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Treatment of waste activated sludge by steam explosion and alkaline acidogenesis

Tratamiento de lodos activados de purga por explosión de vapor y acidogénesis alcalina

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Abstract

A process improvement of the anaerobic digestion of waste activated sludge (WAS) is needed to produce energy and chemicals to compensate the costs of the full wastewater treatment process. Alkaline steam explosion (A-SE) @160°C and 0.67 MPa absolute pressure, followed by thermophilic alkaline acidogenesis (50°C, pH 9) improves the WAS digestibility and the methane yields by breaking the cell walls and the extracellular polymers. This work studies the treatment time and alkalinity effects on the number of destroyed solids measured as cell damage and particle size reduction. To monitor the process a flow cytometer (FCM), through light scattering signals, proved to be an alternative to the measurement of total suspended solids by gravimetry.

The effect of alkaline acidogenesis on the volatile fatty acids (VFA) yield is studied with the A-SE suspension which is cooled down to 50°C and fed to a continuous acidogenic reactor at pH 9 under several organic loads to find the optimum (6 g COD_{VSS}/L·d) with the highest soluble COD and VFA production rates (1.4 and 0.8 g COD/L,d respectively). This effluent can be fed to a methanogenic reactor to produce 0.5 L_{CH4}/L·d or the VFA can be separated for the chemical industry.

Keywords: Anaerobic digestion, flow cytometry, scanning electronic microscopy, particle size reduction, cell wall.

Resumen

Es necesario mejorar la digestión anaerobia de los lodos activados de purga (LAP) para producir energía y productos químicos y así compensar los costos del proceso de tratamiento de aguas residuales. La explosión por vapor alcalina (A-SE) a 160°C y 0.67 MPa absoluta, seguida de acidogénesis alcalina termofílica (50 ° C, pH 9) mejora la digestibilidad de los LAP y los rendimientos de metano al romper la pared celular y los polímeros extracelulares. Este trabajo estudia los efectos del tiempo de tratamiento y la alcalinidad en la cantidad de sólidos destruidos, medidos como daño celular y reducción del tamaño de las partículas. Para monitorear el proceso, un citómetro de flujo (FCM) mediante de señales de dispersión de luz demostró ser una alternativa a la determinación de los sólidos suspendidos totales por gravimetría.

El efecto de la acidogénesis alcalina sobre el rendimiento de ácidos grasos volátiles (AGV) se estudia con material A-SE que se enfría a 50°C y se alimenta a un reactor acidogénico continuo a pH 9 bajo varias cargas orgánicas para encontrar el óptimo (6 g DQOVSS/L·d) con las mayores tasas de producción de DQO soluble y VFA (1.4 y 0.8 g DQO/L·d respectivamente). Este efluente se puede alimentar a un reactor metanogénico para producir 0.5 L_{CH4}/L·d o se pueden separar los AGV para la industria química.

Palabras clave: Digestión anaerobia, citometría de flujo, microscopía electrónica de barrido, reducción del tamaño de partículas, pared celular.

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1 Introduction

Waste activated sludge (WAS) is the byproduct of the most used wastewater treatment process worldwide. It is formed by floccules of aggregated microbial cells bound by extracellular polymeric substances (EPS) which constitute 80% of the floc. It is produced at a yield of 0.6 g of volatile suspended solids/g COD removed and is usually stabilized by anaerobic digestion to produce methane at a yield of 450 L/kg digested VS with 65% volatile solids (VS) removal at 30 day of solids retention time with loading rates (OLR) around 1.45 kg/VSS m^3· d (Appels *et al*., 2008). This low digestion rates are due to the flocs' components, being the EPS as digestible as the cell nuclei (46 to 44%) but more biodigestible than cell membranes (34%) and cell walls (27%) as determined by Xiao *et al*. (2015) (9<pH<12) suggesting that when the floc surfaces are exposed at alkaline conditions get negatively charged creating a strong electrostatic repulsion which releases the EPS, disrupting the sludge floc, saponifying and solubilizing the structural lipids of the cell wall and membrane.

WAS pretreatment by steam explosion (SE) destroys the EPS and the microbial cells' wall and membrane releasing proteins, polysaccharides and other soluble substances. This process: a) improves methane yields (17 to 80%) due to augmented digestibility (up to 35%) of dissolved sludge solids (Carrere *et al*., 2016), b) increases AD rates and thus the solids load to the reactor above5 kgVS/ m^3· d at HRT less than 15 d (Barr *et al*. 2008), d) improve the dewaterability of the residual digested sludge, from 20% to 30-40% (Barber, 2016). In order to be energy efficient, it has to be fed with at least 50 g TSS/L as experienced in the field (Cano *et al*., 2015).

Thus, the combination of SE and alkali (A-SE) adds up to break the EPS and the cell wall fibers. Sani-Shehu *et al*. (2012) found an optimal set of conditions at 88.5°C for, 21 min at pH 12 to obtain a 36% increase in biogas yield while Neyens *et al*. (2003) worked at 100°C for 60 min T pH 10 obtaining a dewatered cake of 46% solids.

Another important concept to improve WAS digestibility is an alkaline acidogenesis to increase the production of volatile fatty acids (VFA). At pH 10 and 8 days reaction time, the VFA yield is 256.2 mg CODVFA/mg VSS (with 50% acetic acid), over 4 times that obtained under uncontrolled pH (Yuan *et al*., 2006). The use of lime also precipitates carbonate and phosphate (Xiao *et al*. 2019) but the alkaline conditions can be detrimental to methanogenesis, mainly the acetoclastic reaction which is an advantage when the objective is to produce VFA (Wang *et al*., 2017).

The thermophilic alkaline acidogenesis can take place in an upflow anaerobic sludge blanket (UASB) reactor where the suspended solids are retained in the lower part, within the sludge blanket (Terreros *et al*., 2009), while the effluent has low suspended solids and high VFA. The physical separation of acidogenesis and methanogenesis in two reactors contributes to the stability of anaerobic digestion (AD) because the high overloads given by the hydrolysed solids are buffered in the alkaline acidogenic UASB reactor accumulating high concentrations of VFA anions to be consumed in the second stage, to produce methane (Vigueras *et al*., 2011) at neutral pH. The hydraulics of this reactor can be studied for scaling up purposes using dimensional relantionships (Monroy. *et al*., 2020).

Flow cytometry (FCM) has been used to study damage to cell structure during pretreatments of WAS because it allows a discriminating cell counting (> 1000 cells/sec) to differenciate characteristics like size by light scattering and physiologic state by fluorescence of the stained DNA. It has been used to determine cell integrity and activity, quantifying intact, permeabilized, organic debris or dead cells (Prorot *et al*., 2008 and Foladori *et al*., 2010). A small light deviation of 0.5 to 5° with respect to the laser axis, known as the forward scatter (FSC), is produced by the cell membrane which reflects a cone, an indicator of the cell size (Macey, 2010). Fluorescence, emited by fluorochromes, indentifies between intact and damaged cells; SYBR Green I penetrates all the cells while propidium iodide (PI:$C_{27}H_{34}I_2N_4$) penetrates only wall damaged cells complexing with the DNA. So in these damaged cells, penetrated by both fluorochromes in an energy transfer phenomena, the emission spectra of SYBR Green I is absorbed and invisibilized by the PI spectra (Ziglio *et al*., 2002). Pang *et al*. (2014), measured the effect of alkaline acidogenesis on cell integrity and found that soluble organic matter (OMS) comes only from the flocs disaggregation (breakage of EPS) during acidogenesis at uncontrolled pH while under alkaline conditions there is also a disruption of the cell wall thus adding to the production of OMS.

The aim of this study was to combine alkaline and SE pretreatments (A-SE) with alkaline acidogenesis to improve, above these processes separated, the degradation of WAS. It was done in a batch SE

reactor at pH 9 and 160°C, followed by a continuous thermophilic (at 50°C) alkaline acidogenesis UASB type reactor to continue the cell destruction and obtain high VFA concentrations. To characterize the A-SE pretreatment, the destruction of TSS, which lumps the microbial cells, was assessed by cell damage and average particle diameter. The alkaline acidogenesis was characterized by the rates of VFA production and the of TSS destruction.

2 Material and methods

The effect of time and alkali on the SE of WAS on the TSS reduction was studied in seven batches. The resulting sludges from each treatment (Three reaction at neutral and alkaline conditions) were analysed for TSS by the gravimetric method and particle size distribution (PSD) and cell integrity in a FCM (by light scattering and stained DNA fluorescence). Then the VFA conversion of the pretreated WAS in a continuous acidogenic UASB reactor at alkaline pH was evaluated.

2.1 Waste activated sludge (WAS)

Samples were obtained from the activated sludge return line (connecting the clarifier to the aeration tank) of the Cerro de la Estrella wastewater treatment plant at Iztapalapa, Mexico City. To get rid of excess water they were let to settle on-site and kept at 4°C for 24 h to concentrate to 33% of their original volume (Ts). Finally, they were centrifuged at 15.3 G for 5 min (Cs) The process produced the sludge which was used in the experiments. All samples were characterized (table 1) according to Rice *et al*. (2012).

2.2 Steam explosion pretreatment (SE)

Batches of 4 kg of centrifuged WAS kept in a stainless-steel basket and 0.5 L of water were exploded in a 4 L reactor heated with saturated vapor (160°C, 0.67 MPa absolute pressure) through an external jacket (García-A. *et al*., 2018). The working pressure was reached in 10 min and the depressurization was instantaneous once the relief valve was opened (figure S1). Retention times of 5, 15 and 20 min under neutral (SE) and alkaline (A-SE, 4.25 g $Ca(OH)_2$/kg WAS) conditions were performed. Exploded WAS samples of 100 mL were analyzed.

2.3 A-SE pretreatment assessment

2.3.1 By gravimetry

Standard methods (Rice *et al*., 2012) were used for the characterization of initial and remaining total suspended solids (TSSi and TSSf) and the solids destruction efficiency was calculated: $\eta_{SST} = 1 - \frac{TSS_f}{TSS_i}$.

2.3.2 By flow cytometry (FCM)

a. Suspended solids size reduction was estimated through particle size distribution (PSD) before and after the pretreatments. To release the maximum number of free cells WAS suspensions were disaggregated with a vortex mixer for several agitations of 1 min at 2000 rpm until no visible flocs were noticed. The resulting suspension was diluted with a phosphate buffer solution (3 g K_2HPO_4, 1 g KH_2PO_4 and 8.5 g NaCl per L, pH=7.2) and filtered through 20 μm membranes to eliminate coarse particles (Foladori *et al*., 2010) and collect the free cells filtrate to inject samples into the FCM to measure the forward scatter (FSC) and to prepare them for the scanning electron microscope.

Table 1. WAS concentration process.

Parameter	Unit	WAS	Ts	Cs
TSS	g/L	6.6 ± 0.8	18.1 ± 4.8	62.1 ± 4.8
VSS		4.7 ± 0.7	12.5 ± 2.7	48.1 ± 5.3
COD		8.8 ± 0.7	18 ± 3.2	76.3 ± 2.2
COD/VSS	-	1.87	1.44	1.58
VSS/TSS		71%	69%	77%
pH	-		7.1 ± 0.3	

Ts: thickened WAS, Cs: centrifuged WAS, n (number of samples) = 7

b. Cell integrity was measured by fluorescence emitted by fluorochromes inside the WAS cells in 1 mL samples of the free cells filtrate with 10 μL of IP (1 mg/mL, Thermo Fisher Scientific, Invitrogen, USA) and SYBR Green I (diluted 1:30 with dimethyl sulfoxide; DMSO, Merck, Germany) and incubated in darkness at 25°C for 15 min. The emitted red fluorescence of IP (λ_{ex} = 536 nm, λ_{em} = 617 nm) indicates damaged and dead cells, while the green fluorescence emitted by SYBR-I (λ_{ex} = 495 nm, λ_{em} = 525 nm) indicates undamaged cells. Both signals are registered, amplified and analyzed with a FACScalibur flow cytometer equipped with an Ar laser (488 nm). Thus, quadrant I (Q_I) corresponds to cells positive to both fluorescences (D + U, damaged and undamaged cells), in which small aggregates or damaged cells with incorporated IP were observed (Ziglio *et al.*, 2002), in Q_{II} red fluorescence was identified, which corresponds to damaged cells (D), Q_{III} was negative to both fluorescences and were considered unidentified pretreatment products or particles (P) and in Q_{IV} corresponding to undamaged cells (U), green fluorescence was identified.

Each analysis used 20,000 cells, data were acquired and processed with the BDCellQuest and Flowing Software 2.5.1 (http://flowingsoftware.btk.fi/).

Destruction efficiency was determined as follows:

$$\eta_P(particle) = [P/(P+D+U)];$$

$$\eta_D(damaged\ cells) = [D/(P+D+U)];$$

$$\eta_{P+D} = (\eta_P + \eta_D)$$

2.3.3 *Scanning electron microscopy of the steam exploded WAS*

Fresh and pretreated WAS were observed through a digital scanning electron microscope (SEM) model DSM 940 (Zeiss, Germany) at 200X amplification. Duplicate samples were: chemically fixed with 3.5% glutaraldehyde for 24 h at 4°C, washed three times with a phosphate buffer, post fixed with 1% OsO_4 for 2 h, dehydrated with an ethanol gradient in 10% increments in 10 min intervals up to 100%, dried with supercritical carbonic gas as transitional fluid and finally, mounted and Au metallized (Taheri *et al.*, 2012). Considering the field length (450 μm) of the obtained micrographs, the particle size distribution (PSD) was obtained with the help of the FCM's FSC.

2.4 *Alkaline acidogenesis*

Volumes of 750 mL of the selected A-SE WAS (pH 9), cooled to 50°C were fed to two 1 L batch reactors for the preliminary selection of reaction time and the effect of an inoculum at a ratio of food to inoculum (S_0/X_0) = 4 g COD/g VSS. Anaerobic sludge (20 g VSS/L) from the UASB reactor treating the University campus wastewater was used as inoculum.

Then, after having selected a reaction time a continuous reactor (in a 7 L glass container, figure S2) was started with the batch conditions selected, until the reaction time was reached and then operated at organic loading rates (B_v) from 3 to 7 g COD/L·d.

3 Results and discussion

3.1 *Gravimetry assessment of the A-SE tests*

The WAS treated by A-SE had a better particle destruction (53%) than the treated in N-SE in 15 min (figure 1). The former followed a first order hydrolysis rate (equations 1), meaning that the decreases linearly with time or that the remaining TSS diminishes exponentially with time. On the other hand, the neutral treatment adjusted better to second order kinetics (equation 2) implying that as the reaction proceeds the rate decreases faster until the concentration becomes asymptote to the time axis (changes little with time).

$$r_A = k_A S, R^2 = 0.998 \quad (1)$$

$$r_N = k_N S^2, R^2 = 0.996 \quad (2)$$

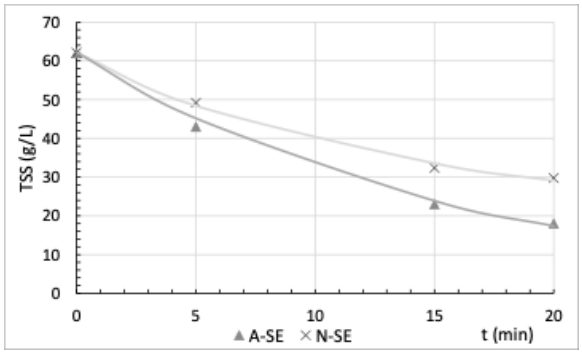

Figure 1. Time and alkali effect on the TSS of centrifuged and steam exploded WAS (N-SE = neutral, A-SE= alkaline) $r_A = k_A S$, $r_N = k_N S^2$.

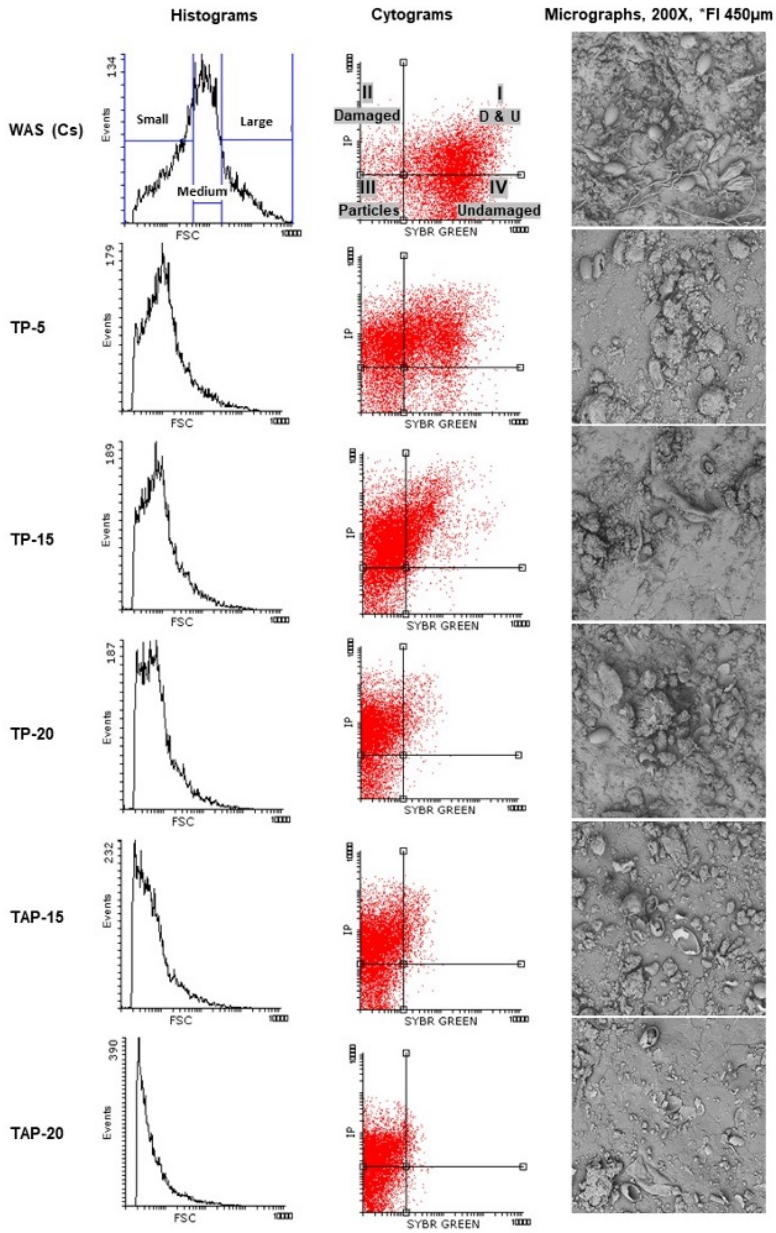

Figure 2. Histograms giving the number of events as a function of the forward scatter, cytograms relating the green and red fluorescences and micrographs *Fl = field length, from the WAS pretreatments.

where r = rate of TSS destruction (g/L·min), S is TSS, k are the specific rate constants: k_A = 0.0637 [min^{-1}] 1st order for A-SE, and k_N = 0.00091 [L g^{-1} min^{-1}] 2nd order for the N-SE.

3.2 FCM assessment of the cells' destruction by A-SE

The samples of the centrifuged WAS (Cs) and those obtained at 5, 15 and 20 minutes, with and without alkali were analyzed for particle size distribution (PSD), as measured with the FSC, the distribution of damaged (D), undamaged (U) cells as measured by FCM fluorescence and the micrographs of the free cell suspensions (figure 2).

Histograms in the 1st column show that the mean PSD moves to the left with increasing treatment time due to particle breakdown. The micrographs (3rd column) show at bare eye the abundance of large particle and the reduction in size and number with time and alkali. To relate the FSC to the particle size an image analysis of the particle micrographs helped to classify them in small (S = 23±13 μm), medium (M = 70±14 μm) and large (L= 98±10 μm) sizes and determine the PSD for each treatment.

Cytograms (2nd column) show the fluorescence signals which assessed the distribution of damaged and undamaged cells (in quadrants (Q) II and IV respectively) together with no DNA debris caused by the SE pretreatments (Q_I). It shows the abundance of U cells in the untreated WAS (WAS Cs). After 5 minutes N-SE (denoted in the figure as TP-5) they have been greatly reduced and almost disappeared in the rest of the treatments, particularly the A-SE treatments (TAP 15 and TAP 20, data of TAP-5 is missing).

With the double staining analysis, it was determined that in the centrifuged WAS (Cs) 44% of the particles are intact (Q_{IV}) and 41% are small aggregates (Q_I), while only 8% are damaged cells (Q_{II}) and 7% are no DNA particles (Q_{III}). At 5 minutes of treatment, the undamaged cells (Q_{IV}) are only 5% and 9% of the total cells until 15 min when there were none but 100% of the cells were damaged (Q_{II}) with small debris associated (Q_{III}). The neutral treatment took 5 more minutes to reach the same results (figure 3). This shows that cell viability is not useful to track the process as they permeabilize faster that the size reduction.

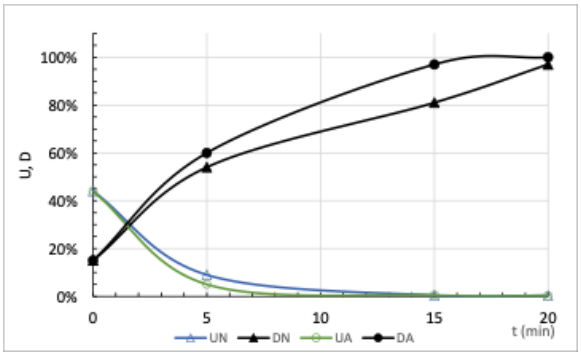

Figure 3. Cell integrity in WAS after SE pretreatments. U = undamaged particles (IV Q), D = damaged and other particles (II+III Q).

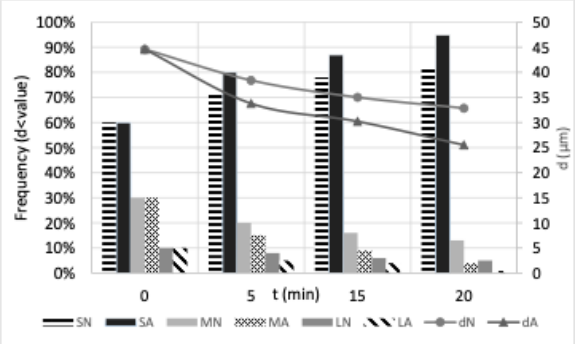

Figure 4. Evolution of the particle size distribution (PSD) after each treatment. S =23±13 μm, M =70±14 μm, L=98±10 μm, d =average diameter, A =A-SE, N=N-SE.

Figure 4 shows how the PSD is displaced in 20 min from 60% small particles (S) to 95% with the A-SE and to 80% with the N-SE treatment, with the concomitant reduction of medium (M) and large (L) sized particles. The weighted average size is also plotted to show the influence of time and alkali on the steam explosion treatments and they follow a first order average diameter (d = μm) reduction with equations 3 and 4.

$$r_{TA} = [0.028 min^{-1}]d \quad (3)$$

$$r_T = [0.016 min^{-1}]d \quad (4)$$

The correlations of the removal efficiencies η_{TSS} with the size reduction and damaged cell efficiencies (η_d and η_D) considering both treatments, with and without alkali (figure 5), obtained from figures 3 and 4, mean that there is a potential use of FCM for the on-line evaluation of the steam explosion treatment instead of the traditional gravimetric method.

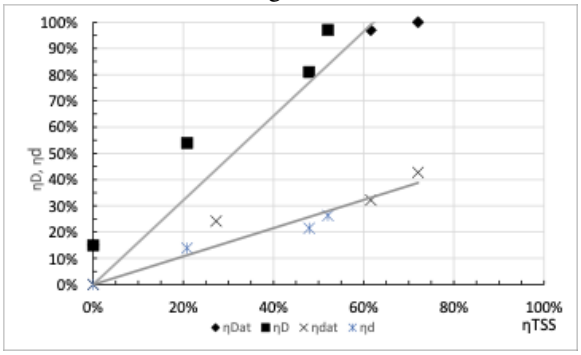

Figure 5. Correlation between η_{TSS} and η_d and η_D by FCM. $\eta_d = 0.537\eta_{VSS}$, $R^2 = 0.99$, $\eta_D = 1.607\eta_{VSS}$ with $R^2 = 0.96$.

Table 2. Characterization of A-SE WAS.

pH	9± 0.1
TSS (g/L)	51 ± 5
VSS (% ST)	73 ± 4
CODT (g/L)	69 ± 5
CODS (g/L)	13 ± 2
n (samples)	15

3.3 Alkaline acidogenesis in UASB reactor (AAR)

3.3.1 Influent preparation

The alkaline steam exploded WAS, 40 batches of approximately 4 kg/run, were stored to 50°C where it settled until it was fed to the continuous alkaline acidogenic reactor (AAR) at 2.5 to three times the concentration obtained from the SE reactor (table 2). The daily feeding flow rate (F = L/d) was calculated according to equation 5

$$F = \frac{B_v V}{S_0} \tag{5}$$

Where V is the reactor volume (7 L) and S_o is the inlet volatile solids COD (g/L).

The pH remained around 9. The soluble COD was low because the supernatant which contained most of the soluble compounds released from the A-SE did not enter the AAR. With this arrangement the full advantage of the alkaline acidogenesis is obtained because with higher VSS concentrations more VFA will be produced with no inhibition because the alkaline media kept them ionized.

3.3.2 Reactor operation

The run was started in batch with a So/X = 4 which meant that 3.25 L of the inoculum (20 g SSV/L) were added to 3.75 L of A-SE WAS (70 g COD/L). After 5 days of batch fermentation, with effluent recirculation, when VFA reached 13 g CODVFA/L (61% acetate) the reactor was started in continuous mode changing the organic loading rates (Bv) from 3 to 7 g COD_{VSS}/L·d (the COD of VSS) by adjusting the hydraulic retention time (HRT) from 20 to 8 days.

During the first OLR run the residual soluble COD (CODs) is 51% and methane is 6% of the VSS solubilization (VSS_0-VSS). Total VFA is 63% of the CODs (figure 6). After an OLR increment, to 6.2, the VSS removal decreases with a concomitant decrease in CODs and VFA_t. A drop of OLR to half and then two successive increments does not stop the leveling of VFA_t to 3 g/L. The individual VFA (figure S3), show in the first run, a high concentration of VFAt peaking to 12 g/L, 62% being acetate and 30% propionate. In subsequent OLR changes the propionic acid is higher than acetic, indicating the presence of H_2. pH is nearly constant at around 8.5 with a minimum period at 7.5 when VFA are at their highest. Methane is low as expected due to the basic pH as suggested by Wang *et al*. (2017).

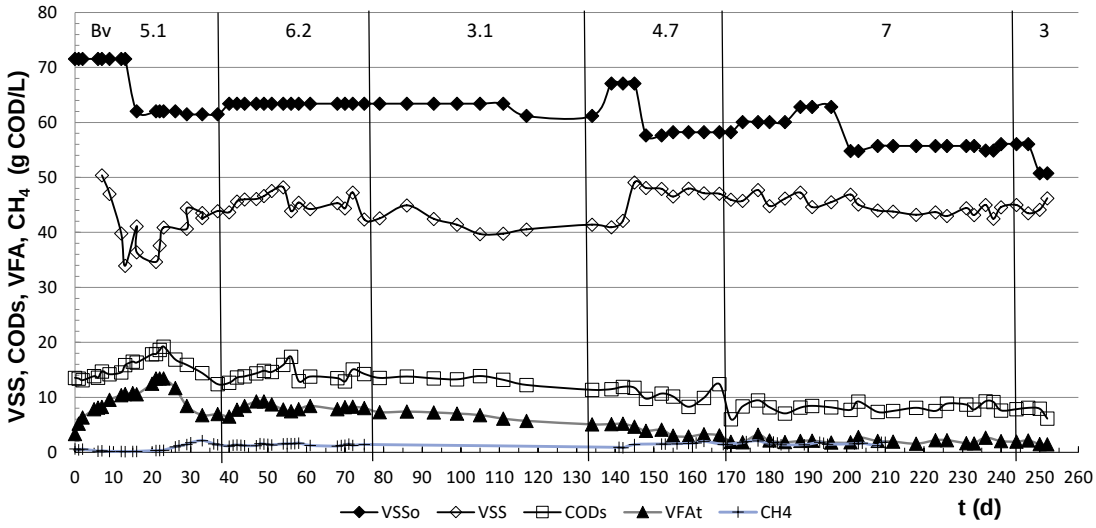

Figure 6. Evolution of alkaline acidogenesis at several Bv (g COD_{VSS}/L·d). VSS_0 (♦), VSS (◊), COD_s (□), total VFA (+), CH_4 (x). Figure S3 shows the VFA in perspective with methane and pH.

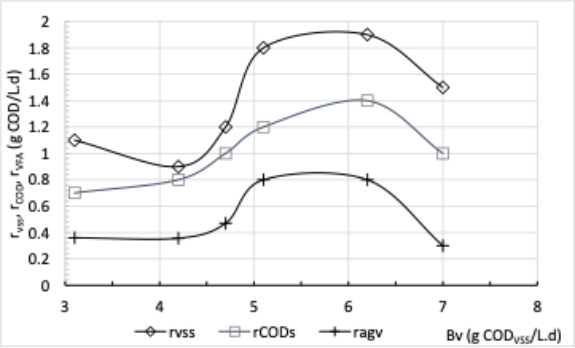

Figure 7. Rates of produced VFA as compared with VSS removal showing an optimal Bv at 5 to 6 g COD_{VSS}/L·d.

By taking five days of constant VSS output as a steady state point for each Bv, the plot of reaction rates as a function of the loading rates is obtained (figure 7). It shows that a OLR of 5 to 6 g COD_{VSS}/g·L is an optimum range to operate an alkaline acidogenesis of WAS with efficiencies of 30% for VSS solubilization and 42% VFA formation from the solubilized VSS. According to these results, WAS treated with A-SE, cooled to 50°C can be fed to a reactor for an alkaline acidogenesis al 60 g TSS/L with 10 days retention time to produce 1.4 g COD_S/L·d with 0.8 g COD_{VFA}/L·d, thus yielding 0.133 g COD_{VFA}/g COD_{VSS} =0.246 g COD_{VFA}/g VSS (table 2), 3 to 4 larger than an acidic acidogenesis (Yuan *et al*., 2006).

Table 3. Material balance of the integrated WAS treatment process in figure 8.

Stream	**1**	**2**	**3**	**4**	**5**	**6**	**7**
V (L)	1000	106	106	condensed steam	60	46	46
TSS (g/L)	6.6	62.1	22		0	51	33.3
CODt (g/L)	9	84	54.3		54.3	69	45
CODs (g/L)	0.05	0.05	13		13	13	15
CODVFA (g/L)	0	0	0		0	0	8

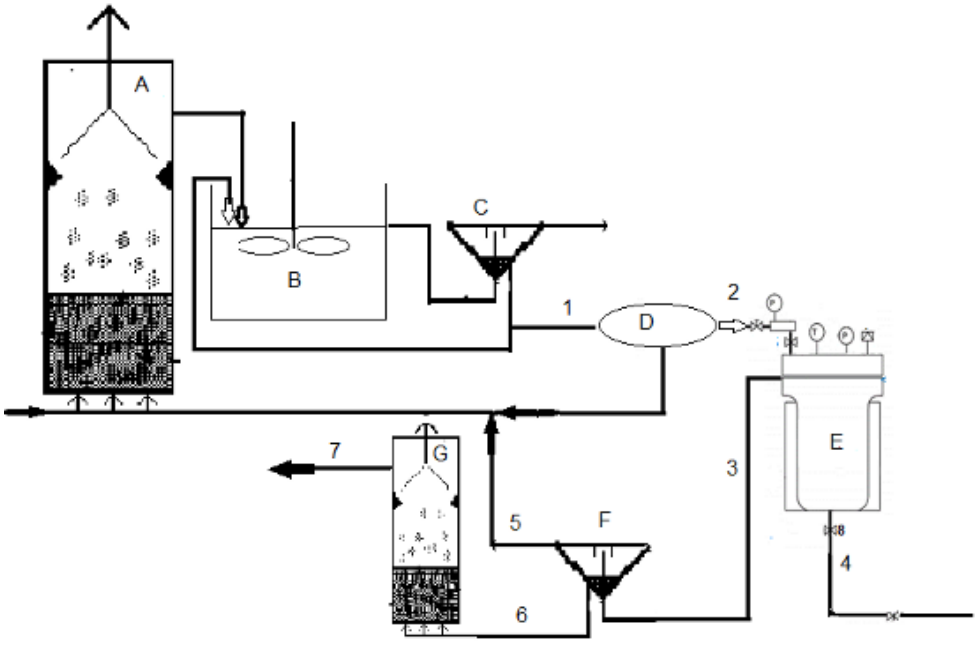

Figure 8. Process flow diagram for the alkaline steam explosion and alkaline acidogenesis of waste activated sludge. A= UASB reactor, B = Activated sludge tank reactor, C = secondary clarifier, D = Centrifuge E = steam explosion reactor, F = consolidation tank, G = alkaline acidogenesis reactor. The streams characteristics are shown in table 3.

Based on these results a process flow diagram is built to appreciate the importance of integrating the alkaline steam explosion with alkaline acidogenesis to reduce the mas of WAS and produce methane and VFA (figure 8, table 3). The process starts at the return line of the clarifier (1) of the activated sludge tank (AST) reactor. The settled sludge (1) is a slurry of about 7 g TSS/L which has to be concentrated about ten times (2) for an efficient heat use in the A-SE reactor which breaks the TSS from 62 to 22 g TSS/L (3). The supernatant which is a secondary effluent is returned to the wastewater treatment process. Stream 3, at 160°C and pH 9 is cooled down to 50°C and concentrated to handle the thermophilic alkaline acidogenic reactor with highest possible load of solids (6). The supernatant can be returned to the WWT process (which if has an anaerobic reactor before the AST, can add to the methane production). Stream 7 can be directed to produce methane, adding to the obtained from stream 5, or for VFA extraction (15 g/L). The alkaline steam explosion alone gives 65% TSS removal and the alkaline acidogenesis yields 35% TSS removal. The overall process produces 77% removal.

Conclusions

A positive effect of the alkaline steam explosion (pH 9, 15 min, 0.67 MPa absolute pressure, 160°C) on the TSS size reduction was found to be concentration dependent (first order rate solids destruction) which is related to a smaller particle size as measured by average particle diameter in a flow cytometer. These results show that the alkaline pre-treatment can double methane production by increasing the WAS digestibility.

Assessing cell integrity by FCM fluorometry did not allow the follow-up of the WAS destruction because with only 5 min of pretreatment, 86% presented damages in their cellular integrity without really breaking down the cells and reducing the particles sizes. On the other hand, light scattering FCM can be implemented on line to assess the VSS destruction of WAS by steam explosion as there is a good correlation with the time-consuming gravimetric analysis of VSS (R^2 = 0.98).

The alkaline steam explosion of waste activated sludge destroys 60% solids in 15 min leaving a stream with high COD concentration and another of solids which needs to be concentrated for high VFA production rates.

The study of the separated unit operations is useful to integrate a process with solid experimental basis.

Supplementary material

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