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Modelling start-up performance of anaerobic digestion of saline-rich macro-algae

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Abstract
Some of the key factors affecting the adaptation of anaerobic digestion processes to increasing levels of salinity were determined in batch tests using brown seaweed as a feedstock. It was found that cultures seeded with non-saline anaerobic inoculum required an adaptation period of up to two months to reach the same level of methane production rate as in those cultures seeded with saline adapted inoculum. The Anaerobic Digestion Model N.1 (ADM1) was modified to include an extra inhibition function to account for the effect of salinity and calibrated using a set of experimental data obtained from batch biochemical methane potential tests. After calibration, the model was able to accurately predict methane production rates. The results thus show that, in the absence of saline-adapted inoculum, non-saline inoculum can be used for the start-up of anaerobic digestion systems treating saline-rich feedstocks.

Keywords
ADM1; cations; inhibition; *laminaria digitata*; saline adapted inoculum; sodium

Introduction

Anaerobic digestion is a well-developed process involving the use of a microbial consortium to breakdown organic matter, the main outputs being methane and carbon dioxide for the gaseous phase and digestate for the liquid phase. The process can potentially treat all types of organic wastes if favourable conditions are provided for microbial activity. Macro minerals such as sodium, calcium, magnesium or potassium play an essential role in microbial activity and development. These minerals are commonly found in varying concentrations in seawater because of mineral washout and erosion of the earth's crust. They also exist in anaerobic digestion systems as a result of either the breakdown of organic matter or being artificially added for pH control (Grady *et al.* 1999). Traces of these nutrients are required in anaerobic systems to stimulate bacterial growth and to ensure process optimisation but too high levels can cause serious microbial inhibition (Chen *et al.* 2008). Sodium, particularly, can be found in toxic concentrations in digesters treating wastewaters originating from food processing industries (Soto *et al.* 1993; Feijoo *et al.* 1995), chemical industries, tanneries or reactors using micro/macro-algae as a substrate.

The methanogenic archaea found in anaerobic reactors are particularly sensitive to sodium concentrations and methanogenesis has been reported to be strongly inhibited at sodium levels exceeding 10 g/l (Lefebvre and Moletta 2006). Accumulation of sodium ions in anaerobic systems can bring about excessive increase in the osmotic pressure regulating the water flow across the cell membrane which can lead to cell death (Ollivier *et al.* 1994). Some authors have reported relatively high accumulation of propionate and acetate in anaerobic systems receiving saline feedstock, indicating that high salinity levels may have a greater impact on the acetogenic bacteria and acetoclastic methanogens than on acidogens (Rinzema *et al.* 1987). Different inhibitory

concentrations of sodium have been reported by various authors, also suggesting that the level of microbial sodium inhibition may be dependent on factors such as system design, operation, and seed inoculum. Soto *et al.* (1993) reported that a sodium ions concentration ranging between 14 to 18 g/l can reduce methanogenic activity by up to 50% (IC_{50}). With granular sludge, Rinzema *et al.* (1987) found an IC_{50} value of about 10 gNa^+/l whilst Feijoo *et al.* (1995) reported an IC_{50} of 16.3 gNa^+/l with saline-adapted seed inoculum. It has also been reported that the use of anaerobic reactor sludge bed fitted with microfiltration membrane systems can increase the IC_{50} to an even higher value of up to 25 gNa^+/l (Jeison *et al.* 2008).

Some microorganisms can tolerate or adapt to a relatively high sodium induced osmotic pressure by accumulating other inorganic ions within their cells which ensures osmotic balance with the saline medium thus preventing sodium from reaching their cytoplasm (Oren 2002). This may result in reduced microbial inhibitory effects of sodium as observed in cultures containing other cations such as Ca^{2+}, Mg^{2+} and K^+ (Appels *et al.* 2008; Chen *et al.* 2008). In contrast, some microorganisms which occur naturally in highly-saline environments require sodium chloride for growth and are categorised as moderately to extremely halophilic according to the level of salt requirement (Ollivier *et al.* 1994). Since the latter, depending on their halophilic category, require certain amounts of salt for their metabolic activities, their use in wastewater treatment may only be limited to wastewaters with the requisite and less variable salt content (Aspé *et al.* 1997).

It can therefore be assumed that halophilic anaerobic microorganisms will be more effective for treating saline rich feedstocks, whilst saline-adapted anaerobic microorganisms will adapt more readily in systems treating feedstocks containing fluctuating salt levels (as often is the case in anaerobic digestion systems receiving diverse types of organic materials). There has been very little literature on the effect of variable level of salts in anaerobic systems treating feedstocks which are classified as non saline-rich such as sewage treatment plant sludge, agricultural and food/beverage residuals, despite the fact that some of these feedstocks can sometimes contain elevated levels of salts usually brought about by operational practices such as effluent recycling, pH control with sodium hydroxide and co-digesting with saline-rich feedstocks like marine biomass (Hierholtzer and Akunna 2012). The aim of this study is therefore to investigate the major factors affecting the adaptation of anaerobic systems treating non-saline rich feedstocks to variable levels of salinity using both experimental and modelling approaches.

Materials and methods

Experimental approach

The aim of the experimental approach was to determine the effects of various levels of feedstock salinity on both saline adapted and non-saline anaerobic digestion cultures.

Inoculum and feedstock

The experiment was carried out in batch, using two types of inoculum: mesophilic anaerobically digested sewage sludge as non-saline inoculum and anaerobic digestate from a laboratory scale mesophilic anaerobic digester treating seaweed as saline-adapted inoculum. The sodium level in the latter was about 15 g Na^+/l. The non-saline inoculum was obtained from a municipal mesophilic anaerobic digester (Hatton, Angus, UK) treating domestic wastewater sludges containing an average of 0.2 g Na^+/l. Both sources of inoculum have been in operation for more than 2 years prior to the commencement of this study. Brown seaweed (*Laminaria digitata*), a saline-rich feedstock, was collected from the Westhaven beach (56° 30′ N, 2° 42′ W) near Dundee, Scotland, UK in October 2010. The seaweed was washed after collection to remove debris and sand, oven-dried at about 75°C for 24 hours and milled in an industrial blender to obtain a particle size of about 1 mm. The dried and milled sample was then stored in sealed containers at room temperature.

Design of batch assays

Batch tests were designed to determine the effect of varying levels of salinity on the kinetic parameters of the anaerobic biodegradation process. Various concentrations of salt in the test cultures were obtained by mixing the prepared feedstock sample and inoculum with either seawater or freshwater. Four sets of batch test cultures were used for the study. The first set consisted of culture bottles (A_{na}), each containing 400 ml of non-saline inoculum, 10 grams of the prepared seaweed, and 90 ml of freshwater. The second set of culture bottles (B_{na}), was similar to the first set, except that 90 ml of seawater, instead of freshwater, was added to each bottle. The third (A_a) and fourth (B_a) sets of culture bottles were similar to A_{na} and B_{na} respectively, except that saline-adapted inoculum replaced the non-saline inoculum. The chemical oxygen demand (COD), total solids (TS) and volatile solids (VS) concentrations of seaweed diluted with freshwater were 66.8 g/l, 88.2 g/l and 21.2 g/l respectively; and 73.2 g/l, 119.3 g/l and 34.4 g/l respectively for seaweed diluted with seawater. All the test cultures were set in duplicates. The bottles were closed with rubber caps, degassed with nitrogen gas and incubated at mesophilic temperature (37°C±1°C) for 50 days. Blanks test cultures containing only inoculum were also incubated alongside the test cultures. To avoid overpressure in the bottles, gas release was carried out routinely by displacement of a syringe piston and the amount of methane gas released was added to cumulative biogas production. Methane production rate was used as the overall indicator parameter. Ultimate methane yields were determined based on the method of Hansen *et al.* (2004).

Analytical methods

Methane production was measured by gas chromatography using a Hewlett-Packard 5890 Series II gas chromatograph with dual thermal conductivity detector and an AT-Alumina stainless steel capillary column. Injector, oven and detector temperatures were 120°C, 50°C and 150°C respectively and helium was used as a carrier gas. Gas samples were collected in a gas tight syringe and 100 µl of sample was transferred to the gas chromatograph. Methane yield results were converted to standard temperature and pressure (STP: 273.15°K; 1013.25 hPa). Total and volatile solids were determined based on standard methods (APHA 1992). Total volatile fatty acids (VFA) were quantified by esterification (Montgomery *et al.* 1962) and colorimetric determination using a DR5000 spectrophotometer (Hach-Lange, USA). Cations concentrations were measured by flame emission spectroscopy with a Flame Photometer 400 (Ciba-Corning, USA) for sodium, calcium and potassium and by using an atomic absorber AAnalyst 200 (Perkin-Elmer, USA) for magnesium. COD was measured using Hach-lange cuvette tests (LCK 014) and samples were centrifuged for the determination of soluble COD. Concentration of ammonium nitrogen was determined by cuvette tests (LCK 304).

Modelling approach

The aim of the modelling approach was to predict the effects of various levels of salinity on the rate of methane production based on the data obtained from the batch experiments.

Model modifications

The implementation of the ADM1 in Matlab/Simulink (MathWorks Inc., USA) carried out by Rosen and Jeppsson (2006) is used as a platform in this study. Sodium inhibition has been reported to affect mainly the acetate utilizers or acetoclastic methanogens, by lowering their maximum specific growth rate and yield (Rinzema *et al.* 1987; Feijoo *et al.* 1995). The original structure of the ADM1 is such that inhibition of acetate utilizers will adversely affect the overall production of methane since acetate is also produced partly from the oxidation of reduced compounds which includes butyric and propionic acids. Hence, implementing an extra inhibition to the rate of acetate uptake can be used to represent the effect of sodium inhibition. Methane production resulting from hydrogenofil methanogenic bacteria might however be overestimated by the model but it is assumed that this pathway is significantly less important than the acetic-methane pathway since

more than two thirds of methane produced during anaerobic digestion comes from the latter (Pohland 1992). An extra inhibition factor $I_{cations}$ can thus be applied to the rate of acetate uptake found in the original model and expressed as in equation (1).

$$I_{acetate} = I_{pH,ac} \cdot I_{IN,\lim} \cdot I_{NH_3} \cdot I_{cations} \quad (1)$$

Where $I_{acetate}$ is the overall inhibition function applied to the rate of acetate uptake, $I_{pH,ac}$ is the pH-inhibition function, $I_{IN,lim}$ is the inorganic nitrogen inhibition function, I_{NH3} is the ammonia nitrogen inhibition function, $I_{cations}$ is a non-competitive function taking into consideration the effect of cations concentration not represented in the original ADM1 model. $I_{cations}$ can be expressed as shown in equation (2).

$$I_{cations} = \frac{1}{1+\left(\frac{S_{Na^+}}{K_{I,Na^+}}\right)+\left(\frac{S_{Mg^{2+}}}{K_{I,Mg^{2+}}}\right)+\left(\frac{S_{Ca^{2+}}}{K_{I,Ca^{2+}}}\right)+\left(\frac{S_{K^+}}{K_{I,K^+}}\right)} \quad (2)$$

Where S_{Na}^{+}, S_{Mg}^{2+}, S_{Ca}^{2+}, and S_{K}^{+} are the concentrations of sodium, magnesium, calcium and potassium respectively found in the system. $K_{I,Na}^{+}$, $K_{I,Mg}^{2+}$, $K_{I,Ca}^{2+}$, and $K_{I,K}^{+}$ are the inhibitory concentrations of sodium, magnesium, calcium and potassium respectively for acetate degrading organisms.

The function, $I_{cations}$, considers that all cations present in saline water have an inhibitory effect on acetoclastic methanogens when found above their respective K_I values. In seawater cations are typically found in ratios of 0.122, 0.039 and 0.037 for Mg^{2+}/Na^+, K^+/Na^+, and Ca^{2+}/Na^+ respectively (Jeison *et al.* 2008) and sodium is found at inhibitory levels before other cations reach their respective inhibitory concentrations. The function is thus valid at cations ratios commonly found in seawater but could be modified if the individual effects of high concentrations of magnesium, calcium or potassium need to be considered. To take into account possible synergism where the presence of Ca^{2+}, Mg^{2+} and K^+ contributes to the reduction of sodium toxicity, a different function could be implemented to decrease the value of the inhibition factor applied to acetate uptake. However, this approach was discarded because it would only be valid if the level of synergism towards sodium inhibition can be experimentally estimated for each group of cations, both individually and as a group. This estimation was not carried out in this study. The parameter S_{CAT} is originally implemented in the ADM1 to represent metallic ions such as Na^+, and can be estimated by the measurement of alkalinity. Sodium, calcium, magnesium and potassium cations were hence not added directly in the charge balance but S_{CAT} was adjusted appropriately to obtain the correct simulation for pH within the model. This modelling approach has been shown to enable model users to obtain satisfying predictions when cation concentrations are not measured individually (Hierholtzer and Akunna 2012). The concentrations of each of the cations can be calculated using equation (3) used individually for Na^+, Ca^{2+}, Mg^{2+} and K^+.

$$\frac{dS_{Cations}}{dt} = \frac{q_{in}}{V_{liq}}\left(S_{Cations,in} - S_{cations}\right) \quad (3)$$

Where q_{in} is the reactor inflow, V_{liq} the effective capacity of the reactor. $S_{Cations}$ and $S_{Cations,in}$ being the initial concentrations of the different cations in the system and in the feedstock respectively.

The degree of acclimatisation of the inoculum used was considered here to impact on the inhibitory sodium concentration tolerated by acetate degrading organisms and on their maximum specific uptake rate ($K_{m,ac}$). By adjusting the values of $K_{I,Na}^{+}$ and $K_{m,ac}$ it was possible to represent the

adaptation of the microbial consortium to sodium over time in the model without specific determination of microbial populations. The pathways of degradation as defined in the ADM1 can thus remain identical and do not need to take into account an alternative population of acetic acid utilizing microorganisms. This approach is considered appropriate for batch tests where the sudden exposure to inhibitory substances and time scale of the study are notably different from full-scale operations. Preliminary experimental work was necessary in order to estimate the values of K_{I,Na^+} and $K_{m,ac}$ and therefore enable the predictive use of the model.

Results and discussion

Experimental results

The experimental and simulated cumulative methane production for all the cultures net of blank results is shown in Figure 1. The cultures seeded with non-saline inoculum, A_{na} and B_{na}, produced relatively small amounts of methane in the first 15 days. The delay in methane production observed for these cultures is believed to be due the process of acclimatisation when the microorganisms were adapting to the higher salt concentration of the culture. After the lag period, the methane increased gradually and eventually reached the same levels as those produced in cultures seeded with saline-adapted inoculum, A_a and B_a. It can also be seen from the figure that, for cultures diluted with freshwater (A_{na}, A_a), methane production rate was higher in cultures seeded with saline-adapted inoculum, A_a, than in those seeded with non-saline inoculum A_{na}. For seawater-diluted cultures (B_{na}, B_a), there was no significant difference between the methane produced by both types of inocula at the end of the experiment. Table 1 shows the different cations concentrations and their ratios in the cultures. It can be seen that the saline-adapted cultures (A_a, B_a) contained relatively high salt concentrations indicating that the addition of freshwater or seawater had little impact on overall salt levels. The similarity in methane production obtained from B_{na} and B_a cultures (and to some extent, A_{na} and A_a) despite the significant differences in their total salt content, shows that microbial adaptation plays a major role in effective digestion of saline-rich feedstock.

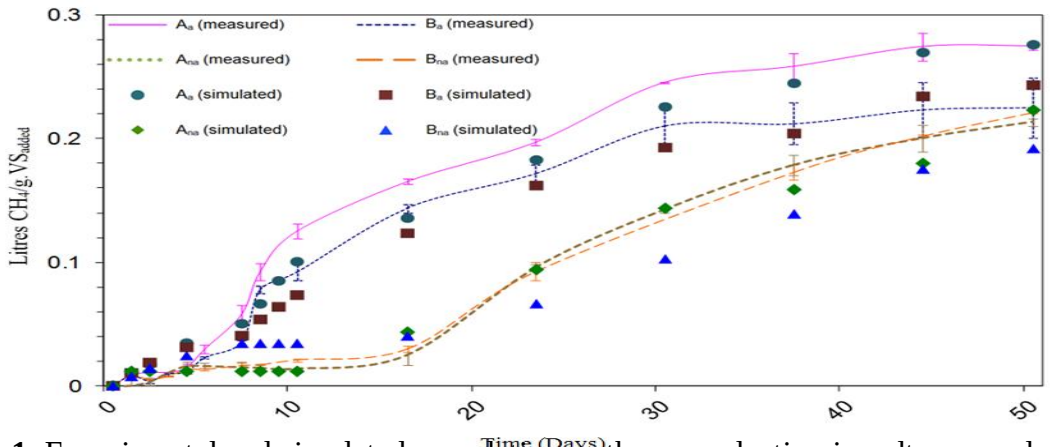

Figure 1. Experimental and simulated cumulative methane production in cultures seeded with non-saline (A_{na}, B_{na}) and saline-adapted (A_a, B_a) inocula, and diluted with freshwater (A_{na}, A_a) and seawater (B_{na}, B_a).

Table 1 - Cations concentrations and ratios

Sample	Cations concentrations (g/L)				Cations ratios		
	Na^+	Ca^{2+}	Mg^{2+}	K^+	Na^+/Ca^{2+}	Na^+/Mg^{2+}	Na^+/K^+
A_{na} (freshwater)	1.22	0.08	0.24	1.23	15.25	5.08	0.99
A_a (freshwater)	13.67	0.48	0.49	2.26	28.48	27.90	6.05
B_{na} (seawater)	5.51	0.2	0.49	1.31	27.55	11.24	4.21
B_a (seawater)	14.87	0.52	0.97	2.34	28.60	15.33	6.35

For cultures seeded with saline-adapted inoculum (A_a, B_a), it was assumed that biodegradation followed first order kinetics and, consequently, the estimation of kinetic parameters was carried out using least square curve fitting of the measured cumulative methane production. Ultimate methane yields were estimated to be 0.332 and 0.289 $L.CH_4/gVS_{added}$, and methane production rate constants 0.055 and 0.061 d^{-1} for A_a and B_a respectively. These values were similar to those reported in literature for *laminaria digitata* which ranged between 0.26-0.29 litres CH_4/gVS_{added} (Carpentier *et al.* 1988; Chynoweth *et al.* 1993). Non-saline adapted cultures (A_{na}, B_{na}) were not considered to follow first-order kinetics during the acclimatisation period which covered the first 15 days. Beyond Day 15, biodegradation in these cultures was considered to follow first order kinetics. Subsequently, ultimate methane yields were estimated to be 0.212 and 0.221 $L.CH_4/gVS_{added}$, and methane production rate constants 0.034 and 0.032 d^{-1} for A_{na} and B_{na} respectively.
These results thus show that in the absence of saline-adapted inoculum, non-saline cultures can be successfully used for the start-up of a digester treating moderately saline feedstocks, although, depending on the operational parameters (hydraulic retention time, organic loading rate, etc) a variable adaptation period would be required before achieving optimum performance.

Modelling results

Influent characterisation and model stoichiometric parameters

The ADM1 requires a careful influent characterisation together with accurate fractionating of intermediates, namely: proteins, carbohydrates, lipids and soluble inerts. Parameters $f_{pr,xc}$, $f_{ch,xc}$, $f_{li,xc}$, $f_{si,xc}$ and $f_{xi,xc}$ were estimated from the average composition of the *laminaria digitata* seaweed used in the study. First order parameters corresponding to the hydrolysis kinetic rates of carbohydrates, lipids and proteins were set at similar rates since their influence is not significant for homogenous substrates (Feng *et al.* 2006). $K_{hyd,ch}$, $K_{hyd,pr}$, $K_{hyd,li}$ were calibrated from the cumulative production of methane obtained after the 50-day test duration and taken equal to 1.2 d^{-1}. The feedstock characterisation was carried out using the transformer model elaborated by Zaher *et al.* (2009), which transforms a set of practical measurements to the input vector required by the ADM1. The concentrations of sodium, potassium, magnesium, and calcium were adjusted from measured values shown in Table 1. The maximum uptake rate for acetate ($K_{m,ac}$) was adjusted from fitting simulation results to experimental results for VFA and methane production, and the values obtained were 17±0.82 $kgCOD.kgCOD^{-1}d^{-1}$ and 7.83±1.55 $kgCOD.kgCOD^{-1}d^{-1}$ for saline-adapted and non-saline cultures respectively. The much lower value obtained for the non-saline inoculum was due to the relatively long period of acclimatisation observed in these cultures as shown in Figure 1. Increasing the half saturation value of acetate users was not considered since it has been reported that different pairs of K_m / K_s could yield similar simulation results, since both parameters are mathematically dependant and cannot be calibrated simultaneously (Girault *et al.* 2011). Hence the values of K_m used in this study were valid only when K_s was set at its default value (0.02 day^{-1}). Similarly, the 50% inhibitory concentration for sodium was estimated from experimental results to be 0.11±0.009 $kmol/m^3$ and 0.61±0.005 $kmol/m^3$ for non-saline and saline adapted microorganisms respectively. Inhibition values for other cations were taken from literature reports, $K_{I,Mg}{}^{2+}$=0.06 $kmol/m^3$ (Appels *et al.* 2008), $K_{I,Ca}{}^{2+}$=0.12 $kmol/m^3$ (Ahn *et al.* 2006) and $K_{I,K}{}^{+}$=0.15 $kmol/m^3$ (Kugelman and McCarty 1965). Initial values for inorganic carbon (S_{IC}), inorganic nitrogen (S_{IN}), and cations concentrations (S_{CAT}) were obtained from experimental results. All other parameters were taken equal to the values used by Rosen and Jeppsson (2006).

Simulation results

Figure 2 shows the correlation between the final methane productions (expressed in normalised ml CH_4/batch) predicted by the modified ADM1 and measured values. A good fit was obtained between the results of the modified model and the experimental data and the model correctly predicted the lag phase observed in some of the experimental tests as shown in Figure 1. The high

correlation coefficients, ranging from 0.925 to 0.985, are indicative of the validity of the modelling approach and variables estimation method used in this study.

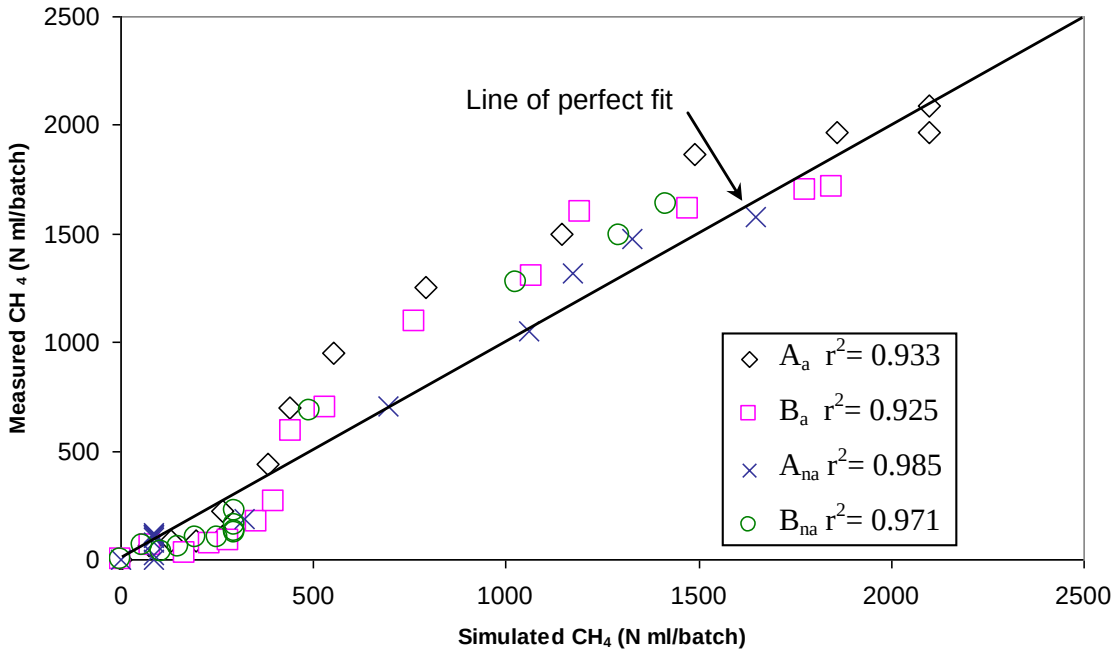

Figure 2. Comparison between experimental and modified ADM1 model results for methane production in cultures seeded with non-saline (A_{na}, B_{na}) and saline-adapted (A_a, B_a) inocula, and diluted with freshwater (A_{na}, A_a) and seawater (B_{na}, B_a).

Following calibration, the modified model can thus be used to determine the effect of increasing salinity on methane production and assist in the operation of anaerobic digesters treating feedstocks with varying salt concentrations. The modifications presented in this work are valid when sodium is found at inhibitory levels before other cations reach their respective inhibitory concentrations and do not consider synergism or antagonism effects with other light metal cations and the impact on microorganisms.

Conclusions

The research has shown that non-saline-adapted anaerobic sludge can effectively be used in the start-up of anaerobic digestion systems treating saline enriched feedstocks, following an appropriate period of adaptation, which depends on initial salt concentration and other operational parameters. The study also showed that the adaption period can be modelled using a modified ADM1 model and parameter estimation methodology.

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