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Anaerobic Digestion of Domestic Wastewater in different Salinity Levels: The Adaptation Process

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ABSTRACT

The effect of osmotic stress was carried out to determine the resistance to salt toxicity using 4 Continuous Stirred Tank Reactor (CSTR). A CSTR digestion study revealed that digesters seeded with an inoculum from a conventional mesophilic digester treating municipal wastewater and fed on domestic wastewater (DW) plus salts were able to acclimate successfully to a final salt concentration of 10 g l^{-1}. The digesters showed some disturbances during the acclimatisation period as indicated by reductions in specific methane production (SMP), specific biogas production (SBP), pH and increases in Intermediate Alkalinity /Partial Alkalinity (1A/PA) ratio and Volatile Fatty Acid (VFA) concentration. This study revealed the order of disturbance was Sodium Chloride (NaCl) > Potassium Chloride (KCl) > KCl + NaCl. The average values for SMP after stabilisation were below those in the controlled digester, at 0.335 (controlled), 0.323 (NaCl), 0.316 (KCl + NaCl) and 0.308 l CH4 g-1 COD added (KCl).

Keywords: Adaptation, chemical oxygen demand, inhibition, osmoregulation theory, specific methane production

INTRODUCTION

Salinity in wastewater is known to lead to low Chemical Oxygen Demand (COD) removals in the anaerobic digestion (AD) due to the loss of activity of organisms (Kargi & Dincer, 1996). Concentrations of salt more than 1% were reported to produce inhibitory or toxic effects on microbes not adapted to high salinity; high salt concentrations (>1%) have been shown to cause plasmolysis and/or loss of activity of cells. Some studies have reported a detrimental effect on COD removal efficiency immediately after addition of salt, due to leading to changes in surface charge leading to alterations in the community Reid

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et al. (2006). This finding is consistent with Abbott et al. (2015), Kumar et al. (2016) and Azman et al. (2016) who reported that shocking with salt caused a decrease in digestion efficiency.

Several studies have looked at the application of anaerobic treatment by ordinary (i.e. non-halophilic) methanogenic consortia for the removal of organic pollutants in highly saline wastewaters. Abbott et al. (2015) studied the effects of metal salt addition on odour and process stability during the anaerobic digestion of municipal waste sludge. Other research which employed non halophilic methanogens was conducted by Rovirosa et al. (2004) using down-flow anaerobic fixed bed reactors for the treatment of piggery effluent. The study revealed that 90% of COD removal at an Organic Loading Rate (OLR) of 0.5 kg COD m^{-3} day^{-1} at a salt concentration of 15 g l^{-1}. The used of non-halophilic groups in an up-flow anaerobic filter has been demonstrated by Guerrero et al. (1997) during the treatment of seafood processing effluent at 15 g l^{-1} of salts and revealed that 83% of COD removal at COD influent of 34 g l^{-1} and OLR of 2.8 kg COD m^{-3} day^{-1}. Furthermore, Astals et al. (2013) also reported thermophilic co-digestion of pig manure and crude glycerol. Kumar et al. (2015) reported correlations between chemical oxygen demand (COD) and pH and (Calcium Chloride) $CaCl_2$ where the reduction of $CaCl_2$ concentration caused pH to increase thus lead to reduction of COD. Anaerobic digestion has not worked well in all such cases, however, and there are reports of saline wastewaters being inhibitory in AD systems when employing non-halophilic methanogens. Gebauer (2004) investigated the recycling of fish farm wastewater with an influent COD of 70.1 g l^{-1} and salt concentration at 35 g l^{-1} using a Completely Stirred Reactor (CSTR). This study found only 50% of COD removal rate at OLR of 2.5 kg COD m^{-3} day^{-1}. Feijoo et al. (1995) demonstrated the effect of antagonism and the adaptation process in treating seafood processing wastewater in an AD system. This experiment was conducted in a continuous digester by introducing three different anaerobic digestates including; (1) sludge from an AF system after 2 years treating mussel wastewater, (2) suspended biomass from a central digester treating a mixture of seafood processing wastewater and (3) sludge from UASB which previously treated potato processing wastewater. This study found that 50% of inhibition at concentration of 3 - 16 g Na l^{-1} but reported adaptation of methanogens to the saline environment, with increased Na tolerance and a shorter lag phase before the onset of methane production.

Microorganisms requiring salt for growth are designated halophilic and halotolerant, and have developed more acidic enzymes that require the ion potassium (K) for optimal activity. Therefore, they can maintain an osmotic balance of their cytoplasm with the external medium by accumulating high concentrations of various organic osmotic solutes (Lai & Gunsalus, 1992). This suggests the potential of using salt tolerant microorganisms in biological saline wastewater treatment. Furthermore, halophilic and halotolerant microorganisms require a high salt concentration, particularly of K, for most biochemical reactions and lower concentrations may lead to cell disintegration (Wood, 2006).

Studies on the treatment of saline wastewater indicate that the salt concentration is not the main indicator in determining the level of inhibition. Apart from pre adapted sludge and population of the bacteria, the presence of other cations and OLR also need to be considered. These statements are supported by Feijoo et al. (1995) who noted that performance in treating saline wastewater in AD system depends on nutrient in the feedstock, previous adaptation of the sludge, antagonistic or synergistic effect (due to the presence of other cations) and lower substrates to biomass ratio used.

Numerous studies on saline wastewater have highlighted the role of Na in contributing to inhibition in AD systems. The effect of other salts, particularly K, has been given relatively little attention. Fernandez and Foster (1994) investigated the threshold of K inhibition using glucose feed substrate (batch study) and observed that low concentrations of K (less than 400 mg l^{-1}) facilitated performance in both the thermophilic and mesophilic conditions, while at higher concentrations (greater than 2500 mg l^{-1}) toxicity of K was significant. Mouneimne et al. (2003) investigated the toxicity of K in an acetate batch assay test and found the toxicity threshold was 0.43 mol l-1. Most of the K toxicity thresholds which have been reported were conducted in batch studies, which implied that further investigation is required in continuous system.

None of the previous research has focused on the potential for anaerobic digestion of wastewater at various types of salts and level of salinity. Recent studies have shown that Na can cause inhibition in AD systems. Only a few studies have been conducted to identify the threshold of K inhibition, and most of these were carried out in batch systems rather than in continuous digesters which are more relevant in practice. Little previous research works has discussed on antagonistic effect (the role of certain ion to reduce the inhibition of other ion) which also required further investigation. Therefore, this study was conducted to investigate the adaptation level and capability of CSTR to withstand at different salinity level in domestic wastewater.

METHODOLOGY

Experimental set up

The continuously stirred tank reactors (CSTR) each had a 5-L capacity and a 4-L working volume as shown in Figure 1. They were constructed of PVC tube with gas-tight top and bottom plates. The top plate was fitted with a gas outlet, a feed port sealed with a rubber bung, and a draught tube liquid seal through which an asymmetric bar stirrer was inserted with a 40 rpm motor mounted directly on the top plate. Temperature was controlled at 35°C by circulating water from a thermostatically-controlled bath through a heating coil around the digesters. Biogas was measured using tipping bucket gas counters with continuous datalogging (Walker et al., 2009) and all gas volumes reported are corrected to standard temperature and pressure (STP) of 0 °C and 101.325 kPa. Semi-continuous operation was achieved by removing digestate through an outlet port in the base before adding feed via the feed port. During this process, a small amount atmospheric air enters the headspace but in insufficient quantities to affect the redox conditions in the digester: any nitrogen detected in the gas composition is corrected for, as this is not normally produced as a result of the digestion process. Feed was added on a daily basis with regular checks to ensure a constant level was maintained in the digesters. The CSTR digesters were initially seeded with digestate from a digester treating municipal wastewater biosolids at Millbrook Wastewater Treatment plant, Southampton, UK and then acclimated to DW until they achieved stable specific methane production of 0.32 l CH_4 g^{-1} $COD_{added.}$

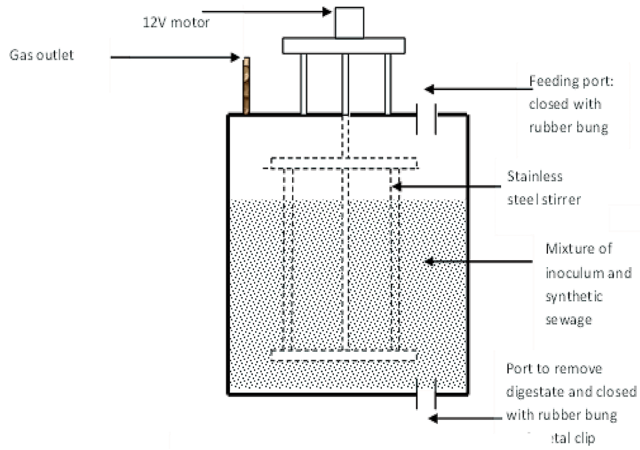

Figure 1. Continuously stirred tank reactors

Analytical Method

Biogas composition (CH_4 and CO_2) was determined using a Varian star 3400 CX gas chromatograph (GC), with a standard of 65.12% (v/v) CH_4 and 34.88% (v/v) CO_2 for calibration. Conductivity was measured using a conductivity meter (LF330, WTW GmbH, Germany); pH was measured using a Jenway 3010 pH meter (Bibby Scientific Ltd, UK) with a combination glass electrode calibrated in buffers at pH 4, 7 and 9 (Fisher Scientific, UK). Suspended solids were determined according to Standard Method 2540 D (APHA, 2005), using glass fibre filter paper with pore size 0.4 µm (Whatman, UK). The COD was measured using a closed tube digestion and titration (APHA, 2005). Total Organic Carbon (TOC) was measured using a Dohrmann TOC (DC-190) based on Standard Method 5310 (APHA, 2005). Ammonia was determined using a Kjeltech block digestion and steam distillation unit according to the manufacturer's instructions (Foss Ltd, Warrington, UK). Potassium (K), Magnesium (Mg), Sodium (Na) and Calcium (Ca) in leachate samples were analysed by first filtering the sample (Whatman No. 1) and then diluting it into 12.5% of nitric acid (HNO_3). The acidified samples were analysed using a Varian Spectra AA-200 atomic absorption spectrometer (Varian Ltd, UK), according to the manufacturer's instructions. The VFA concentrations were quantified in a Shimazdu 2010 gas chromatograph using a flame ionisation detector and a capillary column type SGE BP 21 with helium as the carrier gas.

RESULTS AND DISCUSSION

Two digesters were used, C1 was the control digester and fed only on domestic wastewater (DW) while C2 was fed on DW supplemented with NaCl, C3 on DW with Potassium Chloride (KCl) and Sodium Chloride (NaCl) in a 1:1 ratio by weight and C4 on DW and KCl. The DW was used at a concentration of 48 g COD l^{-1} and the applied organic loading rate was increased during the experiment as shown in Figure 2. The control and digester C3 fed on KCl and NaCl received the same Organic Loading Rate (OLR). Digester C2 was inhibited by

NaCl and required a temporary reduction in loading. Digester C4 with KCl addition also had a slightly reduced load compared to the control. From day 11 salts was added to the DW to give a concentration of 10 g TS l^{-1} of either the individual salt or the mixed NaCl and KCl. No salt additions were made to the inoculum and the increase in salt concentration in the digester therefore occurred by dilution into the reactor. From day 65, additional trace element supplementation was carried out by adding 0.132 ml of oxyanion and cation solutions twice a week for 3 weeks.

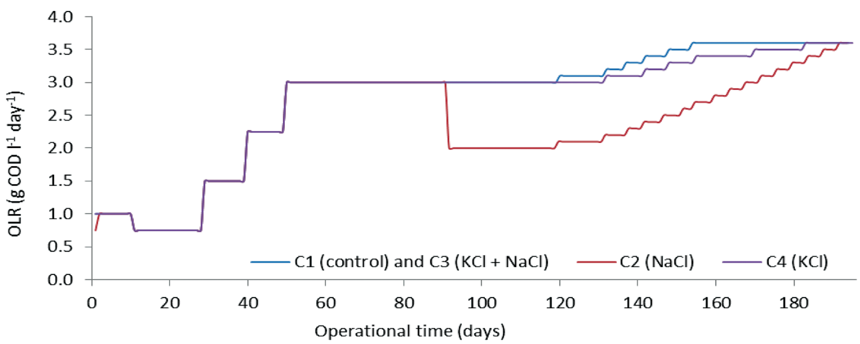

Figure 2. OLR applied to digesters C1-4 during CSTR trial.

On starting to feed the digesters with DW, there were some initial fluctuations in specific biogas production (SBP) and SMP, probably due to the change in feedstock and the gradual depletion of old substrate. By day 25 the SMP had reached a typical value for the DW of around 0.32 l CH_4 $g^{-1.}$ Subsequently, on each occasion when the OLR was increased, there was a fall in SBP and SMP followed by recovery over the next 5-10 days (Figure 3). Until day 45, the behaviour of all four digesters was similar in terms of gas production: by this point, the salt concentration had increased to around 6.5 g l^{-1}, a concentration already higher than that in the UASB trials. From this time, however, the digesters receiving the salt-supplemented feedstock began to show some signs of stress, with a reduction in SBP and SMP relative to the control.

Signs of stress were also seen in the pH, which fell first and most sharply in C2 (receiving NaCl), then less sharply in C4 (KCl) and C3 (KCl + NaCl). The drop in pH was associated with a rise in the alkalinity ratio and in the total VFA concentration (Figure 4). This increase in VFA also occurred in the control digester, and has previously been observed in the laboratory at Southampton following a change of substrate (Zhang et al., 2012, and unpublished data). The change in VFA concentration could therefore not be attributed to salt addition alone: however, the increase in concentration started earlier, lasted longer and gave a higher peak in the following order: C2 (NaCl) >C4 (KCl) > C3 (KCl + NaCl) > C1 (control).

The VFA profiles for each digester are shown in Figure 5. In the control digester C1 the VFA peak was mainly composed of acetic acid, which peaked around day 72. A smaller peak in propionic acid was seen between days ~60 to 90. In C2 (NaCl) propionic acid rose much more rapidly than acetic, reaching a peak around day 72 that declined only slowly in the next 20 days, before falling rapidly. In C3 (KCl + NaCl) acetic and propionic acid increased at similar rates and the propionic peak was both higher and more prolonged than the acetic, but fell rapidly from day 78 onwards. In C4 (KCl) the acetic acid concentration increased first, with a very sharp rise in propionic after day 61 by which time the salt concentration had reached around 9.3 g l^{-1}. The appearance of propionic acid and of other longer-chain VFA (Figure 5 and 6) thus also suggested that the digesters adapted more easily to KCl + NaCl or KCl alone than to NaCl. Addition of Trace Element twice a week for 3 weeks starting from day 65 may also have helped to reduce the VFA concentration and thus improve biogas production particularly in the digester fed on DW+NaCl alone. In terms of inhibition, digester fed on DW + NaCl was badly affected in term of biogas and methane production as compared to digester fed on DW + KCl. This finding showed that Na can affect cell of microbes at lower concentration as compared to K.

Ammonia concentrations in the digesters showed good agreement throughout the experimental period (Figure 4), and in general, remained between 2.5 - 3.0 g N l^{-1}, a level likely to provide some buffering without a strong risk of inhibition.

Because of the particularly high alkalinity ratio and VFA concentration in C2, the OLR on this digester was reduced from day 92 to promote recovery. This lower loading was maintained until day 120, by which time the alkalinity ratio in all digesters was less than 0.3 and the total VFA concentration was below 1000 mg l^{-1}. SBP and SMP values were converging and remained stable from day 140 onwards, during which time the OLR on all digesters was increased to the final value of 3.6 g COD l^{-1} day^{-1}. During the last 30 days of operation (Table 1), all digesters achieved stable operation with methane production of 0.30 l CH_4 g^{-1} COD_{added} and VFA of 100 mg l^{-1}.

Average performance and operating parameters for last 30 days of run are shown in Table 1. From these results, it appears that the inoculum adapted more readily to the mixture of KCl + NaCl than to KCl alone, and to KCl alone better than to NaCl. This circumstance can be explained by the presence of excessive salts alters many cellular properties which include cell volume (dehydration of bacterial cell), turgor pressure and the ionic strength in the cytoplasmic membrane. The alteration of intracellular metabolites concentration can lead to the inhibition of a variety of cellular processes. The phenomenon is known as cell lysis (dehydration under high-osmolarity growth conditions) (Kraegeloh et al., 2005). Despite this difference, in all cases, the digesters were able to adapt successfully to the added salt concentrations.

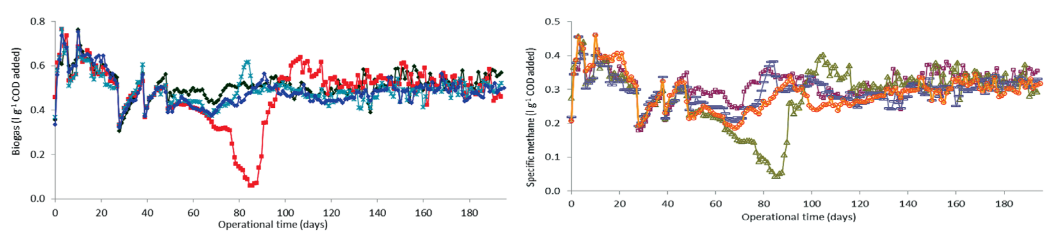

Figure 3. Specific biogas and methane productions for digesters C1-4 during CSTR testing of salt addition

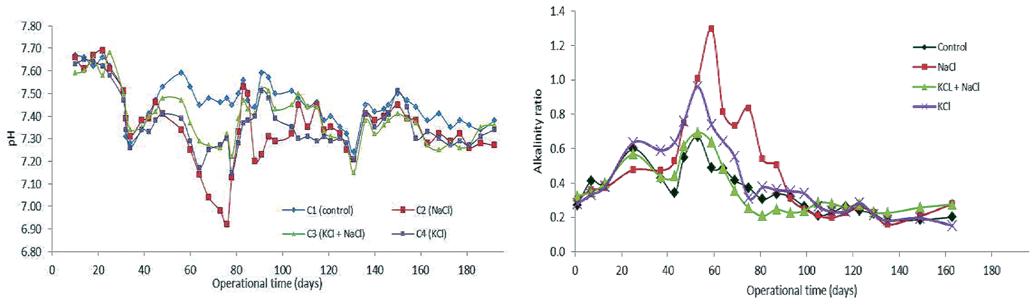

Figure 4. pH and alkalinity in digesters C1-4 during CSTR testing of salt addition

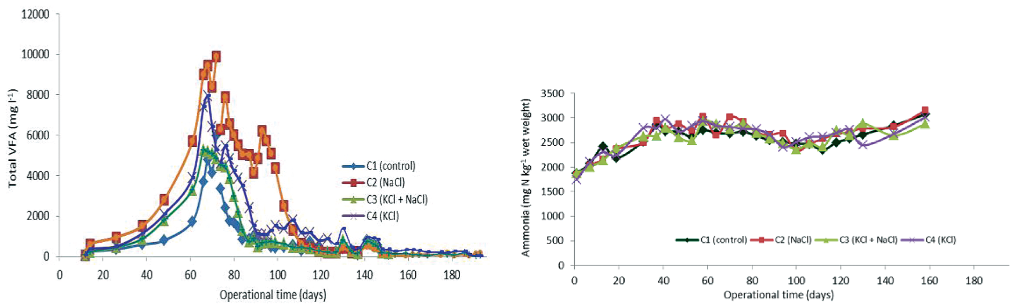

Figure 5. Total VFA and ammonia concentrations in digesters C1-4 during CSTR testing of salt addition

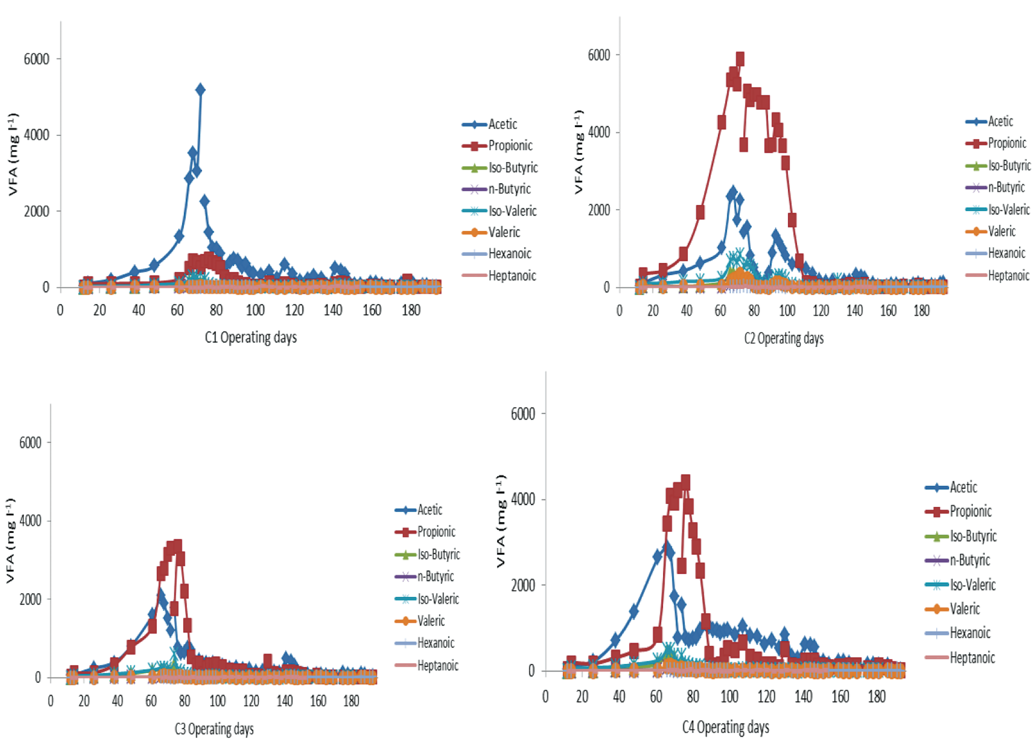

Figure 6. VFA profiles in digesters C1-4 during CSTR testing of salt addition

Table 1
*Average performance over the last 30 days of the experimental run**

	Unit	C1 Control	C2 NaCl	C3 KCl + NaCl	C4 KCl
SBP	l biogas g^{-1} COD $_{added}$	0.531	0.509	0.498	0.490
SMP	l CH_4 g^{-1} COD $_{added}$	0.335	0.323	0.316	0.308
pH	-	7.37	7.29	7.29	7.30
VFA	mg l^{-1}	117	107	91	169

* NB Only C1 control and C4 KCl are at full steady state conditions at this point

CONCLUSION

A CSTR digestion study revealed that that digesters seeded with an inoculum from a conventional mesophilic digester treating municipal wastewater biosolids and fed on DW plus salts were able to acclimate successfully to a final salt concentration of 10 g l^{-1}. This indicated that this source of inoculum would be suitable for start-up of a large-scale CSTR or anaerobic filter plant. The digesters showed some disturbances during the acclimatisation period as indicated by reductions in SBP, SMP and pH and increases in IA/PA ratio and VFA concentration. The order of severity of disturbance was NaCl > KCl > KCl + NaCl. Average values for SBP and SMP after stabilisation were below in the controlled digester, at 0.335 (controlled), 0.323 (NaCl), 0.316 (KCl + NaCl) and 0.308 l CH_4 g^{-1} COD added (KCl). In addition, the role of KCL to reduce the toxicity of NaCl was clearly identified where C3 produced less disturbance compared with C2 at similar amount of final salt concentration.

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