



## Salt inhibition on anaerobic treatment of high salinity wastewater by upflow anaerobic sludge blanket (UASB) reactor

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### ABSTRACT

The adverse effects of salt concentration on anaerobic treatment of synthetic high salinity wastewater were investigated using four lab-scale upflow anaerobic sludge blanket reactors at different salt (NaCl) concentrations. The reactors were inoculated with granular sludge previously not adapted to salinity, and they were fed with synthetic wastewater containing the salt concentrations of 0, 10, 25, and 50 g L<sup>-1</sup> NaCl. Hydraulic retention time and organic loading rate were kept constant at 1 d and 2 kg chemical oxygen demand (COD) m<sup>-3</sup> d<sup>-1</sup>, respectively. Salt inhibition on the COD removal rate and efficiency, methane production were determined. COD removal rate and efficiency significantly decreased when the salt concentration in the feed increased to 50 g L<sup>-1</sup> from 0. The maximum COD removal was obtained at salt concentration of 10 g L<sup>-1</sup>.

*Keywords:* Anaerobic treatment; UASB; High salinity; NaCl; Inhibition

### 1. Introduction

Salinity is one of the most important problems faced in wastewater treatment. High-saline wastewaters that have salt concentrations above 1% are generated from industries such as vegetable, tanning, seafood processing, pickling and cheese processing, chemical manufacturing, pharmaceuticals, and fertilizer [1–4]. Besides, brines containing organic compounds and at least 3.5 w/v total dissolved solids (TDS) are called as hypersaline wastewaters and their sources are chemical manufacturing, oil and gas production operations, meat packing/hidecuring industry, vegetable, vegetable oil, dairy, and landfill leachates [3,5–7].

Biological treatment of saline wastewater usually results in low biological oxygen demand (BOD)

removal performance because of the adverse effects of salt on microbial flora [8]. High salt concentrations disrupt metabolic functions and cause disintegration of cells because of the loss of cellular water (plasmolysis) or recession of the cytoplasm which is induced by an osmotic difference across the cell wall and cause of outward flow of intracellular water resulting in the loss of microbial activity and cell dehydration [4,9,10]. Consequently, at high salt concentrations (>2%), chemical oxygen demand (COD) and BOD removal efficiencies decrease and effluent suspended solid concentration increases [4].

High salt content may cause inhibition and toxicity problems on anaerobic digestion, mainly due to cations of which the most common is sodium [1,11]. The sodium concentration exceeding 10 g L<sup>-1</sup> has been reported as inhibiting methanogenesis [11]. There are

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two fundamental strategies for cells to survive under osmotic stress inherent to the presence of high salt concentrations: (a) cells maintain high intracellular salt concentrations (the “salt-in” strategy) and (b) cells may maintain low salt concentrations within their cytoplasm and the osmotic pressure of the cytoplasm is balanced by organic solutes called compatible solutes (the “compatible solute” strategy) [10,12].

The upflow anaerobic sludge blanket (UASB) reactors became the most widely used high-rate anaerobic treatment system throughout the world [13]. UASB process has advantages such as there is no need support material, its efficiency, low costs of investment and operation and ease of operation. Anaerobic microorganisms are the highly sensitive microorganisms to environmental conditions and inhibitory substances in wastewaters. High salinity in wastewater is one of the most important inhibitory substances [3].

Özalp et al. [3] investigated the effect of high salinity on anaerobic treatment of low strength in a lab-scale UASB reactors using synthetic saline wastewater. They found that there was no significant inhibition at 1.5% salt content. Rinzema et al. [14] reported that 5, 10, and 14 g Na<sup>+</sup> L<sup>-1</sup> concentrations were caused 10, 50, and 100% inhibition on methane production from acetate at neutral pH.

The objective of this study was to evaluate the effect of the salt concentration on performance of UASB reactors treating high salinity synthetic wastewater. For this aim, granular sludge previously not adapted to NaCl as inoculum and the synthetic wastewater with high salinity were used.

## 2. Materials and methods

### 2.1. Experimental setup

The continuous treatment of synthetic wastewater was performed in four lab-scale cylindrical UASB reactors made of fiberglass with an effective volume of 5 L. The inner diameter of reactors was 10 cm. The reactors were placed into a temperature-controlled room at 37°C. The experimental setup is illustrated in Fig. 1. The UASB reactors were equipped with gas–solid–liquid separator. The feeding tanks and the effluent collection tanks were made of plexiglas, and the volume of each tank was 9 L. Influent was continuously provided to the UASB reactors using a peristaltic pump (type 323 S/D, Watson-Marlow, Falmouth, UK).

### 2.2. Wastewater composition

Synthetic wastewater prepared according to Tay and Yang [15] was used through experimental study

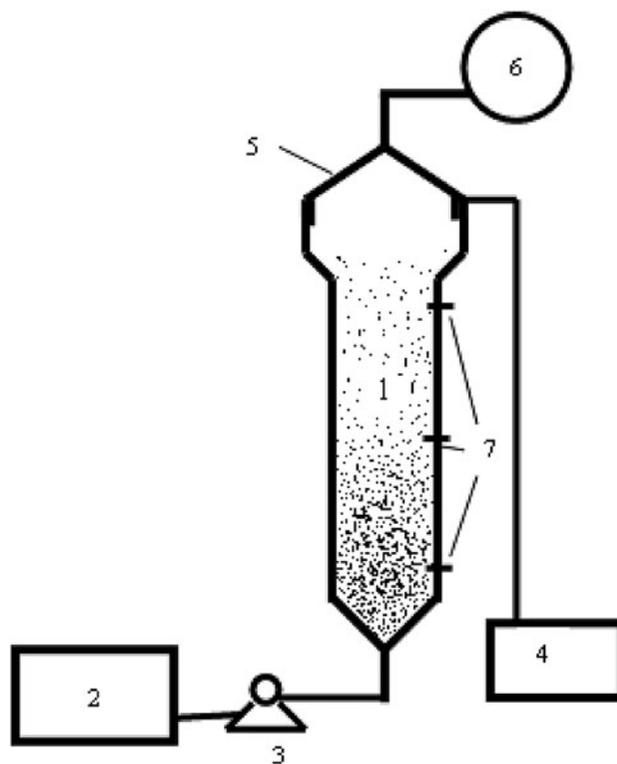


Fig. 1. Schematic diagram of experimental setup ((1) UASB reactor, (2) Influent tank, (3) Peristaltic pump, (4) Effluent tank, (5) Gas–liquid–solid separator, (6) Gas balloon, and (7) Sampling ports).

to maintain uniformity of characteristics of wastewater. Synthetic wastewater composed of macro- and micronutrients required for bacterial growth and its composition is given in Table 1. Glucose was used as the carbon source to give a COD concentration of the feed of about 2,000 mg L<sup>-1</sup>. NaHCO<sub>3</sub> was added to provide the buffering capacity in the anaerobic system. The various concentrations of NaCl (0, 10, 25, and 50 g L<sup>-1</sup>) were added to medium. Demineralized water was used to prepare the influent.

### 2.3. Inoculum characteristics

The reactors were inoculated with granular sludge previously not adapted to NaCl obtained from anaerobic digester of brewery wastewater treatment located at Adana. Total suspended solids (TSS) and volatile suspended solids (VSS) concentration of sludge were 38 and 21 g L<sup>-1</sup>, respectively. The volume of sludge added to each reactor was about 30% of reactor volume.

Table 1  
The composition of synthetic wastewater

Major components	Concentration (mg L <sup>-1</sup> )	Trace elements	Concentration (mg L <sup>-1</sup> )
Glucose	1,400	H <sub>3</sub> BO <sub>3</sub>	0.05
Bacteriological peptone	400	ZnCl <sub>2</sub>	0.05
Meat extract	250	CuCl <sub>2</sub>	0.03
NaHCO <sub>3</sub>	2,000	MnSO <sub>4</sub> ·H <sub>2</sub> O	0.50
NH <sub>4</sub> Cl	200	(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O	0.05
KH <sub>2</sub> PO <sub>4</sub>	45	AlCl <sub>3</sub>	0.05
CaCl <sub>2</sub> ·2H <sub>2</sub> O	300	CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.05
MgSO <sub>4</sub> ·7H <sub>2</sub> O	25	NiCl <sub>2</sub>	0.05
FeSO <sub>4</sub> ·7H <sub>2</sub> O	20		
Na <sub>2</sub> S·7H <sub>2</sub> O	45		

#### 2.4. Experimental conditions

Four UASB reactors used in this study were operated under identical operational conditions during 20 d. Three reactors were fed with synthetic wastewater containing different salt concentrations, while one reactor fed with salt-free synthetic wastewater was used as a control reactor (R4). The salt concentrations (NaCl) were 10 g L<sup>-1</sup> (R1), 25 g L<sup>-1</sup> (R2), and 50 g L<sup>-1</sup> (R3). Hydraulic retention time was kept constant at 1 d. The influent COD concentration was about 2,000 mg L<sup>-1</sup> through experimental period.

#### 2.5. Analytical procedure

COD, alkalinity, total solids (TS), TSS, and VSS were done according to Standard Methods (1989). Mercuric sulfate was added to the diluted samples to precipitate chloride ions in form of HgCl<sub>2</sub> in order to eliminate the interference of chlorides to COD analysis at all salt concentrations. pH and conductivity measurements were done by using ORION 420A pH meter and JENWAY 4075 conductivity meter, respectively. Total volatile fatty acid analysis was carried out with 114791 test kit by using Nova 60 Spectroquant (Merck). Total biogas production was measured with liquid displacement method. The methane percentage in biogas was determined by Geotech GA2000 gas analyzer. The calcium concentration was analyzed by flame photometer (JENWAY-PFP7).

### 3. Results and discussion

#### 3.1. Effect of salt concentration on COD removal and biogas production

The variation of COD removal efficiencies with different salt concentrations in the wastewater is shown in Fig. 2. COD removal efficiencies were calculated by using following equation;

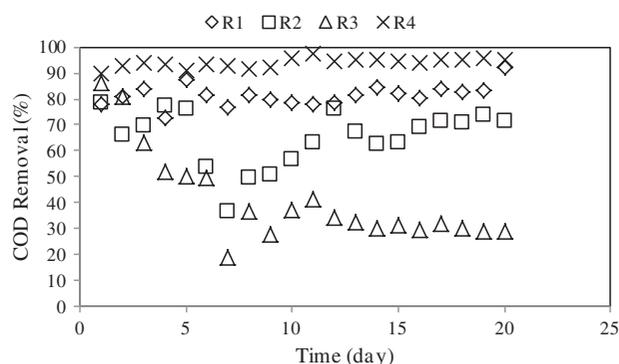


Fig. 2. The variation of COD removal efficiencies of reactors at different salt concentrations in the feed.

$$E = 1 - \frac{S}{S_0} \quad (1)$$

where  $E$  is the COD removal efficiency (%),  $S_0$  is the influent COD concentration (mg L<sup>-1</sup>), and  $S$  is the effluent COD concentration (mg L<sup>-1</sup>). The COD removal efficiency decreased with increasing salt concentration. In particular, COD removal efficiencies of R2 and R3 were significantly decreased. The maximum COD removal efficiency was obtained at R1 and it was between 72 and 92%. COD removal efficiency of non-salt-exposed control reactor, R4, was between 90 and 97.5%. The decrease of COD removal efficiency may be attributed to dehydration of anaerobic bacterial cells resulting in loss of metabolic activity due to osmotic pressure at high salt contents. Lefebvre et al. [11] treated tannery wastewater containing 71 g L<sup>-1</sup> salt and 2.3 g L<sup>-1</sup> COD by UASB reactor and they obtained 78% COD removal. In the study done by Işık [16] was investigated the effect of salt concentration on the performance of UASB reactor treating

simulated cotton textile wastewater. He founded that optimum COD removal efficiency was 80.5% in salt concentration of  $32 \text{ g L}^{-1}$ , and effluent COD concentration increased with increasing salt concentration in the reactor. Habets et al. [17] obtained 65–80% COD removal in treatment by UASB reactor of inuline effluent containing  $10 \text{ g L}^{-1}$  salt concentration and  $7.9 \text{ g L}^{-1}$  COD. Chunshuang et al. [18] used UASB reactor for treating high salinity wastewater from heavy oil production with salinity of  $11.5\text{--}14.6 \text{ g L}^{-1}$  at different organic loading rates (OLRs). They observed that when OLR was increased from  $0.23$  to  $0.61 \text{ kg COD m}^{-3} \text{ d}^{-1}$ , COD removal rate decreased from 65.08 to 55.21%.

COD removal rates were calculated by using following formulation;

$$R_S = \frac{Q(S_0 - S_e)}{V} \quad (2)$$

where  $R_S$  is the COD removal rate ( $\text{mg L}^{-1} \text{ d}^{-1}$ ),  $Q$  is the flow rate ( $\text{L d}^{-1}$ ),  $S_0$  is the COD concentration of synthetic wastewater ( $\text{mg L}^{-1}$ ), and  $S_e$  is the effluent COD concentration ( $\text{mg L}^{-1}$ ).  $R_S$  values are given in Fig. 3. The  $R_S$  values were decreased with increasing salinity.  $R_S$  values for R1, R2, and R3 were 1.52–1.88, 0.73–1.64, and 0.38–1.76, respectively.  $R_S$  values of the control reactor were between 1.83 and 1.94.

The produced biogas and methane volume, and COD conversion rate are illustrated in Fig. 4(a, b, c, and d). As salt concentration increased, the produced biogas and methane volume decreased. High salinity may cause inhibition and toxicity problems in the methanogenic activity [1]. The decrease of methane production under saline conditions may be attributed to two reasons: (a) the consumption of substrate by anaerobic biomass to generate compatible solutes and extracellular polysaccharides to survive under high

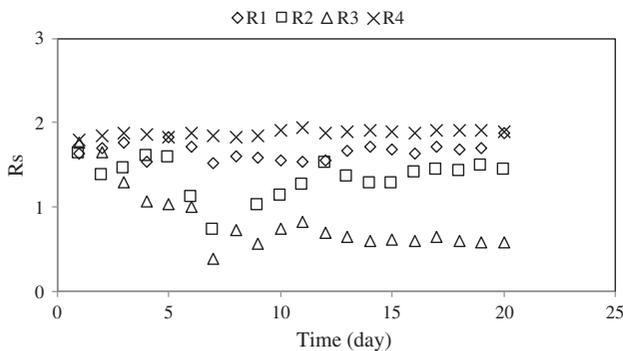


Fig. 3. The  $R_S$  values of reactors at different salt concentrations in the feed.

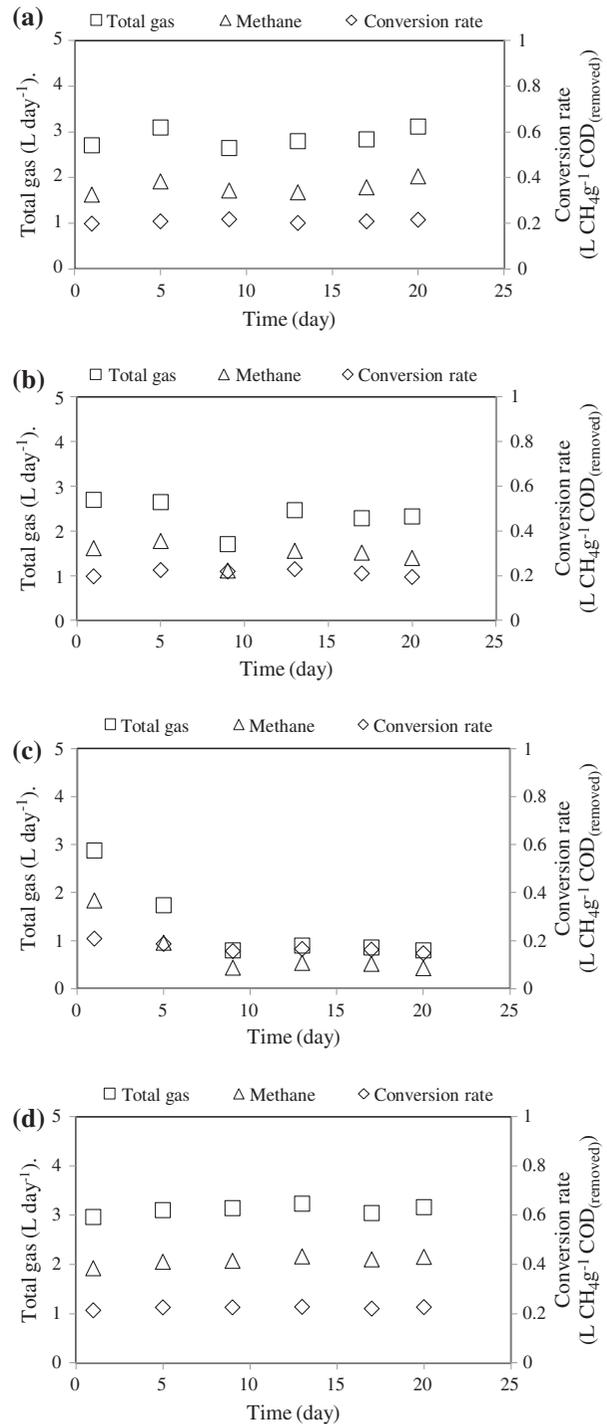


Fig. 4. The total gas volume, methane volume, and conversion rate of reactors at different salt concentrations in the feed. (a) R1, (b) R2, (c) R3, and (d) R4.

osmotic conditions, (b) the production of high molecular weight organics in the reactor possibly due to the release of extracellular compounds during metabolism, enhancement of cell lysis, or stimulation of

efflux mechanisms [19,20]. Rovirosa et al. [21] used down-flow anaerobic fixed bed reactor for treating low-strength saline wastewater. They operated reactors at different HRTs and found that an increase of HRT caused a decrease of biogas and methane production. In their study, biogas and methane productions at HRTs of 12, 24, 48, and 96 h were 0.65, 0.36, 0.19, 0.12 L d<sup>-1</sup> and 0.30, 0.25, 0.14, 0.09 L d<sup>-1</sup>, respectively.

### 3.2. The variation of effluent pH, alkalinity, VFA values

The effluent pH values were remained around 7.0–7.8 for all reactors during experimental study. The alkalinity values of effluent are given in Fig. 5. As seen from Fig. 5, alkalinity value of R3 reactor decreased to 800 mg L<sup>-1</sup> CaCO<sub>3</sub>. Fig. 6 depicts the variation of effluent VFA concentrations depending on salt concentration. While VFA concentration of control reactor was generally between 61 and 84 mg L<sup>-1</sup> CH<sub>3</sub>COOH, VFA concentrations of other reactors were significantly changed compared to that control reactor.

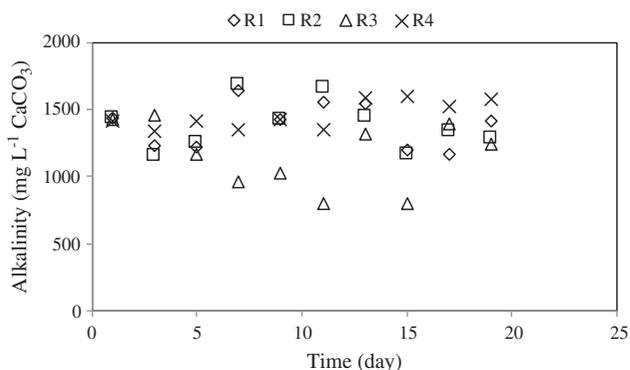


Fig. 5. The effluent alkalinity values of reactors.

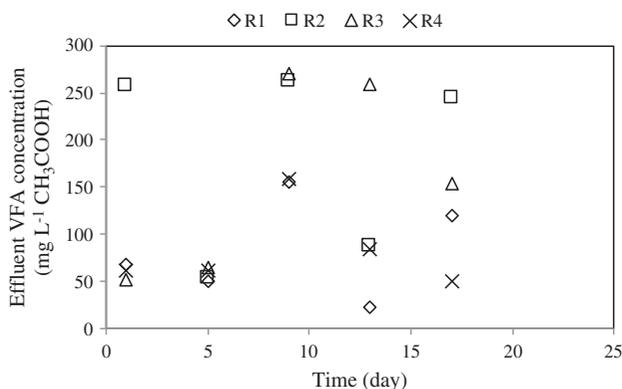


Fig. 6. The effluent VFA concentrations of reactors.

### 3.3. Effect of salt concentration on TS removal

The TS removal efficiency of reactors is given in Fig. 7. At the beginning, the TS removal of reactors fed with salinity wastewater was high, but it decreased with time. High salt concentration induces cell lysis, and therefore, the effluent solids increase [1].

### 3.4. The effluent conductivity values

The effluent conductivity values of reactors are given in Fig. 8. In the non-salt-exposed control reactor, the effluent conductivity was nearly constant. The effluent conductivity values of salt-exposed reactors were increased with increasing salt concentration during experimental period.

### 3.5. The effluent calcium concentrations

It is reported that calcium ion had a positive effect on granulation process and the calcium at concentrations from 150 to 300 mg L<sup>-1</sup> enhanced the mechanical

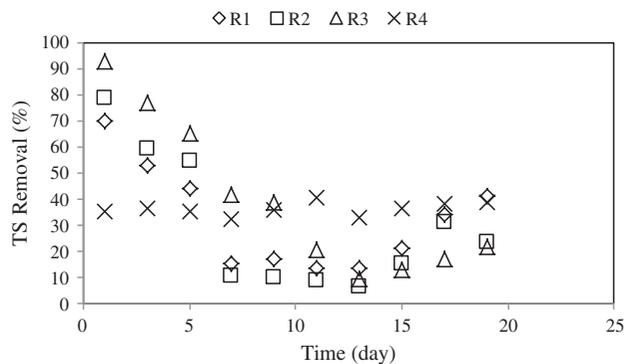


Fig. 7. TS removal efficiency of reactors at different salt concentrations in the feed.

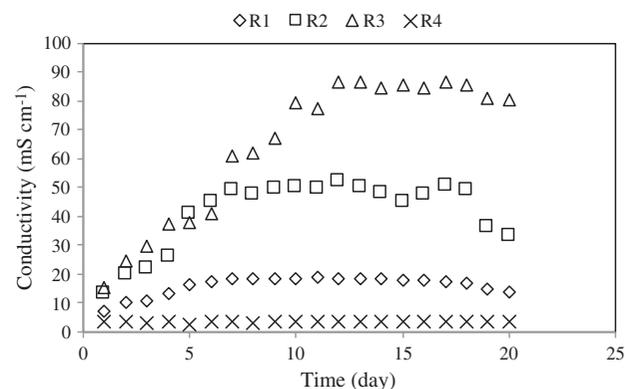


Fig. 8. The effluent conductivity values of reactors.

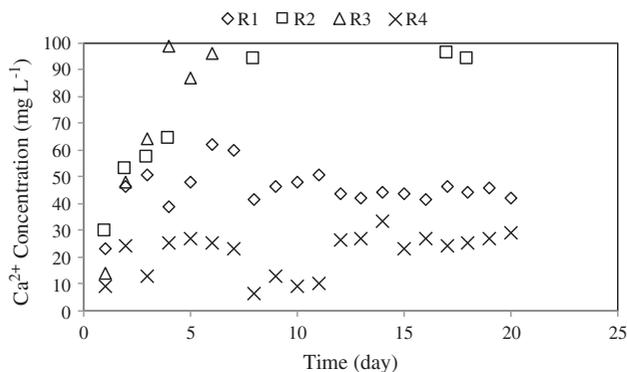


Fig. 9. The effluent  $\text{Ca}^{2+}$  concentrations of reactors at different salt concentrations in the feed.

strength and the settle ability of the granules. At high NaCl concentrations, sodium replaces calcium resulting in increase of the bulk liquid calcium concentration [22]. Fig. 9 represents the effluent  $\text{Ca}^{2+}$  concentrations at different initial NaCl concentrations. The effluent calcium concentration increased with increasing sodium content of medium. Similar results were also obtained in the study done by Ismail et al. [22] who investigated calcium leaching from anaerobic granular sludge at batch experiments. They compared the calcium leaching from anaerobic granular sludge at 0 and 20 g  $\text{Na}^+$   $\text{L}^{-1}$  and observed an increase in the bulk liquid  $\text{Ca}^{2+}$  concentration at  $\text{Na}^+$  concentration of 20 g  $\text{L}^{-1}$ .

#### 4. Conclusions

High salinity wastewaters from various industries can cause inhibition on anaerobic treatment. The performance of UASB reactors treating high salinity wastewater was investigated using synthetic saline wastewater containing different NaCl concentrations between 0 and 50 g  $\text{L}^{-1}$ . The system was inoculated with granular sludge not adapted to salinity, and shock salt loading was applied. COD removal efficiency and COD removal rate, methane production were investigated at different salt contents. The obtained results from this study show that the COD removal efficiency at salt concentration over 10 g  $\text{L}^{-1}$  NaCl was significantly decreased. High salt concentrations caused a decrease of methane production rates. TS removal was decreased with increasing salt content probably due to cell lysis.

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