MNK Publication

International Journal of Latest Research in Science and Technology **ISSN (Online):2278-5299**
Volume 2, Issue 6: Page No.53-57 ,November-December 2013
https://www.mnkpublication.com/journal/ijlrst/index.php

INFLUENCE OF NUTRIENTS ADDITION IN THE START-UP AND STABILIZATION OF AN ANAEROBIC BIOFILM REACTOR WITH STANDARDIZED COMMERCIAL SUBSTRATE

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Abstract*- *In this paper a study about the influence of nutrients and trace elements addition in the performance of anaerobic inverse fluidized bed reactor (IFBR) was conducted. The reactor was adapted for the anaerobic degradation of the organic matter present in commercial substrate. During reactor startup, the substrate consisted only in the diluted commercial apple juice with adjustment of the pH value to neutrality with the addition of sodium bicarbonate. Low density particles of extendosphere® was used as support material and a fraction of previously colonized particles was used as inoculum. Stepwise increments of the Organic Loading Rate (OLR) from 1.2 to 7.5 $g_{COD}L^{-1}day^{-1}$ were tested and biofilm formation was monitored for a period of 170 days alternating stages of addition or absence of nutrients and trace elements with the substrate.

We concluded that the nutrients and trace elements addition to the reactor was essential to reduce the start-up and stabilization time because in the initial phase without them the reactor efficiency only reached 36% of COD removal despite the fact that pH value was stable and near to neutrality. Once the addition of nutrients and trace elements was made the COD removal sharply increased reaching values of 90% in only 30 days. After two more alternating periods of nutrients and trace elements suppression-addition the reactor performance related with the COD removal and biogas production showed a clear interaction between nutrients and trace elements addition and reactor stability.

Keywords – Anaerobic digestion; Biofilm formation; Reactor start-up; Nutrients; Inverse fluidization; Apple juice; Down flow fludization

INTRODUCTION

The microorganisms in the growth phase, made replicas of themselves requiring the elements found in their chemical composition, so it is necessary to give them the nutrients and chemical elements in a form accessible from the metabolic point of view. Also they require energy to synthesize macromolecules and to retain the essential chemical gradients across their membranes.

Microbial growth is influenced by both nutritional and physical aspects. Physical factors include: the hydrogen ion concentration (pH), temperature, oxygen concentration, humidity, and the hydrostatic and osmotic pressure. On the other hand, nutritional factors include: the availability of carbon, nitrogen, sulfur, phosphorus and other minerals and in some cases vitamins (Junco and Rodríguez, 2001).
Anaerobic Digestion is often the preferred biological method to treat effluents with high content of organic matter because the bacteria present in anaerobic reactors, efficiently degrade this kind of contaminants with the advantage of producing biogas that can be used as a renewable energy source.

Although the use of reactors with biomass attached to a carrier material have advantages among which are: ease of separation of the bacteria from the effluent and the use of high loading rates with low hydraulic residence time (HRT), all this in reduced spaces. These advantages are confronted with the slow growth of anaerobic bacteria which represents slow and more problematic reactor start-up in comparison with the aerobic reactors.

In the last decade, the development of new arrangements and fluidized beds for the retention of bacteria inside the reactor has made it possible to shorten HRTs in comparison with classic configurations as the Upflow Anaerobic Sludge Bed (UASB) reactors. Specifically, the inverse fluidized-bed reactors (IFBRs) with a fixed biofilm can treat large volumes of wastewater in a reduced space, as a result of the higher specific surface area available for biomass. Therefore, the IFBR configuration can treat high OLRs of more than 50 Kg COD/m^3·d (Alvarado-Lassman et al., 2008). Also, the IFBR requires less energy compared with traditional fluidization, because the support material has a lower density than wastewater (García-Calderón et al., 1998). However, a serious disadvantage of this system is that 2 to 9 months are required for the formation of an efficient biofilm (Lauwers et al., 1990). Recently some authors have used different strategies to reduce the start-up and stabilization time (Arnaiz et al., 2005; Alvarado-Lassman et al., 2010) however, there are very few reports of the effect of nutrients in these key stages for the proper performance of an anaerobic reactor (Cresson et al., 2006; O-Thong et al., 2007).

The present study, therefore, investigated the effect of nutrients and trace elements presence or absence in the start-

Publication History
Manuscript Received : *26 December 2013*
Manuscript Accepted : *29 December 2013*
Revision Received : *30 December 2013*
Manuscript Published : *31 December 2013*

up and stabilization stages of an IFBR using diluted commercial apple juice as substrate.

MATERIALS AND METHODS

IFBR Reactor

The inverse fluidized bed reactor consisted of a cylindrical acrylic body, two machined Nylamid® parts to close the top and bottom ends and to provide connections for the inlet, recirculation and effluent flow and for biogas collection. The reactor has a total volume of 1.82 L and a working volume of 1.67 L. Feed an liquid recirculation were ensured using peristaltic pumps (Masterflex®) as presented in Fig. 1. The fluidization velocity applied was 2.3 m/h using a liquid distributor located at the top of the column. Reactor temperature was kept at 35°C in a room with temperature control and the pH of the influent was regulated at 7 by addition of sodium bicarbonate.

The reactor was filled with support media up to 30% of the working volume. Small silica particles with an air bubble in the interior (Extendosphere®, PQ Hollowsphere) were used as the support media. The density of support media was 0.69 g/mL, mean particle diameter was 170 µm, and the specific surface area was 20000 m^2/m^3. The fraction of precolonized support used as the sole inoculum source was 8% of the support total volume, based in a previous experience (Alvarado-Lassman et. al., 2010). The colonized support was taken from a pilot anaerobic reactor fed with the organic fraction of municipal solid waste.

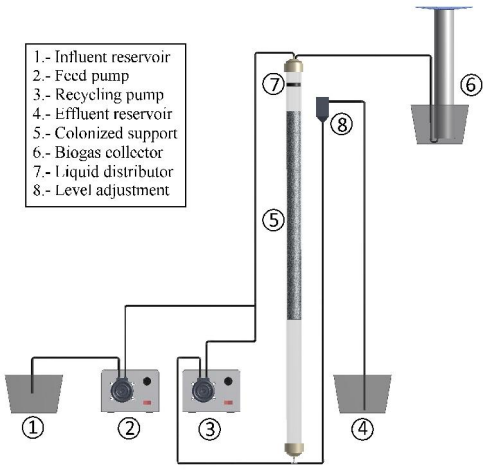

Fig.1 Schematic diagram of the inverse fluidized bed reactor

Substrate and reactor operation

The substrate used in this study was diluted commercial apple juice which is a substrate rich in carbohydrates with an organic matter content measured as COD of 110 g COD/L. The absence of solids in the apple juice allowed the formation of a homogeneous biofilm.

The reactor was operated in continuous mode for a period of 170 days with an HRT of 24 h. The volume supplied was 2200 mL/d, which was obtained through the dilution of apple juice with distilled water in order to obtain the required Organic Loading Rate (OLR). Initially the pH was adjusted with sodium bicarbonate in the stages when the nutrients and trace elements were not supplemented. The parameters measured are presented in Table 1.

Table 1. Measured parameters during reactor operation.

Parameter	Units	Method
COD Soluble	g/L	5220 d (SM)*
TS	g/L	2540 b (SM)
VTST	g/L	2540 e (SM)
pH	[--]	Potentiometric
Gas volume	L	Volumetric
Biogas composition	%	Gas Chromatography

*SM= Standard Methods (APHA, 1995)

The composition of biogas was determined with a Buck 310 gas chromatograph (Buck Scientific) using helium as the carrier gas and a packed column CTR-I.

Biofilm attached to the support was monitored with microscopic observations of samples taken from the reactor at different times.

Nutrient and trace elements addition

The diluted apple juice was complemented with nutrients and trace elements with a composition showed in Table 2.

Table 2. Nutrients and trace elements solution composition.

	Substance	Concentration mg/L
Mineral solution with nutrients	Sodium bicarbonate	1200
	Potassium phosphate monobasic	1500
	Potassium phosphate dibasic	900
	Ammonium chloride	125
	Magnesium sulfate	30
Trace elements	Potassium chloride	0.5
	Cobalt chloride	0.1
	Ferrous chloride	0.5
	Calcium chloride	0.5

Reactor operational scheme

After inoculation, the IFBR was operated with stepwise OLR increments and with the addition or suppression of the nutrients and trace elements solution in accordance with the scheme presented in Table 3.

Table3. Reactor operational stages

Stage	Days	OLR (gCOD/L.d)	Nutrients and trace elements
I	0-70	1.4	Without (WON)
II	71-105	1.6-7.5	With (WN)
III	106-114	7.5	WON
IV	115-127	7.5	WN
V	128-135	6.4	WON
VI	136-170	4.5-7.5	WN

RESULTS AND DISCUSSIONS

The IFBR start-up and stabilization was investigated during 170 days. The OLR was progressively increased from 1.4 to 7.5 g COD/L.d adjusting the concentration of the apple juice determined as 110 gCODs/L.

The pH is one of the most important physical factors to consider for a good performance of the reactor. Anaerobic digestion involves a symbiotic relationship between different types of bacteria. Among them the methanogenic bacteria are the most sensitive to pH changes because they have a narrow optimum pH range (between 6.8 and 7.3), so a careful balance between acid-forming and methane-producing bacteria must be maintained to ensure a complete degradation of the organic matter and a stable biogas production. At start-up the pH value of the influent to the IFBR was adjusted to 7.2 with sodium bicarbonate and then the substrate was fed continuously to the reactor. Because the inoculum comes from a reactor in stable operation that treats a more complex substrate, it is expected that in the initial stage, all the bacterial groups present in the biofilm, will degrade organic matter without acidification of the reactor and at the same time producing more biomass for biofilm formation.

Fig.2 presents the effluent pH and the OLR throughout the experiment. Fig. 3 shows the COD removal expressed as the percentage of the initial soluble COD degraded by the anaerobic bacteria. The biogas production is presented in Fig. 4 contrasted with the theoretical methane that will be produced if the metabolic pathway is oriented toward biogas formation.

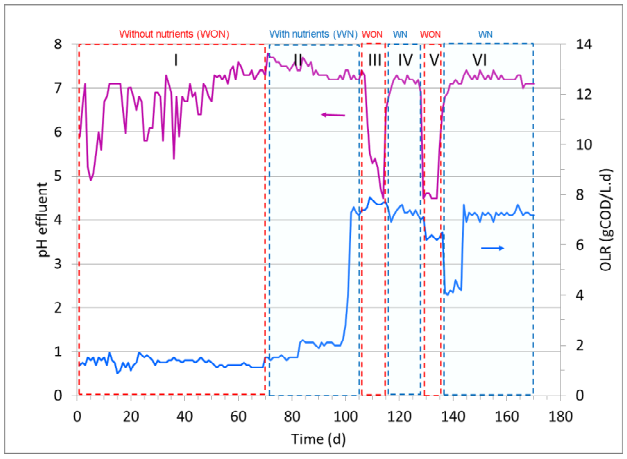

Fig. 2 Effluent pH value and Organic Loading Rate

Stage I: Startup without nutrients supplementation

The start-up period lasted 70 days. The objective of this stage was the colonization of the support media and the evaluation of reactor performance using a low load (1.4 gCOD/L.d) and without nutrients and trace elements addition. According to previous studies and the high substrate biodegradability the reactor should degrade a high proportion of organic matter present in the diluted apple juice, but the reactor only reached a COD removal near to 40% without media acidification.

Stage II: Reactor stabilization with nutrient and trace elements supplementation and organic load increments.
The addition of nutrients and trace elements to the substrate caused a fast response of the reactor performance, which allowed an increase of the organic load by 50% (day 83) and then a more drastic increase on day 100 to 7.5 gCOD/L.d without altering the reactor stability.

Stage III and V: Nutrients and trace elements suppression
These stages are characterized by a drastic fall of the COD removal and the pH value.

Stage IV: Recovery of the previous conditions with nutrients and trace elements supplementation.
Rapid response of the reactor control parameters was achieved when nutrients and trace elements were added once again.

Stage VI: Steady state operation of the IFBR.
With the addition of nutrients and trace elements to the substrate in the final stage, a steady state condition with high COD removal and a methane yield close to the theoretical was achieved.

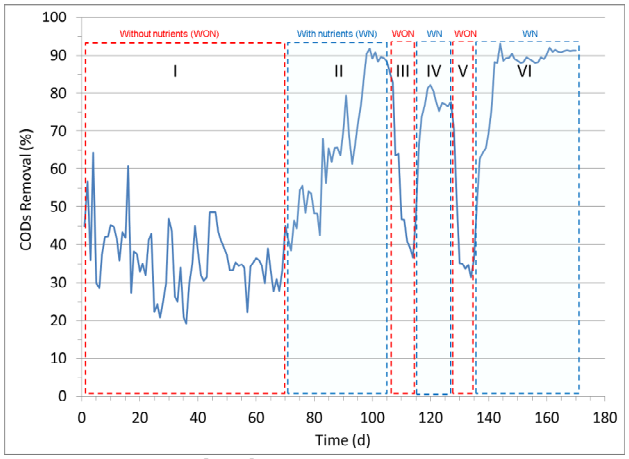

Fig. 3 COD removal in the IFBR

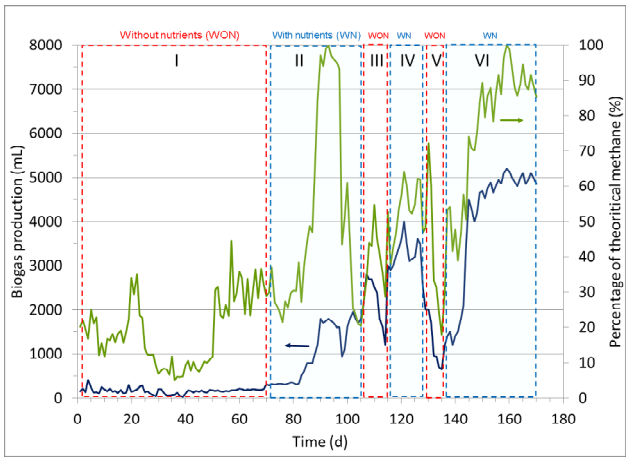

Fig. 4 Biogas and methane production during the experiment

Biofilm formation was stable during all stages as shown in Fig. 5, with a faster growth in the early stages of the experiment because most of the support material was not colonized.

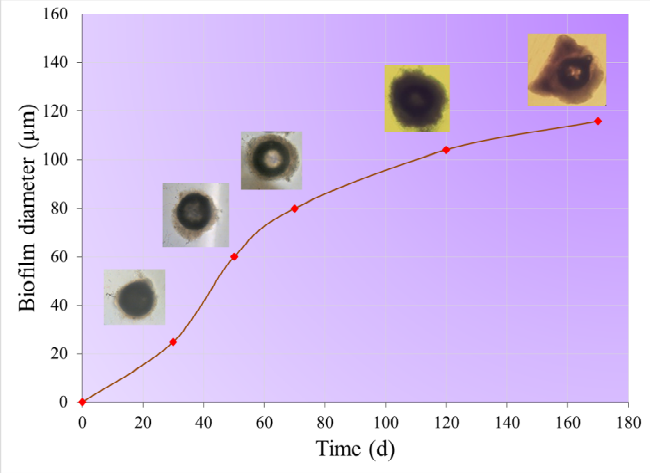

Fig. 5 Biofilm formation in the support media

DISCUSSION

Even when in the start-up stage (stage I) a fraction of precolonized support media with a methanogenic activity of 0.29 L CH_4/g CODremoved was used and also a low OLR was applied, the COD removal was only 36% in average. The substrate pH was adjusted in the influent reservoir, but once inside the reactor, no adjustment was made, resulting in a significant drop in the first 5 days but later a pH near neutrality was observed throughout the stage indicating that no excess Volatile Fatty Acids (VFA) were present.

According to previous experiences on the start-up of an IFBR, this step can be performed within 15 days while achieving high organic loads, similar to the OLR used in the reactor from which the inoculum was removed (Alvarado-Lassman et. al., 2010), however in the present experiment, the reactor did not respond favorably, since in 70 days the COD removal and the methane production were low. In Fig. 4., can be observed a 25 days period (between days 25 and 50) where the methane production was very low in comparison with the achieved COD removal, this fact can be related with the organic matter consumption by bacteria to be used in the anabolic activity of the microorganisms to build the biofilm.

At the end of the start-up stage a more stable period (days 50-70) was observed but with a very low COD removal, which means that the reactor can sustain an stable operation, but the absence of nutrients and trace elements, causes an apparent inability of the reactor to degrade all organic matter present in apple juice.

In stage II once the nutrients and trace elements were provided and also the OLR was increased twice on days 83 and 102, a sustained growth of COD removal up to values greater than 90% at the end of the stage is evident. This means that the bacteria are no longer using organic matter to form biofilm and were ready to assimilate this organic matter in the production of biogas with a methane yield close to the theoretical value. The pH remained near neutrality, which is evidence of a balance between the different bacterial groups of anaerobic digestion process.

After day 102, an increase in OLR to a value of 7.5 gCOD/L.d was implemented to test reactor stability, reaching a COD removal of nearly 90% in the subsequent days, so it was decided to remove the supply of nutrients and trace elements which resulted in a drastic decrease of pH and COD removal (stage III).

Before completely losing the stability of the reactor, nutrients and trace elements were added again (stage IV) and a very fast recovery was observed.

Nutrients and trace elements were again removed from the substrate (stage V), and consequently the values of pH and COD removal fall drastically again, so it was decided to lower the OLR to 6.4 gCOD/L.d. At this stage, is clearly seen in Fig. 4 that the absence of nutrients and trace elements, mainly affects the methanogenic bacteria with a significant decrease in biogas production. Fe and Co are generally considered as micronutrients that increase conversion of acetic acid to methane and also the hydrogen oxidizing methanogens require iron for optimum growth (Pandiyan et. al., 1999).

In stage VI nutrients and trace elements were supplied again, and in the first seven days the OLR was decreased to 4.3 gCOD/L.d to enhance reactor stabilization. The reactor performance fast recovery allowed to establish the OLR value back to 7.5 gCOD/L.d with high COD removal and biogas production close to the theoretical value.

CONCLUSIONS

The use of a substrate rich in organic matter in the form of carbohydrates but deficient in nutrients and trace elements, causes an inefficient reactor start-up in terms of COD removal and methane production, although during this period the reactor showed no acidification which can be misinterpreted as a low biodegradability of the substrate.

Once the substrate was supplemented with a solution of nutrients and trace elements, the system was able to resist fast increments of organic load, with a stable operation confirmed by high COD removal and methane yield near the theoretical. The nutrient suspension in subsequent operational periods, proves that the reactor becomes unstable and the methanogenics bacteria are the most affected among the bacterial groups, as evidence by a significant drop of the pH value and the biogas produced.

Finally, the time required to perform the start-up and stabilization of the anaerobic reactor depends strongly on the substrate and in the presence or absence of key macro and micronutrients.

ACKNOWLEDGEMENTS

The authors want to thank Andrea Alvarado for her contribution in the art design of this paper.

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