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Influence of inoculum on growth and retention of the biomass in anaerobic filters

Influencia del inóculo sobre el crecimiento y la retención de biomasa en filtros anaerobios

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Abstract

In this study we evaluated, on bench scale, two anaerobic filters of equal configuration and using coconut shell as support material, for the treatment of the wastewater generated in cassava's starch extraction process. Considering that in the study area the inoculum available in enough quantity is cow manure, this inoculum was used alone (Reactor 1) and mixed with granular sludge in a ratio of 1:1 (Reactor 2). The influence of inoculum was evaluated in the performance of the anaerobic filters as well as in the biomass adaptation to the substrate and the prevalent growth on support material. Both reactors showed potential for the treatment of this type of wastewater, with a Hydraulic Retention Time (HRT) of 12 hours, an average Organic Loading Rate (OLR) of 7.0 kg COD/m3*d and a Buffer Index (BI) that varied from 0.20 to 0.35. The increase up to 50% in the Specific Methanogenic Activity (SMA) at the end of the start-up in both reactors, confirmed the adequate biomass adaptation to the substrate as a result of the control of buffer capacity, and the higher values in the R2, showed that cow manure mixed with granular sludge improved methane production and process efficiency. It was found that the occluded biomass had greater activity than the attached one; this showed that the support material allowed the development of a greater proportion of active biomass within the interstices.

----------*Keywords:* Anaerobic filter, attached biomass, cassava starch extraction wastewater, occluded biomass, Specific Methanogenic Activity (SMA)

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Resumen

En este estudio se evaluaron dos filtros anaerobios de igual configuración en escala de laboratorio y usando cáscara de coco como material de soporte, para el tratamiento de las aguas residuales del proceso de extracción de almidón de yuca. Considerando que en el área de estudio el inóculo disponible en cantidad suficiente es el estiércol de vaca, se usó este inóculo solo (Reactor R1) y combinado con lodo granular en una proporción 1:1 (Reactor R2). Se evaluó la influencia del inóculo sobre el desempeño de los filtros anaerobios, sobre la adaptación de la biomasa al sustrato y sobre el crecimiento prevalente en el material de soporte. Ambos reactores mostraron potencial para el tratamiento de estas aguas residuales, con un Tiempo de Retención Hidráulico (TRH) de aproximadamente 12 horas, Carga Orgánica Volumétrica (COV) de 7.0 kg DQO/m3*d y un Índice Buffer (IB) entre 0.20 y 0.35. El incremento hasta de 50% en la Actividad Metanogénica Específica (AME) al final del arranque en ambos reactores, confirmó la adecuada adaptación de la biomasa al sustrato como resultado del control de la capacidad buffer; adicionalmente, los mayores valores de AME en el R2 mostraron que la mezcla de estiércol de vaca con lodo granular mejoró la producción de metano y la eficiencia del proceso. Se encontró que la biomasa ocluida tuvo mayor actividad que la biomasa adherida, indicando que el medio de soporte favoreció el desarrollo de una mayor proporción de biomasa activa en los intersticios del material de soporte.

----------*Palabras clave:* Filtro anaerobio, biomasa adherida, aguas residuales del proceso de extracción de almidón de yuca, biomasa ocluida, Actividad Metanogénica Específica (AME)

Introduction

Cassava or tapioca (*Manihot esculenta* Crantz) tuber containing about 25-30% starch is one of the richest sources of starch among the tropical tuber crops [1]. The 80% of the cassava starch production in Colombia comes from the northern region of the Cauca Department in 150 small agricultural industries known as "*rallanderías",* which process from 5 to 12.5 tons of cassava per week [2]. The starch extraction process requires about 4.5-10.0 m^3 of water per ton of roots [1].

The liquid wastes are generated in the washing, peeling and sedimentation steps; in the last step, the starch is separated from the liquid phase and subsequently the starch is fermented [3]. The liquid wastes are deposited in settling tanks, and the supernatant liquid constitutes the main source of wastewater (> 80% of total liquid wastes), which is mainly composed of yellowish starch of low density and quality (commonly known as *"mancha"*) with a high level of organic pollution (3400-5400 mg COD/L) and easily hydrolyzable carbohydrates that promote its rapid fermentation (acidity 400-1500 mg $CaCO_3$/L) [4].

Laboratory and pilot scale studies have demonstrated that the relative simplicity of operation and maintenance of anaerobic filter technology, make it the most appropriate for the socio-economic conditions of this sector, reaching levels of COD removal efficiencies from 77 to 81% of COD and 76% of SS with Hydraulic Retention Times (HRT) within 10 to 12 hours [4-6].

One of the most serious problems associated with anaerobic filters is the difficulty of measuring the biomass quantity and quality, as well as their evolution with time and operating conditions. In these reactors, the biomass immobilization is achieved by adhesion to the surface of the filters (attached biomass) and by retention in the medium interstices (occluded biomass) [7].

In the study developed by [8], three supporting media in anaerobic filters of equal configuration were evaluated, and found that the methane production and hydrodynamic behavior depend on the type of support media, being the rough and porous ones those that exhibit better hydrodynamic performance and contribute to methane production by the occluded biomass. In the smooth and not porous ones, the greater production of methane is achieved by attached biomass and the rough without porosity ones. The production of methane is equal for both types of biomass.

Considering that in the study area the only inoculum available in enough quantity is cow manure, at laboratory scale, we evaluated the influence of the cow manure alone and mixed with granular sludge in a ratio of 1:1 on the performance of two anaerobic filters. Furthermore, the biomass adaptation to the substrate and the predominant growth were evaluated, in terms of methane production at the end of the start-up process.

Material and methods

The reactors were built with acrylic sheets; the volume was 9.5 l and the support media was comprised of Coconut shell with a porosity of 60%. The coconut shells were fractionated into smaller pieces (4 parts) in order to stay in a size suitable for the size of the laboratory scale reactor. A gravel bed was located on the upper portion of the support media to prevent the shell floating. A schematic drawing of the experimental set-up of the anaerobic filters is shown in figure 1.

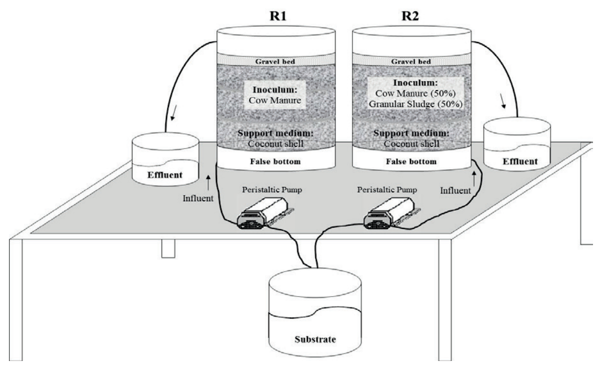

Figure 1 Experimental set up of the anaerobic filters

The substrate used was wastewater from the cassava starch extraction process, particularly the effluent from the settling tank of the *"mancha"* (Table 1). $NaHCO_3$ was added to control the acidity of the substrate, maintaining a $COD:CaCO_3$ ratio between 1.0: 0.6-1.0 [4].

Table 1 Physicochemical characterization of the substrate

Parameter	*Units*	*Range*
pH	Units	4.1 - 4.4
Total Alkalinity	mg/l $CaCO_3$	0 - 10
Volatile Fatty Acids	meq /l	12.6 - 31.8
Acidity	mg/L $CaCO_3$	400 - 1500
Chemical Oxygen Demand (COD)	mg/l	3400 - 5400
Biochemical Oxygen Demand (BOD_5)	mg/l	1876 - 2459
BOD_5/COD Relation	----	0.50 - 0.60
Total Solids - TS	mg/l	2075 - 3500
Total Suspended Solids -TSS	mg/l	330 - 1180
Dissolved Solids	mg/l	1745 - 2320
Sedimentable Solids	ml/l*h	0.8 - 15

Reactor 1 (R1) was inoculated with Cow Manure (CM), and Reactor 2 (R2) was inoculated with CM and a Granular Sludge (GS) from the paper industry in a 1.0:1.0 proportion. The quantity of inoculum added to each reactor was determined to establish a concentration of 15g VS/L and to verify that such addition was enough to fill the equivalent to 40% of the reactor volume [9]. The

quantity of inoculum added was 1.6 kg of CM for R1 and 1.2 kg of GS + 0.8 kg of CM for R2.

The criteria for the start-up of the reactors consisted in the progressive reduction of the Hydraulic Retention Time (HRT) and the increase of the Organic Loading Rate (OLR) in order to promote the biofilm growth and avoid overloading of the systems which could lead to an inhibition of methanogens, and consequently to failure of the start-up process [10].

Samples of the attached and occluded biomass were extracted at the end of the start-up process (150 days). The extraction procedure consisted initially in removing the gravel layer located over the reactor surface. After that, the biomass contained on the support media was separated (attached biomass) and finally the remaining biomass (occluded biomass) was extracted from the reactor once all support media was extracted. In order to separate the attached biomass from the support media, the pieces of support media were washed with distilled water, the biomass (sludge) was allowed to settle and the supernatant was removed.

Volatile Solids (VS) and Total Solids (TS) were measured in the biomass used to inoculate both reactors at the beginning of start-up as well as of the samples of biomass removed at the end of this process in accordance to [11]. Table 2 shows the VS and TS values measured in the inoculum at the beginning and the end of start-up.

The Specific Methanogenic Activity (SMA) test was determined through fluid displacement experiments (on triplicate) at a controlled temperature (30 ±1°C) and the quantity of biomass used as inoculum was 2.5 gVS/L. The determination of SMA was done using the eq. 1 [12].

$$SMA(gCOD/gVS*d)=(m*24)/(433*M) \qquad (1)$$

where:

SMA: Specific Methanogenic Activity

m: slope of the curve of maximum methane production (accumulated volume of CH_4 over time)

M: sludge mass (added sludge volume * Initial sludge concentration)

433: mL of CH_4 per gram of digested COD at a temperature of 30ºC and atmospheric pressure of 0.893 atm for the city of Cali, Colombia; 24: conversion factor from hours to days

Results and discussion

Table 3 shows the results of the BI and the COD removal efficiency reached at different HRT during the star-up on both reactors.

Table 2 Characterization of the inoculum

At the beginning of start-up				At the end of start-up					
Inoculum	TS (g/kg)	VS (g/kg)	Relation VS/ST	TS (mg/l)		VS (mg/l)		Relation VS/TS	
				AB	OB	AB	OB	AB	OB
CM	155.6	113.6	0.73	42.4	51.6	27.6	46.4	0.65	0.90
GS	119.8	81.5	0.68	-	-	-	-		
CM+GS	138.6	94.2	0.68	39.4	45.6	28.8	29.6	0.73	0.65

*TS: Total solids; VS: Volatile Solids; AB: Attached Biomass; OB: Occluded Biomass

Table 3 Variation of the HRT, OLR, BI and COD removal efficiency reached during the start-up of the reactors

Stage	HRT (hours)	OLR (kg/m³*d)	Buffer Index-BI				COD Removal Efficiency (%)			
			R1		R2		R1		R2	
			Average	Min - Max	Average	Min - Max	Average	Min - Max	Average	Min - Max
1	24	4.1	0.33±0.11	0.20 0.49	0.30±0.1	0.14 0.49	35±17	10 55	43±13	23 60
2	20	4.9	0.24±0.03	0.18 0.30	0.16±0.03	0.11 0.23	44±13	15 56	58±13	32 75
3	16	5.7	0.32±0.05	0.22 0.43	0.24±0.07	0.13 0.36	59±10	33 77	57±17	20 86
4	12	7.0	0.33±0.06	0.26 0.48	0.31±0.06	0.19 0.44	63±5	53 71	59±6	49 67
5	10	12.5	0.40±0.06	0.27 0.49	0.33±0.06	0.18 0.43	67±8	56 81	68±10	59 82

*HRT: Hydraulic Retention Time (hours); OLR: Organic Loading Rate (kg/m³*d); COD: Chemical Oxygen Demand
R1: Reactor 1 (Inoculum 100% cow manure); R2: Reactor 2 (Inoculum 50% cow manure and 50% granular sludge)

Both reactors showed an adequate performance against operational changes and although the COD removal efficiencies were favorable with the HRT less than 12 hours, a greater instability in the buffer index was observed, mainly in the reactor R1. It is recommended values of BI between 0.2 - 0.3 and maximum of 0.35 [13], therefore, the most adequate operational condition was achieved with a HRT of 12 hours, which is within the range of 10 to 12 hours found by [6] and it is the recommended design value of the reactors built at a real scale [12, 14].

The recommended HRT for both reactors (12 hours) had associated an OLR of 7.0 kg COD/m³*d and a buffer index from 0.20 and 0.35, which makes possible to reach a maximum COD removal efficiency in the 70% range. Regarding the Buffer Index (BI), the values were greater in R1 than in R2, and R1 required greater amounts of alkaline medium, this behavior shows that the type of inoculum influences the system performance. The combination of cow manure with granular sludge was more efficient and had less risk of acidification than the reactor inoculated with cow manure alone.

The results of the SMA tests performed to evaluate the biomass adaptation to the substrate and the type of prevalent growth rate in terms of methane production are presented in figure 2.

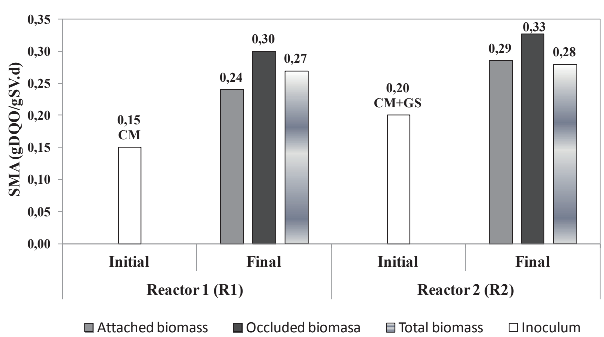

Figure 2 Results of the Specific Methanogenic Activity tests to R1 and R2 reactors

The initial SMA for R1 was less than the R2 because the granular biomass is usually more stable and presents better settling characteristics and methane production, as is also indicated by [9, 15]. The results also agree with the exposed by [16], who indicate that the suspended or flocculent sludges, which have higher specific surface area, suffer greater inhibition than granular sludge. It is precisely for this reason that the incorporation of granular biomass is considered as a favorable strategy to improve the start-up stage of the anaerobic reactors, as also noted [17, 18].

Comparing the initial SMA values with the results at the end of the start-up, a considerable increase is observed in both cases, showing a good adaptation of the biomass to the substrate independently of the type of growth, and showed the formation of active biomass, which is a strategic objective of the start-up stage [10]. This behavior is consistent with the results shown in table 3, which presented a progressive increase of the COD removal efficiencies and stability of the BI.

The SMA increases at the end of the start-up stage and the similarity of the achieved values in both reactors are indicators of an appropriate biomass growth and adaptation to the substrate, which was also improved by proper environmental conditions achieved. In the study, the environmental factor which had a strict control was the buffer index to ensure the neutralization of the volatile fatty acids, being recommended as an excellent control tool for anaerobic processes.

Considering the type of biomass growth, it was observed that in both reactors, the SMA of the attached biomass was less than of the occluded biomass. These results confirm the findings of [19] and [20], which, although they used wastewater with different compositions (paper industry and whey cheese respectively) found higher values of SMA in the occluded biomass. In addition, the results obtained by [12] and [21] show that the greater proportion of microorganisms are suspended inside the interstices of the support media, which can benefit the increase of SMA values in the occluded biomass. Furthermore, [9] indicated that for anaerobic filters with rough and porous support material such as coconut shell, a better hydrodynamic behavior and performance of the occluded biomass occur in terms of methane production.

Conclusions

The evaluation of the two anaerobic filters in bench scale showed that both, the Cow Manure (CM) and the mixture of CM and Granular Sludge (GS), are adequate inoculum for the anaerobic wastewater treatment from the cassava starch extraction process. However, the inoculum mixture (CM and GS) presented great advantages in the reactor performance showing better stability against operational changes and requiring a less control of the buffer index.

The increase in the Specific Methanogenic Activity (SMA) at the end of the start-up (up to 50% in both reactors), confirmed the adequate biomass adaptation to the substrate as a result of the control of buffer capacity.

The addition of granular biomass improves the reactor performance, and greater SMA values of the occluded biomass confirmed that inside in the support media (interstices) is where the greater proportion of active biomass is concentrated, principally when a rough and porous support material, such as coconut shell is used.

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