Modelling Phenol Degradation Under Saline Conditions by AnMBR Biomass

Additional Thesis

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By

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Summary

This study is part of a project titled: “Phenolic compounds degradation in AnMBR under mesophilic and thermophilic operation: BioXtreme-following up”.

Phenol is a toxic contaminant found widely in industrial effluents. It is toxic to humans and animals even at very low concentrations. Anaerobic digestion uses phenol as a carbon source and then to degrade it to non-toxic products for lower costs. Industrial effluents are also likely to have high concentrations of salinity which causes inhibition at high concentrations. Anaerobic membrane bioreactors are an attractive method as it enables biomass retention for biomass. The aim of this study is to understand the effect of Na+ concentration in a batch phenol degradation by phenol adapted mesophilic AnMBR biomass. NaCl concentration ranging from 0-90 g/L were tested on adapted AnMBR biomass. COD, phenol degradation, particle size distribution and methane production of adapted AnMBR biomass were analysed. The results from the batch test were used to model kinetic parameters. The biomass was acclimatized to 30 g/L of NaCl in AnMBR. Phenol removal of 98% was observed at 30 g/LNaCl and it decreased further with elevated salinity. Similarly, biogas production was also highest for 30g/L NaCl and decreased further with higher NaCl concentration. The highest value for SMA of 0.10 ± 0 gCOD-CH4.gVSS-1 d -1 was observed for 30g/L. However, the data did not indicate a specific trend with increasing salinity and showed high variability. The data showed poor fit to both Haldane and Monod growth model as these models were used for substrate inhibition. Modelling with modified Gompertz equation also failed to yield any conclusive results.

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1. Introduction:

Phenol and its derivatives are widely being used in the industries. A large range of products such as herbicides, drugs, paints, cosmetics, and lubricants use phenol as a raw material. It is being used mainly as an intermediate in the production of phenolic resins (Mohammadi, Kargari et al. 2015).As a result of its extensive use, phenol and its derivatives would be present in the effluent of a wide range of industries. The concentration of phenol in the effluent from various industries mentioned in Table 1 ranges from 10 to 17,000 mg/L (Veeresh, Kumar et al. 2005).

Table 1: Phenol concentration in Industrial effluents adapted from Veeresh, Kumar et al. (2005)

Industry	Phenol concentration (mg/L)
Coal gasification	207
Lignite gasification	5500-7260
H-coal liquefaction	4900
Petroleum refinery	6-88
Oil refineries	10 -100
Low temperature carbonization	9250-17500
General petrochemical	50-600
Aircraft maintenance	200-400
Plastics factory	600-2000
Phenolic resin production	1600
Lignite liquefaction	32290-11700

Phenol being soluble in water (9.3 g phenol/100 mL of water) and most of the organic solvents poses severe health implications to humans, animals, and marine systems (Busca, Berardinelli et al. 2008, Mohammadi, Kargari et al. 2015). In humans, exposure to phenol would lead to irregular breathing, muscle weakness, tremor, coma, and respiratory arrest at high concentrations (Villegas, Mashhadi et al. 2016). As low as 1 g of phenol is reported to be deadly for humans(Nuhoglu and Yalcin 2005).Whereas in animals as much as 250-500 mg/kg could be fatal(Medicine 2003). For aquatic life phenol concentration exceeding 1 mg/L would be considered harmful. Based on its wide-ranging

impact on the environment, discharge limit of phenol is set very low in most countries. The discharge limit of 0.5 mg/L was set by European Union and 1 ppb by the US Environmental Protection Agency (Mohammadi, Kargari et al. 2015).

Conventionally, physical and chemical methods such as adsorption and chemical oxidation have been used for the removal of phenol (Nuhoglu and Yalcin 2005) . However, these options tend to be expensive and not completely effective as it leads to secondary effluent problems and higher energy costs. For example, when chlorination is used in phenol degradation chloro-phenols are generated as secondary effluent (Marrot, Barrios-Martinez et al. 2006).The incineration of this activated carbon could lead to release of dioxins and furans that are toxic for humans(Loh, Chung et al. 2000). Although phenol is bactericidal, some microorganisms have developed methods to use phenol as their source of carbon (van Schie and Young 2000). Biological removal methods for phenol are preferred over various techniques available because of its ability to degrade phenol to non-toxic products for lower costs. Biological treatment of phenol can be done aerobically or anaerobically. Anaerobic processes offer advantages such as energy recovery and excess sludge reduction (Pishgar 2011).

The wastewater generated from some industries (Table 1) such as chemical, petroleum, and leather can have high salinity waste streams(Dereli, Ersahin et al. 2012).Industries such as oil refineries also produce hyper saline wastewater with at least 3.5 % w/v of TDS (Aslan and Ekerdag 2016). The salinity can affect the biodegradation efficiencies of pollutants as it can lead to loss of cellular water. This cellular dehydration can disrupt the metabolic activities of the cells(Deng, Wang et al. 2018) . Therefore, the presence of cations such as Na^+ causes toxicity and inhibition in anaerobic processes (Dereli, Ersahin et al. 2012).

The lower growth rate of anaerobic organisms in phenolic wastewater makes biomass retention a desirable feature in high rate reactors (Bornare, Raman et al. 2014). Most high rate anaerobic treatment technologies apply mechanisms such as settling, attachment or granulation for higher biomass retention(Dereli, Ersahin et al. 2012). Under high salinity, granulation and biofilm formation are likely to get affected (Jeison, Rio and Van Lier

2008). In such situations, anaerobic membrane bioreactors (AnMBR) employs membrane filtration for higher sludge retention leading to higher treatment efficiencies. Moreover, it can promote degradation specific of pollutants by retaining respective microbial communities. Therefore, it is an attractive option for the treatment wastewater containing organics under extreme conditions such as salinity (Dereli, Ersahin et al. 2012).

This study is intended to understand the inhibition caused by high salinity (Na^+) on phenol and salinity adapted biomass and to perform a kinetic modelling with increasing salinity.

Objective:

- Effect of Na^+ concentration on batch phenol degradation by salinity adapted AnMBR biomass.
- To find the kinetic parameters for modelling phenol degradation from the batch test (μ_{max}, K_s, K_i)

Research Question

How does salinity inhibition affect the batch phenol degradation in salinity adapted mesophilic AnMBR biomass?

1. What are values of the kinetic growth parameters (μ_{max}, K_s, K_i) for the phenol degradation under saline conditions by AnMBR biomass?
2. To what extent is the biogas production and phenol removal from the phenol acclimatized biomass affected by the increasing salinity?

2. Outline of the report:

Chapter 2 is a literature review involving AnMBR systems, phenol degradation, salinity inhibition and kinetic models. Chapter 3 provides the description of batch test and methodology used. In the following chapter 4, the results from the batch test are analysed and kinetic parameters obtained from the experiment are discussed.

3. Literature Review:

The following section explores the various steps in anaerobic digestion and degradation of phenol.

3.1 Overview of Anaerobic Digestion:

When organic matter is degraded by anaerobic digestion, it follows a number of series and parallel reactions. The degradation of organic matter takes place in four stages, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Figure 1). A wide variety of microbes participate in the successive break down of organic matter, which gets mineralised to methane (CH_4) and carbon dioxide (CO_2).

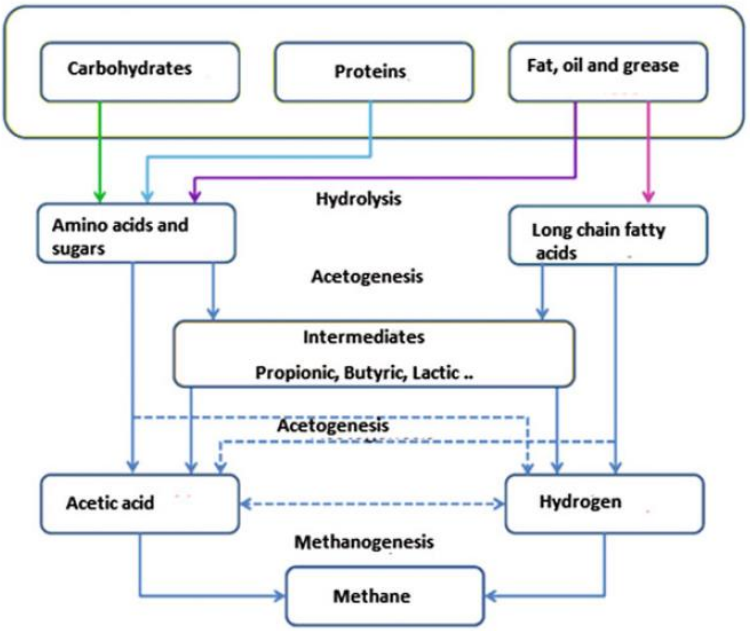

Figure 1:: Anaerobic degradation pathway adapted from (Bajpai 2017)

The balance between different stages in anaerobic digestion are essential for efficient methane production (Mani, Sundaram et al. 2016). In the first step of anaerobic digestion, large organic polymers like carbohydrates and proteins are converted into sugars and amino acids. Acidogenesis which immediately follows hydrolysis step, is the fastest step in anaerobic digestion(Mani, Sundaram et al. 2016). During acidogenesis, the amino acids and sugars from hydrolysis are converted into acids such as propionic acid, butyric acid,

acetic acid, hydrogen and carbon dioxide. These higher organic acids are changed to acetic acid and hydrogen by acetogenesis. Acetate and hydrogen produced in the both these steps are converted during methanogenesis. In this final step of anaerobic digestion, methane is produced. The interaction between acetogens and methanogens are crucial for the efficient performance of anaerobic digester Methanogenic bacteria are central to anaerobic digestion. They are slow growing and are sensitive to environmental changes. Instabilities in the process such as higher concentration of organic compounds in influent, presence of toxins etc. could result in accumulation of VFAs (volatile fatty acids) and alcohols which inhibits methanogenesis(Gujer and Zehnder 1983). Therefore, methanogenesis is considered to be the rate limiting step for anaerobic digestion.

3.2 Phenol Degradation Pathway:

Microbes capable of phenol degradation could be both aerobic and anaerobic. The anaerobic microbes include iron or sulphate reducing bacteria and methanogenic consortia. According to Heider et.al, phenol degradation follows the benzoate pathway as described below. The carboxylation of phenol yields 4-hydroxy benzoate and proceeds in two steps. The conversion of hydroxy benzoate to benzoyl-CoA is done for channelling it to metabolism(Heider and Fuchs 1997). Knoll and Winter (1989) studied phenol degradation that occurs by carboxylation and gets converted to benzoate (Figure 2).

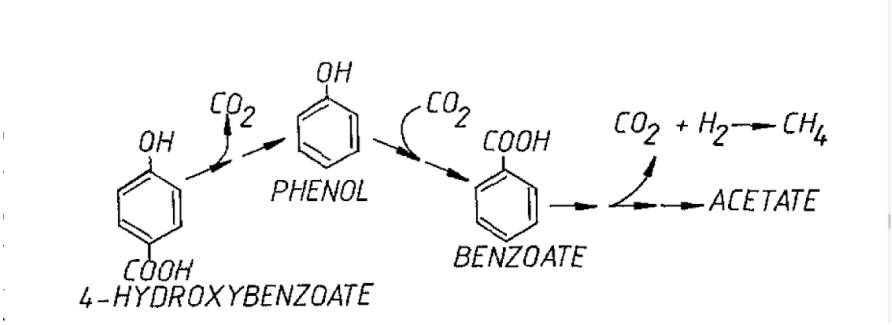

Figure 2:Phenol Degradation Pathway adapted from (Knoll and Winter 1989)

3.3 Effect of salinity inhibition:

Industrial effluents are characterized by the presence organic pollutants like phenol at relatively higher salinity. Along with factors such as pH and temperature, salinity also

affects removal of organic pollutants(Wang, Qu et al. 2009). The efficiency of biomass of anaerobic processes would be affected due to its toxic effect of the biomass. High salinity has been known to induce a negative effect on the biological conversions, as it affects the electrochemical and osmotic interactions(Valentine 2007). Moreover, high salinity could lead to the disintegration of flocs and granules and thus washout of biomass (Ismail, Gonzalez et al. 2008).

The effects of salinity on anaerobic digestion were reported by earlier studies. Sodium concentration above 10 g/L is reported to strongly inhibit methanogenesis (Lefebvre, Vasudevan et al. 2006). Munoz Sierra, Lafita et al. (2017) reported 50% inhibition of methanogenic activity at 23 Na^+ g/L and complete inhibition at 34 g/L. However, it was demonstrated that problems pertaining to inhibition could be mitigated to an extent by adaptation of the biomass(Aloui, Khoufi et al. 2009, Moussavi, Barikbin et al. 2010). Therefore, salinity has the potential to cause inhibition to biological systems which could be mitigated to an extent by long term adaptation of biomass.

3.4 AnMBR system:

Anaerobic membrane reactor (AnMBR), (Fig.1) consists of an anaerobic reactor integrated with a low-pressure ultra-filtration and microfiltration membrane system for solid liquid separation(Chang 2014). This technology offers significant advantages over the conventional anaerobic systems such as UASB (Upflow anaerobic sludge blanket). AnMBR s combine the advantages of anaerobic reactors and membrane technology (Table 2). By the use of membranes, the biomass could be retained in reactors leading to higher biomass concentration and higher substrate removal(Yang, Spanjers et al. 2013). As the membranes allow the bacteria to proliferate without being washed out, AnMBR can treat high strength wastewater.

Table 2: Comparison between aerobic, anaerobic, aerobic MBR and AnMBR. Table adapted from (Lin, et al. 2013)

Feature	Conventional aerobic treatment	Conventional anaerobic treatment	Aerobic MBR	AnMBR
Organic removal efficiency	High	High	High	High

Effluent quality	High	Moderate to poor	Excellent	High
Organic loading rate	Moderate	High	High to moderate	High
Sludge production	High	Low	High to moderate	Low
Footprint	High	High to moderate	Low	Low
Biomass retention	Low to moderate	Low	Total	Total
Energy requirement	High	Low	High	Low

AnMBR s can be applied in two basic configurations: side stream (Figure 1) and submerged. In a side stream MBR, the membrane modules are placed outside the reactor and the mixed liquor is being distributed in a recirculation loop that comprises of the membrane. Whereas, in submerged MBRs the membranes would be in the reactor (Bornare, Raman et al. 2014). The external side stream configuration offers higher control over fouling which is considered to be a major disadvantage for AnMBR s (Wen-Der Liu 2007)

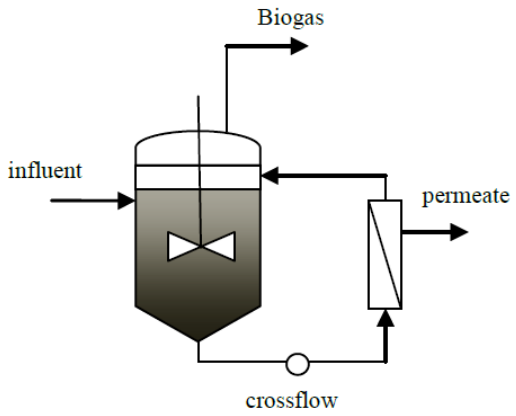

Figure 3: AnMBR with sidestream; source: (Chang 2014)

In mostly used anaerobic technologies such as UASB, anaerobic filters, EGSB (Expanded granular sludge blanket) or IC (internal circulation) reactors, biomass characteristics (like granulation) could get easily affected leading to washout in the presence of toxic

substances. Changes in biomass settling or granulation tends to not affect AnMBR compared to other anaerobic treatment technologies(Muñoz Sierra, Oosterkamp et al. 2018). Due its superior operational stability, AnMBRs can be applied in cases where the sludge technology would likely experience problems such as high/low pH, high salinity, or high temperature(Dereli, Ersahin et al. 2012). Due to the incorporation of membrane technology AnMBRs can also tolerate large fluctuations in organic loading rates(Dvořák, Gómez et al. 2016). The membrane also provides a barrier for the slow growing organisms. The membranes also facilitate the removal specific pollutants from industrial wastewater irrespective of the hydraulic retention time (HRT) (van Lier 2008) (Tao, et al. 2012). When compared to a conventional anaerobic reactor, AnMBRs enable faster start-up of the reactor, production of superior quality of permeate and the ability to withstand high OLR (Dvořák, Gómez et al. 2016).

AnMBR technology had been applied for treatment of water with high organic matter content such as food industry and paper and pulp industry. It has been widely applied in food industry where the wastewater is non-toxic with high COD and suspended solids. The process achieved a COD removal of over 90% in most cases with and organic loading rate (OLR) ranging from 2–15 kg COD/m3/d (Lin, et al. 2013). Xie et.al (2010) used submerged AnMBR for treatment of effleunt from the pulp and paper industry. Using OLR in the range of of 1–24 kg COD/m3/d, a COD removal efficiency of 93–99% were realized. AnMBRs offers a more robust system with high solids and COD removal and production of very high quality permeate (Liao, Kraemer et al. 2006). Long retention time is a strategy that us used to deal with biodegradation of compounds with high salinity. The ability to provide high solid retention time(SRT) also makes it possible to apply AnMBRs for the treatment of different compounds that are present in industries which are not easily biodegradable and toxic. As discussed in Section 3.3 salinity is considered as a major limiting factor in anaerobic systems. AnMBRs can operate in high saline environments achieve high COD removal efficiencies following biomass adaptation (Ismail, Gonzalez et al. 2008, Dereli, Ersahin et al. 2012, Dvořák, Gómez et al. 2016). Therefore, AnMBR could be considered an effective option for the treatment of compounds of toxic nature.

3.5 Kinetic Model for phenol degradation:

When a batch reactor containing an inhibitory substance is subjected to degradation with substrate concentration (S), and biomass (X), the specific substrate concentration(q_s) could be expressed as:

$$q_s = \frac{dS}{dt} = \frac{-\mu X}{Y_{obs}} \quad (2)$$

Where Y is the observed cell mass yield (g/g)

The specific growth rate (μ) can be calculated by substituting it with an established model. If the initial substrate concentration is higher than concentration where μ is maximum, the substrate gets consumed (Wang and Loh 1999). However, in batch processes, cells have been reported to grow at a constant growth rate in the exponential phase and then in the deceleration growth and stationary phase (Allsop, Chisti et al. 1993, L. Shuler and Kargi 2002) . Generally, the kinetic models for phenol degradation are based on Monod or Haldane equations.

3.5.1 Monod model

Monod is a biokinetic model that considers phenol as a non-inhibitory compound. The following equation can be used to describe non-inhibitory degradation by Monod kinetics:

$$\mu = \frac{\mu_{max} S}{K_S + S} \quad (3)$$

where K_s is half saturation coefficient (mg/L), S is the substrate concentration and μ is the specific growth rate (h^{-1}) (Najafpour, Taghizade Firozjaee et al. 2011). However, this model does not take substrate inhibition into account. But in an acclimated culture, phenol could be regarded as a non-inhibitory compound. In that case, Monod model have been used to calculate the kinetic constants satisfactorily by (Pishgar 2011).

3.5.2 Haldane model

Haldane or Andrews equation is a modification in the Monod model where in phenol is considered an inhibitory substance. For a substrate with an inhibitory substance μ (specific growth rate) can be described by Haldane model as:

$$\mu = \frac{\mu_{max} S}{K_S + S + \frac{S^2}{K_i}} \quad (4)$$

Where, and K_i = substrate inhibition constant (mg L^{-1}). For large values of K_i, Haldane models get simplified to Monod equation. The sensitivity of the cells to substrate inhibition could be demonstrated by K_i (Pishgar 2011).

Parameter estimation for models:

In case of both the models (Monod and Haldane), the specific growth rate of the bacteria could be experimentally determined using Malthus law.

$$\mu = \frac{dX}{Xdt} \quad (5)$$

X denotes the concentration of biomass. It can be estimated by dry weight (mg/L). The effect of salinity on phenol degradation could be estimated by calculating the phenol biodegradation rate. The slope of the phenol degradation curves is biodegradation rates (Pishgar 2011).

3.5.3 Modified Gompertz Model:

Modified Gompertz model is a sigmoidal function that correlate the growth of methanogens with methane production. The cumulative biogas production was plotted (H_t) against incubation time (t in hours); where H_{max} (mL)represents the maximum biogas potential, R_{max} (mL/h) and λ shows the lagtime (h) (Shin, Park et al. 2015).

$$H_t = H_{\max} \times \exp\left\{-\exp\left[\frac{R_{\max} \times e}{H_{\max}}(\lambda - t) + 1\right]\right\} \quad (6)$$

4. Methodology:

The following section could be firstly divided into the protocol followed for the batch salinity experiments followed by methods adopted for the chemical analysis.

4.1 Batch Kinetic Experiments:

The phenol acclimated biomass from an AnMBR degrading an acetate- phenol mixture under high Na^+ concentration at mesophilic condition was used as inoculum for the batch experiments. The feed composition of the reactor is given below (Table 3).

Table 3: Feed for AnMBR

	Concentration	
Solution A	51.00	mL/L
Solution B	19.50	mL/L
Macronutrients	9.00	mL/L
Micronutrients	4.50	mL/L
Yeast	1200.0	mg/L
Sodium Acetate	41.15	g/L
Phenol	500.00	mg/L
Sodium Chloride	29.90	g/L

The VSS of the biomass from the reactor was 2.72 g/L. The procedure for the experiment is followed from Lab manual for SMA test (Merle de Kreuk 2012). The micronutrient, macronutrients, solution A, and solution B were added according to protocol in Lab manual SMA.

Substrates:

As substrate for the methanogenic activity, sodium acetate-3hydrate salt and phenol with the same concentration (500 mg/L) in the reactor feed was used. The COD value of sodium acetate-3hydrate salt and phenol are 0.47 $\frac{gCOD}{g\ CH_3COONa.3H_2O}$ and 2.38 $\frac{g\ COD}{g\ C_6H_5OH}$ respectively. The substrate also consisted of phosphate buffers, micronutrients and

macronutrients in the concentrations described in the SMA manual (Merle de Kreuk 2012). (Appendix, Table -1)

Preparation of reaction vessels:

Triplicates were prepared for blank and sample solutions. The sample solutions were varied with Na^+ concentrations (Table 5: Batch kinetic Test- NaCl Concentrations). Based on previous results volume of inoculum and substrate were estimated to be 75 mL and 125 mL respectively.

Table 4: Preparation of reaction vessels

Reaction Vessel	
VSS of ANMBR 3 sludge	2.7 g/L (measured)
NaCl concentration tested	10-90 g/L
No of bottles per test	15
Volume of sludge (per bottle)	75 mL
Volume of substrate	125 mL
Concentration of phenol	500 mg/L
Acetate concentration	41.15 g/L

Once the bottles were filled with the inoculum and substrates, it was mounted on a temperature-controlled shaker. The bottles were stirred continuously at a temperature of 35^{o}C and 120 rpm. The experiments were conducted under anaerobic conditions by using Nitrogen as flushing gas. The experiments were stopped when the bottles stopped producing biogas and COD reduction also did not take place. The tests were conducted in 2 batches. The first batch dealt with NaCl concentration ranging from 10-40 g/L and the second batch tested from 50-90 g/L.

4.2 Analytical methods:

The measurement for all the parameters were done at the beginning and end of the batch test. During the experiment COD, VSS, VFA and phenol concentration measurement were done only in one bottle per triplicate.

4.2.1 COD

The COD of all the bottles were analysed at the beginning and at the end of the batch test. COD concentration was analysed by COD cells Lange, Hach (1000 – 10,000 mg/L). The filtered sample (0.45 µm filter) from the bottles was diluted to the required concentrations as suggested by provider. Following which digestion was performed at 148 ^{o}C for 2 hours. The sample was then cooled and analysed using a spectrophotometric technique.

4.2.2 Particle size Distribution:

Particle size distribution analysis (PSD) were performed using DIPA-2000 Eyetech particle analyser (Donner Technologies). Sludge samples were taken at the beginning and at the end of the batch tests to test the effect of salinity on particle size. The analyses were done in triplicates. The diameters D10, D50, D90 from the analyses were reported.

4.2.3 Suspended solids:

Total and volatile suspended solids were calculated using standard methods (Telliard 2001).

4.2.5 VFA and phenol determination by gas chromatography:

Gas chromatographic analysis was performed using Agilent Technologies 7890A GC system. The filtered samples (0.45 µm filter) were diluted to 1:2 ratio by adding formic acid and pentanol (320 mg/L). The samples were then analysed in the GC for volatile fatty acids and phenol.

5. Results

From the batch tests at varying salinity, a distinct effect of salinity on various parameters could be observed.

4.1 Effect of increasing NaCl concentration on Methane production:

Methane production was found to decrease with increasing Na^+ concentration. As the biomass were acclimatized to 30g/L, it was only natural that it showed the highest cumulative methane production. Likewise, the NaCl concentration lesser than 30 g/L ie, 10g/L and 20 g/L produced 49% and 77% lesser methane when compared to 30g/L. The biogas production higher than 40 g/L NaCl concentration, produced only less than 20% of biogas produced at 30g/L. Feijoo, Soto et al. (1995) reported that Na^+ concentration between 4.4 and 17.7 g Na^+/L resulted in 50% reduction in methane production. Although there is an increasing trend in methane production from 50g/L. High salinity leads to decrease in methane production because of rise in cell lysis or due to the release of high molecular weight organics as extracellular compounds produced during metabolism (Aslan and Ekerdag 2016).Although 33.3 NL of methane was produced at 90 g/L relatively higher than 70 and f NaCl, it could not be conclusive as there was biogas production from only one of the bottles. It is also likely that the composition of gas changed with higher salinity. The results were not conclusive for 60 g/L as well as only two bottles produced biogas and the standard deviation between the values were high. However, it can be concluded that the reduction in methane production happened at higher Na^+ concentrations as the biomass was acclimatized to 18.75 Na^+ g/L (30 g/L).

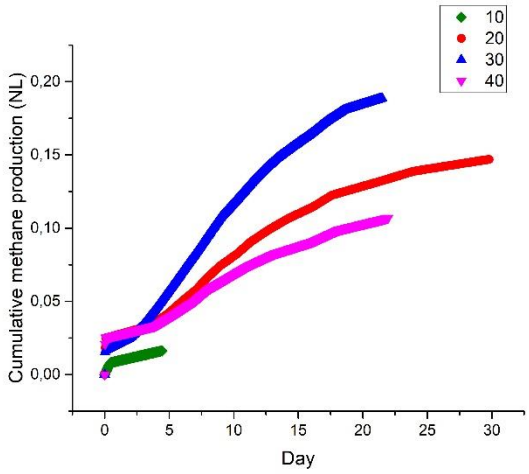

Figure 4: Methane production from batch 1

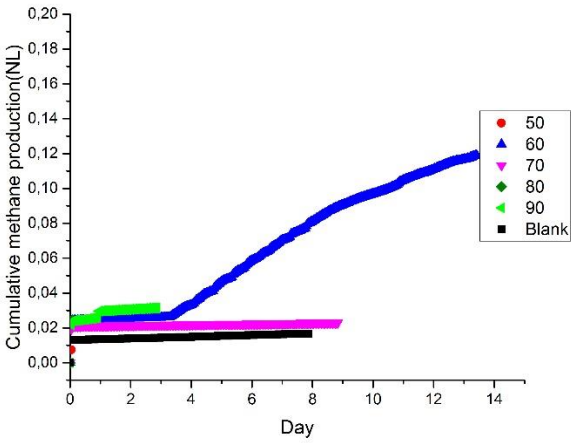

Figure 5: Methane production batch 2

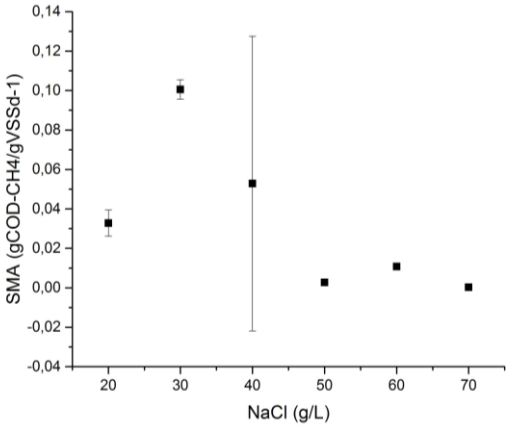

Figure 6: Specific methanogenic activity with increasing Na^+ concentration

The trend in methane production could be corroborated with the specific methanogenic activity which showed the highest activity for the acclimatized concentration of 30g/L. (Figure 6) The SMA for 30g/L was calculated to be 0.10 ± 0 gCOD-CH4.gVSS-1 d -1and 0.01 ± 0 gCOD-CH4.gVSS-1 d -1 60g/L. The specific methanogenic activities decreased by 90 % when NaCl concentration increased from 30 to 60 g/L. The SMA for 80 and 90 g/L of NaCl could not be obtained as there was not sufficient methane production. Previous studies that studied with biomass degrading wastewater with phenol had reported SMAs ranging from 0.15 – 0.66 gCOD-CH4.gVSS-1 d -1 (Hussain and Dubey 2014). The SMA obtained for the batch tests were lower because the biomass used in the test were not

acclimatized to Na+ higher 30 g/L NaCl. It is also to be noted that the large deviation of SMA could also be that samples were withdrawn from bottles for analysis. For lowering the standard deviation in SMA it is better to add another bottle only for analysis without connecting it to AMPTS.

4.1 COD and VSS reduction with increasing Na^+ concentration

In the first batch (Figure 7), containing 10-40 g/l NaCl, highest COD removal could be observed for 30g/L. As the biomass was acclimatised to 30g/L of salinity, the COD removal was highest for this salinity. COD for 40g/L is found to have a steep increase at the end of the batch test probably indication cell lysis.

Figure 8 shows COD reduction in batch 2 with NaCl concentration ranging from 50-90 g/L. All the bottles showed lower COD reduction (<10%) than the blank solution indicating the effect of increasing Na+ concentration on COD removal. After 3 days, 50 and 80 g/L showed an increasing trend in COD. This could be due to the cellular lysis caused by increasing Na+ concentration.

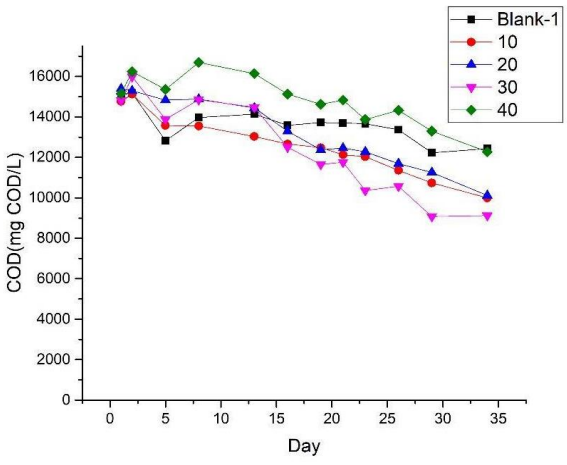

Figure 7: COD from batch 1

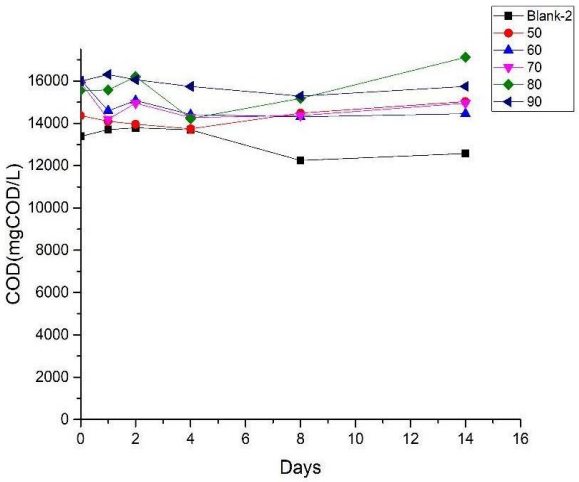

Figure 8: COD from batch 2

For the culture, no lag phase was observed as it had been acclimatized to the conditions in AnMBR (30 g/L salinity). The maximum biomass concentration was obtained for salinity of 30g/L, followed by 40g/L. The inhibition by salinity could not be observed

completely in the first batch. There was shown to be increasing trend in the biomass after 20 days indicating the rupture of cells leading to sudden increase in COD and biomass concentration.

In batch 2 (50-90 g/L NaCl) with higher concentration of NaCl a lag phase could be observed even in the blank. The slope of the plots is significantly lower here as the effect of salinity inhibition could be clearly seen here. The maximum biomass concentration was higher than the previous batch. Also, to be noted is the fact that blank here also has not shown any increase in biomass concentration for the first 3 days. On analysis of VFA it was seen that the total VFA content was high (5547.7 mg/L) (Appendix 4). However, the results did not a specific trend in VSS with increasing salinity.

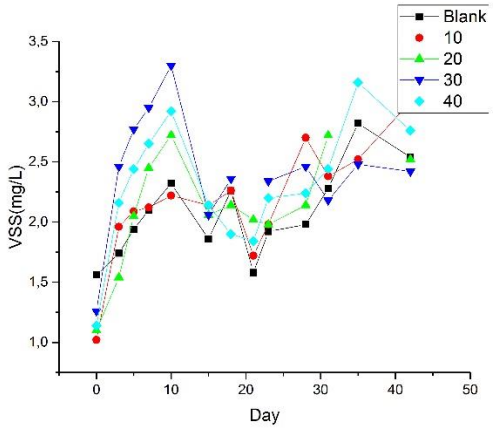

Figure 9: VSS from batch 1

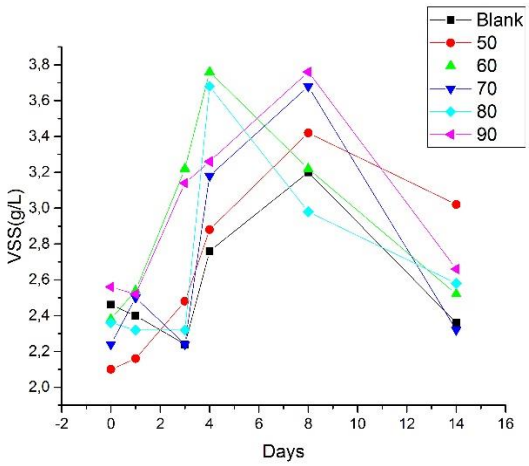

Figure 10: VSS from batch 2

4.2 Phenol removal in batch test:

Higher removal efficiency of 98% was observed for the biomass at 30g/L of NaCl. 10g/L showed higher biodegradation rate in first 5 days and the rate decreased further. Similarly, 40 g/L also showed 53 %removal at lower biodegrading rate. However, 20g/L showed almost no considerable biomass degradation. For NaCl concentration above 40 g/L NaCl the phenol removal remained around 25%.

NaCl Concentration (g/L)	Phenol removal (mg Phenol/mgVSS.h)
10	0.198
20	0.117
30	0.282
40	0.205
50	0.154
60	0.156
70	0.149
80	0.174
90	0.150

For NaCl concentration above 40g/L the efficiency in phenol removal has seemed to decrease indicating the larger NaCl concentration had potentially led to plasmolysis. From table – it can be seen that there is lower phenol removal at higher salinity. However, the data does not suggest a specific trend in phenol removal with increasing salinity.

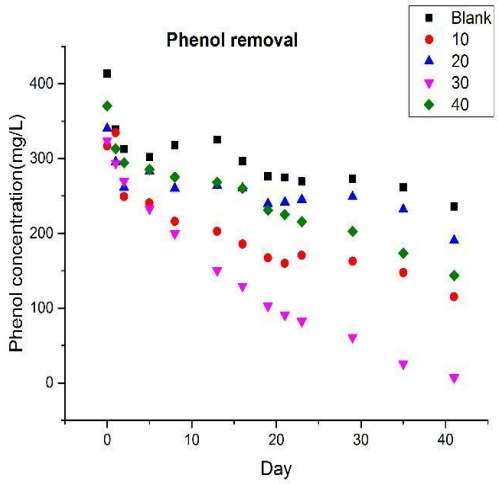

Figure 11: Phenol degradation curve batch 1

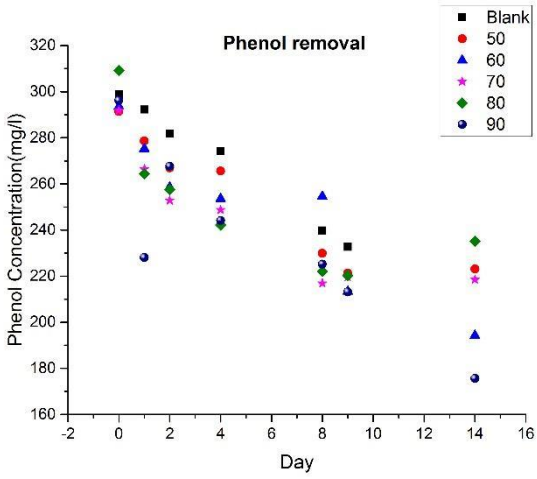

Figure 12: Phenol degradation curve batch 2

4.3 Kinetics:

Using the VSS values during the exponential growth phase, the values for μ were estimated using equation 5. The Matlab code used for curve fitting is provided in

Appendix 2. Haldane and Monod functions were shown in several studies to fit μ vs S data. The calculated μ was considerably lower than μ estimated in Najafpour, Taghizade Firozjaee et al. (2011) i.e. 0.067 h^{-1}. The low growth could be because of the effect of high Na^{+}. The specific growth rate was found decrease when salinity was increased from 30-90 g/L. This trend suggests that high Na^{+} acts as an inhibitory substance for microbial growth. As seen from Figure 17,Monod equation did not show a good fit to the data. The data did not show a good fit for Haldane model as well (Figure 16)

However, Monod and Haldane are growth models pertaining to substrate inhibition and its influence on microbial growth. Since the inhibition was caused by salinity here, the data showed poor fit. The models did not consider the inhibition caused by salinity. Therefore, to model the inhibitory effects of NaCl on microbial growth, different initial substrate concentration has to be tested at each salinity level. The inhibition by salinity could be estimated by modifying the μ_{max} term in Haldane equation to ($\mu_{max} - I_s$), where I_s is the salinity inhibition constant (h^{-1}) (Park and Marchand 2006). Moreover, the substrate concentration on the graph were very close together leading to absence of trend in specific growth rate.

As the data failed to show definite trends in biomass concentration and phenol removal with increasing salinity. The fit of the data was tested with modified Gompertz model. As observed from the table below, not all NaCl concentrations shows good correlation with the predicted value from the model. The maximum methane production rate did not suggest a definite trend as had been discussed earlier. However, on looking at the data that showed a reasonable fit ($r^2 > 0.95$), a reduction in methane production potential with increasing salinity could be observed. Among the NaCl concentrations that showed a good fit, the highest methane potential was indicated at 30g/L. Also, to be noted is that the highest lag time was observed for 30g/L salinity which is in contradiction with the trend in VSS. However, no particular trend could be observed in methane production rate or lag time with increasing salinity.

Table 1: Model prediction from Gompertz model

NaCl (g/L)	Model simulation			
	lag time (h)	Maximum CH4 production rate (ml/h)	CH4 production potential (mL)	R^2
0	0.01	0.45	16.3	0.6
10	0.01	0.3	14.8	0.87
20	0.01	0.3	150.5	0.99
30	5.4	0.5	207.3	0.99
40	0.01	0.2	112.5	0.97
50	0.28	37.2	21.3	0.89
60	0.01	0.4	179.6	0.98
70	0.3	33.5	22.1	0.88
90	0.01	3.03	30.9	0.69

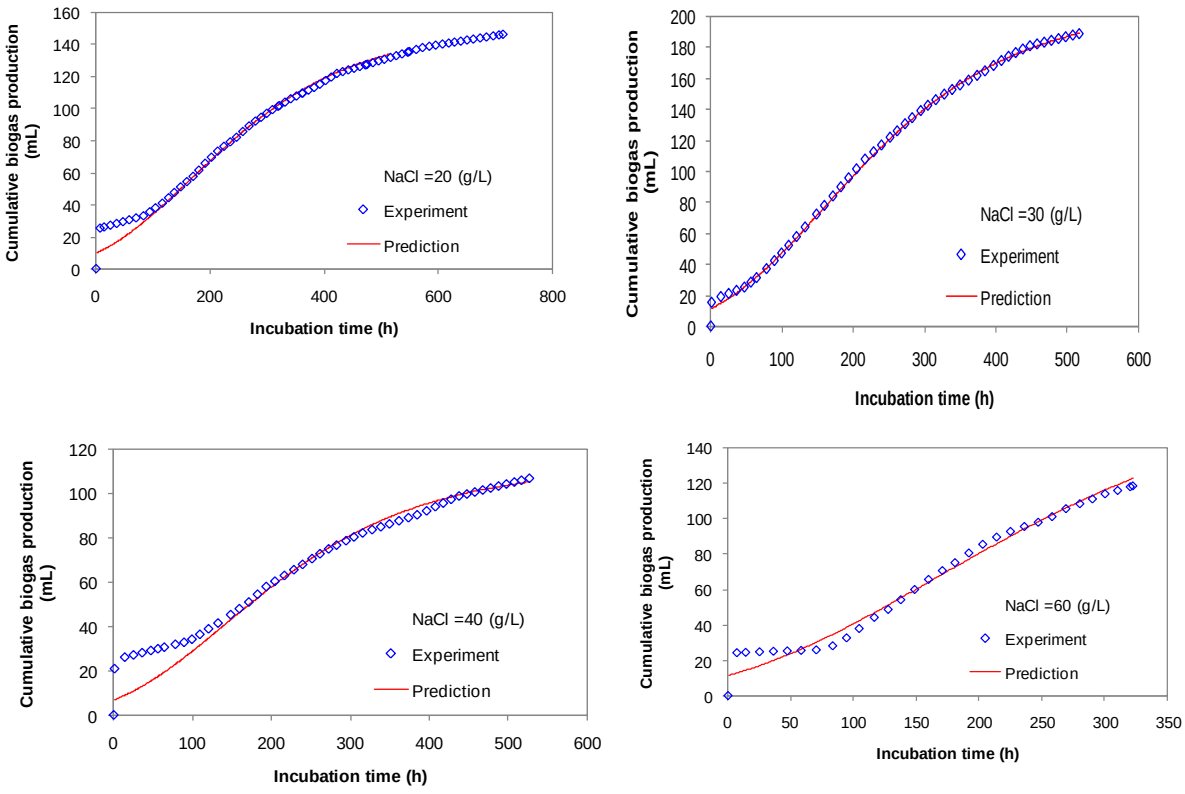

Figure 13: Data fit of 20,30,40 and 60 g/L to Gompertz model

4.4 Particle size distribution:

As salinity increases, the structure of the sludge matrix weakens. The Ca+ is replaced by Na+ (Ismail, Gonzalez et al. 2008, Jeison, Del Rio et al. 2008). A large shift in particle size distribution can be observed at 90g/L of NaCl indicating that the flocs has disintegrated severely. The increasing effect of salinity could be observed from 50 g/L. Higher salinity can cause the disintegration of flocs leading to washout of the biomass (Yang, Spanjers et al. 2013). The occurrence of two peaks indicating that there has been a shift in particle size from ~ 100 µm to ~10 µm (Figure 13). The peak at 10 µm was found to increase with increasing salinity. This indicates that the flocs had started to disintegrate owing to higher salinity.

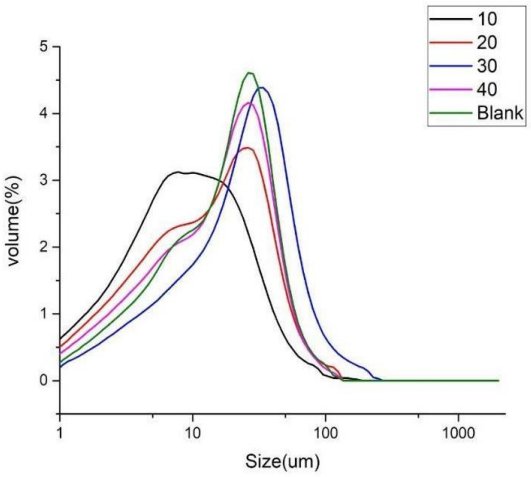

Figure 14:Particle size distribution of Batch 1. PSD of 10,20,30 and 40 g/L NaCl are represented by the curves

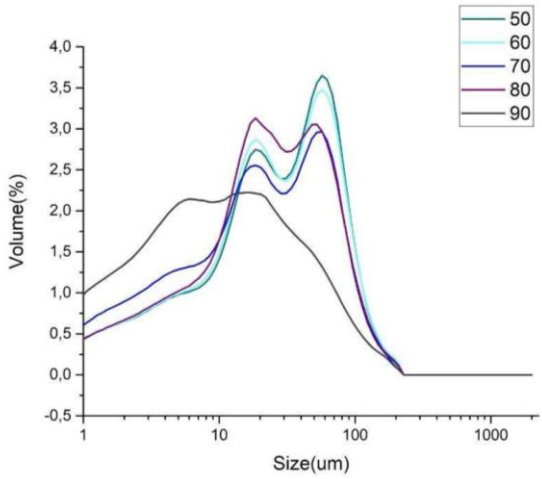

Figure 15:Particle size distribution of Batch 2. PSD of 50, 60,70,80 and 90 g/L NaCl are represented by the curves

The floc structure is largely intact till 50 g/l as the biomass is acclimatized to 30 g/l of NaCl. Therefore, a higher tolerance to salinity could be observed from the salinity acclimatized AnMBR biomass. Also, higher salinity would possibly lead to a deterioration in membrane filtration properties. Moreover, the smaller particles have increased efficiency in mass transfer. Therefore, the smaller particles are more susceptible to toxicity induced by Na^+(Munoz Sierra, Lafita et al. 2017).

Conclusions

The following conclusions could be made from this report:

- The maximum SMA of 0.10 ± 0 gCOD-CH_4.$gVSS^{-1}$ d^{-1} was obtained at 30 gNaCl/L. As the Na^+ concentration increased the activity was seen to have reduced.
- Methane production was highest at 18.87 gNa^+/L; methane production reduced with increasing Na^+ concentration. But the decreasing trend could not be observed above 40 g/L .
- Phenol removal did not a clear trend with increasing salinity. Howver, 30g/L showed the highest phenol removal at 0.282 mgphenol/mgVSS.h
- The kinetic evaluation was not sufficient as the existing kinetic models were based on substrate inhibition and the effects of salinity were not considered. Modelling with Gompertz equation showed fit with some salinities. But on the whole all three models did not indicate any trend with increasing salinity.

Recommendations:

The following recommendations could be made from this study:

- In order to assess the kinetics of biomass at varying Na^+ concentration, varying substrate concentration has to be tested at each level of salinity. The observed kinetic constants need to be corrected for salinity.
- In order to reduce standard deviation observed in the SMA result, it is better to add another bottle to extract the samples for chemical analysis without connecting it to AMPTS.

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Appendix:

1. *Concentration of substrates required in reactor vessels:*

Table 6: Concentration of the stock solution

	Concentration (for 2g COD/L and 1 L of substrate)
SOLUTION A	30.5 mL/L
SOLUTION B	19.5 mL/L
MICRONUTRIENT	0.6 mL/L
MACRONUTRIENT	6 mL/L

2. *Na^+ concentration used in Batch Test*

Table 5: Batch kinetic Test- NaCl Concentrations

Name	Concentration of Na^+ (g/L)
Blank	0
10	10.82
20	14,82
30	18,75
40	22,69
50	26,62
60	30,56
70	34,49
80	38,42
90	42,36

3. *Matlab code for Haldane and Monod function:*

```
% Parameters: mumax = b(1),  Ks = b(2),  Ki = b(3)
HaldaneInhMdl = @(b,S) b(1).*S ./ (b(2) + S + S.^2./b(3));
mu = [0.0012 0.0091 0.0022 0.0093 0.0089 0.0009 0.0033 0.0008 0.0039 0.0008];
S = [6.94 10.89 14.82  18.75 22.69 26.62 30.56 34.49 38.42 42.36];
SSECF = @(b) sum((mu - HaldaneInhMdl(b,S)).^2);        % Sum-Squared-Error Cost Function
B0 = [0.40; 10; 30];                    % Initial Parameter Estimates
options = optimset('MaxFunEvals',2000);
[B, SSE] = fminsearch(SSECF, B0)                % Estimate Parameters
Sp = linspace(min(S), max(S), 50);              % 'S' Vector For Plot
fitmu = HaldaneInhMdl(B,Sp);                    % Calculate Fit
figure(1)
plot(S, mu, 'pg')
hold on
plot(Sp, fitmu, '-r')
```

```
hold off
grid
xlabel('Substrate [S]')
ylabel('\mu')
legend('Data', 'Fit', 'Location', 'NE')
txtlbl = sprintf('\\mu = %.3f \\times S / (%6.0f + S + s^2/%.1f)', B);
text(50, 0.0006, txtlbl)
mdl= fitnlm(S,mu,HaldaneInhMdl,B0)
```

4. *VFA concentration:*

Table 2: Total VFA Batch 2

Total VFA - Batch 1 (mg/l)						
Day	Blank	50	60	70	80	90
1	5741,5	6219	6308,5	353	6209	5945,5
2	6029,5	6345	5815	5631,5	5892	5840,5
3	5520	5724	6277	6074	5705,5	5652
5	5918	5970	5630	5924	6017,5	5968
9	5147	5448	5512	5336	5241,5	5571
10	5676,5	5640	5144	5361	5740	5613,5
15	4804	4656	5160,5	4582,5	5210	5348

Table 3:Total VFA Batch 1

Total VFA - Batch 1					
Day	Blank	10	20	30	40
1	879,015	779	763	817,5	846,5
2	447,5	469,5	425	425,5	444
5	424	374,5	379	405,5	424,5
8	412	380	422	356	429
13	449	345,5	401,5	6222	386,5
16	470	6223,5	6140	5072	6169,5
19	409	311,5	5784,5	5094,5	5562,5
21	416,5	5650	5577,5	4286	5654
23	3409	5558,5	4951,5	4429	5568
26	6327,5	4931,5	5121	4273	5357
29	4587	4416,5	4244	2834	4493,5
34	4107,5	2853	3346,5	2808	4308,5

5 Monod and Haldane model

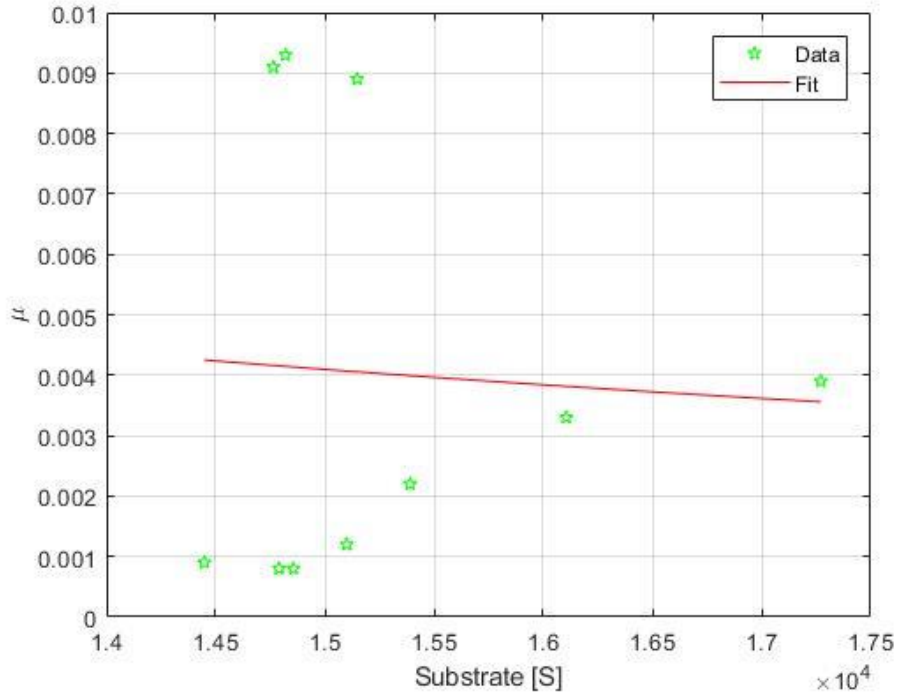


Figure 16: Fitting of growth data to Haldane model

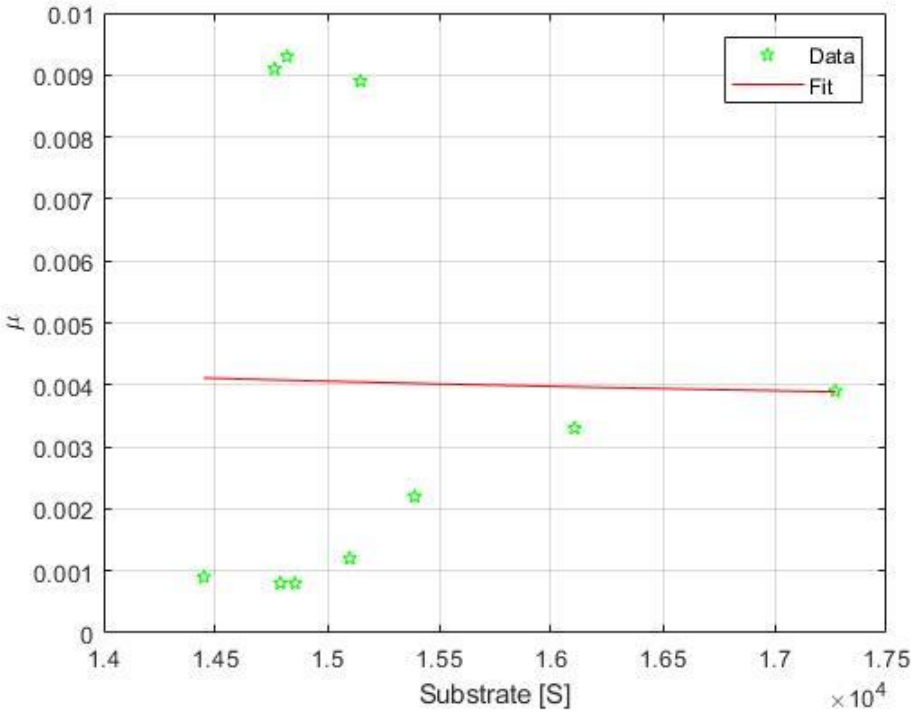
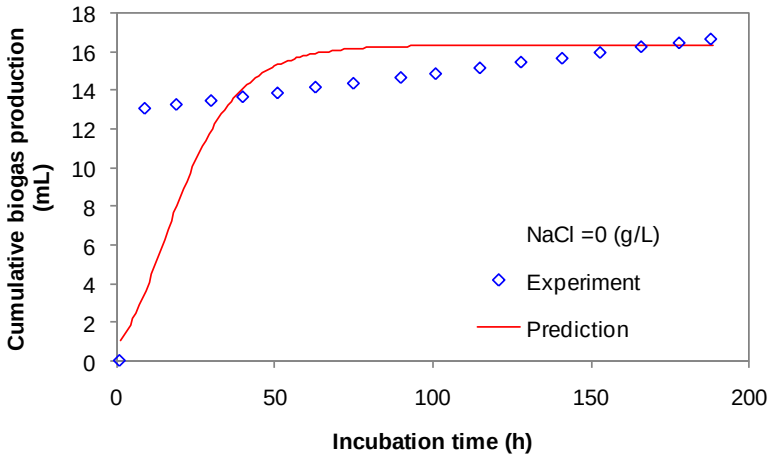
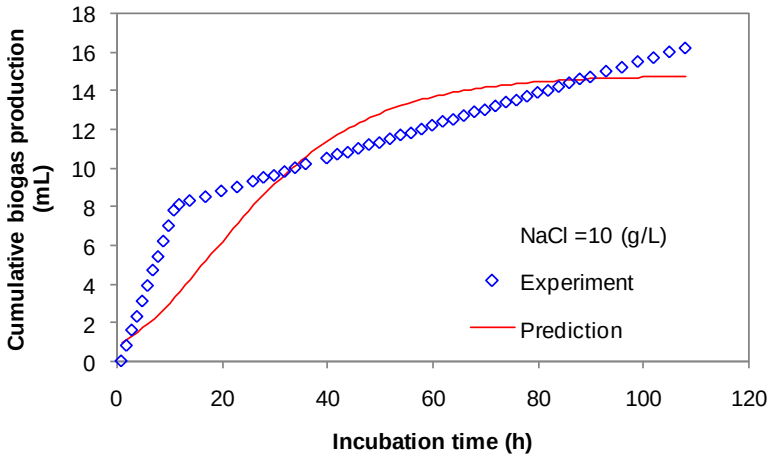
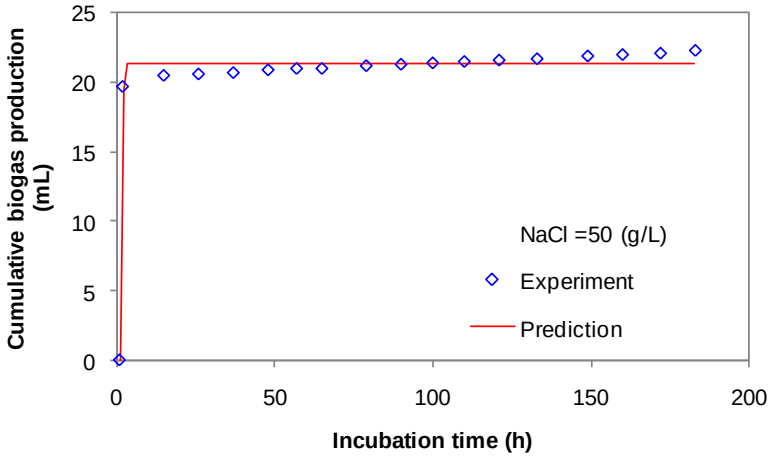


Figure 17: Fitting of growth data to Monod model

6 Data fit for Gompertz model:




Cumulative biogas production (mL)
Incubation time (h)
NaCl =10 (g/L)
Experiment
Prediction
0
2
4
6
8
10
12
14
16
18
0
20
40
60
80
100
120


Cumulative biogas production (mL)
Incubation time (h)
NaCl =50 (g/L)
Experiment
Prediction
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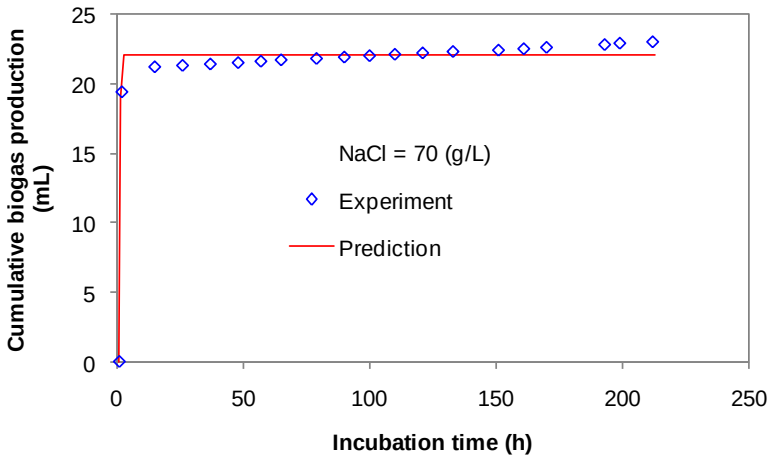
10
15
20
25
0
50
100
150
200


Cumulative biogas production (mL)
Incubation time (h)
NaCl = 70 (g/L)
Experiment
Prediction
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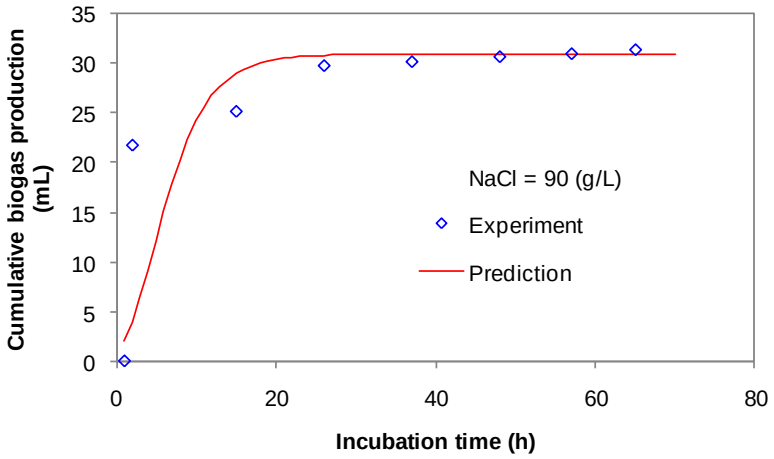
15
20
25
0
50
100
150
200
250


Cumulative biogas production (mL)
35
30
25
20
15
10
5
0
0
20
40
60
80
Incubation time (h)
NaCl = 90 (g/L)
Experiment
Prediction