Anaerobic biodegradation as a treatment of the agro-plastics used in protected agriculture in the Mexican crops.

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ABSTRACT

Purpose: Demonstrate that agricultural plastic waste used in protected agriculture in Mexican crops can be treated through anaerobic biodegradation. **Methods:** Characterization of the organic fraction (OF) and anaerobic inoculum was performed. A system of anaerobic reactors was prepared with agricultural plastic samples (two biodegradable agro-plastic samples, one conventional as negative control and finally cellulose as positive control) simulating the accelerated landfill conditions in accordance with ASTM D 5526-12. For the biogas production and capture, the inverted test tube method was implemented, then the biogas volume produced during ten weeks of experimentation was quantified and a sample of biogas was injected into a gas chromatograph to determine its composition on a daily basis. The molecular weight of the sample with anaerobic behavior was determined before and after the process. **Results:** The ANOVA of the model in "Divided Plots" indicates that there is an interaction of the substrate vs. time. One of the reactors with biodegradable agro-plastics produced a greater amount of biogas (methane and carbon dioxide), while non-biodegradable material has a lower production. The biodegradable samples presented a visible physical alteration, while the conventional sample did not show any change after the process. A low but representative biodegradation percentage was obtained considering the exposure time of the material to the anaerobic process. **Conclusions:** One of the samples produced methane

and carbon dioxide, making it suitable for the anaerobic process compared to the positive control. The anaerobic biodegradation is a safe treatment for the degradation of agricultural plastics used in Mexican crops.

Key words: Protected agriculture, anaerobic process, agricultural plastics waste, biodegradable plastics, soil contamination.

INTRODUCTION

Protected agriculture

Agro-plasticulture is an adaptation strategy to climate change in agriculture. There are institutional public programs that support productive techniques with agricultural plastics [1].

This technique, also called *protected agriculture*, is performed under protective structures that help to exercise a certain degree of control over several factors from the environment, thereby minimizing the impacts that adverse weather conditions and pests cause on crops. Mexico has been incorporating new irrigation and cultivation techniques in order to increase production and insert new crop schemes (Fig1.) and in recent years their use has had a surprising growth of more than 20% per year [2].

Fig1. Protected agriculture in Mexico.

However, generated waste is left outdoors in the fields, discarded in clandestine garbage dumps and landfills. A well-designed and managed *agro-plasticulture* system has immediate advantages: early production, yield and quality increase, efficient use of water, decrease in pesticides and fertilizers application, increase in total production, protection of weather contingencies, as well as a control of pests, diseases and weeds. All the above advantages must balance favorably in short and long terms the higher initial investment cost that implies the use of agro-plastics [3-4].

Agricultural plastics used in Mexico

Gómez and Arellano reported in 2014 that in Mexico the protected agriculture occupied area is 20,000 Ha^2 and the production and abandonment of these agro-plastics in the field causes agricultural soils contamination and a landscaping and visual impact. All of this contributes to increase the bad image that the plastic has as an *environment aggressor*, then the urgent need to develop technologies for their management and their incorporation into the environment is evident (Fig2.).

Fig2. Agro-plastics in San Quintin Valley, Baja California Mexico [5].

The Official Mexican Standard NOM-161-SEMARNAT-2011 classifies the plastic waste generated by agricultural activities as special handling waste [6].

The agricultural plastics used in Mexican crops are greenhouse film, shade mesh and anti-hail materials, quilting, macrotunel, microtunnel, raffia, bags and drip irrigation tape. For the year 2006, it was calculated that at national level there were more than 200,000 Ha with the use of plastics in agriculture [5]. According to SAGARPA [1], it is currently estimated that there are more than 21 million Ha nationally dedicated to agricultural activities and in 70% of this area plastic materials are used.

In 2010, 265 million tons of plastic production was reported worldwide, 2% of this volume was for agricultural use [7]. Gómez and Arellano reported that in 2013 [4], Mexico generated approximately 280 thousand tons of agro-plastics. Only 28,000 tons was recycle, this is 10% of the total waste, the rest ends up in landfills or burns.

Problem of agro-plastics use in Mexico

The incomplete combustion of agricultural plastic waste in open sky scenarios can lead to the release of carbon monoxide, sulfur dioxide and other air pollutants. Another effects are air emissions of heavy metals, dioxins, furans, and polycyclic aromatic hydrocarbons (PAHs) compounds that are highly toxic to health and considered as contaminants with a potential effect on the environment. Specifically, dioxins and furans are a health problem, even in very small amounts and are associated with endocrine disorders, heart disease and cognitive and motor disabilities, as well as being a known human carcinogen [8-10].

Humans can be exposed to dioxins directly by inhalation or through the skin, or by means of plants or meat, as they are concentrated in animal fat. This suggests that the burning of agricultural plastics and the generation of associated dioxins is of particular concern, since this practice occurs on or near active agricultural lands. In addition, most of the human intake of dioxins comes from food sources. Dioxin emissions from the burning of agricultural plastics have the potential to affect the population when they land on crops and are concentrated in the bodies of farm animals. Finally, degraded plastic fragments end up in the sea, contaminating seawater and threatening marine organisms [9].

Based on the above, the purpose of this paper is to demonstrate that biodegradable agricultural plastics can be used in protected agriculture in Mexican fields and can be treated by anaerobic biodegradation.

METHODOLOGY

Initially, two commercial samples of biodegradable agricultural plastic were purchased, both with polylactic acid integrated in their formulation. Also, one conventional sample based on low density polyethylene (LDPE), which is the main material of non-biodegradable agricultural plastics was used as negative control. Cellulose was used as positive control.

Organic fraction and anaerobic inoculum characterization

The organic fraction (OF) was obtained by organic waste accumulation from an apartments unit. The anaerobic inoculum comes from an upflow anaerobic sludge blanket digestion reactor of the wastewater treatment plant from the brewing company Grupo Modelo. For the construction of the 12 reactors and the characterization by the determinations indicated in the ASTM D5526-12 standard, 3 Kg of organic waste and 5 L of anaerobic inoculum was collected. Both samples were stored in a cold room (Fig3.).

Fig3. Organic fraction (left) and anaerobic inoculum (right).

The characterization of the OF and anaerobic inoculum was performed in terms of humidity, total solids (TS), volatile solids (VTS), fixed solids (FTS), Total Organic Carbon (TOC), total N_2 and pH, all of them based on the specifications of the ASTM D 5526-12 standard. The determinations indicate the nutrient content of the OF and the potential to biodegrade during the anaerobic process.

Experimental statistical design

For the experimental design the factors, experimental unit (e.u.), the treatments, the replicates number were considered. The temperature was set at 55 ° C. The organic fraction, inoculum and plastic substrate proportion are determined by the ASTM D5526-12 standard, so they were considered as follows:

- Factor: Conventional / biodegradable agricultural plastic or substrate (Fixed, 4 levels).
- Variable: Biogas volume produced measured through time for ten weeks.
- Small plot or small e.u.: Reactor (OF + Inoculum + Substrate) at time t_k (t_1, t_2, t_3, t_4, ..., t_{10}). Small plot treatment: time, which is expected to give an effect to the response variable.
- Large plot or large e.u.: Reactor (FO + Inoculum). Treatment of large plot: substrate (4 levels), considering that the composition of the plastic (conventional or biodegradable agro-plastic) will give an effect to the response variable.
- Three replicates per factor will be carried out, where the cellulose will be positive control (12 experimental reactors).
- According with the factor, variable, e.u. and nature of the experiment, the model is *Divided Plots*, then the equation is defined by:

$$\boldsymbol{y_{ijk}} = \mu + \boldsymbol{S_i} + \boldsymbol{R_{j(i)}} + \boldsymbol{T_k} + (\boldsymbol{ST})_{\boldsymbol{ik}} + \boldsymbol{\varepsilon_{ijk}}$$

Where:
y= response variable (volume of biogas produced), j-th observation of the i-th treatment in the k-th time.
μ= general average.
S_i= plastic type effect (substrate), large plot treatment (i = 1, 2, 3, 4).
$R_{j(i)}$= error of the jth (random) reactor nested in the i-th treatment (type of agro-plastic).
T_k= effect of time k (1, 2, ..., 10), small plot treatment.
$(ST)_{ik}$= interaction effect (substrate: time).
$\boldsymbol{\varepsilon}_{ijk}$= error associated with the ijk -th observation.

Reactors preparation and anaerobic system construction

Based on the experimental statistical model designed in divided plots, 12 reactors of 1000 mL were prepared. For each reactor, a mixture of 100 g of OF, 106 g of anaerobic inoculum on dry weight and 6 g of agricultural plastic sample was made. The replicates were formulated with the OF + inoculum + substrate mixture as indicated in table 1.

Table 1. Substrates applied as a treatment to the mixture (OF + inoculum + substrate).

Replicates	Substrate
3	S1:Cellulose (positive control)
3	S2: Conventional agro-plastic (commercial brand 1)
3	S3: Biodegradable agro-plastic (commercial brand 2)
3	S4: Biodegradable agro-plastic (commercial brand 3)

For the biogas production and capture, the inverted test tube method was implemented, which works by displacing the volume of water inside the test tube after starting the system.

The system was constructed as shown in figure 4, where the reactors were immersed in a water bath at 55 ° C (simulating the accelerated landfill conditions in accordance with ASTM D 5526-12) and the produced biogas was captured in inverted test tubs.

Fig4. Experimental system of anaerobic reactors.

System start and stop

The biogas volume produced during ten weeks of experimentation was quantified daily. A sample of biogas was taken every third day, which was injected into a gas chromatograph to determine its composition (methane and carbon dioxide proportion). For each reactor a biogas sample was captured with a 1 mL insulin syringe purging the first 3 suctions and staying with the fourth. The produced volume data were introduced to the model for its analysis using the statistical package R.

At the end of the experiment, the supernatant was removed and the test material was separated. The molecular weight of the sample S4 was determined before and after the process in the Center for Research in Applied Chemistry.

Biodegradation percentage calculation

To calculate the percentage of biodegradation (% BD) the following steps were done:

a) CH_4 and CO_2 production quantification by the reactors: The amount of the component (mol) was converted to the equivalent of the reactor volume.

b) Theoretical total carbon determination of the test material (agro-plastic substrates, OF and anaerobic inoculum): Using the total carbon in the test sample (cellulose, conventional and biodegradable agro-plastic), the maximum theoretical gas production (carbon dioxide plus methane) from the anaerobic biodegradation of the test specimen was calculated using the next equation.

$$C_t = \%BM_s * \%C_s * M_s$$

Where:

C_t = Total theoretical carbon in the test material (OF, inoculum, cellulose, conventional agro-plastic and biodegradable) that can be transformed to gaseous carbon by the biodegradation process, g.

$\% BM_s$ = Percentage of biodegradable material in the tested substrate (agro-plastic sample and positive control), %.

$\% C_s$ = Percentage of carbon in the substrate or biodegradable material tested, %.

M_s = Added mass of test material (substrate), g.

For the anaerobic inoculum and OF samples, the carbon percentage in the sample obtained by determining the TOC was used.

c) % BD of anaerobically biodegradable test material estimation: for each type of agro-plastic the %BD was obtained by dividing the average gaseous carbon production of the test material by the average of the total or initial theoretical carbon amount of the test compound (OF + inoculum + agro-plastic substrate) and multiplying by 100.

$$\%BD = \frac{mean\ C_{g\ (test)} - mean\ C_{g\ (blank)}}{C_t} * 100$$

Where:

C_g= amount of gaseous carbon produced, g.

C_t= amount of carbon in test compound added, g.

RESULTS

Organic fraction and anaerobic inoculum characterization.

Table 2 shows the obtained results for organic fraction and anaerobic inoculum characterization:

Table 2. Organic fraction and anaerobic inoculum characterization.

Parameter	OF	Anaerobic inoculum
% Humidity	74.71	67.97
pH	5.06	8.44
TS (mg/L)	30091.25	6812.50
VTS (mg/L)	24485.50	3412.50
FTS (mg/L)	5605.75	3400.00
DQO (mg O_2/L)	285.50	308.42
Total N_2 (%)	0.39	0.49
TOC (%)	40.11	38.86
TOC mixture (%)	43.2	

According to the tests requested by ASTM D5526-12, the organic fraction has a humidity percentage of 74.7%, thus it is within the established limit of 30% of total solids. The parameters, with the exception of the organic fraction pH, are within the established limits and indicate that the OF has the potential for biogas production and biodegradation. An acidic pH can inhibit the anaerobic biodegradation process due to volatile fatty acids presence.

Experimental statistical design.

The statistical model determined that there is a relationship between the amount of biogas produced and the substrate applied. The ANOVA of the model in "Divided Plots" (table 3) indicates that there is a significant difference between the interactions of the substrates vs. time so the p-value of the independent factors must be discarded.

Table 3. ANOVA of the experimental statistical model applied (Divided Plots).

	Componente	gl	SS	CM	F	p-value
Parcela grande	sustrato	3	999032	333011	4.4898	0.039712
	sustrato:reactor	8	593360	74170	30.7911	2.2E-16
Parcela pequeña	ti	9	16334440	1814938	753.4573	2.2E-16
	sustrato:tiempo	27	164384	6088	2.5275	0.000955
	residual	72	173435	2409		

Table 4 shows the comparisons between the substrates obtained by the Student-Newman-Kneuls test (SNK). The *significant differences* (S.D.) indicate the substrates that have important differences, while the *non-significant differences* (N-S D.) are comparisons that represent the substrates with the greatest similarities in terms of average biogas production.

Table 4. Statistical analysis by SNK test for substrate:time interaction.

Week	Biogas production	S.D. ***	S.D. **, *	N-S D.
1	S2<S4<S1<S3	S3-S2, S1-S2, S4-S2	-	S3-S4, S1-S4, S1-S3
2	S2<S4<S1<S3	S3-S2, S1-S2, S4-S2	S3-S4*	S4-S1, S3-S1
3	S2<S4<S1<S3	S3-S2, S1-S2, S4-S2	S3-S4**, S1-S4**	S3-S1
4	S2<S4<S1<S3	S3-S2, S1-S2, S4-S2	S3-S4**, S1-S4*	S3-S1
5	S2<S4<S1<S3	S3-S2, S1-S2, S4-S2	S3-S4*, S3-S1*	S4-S1
6	S2<S4<S1<S3	S3-S2, S1-S2, S4-S2	-	S3-S4, S1-S4, S1-S3
7	S2<S1<S4<S3	S3-S2, S1-S2, S4-S2	-	S3-S4, S1-S4, S1-S3
8	S2<S1<S3<S4	S3-S2, S1-S2, S4-S2	-	S3-S4, S1-S4, S1-S3
9	S2<S1<S4<S3	S3-S2, S1-S2, S4-S2	-	S3-S4, S1-S4, S1-S3
10	S2<S4<S1<S3	S3-S2, S1-S2, S4-S2	-	S3-S4, S1-S4, S1-S3

Code significance degree: (<0.001, ***); (<0.01, **); (<0.05, *); (> 0.05, n-s).

The significance degree indicates that there is no similarity between the production of biogas from S2 and the other substrates (S1, S3 and S4), which remains the same at all time levels. Most of the time levels (weeks) did not show large differences in the average production of the three biodegradable substrates (S1, S3 and S4).

Through the SNK test, the behavior of biodegradable agro-plastic substrates and the positive control (S1, S3 and S4) is the same in terms of average biogas production and the only one that is different is the conventional agricultural plastic (S2), having the lowest average biogas production. This behavior does not consider its biogas composition (CH_4 and CO_2 production).

System stop

After the removal, the material initial appearance was compared to its appearance at the end of the experiment. For cases of biodegradable agro-plastics, there was fragmentation, alteration of material conformation and color change, while the conventional sample did not show any change after the process (Fig5.).

Fig5. Material comparison (experimentation beginning and end, replicate 1).

Only sample S4 and S1 (positive control) had a behavior related to the anaerobic biodegradation process (CH_4 and CO_2 production). The molecular weight of sample S4 before process was 148.978 g/mol ±1.2%, while after the biodegradation process it was 7.204 g/mol ±5.1%. The decrease in molecular weight after the biodegradation process for the S4 substrate can be an indirect indicator of the biodegradation of the material.

Biodegradation percentage calculation.

According to the obtained results through the chromatograph (Fig6.), the reactors with S1 substrate followed by S4 and S3 produced a greater amount of biogas. The S2 has a lower production because it is a non-biodegradable material. The S1 and the S4 gave methane readings in greater proportion, the substrate one (positive control) above the four.

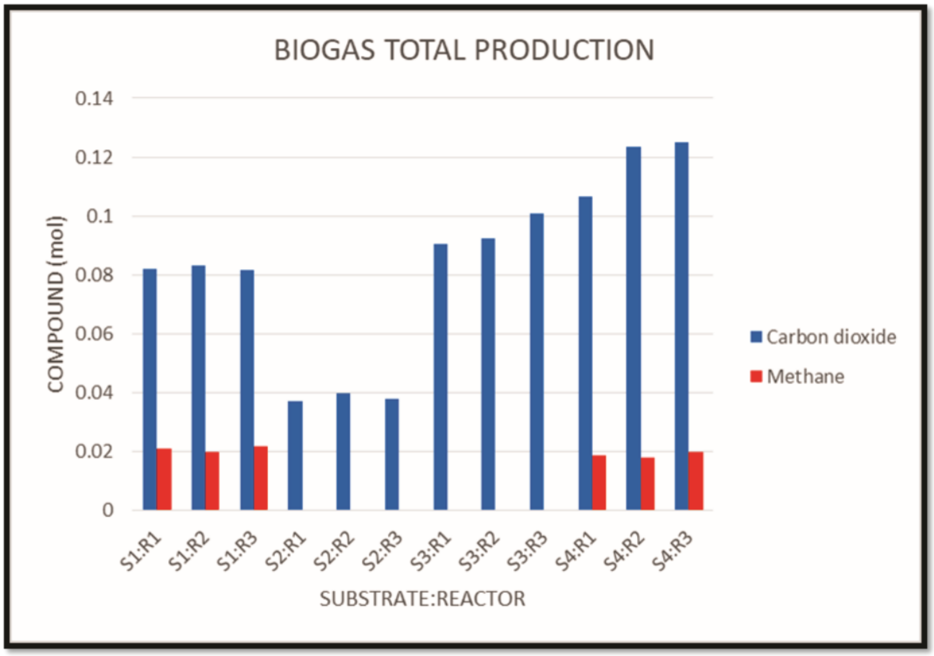

Fig6. Biogas total production.

In the case of the S2 and S3 reactors, it was not possible to read the amount of methane produced because it was not within the detection limit of the chromatograph. The production of methane for both substrates reactors is not ruled out. It is possible that S3 substrate components are mostly compostable and it has biodegradable material in small proportions because it had a high CO_2 production and a physically visible degradation. However, it must have produced methane to be considered anaerobically biodegradable. The S2 substrate had a low CO_2 production and no physical change (non-biodegradable material).

Table 5 shows the calculated total theoretical carbon (C_t) results of each component in the mixture, considering the aggregate mass of the samples (agro-plastic, OF and inoculum), the biodegradable material percentage in the sample and the percentage carbon in the biodegradable material.

Table 5. Carbon balance during anaerobic biodegradation process and calculation of %BD.

REACTOR	C_t OF (g)	C_t inoculum (g)	C_t agro-plastic (g)	Total C_t (g)	Cg produced (g)	C_f or C in reactor (g)	% BD
S1, R1	40.19022	41.184885	1.335422	82.710527	1.23432846	81.4761985	1.49234748
S1,R2	40.114011	41.184885	1.335422	82.634318	1.2373856	81.3969324	1.49742338
S1, R3	40.114011	41.184885	1.3332	82.632096	1.23925133	81.3928447	1.49972152
S2, R1	40.118022	41.184885		81.302907	0.44225213		
S2, R2	40.114011	41.184885		81.298896	0.47572875		
S2, R3	40.15011	41.184885		81.334995	0.45411844		
S3, R1	40.15011	41.184885		81.334995	1.083516		
S3, R2	40.122033	41.184885		81.306918	1.110312		
S3, R3	40.138077	41.184885		81.322962	1.207584		
S4, R1	40.134066	41.184885	2.869	84.187951	1.502004	82.685947	1.78410804
S4, R2	40.118022	41.184885	2.88325	84.186157	1.696128	82.490029	2.01473504
S4, R3	40.126044	41.184885	2.8595	84.170429	1.735188	82.435241	2.06151735

For the S2 and S3 reactors, the theoretical carbon of the agro-plastic sample was not determined because they are commercial products and the percentage of biodegradable material in the product formulation is confidential.

An average %BD of 1.4964 was obtained for the positive control (cellulose) and 1.9534 for the S4 biodegradable agro-plastic (with polylactic acid) in anaerobic reactors simulating accelerated sanitary landfill conditions. This is because the material used as a positive control was sawdust (S1), which contains 50% cellulose which, in turn, contains 44.44% biodegradable organic carbon. In contrast, the agro-plastic used as S4 contains 95% polylactic acid which, in turn, contains 50% available organic carbon. This allows the S4 agro-plastic material to contain more organic carbon available for biodegradation.

Also, this may explain the higher production of CO_2 in S4 substrate than in the positive control. Although S1 had a higher amount of CH_4, the greater proportion of available organic carbon allows its transformation in the biogas components, in addition to the conformation of the polymer chains.

Possibly, the biodegradation process inhibition is due to the presence of volatile fatty acids (VFA's) according to the high pH in OF. The VFA's can cause small changes in the pH levels, thus moving away the reactors from their optimum pH. The methanogenic organisms are more susceptible to the pH variations than the other anaerobic organisms and methanogenesis being a determining step in the process, caused the inhibition of anaerobic biodegradation.

CONCLUSIONS

The biodegradable agricultural plastics presented a higher biogas production compared to the conventional sample, according to the statistical model and the chromatography readings. S4 produced methane and carbon dioxide, making it suitable for the anaerobic process compared to the positive control (S1). According to the determination, the sample S4 reduced its molecular weight after the biodegradation and presented a %BD comparable with the positive control, considering the exposure time of the biodegradable material in the anaerobic process. However, both biodegradable substrates presented a physically visible degradation, which is why we can conclude that anaerobic biodegradation is a safe treatment for the degradation of agricultural plastics used in Mexican crops.

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