Inhibiting factors in the anaerobic digestion process for biogas production

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Abstract
The production of biogas by utilising the anaerobic digestion process is an economically and ecologically interesting process. However, the microbial community and the chemical balances of the anaerobic digestion process are vulnerable to a range of inhibitors. Determining inhibitors and solving the problems that come along is key to increase methane yields in the biogas production. Several methods have been applied to decrease inhibitory effects in anaerobic digesters including the physical-chemical featured separation method called air-stripping. Air-stripping is a method to reduce concentrations of free ammonia which is a known inhibitor of the biogas production. The effects of air-stripping on other components of the anaerobic digestion process are less heavily studied though. This article presents a summary of factors that are reported to inhibit the biogas production and reviews the effects the air-stripping method could have on the following factors: pH, ammonia, sulphate reducing bacteria, long chain fatty acids, humic acids, salinity, heavy metals and nanomaterials.

Introduction
The process of anaerobic digestion is the digestion of biodegradable material by microorganism in the absence of oxygen. Anaerobic digestion can be used to break down organic waste material from food production/consumption, faecal matter from livestock and other organic by-products from households and industry[1]. From the organic materials a range of compounds can be acquired by executing this process. The compounds produced in anaerobic digestion are complimented by the feature of retaining energy in the form of biogas and other biofuels. The utilisation of energy from anaerobic digestion is predicted to make up a sizeable part of sustainable energy generation once optimised[2]. The production of biogas will play the biggest role in this. Biogas is comprised of 50-75% methane (CH_4) which is the main component applicable for energy generation, and carbon dioxide (CO_2) with small amounts of other gases (*e.g.* nitrogen (N_2), ammonia (NH_3) and hydrogen sulphide (H_2S))[3]. The biogas can be put through a desulphurization and drying process to produce a biogas ready for combustion generatosr[4]. The output of that process can be upgraded further to 98% methane for injection into the natural gas network for all applications known to natural gas[5].

The retrieval of energy in the form of biogas from faecal matter and food waste is a process with interesting aspects from both economic and ecological standpoints. Such wastes are regulated due to their environmental impact and increasing storage of waste is an unwanted result of these regulations[6]. Since aerobic digestion leads to extra emission of greenhouse gasses and higher sludge residue, the better option is to feed the waste to a controlled anaerobic digestion setup[7]. To uplift interest, the process has to be optimized as anaerobic digestion is prone to inhibition and failure due to a high sensitivity for a range of toxic substances[8]. Inhibition of the system leading to low methane yield or instability is highly undesirable and delays the implementation of this form of energy generation. As diverse as the starting substrates and the microbiology in the anaerobic digestion system is, such a difference is there in what can give rise to all sorts of problems in the process. Extracting the cause of the problem is a challenging assignment and can be very costly for owners of digesters. Developing techniques to investigate, determine, correct and prevent complications in the system is the ultimate outcome of researching the inhibiting and toxic factors for the anaerobic digestion procedure of biogas production. Known molecules exhibiting the feature of inhibition in the system are ammonia[9], sulphides[10], heavy metals[11], long chain fatty acids[12] and halogenated organic chemicals[13].

Certain methods can be applied to the digestion setup for pre-treatment of the biomass or for removal of target substances such as seperation via physical-chemical properties[14]. The question that has formed the base for this article's subject, originates from the physical-chemical seperation method of air-stripping. In this method the contents of the digester are lead into an added compartment that is heated to temperatures ranging from around thermophillic temperatures up to 90 degrees Celsius. Applying air-stripping to the digester contents leads to a reduction in the concentration of free ammonia diluted in the digester[15]. The volatile and insoluble features of free ammonia on which the physical-chemical separation is based does not just affect the concentration of free ammonia but all chemicals that are both undissolved and volatile under the set temperature of the air-stripping compartment.

Another problem causing hindrance in optimisation derives from the fact that a variable mixed culture of syntrophic growing microorganisms are responsible for the anaerobic digestion process of biogas production from waste[16]. Separately investigating the microorganism has had some setbacks due to the syntrophic nature and the high diversity of the present strains. However, exploiting recent modern techniques does give the ability to more precisely characterise present strains as well as the expressed proteins and a higher quantity of metabolites[17,18].

The aim of this article is to review the inhibiting factors in the anaerobic digestion for biogas production by taking into account the variety of the cultures as well as the variety in substances that is fed to the system. Also viewing from the angle of an anaerobic digestion setup that makes use of the air-stripping method to determine if the effects are overall positive or negative.

1. Anaerobic digestion

Anaerobic digestion of organic material is a multiple step process executed by a variety of microorganism[19]. The first step in this process is referred to as hydrolysis. In hydrolysis the insoluble polymers of the organic waste are hydrolysed by a group comprised mostly of bacterial strains *e.g. Streptococcus* and *Enterobacterium*[19]*.* It is believed that the hydrolysis phase is the most rate limiting in the digestion process as the range of needed reactions is tremendous due to the highly divers composition of the polymers[20]. Because the reactions can differ in many ways, the optimal physiological parameters such as pH and temperature also vary making it near to impossible to optimise every single reaction. Another limitation is the expression levels of the enzymes which depends on the microorganisms present and what their needs are in nutritional and physiological preferences[21]. Temperature as a factor is externally influenced and can therefore be regulated depending on the waste to be digested, present microorganisms and/or desired maximal energy input. Increasing the temperature can increase the rate of the hydrolysis. However, higher temperature will obviously also increase the input energy. The variety in feeding input for the system contributes to the fact that many forms of molecules can be accumulated including some with inhibiting features to the process. The products of hydrolysis in the form of disaccharides, monosaccharides, amino acids, fatty acids and other soluble organic compounds are further digested to short chain organic acids, alcohols, hydrogen, aldehydes and carbon dioxide[22]. This process is titled acidogenesis and is executed by facultative anaerobe microorganisms including *e.g. Pseudomonas, Bacillus, Micrococcus,* and *Flavobacterium* and by some obligatory anaerobe microorganisms including *Clostridium*[23]. This phase also gives rise to some of the most prominent inhibitors of the biogas production, namely ammonia and hydrogen sulphide.

The next phase is the production of acetate and hydrogen from the organic material of the acidogenesis, called the acetogenesis. Methanobacterium, *Syntrophomonas* and *Syntrophobacter* are some of the bacteria known to be responsible for the acetate and hydrogen production[24]. The hydrogen gas that they release is toxic to the acetogenesis bacteria and therefore has to be degraded. The degradation of hydrogen gas is where the methanogenesis occurs. In a syntrophic relation to the bacteria in the acetogenesis phase, the microorganisms in the methanogenesis produce methane from the hydrogen gas and carbon dioxide as well as using up other substrates from previous phases such as acetate, methanol and methylated molecules[25].

The microorganisms responsible for the methane production are methanogens which are predominantly from the Archaea domain, and bacteria make up most of the other phases populations. The microorganisms that are present in bioreactors can also be separated by their temperature preference in the groups of mesophilic (28 to 42°C) and thermophilic (55 to 72°C)[26]. The temperature can therefore highly influence the composition of the methanogens as well as the microorganisms in the other phases. Microbes are highly sensitive to harsh changes in both temperature and pH. A cause of drastic pH change is the overloading of the system which destabilizes the production of the fatty acids leading to acidification of the reservoirs contents[27]. Once methanogens are stressed in any way to a point of seizing the hydrogen gas usage, results in a halt of the acetogenesis. From that moment the system can be fully thrown off balance once the acidogenesis phase overgrows, with more acidification and microbial and/or chemical composition changes as a consequence[28].

2. Biomass degradation

The composition of biomass is divers and can lead to the production of an array of molecules each exhibiting a unique effect on the system. The first process of hydrolysis sees to the degradation of various highly complex structures and is, as mentioned before, probably the rate limiting step in the biogas production. Cell wall components of plant material including celluloses, cell membrane components including phospholipids, fatty acid molecules and other high carbon polymers are the main source of carbon to be hydrolysed resulting in sugars and short chain fatty acids to be used in the acidogenesis[29]. However, not all biomass fed to a digester is degraded because some molecule structures and complexes are not suitable for the enzymes produced by the microorganisms.

Biomass particles that do not degrade and do not dissolve become part of the sludge in the reservoir and are not very reactive to the microenvironment in the digester. It is unfortunate that further application of those carbon sources is not possible but it is not a form of inhibition on the microorganisms as it is a form of inhibition via carbon source limitation. Instead, molecules that are released after hydrolysis and/or acidogenesis that are influential in an inhibiting manner are the main problem for anaerobic digester causing performance problems. Molecules produced during the process are not eligible for separation in a pre-treatment method and therefore have to be taken out in the middle of the process[30]. This has led to the inclusion of *e.g.* air-stripping methods to the setup.

3. Effect of pH

The level of pH is a main factor in the process of anaerobic digestion for biogas production. Preferences in acidity differ for many microorganisms and can therefore lead to unbalanced reactor populations if left uncontrolled. The pH is together with temperature and concentration a crucial factor in most chemical reactions including for those under the catalytic influence of proteins[31]. The

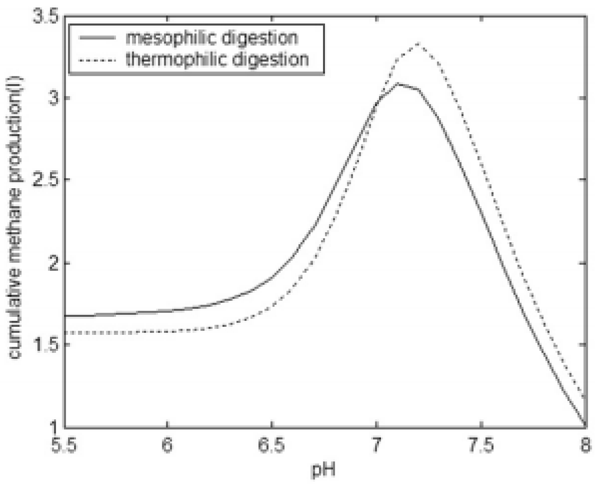

Figure 1: cumulative methane production in liters in anaerobic digestion on mesophilic and thermophilic conditions set out over the pH ranging from 5.5 to 8. As presented by Liu *et al.* (2008)[33].

functionality of proteins is a direct result of the folding of the structure, which is influenced by temperature and pH[32]. In a tank reactor temperature is a consistent factor that can be set externally, yet pH can change uncontrollably causing population shifts and chemical imbalances.

The desirable pH for the methanogens and acetogens in the anaerobic digestion is presented in figure 1 which is between 6.5 and 7.5 and should therefore be controlled as biogas yields decrease rapidly outside of this range[33]. For instance when pH decreases, the environment becomes preferable for bacteria in the acidogenesis phase leading to a population increase of this phase. A higher fraction of acidogenesis phase bacteria results in even further acidification of the environment due to increased production of short chain fatty acids. In an acidic environment microorganisms of the acetogenesis and methanogenesis phase are inhibited in their roles in the anaerobic digestion with severe decrease in biogas production as a consequence[34]. Another problem given rise by pH changes comes from the fact that most chemicals in a solution are present in an equilibrium. If one form of a chemical is (more) toxic and a pH change causes increase of the toxic form this can result in difficulties for the anaerobic digestion process.

4. Ammonia

Nitrogen based organic matter lead to production of ammonia in the acidogenesis phase. Proteins are hydrolysed to amino acids and this together with urea, which is prominent in animal manure, are the main substrates for the ammonia production[35]. In water ammonia is present in two forms, a protonated form of ammonia named ammonium (NH_4^+) and free ammonia (NH_3). The protonated form is not believed to have a negative effect on the anaerobic digestion system. On the contrary, NH_4^+ is actively taken up by some of the microbes as a nitrogen source. The free form of ammonia is the more troubling compound for the system. NH_3 can passively transport over the membrane of the cell due to the nonpolar chemical structure and cause imbalance of the proton potential and/or potassium deficiency[36]. The microorganism of the methanogenesis phase are presumed to be most affected by increasing free ammonia concentrations.

The concentrations of ammonium and free ammonia are heavily connected via an equilibrium. This balance can change, as mentioned before, depending on the pH and temperature. Decreasing the pH to acidic levels tips the balance in favour of ammonium which should decrease the inhibition caused by free ammonia as shown in the plot of figure 2[37]. However, the range in which acetogens and methanogens execute their role in the degradation system efficiently is, as mentioned before, between a pH of 6.5 to 7.5. As pH is not eligible as an interactive factor to decrease free ammonia in the system, another factor that can be altered is the temperature. The anaerobic digestion of biomass with high amounts of nitrogen including for example animal manure, is known to be degraded better under mesophilic conditions than thermophilic conditions[38]. The ratio of free ammonia over ammonium intensifies alongside rises in temperature. This feature can therefore be exploited by dropping the temperature to reduce stress by free ammonia in the system. On the other hand, it is important to maintain a temperature preferable for the microorganisms in the system as this influences the entire metabolism and the cells can be stressed when changing the temperature drastically.

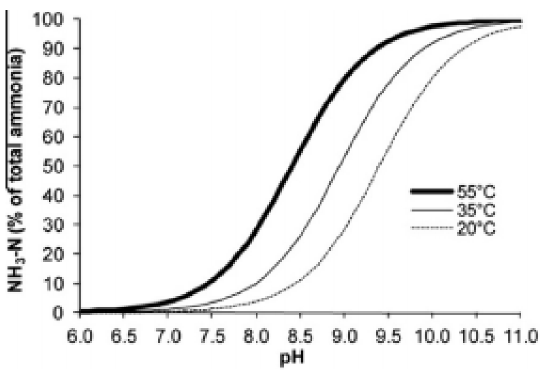

Figure 2: percentage of free ammonia of total ammonia at three different temperatures: 20, 35 and 50°C set out over pH. As presented by Rajagopal *et al.* (2013)[37].

Another factor that is reported to help the system is the antagonistic feature between ammonia and light metal ions[39]. Light metal ions such as sodium, calcium, potassium and magnesium are known to inhibit the biogas production when reaching certain concentration thresholds (this is discussed in a later chapter). The anaerobic digestion system is positively influenced when both light metal ions and ammonia are present in certain concentrations as the toxicity of the chemicals cancels each other out. How ammonia and the light metal ions antagonise their toxicity has not been determined yet.

The concentration at which inhibition by free ammonia differs severely with inhibitions reported at concentrations ranging from 53 to 1450 mg/L free ammonia under numeral different conditions (a more detailed table is presented in appendix 1)[37]. Having trouble in the production of biogas can be due to the inhibition of ammonia and reducing ammonia or nitrogen in the biomass could therefore increase the biogas yield. Reduction of total nitrogen in the biomass can be interesting for manure digesters which can be done by pre-treating the biomass or adding plant material low in nitrogen[40]. However, pre-treatments do not necessarily decrease the ammonia concentration significantly as most nitrogen sources can't be extracted without removing a good portion of biomass and adding plant material to animal manure can elongate the retention time needed for efficient anaerobic digestion. This has led to the demand of methods that reduce ammonia within the digestion setup including the air-stripping method. Using the air-stripping method reduces the total amount of NH_3 as well as NH_4^+ because the chemical balance will result in formation of NH_3 from NH_4^+ to reinstate the equilibrium between the molecules.

5. Sulphate reducing bacteria and sulphide

Sulphur holding organic matter with proteins as the main component lead to the production of sulphate (SO_4^{2-}) in the hydrolysis and acidogenesis phase. Sulphate is also present in many industrial waste and wastewater. As a nutritional source, sulphate can be taken up by sulphate reducing bacteria to produce sulphide (S^{2-})[10]. The growth of the sulphate reducing bacteria exhibits inhibition of the biogas production at two levels. The first inhibition originates from the fact that sulphate reducing bacteria have affinity with using hydrogen and other organic materials including acetate and fatty acids as an energy source for the sulphate reduction[41]. This means that the sulphate reducing bacteria are in competition with the methanogens for their nutritional needs in hydrogen as well as in competition with microbes of the acetogenesis for organic chemicals. Overgrowth of the sulphate reducing bacteria will result in less efficient methane production by the methanogens due to the low availability in necessary substrates. On the other hand, the sulphidogens are crucial for numerous chemical reactions in biomass degradation and therefore a healthy balance should be maintained between the methanogens and the sulphidogens[42].

The second inhibition is initiated by the sulphide production from the sulphate reduction which is toxic to a number of microorganisms present in the anaerobic digester including the sulphate reducing bacteria themselves[42]. The production of its own toxic environment is an important feature for the population regulation as it exhibits a negative feedback function. In a solution sulphide can take three forms: sulphide (S^{2-}), bisulphide (HS^-) and hydrogen sulphide (H_2S). The nonpolar chemical structure of hydrogen sulphide allows it to be passively transported over the membrane and is believed to negatively affect a cell by interacting with disulphide bonds in proteins and thereby inhibiting the enzymes and proteins function[43]. Inhibition by hydrogen sulphide has been reported to reach threshold levels ranging from 50 to 250 mg/L[8] depending on pH and digestion conditions. To reduce the stress caused by sulphide toxicity a range of physical-chemical methods can be applied including air-stripping. In the air-stripping compartment hydrogen sulphide is extracted and therefore reduces sulphide concentrations.

6. Long chain fatty acids

Long chain fatty acids make up a big portion of the biological material and can be released into the compartments in high concentrations when waste consisting mostly of fats is to be digested. The high energy organic compounds are very interesting for degradation to methane and this process has been reported to be achieved[44]. Contrarily, long chain fatty acids have also been reported to largely inhibit the methanogens in the anaerobic digestion[45]. The membrane of methanogens has the feature of being prone to long chain fatty acid binding causing interference in the membrane structure leading to transport problems and other membrane function failures. Concentrations at which long chain fatty acids become toxic depend on their length and saturation. A representation of the effect of lauric acid concentrations on methanogenic activity is presented in figure 3 with inhibition by lauric acid at 4.5 mM leading to around 50% activity. Another inhibiting feature by long chain fatty acids is a consequence of the ability of the fatty acids to bind to sludge particles[46]. The sludge bound by fatty acids can become so insoluble that it will float on the liquid surface. Thus causing the sludge to become unavailable on a physical level. Reducing the portion of long chain fatty acids in the biomass can be achieved by pre-treatment. However, decreasing fatty acids is most undesirable as they provide large amounts of carbon and energy for conversion to methane. Acclimation of methanogens has been key to reduce the inhibitory effect of long chain fatty acids allowing better resistance to it in the anaerobic digestion system[47]. Long chain fatty acids cannot be extracted by using an air-stripper.

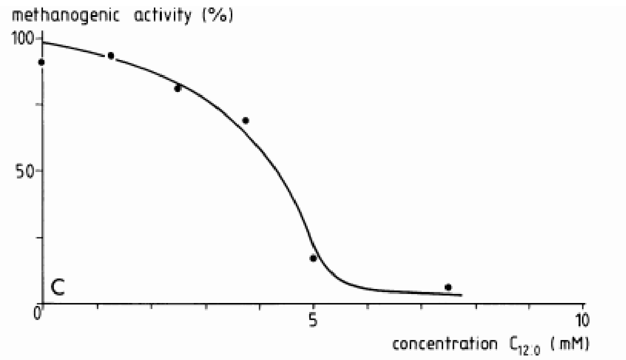

Figure 3: The effect of lauric acid (C12:0) concentrations in mM on the methanogenic activity in percentage. As presented by Koster *et al.* (1987)[12].

7. Halogenated organics and other organic chemicals

The predominantly nonpolar feature of a wide variety of organic chemicals allows the molecules to pass through or attach to a cell membrane same as the aforementioned inhibitors. The molecules binding to the cell membrane can cause irregular behaviour on a structural or functional level leading to loss of protection or membrane leakage followed by chemical imbalances[48,49]. Most organics known to inhibit anaerobic digestion are rarely present in general anaerobic digester tanks excluding industrial waste and (sewage) wastewater reactors where a lot of manufactured organic chemicals can be encountered. The nonpolar and volatile nature of most organics allows it to be extracted from the digester by using the air-stripping method with the extraction amounts proportional to the solubility and size of the molecule. The fraction of organic inhibitors that are more soluble will less likely be extracted in the air-stripping compartment. To counteract inhibition caused by organics it is a possibility to add enzymes or degradation strains to the digester or acclimate the populations to the exposure of the inhibitor.

8. Humic acids

Humic acids has only recently been described as an inhibitor of the anaerobic digestion, particularly the hydrolysis phase is affected by the presence of this substance[50]. It is proposed that humic acids bind to the hydrolytic enzymes which are excreted by the microorganisms and inhibiting the catalytic effects of the enzymes in that manner[50]. Especially, enzymes responsible for cellulose and xylan degradation have been reported to be negatively influenced by humic acids. Humic acids are the products of spontaneous deterioration of organic materials and can therefore be found in most forms of organic waste[51]. Humic acids are very complex and divers molecules and have been described as having the capability of heavily altering the chemical environment surrounding it. Thus it could result in inhibition on other levels as well due to chemical alterations. The results in figure 4 present the effect of adding humic acid holding biomass with 0.5 g/l already showing

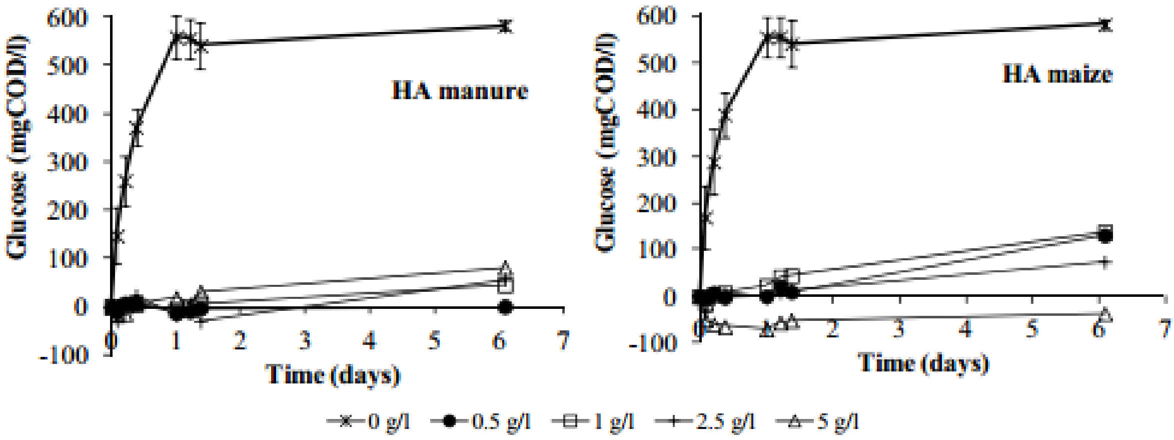

Figure 4: The effect of humic acid holding biomass in g/l on the glucose production in mg of COD/L over 7 days. As presented by Fernandes *et al.* (2015)[50].

severe decreases in glucose production from hydrolysis. To reduce humic acids in the biomass it is necessary to prevent disintegration of the biomass during storage. To increase tolerance of the system to humic acids, addition of hydrolytic enzymes can be applied to overcome the inhibiton caused in the hydrolysis[50]. Another option is the addition of polyvalent ions in the form of calcium and magnesium salts[52]. The ions will bind to humic acids and reduce the interaction with proteins and the influence on the chemical environment. Humic acid structures are soluble at a pH maintained in anaerobic digesters and have a relatively big size compared to volatile organics. Thus it can presumed that humic acids are not extracted when an air-stripping method is applied.

9. Salinity

The maintenance of salt concentration is necessary for good microbial growth and therefore also applies to the care of anaerobic digester cultures. The diluted counterparts of salts interact with the microorganisms as the ionic bound molecules are dissolved into the cation and anion groups. The ionic charge of these particles can cause an ionic imbalance at high concentrations that is accompanied by osmotic pressure which promotes cell dehydration[53,54]. Although, in the anaerobic digestion setup before such extreme concentrations are reached, it is believed that one group of ions already display an inhibitory effect. Of the ion groups from salt, the light metals belonging to the cations are believed to drive this negative effect on the system once concentrations exceed certain thresholds[8]. This includes sodium (Na^{+}), potassium (K^{+}), magnesium (Mg^{2+}), calcium (Ca^{2+}) and aluminium (Al^{3+}) ions as these are the most prominent in biological matter and can be released during degradation. The concentration at which inhibition by the light metal ions occurs differs and acclimation has been known to increase to threshold of inhibition. Mg^{2+} has been reported to cause ceasing of growth at 400 mg/L[55] and Ca^{2+} is reported to have no inhibitory effect up to concentrations of 7000 mg/L[56]. The addition of salts is mentioned as a countering system for some inhibitors and to adjust pH. Addition of salts contributes to concentrations of light metal ions and can eventually lead to inhibition of the biogas production due to precipitation of vital ions, destabilizing membrane potentials and disrupting the function of buffers[57]. To moderate the concentrations of the light metal ions targeted precipitation can be applied. The air-stripping method can potentially increase concentrations of ions over recirculation as water vaporises in the heated air.

10. Heavy metals

Heavy metals such as zink (Zn), iron (Fe), nickel (Ni), cobalt (Co) and cupper (Cu) are severe inhibitors at elevated concentrations[11]. Nonetheless, heavy metals are vital micronutrients to the microorganisms as they are key in numeral intracellular pathways and protein functions[58]. The concentrations of each heavy metal preferable for biogas production are in alignment with the strains of microorganisms present. The same applies to the toxicity of heavy metals that causes inhibition of the anaerobic digestion process when certain concentration thresholds are reached. The microbial community can become more resilient to concentrations of one heavy metal if the other heavy metals used as micronutrients are present in the preferred ratio respectively to the initially toxic heavy metal[59]. In free and soluble form, heavy metals at toxic concentration cause metal substitution in proteins and other structures and thereby disrupting the function[60]. Heavy metals can be released into a tank reactor during the degradation of biomass and when digesting wastes contaminated by heavy metals such as wastewater. Applying the air-stripping method can potentially increase heavy metal concentrations due to the vaporising of water during recirculation. reduction of dissolved heavy metals is done via targeted precipitation.

11. Nanomaterials

The use of nanomaterials has been implemented on a broad scale of industry in recent decades. The particles can be released indirectly into the environment and contaminate water and soil alike[61]. Not all nanomaterials interact on a chemical level in nature but the ones that do can have an ecological effect[62]. Studies investigating the effect of varying nanomaterials in many biological processes have been published recent years including the effects on anaerobic digestion. One reason that nanomaterials can have negative effects is that some hold (heavy) metal ions that can interact with that system in aforementioned manners. As research continues new effects of nanomaterials have been observed including inhibition via direct physical interactions with the microorganisms[63]. Nanomaterials are spreading increasingly and should therefore be taken into account when determining problems in the anaerobic digestion setup. In process extraction of nanomaterials from tank reactors has not been well documented and air-stripping can be presumed to be ineffective for this objective.

Discussion

The anaerobic digestion system for biogas production is an intricate method including various factors that influence the efficiency. Determining and preventing problems that can arise in digesters caused by inhibitors is an important step in increasing the biogas production efficiency and will increase interest. The factors discussed in this article have been documented as inhibitors of the biogas production from biological waste in numeral occasions. Methods for decreasing the effect of the inhibition or reducing the concentration of the inhibitor have been reported and applied to numerous setups of digester. Air stripping is a physical-chemical extraction method aimed to reduce free ammonia concentrations which has an inhibitory effect on the biogas production due to the harmful interaction for the microorganisms. However, other factors than free ammonia concentrations also influence the efficiency of biogas production. This article focuses on what other factors can inhibit the anaerobic process and how applying the air-stripping method influences these factors.

Temperature is an important factor that has to be controlled for a stable anaerobic digestion process. Contents of the main tank reactor are pumped to the input of the air-stripping compartment and will then be subjected to heated air at temperatures ranging from 60 to 90 degrees Celsius.

The higher temperature can cause cells that are processed through the air-stripping compartment to die, reducing the total amount of microorganisms present. A positive trade-off between ammonia extraction needed, cell growth and retention time in the air-stripper leading to cell death has to be implemented for increasing efficiency. Cell death caused by air-stripping is most likely higher in populations growing at mesophilic conditions than for populations growing at thermophilic conditions. Also, as ammonium concentrations are accumulated over time and cell growth is very important at the initiation of a new digestion, delaying the air-stripper to operate later into the digestion might make a difference.

The goal of air-stripping is to reduce free ammonia concentrations by extracting via heated air which is possible due to its volatile property. Free ammonia is reported to be a serious inhibitor of biogas production in anaerobic digesters and air-stripping reduces the stress caused by high concentrations. However, the application of the method could become counteractive for the efficiency of the anaerobic digestion process if extraction of ammonia is too effective. Ammonium is the main nitrogen source for microorganisms and continuously decreasing ammonia eventually will lead to low ammonium levels. The result of low ammonium levels is inhibition on the other end of the scale where nutrient limitation falls into place. Total ammonia nitrogen concentrations of 50 to 400 mg/L are proposed to be beneficial for biogas production[64]. Controlling the levels of nitrogen in the biomass for the digester is advisable when the air-stripping method is applied.

The reduction of ammonia levels can also increase the toxicity of light metal ions as it has been reported that an antagonistic balance is present between the molecules in anaerobic digesters. Light metal ions are not extracted when applying the air-stripping method and can even increase in concentration. During the process of air-stripping the heated air can cause water to vaporise resulting in increased concentration of remaining components. To reduce levels of light metal ions proportional to the reduced ammonia precipitation can be applied.

Air-stripping can extract ammonia due to its volatile and insoluble properties, yet is not the only molecule that will be extracted as more molecules exhibit these properties. Hydrogen sulphide will be extracted when pumping the digestate through the heated air compartment. Hydrogen sulphide is a reported inhibitor and decreasing the concentrations could increase the efficiency of the biogas production. However, hydrogen sulphide can become toxic to methanogens but also keeps the levels of sulphate reducing bacteria populations in balance. By dropping hydrogen sulphide concentrations populations shifts could occur depending on the tolerance of hydrogen sulphide per population. Increases in sulphate reducing bacteria populations acidifies the environment and thus inhibits methanogenesis. On the other hand, increases in methanogens can also occur and might increase the methane yield. However, in a stable process, the toxic effect on both populations remains respectively between the populations. Also, both are limited by hydrogen production and carbon source availability with sulphate reducing bacteria being especially limited by sulphate concentrations. Therefore, the effect of population shifts caused by decreases in hydrogen sulphide depends on the microbial community and nutrient availability. It is advisable to reduce sulphate concentrations when outgrowth of sulphate reducing bacteria inhibits biogas production.

Volatile and insoluble molecules in the organics category could be extracted when an air-stripper is connected to the digester, although this does not make up the whole group. Any of the inhibitors that are not extracted during air-stripping could be slightly concentrated when pumped back into the main tank as water vaporises due to the heated air. Humic acids, long chain fatty acids, metal ions, nanomaterials and the remaining organics could become more concentrated when applying air-stripping and should therefore be taken into account when determining the problems faced in the anaerobic digestion process for biogas production.

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Appendix 1

Summary table of reports on inhibition by free ammonia of the anaerobic digestion process under different conditions and animal wastes from the review by Rajagopal *et al.* (2013)[33].

Ammonia inhibition during the AD of organic feedstock's (animal wastes in particular).

Substrate	Reactor type	Feed TS (VS)	Inoculum	Operating conditions	Inhibition threshold FAN mg/L	Inhibition threshold TAN mg/L	pH	VFA mg/L	Results and comments	References
CM[a]	CFR[e]	6.4% (4.5)	–	Temp: 55 °C; HRT: 15 d	900	>4000	7.9	>3000 (as acetate)	Methane yield decreased to 25%, with both 4000 and 6000 mg N/L added, compared to the controls with 1500 mg N/L ammonia.	Angelidaki and Ahring (1993)
CM[a]	CSTR[f]	(4.2)%	Acc.[i]	Temp: 40–64 °C; HRT: 15 d Ammonia loading: 2.5 and 6.0 g N/L	>700	–	7.4–7.9	>4000	Reduction of temperature below 55 °C resulted in relief from inhibition caused by ammonia and hence biogas yield increased from 0.18 to 0.30 L/g VS, along with a decrease of the VFA.	Angelidaki and Ahring (1994)
CM[a]	UASB[g]	7.0% (5.0)	Acc.[i]	Temp: 55 °C; HRT: 15 d	995 (Initial inhibition)	Max. 7000	7.9	>3000 (as acetate)	SMA[j] of ammonia-inhibited reactors (7000 mg N/L) with acetate or hydrogen as substrate was reduced by 72 and 56%, respectively.	Borja et al. (1996)
SM[b]	CSTR[f]	(4.5)%	Acc.[i]	Temp: 37, 45, 55 and 60 °C; HRT: 15 d	1100	–	8.0	>4000 (as acetate)	Methane yield decreased from 188 (at 37 °C) to 22 (at 60 °C) mL CH_4/g VS.	Hansen et al. (1998)
Soluble non-fat dry milk supplemented with NH_4Cl	CSTR[f]	–	Acc.[i]	Temp: 55 °C; OLR: 4 gCOD/L d; HRT: 7 d	53	5800	6.4	2700 (as acetic acid)	As TAN concentration increased from 3000 to 5800 mg/L, CH_4 production rate reduced from 14 to 7.3 L/d.	Sung and Liu (2003)
SM[b]	ASBR[h]	22 g/L (12.5)	Acc.[i]	Temp: 10 °C Cycle length: 4 weeks OLR: 1.1 gCOD/L d	62	4400	7.89	831 (as acetic acid)	– Methane yield decreased from 0.266 (at 20 °C) to 0.218 (at 20 °C) and 0.080 (at 10 °C) LCH_4/g CODfed. –When temperature increased back to 20 °C, CH4 yield, VFA and SCOD reduction improved –No inhibition by FAN reported	Massé et al. (2003)
				Temp: 15 °C Cycle length: 4 weeks OLR: 1.4 gCOD/L d	96	4500	7.91	357 (as acetic acid)		
				Temp: 20 °C Cycle length: 4 weeks OLR: 1.4 gCOD/L d	185	4600	8.03	183 (as acetic acid)		
CM[a] + digested biomass	CSTR[f]	16.5–27.1 g/L (12–21.2)	–	Temp: 55 °C HRT: 15 d	1200	>10,000	7.6	–	Dilution of biomass with fresh cattle manure resulted in the highest CH_4 production rate during the recovery period	Nielsen and Angelidaki (2008)
SM[b] + solid fraction separated from pig farm	CSTR[f]	15.6% (12.6)	Acc.[i]	Temp: 55 °C HRT: 13.3 d OLR: 9.4 gVS/Ldigester/d	1450	11,000	7.6–8.08	>5700	–50% decrease in CH_4 yield observed at 11,000 mg N/L –Isobutyric, butyric, and isovaleric acids can be useful as process indicators.	Nakakubo et al. (2008)
Ch.M[c]	Batch culture (Dry AD)	25% (14.5)	Acc.[i]	Temp: 37 °C	–	8000–14,000	7.3–8.8	1300–9400	–Acclimation period: 254 d –Total volume of 4.4 LCH_4/kg CM was produced even at high levels of ammonia.	Abouelenien et al. (2009)
SS[d]	Batch study	8.5 g/L (4.4)	–	Temp: 35 °C COD/VS (substrate/inoculum) ratio:1–3	435–757	800–1400	8.0	<2000 (for COD:VS ratio of 1) >4000 (for COD:VS ratio of 2 and 3)	–No inhibition by FAN reported –AD of swine slurry are recommended to carried out at the COD:VS ratio of 1, thereby avoiding reactor imbalances due to VFA accumulation	González-Fernández and García-Encina (2009)

[a] CM,cattle manure.
[b] Swine manure.
[c] Ch.M, chicken manure.
[d] Swine slurry (mixture of nursery, sow and feeder-to finish swine waste).
[e] CFR, continuously fed reactor.
[f] CSTR, continuously stirrer tank reactor.
[g] UASB, up-flow anaerobic sludge blanket reactor.
[h] ASBR, anaerobic sequencing batch reactor.
[i] Acc., inoculum acclimatized to high ammonia concentration.
[j] SMA, specific methanogenic activity.