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Effect of Salt Content on Biogas Production and Microbial Activity: Review Study

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Abstract

Over the years, biogas production technology has advanced with the goal of reducing process costs and greenhouse gas emissions, and increasing biogas production. Several design factors and operational aspects must be taken into account in order to produce a stable and efficient biogas. When the substrates contain high salts, anaerobic treatment will be ineffective due to the disadvantages of high energy input and operating cost, membrane contamination, and low efficiency. This indicates that the treatment of high salinity organic waste is a big challenge. High salinity levels had a negative effect on bacterial growth through bacterial osmotic pressure metabolism. For example, high salinity can alter the course of fermentation and the accumulation of volatile fatty acids at high concentrations, as well as cause a decrease in methane yield and maximum rate of methane production, prolonging the late-stage period. A low level of salt concentration encourages the growth of bacteria since sodium is essential for the growth and metabolism of microorganisms in AD systems. When the sodium salt concentration is less than 8 g/L, there is no significant inhibition in the generation of methane. Addition of >8 g/L NaCl, however, significantly reduced methane production (causing 17-80 percent inhibition). This paper focuses on understanding in detail how NaCl affects methane production and microbial activity, report salt concentrations that improve process efficiency and reduce inhibition, as well as review the modified kinetic model and demonstrate the effect of salt on methane production and delay in methanogenesis.

Keywords: Anaerobic digestion, salt content, methane production, microbial community, kinetic model

Introduction

Anaerobic Digestion of Organic Wastes

An organic waste (OW) generation has garnered public attention due to its amount, odor, and potential for pathogenic microorganism contamination. This is as a result of the population growth and societal changes. Conventional treatments, such as landfills and incineration, have negative environmental impacts (Cheng et al., 2010). The OW contains a high proportion of organic matter and substantial proportions of carbon and nutrients. In order to produce high-value products by anaerobic fermentation, food waste (FW) and an activated sludge produced through biological treatment are commonly considered as alternative substrates (De Gioanniset al., 2013; Pasupuleti et al., 2014). Furthermore, The OW components can be transformed into long-term products such as volatile fatty acids (VFAs), which can be employed as an alternative carbon source in current wastewater treatment facilities for biological nutrient removal and to produce methane and bio-hydrogen (Ye et al., 2013; Jie et al., 2014). Methane and bio-hydrogen are renewable energy sources that can be used as a substitute for fossil fuels (Lin et al., 2013). Rather than using traditional methods, such as dumping, landfilling, anaerobic composting, or conversion into animal feed, anaerobic digestion of OW has been considered as an effective method to cope with the environmental problem caused by the OW. Anaerobic digestion (AD) has a number of advantages, including high energy recovery with a modest amount of energy input, and the creation of renewable and environmentally friendly gas (CH_4) (Ghosh et al., 1974; Vanstarkenburg, 1997). Throughout the last decade, AD of the organic portion of municipal solid waste (OFMSW) has been widespread in Europe. The following are the primary drivers of this rise: (i) European legislation limiting landfill treatment of biodegradable waste (99/31/EC), (ii) an increase in source-sorted trash collection, and (iii) anaerobic treatment of biodegradable fraction resulting in increased energetic valorization (De Baere, 2006). Sludge is produced as a by-product of the physical, chemical, and biological processes employed in wastewater treatment plants. The AD has proven to be an excellent approach for treating the sludge since it can lower the overall load of biosolids to be disposed by up to 0.590 m^3/kg per kg of volatile solids (VSs) (Appels et al., 2011). It is feasible to generate 20 to near 300 kWh of net energy per tonne of garbage when biogas is used to generate electricity (European Commission, 2005). Many estimates have indicated that capturing CO_2 and recovering energy from biogas can significantly reduce greenhouse gas (GHG) emissions (Karagiannidis et al., 2009), while also avoiding ozone depletion and acid rain generation (Khalid et al., 2011). Another advantage of using biomass to generate biogas is that the solid waste product of AD (digestate) contains remineralized nitrogen and phosphorus, making it suitable for use as an organic fertilizer (Ward et al., 2008). Several reports indicated that AD of the organic fraction of solid waste produces promising quantities of biogas as indicated in Table 1.

Table 1. Methane production from AD of various substrates

Substrate type	Methane yield	Methane %	Reference
Municipal solid waste	0.36 m^3 CH_4/kg VS	65	(Kwietniewska et al., 2014)
Vegetable waste	0.16 m^3 CH_4/kg VS	68	(Rajeshwari et al., 1998)
Swine manure	0.33 m^3 CH_4/kg VS	-	(Ahn et al., 2009)
Food waste leachate	0.294 m^3 CH_4/kg VS	-	(Behera et al., 2010)
Straw	(0.27–0.29) m^3 CH_4/kg VS	75.9–78	(Lei et al., 2010)
Swine manure with winery wastewater	0.107 m^3 CH_4/kg VS	-	(Riaño et al., 2011)
Jatropha oil seed cake	0.394 m^3 CH_4/kg TS	66.6	(Chandra et al., 2012)
OFMSW with Sewage Sludge	0.242-0.656 m^3 CH_4/kg VS	-	(Corsino et al., 2021)
Corn stover and pig manure	0.275 m^3 CH_4/kg VS	43	(Qiu et al., 2021)

Anaerobic Digestion Process

AD is carried out by a sequence of metabolic events through several kinds of bacteria, including hydrolysis, acidification, acetogenesis, and methanogenesis. The first group of microorganisms hydrolyzes complex chemical substances enzymatically into monomers (e.g., glucose, amino acids), which are then transformed into higher volatile fatty acids (VFA), hydrogen, and acetic acid. The highly volatile fatty acids generated, such as propionic and butyric acid, are then converted to H_2, CO_2, and acetic acid by Acetogens. Thereafter, the H_2, CO_2, and acetate are eventually converted to CH_4 and CO_2 by methanogenic bacteria (Miyamoto et al., 1997; Khalid et al., 2011). The chemical composition of the feedstock/waste, ambient parameters, and digester operation conditions are important because all influence metabolic activity and the bacteria species, which are active during AD

(Kiener et al., 1983; Fetzer et al., 1993). The interaction between acetogens and methanogens, on the other hand, is extremely complicated. Since these microbes are anaerobes, oxygen poses a threat by disrupting metabolic pathways, resulting in the oxidation of cellular components that are normally present in reduced form. Several methanogens, on the other hand, have been shown to adapt to oxygen due to the inclusion of genes that produce enzymes (e.g., catalase and superoxide dismutase) in their genomes, which aid in the defense against oxygen toxicity (Brioukhanov et al., 2006). Methanogens such as *Methanobacterium thermoautotrophicum, Methanobrevibacter arboriphilus, and Methanosarcina barkerii* have been found to be highly resistant to oxygen and dessication (Kiener et al., 1983; Fetzer et al., 1993). Other studies indicated that with the creation of thick outer cell layers made of extracellular polysaccharide (EPS) and the buildup of cyclic 2,3-diphosphoglycerate, *M. barkeri* had an innate ability to withstand extended periods of exposure to air and deadly temperatures after the desiccation process (a novel metabolite which may be used to stabilize proteins at elevated temperatures). Furthermore, glycerol molecules bound by ether bonds to branched isoprene hydrocarbon molecules in the membrane lipids of archael species cause the organisms to acclimate to such severe temperatures. Acidogens, syntrophic acetogens, and methanogens make up the majority of the microbial community in a digester system (Anderson et al., 2012; Manyi-Loh et al., 2013; McInerney et al., 2009). The literature on AD reveals a wide range of inhibition/toxicity levels for most compounds. The intricacy of the AD process, where mechanisms such as antagonism, synergism, acclimation, and complexing could have a considerable impact on the phenomena of inhibition, is the main cause for these variances. In addition, numerous factors must be regulated to avoid difficulties that cause inhibition of biogas production. Microbial activity is directly influenced by temperature, pH, retention time, salinity, and organic loading rate. Furthermore, the physical characteristics of the feedstock can vary, and it may contain hazardous compounds that affect microbial activity (Refai, 2017; Annibaldi et al., 2019). When food is processed, salt (for example, NaCl), a sort of food flavoring ingredient accumulates in food waste in significant levels. The average concentration of NaCl is between 2% and 5% (in terms of mass fraction), and the content might change significantly depending on regional eating customs. Na^+ is a crucial component for anaerobic digestion system's cell creation, development, and metabolism (Zhao et al., 2017). Pang et al. reported in their study that NaCl (sodium chloride) is an inexpensive chemical with a wide range of sources. They also mentioned that the concentration of NaCl with appropriate doses could lead to the dissolution of the sludge and the deterioration of the blocks structure. Extracellular polymeric materials (EPS) release carbohydrates and proteins (Pang et al., 2020). However, high concentration of NaCl can reduce microbial activity and result to negative effects on AD to some extent (Li et al., 2020). Ammonia, heavy metals, fatty and lipid molecules, and excessive salinity are just a few of the components that have been found to hinder the AD process. High salinity, which could severely limit AD, primarily contains cations of Na, K, Ca, Mg, and Fe (Oh et al., 2013; Chen et al., 2008; Ngan et al., 2020). Also, in the study conducted by Yin et al. (2022), they found that increased salinity could trigger an expensive stress response for bacteria to balance the osmotic pressure in the cellular cytoplasm and reduce the energy available for metabolism. They further mentioned that methananositas, which belong to aceto-clastic methanogens, are subject to salt stress and their relative abundance is low. This is attributed to the inhibition of methane process at salt concentration higher than 25 g-NaCl/L (Yin et al., 2022). Biogas generation from solid OW is often carried out by several different anaerobic bacteria. The acid-forming and methane-forming microorganisms in AD have vastly different physiologies, dietary requirements, growth kinetics, and environmental sensitivity (Pohland et al., 1971). The principal cause of reactor instability is a failure to maintain the equilibrium between these two groups of bacteria. Therefore, this reference study aims to identify three things: to know the effect of high salinity levels on the process of AD and microbial activity, to reveal the effect of inhibition on acidification and methane processes at different salinity levels, and to review the modified kinetic model during anaerobic digestion of salt wastes, respectively.

Effect of Salt Content on Biogas Production

Although the composition of FW varies greatly depending on the source of collection, it usually contains a high level of salinity. The NaCl-added FW had 10 to 35 g/L NaCl, while the non-washed FW included 11.6 g/L NaCl (Shetty et al., 2008). It was reported that the FW from Shanghai cafeterias with an NaCl concentration of 8.0 g/L (Dai et al., 2013). The NaCl concentration in FW anaerobic digestate can reach 13.8 g/L (Wang et al., 2016). This increased salinity could produce an osmotic stress imbalance in cells, leading to plasmolysis and/or cell activity loss, which impedes and perhaps result to failure of the AD process (Lefebvre et al., 2007). According to a previous study, despite its highly nutritious biomass (Nagai et al., 2002), the consumption of FW from soy sauce was problematic due to its high salinity of 10% (w/w). Another study looked at the impact of salinity on biogas generation from food waste leachate and discovered that 0.52 g/L NaCl increased methane yield whereas 5 and 10 g/L NaCl reduced methane yield by 36 and 41 %, respectively (Lee et al., 2009). Rinzema et al. reported that at Na^+ concentrations of 5, 10, and 14 g/L, the synthesis of methane from acetate is hindered by 10, 50, and 100%, respectively (Rinzema et al., 1988). Another

study found that Na^+ concentration of 2 to 10 g/L inhibited methanogenic activity moderately, while a concentration exceeding 10 g/L inhibited strongly (Gourdon et al., 1989). It was reported that methanogenesis was impaired at an NaCl concentration of 5 g/L, while acidogenesis was significantly damaged (Lefebvre et al., 2007). At Na^+ of 4.42 g/L, the greatest methane output of 290.41 ± 34.21 mL of CH_4/ gVS was obtained. Meanwhile, at a salt content of 4.42 g/L, greater VFA synthesis was found. In the same investigation, inhibitory concentration values of 10%, 50%, and 90% were found at Na^+ concentrations of 6.3, 11.3, and 18.7 g/L respectively (Zhang et al., 2017).
Salt is harmful to bacteria and due to osmotic pressure, high quantities of salt concentrations dry the cells (Elefsiniotis et al., 2007; Feijoo et al., 1995). When the sodium content was ≤ 6.0 g/L, VFA production improved because the osmotic pressure was too low to change (Appels et al., 2011; Patel et al., 1997). A previous study found that the level of electrical conductivity (EC) at 35 mS cm^{-1} (19 mg/L NaCl) hindered CH_4 production. The EC level with a greater salt concentration of 80 mS cm^{-1} (44 mg/l NaCl) suppressed not only CH_4 and CO_2 production, but also organic compounds breakdown (Ogata et al., 2016). Increased salt concentration (0, 13, 30, and 60 g NaCl/L) had a negative influence on biogas volume produced from a co-digestion of food waste (Alhraishawi & Alani, 2018). Excessive salinity (NaCl >4.4 g/L) decreased AD performance. Additionally, the high salinity led to decreased microbial Ca^{2+} Mg^{2+} - ATPase activity, subpar EPS secretion, and the greatest variation in microbial operational taxonomic units, which together impeded AD process (Shi et al., 2021).

Effect of Salt Content on Microbial Community

Low salinity concentrations of 350 mg Na^+/L (0.8 g/L NaCl) were advantageous for methanogen growth, while 8-13 g/L NaCl caused significant inhibition and values exceeding 20 g/L NaCl led to severe impairment (Appels et al., 2008; Chen et al., 2008; Omil et al., 1996). It was reported that the specific CO_2 production rate in the high concentration of NaCl (High group) was much higher than in the blank group. Increasing NaCl concentrations up to a certain level had no negative impact on the bacteria's capacity to degrade organic compounds in acidogenesis. However, when the NaCl content reaches 20 g/L, it might increase the acidogenic impact. In contrast to acidogenesis, the specific CH_4 generation rate in methanogenesis showed a considerable suppression when NaCl concentrations were increased from 5 to 20 g/L, resulting in a 37.12 % decrease in the specific CH_4 generation rate (Wang et al., 2017). A previous study by Pang et al. (2022) indicated that the protease activity significantly increased at the NaCl concentrations within the range of 10–30 g/L, while the α-glucosidase activity decreased. It could be inferred that the hydrolysis of proteins improved and the hydrolysis of carbohydrates inhibited in the NaCl helped promote anaerobic fermentation (Pang et al., 2020). Acclimatization to high salt concentrations might result in the succession of halotolerant or even halophilic bacteria, thereby allowing the bioreactor to progressively restore its functionality (Luo et al., 2016). Increased salinity causes a shift in bacterial and hydrogenotrophic methanogen populations (Sudmalis et al., 2018). When salinity rises from low to high levels, archaea abundance and genes involved in methanogenesis decrease considerably. Similarly, gene abundance in the hydrogenotrophic pathway decreases (Wu et al., 2017). Acetoclastic methanogens, on the other hand, are more resistant to high salinity than hydrogenotrophic methanogens (Wang et al., 2017). The relative abundances of gram-negative *Pseudomonadaceae sp.* decreased, while salt-tolerant *Thermovirgaceae* and gram-positive *Clostridium sp.* increased 26% and 31%, respectively (Sierra et al., 2018). The hydrogenotrophic *Methanobacterium sp.* grew increasingly dominant among archaea. Another study also indicated that at high salinity, the dominance of *Methanobacterium* and *Methanosaeta* was observed. It was revealed that while the *Methanosaeta sp.* were dominant, they did not have a high salt tolerance (Onodera et al., 2017). A previous study conducted by Zhang et al. (2017) indicated that the dominant phyla of bacteria *Bacteroidetes, Firmicutes*, and *Proteobacteria* and the *Methanobacterium*, *Methanosaeta*, and Methanosarcina genera in archaea were predominant at different salinities. Hydrogenotrophic methanogens such as *Methanobacterium* can tolerate salinity up to 85 g/L, whereas acetoclastic methanogens, *Methanosaeta*, and Methanosarcina were severely inhibited at salinity greater than 65 g/L (Zhang et al., 2017).

Kinetic Equations on the Effect of the Salt Content on Biogas Production

There are several kinetic models that have been applied during the AD process. Among these kinetic models is the modified Gompertz model, which provides information on the lag phase and the maximum rate of specific methane production (Pramanik et al., 2019), as shown in the equations below:

$$M = P.exp\left(exp\left[\frac{R_M.E}{P}(\lambda - t)\right]\right) \quad \textbf{(1)}$$

M = cumulative methane production (ml), R_m = maximum methane production rate (ml/day), P = methane production potential (ml), λ = lag phase (days) and t = time of digestion (days), E: methane production potential (mL) constant (2.7182).

The first-order model showed a better fit than the modified Gompertz. Nonetheless, when a lag phase was reported, the modified Gompertz model better predicted the BMP compared to the first order (Strömberg et al., 2015).

From the original form, the modified Gompertz equation is established as shown in equation (2):

$$M = P.exp\left(\left[\frac{-r_0}{\alpha}\ .exp(-\alpha.t)\right]\right) \quad \textbf{(2)}$$

where r_0 and 〈 are parameters in Gompertz which is directly related to R_m and λ in Eq. (1) (Jijai et al., 2017). According to the study by Anwar et al. (2016), the modified Gompertz model predicted cumulative methane yield (CMY) accurately ($R^2 > 0.99$) under low salt concentrations. When the sodium salt concentration was increased, the lag period showed a relative increase, with λ being around 5 days for 2-8 g/L. However, it extended with higher sodium salt concentrations. λ was about 19.2 days for the reactor (with the addition of 16 g/L NaCl). Conversely, the methane production potential at 16 g/L decreased from 591 to 212 mL/g VS added, while the maximum methane production rate decreased from 39.4 mL/g VS added to 3.9 mL/g VS added (Anwar et al., 2016). In another study, the results showed that adding salt 2-4 g/L, which is the appropriate salt addition according to the results of the modified Gompertz model, could accelerate biogas production and improve the maximum biogas production rate (R_{max}). The delay periods were also very low with the exception of high doses of salt. More so, the researchers proved that the interaction of salt concentration and fermentation was significant for FW characterized by carbohydrates and protein ($p < 0.05$). High salt concentration and fermentation could break the AD system when the feed material is FW marked with carbohydrate. On the other hand, for FW marked with protein, the interaction of fermentation concentrations and addition of salt could mitigate the degrees of inhibition (Li et al., 2019). In another study, it was also shown that adding 4g/L of salt had a positive effect on gas production since the kinetic parameters obtained from the modified Gompertz equation were: P = 328.8 ml CH_4/gVS, R_{max} = 13.15 ml CH_4/(gVS.day), and λ = 2.1 day. A short lag phase of 2.1 day was observed for methane production. A sharp increase in methane production was observed from 2.1 to 6 days and methane production gradually increased (Han et al., 2012).

Conclusion

AD is a waste treatment technique that uses natural anaerobic decomposition to minimize waste volume while also producing biogas. For a long time, it has been used to treat waste from agricultural and industrial processes. The waste stream may contain inhibitory or even hazardous elements, such as salt content, depending on the source. Reduced biogas output and/or methane concentration in the biogas, as well as the possibility of reactor failure, could result from the accumulation of these compounds. The results of earlier investigations on the inhibition of anaerobic processes vary significantly due to differences in anaerobic microorganisms, waste composition, experimental methodologies, and circumstances. Obtaining information on waste components is critical for AD to work properly. It has been discovered that the right amount of salt can boost microbial activity and nutrient release, thereby increasing biogas generation. To avoid severe methane inhibition and poor decomposition performance, it is recommended that the sodium salt concentration in AD be kept below 8 g/L.

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