

# Performance of an anaerobic biofilm reactor through the application of different operational conditions

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**Abstract:** Inadequate management of Municipal Solid Waste (MSW) and the energy crisis due to dependence on fossil fuels (carbon and hydrocarbons) are growing problems in Latin American countries such as Mexico. These problems are caused by various factors, including the lack of infrastructure and the limited development of technologies focused on addressing these areas. In Mexico, between 37.55 and 43.84 million tons of MSW are generated annually, while 5,896 PJ of fossil fuels are produced in the same period, contributing strongly to environmental pollution due to inadequate management and procurement-use processes respectively. In order to mitigate these problems, it is necessary to propose dual-purpose strategies, such as anaerobic digestion, that can help in the treatment of the organic fraction of MSW (OFMSW) and at the same time producing biogas as a renewable energy source. For these reasons, the aim of this work was to evaluate the performance of an anaerobic biofilm reactor through increases in the Organic Loading Rate (OLR) using the liquid fraction of the OFMSW as a substrate for biogas production. An anaerobic biofilm reactor called the Anaerobic Hybrid Reactor (AHR) was used to carry out this stud. The performance of the AHR in four stages applying different OLR values from 5 to 21 gCOD/L.d was analyzed. Anaerobic biofilm formation, pH, %COD and %solids removal, alkalinity, biogas production, and methane yield were evaluated. After 119 days of reactor operation, 93.45% colonization of the inverse fluidized bed, 85% total and soluble COD and removals greater than 80% for TS and VS, alkalinity less than 0.3, around 20 L of biogas per day with methane yields of 0.31 LCH<sub>4</sub> at STP/gCOD<sub>rem</sub> were obtained. The use of high OLR allows a larger volume of OFMSW liquid fraction to be treated producing a greater quantity of biogas with an excellent methane yield, thus demonstrating the high efficiency of the AHR.

Keywords: anaerobic biofilm reactor, anaerobic digestion, organic loading rate, organic fraction of municipal solid waste, renewable energy

#### Introduction

In Latin American countries, including Mexico, which are listed as developing countries, the management of Municipal Solid Waste (MSW) stands out as a growing problem due to the uncontrolled growth of cities, inadequate recycling of solid waste, poor management systems and infrastructure deficiencies. In Mexico, between 37.55 and 43.84 million tons of MSW are generated per year, of which the state of Veracruz contributes 6.5% with 2.9 million tons per year and the City of Orizaba produces around 30 thousand tons per year (SEMARNAT, 2017; El Sol de Orizaba, 2020). Specifically in Orizaba, the Emiliano Zapata Municipal Market is one of the main areas of Municipal Solid Waste (MSW) generation, whose composition consists of 84.17% organic waste of agricultural origin (fruits, vegetables, legumes, tubers, citrus, etc) and the remaining 15.83% corresponds to inorganic waste and organic waste that is very difficult to biodegrade (Apanco-Rosas, 2018). The organic part is known as the Organic Fraction of MSW (OFMSW) and it is necessary to propose strategies to facilitate its handling and final disposal due to the large volumes generated. One strategy is through mechanical pretreatment which helps to separate OFMSW into its solid and liquid components, leaving an available liquid, balanced and rich in organic matter and nutrients, which is suitable for treatment and recovery through biological processes such as anaerobic digestion. Therefore, the high liquid content in OFMSW makes it convenient to mechanically separate these wastes into their liquid and solid fractions (Alvarado-Lassman et al., 2016). In this way, the mechanical pretreatment helps to improve the degradation of organic matter through the activity of anaerobic microorganisms, in such a way that it increases the contact surface and reduces the crystallinity of the cellulose, which causes the sugars to hydrolyze, monomeric in less time (Mlaik et al., 2017; Ariunbaatar et al., 2014). The separation of MSW is carried out in a series of steps that include: 1) Manual separation of organic complex residues and inorganic residues from the usable organic fraction, that is, residues of agricultural origin, 2) Size reduction by crushing in a mill, 3) The dilution of the organic matter, and 4) Filtration to separate the solid organic part and thus obtain a liquid fraction (Apanco-Rosas, 2018). This separation and individual processing of the liquid fraction of solid waste could significantly reduce the amount of waste that needs to be transported and disposed of, which causes a reduction in the capital that





the municipal government would spend on its treatment, since it subtracts the use of space in landfills and it is possible to take advantage of the biogas produced and instead of emitting it into the atmosphere (Alvarado-Lassman *et al.*, 2016).

Another area of priority attention at the global, Latin American and Mexican levels is that the main sources of energy used are derived from non-renewable resources such as fossil fuels, whose production in Mexico 2020 was 5,896 PJ (SENER, 2020), which, together with the increase in population and continuous urbanization have generated serious environmental problems. At the same time, the state of Veracruz has a high potential as a biomass generator that can be used for bioenergy production (Nava-Pacheco et al., 2020). For this reason, it is necessary to develop strategies to increase the use of renewable energy sources, such as biogas produced through biological processes such as anaerobic digestion (Díaz-Trujillo and Nápoles-Rivera, 2019). Biogas production has proven to be a viable way to partially substitute fossil fuels, since its use has important economic, environmental, and social benefits (Garbs and Geldermann, 2018). According to the National Energy Balance, in Mexico during 2020 biogas contributed with 2.53 PJ and it was used for the generation of electrical energy (SENER, 2020). In this respect, it is feasible for Mexico to reach a production and use of biogas of approximately 200 to 350 million m<sup>3</sup> by the year 2024, and from 600 to 900 million m<sup>3</sup> by 2030. According to these data, it is feasible to practically quadruple the pattern of biogas production with a normal financial expense of 23 million USD per year in 2024 and around 46.5 million USD per year in 2030 (Gutiérrez, 2018; Weber et al., 2012). The production of biogas is the result of a series of processes that are carried out under anaerobic conditions by groups of microorganisms (Vega-De Kuyper and Ramírez-Morales, 2014), so it is important to consider the composition of the material to be used as input, since it provides the complements the metabolism of the microorganisms in question and has a direct influence on the biogas production (Wid and Horan, 2018).

In this context, some of the alternatives and infrastructure explored to minimize environmental pollution and promote the generation of renewable energy sources have been applied to OFMSW treatment, industrial effluent treatment, wastewater treatment and various liquid substrates through of biological processes. All of this has become of essential importance, so it is necessary to promote options that consolidate high treatment efficacy with low operation and maintenance costs (Lettinga et al., 1983; Cristaldi et al., 2020). Worldwide, the types of biological reactors mostly used for handling high Loading Rates (OLR) such as those presented by the liquid fraction of the OFMSW are: 1) Upflow Anaerobic Reactor and Sludge Blanket (UASB), which is presented as a suitable option for the treatment of organic effluents (Lorenzo-Acosta and Obaya-Abreu, 2005; Utino et al., 2022), 2) Expanded Granular Sludge Bed Reactor (EGSB) developed from UASB reactors, and 3) Anaerobic Biofilm Reactor, whose advantages are that it offers a greater contact surface between the substrate and the biomass, as well as the simple separation of the treated water from the biomass and a compact design (Houbron, 2012, Karadag et al., 2015). Therefore, to evaluate the efficiency of these devices, it is essential to determine the production and quality of biogas, methane yield, and relate these data to the OLR used (Lebuhn, et al., 2015). An anaerobic biofilm reactor that has shown excellent performance is a device called Anaerobic Hybrid Reactor (AHR). Rosas-Mendoza et al. (2018), carried out the anaerobic digestion process of effluents from the citrus industry using an AHR, where the production and use of biogas as an alternative energy source was evaluated. AHR is made-up of two sections: a Fixed Bed (FB) and an Inverse Fluidized Bed (IFB), that allow high OLRs to be handled minimizing the inhibition effects caused by the D-limonene of the citrus effluent.

Other outstanding works using high load reactors are: Doloman *et al.* (2017), evaluated the changes in the composition of the microorganisms presents in the anaerobic co-digestion of biomass of microalgae cultivated in municipal wastewater. They used two UASB reactors and the operating time of the reactors was 87 days with an OLR between 0.9 and 5.4 gCOD/L.d; Arreola-Vargas *et al.* (2018), estimated the performance of a Packed Bed Reactor (PBR) with a capacity of 445 L, in the anaerobic digestion process of tequila vinasse applying different OLRs for 231 days; Botello-Suárez *et al.* (2018), studied the effects of increasing the OLR (6.1 to 18.2 gCOD/L.d) of coffee vinasse on the yield and microbial composition in two-stage 20 and 10 L UASB reactors. The system operated for 306 days under mesophilic conditions. Patel *et al.* (2021), focused on hydrodynamics of the hybrid upflow anaerobic sludge blanket reactors with different inert media and the obtained results for both the reactors indicates the intermediate degree of dispersion seems to be best for max substrate conversion. Cecconet *et al.* (2022), studied the performance evaluation of a pilot-scale UASB reactor with a volume of 2.75 m<sup>3</sup> for the treatment of urban wastewater at sub-mesophilic temperature of 25 °C and the results showed that, despite lower methane production and COD removal efficiency compared to operation under ideal conditions, a UASB can still achieve satisfactory performance.



In order to provide an alternative for the reduction of MSW contributing to the generation of a renewable energy source such as biogas, the objective of this work was to evaluate the performance of an anaerobic biofilm reactor through increases in the organic loading rate using as substrate the liquid fraction of the OFMSW.

## **Materials and Methods**

#### Experimental device

An anaerobic biofilm reactor was used, called Anaerobic Hybrid Reactor (AHR) (Rosas-Mendoza *et al.*, 2018). The AHR has two sections: 1) a Fixed Bed (FB) in the upper section, as shown in Figure 1, constructed with an acrylic tube containing polymeric rings with an average length of 2.76 cm as a support media and 2) an Inverse Fluidized Bed (IFB) in the bottom section, as shown in Figure 2, constructed with an acrylic tube 80 cm long and with a nominal diameter of 8.89 cm, where a silica sand called Extendosphere<sup>TM</sup> with a diameter of 170  $\mu$ m, a specific area of 20,000 m<sup>2</sup>/m<sup>3</sup> and density of 0.69 kg/m<sup>3</sup> is used as support media.



Figure 1. Fixed Bed of the AHR.



Figure 2. Inverse Fluidized Bed of the AHR.

# Inoculum and substrate

A volume of 1,500 mL of pre-colonized Extendosphere<sup>™</sup> support media was used as inoculum of the IFB within the AHR, equivalent to 36.35 ± 2.01 g of biomass (The analyzes were done in triplicate) and with a colonization of 35.93% on average, using as reference the maximum colonization value of 0.21 gBiomass/gSupport (Buffière *et al.*, 2000). The inoculum was obtained from an anaerobic reactor that was fed with the liquid fraction of OFMSW, located in the Pilot Plant for Processing and Valorization of Solid Waste of the Instituto Tecnológico de Orizaba. Once the inoculum was obtained, it was immediately introduced into the AHR.

The liquid fraction of OFMSW from fruits and vegetables was used as substrate. The OFMSWs were collected at the Emiliano Zapata Municipal Market in Orizaba, Veracruz, Mexico. Afterwards, the OFMSWs were separated and crushed in such a way that a pasty semi-solid was obtained, following the procedure of Alvarado-Lassman *et al.* (2016). From this semi-solid, the liquid part was separated and filtered to obtain a liquid fraction, which was used as a substrate in a pilot scale hydrolysis reactor, located in the Pilot Plant for Processing and Valorization of Solid Waste of the Instituto Tecnológico de Orizaba. Finally, a hydrolyzed effluent was obtained that was used as a substrate during the first days of the AHR start-up. After the start-up period, the liquid fraction of the OFMSW without hydrolyzing was used. In Table 1, the characterization of the substrate used in this research is presented and the analyzes were done in triplicate.



Table 1. Characterization of the hydrolyzed liquid fraction and liquid fraction without hydrolyzing as substrates used.

Parameter	Unit	Hydrolyzed liquid fraction	Liquid fraction without hydrolyzing
Total COD	gCOD/L	$8.63 \pm 0.07$	$39.57 \pm 0.98$
Soluble COD	gCOD/L	7.99 ± 0.28	$35.48 \pm 0.49$
TS	g/L	5.24 ± 0.18	$10.6 \pm 0.73$
VS	g/L	1.08 ± 0.39	$9.48 \pm 0.69$
pH	-	7.9 ± 0.60	$5 \pm 0.2$

# Experimental methodology

The AHR was operated for 119 days applying variations of the operational conditions was operated, through increases of the OLR from 5 to 21 gCOD/L.d. This process was carried out in four stages as mentioned in Table 2. The reactor was started up in stage 1, applying an OLR from 5 to 8 gCOD/L.d with the hydrolyzed substrate. While the stabilization was achieved in stage 2, an OLR around 8 gCOD/L.d was fed, using the substrate without hydrolyzing. Once the AHR was stabilized, two increases were made in the OLR with the substrate without hydrolyzing, i.e., in stage 3 an OLR from 15 to 16 gCOD/L.d and in stage 4 an ORL from 20 to 21 gCOD/L.d were employed.

Table 2. Duration of the four stages during the operation of the Anaerobic Hybrid Reactor.

Stage	Description	cription OLR (gCOD/L.d)	<b>Operational days</b>		Duration
Stage Description	Description		Beginning	Final	(days)
1	Start-up	5-8	0	26	26
2	Stabilization	7-8	27	83	57
3	Increment 1	15-16	84	109	25
4	Increment 2	20-21	110	119	10

The AHR was operated under mesophilic conditions of 35 °C  $\pm$  2. The substrate was monitored at the inlet and the outlet of the AHR. It is important to mention that during the start-up stage, the hydrolyzed liquid fraction had a pH higher than 7.5, since according to Jáuregui-Jáuregui *et al.*, (2014) a substrate with alkaline pH works as a strategy to favor the formation of the anaerobic biofilm. However, for the three subsequent stages, the non-hydrolyzed liquid fraction was used, regulating the pH between 6.8 and 7.2 with a 3M NaOH solution.

# Analytical methods

The biofilm formation development of the anaerobic biofilm was observed at 40x using a Zeiss Primo Star Binocular Microscope. The pH was measured with an Orion Model 250A potentiometer. Alpha alkalinity was determined by the method described by Bernard et al., (2000). Total Chemical Oxygen Demand (COD<sub>T</sub>) and soluble COD (COD<sub>S</sub>) were determined by the colorimetric method (APHA, 2005) using a HACH spectrophotometer at 620 nm. Total solids (TS) and volatile solids (VS) by the gravimetric method (APHA, 2005) were determined, using a Riossa oven and a Barnstead/Themolyne muffle. Similarly, according to common laboratory practices, the content of biomass present in the FB and in the IFB was measured as VS. The biogas composition was measured on a Buck 310 gas chromatograph equipped with an AllTech CRT I packed column (6" long and 0.25" diameter), and it detected CH<sub>4</sub>, CO<sub>2</sub>, O<sub>2</sub>, and N<sub>2</sub>. A 2-mL sample was injected directly into the packed column. The operating conditions were as follows: Helium at 70 psi was used as the carrier gas, the temperature of the column was 36 °C, and the temperature of the detector was 121 °C (Rosas-Mendoza et al., 2018).



# AHR performance evaluation

The performance of the AHR in each stage was evaluated through the removal percentages of parameters such as: total and soluble COD, TS and VS. Similarly, the pH, alkalinity and methane yield were monitored. The criteria to know if each stage had reached its stable phase were: removal of COD and solids greater than 80%, pH close to 7, alkalinity less than 0.3, and methane yield close to the theoretical value of 0.35 LCH<sub>4</sub> at STP/gCOD<sub>rem</sub>.

## **Results and Discussion**

The results obtained for the performance evaluation of the AHR are described below:

## Biofilm development of the AHR

As mentioned above, samples of Extendosphere<sup>™</sup> support media were taken from the upper, middle and bottom sections of the IFB and the content of volatile matter adhered to the support media was determined, that is; the amount of biomass that makes up the anaerobic biofilm. At the beginning of stage 1, the upper, middle and bottom sections had 0.0248, 0.0227 and 0.0252 gBiomass/mLSupport, respectively, which are equivalent to 36.35 g biomass on average, which corresponds to a 35.93% colonization on average. At the end of stage 1, there was an increase in the development of the anaerobic biofilm reaching 46.25% colonization on average. Continuing with the AHR operation, by the end of stages 2 and 3, 50.83 and 82.48% colonization were reached on average, respectively. After 119 days of operation, to conclude with stage 4, the content of gBiomass/mLSupport for the upper, middle and bottom sections was 0.0474, 0.0464 and 0.0423, respectively. The above is equivalent to 94.63, 90.37 and 95.37% colonization for each section of the IFB. In Table 3, the anaerobic biofilm formation is presented.

Table 3. Monitoring of biofilm development inside the three sections of the IFB during the operation of the AHR.

Section of the IFB	Time (d)	Biomass/Support (g/g)	Biomass/Supoort (g/mL)	Colonization (%)
	1	0.0760	0.0248	36.19
	25	0.1050	0.0247	50.00
Upper section	83	0.1198	0.0341	57.03
	109	0.1820	0.0399	86.70
	119	0.1987	0.0474	94.63
	1	0.0744	0.0227	35.44
	25	0.0853	0.0269	40.60
Middle section	83	0.0864	0.0262	41.15
	109	0.1671	0.0273	76.93
	119	0.1898	0.0464	90.37
	1	0.0760	0.0252	36.18
	25	0.1011	0.0279	48.16
Bottom section	83	0.1140	0.0277	54.30
	109	0.1761	0.0211	83.80
	119	0.2003	0.0423	95.37

The fact that the upper and bottom sections presented higher colonization is due to the direct contact of the anaerobic biofilm with the substrate, while the middle section showed less colonization due to low fluidization velocities. In this sense, Trinet *et al.* (1991), mention that this benefits the formation of a thick biofilm with a greater amount of active biomass, in the outer layers such as the upper and bottom sections, which consume large amounts of substrate.

The anaerobic biofilm, being a microscopic structure, can be observed through micrographs, whose evolution is shown in Figure 3. The structure of the anaerobic biofilm is observed as microbial biomass irregularly adhered to the surface



of Extendospheres, this structure benefits the mass transfer towards the microbial populations of the lower sections and that, in addition, makes it more resistant to the occurrence of washing out of the microorganisms due to the fluidization of the substrate (Okabe *et al.*, 1998). In Figure 3, a more populated and thicker layer can be observed in the micrographs of the upper and bottom sections after 119 days of the AHR operation, which corresponds to the results mentioned above, where these sections are the ones with the highest colonization. A thick biofilm enables a combination of anaerobic metabolisms that can give a greater efficiency to the AHR. In contrast, the literature mentions that a thin biofilm, such as those observed in the middle section, favors the treatment of the substrate due to a greater exchange of nutrients (Beyenal and Lewandowsky, 2000).



Figure 3. Evolution of the anaerobic biofilm adhered to the support media Extendosphere<sup>™</sup> inside the inverse fluidized bed.

#### AHR performance evaluation

During stage 1, from days 1 to 27, the hydrolyzed substrate was used, which presented a high pH, this is attributed to the fact that the organic matter of the liquid fraction of the OFMSW was apparently already degraded. Lohani and



Havukainen (2018), mention that pH values higher than 8 inhibit biological functions for anaerobic microorganisms, since during this stage there was no biogas production. However, as mentioned above, Jáuregui-Jáuregui *et al.* (2014) highlight that a substrate with alkaline pH works as a starting strategy to favor biofilm formation, which was observed in the increase in colonization from 35.93 to 46.25%.

To mitigate the effect of high pH, Ostrem *et al.* (2004), mention that the addition of fresh substrate regulates the pH levels. So, from stage 2 onwards substrate without hydrolyzing was used, but ensuring the pH control close to neutrality through the addition of a 3M NaOH solution, because the fresh substrate had pH values between 4.9 and 5.2. Table 4 shows that for stages 2, 3 and 4 the average pH values were 7.33, 7.74 and 7.44, respectively, which also favored the production of biogas. Hussain *et al.* (2021), report that the appropriate pH level to favor biogas production should be in a range of 6.4 to 7.6.

Parameter	Stage 1	Stage 2	Stage 3	Stage 4
рН	8.31	7.33	7.74	7.44
Total COD removal (%)	18.80	96.10	86.70	92.80
Soluble COD removal (%)	3.33	98.50	88.90	97.80
TS removal (%)	31.90	90.60	82.80	60.90
VS removal (%)	27.80	94.80	85.20	66.50
Alkalinity (factor $\alpha$ )		0.143	0.230	0.067
Biogas production (L/día)		6.60	15.30	20.70
Methane yield (LCH <sub>4</sub> /gCOD <sub>rem</sub> )		0.33	0.33	0.31

Table 4. Results of the Anaerobic Hybrid Reactor performance at the end of each stage.

The values of the COD<sub>T</sub> and COD<sub>S</sub> removal percentages during the first days of operation ranged from 18.80 and 3.33%, respectively, which made this stage quite unstable. After this, in stage 2, the biological system began to show significant improvements, obtaining 96.10% removal of COD<sub>T</sub> and 98.50% removal of COD<sub>S</sub>. With the stable pH and COD removals, the OLR was increased on stage 3 and the removals decreased to 86.70 and 88.90%, respectively, however, it is shown that the AHR can tolerate high organic concentrations and maintain high levels of COD removal. Despite this fact, another OLR increase for stage 4 was made and at the end of this, 92.80% COD<sub>T</sub> removal and 97.80% COD<sub>S</sub> removal were obtained, as shown in Table 4. Akunna (2019), highlights that the growth of microorganisms is influenced by factors such as pH, the variable composition of the inlet substrate and variations of OLR. On the other hand, the total and volatile solids removal percentages showed a similar trend to the total and soluble COD removal percentages as shown in Table 4. It is important to mention that for stage 4 this percentage decreased due to the fact that the OLR between 20 and 21 gCOD/L.d was handled, so the substrate had a higher presence of solids.

The alkalinity factor  $\alpha$  is a parameter that indicates the stability of the process given by the ratio of intermediate and total alkalinities, which must be less than 0.3. Thanks to this factor, it is possible to prevent an acidification of the system due to the presence of volatile fatty acids (Bernard *et al.*, 2000). From stage 2 to stage 4 there was an alkalinity value lower than 0.3, which indicated stability during the AHR operation. Foresti (1994) and Liu *et al.* (2021), also mention that this index, being strongly related to the type of substrate, makes it possible to achieve process stability with values different from the reference value ( $\alpha$ <0.3).

Finally, the consumption of organic matter by the anaerobic biofilm, in addition to influencing the increase in the colonization percentage of the IFB, also influenced the biogas production and better methane yields, as shown in Table 4. It was observed a direct relationship between the increase of OLR with the production of biogas, since increasing the OLR at the end of stages 2, 3 and 4, 6.60, 15.30 and 20.70 LBiogas at STP per day were obtained. It had previously been mentioned that during stage 1 there was no detectable biogas production. Regarding the methane yield, in Table 4 values very close to the theoretical one of  $0.35 \text{ LCH}_4$  at STP/gCOD<sub>rem</sub> can be observed, with a slight decrease being evident for stage 4.



## Conclusions

The performance of an Anaerobic Hybrid Reactor, which is made-up of a Fixed Bed and an Inverse Fluidized Bed, was evaluated. The increases of the OLR, from 5 to 21 gCOD/L.d, in four stages were carried out. The AHR, in stage 1, with pre-colonized Extendosphere<sup>™</sup> support with 36.35 g biomass, equivalent to 35.93% colonization on average, was inoculated. After 119 days of operation, at the end of stage 4, the AHR reached 68.05 g biomass in the inverse fluidized bed, equivalent to a 93.45% colonization on average. Once the start-up of the AHR was completed, at the conclusion of stages 2, 3 and 4, there were removals greater than 85% of total and soluble COD, removals larger than 80% of TS and VS (except during stage 4), and alkalinities less than 0.3. Regarding biogas production, 6.60, 15.30 and 20.70 L for stages 2, 3 and 4, respectively, was achieved, observing a close relationship with the increases in OLR. Finally, the methane yield 0.33 LCH<sub>4</sub> at STP/gCOD<sub>rem</sub> for stages 2 and 3 was obtained, while for stage 4 this value had a slight decrease, about 0.31 LCH<sub>4</sub> at STP/gCOD<sub>rem</sub>, observing that these results were close to the theoretical value. The AHR, being a biofilm reactor, proved to be a device that withstood large increases in OLR, and the anaerobic biofilm presented a quick recovery from the stress suffered by operational changes. The management of high organic loading rates allows treating a greater amount of the liquid fraction of OFMSW, contributing to the reduction of waste in landfills, in addition to the production of a renewable energy source such as biogas rich in methane.

As future work, the study of the dynamics of microbial populations of the anaerobic biofilm will be carried out through the aforementioned operational changes.

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# References

Akunna, J. C. (2019). Anaerobic Wastewater Treatment and Biogas Plants. Taylor & Francis Group. First edition.

- Alvarado-Lassman, A., Méndez-Contreras, J. M., Martínez-Sibaja, A., Rosas-Mendoza, E. S., & Vallejo-Cantú, N. A. (2016). Biogas production from the mechanically pretreated, liquid fraction of sorted organic municipal solid wastes. Environmental Technology 3330.
- Apanco-Rosas, V. (2018). Comparación del desempeño de un reactor anaerobio piloto en configuración en paralelo, con 2 diferentes sustratos. Tesis de Maestría. Instituto Tecnológico de Orizaba. México.
- APHA. (2005). Standard Methods for the Examination of Water and Wastewater. 21st Edition, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC.
- Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F. & Lens, P. (2014). Pretreatment methods to enhance anaerobic digestion of organic solid waste. APPLIED ENERGY 123: 143–56.
- Arreola-Vargas, J., Snell-Castro, R., Rojo-Liera, N. M., González-Álvarez, V. & Méndez-Acosta, H. O. (2018). Effect of the organic loading rate on the performance and microbial populations during the anaerobic treatment of tequila vinasses in a pilot-scale packed bed reactor. J Chem Technol Biotechnol 93(2):591–99.
- Bernard, O., Polit, M., Hadj-Sadok, Z., Pengov, M., Dochain, D., Estaben, M. & Labat, P. (2000). Advanced monitoring and control of anaerobic wastewater treatment plants: III-Software sensors and controllers for an anaerobic digester. In: 5th International Symposium in System Analysis and Computing in Water Quality Management, 3.65–3.72. Gent, Belgium.
- Beyenal, H. & Lewandowski, Z. (2000). Combined effect of substrate concentration and flow velocity on effective diffusivity in biofilms. Water research. 34(2): 528-538.
- Botello-Suárez, W. A., Da Silva-Vantini, J., Duda, R- M., Giachetto, P. F., Carrijo-Cintra, L., Tiraboschi Ferro, M. I. & Alves-De Oliveira, R. (2018). Predominance of syntrophic bacteria, Methanosaeta and Methanoculleus in a two-stage up-flow anaerobic sludge blanket reactor treating coffee processing wastewater at high organic loading rate. Bioresour Technol 268, 158–68.
- Buffière, P., Bergeon, J. P. & Moletta, R. (2000). The inverse turbulent bed: a novel bioreactor for anaerobic treatment. Water Research, 34(2), 673-677.
- Cecconet, D., Callegari, A. & Capodaglio, A.G. (2022). UASB Performance and Perspectives in Urban Wastewater Treatment at Sub-Mesophilic Operating Temperature. *Water*, *14*(1), 115.
- Cristaldi, A., Fiore, M., Zuccarello, P., Oliveri Conti, G., Grasso, A., Nicolosi, I., Copat, C. & Ferrante, M. (2020). Efficiency of Wastewater Treatment Plants (WWTPs) for Microplastic Removal: A Systematic Review. Int J Environ Res Public Health, *17*(21), 8014.
- Díaz-Trujillo, L. A. & Nápoles-Rivera, F. (2019). Optimization of biogas supply chain in Mexico considering economic and environmental aspects. Renewable Energy, 139, 1227–1240.
- Doloman, A., Soboh, Y., Walters, A. J., Sims, R. C. & Miller, C. D. (2017). "Qualitative Analysis of Microbial Dynamics during Anaerobic Digestion of Microalgal Biomass in a UASB Reactor". Int J Microbiol 2017(5291283):12.



- El Sol de Orizaba. (2020). <u>https://www.elsoldeorizaba.com.mx/local/se-inaugura-planta-ecori-ayuntamiento-firma-convenio-con-geocycle-medio-ambiente-basura-reciclaje-orizaba-5518152.html</u>.
- Foresti, E. (1994). Fundamentos de proceso de digestión anaerobia. III Taller y Seminario Latinoamericano sobre Tratamiento anaerobio de aguas residuales, Montevideo, Uruguay, 97-110.
- Garbs, M. & Geldermann, J. (2018). Analysis of selected economic and environmental impacts of long-distance manure transports to biogas plants. Biomass and Bioenergy, *109*(December 2017), 71–84. <u>https://doi.org/10.1016/j.biombioe.2017.12.009</u>
- Gutiérrez, J. P. (2018). Situación actual y escenarios para el desarrollo del biogás en México hacia 2024 y 2030. Red Tematica De Bioenergia De Conacyt, 22.
- Houbron, E. (2012). Methane yield and microscopic observation as monitoring biofilm behaviour parameters, during start up phase of anaerobic inverse fluidized bed reactor. Afr J Biotechnol 11(78), 14392–98.
- Hussain A., Johain J. & Fawziea M. (2021). Effect of pH on biogas production during anaerobic digestion. Journal of University of Shanghai for Science and Technology, 23(8), 224-231.
- Jáuregui-Jáuregui, J. A., Méndez-Acosta, H. O., González-Álvarez, V., Snell-Castro, R., Alcaraz-González, V. & Godon, J. J. (2014). Anaerobic treatment of tequila vinasses under seasonal operating conditions: Start-up, normal operation and restart-up after a long stop and starvation period. Bioresource Technology, *168*, 33–40.
- Karadag, D., Köroğlu, O., Ozkaya, B. & Cakmakci, M. (2015). A review on anaerobic biofilm reactors for the treatment of dairy industry wastewater, Process Biochemistry, 50(2), 262-271.
- Lebuhn, M., Weib, S., Munk, B. & Guebitz, G. M. (2015). Microbiology and Molecular Biology Tools for Biogas Process Analysis, Diagnosis and Control. Biogas Sci Technol 1–200.
- Lettinga, G., Roersma, R. & Grin, P. (1983). Anaerobic treatment of raw domestic sewage at ambient temperatures using a granular bed UASB reactor. Biotechnol and Bioeng, 25(7), 1701–1723.
- Liu, S., Cao, L., Xu, F., Yang, L., Li, Y. & Inalegwu, O. (2021). Chapter Five Integration of algae cultivation to anaerobic digestion for biofuel and bioenergy production, Advances in Bioenergy, Elsevier, 6(1), 199-300,
- Lohani, S. P. & Havukainen, J. (2018). Anaerobic Digestion: Factors Affecting Anaerobic Digestion Process. Waste Bioremediation, Energy, Environment, and Sustainability.
- Lorenzo-Acosta, Y. & Obaya-Abreu, M. C. (2005). La digestión anaerobia. Aspectos teóricos. Parte I. ICIDCA. Sobre los Derivados de la Caña de Azúcar, 39(1, 2005), 35-48.
- Mlaik, N., Khcharem, M., Kouas, M., Sayadi, S. & Khoufi, S. (2017). Improvement of anaerobic biodegradability of organic fraction of municipal solid waste by mechanical and thermochemical pretreatments. Inter J Environ Sci Technol.
- Nava-Pacheco, D., Juárez-García, I.A., Landeta-Escamilla, O., Del Moral, S. & Rosas-Mendoza, E.S. (2020). Bioenergy potential from agroindustrial wastes of the state of Veracruz. Renewable Energy, Biomass & Sustainability (REB&S), 2(2), 1-6.
- Okabe, S., Kuroda, H. & Watanabe, Y. (1998). Significance of biofilm structure on transport of inert particulates into biofilms. Water Science and Technology. *38*(8,9): 163-170.
- Ostrem M.K., Millrath K., & Themelis N.J. (2004). Combining anaerobic digestion and waste to energy. In: 12th North America waste to energy conference. Columbia University, New York.
- Patel, B.B., Gundaliya, P., Patel, U. & Umeshkumar Khare, U. (2021). Hydrodynamics of hybrid upflow anaerobic sludge blanket reactors using tracer studies. Environmental Challenges, 5.
- Rosas-Mendoza, E. S., Méndez-Contreras, J. M., Martínez-Sibaja, A., Vallejo-Cantú, N. A. & Alvarado-Lassman, A. (2018). Anaerobic digestion of citrus industry effluents using an Anaerobic Hybrid Reactor. Clean Technol Environ Policy 20(7), 1387–97.
- SENER (Secretaría de Energía). (2020). Balance Nacional de Energía 2020. https://www.gob.mx/cms/uploads/attachment/file/707654/BALANCE NACIONAL ENERGIA 0403.pdf.
- SEMARNAT (Secretaría de Medio Ambiente y Recursos Naturales). (2017). Información sobre residuos sólidos urbanos. https://www.gob.mx/semarnat/acciones-y-programas/residuos-solidos-urbanos-

rsu#:~:text=En%20M%C3%A9xico%20se%20generan%20diariamente,9.63%25%20de%20los%20residuos%20generados.

- Trinet, F., Heim, R., Amar, D. & Chang, H. (1991). Study of biofilm and fluidization of bioparticles in a three-phase liquid fluidized-bed reactor. Water Science and Technology. 23, 1347-1354.
- Utino, B.M.Y., Alves, I., Del Nery, V., Kimiko, S.I., Pozzi, E. & Rissato, M.H. (2022). Methane production in a UASB reactor from sugarcane vinasse: shutdown or exchanging substrate for molasses during the off-season? Journal of Water Process Engineering, *47*.
- Vega-De Kuyper, J. C. & Ramírez-Morales, S. (2014). Fuentes de energía, renovables y no renovables. Aplicaciones. México: Alfaomega.
- Weber, B., Rojas Oropeza, M., Torres Bernal, M. & Pampillón González, L. (2012). Producción de biogás en México estado actual y perspectivas. Cuaderno Temático No. 5 Red Mexicana de Bioenergía, 48.
- Wid, N. & Horan, N. J. 2018. Anaerobic digestion of screenings for biogas recovery. Springer Singapore.