



**COD AND AMMONIUM REMOVAL IN SBR OPERATED UNDER DIFFERENT COMBINATIONS USING PRE-TREATED SLAUGHTERHOUSE WASTEWATER**

**REMOCIÓN DE DQO Y AMONIO EN UN REACTOR SBR OPERANDO BAJO DIFERENTES CONDICIONES USANDO AGUA RESIDUAL DE RASTRO PRE-TRATADA**

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Received December 8, 2017; Accepted February 6, 2018

**Abstract**

The slaughterhouse wastewater contains high amount of organic matter; therefore, a pre-treatment is necessary to hydrolyze the protein and diminish the COD concentration in order to produce wastewater of easy degradation. Sequencing batch reactor (SBR) technology has been developed to completely remove nitrogen in one single reactor combining aerobic and anoxic stages. In this work, pre-fermentation of 7 days under anaerobic conditions was carried out; achieving 78% of protein hydrolysis, 139 mg NH<sub>4</sub><sup>+</sup>-N/L was produced and 53% of COD<sub>soluble</sub> was diminished. Bio-degradability assays under oxic/anoxic (O/A) and anoxic/oxic (A/O) conditions using pre-fermented wastewater were performed; raw slaughterhouse wastewater was used as control assay. Low values of protein hydrolysis and removal efficiencies of COD were obtained with pre-fermented wastewater: (O/A) 44% and 46%, and (A/O) 34% and 54%, respectively; whereas with the raw wastewater the hydrolysis and COD removal efficiencies were above 60%. Higher removal efficiencies of total nitrogen were obtained with pre-fermented wastewater under O/A conditions (60%). The pre-fermentation was not able to improve the biodegradability; however, the treatment of raw wastewater under A/O conditions produced an effluent with quality to be discharged in according to Mexican Normative.

*Keywords:* anoxic-oxic, COD, oxic-anoxic, protein, removal, slaughterhouse.

**Resumen**

El agua residual de rastro contiene alta cantidad de materia orgánica; por lo que, con la finalidad de producir un agua residual de fácil degradación es necesario un pre-tratamiento para hidrolizar las proteínas y disminuir la DQO. Los reactores secuenciales discontinuos (SBR) son una tecnología desarrollada para eliminar completamente el nitrógeno en un sólo tanque, combinando etapas óxicas y anóxicas. En este trabajo, agua residual de rastro fue sometida a una pre-fermentación anaerobia durante 7 días, hidrolizando 78% de la proteína, produciendo 139 mg N-NH<sub>4</sub><sup>+</sup>/L y reduciendo 53% de la DQO soluble. Se implementaron ensayos de biodegradabilidad del agua pre-fermentada bajo condiciones óxicas/anóxicas (O/A) y anóxicas/óxicas (A/O), y se utilizó agua cruda como control. Con agua pre-fermentada la hidrólisis de proteína y remoción de la DQO fueron bajas: (O/A) 44% y 46%; (A/O) 34% y 54%, respectivamente; mientras con agua cruda ambos parámetros superaron el 60%. Bajo condiciones O/A con agua pre-fermentada se obtuvieron altas eficiencias de remoción de nitrógeno total (60%). La pre-fermentación no fue capaz de mejorar la biodegradabilidad; sin embargo, el tratamiento con agua residual cruda bajo condiciones A/O produjo un efluente con calidad para ser descargado de acuerdo a la Normatividad Mexicana.

*Palabras clave:* anóxico/óxico, DQO, óxico/anóxico, proteína, remoción, aguas residuales de rastro.

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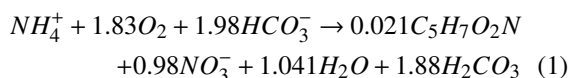
doi: 10.24275/10.24275/uam/izt/dcibi/revmexingquim/2018v17n2/Hernandez

issn-e: 2395-8472

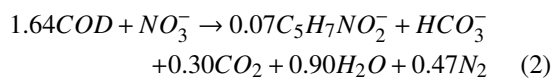
## 1 Introduction

The slaughterhouse industry is an ancient human activity and its environmental impact has grown considerably due to the increase in plant production (Jia *et al.*, 2012). Various solid and liquid wastes such as offal, hair, hooves, ruminal and intestinal contents, blood and washing-water are generated in slaughterhouses (Ramírez-Guerrero *et al.*, 2013). These residues by their nature are considered to be special handling wastes, and the slaughterhouse wastewaters discharged without any treatment into sewage or receiving water bodies are creating serious ecological and public health concerns (Espinosa, 2011). In Mexico, municipal slaughterhouses are requiring about 22,000 m<sup>3</sup>/d of water, and the wastewater generated contains high amounts of pollutants, usually varying from 1,000-20,000 mg/L in terms of COD; 150-10,000 mg/L of total nitrogen; 22-217 mg/L of total phosphorous; 250-5,000 mg/L of suspended solids, and 100-800 mg/L of fats and oils (Zhan *et al.*, 2008). The discharge of these wastewaters into water bodies contributes approximately in 5.8 thousand tons annually of BOD<sub>5</sub> (Signorini *et al.*, 2006). Most slaughterhouse wastewater treatment systems utilize activated sludge, stabilization ponds and anaerobic reactors as main biological treatment technologies (Jia *et al.*, 2012). The anaerobic biological treatment is usually used to treat wastewater with high organic load. This biological process removes organic matter; however, the ammonia content is increased, due to the hydrolysis of proteins. Therefore, a post-treatment is necessary for the ammonium removal produced during anaerobic digestion. The nitrogen cycle, is a good alternative to treat wastewaters polluted with organic and nitrogen compounds (Téllez-Pérez *et al.*, 2017), since can be coupled the nitrification to denitrification process, having as end product, molecular nitrogen (equations 1 and 2).

*Nitrification:*



*Denitrification:*



The amount of protein present in slaughterhouse wastewater is very high; it is therefore necessary

the ammonification in order to increase the amount of NH<sub>4</sub><sup>+</sup> to be oxidized by nitrification. Several biological reactors can be used to treat this type of wastewater; nonetheless, Sequencing Batch Reactor (SBR) is preferred for its small space requirement, low engineering investment and operational cost, effective treatment and convenient operation and management (Obaja *et al.*, 2005). In a full-scale SBR, 82% of BOD and 75% of nitrogen from swine wastewater were also biological removed after a cycle period of 4.6 hours (Lo and Liao, 2007). Previous researchers suggested that a pre-treatment in order to hydrolyze COD partially into fat molecules, might accelerate the anaerobic treatment of slaughterhouse wastewater. Jia *et al.* (2012) proposed acidogenic pre-fermentation of slaughterhouse wastewater, in anaerobic conditions, in order to diminish the concentration of fats and oils and COD and increase the amount of easily biodegradable COD in terms of volatile fatty acids (VFA). The pre-fermentation was later treated in an SBR under Anaerobic/Anoxic/Oxic conditions, achieving higher removal efficiencies for COD and ammonium. This research demonstrated that pre-fermentation could be a good alternative for treating slaughterhouse wastewaters. In literature, the research about combining oxic-anoxic conditions or vice versa to treat slaughterhouse wastewater is even scarce in America, so that the focus on this topic might improve the metabolic and kinetic information that can be used for treating efficiently this type of wastewater.

In the present investigation, an attempt has been made to explore the effect of pre-fermented slaughterhouse wastewater for simultaneous removal of organic matter and ammonium nitrogen under different combinations (Oxic/Anoxic and Anoxic/Oxic).

## 2 Materials and methods

### 2.1 Chemical characterization of slaughterhouse wastewater

Three samplings were performed at three different dates (Table 1). The wastewater was taken from the internal sewer system of a municipal slaughterhouse during different slaughtering processes (scalding, evisceration, slaughter and channel washing). The samples were filtered through a 5 mm mesh screen and were maintained at 4 °C until the analysis.

Table 1. Characterization of slaughterhouse wastewater (n = 3)\*

Compound	Range (mg/L)
$P_{Soluble}$	1,000 - 4,102
$NH_4^+$ -N	41 - 127
$COD_{Soluble}$	2,250 - 5,569

\*n = number of samples

The concentration of ammonium nitrogen ( $NH_4^+$ -N) was quantified using the Phenate Method 4500- $NH_3$ ; the Chemical Oxygen Demand ( $COD_{Soluble}$ ) by Closed Reflux 5220 D and Volatile Solids (VS) by Fixed and Volatile Solids Ignited at 550°C 2540 E according to *Standard Methods* (APHA-AWWA-WEF, 2005). The soluble protein was quantified ( $P_{Soluble}$ ) by the Lowry method (García and Vázquez, 1998).

## 2.2 Pre-fermentation

The pre-fermentation was carried out in a reactor of 500 mL, with operational volume of 400 mL. Two assays were performed; for instance, the first assay consisted of inoculating raw wastewater with 20 mL of methanogenic sludge into anaerobic reactor; the anaerobic sludge inoculated had specific activity of 7.69 mL  $CH_4$ /g VS h. The second assays were carried out using raw wastewater without inoculum. Both treatments were carried out in triplicate and were incubated at 30 °C for a period of 5, 7 and 10 days.  $NH_4^+$ -N,  $P_{Soluble}$ , and  $COD_{Soluble}$  were determined. The  $NH_4^+$ -N recovery was calculated using next equation (Palatsi et al., 2011):

$$\text{Theoretical } NH_4^+ - N = \frac{\text{Protein produced}}{6.25} \quad (3)$$

The Wilcoxon signed rank sum test was used to identify the best pre-fermentation treatment. In

order to select the optimal pre-fermentation time, an Analysis of Variance (ANOVA) was applied with Kruskal-Wallis by ranks. The analyses were performed using the JMP ver. 8.0 statistical software program.

## 2.3 Biodegradability assays

The pre-fermented wastewater (PW) from assays with inoculum and a pre-fermentation time of 7 days was used in order to evaluate its biodegradability, in batch tests. The Raw Water (RW) was used as control assay. The chemical composition of the PW and RW is shown in Table 2. Two SBR reactors of 500 mL were used. The biological reactors were inoculated with 60 mL of mixture containing nitrifying and denitrifying sludge (1.76 g VS/L). The nitrifying sludge was taken from Industrial Wastewater Treatment Plant located in Mexico, which is treating more than 200 factories; this sludge had specific activity of 0.6 g  $NH_4^+$ -N/g VS d. The denitrifying sludge was taken from Industrial Wastewater Treatment Plant installed in the Costeña Food Company, in Mexico. The denitrifying sludge had specific activity of 1.2 g  $NO_3^-$ /g VS d. The nitrifying and denitrifying sludges were acclimated to conditions: O/A and A/O, for 40 days before to be evaluated in batch assays.

## 2.4 Operational conditions

The biodegradability assays were carried out in one cycle of 6 hours under Oxidic/Anoxic (O/A) and Anoxic/Oxidic (A/O) conditions at  $25 \pm 2$  °C. The O/A cycle consisted of three following periods: 210 min of aeration at 0.5 mL/min keeping a dissolved oxygen concentration of 6 mg/L. and stirring at 100 rpm (nitrifying stage); 135 min of stirring without aeration (denitrifying stage), and 15 min of sedimentation.

Table 2. Chemical composition of pre-fermented wastewater and raw water.

Culture conditions	Parameters (mg/L)	RW	PW
O/A	$NH_4^+$ - N	47 ± 0.6	191 ± 0.3
	$NO_3^-$ - N	6 ± 0.01	8 ± 0.01
	$COD_{soluble}$	577 ± 3.5	431 ± 1.8
	$P_{Soluble}$	71 ± 0.8	78 ± 1.6
A/O	$NH_4^+$ - N	47 ± 0.3	188 ± 1.6
	$NO_3^-$ - N	97 ± 0.01	76 ± 0.01
	$COD_{soluble}$	598 ± 12.4	432 ± 5.3
	$P_{Soluble}$	76 ± 1.3	79 ± 1.6

The A/O cycle was operated at 210 min of stirring without aeration (denitrifying stage); 135 min with aeration at 0.5 mL/min and stirring at 100 rpm (nitrifying stage), and 15 min of sedimentation. For the A/O assays, wastewater was supplemented with  $\text{NO}_3^-$ , in according to equation 2. Initial pH in the reactors, in both combinations (A/O and O/A), were around 7.5, and the end of the assays were around 8.5. The number of cycles was controlled by PLC program (SBE-OP380300170 model Tech, Dataloger). The SBR reactors were fed as batch cultures. For the assays with PW, influent was taken from the pre-fermentation tank, while for the assays with RW it was taken from the storage tank and diluted with tap water, in order to adjust the COD with the purpose of achieving the same concentration contained in PW (Table 2). Samples were withdrawn at different times;  $P_{\text{Soluble}}$ ,  $\text{COD}_{\text{Soluble}}$ ,  $\text{NH}_4^+\text{-N}$ , nitrite ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ) were determined.  $\text{NO}_2^-$  and  $\text{NO}_3^-$  were analyzed by liquid chromatography (HPLC), with an anion exchange column (Dionex IonPac AS16, 4 x 250 mm), UV detector at 230 nm and a mobile phase (NaOH 20 mM). Finally, the slopes of the lineal regression graphics for each experiment were used for calculating specific rates; the correlation coefficient ( $R^2$ ) was above 95% for all experiments.

### 3 Results and discussion

#### 3.1 Chemical characterization of slaughterhouse wastewater

The slaughterhouse wastewater presented high variability (Table 1); this was due to the amount of slaughtered animals during the water collection. The wastewater was collected directly from the drainage system without any previous treatment, in contrast with other studies. For example, Cassidy and Belia (2005) reported values of  $\text{COD}_{\text{Soluble}}$  and  $\text{NH}_4^+\text{-N}$  of 5,163 mg/L and 50 mg/L, respectively. On the other hand, Sindhu and Meera (2012) analyzed wastewater collected from the sedimentation tank of a municipal slaughterhouse, determining total protein concentrations of 2,523 mg/L, and 102 mg  $\text{NH}_4^+\text{-N/L}$ . The values reported above are within the ranges determined in the presented work.

#### 3.2 Pre-fermentation of slaughterhouse wastewater

Pre-fermentation assays were carried out in order to increase the protein hydrolysis into  $\text{NH}_4^+\text{-N}$  and diminish the organic matter concentration, at 10 days of incubation period. Figure 1 shows the consumption and production profiles during pre-fermentation. The assays with inoculum showed the greatest protein hydrolysis, since it diminished from 1,451 mg/L to 105 mg/L. The percentage of  $\text{NH}_4^+\text{-N}$  recuperation and organic matter removal were 50 and 83%, respectively (Table 3). The treatment without inoculum presented lower efficiencies on  $\text{NH}_4^+\text{-N}$  recuperation (approx. 38%), as well as in the organic matter removal (approx. 69%). These values are in accordance with Jia *et al.* (2012), who, in 7 days of pre-fermentation, without inoculum and at temperature of 30 °C, achieved organic matter removal efficiencies of 65%, with the production of 98 mg/L of  $\text{NH}_4^+\text{-N}$ .

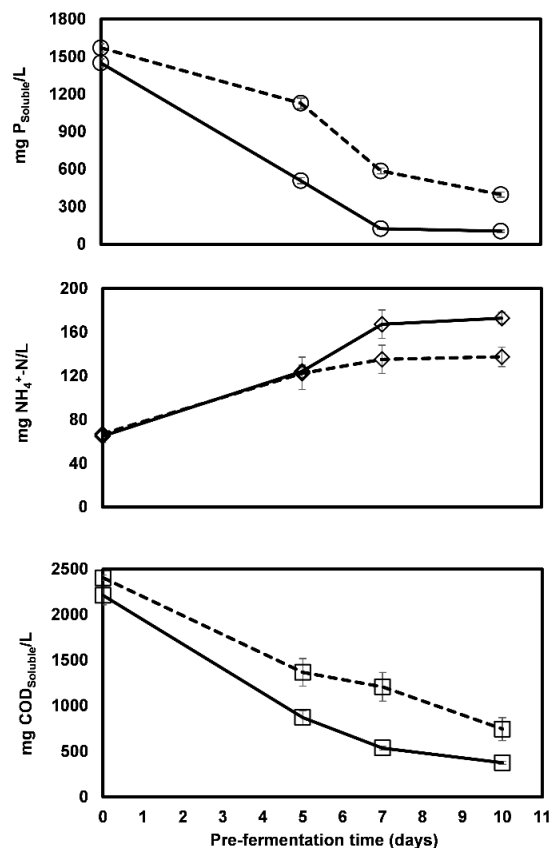


Fig. 1 Concentration profiles for protein, nitrogen and COD during pre-fermentation of slaughterhouse wastewater. Comparative assay: Methanogenic inoculum (-) without inoculum (- -).

Table 3. Ammonium recuperation and COD removal in the pre-fermentation assays (10 days).

Compound	Treatment	
% Recuperation of $\text{NH}_4^+\text{-N}$	Inoculum	$50 \pm 2$
	Without inoculum	$38 \pm 5$
% Removal $\text{COD}_{\text{Soluble}}$	Inoculum	$83 \pm 1$
	Without inoculum	$69 \pm 5$

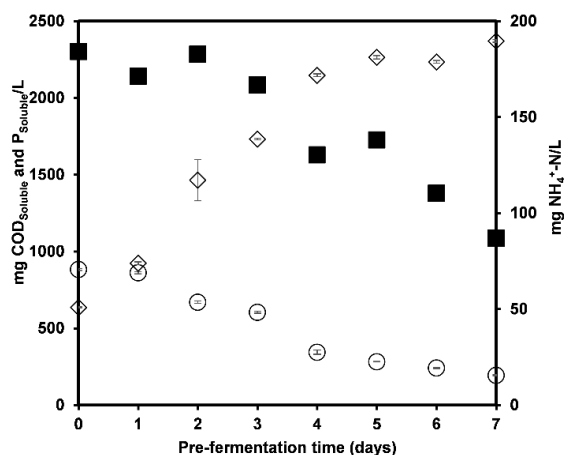


Fig. 2 Profile of protein hydrolysis, ammonium formation and organic matter degradation in 7 days of pre-fermentation with inoculum. COD (■), Ammonium (◇), Protein (○).

According to the statistical analysis and taking account the formation of  $\text{NH}_4^+\text{-N}$  as response variable, significantly differences were found for pre-fermentation days 5, 7 and 10 ( $p = 0.0006$ ), however between 7 and 10 days the formation  $\text{NH}_4^+\text{-N}$  was the same ( $p = 0.8247$ ). A significantly effect for the treatments with inoculum and without inoculum was not observed ( $p = 0.2481$ ). Therefore, the treatment with inoculum was selected where the greatest organic matter removal and protein hydrolysis were achieved and 7 days of pre-fermentation.

To obtain a complete pre-fermentation profile, pre-fermentation during 7 days with inoculum was performed. Protein hydrolysis, ammonium formation and organic matter consumption were daily monitored. The initial concentrations spiked were 2,300 mg  $\text{COD}_{\text{Soluble}}/\text{L}$ , 884 mg  $\text{P}_{\text{Soluble}}/\text{L}$  and 51 mg  $\text{NH}_4^+\text{-N}/\text{L}$ . It is noteworthy that the initial concentration of  $\text{P}_{\text{Soluble}}$  in this pre-fermentation was inferior to that reported in the first pre-fermentation, this was due that wastewater samples were refrigerated, and under these environmental conditions, the chemical

composition of the wastewater changes, affecting mainly the soluble protein (data not shown). The results are plotted in the Fig. 2. The protein was hydrolyzed at rate of 0.01 mg  $\text{P}_{\text{Soluble}}/\text{min}$ ; the conversion efficiency was of 78% with production of 139 mg  $\text{NH}_4^+\text{-N}/\text{L}$ . The organic matter removal efficiency obtained was 53%, this value differed regarding to the first pre-fermentation, in which an efficiency of 83% was attained; this can be due to the fact that initial concentration in this pre-fermentation assay was slightly higher. Finally, the experimental results showed that 7 days of pre-fermentation was enough to produce wastewater with high amount of ammonium and low organic matter content.

### 3.3 Biodegradability assays under oxic/anoxic conditions

#### 3.3.1 Assays with raw water

The organic matter is the energy source for heterotrophic activity (aerobic phase) and denitrifying bacteria (anoxic phase), and it was estimated as chemical oxygen demand (COD). SBR was spiked with initial concentrations of 577 mg  $\text{COD}_{\text{Soluble}}/\text{L}$ , 47 mg  $\text{NH}_4^+\text{-N}/\text{L}$  and 71 mg  $\text{P}_{\text{Soluble}}/\text{L}$ . The results are plotted in the Fig. 3a. The major fraction of COD removal took place within 3.5 h of aerobic phase. The organic matter was consumed at rate of 1.72 mg  $\text{COD}_{\text{Soluble}}/\text{min}$ , with specific rate of 1.41 g  $\text{COD}_{\text{Soluble}}/\text{g VS d}$ . The COD removal efficiency obtained was of 61% as can be seen in Table 4. Pan *et al.* (2014) showed COD removal efficiencies of 98.2% treating slaughterhouse wastewater at 11°C in an intermittently aerated sequencing batch reactor (IASBR) with an aeration rate of 0.6 L air/min. The removal efficiency also depends on the reaction time. For instance, Kundu *et al.* (2013) indicated that the carbon utilizing bacteria are able to degrade more COD with production of new cells due to enhancement of aeration time. In the present work, the reaction time was around 6 h, so that the increase of time might improve the COD removal efficiency.

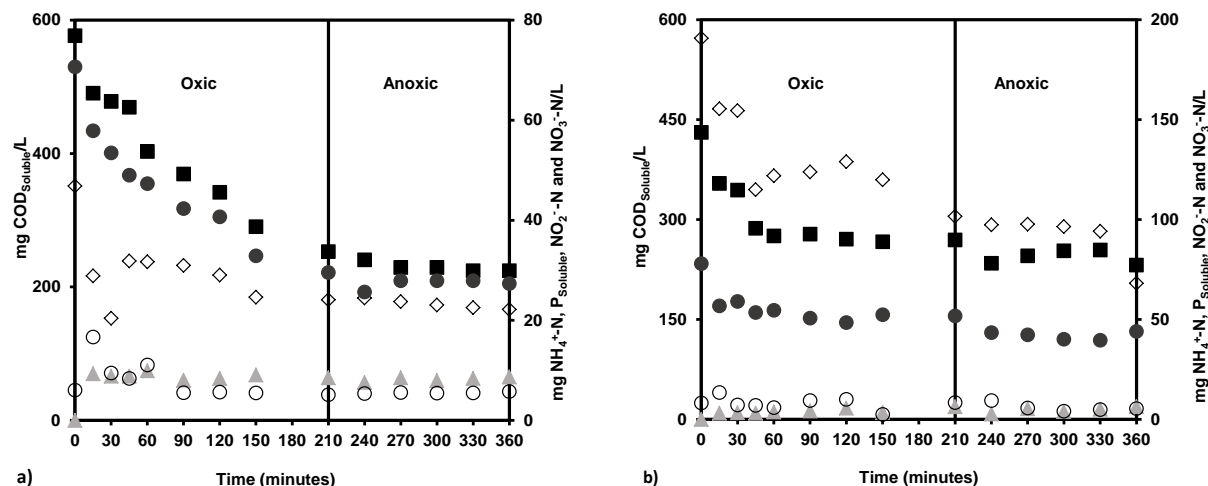


Fig. 3 Biodegradability assays under O/A conditions. a) raw water, b) pre-fermented water: COD (■), Ammonium (◇), Protein (●), Nitrite (▲), Nitrate (○).

Table 4. Removal efficiencies achieved in the biodegradability assays.

Condition	RW (%)	PW (%)
O/A	TN = 31	TN = 60
	COD <sub>soluble</sub> = 61	COD <sub>soluble</sub> = 46
A/O	TN = 50	TN = 36
	COD <sub>soluble</sub> = 71	COD <sub>soluble</sub> = 54

Table 5. Specific rates for the biodegradability assays in the different cycles tested.

Cycle	(RW)		(PW)	
	Rates (mg/min)	Specific rates (g/g VS d)	Rates (mg/min)	Specific Rates (g/gVS d)
Oxic/Anoxic				
NH <sub>4</sub> <sup>+</sup> -N	0.88	0.72	1.52	1.24
COD <sub>Soluble</sub>	1.72	1.41	2.95	2.41
Anoxic/Oxic				
NO <sub>3</sub> <sup>-</sup> -N	0.38	0.31	0.28	0.23
NH <sub>4</sub> <sup>+</sup> -N	0.13	0.10	0.49	0.39
COD <sub>Soluble</sub>	1.72	1.40	0.63	0.51

In oxic phase, in the first 30 min ammonium started to be produced linked to protein consumption, reaching 25 mg/L of NH<sub>4</sub><sup>+</sup>-N/L at the end of the experiment. Nitrite and nitrate was detected, showing values below 10 mg N/L. A partial nitrifying activity was observed, since 10 mg/L of nitrite nitrogen was produced. This metabolic behavior was observed in the first 30 min, after that, nitrite production ceased. The respiratory process of nitrifying could have been stopped due to the presence of certain organic inhibitors. For instance, in slaughterhouse

wastewater there are volatile fatty acids (VFA) usually varying from 152-265 mg/L of VFA in terms of COD, as was reported by Jia *et al.* (2012). Oguz *et al.* (2006) showed evidences that nitrite removal and nitrate formation can be affected by the presence of VFAs, but to different degrees. The rate obtained for ammonium was 0.88 mg NH<sub>4</sub><sup>+</sup>-N/min, with specific rate of 0.72 g NH<sub>4</sub><sup>+</sup>-N/g VS d (Table 5), which is lower than that reported by Campos *et al.* (2002), whose obtained an activity of 1.13 g NH<sub>4</sub><sup>+</sup>-N/g VS d under nitrifying conditions. In the present work, total

nitrogen removal efficiency was of 31% (Table 4). Similar results for nitrogen removal were reported by Figueroa *et al.* (2008), who worked with an effluent of fish conserves in a granular aerobic reactor, and the removal efficiencies did not exceed 45%. It is important to mention that there are several factors that can affect the kinetics and metabolism of nitrification, such as, type of inoculum, kind of wastewater, initial inoculum concentration, and dissolved oxygen, among others.

In anoxic phase, two biological process were expected, methanogenesis or denitrification. Nonetheless, methanogenic activity can be inhibited by the presence of nitrite or nitrate. Banihani *et al.* (2009) showed 95% of inhibition of the methanogenesis at lowest concentrations of  $\text{NO}_2^-$  or  $\text{NO}_3^-$  added, 7.6-10.2 mg N/L and 8.3-121 mg N/L, respectively. In the present work, in anoxic phase, around 20 mg/L of nitrite plus nitrate nitrogen were present, which might be inhibiting the methanogenic activity. Regarding to denitrification, organic matter can be oxidized using nitrate or nitrite as electron acceptor; but denitrification with nitrite is a very slow biological reaction, as was reported by González-Blanco *et al.* (2013). In anoxic phase, denitrification with nitrite could have taken place; however, organic matter was not consumed. Perhaps, it was required more reaction time in order to denitrification takes place.

### 3.3.2 Assays with pre-fermented water

The initial concentrations spiked were 431 mg  $\text{COD}_{\text{Soluble}}/\text{L}$ , 191 mg  $\text{NH}_4^+\text{-N}/\text{L}$  and 78 mg  $\text{P}_{\text{Soluble}}/\text{L}$  (Fig. 3b). Under these operating conditions, the organic matter was consumed in the first 60 minutes, at rate of 2.95 mg  $\text{COD}_{\text{Soluble}}/\text{min}$ , with specific rate of 2.41 g  $\text{COD}_{\text{Soluble}}/\text{g VS d}$ . After 60 min, the organic matter consumption ceased, probably it was due to the presence of organic matter of recalcitrant type. The COD removal efficiency was low, around 46%. For instance, Val del Río *et al.* (2013) reported specific rates of 2.07 g  $\text{COD}_{\text{Soluble}}/\text{g VS d}$  and COD removal efficiencies of 90%; in an aerobic granular biomass reactor treating wastewater coming from seafood industry. In the present work, the COD specific consumption rate was 15% faster than reported by those authors. Regarding to nitrogen compounds behavior, for example the ammonium, it was consumed relatively slow during both phases, but the greater consumption was in anoxic phase. The fate

of ammonium nitrogen consumption could be used for synthesis of new cells. Ammonium was consumed at rate of 1.52 mg  $\text{NH}_4^+\text{-N}/\text{min}$ , with specific rate of 1.24 g  $\text{NH}_4^+\text{-N}/\text{g VS d}$ , as can be seen in Table 5. In oxic phase, neither nitrite nor nitrate were produced. In anoxic phase, nitrite and nitrate concentrations were kept without change. Finally, the total nitrogen removal efficiency was of 60%.

## 3.4 Biodegradability assays under anoxic/oxic conditions

### 3.4.1 Assays with raw water

Initial concentrations evaluated were 598 mg  $\text{COD}_{\text{Soluble}}/\text{L}$ , 47 mg  $\text{NH}_4^+\text{-N}/\text{L}$ , 76 mg  $\text{P}_{\text{Soluble}}/\text{L}$  and 97 mg  $\text{NO}_3^-\text{-N}/\text{L}$  (Fig. 4a). Nitrate was supplemented as electron acceptor to oxidize the organic matter in anoxic phase. In anoxic phase, in the first minutes, nitrate started to be consumed, reaching a rate of 0.38 mg  $\text{NO}_3^-\text{-N}/\text{min}$ , with specific rate of 0.31 g  $\text{NO}_3^-\text{-N}/\text{g VS d}$ . At the end of anoxic phase, nitrate nitrogen was recovery, in part, as nitrite nitrogen, around 50 mg  $\text{NO}_2^-\text{-N}/\text{L}$ . Regarding to COD profile, it was consumed at rate of 1.72 mg  $\text{COD}_{\text{Soluble}}/\text{min}$ , with specific rate of 1.40 g  $\text{COD}_{\text{Soluble}}/\text{g VS d}$ . The efficiency of COD removal was of 71%. An electron balance indicated that 300 mg/L of COD consumed donated about 37.5 mEq of  $e^-/\text{L}$ . Taking account that nitrate was reduced to nitrite, mainly, and the other fraction to  $\text{N}_2$ , so that the electrons accounted were 12.49 mEq of  $e^-/\text{L}$ . This means that COD consumed was not totally coupled to denitrification, suggesting that the other fraction of COD consumed might be followed another fate, for instance, methanogenesis. Mosquera-Corral *et al.* (2001) in a lab-scale hybrid upflow bed-filter reactor, treating effluents from a fish canning industry, showed the simultaneous methanogenesis and denitrification.

In oxic phase, COD, ammonium, nitrate and nitrite presented a consumption profile. The nitrite and nitrate consumption, under these aerobic conditions, was not expected. These experimental results might be suggesting that COD was coupled to nitrite and nitrate consumption, but for aerobic denitrification. For example, supporting this theory, Chen and Strous (2013) showed the biochemical mechanism for aerobic denitrification, and Yao *et al.* (2013) showed evidences of heterotrophic nitrification and aerobic denitrification, with efficient removal of COD and TN from synthetic wastewater, in aerated batch tests.

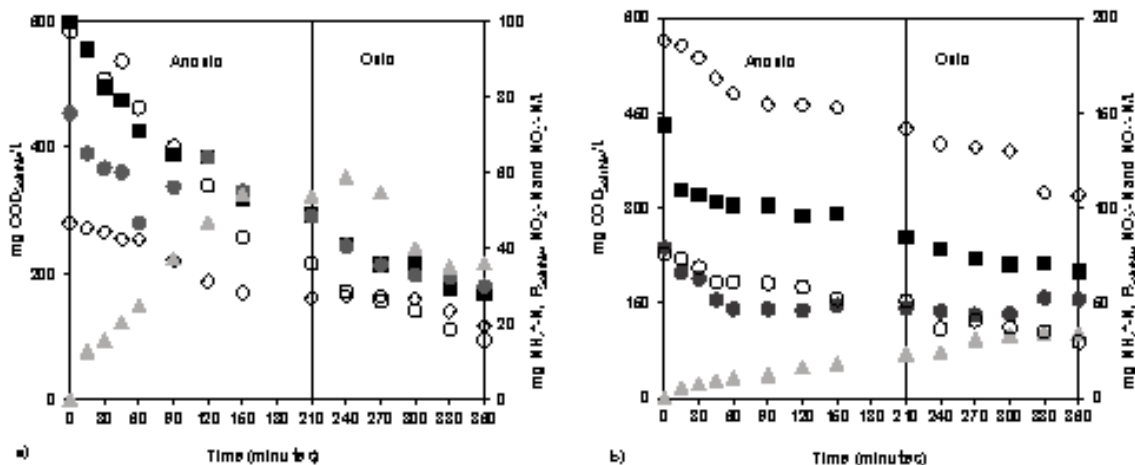


Fig. 4 Biodegradability test A/O. a) raw water, b) pre-fermented water: COD (■), Ammonium (◇), Protein (●), Nitrite (▲), Nitrate (○).

### 3.4.2 Assays with pre-fermented water

Initial concentrations evaluated were 432 mg COD<sub>Soluble</sub>/L, 188 mg NH<sub>4</sub><sup>+</sup>-N/L, 79 mg P<sub>Soluble</sub>/L and 76 mg NO<sub>3</sub><sup>-</sup>-N/L (Fig. 4b). COD and nitrate were consumed from the beginning. The organic matter was consumed at 1.72 mg COD<sub>Soluble</sub>/min, with specific rate of 1.40 g COD<sub>Soluble</sub>/g VS d. NO<sub>3</sub><sup>-</sup> was consumed at rate of 0.38 mg NO<sub>3</sub><sup>-</sup>-N/min, with specific rate of 0.31 g NO<sub>3</sub><sup>-</sup>-N/g VS d. The COD removal efficiency was of 54%. The COD consumption was equivalent to 13 mEq of e<sup>-</sup>/L donated. Nitrate was completely reduced to Nitrite, accepting about 9 mEq of e<sup>-</sup>/L. The electron balance in terms of milliequivalents (mEq) suggested that COD oxidation was coupled to partial denitrification. On the other hand, ammonium consumption could be used for microbial growth, since heterotrophic denitrifiers can have doubling times of less than 12 hours (Komor and Fox, 2002).

In oxic phase, ammonium was oxidized, with increase of nitrite, suggesting partial nitrification, since nitrate was not produced. Regarding to COD, it was also in part consumed. The low efficiency of organic matter removal might be due to the COD/NO<sub>3</sub><sup>-</sup> ratio. Chiu *et al.* (2007) reported that to achieve good denitrification in coupled systems, COD/NO<sub>3</sub><sup>-</sup> ratio should be ranging between 6.0 and 8.0. In the present work, the ratios obtained for the O/A cycle with RW and PW were 10.24 and 10.65; while for the A/O cycle were 1.38 and 1.27, respectively. The C/N ratio in the wastewater links the availability and competition for COD and NH<sub>4</sub><sup>+</sup>. For a high C/N ratio, heterotrophic bacteria outcompete with nitrifiers

for available oxygen, but when the C/N ratio is relatively low nitrifiers are able to outcompete with heterotrophic bacteria. However, the critical C/N ratio affecting the nitrification rate is related to the chemical characteristics of the COD available (Michaud *et al.*, 2006).

### 3.5 Comparison between raw water and pre-fermented water

In the experiments with pre-fermented water, under Oxidic-Anoxic conditions, the specific rates for ammonium and COD were 1.72 g NH<sub>4</sub><sup>+</sup>-N/g VS d and 1.70 g COD<sub>Soluble</sub>/g VS d fold faster than raw water, respectively. For the experiments for pre-fermented water, under Anoxic-Oxidic conditions, the specific rate for ammonium was 3.9 g NH<sub>4</sub><sup>+</sup>-N/g VS d fold faster than raw water. However, with raw water, the COD consumption specific rate was 2.74 g COD<sub>Soluble</sub>/g VS d fold faster than pre-fermented water. In all experiments the proteins were consumed as part of the COD. In both studies, the removal efficiencies for COD and NH<sub>4</sub><sup>+</sup>-N were low values. In the studies with RW, the COD removal efficiencies were approximately 32% better than the studies with PW. In the case of the ammonium removal efficiencies, under O/A conditions, the better removal efficiency was presented in the pre-fermented water. For the A/O assays, the better ammonium removal efficiency was presented in the raw water, and in this condition was produced an effluent with quality to be discharged in according to the NOM-CCA-022-ECOL/1993 (DOF, 1993).



## Conclusions

The present experimental investigation demonstrated that sequential batch reactor is feasible to treat slaughterhouse wastewater in a single unit. 7 days of pre-fermentation using methanogenic sludge was enough to get higher COD removal and protein hydrolysis, 53% and 78%, respectively. The better kinetic behavior was presented in the experiments with raw water, since in the Oxic-Anoxic conditions, specific rates for ammonium and COD were 1.72 g  $\text{NH}_4^+$ -N/g VS d and 1.70 g  $\text{COD}_{\text{Soluble}}$ /g VS d fold faster than pre-fermented water, respectively. Higher removal efficiencies of total nitrogen were obtained with pre-fermented wastewater under O/A conditions (approx. 60%). Contrary to the literature this study shows that the pre-fermentation was not able to improve the biodegradability; however, the treatment with raw water under A/O conditions produced an effluent with quality to be discharged in according to Mexican Normative.

## Acknowledgements

The first author thanks the support of the Consejo Nacional de Ciencia y Tecnología (CONACYT) for studying the master's degree in Ciencias Agropecuarias, within the program at the Universidad Autónoma Metropolitana-Xochimilco, included in the pattern of excellence with the agreement 000357. To Beatriz Schettino Bermúdez for the support in analytical techniques.

## Abbreviations

A/O	Anoxic/Oxic
$\text{COD}_{\text{Soluble}}$	soluble chemical oxygen demand
mEq	milliequivalents
$\text{NH}_4^+$ -N	nitrogen of ammonium
$\text{NO}_2^-$ -N	nitrogen of nitrite
$\text{NO}_3^-$ -N	nitrogen of nitrate
O/A	Oxic/Anoxic
PW	pre-fermented water
RW	raw water
$\text{P}_{\text{Soluble}}$	soluble Protein
VS	volatile solids
VFA	volatile fatty acids

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