 10 times the energy
required to treat it. It has been proven to be technically feasible to recover energy from the

sludge, which can be directly used in wastewater treatment or be sold to the network, reducing the facility's dependency on conventional electricity and helping the stressed public budgets. Nevertheless, the anaerobic digestion of sewage sludge has several disadvantages such as relatively low methane production, 30 - 50 % biodegradability and the presence of some inhibitory compounds that make necessary the use of high retention times in the digesters with high mixing costs.^[7-9] These limiting factors are generally associated with the hydrolysis stage.^[10-11] During hydrolysis, cell walls are ruptured and extracellular polymeric substances (EPS) are degraded resulting in the release of readily available organic material for the acidogenic microorganisms. This mechanism is particularly important in mixed sludge digestion, since the major constituent of its organic fraction are cells, being a relatively unfavorable substrate for microbial degradation.

In order to avoid or reduce the existence of the hydrolysis process in the sludge anaerobic digestion several methods have been studied, alone or in combination such as thermal^[12-14], physical-chemical^[15-16] and mechanical.^[17-18] Some biological methods have been also investigated.^[19-20]

Among biological, the use of micro aeration as a treatment prior to anaerobic digestion has received less attention, even though it has been reported that the limited oxygen supply caused an increase of the enzymatic hydrolysis rates for the case of complex organic matter in batch tests.^[21] Results from some micro aerobic research carried out with municipal solid wastes and other wastes suggest that hydrolysis, and therefore, the COD solubilization might be improved by micro aeration.^[22-24] However, the presence of oxygen in the anaerobic digestion can also produce a problem regarding to the use and degradation of organic matter. In presence of oxygen, there is a competition by the substrate between facultative and strict anaerobes^[25], which could reduce the organic matter available for

methanogenic archaea, because part of the substrate is used for the production of CO₂ and biomass by facultative microorganisms. Therefore, the management of micro-aeration should be carefully carry out in order to improve the hydrolytic rate with a minimum consume of organic matter by facultative microorganisms with CO₂ and biomass production.

Regarding the effect of micro-aeration on sewage sludge anaerobic digestion, few studies have been reported. Jenicek et al.^[26], using a continuous bioreactor and pumping air continuously into the sludge recirculation stream, showed that VSS/TSS ratio of the digested sludge, soluble COD concentration, ammonia nitrogen and phosphate concentration decreased in all experiments with micro aerobic conditions. Jenicek et al.^[27] working with a continuous stirred tank reactor (CSTR) under continuous micro aeration, indicated that efficient H₂S removal, higher specific methane production, lower methane concentration in biogas due to dilution by nitrogen remained from the air dosed were achieved in the anaerobic digestion of the pre-aerated sludge, while better sludge liquor quality and lower foaming potential and foam stability were observed in the pre-treated sludge after using micro aeration. Montalvo et al.^[28] determined that applying 0.3 vvm, and at temperature of 35 C were the best operational conditions increasing hydrolysis rate of mixed sludge. However they did not find the optimum amount of air for hydrolysis nor tested the effect of aerobic hydrolysis to different aeration rates on anaerobic digestion sludge

Given this background, the behavior and performance of the micro aerobic hydrolysis of mixed sewage sludge looking for the optimum level of aeration were evaluated in the present research work with the aim of achieving maximum efficiency of hydrolysis. The effect of the micro-aeration level of the mixed sludge on the methane yield was also

evaluated in biochemical methane potential tests carried out at 35 °C in batch mode, comparing the results obtained with the pre-aerated sludge with those achieved for control sludge without pre-aeration.

MATERIALS AND METHODS

Three experimental runs were carried out at laboratory-scale. A summary of the operating conditions assayed in each experimental run is shown in Table 1.

The substrate used in all the assays was a mixed sludge (mixture of waste activated sludge and primary sludge). This mixed sludge was obtained from the urban wastewater treatment plant (UWTP) called “La Farfana”, which is located in Santiago de Chile city. Each studied experimental run was carried out by duplicate.

The experimental run I was performed with one sample of mixed sludge while the experimental series II and III were carried out with four samples of mixed sludge taken during different times along the day with the aim of obtaining more representative results.

In the experimental run I, batch reactors of 5 L of total volume and 4 L of working volume were used for the micro aeration process. After micro aerobic pretreatment, 200 mL of pretreated sludge is passed into batch anaerobic digesters of 280 mL of total volume. After that, 50 mL of anaerobic inoculum is added to each anaerobic digester. The anaerobic inoculum came from the anaerobic plants that digest waste activated sludge at “La Farfana” UWTP. This anaerobic inoculum had a VSS concentration of 10 g/L and a specific methanogenic activity of 0.32 g COD-CH₄/(g VSS·d). The anaerobic digesters were sealed and headspace flushed with N₂ at the beginning of the test. The produced biogas was measured by liquid displacement after going through a 3% (w/w) NaOH solution to capture

the produced CO₂; the remaining gas was assumed to be only methane. The anaerobic tests were run for a period of c.a. 20-25 days until the accumulated gas production remained essentially unchanged, i.e. on the last day production was lower than 3% of the accumulated methane produced. The main objective of this experimental run was to obtain the best aeration level within the range from 0.1 to 0.5 vvm (volume of air/(volume of reactor · minute)) taken into account as criterion the methane production achieved in the batch anaerobic digestion experiment of the pre-aerated sludge. The experiment corresponding to an aeration level of 0.3 vvm was extended until 60 h with the aim of assessing the evolution of proteins and total sugars with time.

The experimental run II had two main objectives: firstly, to obtain the optimum aeration level and, secondly, to assess the influence of aeration time on the hydrolysis process. After this run, no batch anaerobic digestion experiments of the pre-aerated sludge were made.

Finally in the experimental run III, the performance of the micro-aerated hydrolysis was evaluated from the evolution of different parameters such as soluble proteins and sugars with time and its influence on the methane production of the pre-aerated sludge. In this run II, it was used the best aeration level obtained in the second experimental run (0.35 vvm).

The operating temperature selected for the micro aerobic hydrolysis experiments was 35 °C according to previous results reported in the literature.^[28] This temperature was also used for batch anaerobic digestion experiments of the pretreated sludge because it coincides with the optimum temperature reported for achieving maximum methane production within the mesophilic interval.^[7]

All chemical analysis were determined according to American Public Health Association Standard Methods.^[29] Specifically, chemical oxygen demand (COD) and volatile suspended solids were analysed according to the closed digestion and colorimetric 5220D method and

2540B method, respectively of the Standard Methods (APHA, 2012).^[29] pH was determined using a pH-meter model Crison 20 Basic.

Soluble proteins and total sugars were determined using the ASTM D5712 and ASTM D6406 standard methods, respectively.

For the statistic processing and analysis of the data, the software Minitab 8 was utilized. An ANOVA analysis was done and a comparison of confidence intervals for mean values was made with a confidence of 95%.

RESULTS AND DISCUSSION

No significant differences in the behavior of duplicate reactors were observed in all assays. Therefore, only the results of one of the duplicate reactors will be shown.

Assay I

Figure 1 shows the evolution of the methane production (measured as mL methane/g of volatile suspended solids (VSS) added), which will allow to assess the variation of the accumulated methane with time per each gram of VSS added at the beginning of the anaerobic digestion process.

As can be seen for an aeration level of 0.1 vvm only a small increase in methane production was observed compared to the control (not subjected to aeration). An ANOVA analysis of the data revealed that this difference in production of methane was not statistically significant with a confidence level of 95%. In this case, the following values of statistical parameters were obtained: $F=0.141$; $p=0.709$; $F_{critical}=4.130$ ($F_{critical}$ value was higher than

F). By contrast, for an aeration level of 0.3 vvm an increase of 114% in the methane production of the pre-aerated sample was achieved compared to the control without aeration. In this case, the difference was statistically significant as was shown in the statistical analysis ($F = 6.789$; $p = 0.013$; $F_{\text{critical}} = 4.149$). Finally, for the experimental run of 0.5 vvm, no significant differences between the methane production of the pre-aerated sludge and the control were detected ($F=0.044$; $p=0.834$; $F_{\text{critical}}=4.130$), which demonstrates that a high aeration may not be beneficial, and, even, harmful for the anaerobic digestion process.

Therefore, according to these results an aeration level of 0.3 vvm contributes to better performance of methanogenic microorganisms confirmed by the methane production obtained in this case. When the aeration level increased up to 0.5 vvm a decrease in methane production was observed as consequence of the higher concentration of dissolved oxygen, which inhibited the metabolism and activity of methanogens. Gerritse and Gottschal^[30] demonstrated that an aeration level of 0.1 vvm did not increase the methane formation compared to a control sample. However, a short time of exposure to oxygen did not reduce the methanogenic activity and under these conditions the methanogens can survive more time than previously expected.^[31] For instance, oxygen concentration levels in the range of 4.9-6.4 mg/L are required to inhibit 50% of the methanogenic activity.^[32] All these results clearly indicated that an adequate optimization of oxygen supply to aeration is required in all cases to avoid the inhibition of methanogenic activity.

As was shown in Figure 1, the maximum methane yield (35 mL CH₄/g VSS added) was obtained for an aeration level of 0.3 vvm. This value represents an increase of 114% in methane production compared to that obtained with the non-aerated sludge. This increase in the methanogenic activity is attributed to an improvement in the growth of facultative

anaerobic microorganisms, which can keep a low redox potential, providing the best conditions for the growth of strict anaerobes.^[23]

Table 2 shows the effect of aeration on the final pH of the reactors. As can be seen, a pH increase of 7.1% and 3.2% was achieved in the reactors with aeration and non-aeration, respectively. This pH increase can be due to two main factors: firstly, the hydrolysis of proteins produces the liberation of amino groups ($-\text{NH}_2$), which are converted into ammonia (NH_3), which when is dissolved generates ammonium (NH_4^+) and hydroxyl ions (OH^-), causing a pH increase.^[33] Second, when micro-aeration is applied, a CO_2 stripping takes place by the air supplied to the process.

Figures 2 shows the evolution of the soluble proteins (measured as equivalent concentration of bovine serum albumin, BSA) during the micro-aeration process at 0.3 vvm of the three samples of sludge.

As can be seen in Figure 2, an increase in the soluble proteins of 185% was observed after 48 h of aeration (at 0.3 vvm), while that an increase of only 7.8% was detected in the non-aerated reactor. The evolution of the soluble proteins with time had a behavior of bell-shaped, achieving a maximum value at 48 h of aeration. This behavior can be explained taking into consideration that a part of the mixed sludge is composed by waste activated sludge, which consisted of flocs formed by clusters of cells and organic matter within a viscous material. For this reason, there was an increase in soluble proteins until 48 h, which were released and at the same time the insoluble proteins retained in the flocs could also be solubilized. This process is called de-flocculation, and is caused, among other factors, by a decrease in the aeration of the activated sludge process, reducing the amount of dissolved oxygen, which coincides with the operating parameters in the present work, where low air

flows were injected, causing the low amount of dissolved oxygen in the reactors, its concentration was found to be zero in all measurements carried out.

Another possible explanation of the increase in the protein concentration during the first 48 h of aeration is the solubilization of insoluble proteins found in the organic matter of the sludge by proteases enzymes.

Finally, once the maximum protein concentration was achieved at 48 h, a decrease in the protein content was observed due to its degradation by non-hydrolytic bacteria.

Figure 3 shows evolution of total sugars (measured as equivalent concentration of glucose).

As it is known hydrolysis is the rate-limiting step for the carbohydrates conversion.^[34]

Therefore, the total sugar concentration also increased in a similar manner during the first 48 h of aeration, obtaining an increase of 192% with respect of its initial value (Figure 3).

This rise can be mainly attributed to the hydrolysis of polysaccharides to monosaccharides when micro-aeration is applied, which is extremely beneficial for anaerobic digestion. A maximum value in the sugar concentration was achieved at 48 h of aeration, after which a soft decrease was detected. This may be attributed to a certain conversion of polysaccharides after this time and the beginning of the transformation of the monosaccharides. By contrast, the increase in the total sugars in the non-aerated reactor was only of 3%.

Table 3 summarizes the values of the concentration of total ammonia (TAMON), total COD (COD_T), soluble COD (COD_S) and VSS (average of the three samples of mixed sludge) after micro-aeration (0.3 vvm) during 48 h and after non-aeration conditions.

The results shown in Table 3 indicate that the micro aeration process of mixed sludge influence the significant increase of ammonia nitrogen, which is due to the hydrolysis of proteins and their further conversion to amino-acids and ammonia nitrogen. This agrees

with the increase of pH from 5.8 to 6.0-6.2, which results beneficial for the anaerobic digestion process of the pre-aerated sludge. Despite the increase in ammonia concentration up to values of 920 mg/L, this value cannot be considered as toxic or inhibitory for anaerobic process, whose values range from 1500-3000 mg/L^[35-36] The COD_S also increased both in the non-aerated as in the micro-aerated reactors. However, in the reactors with micro-aeration, the COD_S increased 150% with respect of its initial value, while in the non-aerated ones this increase was only of 27%. These values showed that micro-aeration promotes the hydrolysis of the complex organic matter and its conversion to soluble matter with a considerable rise of the COD_S as a result of the enzymatic reaction.^[37]

On the other hand, the COD_T experimented a relatively small decrease in both cases studied, being this decrease quite less for the non-aerated reactor. This implies that the complex organic matter can be transformed into more simple matter but never to develop a removal process of organic matter aerobically, which would imply a much higher energy expenditure.

Finally, the VSS concentration also decreased in all reactors, although this decrease was quite higher in the micro-aerated reactors (40%) compared to that achieved in the control reactors (11%). This again shows the micro-aeration promote the hydrolysis of the complex organic matter, and this matter is precisely the substrate of enzymatic reaction.^[38]

Assay II

Figure 4 shows the evolution of the COD_S with time for each one of the four samples of mixed sludge (A, B, C and D) subjected to micro-aerobic hydrolysis using aeration levels of 0.3, 0.35, 0.4, 0.45 and 0.5 vvm. As can be seen, an increase in COD_S with the aeration

time was observed in most cases. Specifically, increases in COD_S of between 100% and 383% were observed for the three experimental runs carried out (Figures 8 B, C and D) when the aeration intensity increased from 0.3 to 0.5 vvm. Therefore, when aeration was increased between the mentioned values, the COD_S was at least doubled. For the best aeration level tested (0.35 vvm) an increase in the solubility from 8% to 23% was obtained when the aeration time increased from 48 to 60 h.

The variation of the VSS with time for the four experimental runs carried are with different aeration intensities is shown in Figure 5. A decrease in the VSS content was observed for all the cases studied. VSS reduction percentages in the range from 11.5% to 23.6% were achieved after 60 h of aeration. This reduction represents the decomposition of the complex organic matter that is transformed in the substrate of the hydrolytic reactions. The highest VSS removal was reached with an aeration level of 0.35 vvm. Therefore, the use of a controlled aeration with a specific aeration level allow eliminating certain non-biodegradable compounds or compounds more difficult to biodegrade using only anaerobic digestion.^[39] VSS reductions of between 2-5% were only reached between the 48 and 60 h of aeration for the experiments carried out with an aeration level of 0.35 vvm.

Finally, the VSS removal percentages obtained at an aeration level of 0.35 vvm (23.6%) were higher than others reported in the literature using an increase of temperature between 40 and 50°C (20% VSS reduction) instead of a micro-aeration step.^[40]

Assay III

Figure 6 shows the variation of the COD_T with time during the anaerobic digestion processes of the non-aerated sludge (control) and micro-aerated sludge at 0.35 vvm. As can

be seen a higher COD_T removal efficiency was always observed for the micro-aerated sludge compared to the control, being the final COD_T removal efficiency for the pre-aerated sludge at 23th day of digestion time 17% higher than for the control, difference that can be considered statistically significant.

Figure 7 illustrates the variation of the VSS with digestion time for the anaerobic digestion of micro-aerated sludge and control. As can be seen a clear decrease in the VSS content with time was observed from the beginning of the process, in which the hydrolytic stage takes place, achieving low values of this parameter, especially in the case of the pre-aerated sludge. Similar trends in the evolution of VSS with time were reported by Diak et al.^[41], although final VSS values close to 15000 mg/L were obtained in this case, being this value much higher than those reached in the present work

Figure 8 shows the variation of methane production (measured as accumulated CH₄ in mL/g VSS added) as a function of the digestion time, which allows evaluating the volume of methane produced per each gram of VSS added at the beginning of the anaerobic digestion process. According to the data shown in this Figure, a significant increase (110%) in the production of methane was obtained when applying to the mixed sludge aerobic hydrolysis compared to the digestion process of non-aerated sludge. These results are consistent with the evolution of the COD and VSS presented previously. Lower increments in the methane yield (21%) were previously reported during the anaerobic co-digestion of brown water and food waste (during 45 day operation time) previously subjected to an micro-aerobic pretreatment with an aeration intensity of 0.0375 L O₂/L_{reactor}/day.^[41]

CONCLUSIONS

This study demonstrated that biological hydrolysis by micro aeration of mixed sludge from urban wastewater treatment plants is an effective pretreatment of this waste for its subsequent anaerobic digestion. The most efficient aeration level to increase significantly the methane yield of the micro aerated sludge compared to a control without pre-aeration was 0.35 vvm. Although higher COD increases and VSS decreases were found at 60 h of micro aeration, the above parameters did not vary significantly with respect to the values found at 48 h. Therefore, it would be necessary carefully evaluate the need to extend the aeration for more than 48 h since the process improvement was not significantly increased after 2 days of aeration. The application of a hydrolysis by micro aeration also allowed stimulating the production of exoenzymes that carried out the degradation of slowly biodegradable compounds which were otherwise resistant to degradation under completely anaerobic conditions.

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REFERENCES

- [1] Appels, L.B. Principles and potential of the anaerobic digestion of waste-activated sludge. *Prog. Energy Combust. Sci.* **2008**, *34*(6), 755-781.

- [2] Fonoll, X.; Astals, S.; Dosta, J.; Mata-Alvarez, J. Anaerobic co-digestion of sewage sludge and fruit wastes: Evaluation of the transitory states when the co-substrate is changed. *Chem. Eng. J.*, **2015**, *262*, 1268–1274,
- [3] Huiliñir, C.; Montalvo, S.; Guerrero, L. Biodegradability and methane production from secondary paper and pulp sludge: Effect of fly ash and modeling. *Water Sci. Technol.* **2015**, *72*(2), 230–237.
- [4] Pilli, S.; Bhunia, P.; Yan, S.; LeBlanc, R.J.; Tyagi, R.D.; Surampalli, R.Y. Ultrasonic pretreatment of sludge: a review. *Ultrason. Sonochem.* **2011**, *18*(1), 1-18.
- [5] Zhang, L.; Zhang, Y.; Zhang, Q.; Verpoort, F.; Cheng, W.; Cao, L.; Li, M. Sludge gas production capabilities under various operational conditions of the sludge thermal hydrolysis pretreatment process. *J. Energy Inst.* **2014**, *87*(2), 121–126.
- [6] Garrido-Baserba, M.; Molinos-Senante, M.; Abelleira-Pereira, J. M.; Fdez-Güelfo, L. A.; Poch, M.; Hernández-Sancho, F. Selecting sewage sludge treatment alternatives in modern wastewater treatment plants using environmental decision support systems. *J. Cleaner Product.* **2015**, *107*, 410–419.
- [7] Bolzonella, D.; Pavan, P.; Battistoni, P.; Cecchi, F. Mesophilic anaerobic digestion of waste activated sludge: influence of the solid retention time in the wastewater treatment process. *Process Biochem.* **2005**, *40*(3-4), 1453-1460.
- [8] Lee, I-S.; Parameswaran, P.; Rittmann, B.E. Effects of solids retention time on methanogenesis in anaerobic digestion of thickened mixed sludge. *Bioresour. Technol.* **2011**, *102*(22), 10266–10272.
- [9] Pérez – Elvira, S.I.; Fernández-Polanco, M.; Fernández-Polanco, F. Enhancement of the anaerobic digestion of sludge: comparisons of four different strategies. *Water Sci. Technol.* **2011**, *64*(2), 375–383.

- [10] Tiehm, A.; Nickel, K.; Zellhorn, M.; Neis, U. Ultrasonic waste activated sludge disintegration for improving anaerobic stabilization. *Water Res.*, **2001**, 35(8), 2003-2009.
- [11] Zhu, M.; Lu, F.; Hao, L.P.; He, P.J.; Shao, L.M. Regulating the hydrolysis of organic wastes by micro-aeration and effluent recirculation. *Waste Manage.* **2009**, 29(7), 2042-2050.
- [12] Ruíz, J.E.; Méndez, J.M.; Alvarado, A.; Martínez, S.A. Effect of low temperature thermal pre-treatment on the solubilization of organic matter, pathogen inactivation and mesophilic anaerobic digestion of poultry sludge. *J. Environ. Sci. Health Pt. A.* **2005**, 47(12), 1795-1802.
- [13] Carrère, H.; Bougrier, C.; Castets, D.; Delgenès, J.P. Impact of initial biodegradability on sludge anaerobic digestion enhancement by thermal pretreatment. *J. Environ. Sci. Health Pt. A.* **2008**, 43(13), 1551-1555.
- [14] Bien, J.B.; Malina, G.; Bien, J.D.; Wolny, L. Enhancing Anaerobic Fermentation of Sewage Sludge for Increasing Biogas Generation. *J. Environ. Sci. Health Pt. A.* **2004**, 39(4), 939-949.
- [15] Prince-Pike, A.; Wilson, D.I.; Baroutian, S.; Andrews, J.; Gapes, D.J. A kinetic model of municipal sludge degradation during non-catalytic wet oxidation. *Water Res.* **2015**, 87, 225–236.
- [16] Zhen, G.; Lu, X.; Li, Y-Y.; Zhao, Y. Combined electrical-alkali pretreatment to increase the anaerobic hydrolysis rate of waste activated sludge during anaerobic digestion. *Appl. Energy* **2014**, 128, 93-102.
- [17] Braguglia, C.M.; Gianico, A.; Mininni, G. Comparison between ozone and ultrasound disintegration on sludge anaerobic digestion. *J. Environ. Manage.* **2012**, 95, 139–143.

- 382 [18] Cesaro, A.; Belgiorno, V. Pretreatment methods to improve anaerobic
383 biodegradability of organic municipal solid waste fractions. *Chem. Eng. J.*, **2014**, *240*,
384 24-37.
- 385 [19] Guellil, A.; Boualam, M.; Quiquampoix, H.; Ginestet, P.; Audic, P.; Block, J.C.
386 Hydrolysis of wastewater colloidal enzymes extracted from activated sludge flocs.
387 *Water Sci. Technol.* **2001**, *43*(6), 33–40.
- 388 [20] Whittington-Jones; K.J., Molwantwa, J.B.; Rose, P.D. Enhanced hydrolysis of
389 carbohydrates in primary sludge under biosulfidogenic conditions. *Water Res.* **2006**,
390 *40*(8), 1577-1582.
- 391 [21] Botheju, D.; Bakke, R. Oxygen Effects in Anaerobic Digestion – A Review. *The Open*
392 *Waste Manage J.* 2011, *4*, 1-19.
- 393 [22] Kato, S.; Haruta, S.; Cui, Z. J.; Ishii, M.; Igarashi, Y. (2005), Stable coexistence of
394 five bacterial strains as a cellulose-degrading community. *Appl. Environ. Microbiol.*
395 **2005**, *71*(11), 7099- 7106.
- 396 [23] Mshandete, A.; Bjornsson, L.; Kivaisi, A.K.; Rubindamayugi, S.T.; Mattiasson, B.
397 Enhancement of anaerobic batch digestion of sisal pulp waste by mesophilic aerobic
398 pretreatment. *Water Res.* **2005**, *39*(8), 1569-1575.
- 399 [24] Nguyen, P.H.L.; Kuruparan, P.; Visvanathan, C. Anaerobic digestion of municipal
400 solid waste as a treatment prior to landfill. *Bioresour. Technol.* **2007**, *98*(2), 380-387.
- 401 [25] Gerritse, J.; Schut, F.; Gottschal, J.C. Mixed chemostat cultures of obligately aerobic
402 and fermentative or methanogenic bacteria grown under oxygen limiting conditions.
403 *FEMS Microbiol. Lett.* **1990**, *66*(1-3), 87-89.

- 404 [26] Jenicek, P.; Keclik, F.; Maca, J.; Bindzar, J. Use of micro aerobic conditions for the
405 improvement of anaerobic digestion of solid wastes. *Water Sci. Technol.* **2008**, 58,
406 1491-1496.
- 407 [27] Jenicek, P.; Celis, C.A.; Koubova, J.; Ruzickova, I. Change in the digested sludge
408 quality at microaerobic digestion. *J. Residuals Sci. Technol.* **2011**, 8(2), 39-44.
- 409 [28] Montalvo, S.; Huiliñir, C.; Ojeda, F.; Castillo, A.; Lillo, L.; Guerrero, L. Microaerobic
410 pretreatment of sewage sludge: Effect of air flow rate, pretreatment time and
411 temperature on the aerobic process and methane generation *Int. Biodeterior.*
412 *Biodegrad.* **2016**, 110, 1 – 7.
- 413 [29] American Public Health Association (APHA). Standard Methods for the Examination
414 of Water and Wastewater, 20th ed.; American Public Health Association/American
415 Water Works Association/Water Environment Federation, Washington DC, **2012**.
- 416 [30] Gerritse, J.; Gottschal, J.C. Two-membered mixed cultures of methanogenic and
417 aerobic bacteria in O₂ limited chemostats. *J. Genetic Microbiol.* **1993**, 139(8), 1853-
418 1860.
- 419 [31] Conklin, A.; Bucher, R.; Stensel, H.D.; Ferguson, J. Effects of oxygen exposure on
420 anaerobic digester sludge. *Water Environ. Res.* **2007**, 79(4), 396-405.
- 421 [32] Celis-Garcia, M. L.; Ramirez, F.; Revah, S.; Razo-Flores, E.; Monroy, O. Sulphide
422 and oxygen inhibition over the anaerobic digestion of organic matter: influence of
423 microbial immobilization type. *Environ. Technol.* **2004**, 25(11), 1265-1275.
- 424 [33] Gerardi, M.H. *The Microbiology of Anaerobic Digesters*; Wiley- Interscience, John
425 Wiley & Sons, Inc., Publication: New Jersey, 2003; 177 pp.

- [34] Miron, Y.; Zeeman, G.; van Lier J.; Lettinga, G. The role of sludge retention time in the hydrolysis and acidification of lipids, carbohydrates and proteins during digestion of primary sludge in CSTR systems. *Water Res.* **2000**, *34*(5), 1705-1713.
- [35] Markou, G. Improved anaerobic digestion performance and biogas production from poultry litter after lowering its nitrogen content. *Bioresour. Technol.* **2015**, *196*, 726-730.
- [36] Yenigün, O.; Demirel, B. Ammonia inhibition in anaerobic digestion: a review. *Process Biochem.* **2013**, *48*(5-6), 901–911.
- [37] Burgess, J.E.; Pletschke, B.I. *Hydrolytic enzymes in sewage sludge treatment*. Grahamstown: Department of Biochemistry and Biotechnology, Rhodes University, South Africa, 2008.
- [38] Lim, J.W.; Wang, J.Y. Enhanced hydrolysis and methane yield by applying microaeration pretreatment to the anaerobic co-digestion of brown water and food waste. *Waste Manage.* **2013**, *33*(4), 813-819.
- [39] Johansen, J.E.; Bakke, R. Enhancing hydrolysis with microaeration. *Water Sci. Technol.* **2006**, *53*(8), 43-50.
- [40] Diak, J.; Örmeci, B.; Kennedy, K.J. Effect of micro-aeration on anaerobic digestion of primary sludge under septic tank conditions. *Bioprocess Biosyst. Eng.* **2013**, *36*(4), 417-424.

FIGURE CAPTIONS

Figure 1. Variation of the methane production with time for the different experiments carried out in the assay I

Figure 2. Evolution of the soluble protein concentration during the micro-aeration process at 0.3 vvm

Figure 3. Evolution of the total sugars concentration during the micro-aeration process at 0.3 vvm

Figure 4. Effect of the aeration level on the COD_S concentrations during the micro-aerobic hydrolysis of the four samples of mixed sludge from urban wastewaters

Figure 5. Effect of the aeration level on the VSS contents during the micro-aerobic hydrolysis of the four samples of mixed sludge from urban wastewaters

Figure 6. Evolution of the total COD (COD_T) with time in the anaerobic digestion processes of the non-aerated and micro-aerated mixed sludge

Figure 7. Evolution of the VSS with time in the anaerobic digestion processes of the non-aerated and micro-aerated mixed sludge.

Figure 8. Variation of the methane production (mL CH₄/g VSS) with time in the anaerobic digestion processes of the non-aerated and micro-aerated mixed sludge.

Figure 1.

Figure 2.

Figure 3.

(A)

(B)

(C)

(D)

Figure 4.

(D)

(B)

(C)

—◇— 0,3 vvm —□— 0,35 vvm —△— 0,4 vvm —×— 0,45 vvm —*— 0,5 vvm

Figure 5.

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Figure 7.

Figure 8.

541 **Table 1.** Operational conditions of the three experimental runs carried out

# Assay	Aeration level (vvm)	Aeration Time (hours)	Operating Temperature (°C)	Batch anaerobic digestion at 35°C
I	0	48	35	YES
	0.1	48	35	YES
	0.3	48	35	YES
	0.5	48	35	YES
III	0.3	24 – 48 - 60	35	NO*
	0.35	24 – 48 - 60	35	NO*
	0.4	24 – 48 - 60	35	NO*
	0.45	24 – 48 - 60	35	NO*
	0.5	24 – 48 - 60	35	NO*
III	0	48	35	YES
	0.35	48	35	YES

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Table 2. Influence of aeration on the final pH of the reactor

Aeration level [vvm]	Initial pH (t=0 h)	Final pH (t=48 h)
0	5.80 ± 0.05	6.00 ± 0.04
0.35	5.80 ± 0.05	6.20 ± 0.03

Table 3. Variation of the concentration of total ammonia (TAMON), COD_T, COD_S and VSS after the micro-aeration and non-aeration processes

Parameter (mg/L)	Aeration level (vvm)	Aeration time (hours)		Increase (+) o decrease (-) (%)
TAMON	0	0	48	25 (+)
		300	375	
	0.3	300	920	207 (+)
COD _T	0	48900	47433	3 (-)
	0.3	48900	39609	19 (-)
COD _S	0	2500	3175	27 (+)
	0.3	2500	6250	150 (+)
VSS	0	33457	29776	11(-)
	0.3	33457	20074	40 (-)