

Anaerobic Digestion of Wheatgrass Under Mesophilic and Thermophilic Conditions and Different Inoculum Sources

Igor M. O. Silva*, Davide Dionisi

Materials and Chemical Engineering Group, School of Engineering, University of Aberdeen, Aberdeen, UK AB24 3UE

igormosilva@abdn.ac.uk

Anaerobic digestion of lignocellulosic biomass can be used to produce in one single step different products with applications as fuels or as bulk chemicals such as methane, ethanol and other particular volatile fatty acids: acetic, propionic and butyric acids. This study aims to investigate the anaerobic digestion of lignocellulosic biomass (wheatgrass) in batch condition under mesophilic (40°C) and thermophilic (50°C) conditions, two inoculum concentrations and different microbial sources: anaerobic digester sludge and soil. Vials of wheatgrass powder (20 g/l) were inoculated and maintained under anaerobic condition in a water bath shaker with temperature control. The results showed that both inocula were able to hydrolyse lignocellulose biomass without physicochemical pre-treatment, but higher VSS and TC removals were achieved when anaerobic sludge inoculum was used. Acetic acid was the main product for the different batch conditions and corresponded to around 60% of the COD_{products}. Butyric acid, propionic acid and ethanol were identified in lower concentration. The maximum volatile suspended solids (VSS) and total carbohydrates (TC) removal were (44 ± 5) % and (50 ± 11) % respectively. The maximum yield of products in the liquid phase was 37% in COD basis.

1. Introduction

Anaerobic digestion (AD) has gained increasing interest since it can convert organic matter, including lignocellulosic biomass, into added valuable products under minimal pre-treatment (Pesta 2007). Although this process is a classic technology to produce methane, an energy fuel source, the production of other green added value products such as organic acids and alcohols has received research attention (Kleerebezem, Jooisse et al. 2015). This technology presents various advantages over conventional processes since it does not require pre-treatment, sterilization and can use organic waste as feedstock (Dionisi, Silva 2016).

The AD process is composed of four stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. During these stages, the substrate is hydrolysed and converted to volatile fatty acids (VFAs) and alcohols, before further conversion to methane and carbon dioxide. However, the recalcitrant properties of lignocellulosic material have been a challenge to the hydrolysis stage (Dionisi, Anderson et al. 2015). Some researchers have been focusing on the pre-treatment of the feedstock under physicochemical conditions, but the high temperature, pressure, amount of chemicals/enzymes required may lead the process to be a more expensive and complex technology. This paper investigates the anaerobic digestion of lignocellulosic substrate (wheatgrass) without pre-treatment for two different temperatures, inoculum concentrations and different inoculum sources. The study aims to analyse the effect of these conditions on the hydrolysis of lignocellulosic biomass and the value-added products that can be produced during the fermentation process. The experiment was carried out under batch condition and the concentration of products, volatile suspended solids (VSS), total and soluble carbohydrates (TC and SC), chemical oxygen demand (COD) and pH were monitored throughout the experiment.

2. Materials and methods

2.1 Media composition

The lignocellulosic biomass used for the fermentation process was wheatgrass powder (Bulk Powders™). This substrate is typically composed of 33-40% cellulose, 10-18% hemicellulose and 9-17% lignin (Levakhin, Duskaev et al. 2015). The fermentation media was comprised of: wheatgrass powder (20.0 g/l), K_2HPO_4 (17.4 g/l), NaH_2PO_4 (12.0 g/l), NH_4Cl (2.0 g/l), $MgCl_2 \cdot 6H_2O$ (0.125 g/l) and $CaCl_2 \cdot 6H_2O$ (0.09 g/l). The phosphate buffer (200 mM) was used to maintain the pH in the range 6-7 throughout the experiment, since no external pH controller was used.

2.2 Inoculum and batch vials set-up

Two different inoculum sources were used for the fermentation process: 1) sludge from an anaerobic digester (Gask, Turriff, Aberdeenshire, UK), whose VSS concentration was (22.7 ± 0.4) g/l; 2) soil collected from a controlled site (Craibstone in Aberdeenshire, UK) (0.115 ± 0.003 g of VSS / g of soil). The sludge was filtered through a Buchner funnel to remove large solid particles ($d_p > 0.12$ mm). The soil was sieved with a 150 μ m mesh prior to inoculation to obtain homogenous and thin particles.

Four media solutions of 200 ml were prepared and nitrogen (free of oxygen) was sparged for 5 minutes before inoculation. Two solutions were inoculated with 10 ml of anaerobic sludge (1 g VSS/l of inoculum concentration) (Batch 1 and 2). One solution was inoculated with 40 ml of anaerobic sludge (4 g VSS/l of inoculum concentration) (Batch 3). The final solution was inoculated with 2 g of soil (1 g VSS/l of inoculum concentration) (Batch 4). All the different solutions were homogenized and divided in samples of 10 ml. The vials were sealed to guarantee anaerobic condition and inserted in a water bath shaker with the respective temperatures: Batch 1, 3 and 4 at 40°C; Batch 2 at 50°C.

2.3 Analytical methods

The batch experiment was carried out in duplicate and two vials were sacrificed for the analytical methods. The vials were opened and homogenized with magnetic stir bar to perform the following analysis: volatile suspended solids (VSS) concentration, total and soluble carbohydrate (TC and SC) concentration and volatile fatty acids (VFAs) and ethanol concentration. VSS concentration was measured according to the Standard Methods (Rice, Bridgewater et al. 2012). The total and soluble carbohydrate concentrations were estimated using the Anthrone reagent method described by Sadasivam et al. (1996). The concentration of VFAs such as acetic, propionic and butyric acids and ethanol was determined by gas chromatography (GC), using a capillary column (30m x 0.25mm, TG-WaxMS A), from Thermoscientific, coupled to a Flame ionization detector (FID). The initial temperature of the column was 80 °C for 2 min followed with a ramp of 20 °C/min and a final temperature of 200 °C for 1 min; the injector and detector temperatures were 200 °C and 250 °C respectively. Hydrogen was used as the carrier gas at a flowrate of 35 ml/min. The samples were acidified with H_3PO_4 (30% (v/v)) and 2-ethyl-butyrac acid was used as internal standard as described by Raposo et al. (2015). The initial chemical oxygen demand (COD) was measured using a COD assay kit (Merck Darmstadt, Germany). All the experiments were done in duplicate and the average values are presented.

3. Results and discussion

The Figures 1, 2 and 3 presents the results of VSS, TC and $COD_{products}$ concentrations for the different batch experiments. For comparison and to observe the influence of the different conditions, Batch 1 (at 40 °C and 1 g of inoculum VSS/l of AD sludge) was used as a baseline control. Since the thermophilic condition showed to reach a stable VSS value more rapidly, this batch condition was carried out for only 30 days whereas the mesophilic condition was carried out for more than 100 days. The initial total COD and SC concentrations (without inoculation) were (24.3 ± 0.3) g/l and (1.3 ± 0.2) g/l. Soluble carbohydrates were totally consumed in the beginning of the experiments, the pH was maintained around 6.0-7.0 and their profiles are not reported.

According to Figure 1, it is possible to observe the VSS removal due to the partial hydrolysis of the organic solids into soluble products. The reduction is more evident in the beginning of the experiments in which microorganisms are more active and/or easily biodegradable substrate is hydrolyzed. The VSS seems to stabilize at a constant value for the different batch conditions. The VSS removal was higher when AD sludge was used as inoculum if compared to the soil (Figure 1.a). This effect confirms the high activity of AD sludge for lignocellulosic substrate also identified in other studies (van Aarle, Perimenis et al. 2015). Increasing inoculum concentration showed to raise the initial VSS removal rate in the beginning of the experiment, but no significant difference on the final VSS concentration was observed (Figure 1.b). Under thermophilic condition

at 50 °C, the VSS concentration seems to rapidly stabilize to a lower VSS removal if compared to the mesophilic condition (40 °C) (Figure 1.c).

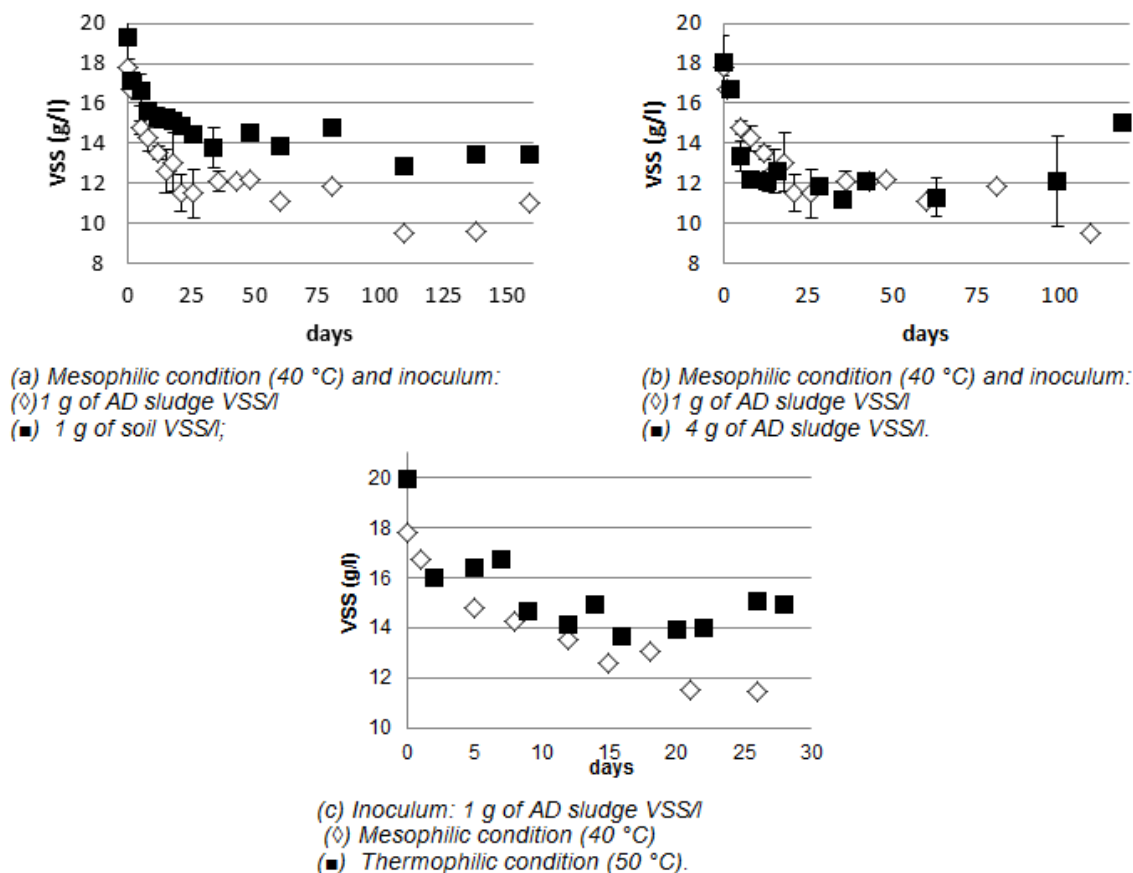
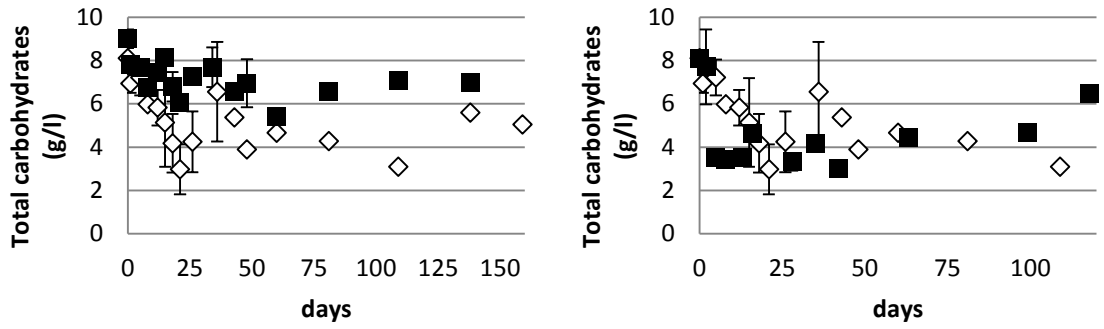


Figure 1 – VSS removal for the different batch conditions.

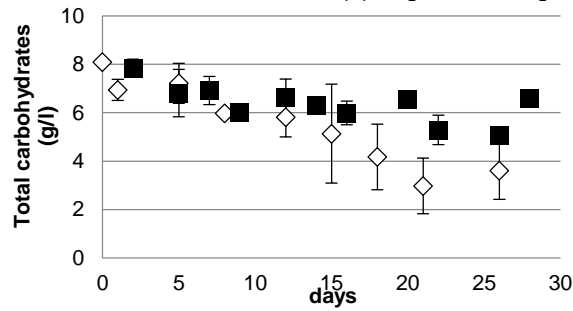
In Figure 2, the total carbohydrate concentration is shown for the different batch conditions. It is noticed that the reduction in the carbohydrate concentration presented similar behavior to the VSS removal results. This fact was expected since the VSS composition is mainly composed of carbohydrates (around 50%). Carbohydrates consumption was more significant when AD sludge was used as inoculum than when soil was used (Figure 2.a). The consumption of carbohydrates was more rapid for high inoculum concentration but no significant difference was observed on the final TC concentration (Figure 2.b). The thermophilic condition at 50 °C showed not to increase the consumption of carbohydrates.

For all the experiments, the main metabolic end product of the fermentation process was acetic acid. Butyric, propionic and ethanol were the other products identified in the fermentation broth in lower concentrations. In Figure 3, the profiles of the total concentration of products in COD basis are illustrated for the different conditions. A peak of COD_{products} concentration was observed for the initial 12 and 30 days for the thermophilic and mesophilic conditions respectively. The further decrease in the COD_{products} after these days may be related to the conversion of acetic acid into methane by acetotrophic methanogens. Some production of gas was detected inside the vials, but the quantification and characterization of this gas was not possible due to the experimental setup applied. This effect, nonetheless, was only observed when AD sludge was used as inoculum for the period analyzed (Figure 3.b and 3.c). No decrease in the COD_{products} concentration was observed for the batch inoculated with soil (Figure 3.a). This factor might be considered favorable for process that are more interesting in VFA production rather than methane.



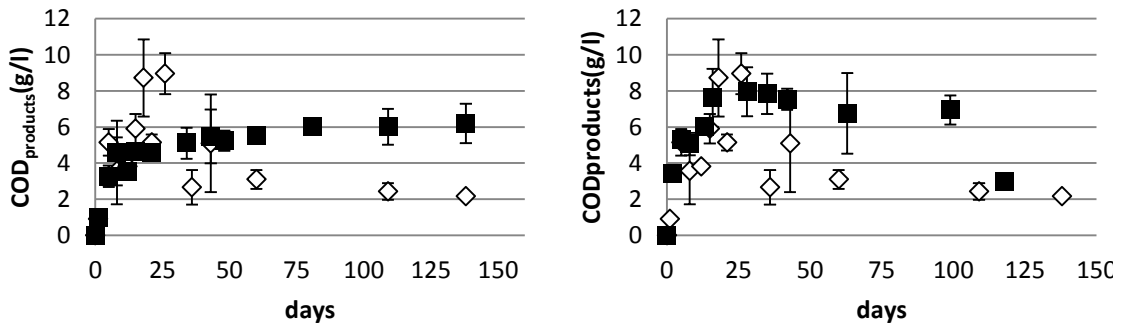
(a) Mesophilic condition (40 °C) and inoculum:
 (◇) 1 g of AD sludge VSS/l
 (■) 1 g of soil VSS/l;

(b) Mesophilic condition (40 °C) and inoculum:
 (◇) 1 g of AD sludge VSS/l
 (■) 4 g of AD sludge VSS/l.



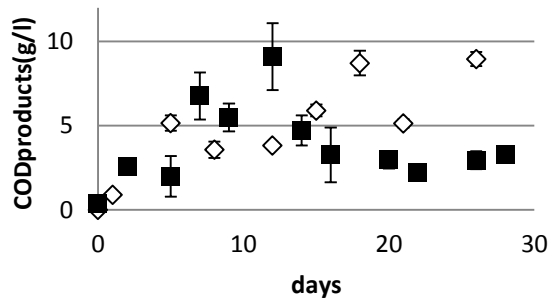
(c) Inoculum: 1 g of AD sludge VSS/l
 (◇) Mesophilic condition (40 °C)
 (■) Thermophilic condition (50 °C).

Figure 2 – Total carbohydrates consumption for the different batch conditions.



(a) Mesophilic condition (40 °C) and inoculum:
 (◇) 1 g of AD sludge VSS/l
 (■) 1 g of soil VSS/l;

(b) Mesophilic condition (40 °C) and inoculum:
 (◇) 1 g of AD sludge VSS/l
 (■) 4 g of AD sludge VSS/l.



(c) Inoculum: 1 g of AD sludge VSS/l
 (◇) Mesophilic condition (40 °C)
 (■) Thermophilic condition (40 °C).

Figure 3 – Products formation in COD basis for the different batch conditions.

3.1 Summary of batch experiments

The VSS and TC removals were calculated from the average of the three final points and their respective initial concentration (Equation 1 and 2). The maximum yield of products in the liquid phase was calculated from the initial media COD and the maximum concentration of products in COD basis (Equation 3). The selectivity of acetic acid was measured according to the ratio between the concentration of acetic acid and the total concentration of products in COD basis (Equation 4). The rate of products formation was obtained from the slope of the curve adjusted for the initial 12 and 30 days for the thermophilic and mesophilic conditions respectively. The measurements for the different batch conditions are presented in Figure 4.

$$VSS \text{ removal } (\%) = 1 - \frac{VSS_{final} \left(\frac{g}{l}\right)}{VSS_{initial} \left(\frac{g}{l}\right)} * 100\% \quad (1)$$

$$TC \text{ consumption } (\%) = 1 - \frac{TC_{final} \left(\frac{g}{l}\right)}{TC_{initial} \left(\frac{g}{l}\right)} * 100\% \quad (2)$$

$$\text{Maximum products formation yield } (\%) = \frac{\max \sum COD_{products} \left(\frac{g}{l}\right)}{COD_{initial} \left(\frac{g}{l}\right)} * 100\% \quad (3)$$

$$\text{Acetic acid selectivity } (\%) = \frac{COD_{acetic \text{ acid}} \left(\frac{g}{l}\right)}{COD_{products} \left(\frac{g}{l}\right)} * 100\% \quad (4)$$

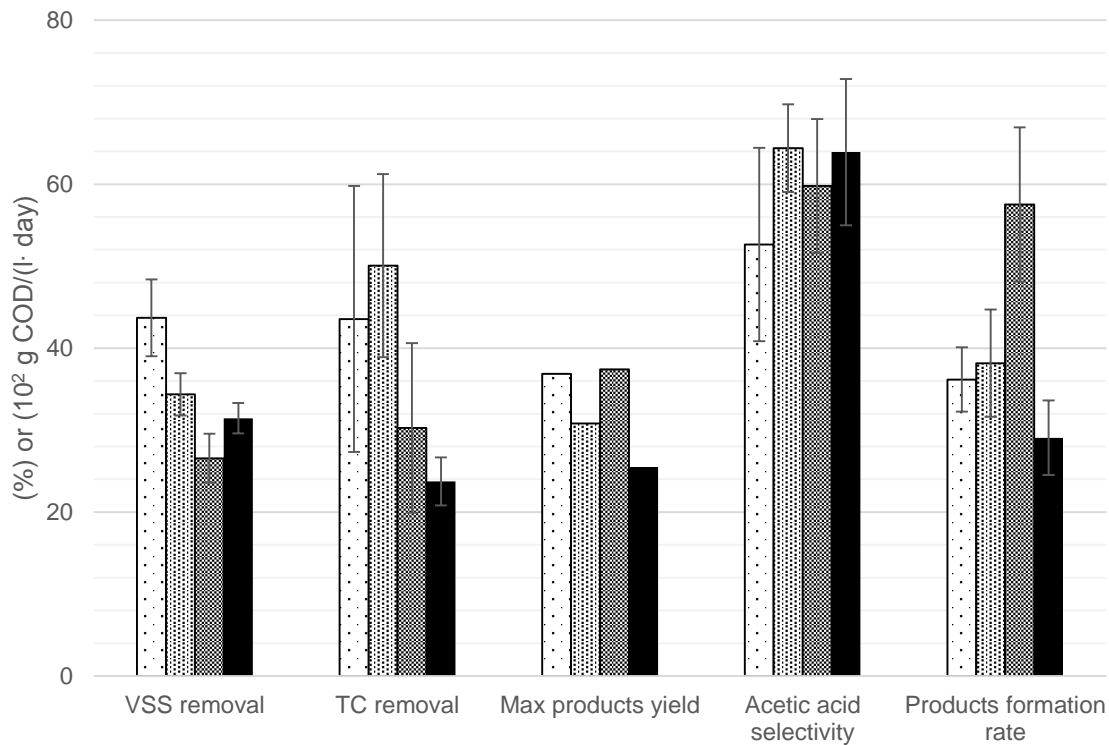


Figure 4 - VSS/TC removal, maximum products yield and acetic acid selectivity for the different batch conditions.
 □ AD sludge (1 g of VSS/l) (40 °C) ▨ AD sludge (4 g of VSS/l) (40 °C) ▩ AD sludge (1 g of VSS/l) (50 °C)
 ■ Soil (1 g of VSS/l) (40 °C)

In Figure 4 it can be observed that the VSS removal was not significantly increased when the batch was performed with a higher inoculum concentration or different source (soil). For all different conditions, acetic acid was the main product and corresponded to around 60% of the COD_{products}. The TC consumption was slightly higher with increasing inoculum concentration, but the products formation yield was relatively the same for the different condition. The thermophilic condition presented a higher rate of products formation but did not increase the VSS and TC removal throughout the fermentation process. Therefore, other conditions should be analyzed in further studies to understand the limited removal of VSS and TC. Although this effect can be related to the presence of hardly biodegradable fraction of lignocellulosic substrate, other factors might influence the limited hydrolysis such as: hydrogen partial pressure inhibition, microbial biomass decay and limiting substrate (Chen, Cheng et al. 2008).

Conclusion

Anaerobic digestion is a potential technology to hydrolyze lignocellulosic biomass and produce value-added products in a consolidated bioprocessing. The process is advantageous over other conventional process since biomass pre-treatment and sterilization are not required and organic and lignocellulosic waste can be used as feedstock. This experimental study investigated the different conditions that could increase the conversion of the lignocellulosic biomass: inoculum source, concentration and temperature. Although no significant difference in the maximum yields was identified, the thermophilic condition (50 °C) reached its maximum yield more rapidly than under mesophilic condition (40 °C). Anaerobic sludge inoculum presented a higher total carbohydrate consumption when it was used as inoculum. Both inocula presented capability to hydrolyze the lignocellulosic substrate. The experimental results, therefore, could provide insights towards understanding the bioprocessing in a biomass-derived VFA platform. The next step of the study is to investigate the anaerobic digestion of lignocellulosic biomass with reactor under continuous mode and other batch conditions that could increase the lignocellulosic degradability.

Acknowledgments

The authors wish to acknowledge CAPES (Coordination for the Improvement of Higher Education Personnel) for the financial support and Mrs Elizabeth Hendrie and Ms Gabriela Paupitz for the technical collaboration.

References

- CHEN, Y., CHENG, J.J. and CREAMER, K.S., 2008. Inhibition of anaerobic digestion process: a review. *Bioresource technology*, 99(10), pp. 4044-4064.
- DIONISI, D. and SILVA, I.M., 2016. Production of ethanol, organic acids and hydrogen: an opportunity for mixed culture biotechnology? *Reviews in Environmental Science and Bio/Technology*, , pp. 1-30.
- DIONISI, D., ANDERSON, J.A., AULENTA, F., MCCUE, A. and PATON, G., 2015. The potential of microbial processes for lignocellulosic biomass conversion to ethanol: a review. *Journal of Chemical Technology & Biotechnology*, 90(3), pp. 366-383.
- KLEEREBEZEM, R., JOOSSE, B., ROZENDAL, R. and VAN LOOSDRECHT, M.C., 2015. Anaerobic digestion without biogas? *Reviews in Environmental Science and Bio/Technology*, 14(4), pp. 787-801.
- LEVAKHIN, G., DUSKAEV, G. and DUSAEVA, H., 2015. Assessment of Chemical Composition of Grain Crops Depending on Vegetative Stage for Feeding. *Asian Journal of Crop Science*, 7(3), pp. 207.
- PESTA, G., 2007. *Anaerobic digestion of organic residues and wastes. Utilization of by-products and treatment of waste in the food industry*. Springer, pp. 53-72.
- RICE, E.W., BRIDGEWATER, L. and AMERICAN PUBLIC HEALTH ASSOCIATION, 2012. *Standard methods for the examination of water and wastewater*. American Public Health Association Washington, DC.
- SADASIVAM, S. and MANICKAM, A., 1996. *Biochemical methods*. New Age International.
- VAN AARLE, I.M., PERIMENIS, A., LIMA-RAMOS, J., DE HULTS, E., GEORGE, I.F. and GERIN, P.A., 2015. Mixed inoculum origin and lignocellulosic substrate type both influence the production of volatile fatty acids during acidogenic fermentation. *Biochemical engineering journal*, 103, pp. 242-249.