Vol. 17, No. 3 (2018) 999-1009
Revista Mexicana de Ingeniería Química

# INFLUENCE OF SODIUM HYPOCHLORITE ON THE ANAEROBIC TREATMENT OF BROWN WATER

# INFLUENCIA DEL HIPOCLORITO DE SODIO EN EL TRATAMIENTO ANAEROBIO DE AGUAS CAFÉS

C.F. Moreno-Cruz[1], V.H. Lara[2], F. Thalasso[3], O. Monroy[1], F. Ramírez-Vives[1]*

[1]*Departamento de Biotecnología, Universidad Autónoma Metropolitana-Iztapalapa, Av. San Rafael Atlixco 186, Col. Vicentina, Iztapalapa, C.P. 09340, D.F., México.*
[2]*Departamento de Química, Universidad Autónoma Metropolitana-Iztapalapa, Av. San Rafael Atlixco 186, Col. Vicentina, Iztapalapa, C.P. 09340, D.F., México.*
[3]*Departamento de Biotecnología y Bioingeniería, Centro de Investigación y Estudios Avanzados, Av. Instituto Politécnico Nacional 2508, Col. San Pedro Zacatenco, Gustavo A. Madero, C.P. 07360, D.F., México*

Received November 17, 2017; Accepted March 12, 2018

## Abstract

Effluent separation at the origin (brown water (BW), gray water and yellow water) improves sewage treatments. However, the BW can contain disinfectants such as sodium hypochlorite (NaOCl). This disinfectant has a high oxidant potential that could reduce the methane production and affect the granular sludge physiology. Therefore, the initial concentration of volatile solids (VS) from BW and the NaOCl effect on the anaerobic digestion (AD) were evaluated in this work. Variables analyzed were specific methanogenic activity (SMA), structural morphology and mineral composition of granular sludge. A VS removal of 60% was obtained for 6, 9 and 14.6 g/L of VSo. At low concentrations of NaOCl (< 125 mg/L), a VSo removal of up to 55 % and methane production of 125 $mL_{STP}CH_4/L$ were observed. While at NaOCl high concentrations (>250 mg/L), the rupture of the granules was observed releasing exopolymeric substances (EPS), the SMA decreased to 0.004 $gCODCH_4/gVS.d$. Our findings reveal that the NaOCl (> 125 mg / L) decreases the efficiency of BW treatments, and its effect must be considered to implement the anaerobic digestion of BW in low-flow toilet systems.

*Keywords*: anaerobic granules, brown water, sodium hypochlorite, specific methanogenic activity.

## Resumen

La separación de efluentes en el origen (agua café (BW), agua gris y agua amarilla) mejora los tratamientos de las aguas residuales. Sin embargo, el BW puede contener desinfectantes como el hipoclorito de sodio (NaOCl). Este desinfectante al tener un alto potencial oxidante podría reducir la producción de metano y afectar la fisiología del lodo granular. Por lo tanto, en este trabajo se evaluó la concentración inicial de sólidos volátiles (VS) del BW y el efecto del NaOCl sobre la digestión anaerobia (AD). Las variables analizadas fueron la actividad metanogénica específica (SMA), la morfología estructural y la composición mineral del lodo granular. Se obtuvo una remoción del 60 % de VS para concentraciones de VSo de 6, 9 y 14.6 g/L. A bajas concentraciones de NaOCl (<125 mg/L), se observó una remoción del 55% de VS y una producción de metano de 125 $mL_{STP}CH_4/L$. Mientras que a altas concentraciones de NaOCl (>250 mg/L), se observó la ruptura de los gránulos liberando sustancias exopoliméricas (EPS), la SMA disminuyó a 0.004 $gCOD_{CH_4}/gVS.d$. Nuestros hallazgos revelan que el NaOCl (>125 mg/L) disminuye la eficiencia de los tratamientos de BW, y su efecto debe considerarse para implementar la digestión anaerobia del BW en inodoros de bajo flujo.

*Palabras clave*: gránulos anaerobios, aguas cafés, hipoclorito de sodio, actividad metanogénica específica.

* *Corresponding author. E-mail*: frav@xanum.uam.mx

# 1 Introduction

Different types of domestic effluents are obtained with the separation of wastewater streams at the origin, such as black waters (faeces with urine), brown water (faeces without urine), yellow waters (urine) and gray waters (laundry, shower and kitchen). Effluents are generated in different quantities and qualities, resulting in specific treatments for degradation and/or reuse (de Graaff *et al*., 2010; Rajagopal *et al*., 2013). Effluent separation in situ can be applied in homes, housing complexes and small communities. Additionally, the facilitation of the recovery of nutrients, energy production and the efficient removal of contaminants and pathogenic microorganisms can be obtained through wastewater treatments (de Graaff *et al*. 2010). In the case of black and brown water effluents, the volumes generated are dependent on the water consumption required for cleaning the toilet system type. For example, conventional flush toilets (without separation of domestic water) consume 36 to 72 L/(p.d.). Vacuum and gravity toilets require only up to 1.5 and 0.5 L per flush, respectively. Notably, without urine separation the water consumption is 6-9 and 2-3 L/(p.d.) for vacuum and gravity toilets respectively, reducing the tap water use by more than 80% compared to conventional toilets. BW is particularly characterised by its high content of organic matter, nutrients, pathogens, pharmaceutical residues and hormones (Kujawa and Zeeman, 2006). Several studies have focused on the anaerobic degradation of black and brown waters. For example, Lim and Rajagopal *et al*. mixed brown water with municipal solid organic wastes using a continuous stirred-tank reactor (CSTR), and obtained yields of 0.3 to 0.5 $L_{CH_4}$/g$VS_{fed}$ (Lim, 2011; Rajagopal *et al*., 2013). The recovery of nutrients (phosphorus) and energy production from black water has also been reported (Tervahauta *et al*., 2014).

Several factors affect the anaerobic degradation of BW. Disinfectants that contain NaOCl could decrease wastewater treatments when they are used in an uncontrolled manner (Bodik *et al*., 2008). In Mexico, the main chemical components present in disinfectants contain NaOCl, sodium hydroxide, ammonium hydroxide, anionic surfactants and nonionic surfactants.

Particularly, the NaOCl is an inexpensive strong oxidant that presents antimicrobial action and is often used in the toilet cleaning processes. The disinfection property of NaOCl depends on the available chlorine concentration and the pH of the solution (Estrela *et al*. 2002). NaOCl in contact with water becomes hypochlorous acid (HOCl), which is a very effective biocidal species (Von Gunten and Oliveras, 1998) due to its ability to penetrate the cell membrane by passive diffusion and denature proteins. Furthermore, the concentration of hypochlorite ions ($ClO^-$) is a key indicator of the cleaning efficiency (Fukuzaki, 2006); although it also oxidizes organic matter which results in reduced disinfectant efficiency (Yoon *et al*., 2013). An inhibitory effect of $ClO^-$ has been observed in the anaerobic treatment of pig manure (Yoon *et al*., 2013). A viable alternative for efficient treatment is to use fermentation times up to 20 days and high organic matter concentration (43 g/L), in order to reduce the inhibitory effect of $ClO^-$ on the methanogenic archaea (Yoon *et al*. 2013). Main effects of NaOCl on the biological sludge include solubilization of sludge, increase in the EPS caused by floc disruption or destruction of microbial cells, and smaller floc sizes decreasing the sludge settling properties (Chang *et al*., 2004; Navarro *et al*., 2016; Liao *et al*., 2004).

Therefore, the objective of this work was to evaluate the initial concentration of BW volatile solids (VSo) and different concentrations of NaOCl (ranging from 125 mg/L to 7.5 g/L) on anaerobic digestion of BW analysing the AME, degradation of solids and morphology of granular sludge.

# 2 Materials and methods

## 2.1 *Sampling and BW characterisation*

Faeces samples (without urine) were collected and diluted with 1.5 L of tap water to simulate samples from vacuum toilets. Samples were homogenised and stored at 4 °C until used. The parameters measured were: pH, conductivity, soluble chemical oxygen demand (CODs), total chemical oxygen demand (CODt), total solids (TS), volatile solids (VS) and ammonium ($NH_4^+$).

## 2.2 *Inoculum*

Granular anaerobic sludge from reactor samples sourced from the food processing industry was used as the inoculum. Granular sludge showed a specific methanogenic activity (SMA) of 2.23 ± 0.073 g$CODCH_4$/gVS.d and a VS concentration of 43 g/L.

## 2.3 Biodegradability assays

The batch assays were conducted at 35 ± 2 °C using serological bottles (120 mL) containing 80 mL of substrate and 6.9 g/L of VS as inoculum for each experimental unit. The air was displaced with nitrogen gas over 1 min to obtain anaerobic conditions. Analyses were performed in duplicate and brown water samples without inoculum were used as the controls. Methane production was quantified by the displacement of a solution of 3% (w/v) NaOH.

### 2.3.1 Biodegradability assays of concentrated BW

The effect of VS concentration on the anaerobic degradation of BW was evaluated in this study through batch assays using different concentrations of VSo (5.98, 9.21, 14.58, 17.00 g/L). In particular, the assay with 17 g/L of VS was monitored over time to determine the degradation rate of VS. Different response variables were analysed after 25 days: the methane production, degradation of VS, CODt, CODs, solubilisation of organic matter, pH, ammonia, and volatile fatty acids (VFA).

### 2.3.2 Effect of sodium hypochlorite on BW biodegradability

Batch assays were conducted adding NaOCl to evaluate its effect on the anaerobic biodegradability of BW. A completely randomised design was used to develop the experiments using six levels of NaOCl concentration as the factor. All analyses were performed in triplicate. Different concentrations of NaOCl (reagent grade, available chlorine 14 %) were evaluated (50, 125, 250, 500, 1000, 2500 and 7500 mg/L). 1.5 L of tap water was used to dilute samples of stool to simulate samples from vacuum toilets. CODt concentrations of 14.5 g/L and 6.8 g/L of VS (inoculum) were used to develop the batch assays. Samples of BW without NaOCl were used as controls. The evaluated variables were: methane production over 30 days, VS degradation, total and soluble COD, and inhibition percentage of methane production (ratio between maximum methane production (exposed to NaOCl) and controlled methane production (without NaOCl)) were calculated using the equation 1.

$$\%I = \left(1 - \frac{P_t}{P_c}\right) \times 100 \quad (1)$$

Where:

$P_c$ is the rate biogas production in the control bottles

$P_t$ is the rate biogas production in the test bottles

## 2.4 Analysis of granular sludge exposed to NaOCl (morphology and EPS)

The influence of NaOCl on the specific methanogenic activity (SMA) of granular sludge was evaluated using different concentrations of NaOCl. SMA was determined using the method described by Duran *et al*. (2008). Serological bottles were used and 80 mL of Visser mineral medium and glucose were used as the carbon and energy source. Serological bottles without substrate were used as the controls. Biogas production was determined by the displacement of a pH 2 saline solution, to prevent carbon dioxide dissolution (Gonzalez Blanco *et al*., 2017). In order to evaluate the effect of NaOCl on the structure of granular sludge, assays were conducted using granular sludge exposed to different concentrations of NaOCl (50, 125, 250, 500, 1000, 2500 and 7500 mg/L). After 25 days of exposure to NaOCl, granule morphology was observed under an optical microscope (Olympus BX50, 4X objective, Evolution$^{TM}$ VF camera). Subsequently, the concentrations of the soluble exopolymeric compounds (proteins and carbohydrates) were determined. Granular sludge samples without exposure to NaOCl were used as the controls.

### 2.4.1 Analysis of granular sludge mineral composition

Changes in the mineral composition of granular sludge that was exposed to NaOCl were determined by X-ray diffraction analysis. Sludge samples were dried at 100 °C for 24 h and calcined at 500 °C for 50 min. Then, samples were sieved through a mesh of 0.100 mm. Diffraction profiles were obtained using an X-ray diffractometer (Siemens Brand D500) with a Cu-Ka radiation source ($\lambda$ = 1.5406 Å) and a secondary beam monochromator. Identification was determined by comparing the relative intensities using the JCPDS program "Joint Committee on Powder Diffraction Standards".

## 2.5 Analytical methods

pH and conductivity were measured using potentiometric analysis. TS and VS were quantified by methods described by Ferreira *et al*. (2014). COD concentrations were determined using standard methods at the initial and final time of assays (APHA, AWWA, WPFC. 2005). Biogas composition was

determined by gas chromatography, equipped with a thermal conductivity detector (Gow-Mac Serie 580) and a carbosphere 80/100 packed column. Helium was used as the carrier gas with a flow rate of 25 mL/min. The temperatures of the column, injector and detector were set to 140, 170 and 190 °C, respectively. The compounds detected were methane and carbon dioxide. The methane volume was corrected to standard pressure (760 mm Hg) and temperature (0 °C). Carbohydrates and proteins were determined by methods described by Dubois *et al*. (1956) and Lowry *et al*. (1951), respectively. Ammonium was determined using an ion selective electrode (Thermo Scientific Orion 9512HPBNWP). VFAs were analysed by gas chromatography. A flame ionisation detector (HP 5890) and an AT-1000 column were used (0.53 mm × 1.2 mm × 10 m). The temperature of the oven was increased to 120 °C (10 °C $min^{-1}$), and the temperature of the injector and detector were set to 250 °C and 200 °C, respectively. Nitrogen was used as the carrier gas with a flow rate 3 mL/min and the sample volume injected was 2 $\mu$L.

### 2.6 *Statistical analysis*

The one-way analysis of variance (ANOVA) was applied to estimate significant differences ($p$ <0.05). Number cruncher statistical system (NCSS) software (Kaysville, Utah, USA) was used for the analyses of the statistical experimental design in the batch assays. The Duncan test was used for multiple comparisons of means.

## 3 Results and discussion

### 3.1 *Characterisation of concentrated brown water samples*

Samples of brown water were characterised and simulated as discharges from vacuum toilets (Table 1). The total (CODt) and soluble (CODs) chemical oxygen demand measurements were determined, 25.25 ± 10.58 g/L and 6.80 ± 3.58 g/L, respectively. Based on the concentrations of VS and TS, a ratio of VS/TS was calculated (85%). Similar results (VS/TS ratio of 87%) were previously reported (Rajagopal *et al*., 2013), indicating that these samples are suitable substrates for anaerobic digestion (AD).

High variability between sample parameters taken in different studies of brown water can be attributed to several factors including the human source-food composition (Rose *et al*., 2015). Furthermore, the composition of human excreta varies according to the geographical region, age and time of day (Ronteltap *et al*., 2009).

### 3.2 *Biodegradability assays of concentrated brown water*

The influence of VS concentration (6, 9 and 14.6 g/L) on the anaerobic digestion of brown water was evaluated in terms of solid degradation, solubilisation of organic matter and methane production. As shown in Fig. 1a, high solid degradation (60%) was obtained with a concentration of 6 g/L VS compared to the controls and other treatments. However, the solubilisation of organic matter increased in all of the controls: 6 g/L VS (56.5 ± 4.2%), 9 g/L VS (49 ± 2.2%) and 14.6g/L VS (45 ± 1.8%). These results can be attributed to methane production from the organic compounds. For example, the methane production was higher at a concentration of 14.6 g/L VS (139.5 ± 23.6 $mLCH_4$/(L.d)). In contrast, the concentration of VFA in all of the controls was up to 3 gCOD/L. Also, the pH decreased to 5.6, resulting in low methane production and a high solubilisation percentage Figure 1b. Moreover, the ammonia production was higher in the treated samples than the controls, which resulted in increased sample pH values of close to 7. High concentration of VSo, caused the ammonium accumulation and increased pH (Fig. 1b). Specifically, 1800 $mgNH_4^+$/L was obtained for concentrations of 14.6 g/L VS, which is equivalent to 1.4 g/L of nitrogen in brown water. This behaviour could be explained by influence of the ratio of initial substrate concentration to biomasss concentration (0.8 to 2.5 $gVS_{BW}/gVS_{inoculum}$) obtained in this study (Lopez-Avilés *et al*., 2017). The VS removal levels obtained in our study are slightly higher than 56 % that was reported by Lim (2011).

In order to determine the degradation rate of VS, assays were conducted at a VS concentration of 17 g/L (Fig. 2). Hydrolysis was expressed in terms of soluble COD concentration and VS reduction. As can be seen from Fig. 2, the total COD decreased to 50% after 30 days and the soluble COD increased up to 88% (over a time-period of 2 to 16 days). During the same period a VS removal of 50 % was obtained. A VS degradation rate of 0.49 g/(L.d) was reached. In addition, the methane production increased from day 17 to day 30 (Fig. 2).

Table 1. Characterisation and comparison of brown water (BW) samples.

| Parameter | Units | This Study (n=5) | BW* | BW+FW ** | BW+FW*** |
|---|---|---|---|---|---|
| pH | - | 6.40 ± 0.51 | 6.70 | 6.2 ± 0.6 | 6.23 ± 0.07 |
| Conductivity | $\mu$S/cm | 2211 ± 748.0 | - | - | - |
| [*Ammonium*] | mg$NH_4^+$/L | 136.20 ± 8.03 | - | - | - |
| [$CODt$] | g/L | 25.25 ± 10.58 | 8.16 ± 0.56 | 35.0 ± 10.4 | - |
| [$CODs$] | g/L | 6.80 ± 3.58 | - | 13.0 ± 4.4 | 12.29 ± 5.27 |
| [$VS$] | g/L | 15.05 ± 5.76 | 4.41 ± 0.90 | 19.89 ± 3.74 | 22.19 ± 8.08 |
| [$TS$] | g/L | 17.60 ± 5.76 | 5.19 ± 0.07 | 21.41 ± 4.09 | 24.44 ± 10.11 |
| VS/TS | % | 85.66 ± 2.70 | 85 | 93 | 90 |

*Lim (2011), **Rajagopal *et al*. (2014), 2 L BW +150 g of food waste (FW); *** Lim *et al*. (2013), 2L BW+ 300 g FW

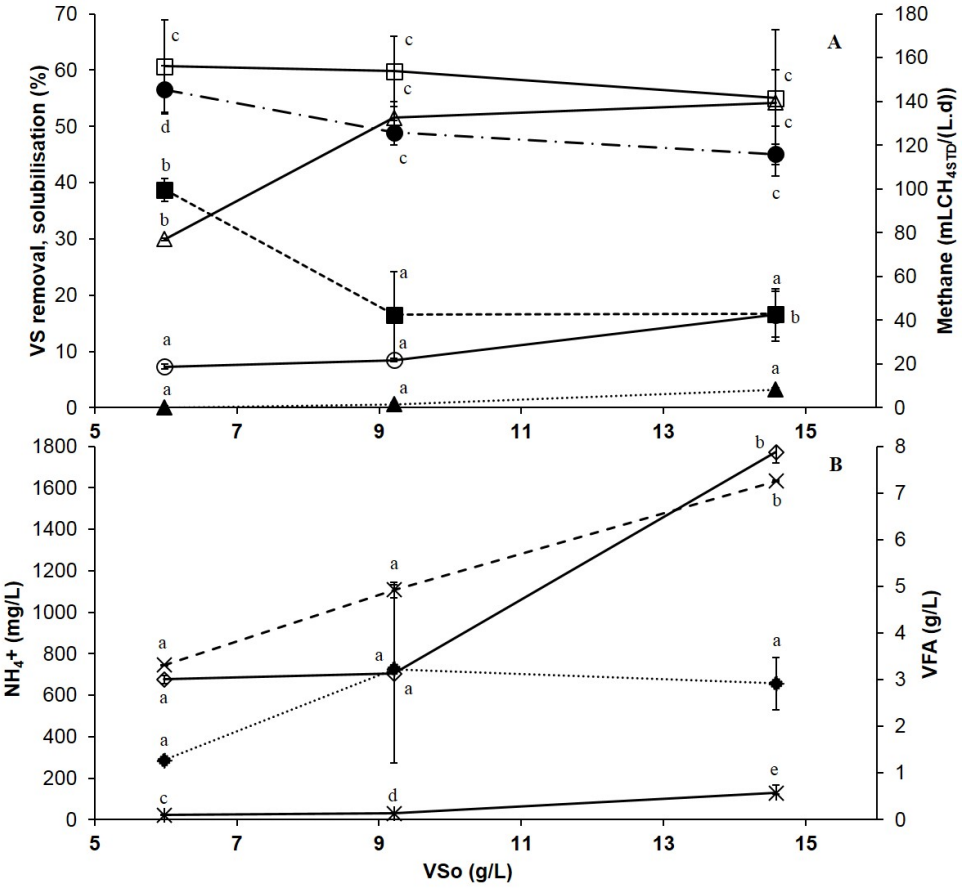


Fig. 1. Biodegradability assay plots of brown water at different volatile solids concentrations. (a) Volatile solids removal (control –■– and treated –□–), solubilisation of organic matter (control -●– and treated –o–) and methane production (control –▲– and treated –Δ–). (b) Ammonium production (control –♦– and treated –◊–) and volatile fatty acids production (control –×– and treated –*–), after 30 days of digestion. Means with same letter are not significantly different (Duncan test, $\alpha \leq 0.05$, $n = 2$).

These findings suggest that the anaerobic digestion of VS occurs over two stages (i) hydrolysis and fermentation (during the initial 17 days) and (ii) a methanogenic stage (last 13 days). Generally, the anaerobic digestion is developed in two reactors with separate stages (hydrolysis-fermentation and methanogenic) to overcome problems of inhibition.

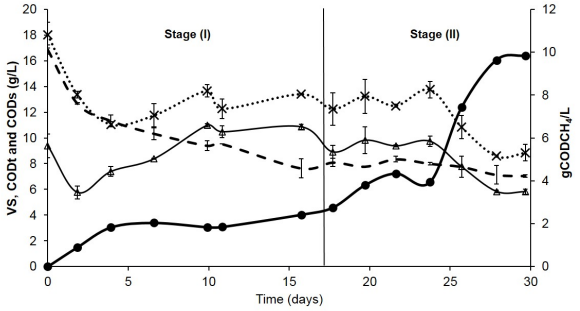


Fig. 2. Anaerobic degradation of brown water. Volatile solids (—), total chemical oxygen demand (×), soluble chemical oxygen demand (–Δ–) and methane production (–●–).

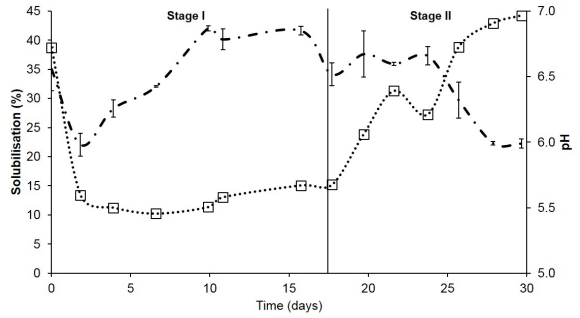


Fig. 3. Solubilisation of organic matter and pH profiles during anaerobic degradation of brown water. Soluble chemical oxygen demand (- · - · -) and pH (· · □ · ·).

However, this study showed the two stages at the same reactor. These results could be explained by the interaction among nitrogen compounds, VFA, pH and the activity of biological sludge during the anaerobic digestion of BW. Similar studies have reported single-stage reactor using human excreta (Colon *et al*., 2015). Although, other authors consider studying the codigestion of faeces with other substrates to avoid phenomena of inhibition and obtain high efficiencies of degradation and methane production (Lalander *et al*., 2018; Rajagopal *et al*., 2013).

Moreover, solubilisation of the organic matter was monitored during the anaerobic digestion of brown water (Fig. 3). The results showed that from day 4 to day 15 the soluble COD increased to 41%, which is over the same time-period that VS degraded at its fastest rate. The pH was not controlled in the batch reactors. Over the anaerobic digestion period two distinct pH stages could be observed (i) the pH decreased to 5.6 (during the first three days of treatment) and remained constant (between 5.5 and 5.7) until approximately day 16 after which can be attributed to the hydrolysis of the solids contained in the brown water observed in Fig. 2; (ii) the pH increased up to 7 (after 30 days of treatment). These results indicate that the activity of methanogenic archaea was favoured rather than fermentative bacteria. Furthermore, the buffering capacity could be explained by protein hydrolysis ($NH_4^+$ production) and VFA formation from the carbon source. Notably, this contributes to increased efficiency in different stages of anaerobic digestion. The production of $NH_4^+$ and VFA by hydrolytic acidogenic bacteria creates a weak buffer system (Venkata *et al*., 2009; Wang *et al*., 2013). Additionally, in the presence of $CO_2$, products from protein hydrolysis result in $NH_4^+$ formation and ammonium bicarbonate (Eq. 2 and Eq. 3). The buffering capacity depends on the balance between $CO_2$, bicarbonate and VFA produced (Dahiya *et al*., 2015).

$$CO_2 + H_2O \leftrightarrow H_2CO_3^- \quad (2)$$

(pKa=6.1,bicarbonate buffer)

$$CO_2 + H_2O \leftrightarrow H_2CO_3^- + NH_4^+ \leftrightarrow NH_4HCO_3 + H^+ \quad (3)$$

(pKa=6.35 / 9.35)

### *3.3 Effect of sodium hypochlorite on brown water biodegradability*

The effects of NaOCl on SMA and VS removal were evaluated during brown water biodegradation. The results indicate that the VS degradation levels were similar when the concentrations of NaOCl were 50 mg/L and 125 mg/L (Fig. 4). Notably, 51% of VS degraded in the presence of 250 mg/L NaOCl due to fermentative bacteria activity. However, the VS degradation significantly decreased at concentrations of 7500 mg/L NaOCl close to 15%, indicating that the NaOCl causes the chemical oxidation of VS (Fig. 4). Up to 92% methanogenic activity was inhibited when NaOCl concentrations were higher than 250 mg/L. Moreover, SMA decreased to 50% in the presence of 190 mg/L NaOCl. These results indicate that SMA was not affected by NaOCl concentrations ranging from 50 to 125 mg/L when compared with the control group (0.056 $gCOD_{CH_4}$/(gVS.d)) with brown water as the substrate (Fig. 5). The inhibition of SMA in granular sludge was evaluated under different concentrations of NaOCl (250, 500, 2500 and 7500 mg/L) with glucose used as substrate.

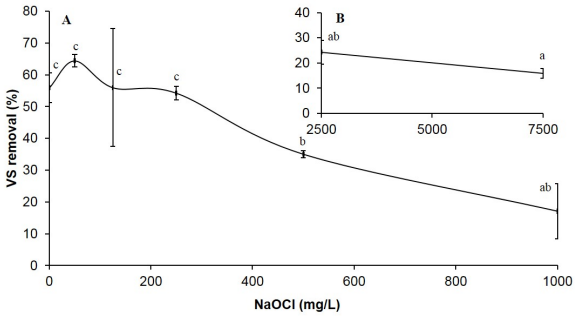


Fig. 4. Effect of NaOCl on volatile solids removal at NaOCl different concentrations. (a) 50, 125, 250, 500, 1000 mg/L; (b) 2500 and 7500 mg/L. Means with same letter are not significantly different, Duncan test ($\alpha \leq 0.05$, $n = 2$).

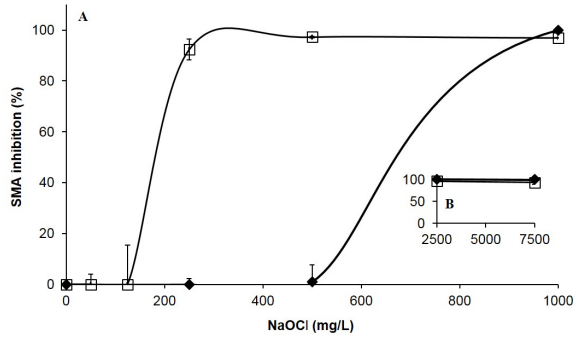


Fig. 5. Effect of NaOCl on specific methanogenic activity (SMA) inhibition during anaerobic degradation of brown water (–□ –) and glucose (–♦–). NaOCl concentrations: (a) 50, 125, 250, 500, 1000 mg/L; (b) 2500 and 7500 mg/L. The SMA without NaOCl: brown water (0.056 $gCODCH_4/(gVS.d)$) and glucose (0.10 $gCODCH_4/(gVS.d)$).

The SMA test was used to evaluate the inhibitory concentration of NaOCl in the anaerobic digestion of BW. As shown in Fig. 5, the SMA decreased significantly as the NaOCl concentration increased. The SMA inhibition reached 90 % at 250 mg/L NaOCl using BW as substrate. While, the SMA inhibition reached 100 % at 1000 mg/L NaOCl using glucose as substrate. These results can be explained due to the BW contains a solid high concentration causing problems of mass transfer and decreasing the inoculum activity compared with glucose. Previous studies have reported that NaOCl affect biological treatments when NaOCl is used uncontrolled manner (Bodik *et al*., 2008). In addition, values of the SMA inhibition near 100 % indicate a negative effect on the biological activity of methanogenic archaea as

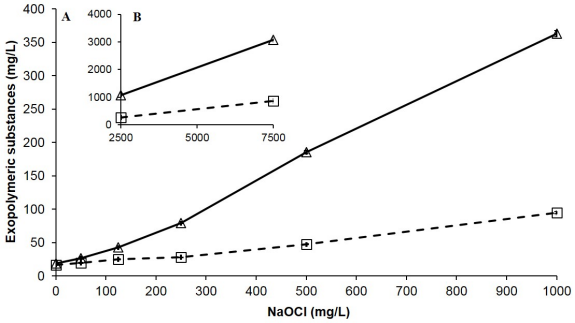


Fig. 6. Effect of NaOCl on granular sludge structure, release of exopolymeric substances carbohydrates (–□–) and proteins (–Δ–). NaOCl concentrations: (a) 50, 125, 250, 500, 1000 mg/L; (b) 2500 and 7500 mg/L.

previously observed by Yu *et al*. (2016). Therefore, our findings indicate that the BW is a substrate difficult to degrade and the presence of NaOCl decrease significantly its removal.

### *3.4 Analysis of granular sludge exposed to NaOCl (morphology and EPS)*

Exopolymeric substances (proteins and carbohydrates) were analysed to evaluate the changes in granular sludge structure due to the presence of NaOCl. As shown in Fig. 6, exopolymeric substances increased as NaOCl concentration was increased (3080 mg/L of EPS). The results suggest that the structural damage of granular sludge is due to the release of proteins and carbohydrates. In particular, EPS contribute to stabilise the anaerobic granules (matrix microbial structure) and improve the granulation process. Therefore, if EPS are released, granular sludge will lose its conformational integrity, which in-turn decreases the efficiency of water treatment systems (Liu *et al*., 2004; Ismail *et al*., 2010). These results are evidence that solubilization of granular sludge was caused by oxidation of solid sludge, granular disruption or destruction of microbial cells as previously reported studies (Chang *et al*., 2004; Navarro *et al*., 2016; Liao *et al*., 2004).

Samples of granular sludge exposed to NaOCl (up to a concentration of 7500 mg/L) were observed under an optical microscope in order to determine structural damage (Fig. 7). The rupture of granular sludge was observed as the NaOCl concentration was increased. For example, damaged zones in the granular sludge contour were observed at 1000 mg/L NaOCl (Fig. 7b); while a clear rupture of sludge was observed at 7500 mg/L NaOCl (Fig. 7d).

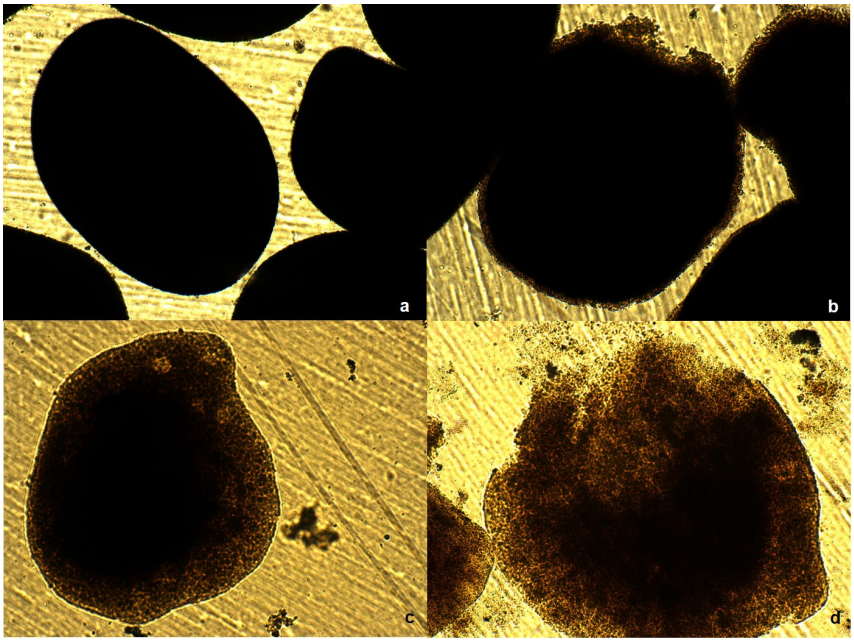


Fig. 7. Micrographs of granular anaerobic sludge exposed to different concentrations of NaOCl: (a) control (without NaOCl); (b) 1000 mg/L; (c) 2500 mg/L and (d) 7500 mg/L.

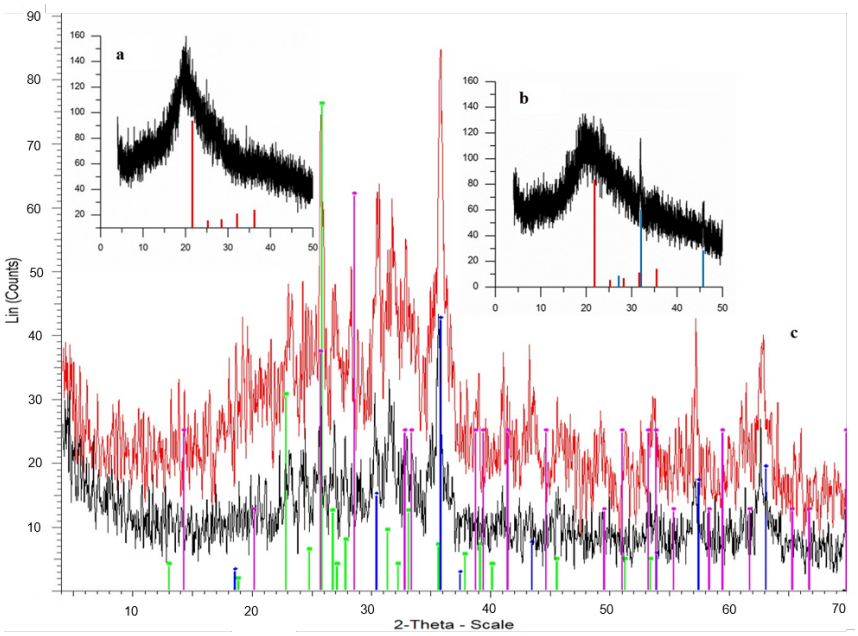


Fig. 8. Diffractograms of granular sludge exposed to NaOCl. (a) X-ray diffraction (XRD) profile of control sludge (without NaOCl), red line 77-1316 - cristobalite low, syn - $SiO_2$; (b) XRD profile of sludge exposed to 7500 mg/L NaOCl, blue line 05-0628 - halite, syn - NaCl; (c) XRD profiles of control (red) and sludge exposed to NaOCl (black). Green line 27-1280 - manganese molybdenum oxide ($MnMoO_4$), blue line 24-0354 - copper chromium manganese oxide ($CuCr_{0.5}Mn_{1.5}O_4$), pink line 08-0018 - coesite ($SiO_2$).

These results can be assigned to the loss of granular sludge mass (3000 mg/L of exopolymeric protein). According to previous reports, NaOCl can increase the specific surface area of soil particles (Kaiser and Guggenberger, 2003), suggesting that NaOCl could rupture the granular sludge. Our findings indicate that NaOCl is involved with the rupture of granular sludge (bacteria associations, organic matter, inorganic compounds and exopolymeric), which in-turn decreases the transport of substrates to various microbial consortia limiting methane production.

### *3.5 Analysis of mineral composition of granular sludge by X-ray diffraction*

X-ray diffraction (XRD) analyses were performed to determine the structural changes of granular sludge exposed to different NaOCl concentrations. According to the results, granular sludge is mainly composed of amorphous and crystalline fractions. A cristobalite phase ($SiO_2$) with identification number 77-1316 and an amorphous phase, mainly composed of organic material, were observed in the granular sludge that was not exposed to NaOCl (control) (Fig. 8a).

Previous studies reported similar granular sludge profiles, with a crystalline silica fraction associated to the sludge matrix and an amorphous silica fraction associated to diatoms, which are embedded in the silica matrix (Certucha *et al*., 2010). However, different results were observed for granular sludge exposed to 7500 mg/L NaOCl (Fig. 8b). In this case, a microcrystalline arrangement was observed in the region $2\theta$ : 5 – 25 degrees, which is indicative of the presence of NaOCl oxide organic matter. Furthermore, precipitates of NaCl were observed (05-0628). Zimmermann (2007) worked with soil samples exposed to NaOCl, no changes in the structures of soil minerals were observed. Similar results were observed in this study (Fig. 8c) for control and sludge samples exposed to NaOCl, where the mineral composition was observed to be similar. Therefore, our findings confirm that while NaOCl has an effect on the oxidation of organic matter it does not modify the mineral structure of the granular sludge.

## Conclusions

The results presented herein show that anaerobic digestion is a viable alternative for concentrated BW treatment resulting high rates of solid degradation (0.49 gSV/L.d), VS removal efficiencies (60 %) and methane production (139.48 $mLCH_4$/L.d ). Anaerobic biodegradability assays suggest that this process can be implement in a single state. In addition, this work was conducted to understand the NaOCl effect on BW treatment (commonly found in toilet cleaning products). As result, the NaOCl decrease the efficiency of anaerobic treatment systems. Particularly, the VS degradation was decreased by the effect of NaOCl (>500 mg/L). The SMA was inhibited by over 90% at NaOCl concentrations of higher than 250 mg/L. In conditions where the NaOCl concentration exceeds 500 mg/L, the anaerobic granules disintegrate due to the release of exopolymeric substances. X-ray diffraction analyses showed a loss of granular organic material due to NaOCl exposure, but no alteration to the mineral composition was observed. This study shows important information regarding the factors that affect the anaerobic degradation of brown water using disinfectants with sodium hypochlorite for toilet cleaning.

### *Acknowledgements*

The authors gratefully acknowledge the National Council for Science and Technology (CONACyT) México for the scholarship (211557), Mexico City's Science, Technology and Innovation Secretary (SECITI DF) (PICSO12-106) and Dra. Olivia Tzintzun Camacho for her invaluable support in the translation and critical review of this manuscript.

## References

APHA, AWWA, WPFC. (2005). *Standard Methods for the Examination of Water and Wastewater*, 21st edn. American Public Health Association/ American Water Works Association/Water Environment Federation, Washington, DC, USA.

Ferreira-Rolón, A., Ramírez-Romero, G., Ramírez-Vives, F. (2014). Granular sludges methanogenica activity increase due to $CO_2$ bubbling calcium precipitation over nejayote. *Revista Mexicana de Ingeniería Química 13*, 517-525.

Bodík, I., Gaspariková, E., Dancová, L., Kalina, A, Hutnan, M., and Drtil, M. (2008). Influence of disinfectants on domestic wastewater treatment

plant performance. *Bioresource Technology 99*, 532-539.

Certucha Barragán, M.T., Duarte Rodríguez, G., Acedo Félix, E., Almendrariz Tapia, F.J., Monge Amaya, O., Valenzuela García, J.L., and Leal Cruz, A. L. (2010). Bioadsorción de cobre utilizando lodo anaerobio acidogénico. *Revista Internacional de Contaminación Ambiental 40*, 101-108.

Colon, J., Forbis-Strokes, A., Deshusses, M.A. (2015). Anaerobic digestion of undiluted simulant human excreta for sanitation and energy recovery in less-developed countries. *Energy for Sustainable Development 29*, 57-64.

Chang, W.C., Jou, S.J., Chien, C.C., and He, J.A. (2004). Effect of chlorination bulking control on water quality and phosphate release/uptake in an anaerobic-oxic activated sludge system. *Water Science and Technology 50*, 177-183.

Dahiya, S., Sarkar, O., Swamy, Y.V., and Venkata, Mohan S. (2015). Acidogenic fermentation of food waste for volatile fatty acid production with co-generation of biohydrogen. *Bioresource Technology 182*, 103-113.

Dubois, M., Gilles, K.A., Hamilton, J.K., Roberts, P.A., and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry 28*, 350-356.

Durán, U., Monroy, O., Gómez, J., Ramírez, F. (2008). Biological wastewater treatment for removal resins in UASB reactor: influence of oxygen. *Water Science and Technology 57*, 1047-1052.

de Graaff, M.S., Temmink, H., Zeeman, G., and Buisman, C.J.N. (2010). Anaerobic treatment of concentrated black water in a UASB Reactor at a short HRT. *Water 2*, 101-119.

Estrela, C., Estrela, C.R., Barbin, E.L., Spano, J.C., Marchesan, M.A., and Pecora, J.D. (2002). Mechanism of action of sodium hypochlorite. *Brazilian Dental Journal 13*, 113-117.

Ismail, S.B., de La Parra, C.J., Temmink, H., and van Lier, J.B. (2010). Extracellular polymeric substances (EPS) in upflow anaerobic sludge blanket (UASB) reactors operated under high salinity conditions. *Water Research 44*, 1909-1917.

Fukuzaki, S. (2006). Mechanisms of actions of sodium hypochlorite in cleaning and disinfection processes. *Biocontrol Science 11*, 147-157.

Gonzalez Blanco G., Valencia Nava A., Beristain Cardoso R., Hernández Jaimes C., Orozco Villafuerte J. and Buendía Gonzalez L. (2017). Anammox activity of sludge coming from wetland monocots (*Typia sp*): kinetic study. *Revista Mexicana de Ingeniería Química 16*, 555-561.

Kaiser, K., and Guggenberger. G. (2003). Mineral surfaces and soil organic matter. *European Journal of Soil Science 54*, 219-236.

Kujawa-Roeleveld, K., and Zeeman, G. (2006). Anaerobic treatment in decentralized and source-separation-based sanitation concepts. *Reviews in Environmental Science and Bio/Technology 5*, 115-139.

Lalander, C., Nordberg, A., Vinneras, B. (2018). A comparison in product-value potential in four treatment strategies for food waste and faeces - assessing composting, fly larvae composting and anaerobic digestion. *Global Change Biology Bioenergy 10*, 84-91.

Liao, B.Q., Catalan, L.J.J., Droppo, I.G., and Liss, S.N. (2004). Impact of chemical oxidation on sludge properties and membrane flux in membrane separation bioreactors. *Journal of Chemical Technology and Biotechnology 79*, 1342-1348.

Lim, J.W. (2011). Anaerobic co-digestion of brown water and food waste for energy recovery. 11th edition of the World Wide Workshop for Young Environmental Scientists (WWWYES-2011) - Urban Waters: resource or risks? Arcueil: France. https://hal.archives-ouvertes.fr/hal-00607958/document. (Accessed 24 August 2017)

Lim, J.W., and Wang, J.Y. (2013). Enhanced hydrolysis and methane yield by applying microaeration pretreatment to the anaerobic co-digestion of brown water and food waste. *Waste Management 33*, 813-819.

Liu, Y.Q., Liu, Y., and Tay, J.H. (2004). The effects of extracellular polymeric substances on the formation and stability of biogranules. *Applied Microbiology and Biotechnology 65*, 143-148.

Lopez Avilés, G., Almendariz Tapia, F.J., Meza Escalante, E.R., Monge Amaya, O., Certucha Barragan, M.T. (2017). Simultaneous denitrification and methanogenesis in an anaerobic expanded bed reactor. *Revista Mexicana de Ingeniería Química 1*, 281-290.

Lowry, O. H., Rosenbroug, H. J., Lewis, A., and Randall, K. J. (1951). Measurements with the folin-phenol reagents. *Journal of Biological Chemistry 193*, 265-275.

Navarro, R., Hori, T., Inaba, T., and Matsuo, K. (2016). High-resolution phylogenetic analysis of residual bacterial species of fouled membranes after NaOCl cleaning. *Water Research 94*, 166-175.

Rajagopal, R., Ahamed, A., and Wang, J.Y. (2014). Hydrolytic and acidogenic fermentation potential of food waste with source segregated feces-without-urine as co-substrate. *Bioresource Technology 167*, 564-568.

Rajagopal, R., Lim, J.W., Mao, Y., Chen, C.L., and Wang, J.Y. (2013). Anaerobic co-digestion of source segregated brown water (feces-without-urine) and food waste: For Singapore context. *Science of the Total Environment 443*, 877-886.

Ronteltap, M., Khadka, R., Sinnathurai, A.R., and Maessen, S. (2009). Integration of human excreta management and solid waste management in practice. *Desalination 248*, 369-376.

Rose, C., Parker A., Jefferson, B., and Cartmell, E. (2015). The characterization of faces and urine; a review of the literature to inform advanced treatment technology. *Critical Reviews in Environmental Science and Technology 45*, 1827-1879.

Tervahauta, T., van der Weijden, R.D., Flemming, R.L., Hernández Leal, L., Zeeman, G., and Buisman, C.J.N. (2014). Calcium phosphate granulation in anaerobic treatment of black water: A new approach to phosphorus recovery. *Water Research 48*, 632-642.

Venkata, Mohan S., Mohanakrishna, G., Goud, R.K., and Sarma, P.N. (2009). Acidogenic fermentation of vegetable-based market waste to harness biohydrogen with simultaneous stabilization. *Bioresource Technology 100*, 3061-3068.

Von, Gunten U., and Oliveras, Y. (1998). Advanced oxidation of bromide-containing waters: bromate formation mechanisms. *Environmental Science & Technology 32*, 63-70.

Wang, Q., Peng, L., and Su, H. (2013). The effect of a buffer function on the semi-continuous anaerobic digestion. *Bioresource Technology 139*, 43-49.

Yoon, Y., Kim, H., So, K., and Kim, C. (2013). Effects of residual hypochlorite ion on methane production during the initial anaerobic digestion stage of pig slurry. *Asian-Australasian Journal of Animal Sciences 26*, 122-127.

Yu, H., Wang, Z., Wu, Z., and Zhu, Chaowei. (2016). Enhanced waste activated sludge digestion using a submerged anaerobic dynamic membrane bioreactor: performance, sludge characteristics and microbial community. *Scientific Reports 6*, 1- 10.

Zimmermann, M., Leifeld J., Abiven, S., Schmidt, M.W.I., and Fuhrer, J. (2007). Sodium hypochlorite separates an older soil organic matter fraction than acid hydrolysis. *Geoderma 139*, 171-179.