ISSN 0370-5404
NOTA TÉCNICA

Rev. Ind. y Agríc. de Tucumán
Tomo 96 (1): 55-63; 2019

Granulation of dispersed anaerobic sludges fed with lemon industry effluent

Walter Daniel Machado*, Marcos N. Russo*, César F. Molina*, Eugenio A. Quaia* y Luis A. Núñez Recio**

ABSTRACT

UASB reactors excel in the treatment of high-load effluents, combining their efficiency with the possibility of using and maintaining the granular state of the consortia of anaerobic microorganisms. However, when it is not possible to start the reactor with already formed anaerobic granules, flocculent or dispersed slurry can be used to promote their clustering in granular form. This work aimed to monitor the biodigestion process and to establish such operating conditions that would cause granulation of dispersed anaerobic slurry in a pilot scale UASB reactor fed with industrial lemon processing effluent. A dispersed sludge obtained from anaerobic lagoons of a textile industry, was inoculated in a 30 liter reactor, with a cross-section 225 cm^2 and 1.5 meters in height. This system incorporated effluent recirculation that provides the necessary upflow velocity. The substrate, a lemon processing effluent was pretreated by sedimentation. During the period studied, its organic matter concentration ranged from 9.0 to 15.0 g O_2/l of COD. The average COD removal was 75%, at upflow velocities between 0.6 and 0.9 m/h, granules of 2 mm diameter were observed after the third month. The average particle size grew from 0.36 to 0.69 mm.

Key words: UASB, granulation, dispersed sludge, citrus effluent, upward velocity.

RESUMEN

Granulación de lodos anaerobios dispersos alimentados con efluentes de la industria del limón

Los reactores UASB se destacan en el tratamiento de efluentes de alta carga, asociando su eficiencia con la posibilidad de usar y mantener el estado granular de los consorcios de microorganismos anaerobios. Sin embargo, cuando no es posible iniciar el reactor con gránulos anaeróbicos ya formados, se puede proyectar el uso de una suspensión floculenta o dispersa con la idea de promover su agrupamiento en forma de gránulos. Este trabajo tiene como objetivo monitorear el proceso de biodigestión y proponer condiciones de operación suficientes para causar la granulación de la suspensión anaeróbica dispersa en un reactor UASB a escala piloto alimentado con efluente industrial de procesamiento de limón. Se utilizó un lodo disperso obtenido de las lagunas anaerobias de una industria textil, inoculado en un reactor de 30 litros, con una sección cuadrada de 225 cm^2 y 1,5 metros de altura. Este sistema incorporó la recirculación de efluentes que permite manejar la velocidad del flujo ascendente. Como sustrato, se usó el efluente de procesamiento de limón pretratado mediante sedimentación. Sus concentraciones de materia orgánica oscilaron entre 9.0 y 15.0 g de O_2 / l de DQO, durante el período estudiado.

Trabajando con velocidades de flujo ascendente entre 0.6 y 0.9 m / h, se observaron gránulos de 2 mm de diámetro después del tercer mes. El tamaño medio de partícula creció de 0,36 a 0,69 mm.

Palabras clave: UASB, granulación, lodos dispersos, efluentes cítricos, velocidad ascendente.

Fecha de recepción: 26-02-18 - Fecha de aceptación: 8-10-18

**Sección Ingeniería y Proyectos Agroindustriales – Estación Experimental Agroindustrial Obispo Colombres.*
***Universidad de Burgos, Área de Ingeniería Química.*
Email: daniel.machado@eeaoc.org.ar

INTRODUCTION

Lemon (*Citrus lemon*) is grown both for marketing as fresh fruit and industrial processing. Industrial processing produces concentrated juices, essential oil, dehydrated peel and essences. This industrial activity entails the generation of between 2.6 and 5.0 cubic meters of effluent per ton of processed fruit, depending on a variety of operating factors or commercial requirements. In the same way, the concentration of organic matter in the raw effluent is also variable, which, expressed in terms of chemical oxygen demand (COD), is usually between 12 g/l and 16 g/l although on certain occasions it can reach 20 g/l.

Raw effluent presents a variable content of suspended solids, generally composed of pulp and peel remains. This effluent, due to its biodegradable nature and its high organic load, is adequate to be treated by means of anaerobic digestion (Navarro *et al*., 2013). In addition, anaerobic treatments are advantageous because of their relatively low capital requirements and the possibility of generating biogas that can be used as an energy source.

Some lemon processing plants use anaerobic reactors like CSTR (complete stirring tank reactor) for the treatment of their effluents. For this class of reactors a reduction of suspended solids in the influent is not required. Other industrial plants opt for UASB reactors, which in order to increase its efficiency require a reduction of suspended solids in the influent.

UASB has been used for treating various high-strength wastewaters. Their capital and operating costs make them very competitive against another biotechnological processes (Lim,S. and T. Kim, 2013).

According to Kassam *et al.* (2003) the trend to use anaerobic technology exhibited an exponential growth between 1990 and 1994, then it declined and after that remained constant.

This technology was successfully tested in wastewaters containing easily hydrolysable substrates such as those from rye, barley and wheat breweries (Blonskaja *et al*., 2003). On the other hand, the observed performance was lower when it was operated with difficult substrates to hydrolyze, like phenols, food and dairy product factory effluents animal slaughter facilities, etc. (Tiwari *et al*., 2006).

UASB reactors can partially or totally fulfill some of the unitary processes required in conventional aerobic systems, such as primary settlers, sludge digester and secondary settlers (Fernández-Polanco and Seghezzo, 2015).

One of the drawbacks of anaerobic technologies is the long start-up period if they do not start with the sludge already in granular state. Although UASB reactors can work with some efficiency using flocculated or dispersed inoculums, organic loading can be increased if granular sludge is used from the beginning of the process.

Biogranulation process involves different interactions between cells and involves biological, physical and chemical phenomena. These phenomena were observed in both aerobic and anaerobic processes (Liu and Tay, 2004).

Granular sludge formation can be considered the most important reason for the success achieved by UASB reactors in the treatment of industrial effluents, due to their higher settling velocity of about 60 m/h and their specific methanogenic activity (Hulshoff Pol *et al*., 2004).

The nature of the effluent to be treated and the reactor's hydrodynamics are among the most influential factors in the development and stability of anaerobic granular sludge. In fact, granules grown in a complex substrate are bigger in size than those grown in a simple substrate. (Lim and Kim 2018), Arcand *et al*., (1994) suggests that the upflow velocity seems to have the greatest influence on granulation process and on the granule size, by classifying particles according to their settling velocity, thus affecting growth rate and biomass activity.

Alphenaar *et al*., (1993) argued the importance of maintaining a high upflow velocity of the influent in combination with short hydraulic retention times. The inverse situation, low upflow velocities and high hydraulic retention times may contribute to keeping microorganisms in a dispersed state (Liu and Tay, 2004).

It is important to maintain a balance when selecting initial organic load, which should be as high as possible to ensure rapid granulation but not exceed an upflow velocity that would lead to washing the granules off the reactor. A simple strategy proposed by H. H. Fang and Chui, (1993) is to increase the organic loading rate (OLR) only until 80% COD degradation is achieved.

It is necessary to consider that application of high OLR during the start-up of a UASB reactor implies an increase in the biogas generation rate and an increase in the flow turbulence, which could lead to the reactor washing.

Starting

In order to start a UASB reactor it is convenient to inoculate a granulated sludge from another similar reactor. However, the availability of granulated sludge is usually limited and its acquisition and transportation can be very expensive (Liu and Tay, 2004).

As for the acidity aspects of the medium, pH range of methanogenic bacteria is quite narrow (6.7 to 7.4), while the acidogenic bacteria tolerate values around 5.0.This explains the greater susceptibility to acidification of methane-producing bacteria compared to acid-producing bacteria. Acids produced by acidogenic bacteria are buffered by the bicarbonate generated by the metabolism of methanogenic bacteria (Liu and Tay, 2004).

The objective of this work was to propose a scheme to start and operate a pilot UASB, initially loaded with flocculent sludge, so as to allow its granulation, using effluent from lemon industrial processing as a substrate.

MATERIALS AND METHODS

2.1. Laboratory analysis

Samples were taken at the points indicated in Figure 1 and referenced in Table 1.

Table 1. Sampling points and parameters analyzed

Parameters	Sampling points (See Figure 1)
COD	1 - 3 - 4
pH	1 - 3 - 4
Alpha ratio	1 - 3 - 4
Solids	1 - 3
Flow	1 - 3 - 4
Granulometry	2

Analytical techniques to determine total, fixed and volatile solids (TS, FS, VS), total suspended solids, fixed and volatile (TSS, FSS and VSS), chemical oxygen demand (COD) and pH were performed according to procedures described in Standard Methods for the Examination of Water and Wastewater (APHA, 2005).

To control problems by acidification of the system, the alpha ratio was used, which is obtained by a volumetric analysis that determines total alkalinity of the system by titrating up to pH 5.75 and pH 4.3 using a sulphuric acid solution, recording the volumes V1 and V2, respectively (Rojas, 1987).

Alpha ratio is defined as:

$$\alpha = \frac{V_2 - V_1}{V_2}$$

Analysis of count and distribution of particles or suspended granules in a sludge sample (granulometry) were performed on agar-agar plates. The technique consisted in preparing square agar-agar plates of 20 cm of side, with a suspension of 1 ml of sludge taken from the lower sampling point. After the agar got solid, the image of the plate was digitized by means of a scanner and a count and classification of granules with computer software was made (Image Tool 3.0). This software can be configured to only consider certain particle size ranges. For example, in case of sludge, this range was limited to a diameter between 0.2 mm and 4.0 mm.

2.2. Pilot reactor.

The test was carried out on the premises of a lemon processing industrial plant, with adequate capacity to provide electricity, water and air pressure necessary for the operation of the pilot plant, in addition to the influent to feed the reactor.

Pilot plant (Figure 1), which was provided by a

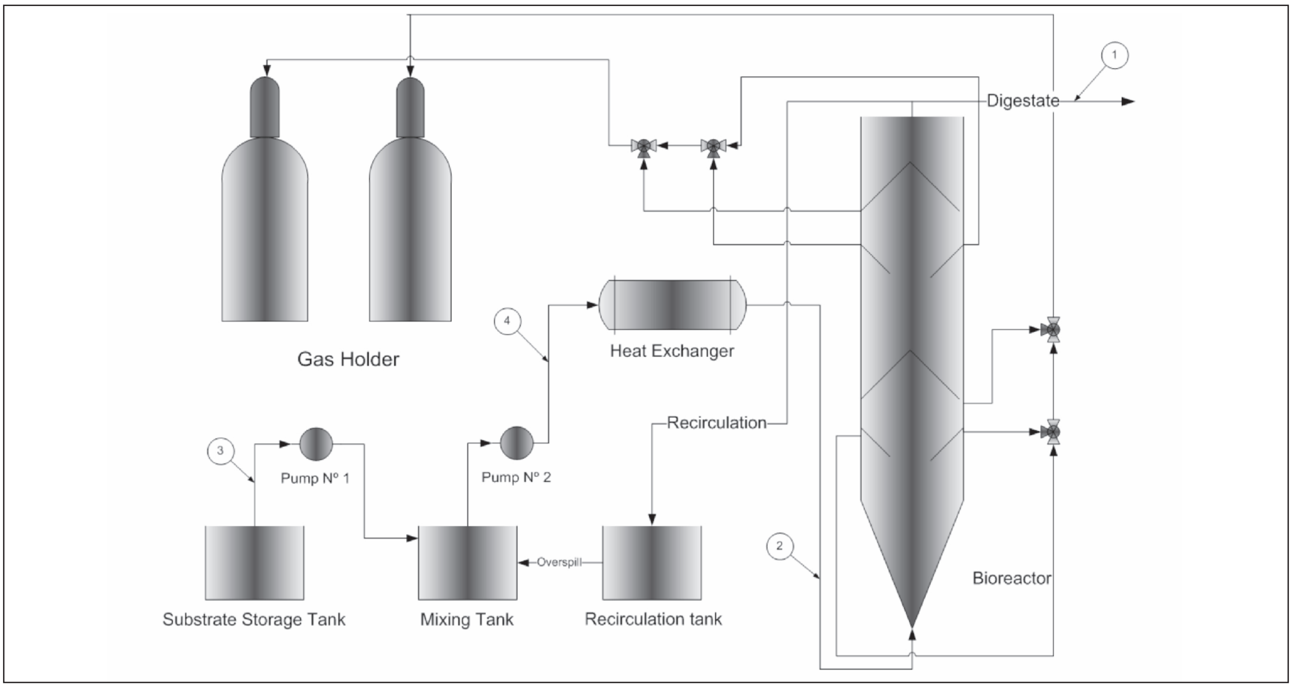

Figure 1. Scheme of pilot plant and its sampling points.

private company, consisted of a module composed of a set of devices designed to operate under different operating conditions.

A 30 liter stainless steel reactor was used. It had a square section of 225 cm^2 and 1.5 meters in height. Inside of the reactor were six gas-liquid separators, to collect and direct the generated biogas into two stainless steel cylinders (biogas manifolds), arranged vertically, which measured its volume by displacement of the liquid.

Feed and recirculation system consisted of two diaphragm pumps and three stainless steel tanks with the following functions:

a) Fresh effluent tank, or substrate storage tank, received raw industrial effluent, previously subjected to a primary treatment to reduce suspended solids.

b) Recirculation tank received a fraction of the treated effluent leaving the reactor (the other part of the treated effluent was eliminated).

c)Mixing tank, for mixing the fresh and recirculated effluent and feeding the reactor.

Temperature, measured in effluent at outlet point of reactor was, on average 25º C.

For the initial three months of the trial, the reactor was fed with 9 g/l of COD citric industrial effluent saved from the last manufacturing season that was had been stored at low temperature (4º C) in a cold chamber. When the current season began, fresh effluent was incorporated with a monthly average values of up to 15 g/l COD and pH of 4.2. In special situations, values of the factory effluent reached just over 19 g/l of COD.

Reactor input flow (Q_e), an upflow velocity (V_{up}) were established according to literature suggestions (Chernicharo de Lemos, 2007).

At this point it should be borne in mind that, although a certain upflow velocity is required as a selection pressure to favor granulation, a very high value would produce the evacuation of the microorganisms with subsequent reactor´s washing.

In order to avoid the lloss of sludge and taking into account that dispersed one was used as inoculums, started up was done at V_{up} of about 0.2 m/h, reaching, at the end of the test, a V_{up} of 0.9 m/h.

The modifications of the organic loads were made according to process stability criteria, taking alpha ratio as an indicator parameter. When the alpha ratio values were ≥ 0.5 feed was cut off and reactor contents were recirculated until this parameter was below 0.3.

Table 2. Characterization of dispersed sludge for pilot granulation test.

TS [g/l]	FS [g/l]	VS [g/l]	TSS [g/l]	FSS [g/l]	VSS [g/l]	Specific Methanogenic Activity (SMA) [gCOD/gVSS. d]
16.18	4.79	11.39	12.95	2.92	10.03	0.462

As inoculums sludge obtained from the anaerobic pond of a textile industry was used. Table 2, shows its characterization.

Reactor was inoculated with 10 liters of sludge and water to complete its volume. The concentration of biomass inside the reactor reached 3.34 gVSS/l. As it was calculated in equation 1:

$$X_f = \frac{X_i \times X_f}{V_f} \quad \text{Ec. 1}$$

X_i = initial sludge concentration [g VSS/l].

X_f = final sludge concentration in the reactor [gVSS/l].

V_i = initial volume of concentrated sludge loaded into the reactor [l].

V_f = effective volume of reactor [l].

Replacing in equation 1:

$$X_f = \frac{10.03\ \frac{g}{l} \times 10\ l}{30\ l} = 3.34\ \frac{gVSS}{l}$$

Daily flow, temperature and pH controls and periodic COD determinations of the raw, feed and recycle effluent were performed. Sludge in reactor was analyzed monthly. The operation time was 224 days, between march and october.

Maximum theoretical OLR that could support the reactor considering this concentration of microorganisms and their biological activity was calculated as forward:

$$\text{OLR} = \text{Xf} \times \text{SMA} \quad l \quad \text{Ec. 2}$$

Where:

OLR: organic loading rate [gCOD/l.d]

X_f: sludge concentration in the reactor [gVSS/l]

SMA: specific methanogenic activity [gCOD/gVSS.d)

Replacing in equation 2:

$$\text{OLR} = 3.34\text{gVSS/l} \times 0.462\ \text{gCOD/(gVSS.d)} = = 1.54\ \text{gCOD/(l.d)}$$

To calculate feed flow, we used the following expression:

$$Qa = \frac{OLR \times V}{COD} \qquad \textbf{Ec. 3}$$

Where:

$$Qa = \text{ feed flow (l/d)a}$$

Q_a = feed flow (l/d)

OLR = organic loading rate (gCOD/l.d)

COD = organic matter concentration of influent to be treated (gCOD/l)

V = effective volume of the reactor (l)

Replacing in equation 3:

$$Qa = 1.54\frac{gCOD}{l.d} \times \frac{30\ l}{10\ \frac{gCOD}{l}} = 4.62\frac{l}{d}$$

$$Qa = 4.6\frac{l}{d} \times \frac{1\ d}{24\ h} = 0.19\frac{l}{h}$$

Feed flow (Q_a) represented the maximum flow rate of influent to be treated. In occasion, due to operating circumstances, a lower value could be used.

The reactor inlet flow was calculated as follows:

$$Qe = V up \times A \qquad \textbf{Ec. 4}$$

Where:

Q_e = reactor inflow rate (m^3/d)

V_{up} = upflow velocity (m/h)

A = reactor area (m^2)

Replacing in equation 4:

$$Qe = 0.2\frac{m}{h} \times 0.0225\ m^2$$

$$Qe = 0.0045\frac{m^3}{h} Qe = 0.0045\frac{m^3}{h} \times 1000\frac{l}{m^3} = 4.5\frac{l}{h}$$

Figure 2 shows the calculated flows and its directions.

Qa: feed flow.

Qe: inflow to the UASB reactor.

Qte: treated effluent flow rate or outflow = Qa.

RESULTS AND DISCUSSIONS

Table 3 shows the monthly averages of the operating variables recorded during the granulation process in the pilot reactor.

Figure 3 shows daily measurements of pH in feeding and effluent currents of the reactor, they give an idea of the process stability. Variations observed on pH values in feed current are due, mainly, to the lack of homogeneity in the aggregate of lime during primary treatment of the industrial effluent.

Feed flows were handled according to the influent COD values. Figure 4 shows that the hydraulic retention time (HRT) decreased in the first 50 days, from a value of 35 days to values around 2 days, but there were also variations of HRT due to fluctuations in the pump flow rate. The high dispersion observed in organic loading rate (OLR) values (figure 4), are attributed both to the difficulties in maintaining a constant flow rate and to the COD variations in the influent.

Figure 5, shows the evolution of COD values on

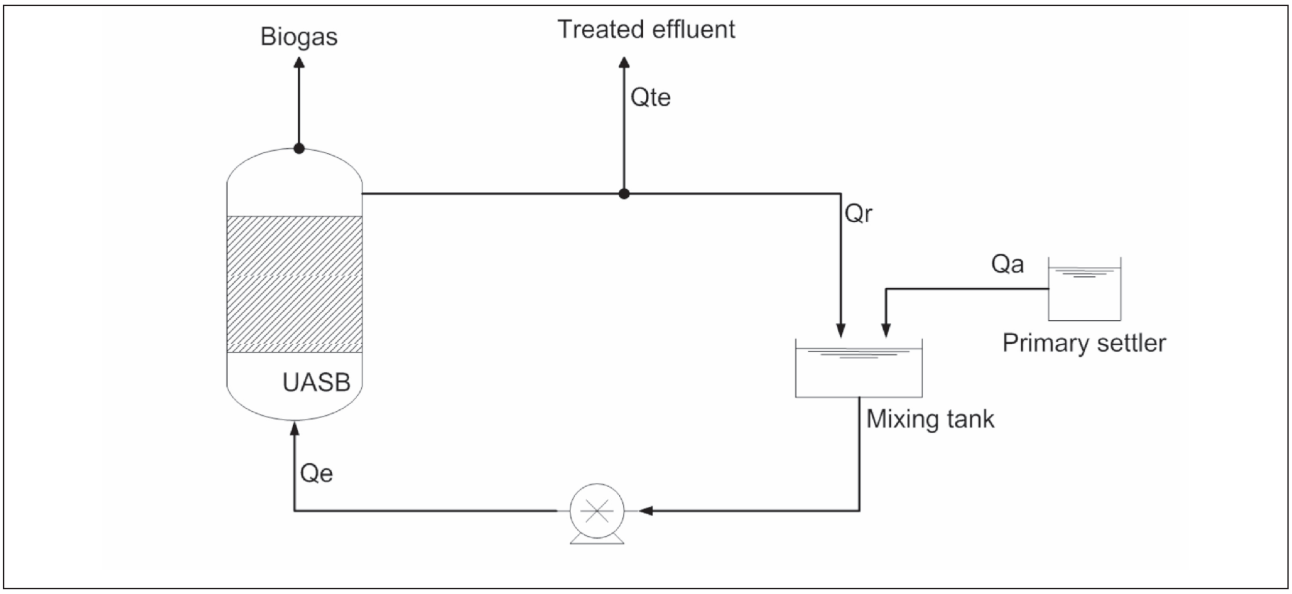

Figure 2. Reactor flow diagram

Table 3. Operational variables of the pilot reactor during the test.

	COD feeding [mg/l]	OLR Aver. [gCOD/l.d]	T [ºC] recycle	pH recycle	Qa Aver. [ml/h]	As. Aver. [m/h]
Start	9040	0.56	27.60	7.60	78.00	0.20
Month 1	9640	1.85	26.40	7.60	240.00	0.67
Month 2	9420	3.15	25.90	7.40	417.60	0.77
Month 3	14470	4.20	24.60	7.24	362.40	0.79
Month 4	13535	4.85	24.10	7.11	447.60	0.79
Month 5	11928	2.83	24.29	7.56	296.40	0.84
Month 6	12986	7.47	24.69	7.42	718.80	0.86
Month 7	13188	7.45	24.70	7.42	720.10	0.92
Month 8	14754	1.97	25.40	7.95	380.00	0.88

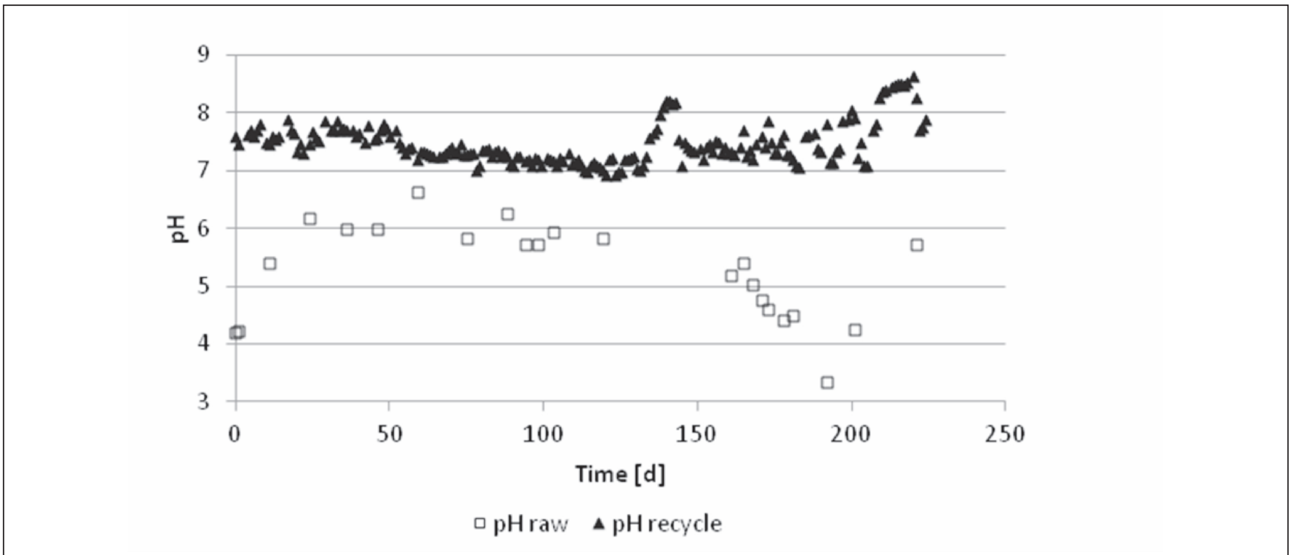

Figure 3. Evolution of the inlet (raw) and outlet (recycle) pH.

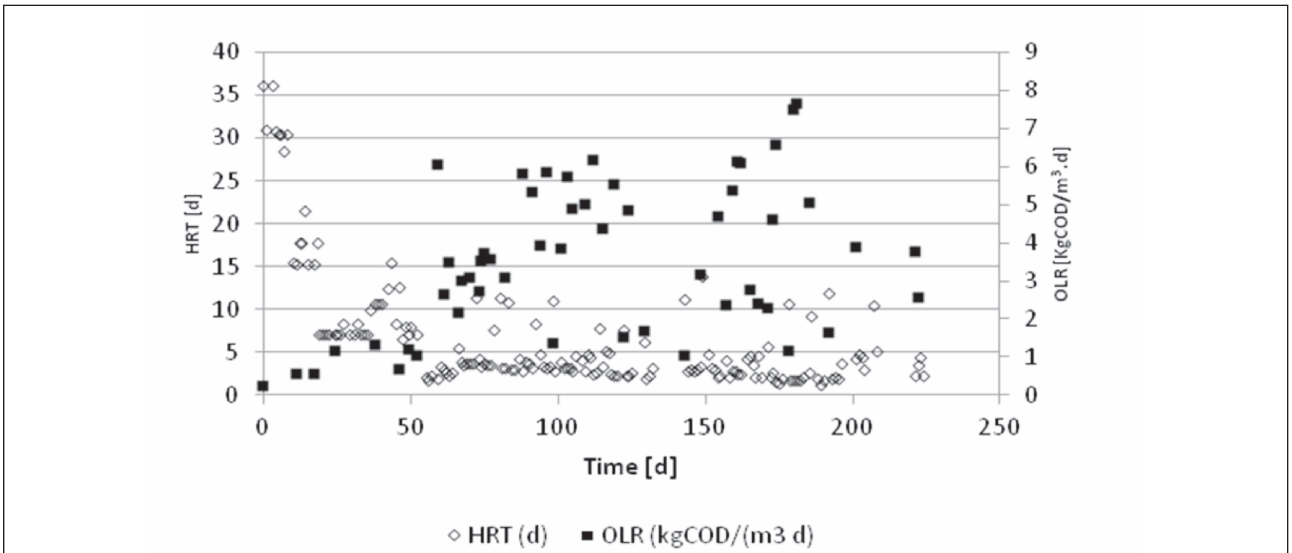

Figure 4. Evolution of HRT and OLR.

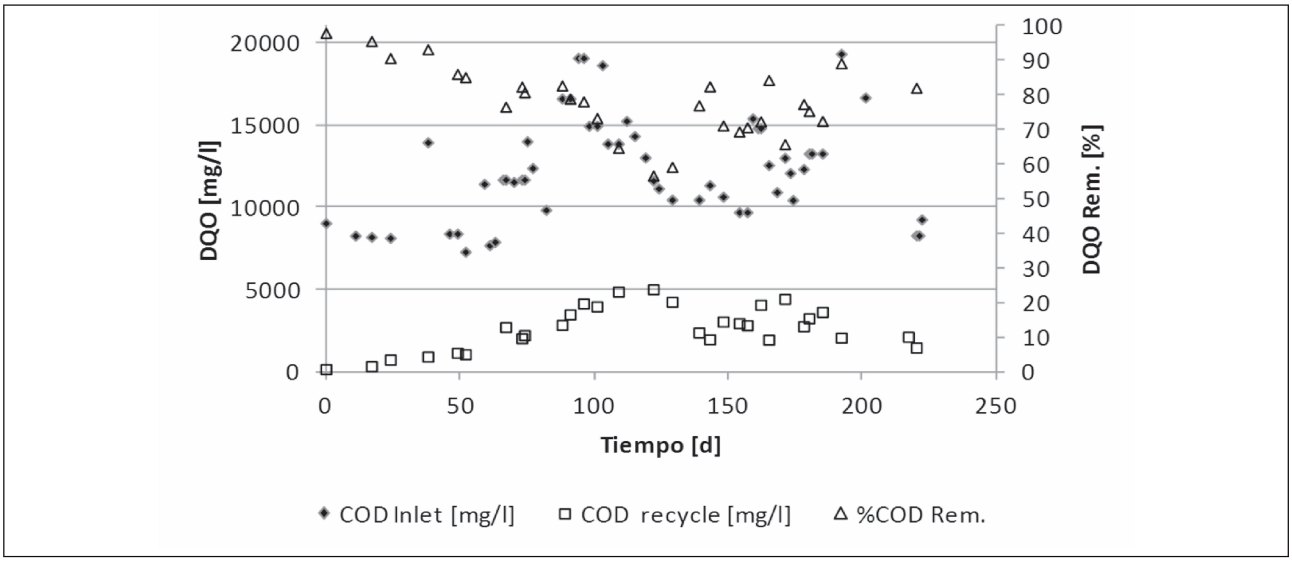

Figure 5. Inlet and outlet (recycle) COD evolution on time and removal percentage.

feed and outlet currents of the reactor, associated with the removal percentage COD. Removal Average was around 75%, lower than the 90% obtained in experiments carried out by Russo *et al*. (2010).That difference is attributed to the type of sludge (dispersed sludge against granular sludge). However, similar OLR could be reached without other critical problems. This situation is also reflected in the graph in Figure 6.

Figure 7 shows in chronological order, the sequence of granulation progress in sludge into the reactor, from the firth month until the sixth.

Figure 8 shows the average particle size evolution inside the reactor through the time.

Tiwari *et al*., (2006) mentioned, in a review, that the OLR and Vup among others were determinant parameters to obtaining granules. There is a shortage of reported works that used industrial effluents, particularly effluents from the lemon processing, to reach granular sludge. That makes it difficult to compare the methodology used in the present study with other modalities. Instead of that, several works of granulations that used flocculent sludge and synthetic substrates were found. One of them, in which a solution made of glucose and nutrients was used as a feed, reported appearance of granules two months after the reactor's start up, working at temperatures close to 30ºC, OLR between 2 and 8 kg COD/m^{3}d and upflow

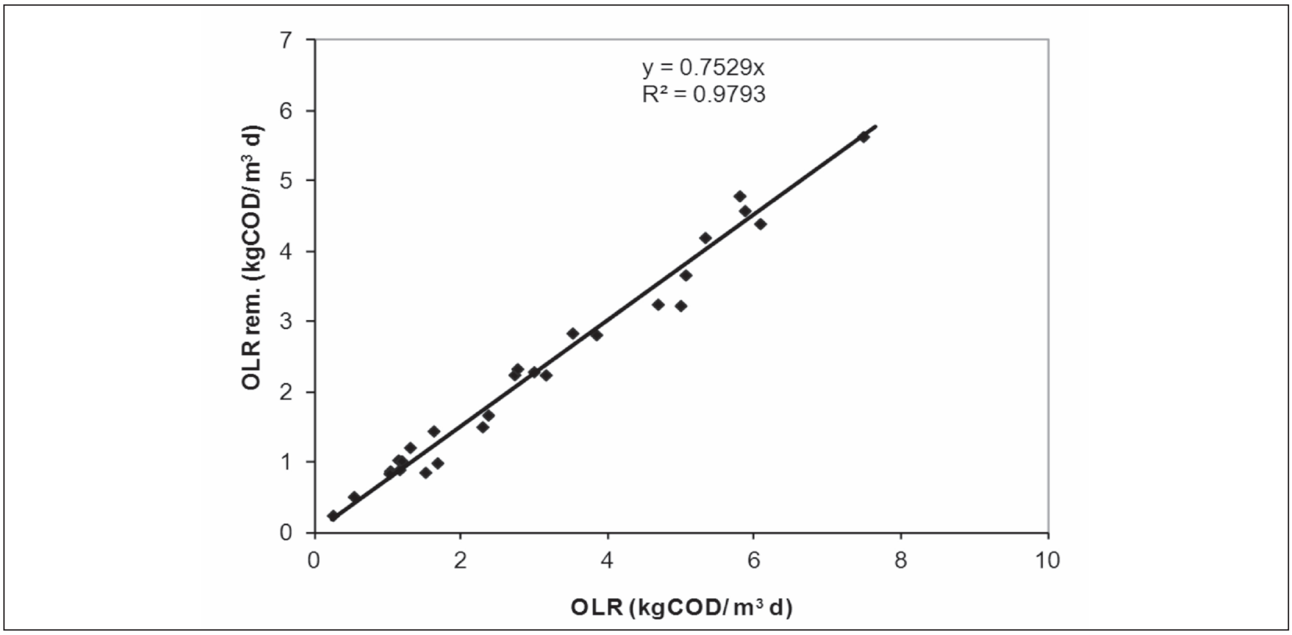

Figure 6. Relation between inlet OLR and removed OLR.

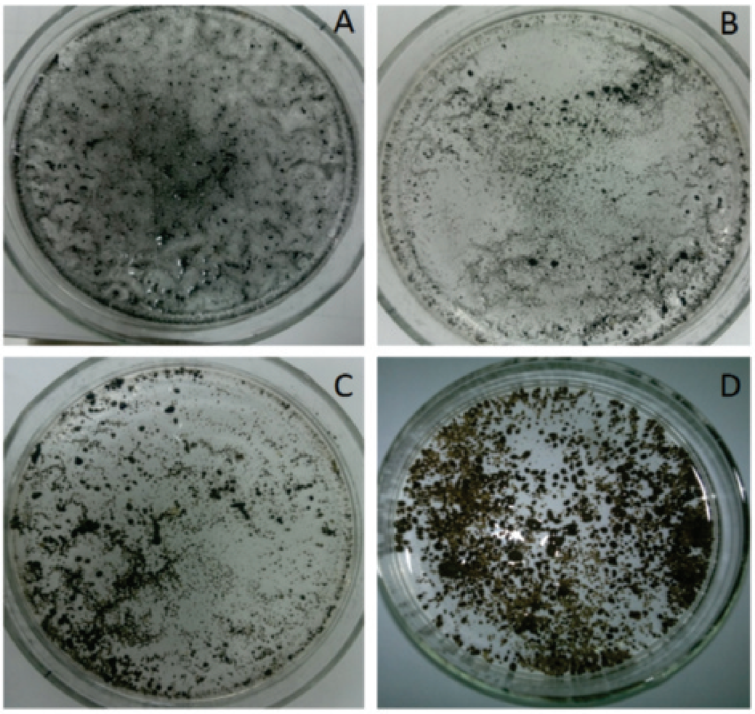

Figure 7. Photographs of the reactor sludge corresponding to the first month (A), second month (B), third month (C) and sixth month (D).

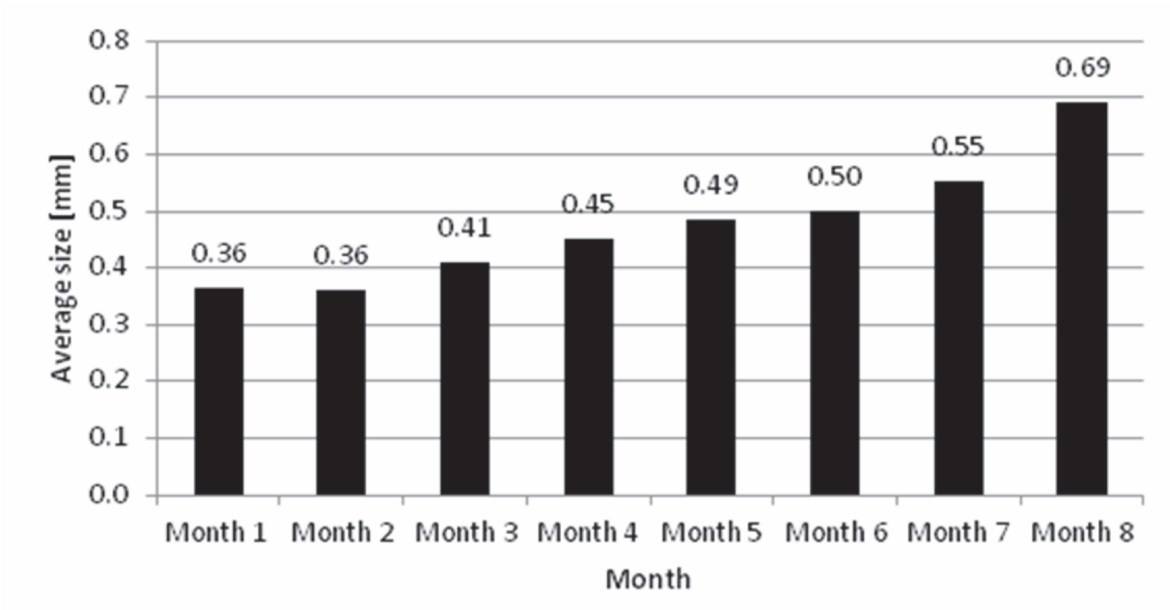

Figure 8. Average particle size evolution in time.

velocities above 0.2 m/h (Francese, 1993). Ghangrekar *et al.*, (2005), reported effective granulation at 80-90 days, using glucose and nutrients as a substrate, although working at low upflow velocities, between 0.05 and 0.125 m/h, organic loads varying between 1.43 and 9.50 kg COD/m^3d and temperatures between 24 and 32°C.

CONCLUSIONS

In the present work, after three months it was possible to confirm the existence of true granules of about 2 mm in diameter. The average size of the particles was 0.41 mm and its growth continued until the end of the test.

The solids in suspension inside the reactor, both from the dispersed sludge used for reactor start-up and from the feed influent, could have acted as growth nuclei for the development of the granules, according to the pressure selection theory.

Organic matter removal was lower than that obtained in other tests initiated with granular sludge. This is attributed precisely to the type of flocculent sludge with which the process was initiated. However, organics loads similar to the reached by granular sludge were achieved without critical problems.

The proposed start-up and operation of the reactor inoclutaed with flocculent sludge of course, took longer times and greater development of the granules was required to achieve higher organic loading rates.

It is emphasized that the initial concentration of suspended solids was not as high as some authors suggest for start-up.

The proposed starting and operating scheme was at least sufficient to reach the formation of defined anaerobic granules and to promote their growth. This result may be relevant in regions where the anaerobic treatment of the lemon processing effluents is carried out and where there are few possibilities of having industrial volumes of granular sludge.

BIBLIOGRAPHY CITED

Alphenaar, P.A., Visser, A., Lettinga, Gatze, 1993. The Effect Of Liquid Upward Velocity And Hydraulic Retention Time On Granulation in UASB Reactors Treatring Wastwater With High Sulphate Content. Bioresource Technology 43, 249–258.

Arcand, Y., Guiot, S.R., Desrochers, M., Chavarie, C., 1994. Impact of the reactor hydrodynamics and organic loading on the size and activity of anaerobic granules. The Chemical Engineering Journal 56, B23–B35.

Blonskaja, V., Menert, A., Vilu, R., 2003. Use of two-stage anaerobic treatment for distillery waste. Advances in Environmental Research 7, 671–678.

Chernicharo de Lemos, C.A., 2007. BiologicalWastewater Treatment Series: Anaerobic Reactors. IWA.

Fang, H.H., Chui, H.K., 1993. Maximun COD Loading Capacity In UASB Reactors At 37oC. Journal of Environmental Engineering 119, 103–119.

Fernández-Polanco, F., Seghezzo, L., 2015. Diseño De Reactores Upflow Anaerobic Sludge Blanket (UASB).

Francese, A., 1993. Puesta En Marcha De Reactores Anaerobicos De Manto De Barros Y Flujo Ascendente. Universidad Nacional de San Luis.

Ghangrekar, M.M., Asolekar, S.R., Joshi, S.G., 2005. Characteristics of sludge developed under different loading conditions during UASB reactor start-up and granulation. Water research 39, 1123–33.

Hulshoff Pol, L.W., De Castro Lopes, S.I., Lettinga, G, Lens, P.N.L., 2004. Anaerobic sludge granulation. Water research 38, 1376–89.

Kassam, Z.A., Yerushalmi, L., Guiot, Serge R, 2003. A Market Study On The Anaerobic Wastwater Treatment Systems. Water, Air, and Soil Pollution 143, 179–192.

Liu, Y., Tay, J., 2004. State of the art of biogranulation technology for wastewater treatment. Biottechnology Advances 22, 533–563. doi:10.1016/j.biotechadv.2004.05.001

Navarro, A.R., Lopez, Z., Salguero, J., Maldonado, M.C., 2013. Anaerobic treatment of residual lemon pulp in digesters with semi-continuous feed. water Science & Technoiogy | 67, 514–520.

Tiwari, M.K., Guha, S., Harendranath, C.S., Tripathi, S., 2006. Influence of extrinsic factors on granulation in UASB reactor. Applied microbiology and biotechnology 71, 145–54.