RESEARCH ARTICLE

Effect of free ammonia nitrogen on the methanogenic activity of swine wastewater

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Abstract Swine wastewater is characterized by high organic matter content, solids, nitrogen (expressed as total ammonia and protein) and heavy metals. This work determines the methanogenic toxicity effect of free ammonia contained in swine wastewater comparing raw swine wastewater (RW) and the liquid fraction of swine wastewater (TW). The values of IC_{50} (50% of inhibition) obtained for methanogenic bacteria ranged between 56 and 84% for RW, meanwhile IC_{50} for TW was ranged between 84 and 94%. Such inhibitory effects can be related to the free ammonia nitrogen concentration (> 40 mg NH_3-N/L) contained in swine wastewater.

INTRODUCTION

Pollution caused by livestock wastes and by-products (including slurries, manure, air emissions, pigs and veterinary wastes) is a growing concern in many countries (Babot and Andres, 2004). Due to the environmental impacts on water (eutrophization), soil (acidification and toxicity) and air produced by the intensive livestock explotation (Choi, 2007; González-Fernández et al. 2008a). The sustainability of this activity is becoming a priority and environmental management of intensive livestock production is observed as the solution.

Swine wastewaters are characterized by the presence of the animal feces (55%), urine (45%) and wash water (Babot and Andres, 2004). The animals' physiological state and diet as well as the buildings and waste management period result in different physical-chemical characteristics (Magrí et al. 2009). In general, swine wastewater characterization shows high organic matter (4.2-12.3 g BOD_5/L), electric conductivity (5150-8425 µS/cm), solids (total: 12.6-53.9 g/L; volatile: 7.8-21.7 g/L), fecal coliforms (4.2 x 10^7-1.9 x 10^8 MPN 100/mL) and nutrients (4.8-720 mg/L of total nitrogen, 486-954 mg NH_4^+-N/L, 266-1600 mg P/L) (Teira-Esmatges and Flotats 2003; Ahn et al. 2004; Boursier et al. 2005; Vanotti, et al. 2007; González-Fernández et al. 2008a; Rodríguez et al. 2011). In addition to heavy metals (*e.g.* 30-40 mg Cu/L; 60 mg Zn/L), hormones and antibiotics are present in swine wastewater (Choi, 2007; Ben et al. 2008; Flotats et al. 2009).

Due the high waste production and the low land availability on intensive pig farms, pig slurry must be treated to comply with the environmental regulations. In this sense, anaerobic digestion is a well-known wastewater treatment that allows simultaneously the reduction of organic matter in swine wastewater and methane production (Campos et al. 2008). In order to apply this process, two different strategies are possible: a) To treat raw swine wastewater in one single reactor or; b) To separate previously both solid and liquid fractions and to treat each one in two different kinds of anaerobic systems. When solid

and liquid fractions are not separated, anaerobic digesters with high hydraulic retention times are required due to the low rate of solids hydrolysis. Nevertheless, if solids are previously separated, the liquid fraction, which supposes around 80% of the flow rate, could be treated in high rate systems such as upflow anaerobic sludge blanket (UASB) reactors (Karakashev et al. 2008) and, therefore, the volume needed for the anaerobic sludge digesters is reduced.

On the other hand, the solid fraction of slurry contains around 30-50% of the Kjeldahl nitrogen and 80% of both copper and zinc (Burton, 2007; Moral et al. 2008). Therefore, during its hydrolysis under anaerobic conditions the generation of ammonia and/or release of heavy metals could cause the inhibition of methanogenic bacteria (Koster and Lettinga, 1988; Demirel and Yenigün, 2002; Karri et al. 2006; Chen et al. 2008). Until now, most of the studies about anaerobic digestion of the solid fraction were focused on improving the rate of the hydrolysis step (Rodríguez-Andara and Lomas-Esteban, 1999; Menardo et al. 2010) or determining its biodegradability (Campos et al. 2008; González.-Fernández, 2008b). Several authors have studied the anaerobic digestion to treated swine wastewater. Nevertheless, the possible differences of methanogenic activity treating raw swine wastewater or liquid fraction swine wastewater were not yet studied. For this reason, the main objective of the work is to determine the effect of the free ammonia nitrogen (NH_3-N) on the methanogenic activity contained in both raw (RW) and the liquid fraction (TW) of swine wastewater.

MATERIALS AND METHODS

Swine wastewater

Slurry was obtained from a piggery farm located in southern Chile. Raw wastewater (RW) was sampled after the primary settling treatment and was keeping refrigerated at 4ºC. Treated wastewater (TW) had been previously filtered using a membrane (0.45 µm) to remove solid content. RW and TW were used as influent for feeding the methanogenic toxicity assays. Table 1 shows the physicochemical characteristics of RW and TW.

Inoculum

Two different kinds of anaerobic biomass were used to carry the methanogenic toxicity assays: a) anaerobic flocculent sludge with a maximum specific methanogenic activity of 0.1 g COD/g VSS·d which was collected from an anaerobic digester located in the municipal wastewater treatment plant and; b) granular sludge with a maximum specific methanogenic activity of 0.3 g COD/g VSS·d which was collected from an anaerobic digester treating brewing effluent.

Table 1. Characteristics of swine wastewater.

Parameters	Average ± S.D.	
	Raw Wastewater (RW)	Treated Wastewater (TW)
pH	7.3 ± 0.5	7.5 ± 0.5
Total COD(g/L)	25.2 ± 4.1	16.0 ± 2.5
TS (g/L)	20.4 ± 2.9	11.2 ± 1.5
VS (g/L)	14.6 ± 1.7	8.9 ± 0.9
TN (g TN-N/L)	3.2 ± 0.1	2.1 ± 0.1
NH_4^+-N (g NH_4^+-N/L)	2.7 ± 0.1	1.9 ± 0.1
TA (g $CaCO_3$/L)	5.0 ± 0.1	4.7 ± 0.1
PA (g $CaCO_3$/L)	3.9 ± 0.1	3.1 ± 0.1
IA (g $CaCO_3$/L)	1.1 ± 0.1	1.6 ± 0.1
VS/TS	0.7 ± 0.3	0.8 ± 0.4
COD/VS	1.7 ± 0.5	1.8 ± 0.5
Copper (mg/L)	1.83	0.12
Zinc (mg/L)	7.53	0.89

Methanogenic toxicity assays

The methanogenic toxicity assays were carried out in 100 mL of total volume (the glass serum bottle volume was 125 mL) using a volatile fatty acid (VFA) as described by Vidal and Diez (2003) and Soto et al. (1993). Methane production was measured by displacement of an alkaline solution (2.5% NaOH). A neutralized VFA stock solution was used to provide a final concentration of acetic acid of 2 g/L, propionic acid of 0.5 g/L, and n-butyric acid of 0.5 g/L (total COD from VFA was 3.8 g COD-VFA/L). The media contained also the following nutrients (per liter): NH_4Cl, 0.14 g; K_2HPO_4, 0.125 g; $MgSO_4$, $7H_2O$ 0.1 g; $CaCl_2$ $2H_2O$, 0.01 g; $NaHCO_3$, 0.2 g. In order to reduce the medium, 100 mg Na_2S $9H_2O/L$ was added. Diluted HCl or NaOH solutions were used to adjust the initial pH to 7.0 ± 0.1. 2.0 mL of the neutralized VFA stock solution, 10.0 mL of the nutrients, 1.0 mL of the Na_2S $9H_2O$ and 21 mL of biomass (to obtain the desired biomass concentration), were added to obtain a total volume of 34 mL. Then, the liquid volume was completed until 100 mL with: a) 66 mL of water (control test); b) 66 mL of RW (RW 100%); c) 66 mL of TW (TW 100%); d) 33 mL of water and 33 mL of RW (RW 50%) or; e) 33 mL of water and 33 mL of TW (TW 50%). Later nitrogen gas was bubbled up into each flask to remove air from the headspace for 3 min at a pressure of 1.5 Pa. The assays were carried out at 37°C.

Three successive feedings were made during each experiment. During the first feeding, the sludge was exposed to media containing effluent and VFA substrate. At the end of the first feeding, the spent medium (liquid phase) was carefully decanted and the sludge was again exposed to effluent and VFA substrate. At the end of the second feeding, the spent medium was removed. In order to test the residual sludge activity after the first and second exposure, a third feeding containing only the VFA stock solution as substrate was added to the culture bottles (total volume 100 mL).

These experiments were carried out with both kinds of biomass and using two different biomass concentrations (2.0 and 5.0 g VSS/L) (Table 2).

The methanogenic activity, AC_m (g COD/g VSS·d), was determined according to Equation 1 (Campos et al. 2008).

$$AC_m = \frac{R}{f \cdot V \cdot [VSS]}$$

Equation 1

Where: R, methane production rate (mL CH_4/d), f is the conversion factor from CH_4 to g COD (385 mL CH_4/g COD), V is the liquid phase volume (0.1 L) and $[VSS]$ is the biomass concentration (g VSS/L).

The results are reported as the percentage of methanogenic activity of the each assay in relation to the methanogenic activity of the control ($\%AC_m$), considered according to Equation 2.

$$\%AC_m = \frac{AC_{m(Assays)}}{AC_{m(Control)}} \cdot 100\%$$

Equation 2

Where: $AC_{m(Assays)}$: methanogenic activity of each assay, $AC_{m(Control)}$: methanogenic activity of the control. The $\%AC_m$ -obtained values were plotted against the wastewater COD concentration used in each assay in order to calculate the IC_{50} (%) by data interpolation (Vidal et al. 1997).

Analytical methods

The COD (chemical oxygen demand), Total Suspend Solids (TSS), Volatile Suspend Solids (VSS), ammonium (NH_4^+-N), total nitrogen (TN) and total phosphorus (TP) were determined according to the protocols established in Standard Methods (APHA, 1985). The pH was evaluated using electrodes.

Table 2. Batch assays: experimental conditions.

Series	Biomass		Kind of slurry	
	Type	Concentration (g/L)	RW	TW
A	Flocculent	2.0	No dilution (RW 100%) Dilution 1:2 (RW 50%)	No determined
B	Flocculent	5.0	No dilution (RW 100%) Dilution 1:2 (RW 50%)	No dilution (TW 100%) Dilution 1:2 (TW 50%)
C	Granular	2.0	No dilution (RW 100%) Dilution 1:2 (RW 50%)	No dilution (TW 100%) Dilution 1:2 (TW 50%)
D	Granular	5.0	No dilution (RW 100%) Dilution 1:2 (RW 50%)	No dilution (TW 100%) Dilution 1:2 (TW 50%)

The Total Alkalinity (TA), Intermediate Alkalinity (IA, which is the alkalinity due to VFA) and Partial Alkalinity (PA) were determined by titration (Ripley et al. 1986). To evaluate the effect of the solids on methanogenic toxicity, the swine wastewater was filtered through a 0.45 µm pore size nitrocellulose membrane. Heavy metals, such as copper and zinc, were determined by atomic absorption flame spectrometry (NCh 2313/10 Of. 96).

RESULTS AND DISCUSSION

RW and TW characterization

Table 1 shows the physicochemical characterization of the RW and TW. Total and volatile solid of RW and TW were 20.4 and 11.2 g TS/L, and 14.6 and 8.9 g VS/L, respectively. Organic matter measured as total COD was 25.2 and 16.0 g COD/L for RW and TW, respectively. On the other hand, total nitrogen and NH_4^+-N were 3.2 g TN/L and 2.7 g NH_4^+-N/L and 2.1 g TN/L and 1.9 g NH_4^+-N/L for RW and TW, respectively. Similar concentrations for total solids (26.5-24.9 g TS/L) and total COD (18.7-30.1 g/L) were reported by Magrí et al. (2009).

In this study, the filtration treatment caused a removal efficiency of total and volatile solid around 55 and 61%, respectively. Similar results have been reported by González-Fernández et al. (2008b), who achieved efficiencies of 59% and 57% for total and volatile solids, respectively. In this study, the efficiency of COD reduction was about 64%, while González-Fernández et al. (2008b) obtained values about 57%. Moreover, a reduction of TN around 40% was obtained. This percentage agrees with that found by González-Fernández et al. (2008b) when applied polyacrylamide to remove solids from swine wastewater. These authors also a screen with a pore size of 0.25 mm but, in this case, they only achieved a reduction of TN around 10-15%. Therefore, it can be inferred that the efficiency of TN reduction is closely related to the percentage of solids removed. Typical in this type of effluent is the alkalinity presence, regulating the pH according to several authors (Bonmatí and Flotats, 2003; González-Fernández and García-Encina, 2009) as in this study.

Heavy metal concentrations showed that only 6.6% of cooper and 11.9% of zinc are related to the liquid fraction (TW). These results agree with those found by Marcato et al. (2008) who found that most of Cu and Zn (86%) are trapped within particles between 3 and 25 µm.

Methanogenic activity assays

Figure 1 shows the evolution of the methane production of the different assays. In all the cases, the steady state was reached after 150 hrs of assays. Nevertheless, in those assays with flocculent sludge (Figure 1 A.1, B.1, B.2) was observed a lag-phase during the first 25 hrs of operation. On the other hand, in assays using granular sludge as inoculum lag-phase was not observed (Figure 1 C.1, C.2, D.2). On the contrary, Vidal and Diez (2005) show a lag-phase > 5 days for sludge flocculent and fiberboard effluents. For this study, methanogenic inhibition was evident in all the assays (Figure 1). Using RW as substrate and flocculent sludge as biomass a reduction of the methanogenic activity was observed expressed as a methane reduction attain 10% respect to the control assays. So, methane production by RW assay was even lower of 15 mL CH$_4$ regarding 100 mL CH$_4$ approximately of the control in the operation time (Figure 1 A.1, C.1, C.2). However, granular sludge was generated a 20-25% more of methane production than when using flocculent sludge.
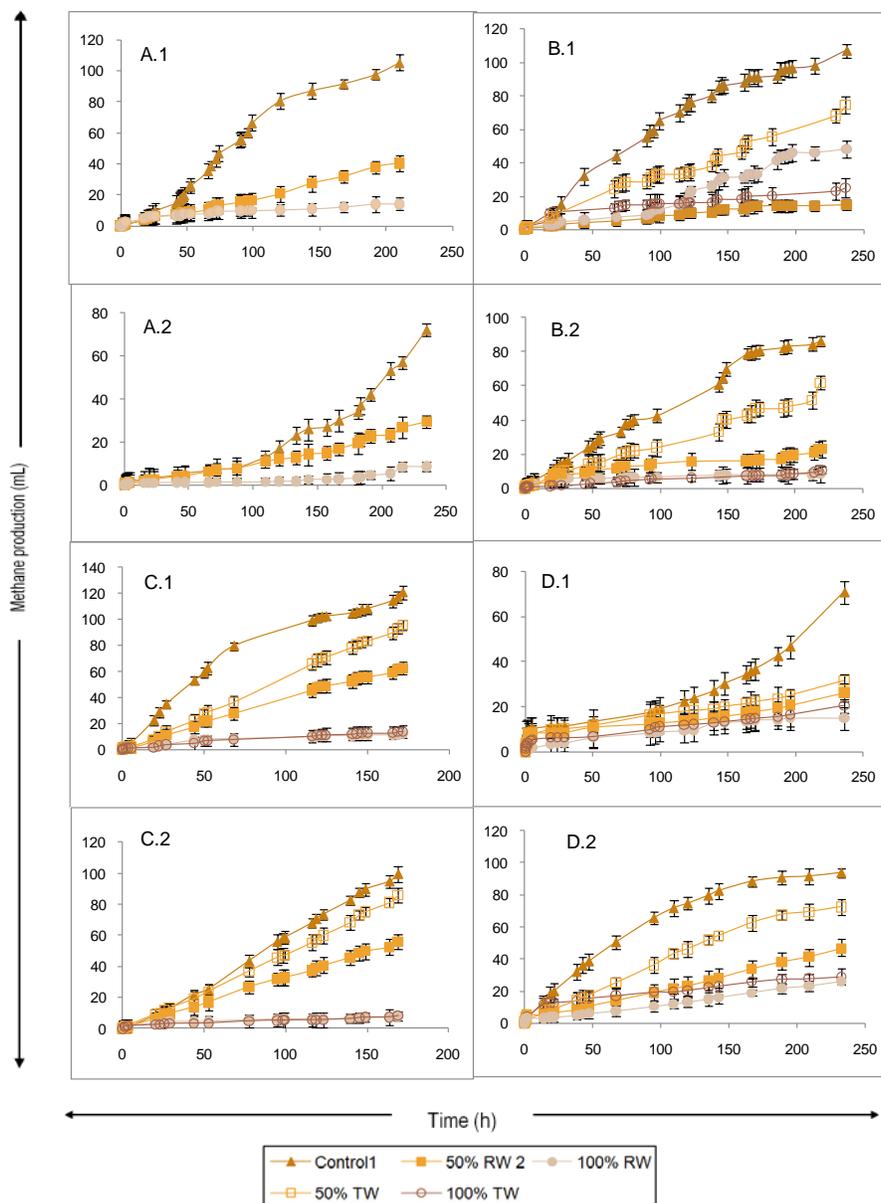
Fig. 1 Methane production in the methanogenic toxicity assays of piggery wastewater (1) first feeding and (2) second feeding. Assays corresponding: A) serie A; B) serie B; C) serie C; and D) serie D.

Table 3 shows the AC_m evaluated from Equation 2. High nitrogen concentrations (> 2.0 g N/L) characteristic of this type of wastewater and a high pH (> 7.5) causes the presence of free ammonia that can bring about the inhibition of some bacterial populations methanogenics (aceticlastic and hydrogenotrophic metanogens) (Guerrero et al. 1997; Chen et al. 2008). In this study, free ammonia nitrogen (NH_3-N) concentration was calculated for each test according to Anthonisen et al. (1976) and was correlated to the percentage of methanogenic activity obtained (Figure 2). There is a clear tendency between both parameters which would indicate that the inhibitory effect should be caused by NH_3-N for RW and TW (Figure 2). Moreover, Figure 2a shows that 40 mg NH_3-N/L contained in RW produce a reduction until 20% of the methanogenic activity under this conditions the study. Chen et al. (2008) are indicated that anaerobic digestion process can be inhibited due to concentrations at 50% by a total ammonium nitrogen concentration above 1.7 g/L. Regarding this, Guerrero et al. (1997) show that methanogenic toxicity is produced by concentration from 25 to 140 mg NH_3-N/L in mesophilic process. However, Nielsen and Angelidaki (2008) show that, the inhibition anaerobic digestion of livestock manure are caused by concentrations between 80-100 mg NH_3-N/L. Although adapted biomass can support free ammonia concentrations up to 3 g NH_3-N/L (Guerrero et al. 1997; Nielsen and Angelidaki, 2008; González-Fernández and García-Encina, 2009). Furthermore, Rodríguez et al. (2011) observed a very good performance of the anaerobic continuous system under concentration of ammonia higher than 375 mg NH_3-N/L.

Table 3. Maximum specific methanogenic activity (1st and 2nd feeding).

Inoculum		Substrate	% AC_m			
			1º Feeding		2º Feeding	
			$COD_{effluent}$ (%)		$COD_{effluent}$ (%)	
Type	Concentration (g VSS/L)		50	100	50	100
Flocculent	2.0	RW	95 ± 1	75 ± 1	90 ± 1	25 ± 1
		TW	n.d.	n.d.	n.d.	n.d.
Granular	2.0	RW	38 ± 2	7 ± 2	39 ± 2	7 ± 1
		TW	55 ± 2	11 ± 2	62 ± 4	8 ± 1
Flocculent	5.0	RW	66 ± 4	56 ± 1	55 ± 4	45 ± 1
		TW	98 ± 2	73 ± 10	90 ± 5	28 ± 1
Granular	5.0	RW	46 ± 1	20 ± 1	59 ± 7	34 ± 1
		TW	41 ± 1	25 ± 2	91 ± 9	52 ± 1

n.d.: no determined.

On the other hand, heavy metals (Cu and Zn) contained on the TW (0.12 mg Cu/L and 0.89 mg Zn/L) or RW (Cu: 1.83 mg/L and Zn: 7.53 mg/L) were found as a potential inhibitor to the AC_m. Although these concentrations are lower in comparison with those reported by Choi (2007) for swine wastewater (Cu: 30-40 mg/L, Zn: 60 mg/L), evidence of the methanogenic toxicity was not found when RW was feeding at 100%. On the other hand, Karri et al. (2006) found that Cu^{2+} concentrations (< 20.0 mg/L) could cause the reduction in the hydrogenotrophic methanogenic bacteria activity in 50% (IC_{50}) with 8.9 mg Cu^{2+}/L. Additionally, zinc concentrations between 5.0 and 40.0 mg/L can be inhibitory to the methanogenic activity (Demirel and Yenigün, 2002).

Table 4 shows the IC_{50} expressed as dilution of the effluent (%) during the second feeding for the different assays. So, the IC_{50} for RW in generally was ranged between 56-84%, meanwhile IC_{50} for TW was determined only for the granular sludge, was ranged between 84-94% found for the granular sludge. Similar results were found by Vidal and Diez (2005) in similar aggregates sludge structures but for different compounds. All the previous results show that TW is less toxic for methanogenic bacteria in comparison with RW.
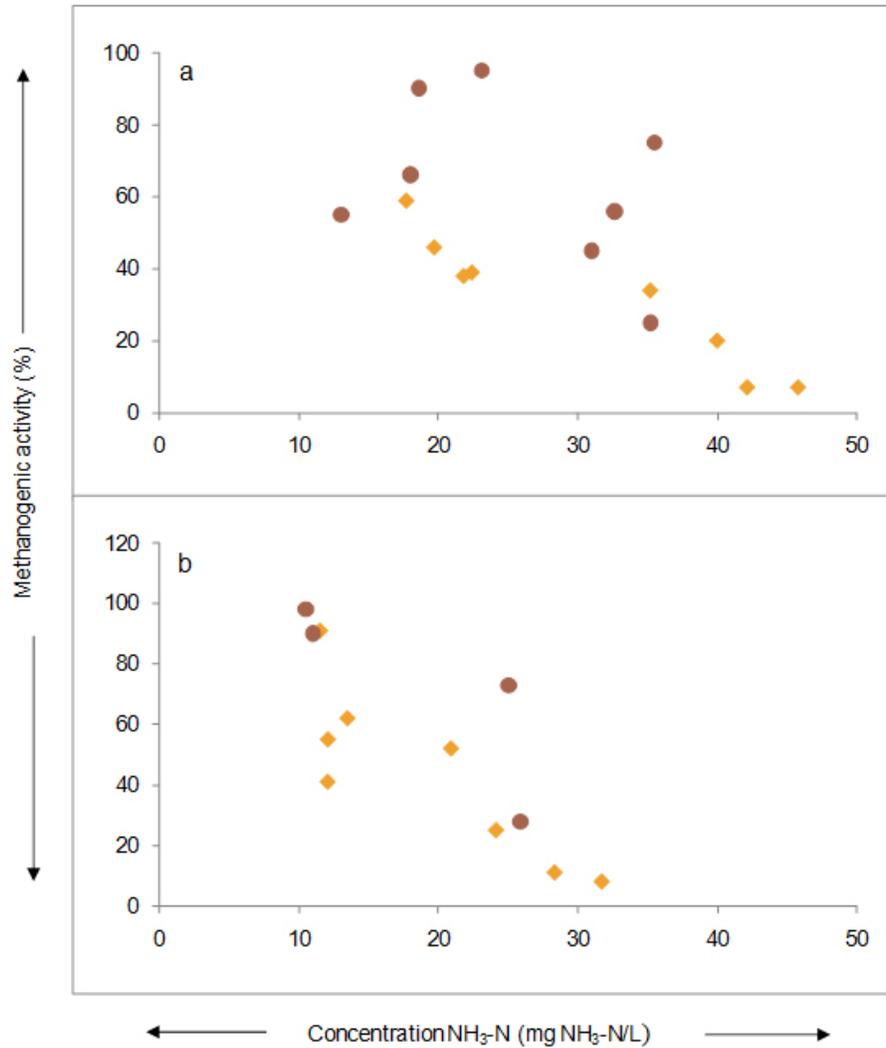
Fig. 2 Behavior methanogenic activity and free ammonia concentration for RW (a) and TW (b). Sludge flocculent (●) and granular (♦).

Table 4. IC$_{50}$ values obtained for the raw and treated effluents during the second feeding.

Biomass			
Type	**Concentration (g VSS/L)**	**Substrate**	**IC$_{50}$ ± S.D. (%)**
Flocculent	2.0	RW	85 ± 1
		TW	n.d.
Granular	2.0	RW	67 ± 2
		TW	84 ± 2
Flocculent	5.0	RW	56 ± 2
		TW	*
Granular	5.0	RW	72 ± 1
		TW	94 ± 1

n.d.: no determined. *Inhibition was lower than 50% even for the effluent without dilution.

CONCLUDING REMARKS

This study shows that methanogenic bacteria -under this conditions investigated, were inhibited by RW (IC_{50}: 56 and 84%). This reduction in the methanogenic activity is associated to the presence of free ammonia nitrogen (NH_3-N) (> 40 mg NH_3-N/L) in the system. On the other hand, a lower reduction of the methanogenic toxicity effect (IC_{50} for TW: between 84 and 94%) was evaluated by TW.

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