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Microbiological Handbook for Biogas Plants

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FOREWORD

In Sweden, there is currently great interest in the biogas process, since it can stabilize and reduce various types of organic waste whilst producing renewable and environmentally friendly energy in the form of biogas. In recent years, increasing interest in this process has led to the establishment of a dozen full-scale plants, and a few more are being planned. There is also increasing interest in both the production of biogas from municipal sewage treatment plants and on-farm biogas production within agriculture.

Efficient production of biogas relies on a complex microbiological process. Controlling the biogas process in an efficient manner to ensure maximum yield requires some advanced knowledge of how microorganisms work and of the microbiology underlying the biogas process. To date, there has been a lack of easily accessible literature in Swedish that is specifically written for the staff responsible for biogas production plants. This type of literature can be used to support training and as a separate guide for staff at the plants. This guide serves these purposes.

The guide was compiled by Anna Schnürer (Dept. of Microbiology, Swedish University of Agricultural Sciences, Uppsala) and Åsa Jarvis (Jarvis Biowrite, Uppsala). Both authors have written doctoral dissertations on the subject of the biogas process and its microbiology and have extensive experience in the field.

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This is a translation of a Swedish document published by Avfall Sverige [Swedish Waste Management] and Swedish Gas Centre (SGC). Some of the contents of this document pertain specifically to Sweden but the vast majority of the information is generic and highly valuable as guidance for proponents and operators of anaerobic digestion facilities around the world. Since the translation is quite literal, both the credit and the responsibility for the accuracy and appropriateness of the information contained within this document lies completely with the original authors and the publishing organization. The initial translation of the document was translated by the Vetter Group, Vancouver, Canada.

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1. MICROBIOLOGY OF THE BIOGAS PROCESS

A complex microbiological process lies behind the efficient production of biogas. Many different species of microorganisms need to be active in order for biogas to form. In addition, these organisms have to work closely together. A disturbance of this teamwork results in reduced biogas production and, in the worst case scenario, a breakdown of the process. Controlling the biogas process in an efficient manner requires knowledge of the microbiology behind the biogas process and of how microorganisms function.

1.1 What is Required for the Function and Growth of Microorganisms?

In order to function and grow, a microorganism needs access to an appropriate culture medium, i.e. a substrate. A substrate is "food" for the organism and must contain several different elements: a source of energy, electron acceptors, building blocks for building new cells, and different types of vitamins and trace elements (metals). With access to a substrate, microorganisms can metabolize, that is build up new cells (anabolism) and produce energy (catabolism) for this growth. The organic waste treated in the biogas process represents the substrate for various microorganisms. The more varied the composition of the organic material, the more components are available for growth, and thus the greater diversity of organisms that can grow. However, it is not good if the composition varies too much with time because many of the microorganisms that develop during the process are specialists, i.e. they grow best on a specific substrate.

In addition to the substrate, the microorganisms require a suitable environment in order to thrive and function. Examples of important environmental factors for growth are: temperature, pH, oxygen content, and salt concentration. Different organisms have different requirements for these environmental factors in order to be able to grow optimally. Typically, microorganisms adapt to their environment. For example, microorganisms that live in, and are adapated to, high temperature environments often grow best at these temperatures. In a biogas process where many different microorganisms may be active, the reactor environment has to be compatible with the requirements of as many microorganisms as possible. This means that the environment may not be perfect for each microorganism, but still good enough to allow the organisms to grow.

When microorganisms utilize substrate, they form new cells, but also various types of waste products (decomposition products). Normally, the waste product excreted by a specific organism cannot be used by it any longer, but it can serve as a substrate for another microorganism. This is typical of the biogas process, namely a series of different microorganisms utilize each other's decomposition products as substrate. Examples of microbial waste products in a biogas process are fatty acids (acetic, propionic acid, etc.), carbon dioxide, and hydrogen. Methane, which is the end product of a biogas process, is also a microbial waste product.

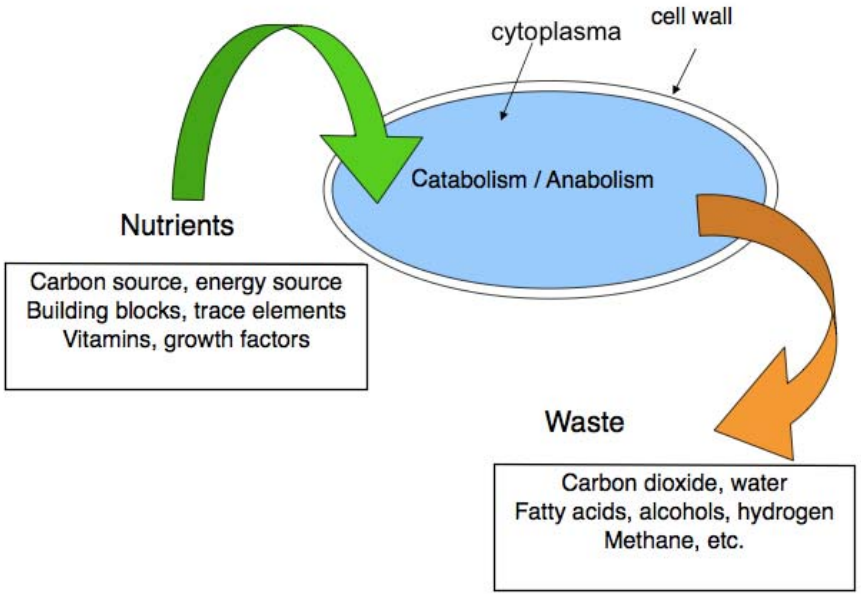

Figure 1. Cell Metabolism

Energy Source

The energy source is the material that the organism uses to get energy for both its growth and function, such as movement or the intake of substrate. It can be compared to petrol for a car engine or the sun for a plant. The energy source for a microorganism can either be a chemical compound or solar energy. The organisms in a biogas process use various chemical compounds as energy sources. These can be either inorganic compounds like hydrogen, or organic compounds such as various types of sugars, fats, and proteins. When organisms use a chemical compound as a source of energy, the compound oxidizes and electrons/protons are transferred via a number of so-called intermediate carriers to a final electron acceptor. Energy is formed during this transfer of electrons. The type of energy used by microorganisms is often the chemical compound ATP (Adenosine Triphosphate).

Electron Acceptors

Oxygen is the final electron acceptor (sometimes called an electron receiver) in aerobic respiration (breathing oxygen). In the absence of oxygen, either fermentation or a so-called anaerobic (oxygen-free) respiration takes place. Fermentation mainly uses various organic substances as electron acceptors. The end products formed are primarily various acids and alcohols, as well as hydrogen and carbon dioxide. Anaerobic respiration primarily uses inorganic compounds as electron acceptors. Substances that can be used for anaerobic respiration include, for example, sulphate (SO_4^{2-}), iron (Fe^{3+}), manganese (Mn^{4+}), nitrate (NO_3^-), and carbon dioxide (CO_2). Some microorganisms can only use a single type of acceptor, while others can use several different types. Some electron acceptors are more advantageous than others because they enable

the formation of more energy, in the following order: $O_2 > Fe^{3+} > Mn^{4+} > NO_3^- > SO_4^{2-} > CO_2$, where oxygen ($O_2$) provides the most energy and carbon dioxide (CO_2) the least (Zehnder 1988).

If several electron acceptors are available in the same process, the organisms that utilize the most energy-generating compounds will dominate. This is exemplified by the biogas process, where there is normally a large amount of carbon dioxide (and carbonates). The methane-producing microorganisms dominate here, and they use carbon dioxide as the final electron acceptor. The process also includes a small number of sulphate-reducing bacteria. These form hydrogen sulphide (H_2S) with sulphate (SO_4^{2-}) as the final electron acceptor. If large amounts of sulphate were added to a biogas process, the relationship would be reversed, i.e. sulphate reducers would grow at the expense of methane producers that would decrease in number. This is because the sulphate-reducers generally obtain more energy in their metabolism and thus can grow better.

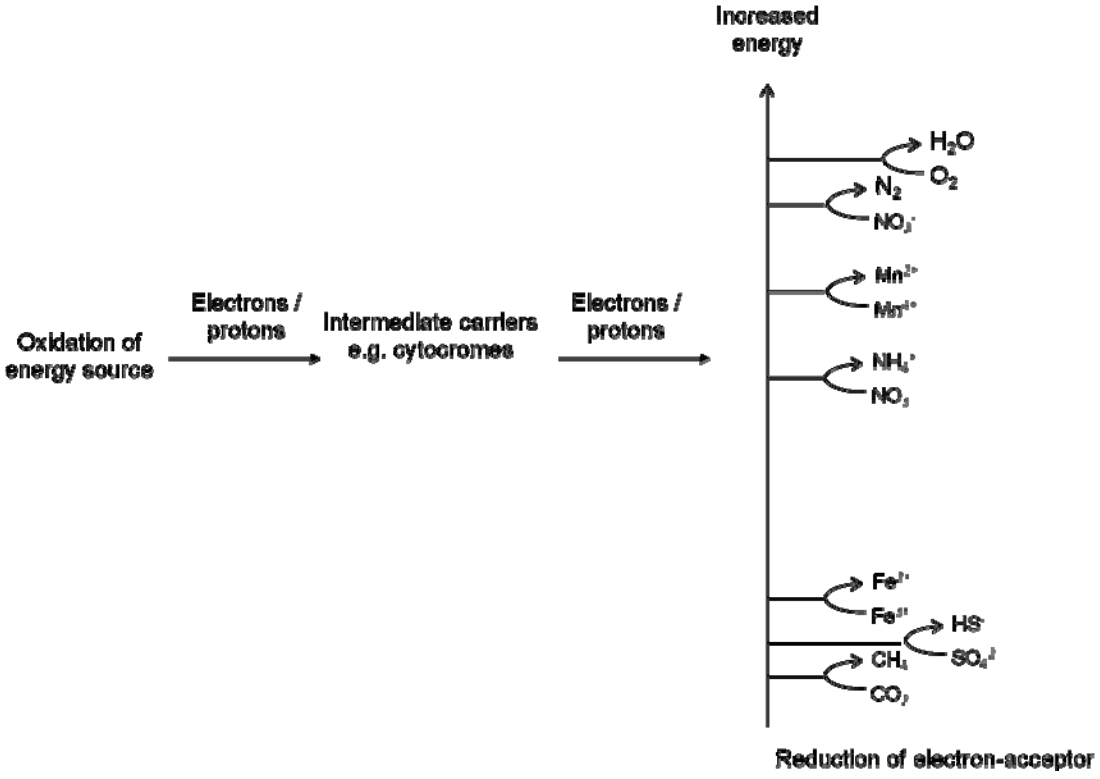

Figure 2. The flow of electrons to various electron acceptors during anaerobic respiration

Building Blocks

The most important building blocks are carbon, which provides about 50% of the microorganism biomass, and oxygen, nitrogen, and hydrogen (Table 1). Other important building blocks are sulphur, phosphorus, sodium, potassium, magnesium, calcium, and chlorine. When the energy source is organic, it is also common to use it as a source for the building blocks. When the energy source is inorganic, carbon dioxide (CO_2) is the most common source of carbon, and ammonia (NH_3) is the most common source of nitrogen. Energy formed by oxidation of the energy source

is used to form new cells. The design of synthetic nutrient solutions for growing microorganisms is often based on the structure of the cells (Table 1). The structure of the cells can also be used as a guideline for the approximate composition of an optimal substrate.

Component	C	O	N	H	P	S	K	Na	Ca	Mg	Fe	Other
% of dry weight	50	20	14	8	3	1	1	1	0.5	0.5	0.5	0.5

Table 1. Approximate composition of a bacterial cell (Modified based on Madigan and Martinko, 2006)

Trace Elements and Vitamins

Just like other living organisms, microorganisms need different trace elements and vitamins to function. Different organisms have different requirements for these substances. Some organisms can form vitamins themselves, while other organisms need to absorb a number of vitamins from their environment. Trace elements are always taken up from the surrounding environment. In a biogas process, the substrate should supply these substances to the microorganisms. However, the occurrence of these substances varies greatly between various types of substrates.

Numerous scientific articles have demonstrated the importance of trace elements for the function of the biogas process and specifically the methane-producing organisms. Despite the importance of trace elements for process stability and the production of biogas, it is very clear that there is still no formula for optimum composition. Trace elements that have been found to be important to methane-producing organisms are iron, zinc, nickel, copper, cobalt, molybdenum, and in some cases selenium and tungsten (Jarrell and Kalmokoff 1988, Zhang et al 2003). Several studies have also shown that the addition of trace elements can stimulate the biogas process and enable higher organic loadings (Florencio et al 1993, Nordberg and Edström 1997, Nordberg et al 1997, Jarvis et al 1997, Osuna et al 2003, Climenhaga and Banks 2008). Substrate characteristics determine whether trace element additions are needed. For example, plant-derived materials may limit the biogas process due to the low content of certain trace elements. Several biogas plants in Germany that digest plant-based materials (and do not use manure) add trace elements to achieve stable operation (personal communication Ralf Winterberg, Elbe bioenergie®).

Environmental Factors

Temperature

The optimum temperature, i.e. the temperature at which the organism grows fastest and works most efficiently, varies among species. Microorganisms can be divided into different groups depending on the temperature at which they best thrive and grow: psychrophilic, mesophilic, thermophilic, and extremophilic/hyperthermophilic (Noha and Wiegel 2008). Typically, the optimum temperature for a specific organism is strongly linked to the environment from which it originates. For example, microorganisms that live in marshland, tundra, or in a septic tank, may

have a low optimum temperature (around 10°C) (psychrophilic temperature range), whereas human intestinal bacteria, such as Esherichia coli, grow best at 37°C (mesophilic temperature range).

Organisms with an optimum temperature above 50°C are called thermophiles, and those that grow above 65°C are called extreme thermophiles (Noha and Wiegel 2008). Some microbial communities are adapted to grow at even higher temperatures. Microorganisms that have very high optimum growth temperatures (above 85°C) live in hot springs and submarine volcanoes. The latter belong to the so-called hyperthermophiles, in which the cell proteins and other components are intact even at these high temperatures (Wagner and Wiegel 2008).

Common to all growth intervals is that the temperature that allows the highest rate is close to the so-called maximum temperature, which results in cell death. If the temperature increases above this maximum temperature, the cell's proteins and other components are quickly inactivated, causing the organism to die. The maximum temperature varies depending on which temperature range the microorganism is adapted to.

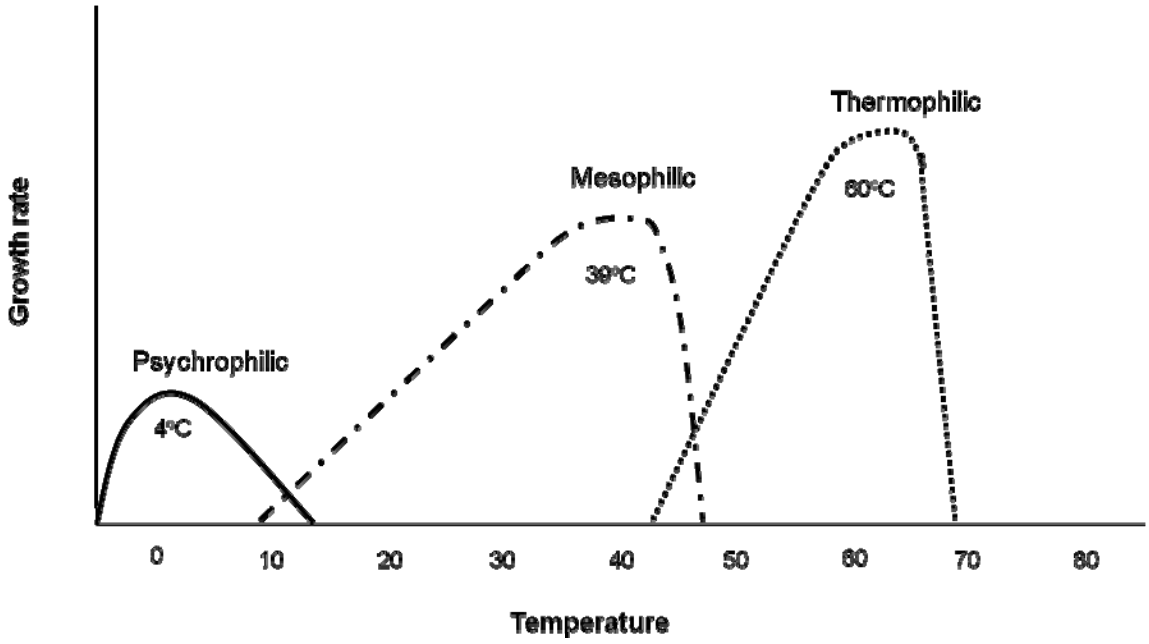

Figure 3. Growth of microorganisms at different temperatures (Modified based on Madigan and Martinko 2006)

A biogas process contains many different organisms, and to some extent, they differ in how they respond to temperature. However, the biogas process usually operates at a temperature range of around 30°C-40°C or 50°C-60°C (Nordberg 2006). Biogas production is possible at psychrophilic temperatures but may also result in a lower methane production rate depending on the type of process (Hesselgren et al 2005, Collins et al 2006, Bohn et al 2006). In the case of high temperatures, there are examples of methane-producing organisms that can handle 110°C

(Chaban et al 2006), but stable biogas processes do not seem to operate above 60°C-70°C (Scherer et al 2000). At temperatures above 60°C, the activity of methane producers is reduced to a greater degree than that of acid-forming organisms, which often results in the accumulation of fatty acids in the biogas process (Nozhevnikova et al 1999, Scherer et al 2000).

The growth of some microorganisms does not follow the curves in the figure above. Examples include the so-called thermo-tolerant organisms. They survive at high temperatures (up to about 60°C) despite the fact that their optimum growth takes place in the mesophilic temperature range. There are also organisms that can survive at mesophilic temperatures despite growing best at higher temperatures. Research has shown that about 10% of the microorganisms in a mesophilic biogas process are actually thermophilic (Chen 1983). The presence of these organisms makes it possible to convert a mesophilic biogas process to a thermophilic process. Chapter 2 contains more information about this. In principle, the wide range of organisms present in a biogas process also makes it possible to produce biogas at intermediate temperatures such as 45°C (Lindorfer et al 2008).

Oxygen

The importance of oxygen concentration varies greatly for the different microbial communities that comprise the biogas process. Some of the organisms, such as those that produce methane, are very sensitive to oxygen and die if they come in contact with air. Others can survive quite low concentrations of oxygen, while others grow better if oxygen is present. The free radicals of oxygen are strong oxidising agents that can destroy cells by oxidizing various cell components. Microorganisms that can live in the presence of oxygen have different defence systems, that is, various enzymes that can protect the cell against oxidation by oxygen. The organisms that are sensitive to oxygen do not have this enzymatic defence system and are destroyed in the presence of air. Microorganisms are usually divided into different groups depending on their relationship with oxygen. Both strict anaerobes and so-called facultative aerobes are found in the biogas process. Strict anaerobes only grow in the absence of oxygen. This group includes the methane-producing organisms. On the other hand, facultative aerobes grow in both the presence and absence of oxygen. This group includes numerous fermentative microorganisms. In the presence of oxygen, they can grow by aerobic respiration, but then they switch to fermentation when oxygen is depleted. This means that a temporary air leakage to a biogas process need not be a problem because there are microorganisms that can rapidly consume the incoming oxygen. There are even studies that show that a brief aeration during the biogas process can be a way of reducing the concentration of fatty acids (Agdag and Sponza 2004).

Strictly aerobic	Facultative aerobic	Oxygen Tolerant	Micro aerophilic	Strictly anaerobic
Always respire with oxygen	Respire with oxygen, but can switch to	Can live in the presence of oxygen, but always carry out	Respire with oxygen, but only at lower oxygen	Do not require oxygen for their growth and may

	fermentation or anaerobic respiration in the absence of oxygen	fermentation	concentrations than in the atmosphere (<20%)	even die in its presence. Always perform anaerobic respiration or fermentation

Table 2. The importance of oxygen to different microbial communities

pH

Most microorganisms prefer a neutral pH range, i.e. about pH 7.0-7.5. However, some organisms are active at both lower and higher pH values. There are several different organisms in the biogas process, and their pH requirements for optimal growth vary greatly. While fermenting, acid-producing microorganisms manage to live in relatively acidic conditions, down to pH 5.0, most methane producers generally require neutral pH values to be active. Although most methane producers thrive best at neutral pH values, they remain active outside this pH-range (Whitman et al 2006). There are known examples of acidophilic methane producers that grow down to pH 4.7 (Bräuer et al 2006) and alkaliphilic methane producers that grow at pH values of up to 10 (Mathrani et al 1988). Several biogas processes are currently operating in Sweden at pH values around 8 (personal communication, Anders Ek, Swedish Biogas) and the literature also contains examples of biogas processes operating at a pH values below 6 (Savant et al 2002). The fact that acid-forming organisms can handle a lower pH is illustrated by the fact that decomposition of the substrate often begins already in the substrate tank, with acid formation and low pH as a result. However, methane production does not usually occur here because the pH is too low. Instead, it starts in the digestion tank where the pH is significantly higher. The growth of microorganisms at various pH ranges often follows the same pattern as the growth at various temperatures. That is, at all growth intervals, the pH value that generally results in the greatest rate is closest to the pH value that results in cell death.

Salts

All microorganisms require salts to function. The salts contain essential building blocks for the microorganisms, such as sodium, potassium, and chlorine. These substances are available in many substrates and do not need to be added to the biogas process separately. However, some waste has a high salt concentration or results in the release of excess salt, which can inhibit the microorganisms in the biogas process. Salts (and sugars) generally have a preservative effect, that is, they inhibit bacterial growth. Too much salt (or sugar) causes the cell to pump out water and lose both form and function. Some organisms can adapt to high salt concentrations if they are allowed to adjust slowly. They often form so-called osmolytes: compounds that help them maintain their function, even in the presence of salt. Organisms that can handle relatively high salt concentrations are called halotolerant, and those that grow even better at high salt concentrations are called halophiles. The most extreme forms of halophile grow best at salt concentrations above 20%-30% sodium chloride (> 3.4mol/L-5.1mol/L) and this group also includes some methane producers (Chaban et al 2006). Examples of materials that could lead to

increasing salt concentrations in biogas processes are waste from the food and fisheries industries, or different types of protein-rich materials that lead to the release of ammonia. Typically, methane-producing microorganisms are usually the ones most affected by increasing salt concentrations in a biogas process (see Chapter 4).

1.2 Decomposition of Organic Compounds in the Biogas Process

In a biogas process, large organic molecules (proteins, sugars and fats) are successively broken down into methane and carbon dioxide, a gas mixture called biogas (Figure 4). The presence of several different microbial communities is required for the biogas process to work. In order to form biogas as an end product, these active microorganisms also have to work together (Zinder 1984, Dassonville and Renault 2002). This means that both the nutritional and the environmental requirements of a large number of microorganisms have to be met for the biogas process to function as a whole. The various stages of decomposition and the microorganisms that are active at each stage are described below.

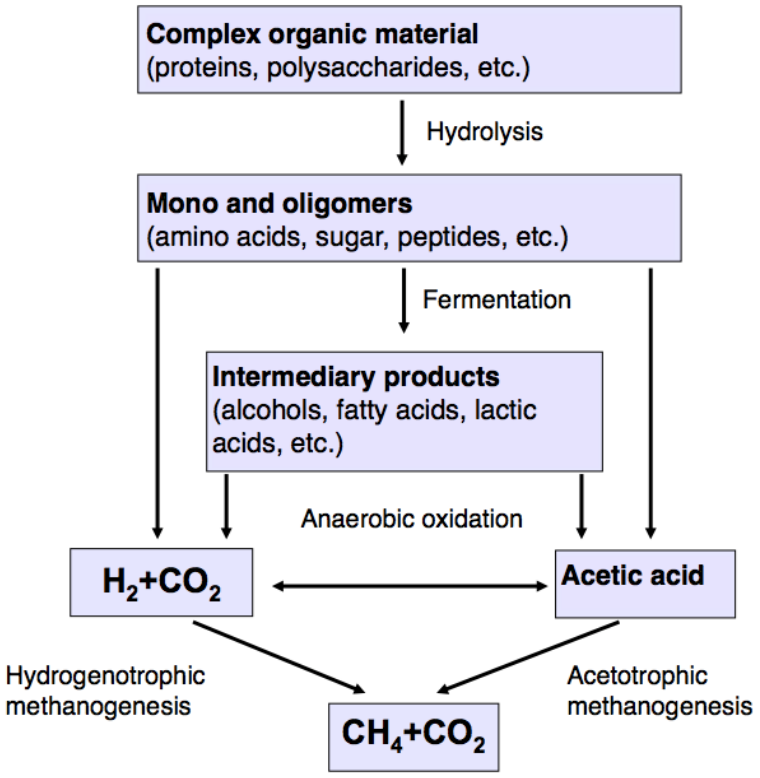

Figure 4. Stepwise decomposition of organic matter into biogas.

1.2.1 Stage 1. Hydrolysis

Hydrolysis is the first stage of the biogas decomposition process. In this stage, sugars, fats, and proteins are converted into smaller organic compounds such as amino acids, simple sugars, fatty acids, and some alcohols. This first stage is very important because large organic molecules are

simply too large to be directly absorbed and used by microorganisms as a substrate/food source. To accomplish biodegradation, certain microorganisms secrete different types of enzymes, called extracellular enzymes, which "cut" the larger molecules up into smaller pieces that the microorganism can then take into the cell and use as a source of energy and nutrition. Some microorganisms secrete several different enzymes, which allow them to break down different types of organic materials. Other microorganisms are specialised. For example, they secrete enzymes that break down either sugar or protein. Microorganisms that break down different sugars are called saccharolytic, while those that break down proteins are called proteolytic. There are different enzymes for sugars, proteins, fats etc. The table below contains examples of some different groups of extracellular enzymes. Each group contains several enzymes that are specialised in various substrates, such as different proteins. The rate of decomposition during the hydrolysis stage depends greatly on the nature of the substrate. The transformation of cellulose and hemicellulose generally takes place more slowly than the decomposition of proteins. Chapter 3 contains more information about the importance of substrates to the biogas process.

Enzymes	Substrate	Breakdown Products
Proteinase	Proteins	Amino acids
Cellulase	Cellulose	Cellobiose and glucose
Hemicellulase	Hemicellulose	Sugars, such as glucose, xylose, mannose and arabinose
Amylase	Starch	Glucose
Lipase	Fats	Fatty acids and glycerol
Pectinase	Pectin	Sugar, for example, galactose and arabinose, and polygalactic uronic acid

Table 3. Some important groups of hydrolytic enzymes and their functions.

Hydrolysis of polysaccharides

Polysaccharides are compounds that contain chains of linked sugars. Common polysaccharides are cellulose, hemicellulose, starch, pectin, and glycogen. Cellulose, hemicellulose and starch are important components in plant material and are found in fruit, grains, vegetables, and root crops among many others. Glycogen is a polysaccharide that functions as a sugar reserve, primarily in animals. Pectin is common in fruit, and its structure, which is very complex, varies between

different fruits and degrees of ripeness. Polysaccharides may be linear (cellulose, starch) or branched chains of sugars (hemicellulose, starch, glycogen, pectin). Hydrolysis of cellulose results in the formation of cellobiose (two interconnected glucose molecules) and glucose. Starch and glycogen are cleaved into glucose units, and several different sugars are formed from the hemicellulose and pectin. Organisms that are active in a biogas process during the hydrolysis of polysaccharides include various bacterial groups in, for example, the genera Bacteriodes, Clostridium, and Acetivibrio (Cirne et al 2007, Doi 2008). Some of these organisms have several different enzymes combined into so-called cellulosomes that are situated on the organism's cell wall. In addition to enzymes, these cellulosomes also contain proteins that have the ability to bind to cellulose (Ding et al 2008). Binding to its substrate makes decomposition more efficient because the enzymes can work directly 'on-site'.

Hydrolysis of proteins

Proteins are chains of amino acids that are found in high concentrations in, for example, meat-derived substrates and in chicken and swine manure. Shorter chains (<50 amino acids) are also called peptides or peptide chains. Amino acids are the primary end products of the hydrolysis of proteins and peptides. Which amino acids form depends on which proteins are found in the organic matter. Certain proteins, so-called glycoproteins, also contain carbohydrates. These are commonly found in cell membranes and on the surface of cells and the carbohydrate portion of these may correspond to as much as 80 percent of the weight. In addition to amino acids, the decomposition of glycoproteins also produces various carbohydrates. Proteolytic organisms in the biogas process include, among others, the genera Clostridium, Peptostreptococcus, and Bifidbacterium (Örlygsson 1994, Ramsay and Pullammanappallil 2001).

Hydrolysis of fats

There are several different fats, with a varying composition depending on their origin. Generally, these fats consist of glycerol (an alcohol) and different fatty acids, all of which are released by biodegradation (McInerney 1988). Enzymes that break down fats are called lipases. Examples of materials that are high in fat are slaughterhouse waste and grease-separation sludge. Most of the known lipases are produced by aerobic or facultative aerobic microorganisms. Strict anaerobes that secrete lipases include, among others, the genus Clostridium (Gupta et al 2004, Petersen and Daniel 2006).

1.2.2 Stage 2. Fermentation

The fermentation stage in a biogas process consists, just as the hydrolysis stage, not of one reaction but of several. Exactly which reactions occur depends on which organisms are present and on which substrate is treated during the process. Many different organisms are active during this stage, more than during the other stages (McInerney 1988, Colberg 1988). Many of the organisms that carry out fermentation are the same ones that carried out hydrolysis during the first stage, but other organisms in other genera that are also active include, for example, Enterobacterium, Bacteriodes, Acetobacterium and Eubacterium. During fermentation, the products from the previous hydrolysis stage (carbon and energy sources) are used as substrate by a number of different microorganisms. Sugars, amino acids, alcohols, etc. can be used as

substrates by fermenting microorganisms (Madigan and Martinko 2006). On the other hand, the fermenting organisms do not use the fatty acids released during the breakdown of fats and aromatic structures. Instead they are not broken down until the next stage in the breakdown chain (anaerobic oxidation).

Through various fermentation reactions, the products from hydrolysis are converted mainly into various organic acids (acetic, propionic acid, butyric acid, succinic acid, lactic acid etc.), alcohols, ammonia (from amino acids), carbon dioxide and hydrogen (Table 4). Exactly which compounds are formed depends on the substrate and environmental process conditions, as well as on what organisms are present.

Typical of the acids that are formed, is that the charged form (without protons) is in equilibrium with the uncharged form (with protons, Equation 1). The acid constant (pKa) indicates how easily the acid releases its proton. If the pH is below the pKa-value, the majority of the acid is in its uncharged form, while at a pH above the pKa-value it is mainly in the charged form. In a biogas process at pH> 7, acids are mainly in the charged form (anion). At this stage, they tend to form salts with different metals such as sodium and potassium. The acid form and anion have different names (for example acetic acid (acid) and acetate (anion, Table 4).

$CH_3COOH \leftrightarrow CH_3COO^- + H^+$

Equation 1. Acetic acid is in equilibrium with its anionic form, acetate

Common Name	Systematic name	Anion	pKa	Chemical structure (acid form)
Formic acid	Methanoic Acid	Formate	3.77	HCOOH
Acetic acid	Ethanoic acid	Acetate	4.76	CH_3COOH
Propionic acid	Propanoic acid	Propionate	4.80	CH_3CH_2COOH
Butyric acid	Butanoic acid	Butyrate	4.83	$CH_3CH_2CH_2COOH$
Valeric acid	Pentanoic acid	Valeriate	4.84	$CH_3CH_2CH_2CH_2COOH$
Caprylic acid	Hexanoic acid	Capronate	4.85	$CH_3CH_2CH_2CH_2CH_2COOH$

Table 4. Names of some common acids and their pKa values and chemical structure. The values apply to aqueous solutions at 25°C

Fermentation products from the same compound may be different in different organisms. Even organisms within the same genus or species can form different products from the same compound (Table 5). In some cases, one and the same organism can also change its fermentation pattern depending on prevailing conditions (the presence of other organisms, environmental factors). For the organism that produces them, fermentation products are waste products that are excreted and are of no further use. Instead, they serve as a substrate for other microorganisms in the biogas process, including other fermenting microorganisms, which further decompose them.

Products	Clostridium butyricum	Clostridium acetobutylicum
Butyric acid	76	4
Acetic acid	42	14
Lactic acid	-	-
CO_2	188	221
H_2	235	135
Ethanol	-	7
Butanol	-	56
Acetone	-	22

Table 5. Fermentation products of glucose formed by two different species of bacteria of the related genus Clostridium (modified based on Gottschalk 1986). The figures represent the amount formed (mol) per 100 mol glucose.

1.2.3 Stage 3. Anaerobic Oxidation

The products formed during the fermentation stage are further broken down by various anaerobic oxidation reactions. This is a very important step in the biogas process that requires close

cooperation between the organisms that carry out oxidation and the methane-producing organisms that are active in the next stage, the actual formation of methane. The reason that two different groups of organisms have to work together is very complex, but in brief it can be said that the phenomenon is strongly linked to the concentration of hydrogen gas. During anaerobic oxidation, protons are used as final electron acceptors and this produces hydrogen gas. For thermodynamic reasons, formation of hydrogen gas will only take place if the concentration of hydrogen gas is constantly kept at a very low level. If the hydrogen gas formed is not continuously removed, anaerobic oxidation will stop because the microorganisms will then no longer get enough energy for growth (Figure 5, Schink 1997, 2002).

This is where the formation of methane comes into the picture. This process constantly consumes hydrogen gas, thus keeping the concentration of hydrogen gas at a sufficiently low level. In biological systems other than the biogas process, there are other hydrogen gas-consuming organisms that can drive anaerobic oxidation, such as sulphate-reducing or nitrate-reducing microorganisms.This cooperation between microorganisms is called syntrophy and the transfer of hydrogen gas is called "Inter-species Hydrogen Transfer" (IHT) in the literature, meaning the transfer of hydrogen gas between species (Schink 1997, 2002).

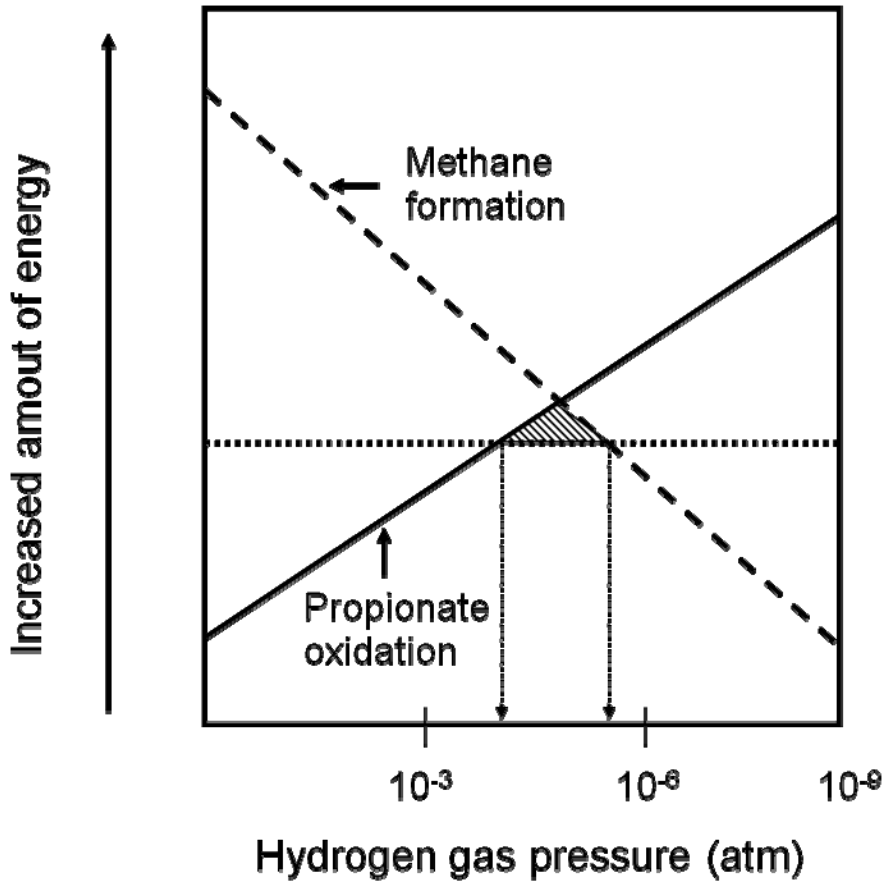

Figure 5. The importance of hydrogen gas for the anaerobic oxidation of propionate to hydrogen gas and acetate (solid line) and for the formation of methane from hydrogen gas (dashed oblique line drawn). The horizontal line represents the level at which the organisms get energy for growth. Only when the hydrogen gas pressure is such that the oblique line goes above this horizontal line does the organism get enough energy to allow for growth. For the propionate-oxidising organism, this means that it is advantageous to have low hydrogen gas pressure, but for methane-producing organisms, the opposite is true, namely that it grows better at high hydrogen gas pressure. The shaded triangle in the middle shows the area of hydrogen gas concentration where both organisms can grow at the same time. (Modified based on Dolfing 1988)

It is worth noting that hydrogen gas can form in different ways and not all hydrogen gas-producing microorganisms are dependent on a partner organism and IHT (Gottschalk 1986, Dolfing 1988). Several fermentative organisms produce hydrogen gas even in the absence of a hydrogen gas-consuming organism, but at much lower concentrations. Many syntrophs that form hydrogen gas can also use alternative breakdown pathways in the absence of a hydrogen gas-consuming partner, which does not lead to hydrogen gas formation. They can then adapt to the prevailing hydrogen gas concentration. Others always form hydrogen gas and in that case they are absolutely dependent on the hydrogen gas-consuming organism. Generally, the organisms that can switch metabolism are the ones that, when they cannot form hydrogen gas, produce more of various types of fatty acids and alcohols instead (Thauer et al 1977).

Substrates for anaerobic oxidation consist of various fatty acids, alcohols, some amino acids and aromatics (Dassonville and Renault 2002). Aromatics are compounds with ring structures, such as benzoic acid, phenols or certain amino acids that occur, for example, in plant materials and pig manure. Fatty acids and alcohols are products of different hydrolysis and fermentation reactions. In addition to hydrogen gas, these compounds primarily form acetate and carbon dioxide by anaerobic oxidation (Fuchs 2008, Sousa et al 2008). Syntrophomonas, Syntrophus, Clostridium, and Syntrobacter are examples of genera in which there are numerous organisms that can perform different anaerobic oxidations in syntrophy with an organism that uses hydrogen gas (McInerney et al 2008). Many of these organisms are known as acetogens, that is, in addition to hydrogen gas and carbon dioxide they also form acetate as their main product (Drake et al 2008).

1.2.4 Stage 4. Methane Formation

Methanogenesis is the final stage of the biogas process. In this stage, methane and carbon dioxide (biogas) are formed by various methane-producing microorganisms called methanogens. The most important substrates for these organisms are hydrogen gas, carbon dioxide, and acetate, which are formed during anaerobic oxidation. But other substrates such as methyl amines, some alcohols, and formates can also be used for the production of methane (Liu and Whitman 2008). Just like in other stages of the biogas process, not just one, but several different types of microorganisms are active in this stage. The methane-producing group that usually dominates in a biogas process is the so-called acetotrophic methanogens, which use acetate as substrate. In their metabolism, acetate is cleaved into two parts. One of the carbons is used to form methane and the other to form carbon dioxide.

Thus, acetotrophic methane producers are sometimes also called acetate-splitting methanogens. Acetate is the source of about 70% of the biogas produced in a digestion tank (Zinder 1993).

The hydrogenotrophs are another important group of methanogens, for which the primary substrate for the formation of methane is hydrogen gas and carbon dioxide. Today there are only two known groups of methanogens that break down acetate: Methanosaeta and Methanosarcina, while there are many different groups of methanogens that use hydrogen gas, including Methanobacterium, Methanococcus, Methanogenium and Methanobrevibacter (Garcia et al 2000, Liu and Withman 2008). Methanosaeta and Methanosarcina have different growth rates and also differ concerning their ability to utilize acetate (Westermann et al 1989). Methanosarcina grows faster, but finds it difficult to use acetate at low concentrations, when Methanosaeta has an advantage. However, the presence of these organisms is affected not only by the acetate concentration, but also by factors such as loading frequency and mixing (Liu and Withman 2008).

Metanogens	Doubling time	Lowest acetate concentration used
Methanosarcina	1 day	~ 20 mg/L
Methanosaeta	2-12 days	~ 4 mg/L

Table 6. Doubling time and the lowest acetate concentrations used with Methanosarcina and Methanosaeta

Because methane producers generally grow very slowly, this is often the rate-limiting stage of the biogas process (Liu and Withman 2008). Generation time, i.e. the time required for a microorganism to divide itself in two, is between 1 and 12 days for methane producers. Methanosaeta grow the slowest. The growth rate of methanogens often sets the limit for how short the retention time in continuous biogas process can be (see Chapter 2). Too short retention time (less than 12 days) increases the risk that these organisms will be washed out of the process, because they do not have sufficient time to increase at the same rate as the material is pumped into and out of the digestion tank.

Methanogens differ from the other organisms in the biogas process, because they are not common bacteria. Instead methanogens are part of a group of organisms called Archaea (Garcia et al 2000). The Archaea are a separate group of organisms that have evolved in parallel with the bacteria (prokaryotes) and fungi (eukaryotes). Because of their unique character, methanogens are easily distinguished from other "common" bacteria in the microscope. Methanogens contain a compound (F420) that allows them to fluoresce with a green-blue colour when illuminated in the wavelength range of around 350-420 nanometres (Liu and Whitman 2008; Figure 6). The fact that methanogens do not resemble other organisms also means that they are not as robust as

many other microbes in the process. The methanogens are often the first to be affected by various disturbances such as pH changes or the presence of toxic compounds such as heavy metals or organic pollutants (Chen et al 2008, Liu and Withman 2008). Because these organisms are also of great importance to the function of anaerobic oxidation, inhibition/disruption of methanogens can seriously affect the entire process.

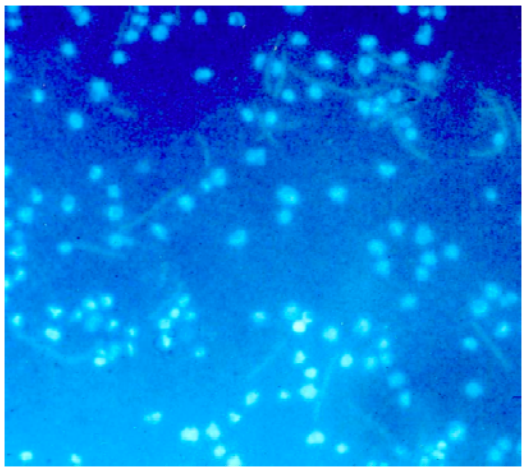

Figure 6. Fluorescent methanogens. Photo Anna Schnürer

Alternative Methane Production Pathway from Acetate

An alternate pathway for methane production from acetate is increasingly being described in scientific articles (fig.7; Schnürer 2007, Hattori 2008, Schnürer and Nordberg 2008). The importance of this route of decomposition is currently unknown. Not considering reactions occurring in the natural environment, this reaction pathway has only been demonstrated for some Danish biogas plants and a few Swedish co-digestion plants (Schnürer et al 1999, Karakashev et al 2006, Schnürer and Nordberg 2008). Factors that are considered to influence the development of this path in a biogas process are the content of ammonia and acetate, and the types of active methanogens. Retention time in the biogas process has also proven to be significant, along with temperature. With this methane formation pathway, biogas is not directly generated from acetate by acetotrophic methane production (so-called acetate splitting). Instead, acetate is first converted by non-methane-producing bacteria into hydrogen gas and carbon dioxide. These products are then used by hydrogenotroph (hydrogen gas-consuming) methane producers to form biogas. This cooperation between two different groups of organisms is called syntrophic acetate oxidation (SAO). For the conversion of acetate to hydrogen gas/carbon dioxide to take place, the hydrogen gas pressure must be kept low, which is taken care of by the methane producers. This methane formation path from acetate is slower than that of the acetotrophic (acetate splitting) methane producers, which results in slower breakdown of organic matter and biogas production when the SAO path is used.

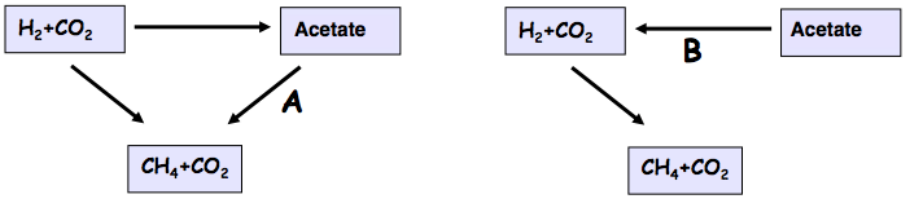

Figure 7. Two different methane production pathways from acetate are known: splitting of acetate by an acetotrophic metanogenesis (A) or oxidation of acetate to hydrogen gas and carbon dioxide by a non-metanogenic bacteria (B) followed by a reduction of carbon dioxide to methane by a hydrogenotrophic methane producer.

CHECK YOUR KNOWLEDGE

- What does a microorganism need for its function and growth?
- Where do the microorganisms in the biogas process find their nutrients?
- What environmental factors are important for the microorganisms to thrive in the biogas process?
- Why is it important to have trace elements in the biogas process?
- Why don't microorganisms like high salinity?
- Why is a varied substrate good?
- How many different microbial communities exist in a biogas process?
- What are the different stages of decomposition of a biogas process?

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2. THE IMPORTANCE OF TECHNOLOGY TO MICROBIOLOGY

As the previous chapter shows, the interaction between different microorganisms controls the biogas process. Therefore, in order to achieve a functioning and stable process with high methane production, it is important to create and maintain as beneficial an environment as possible for these microorganisms. This is where technology comes into the picture. With the help of technology, we can shape the working environment for the microbes and thus get them to work and produce at maximum efficiency. However, the microorganisms in the biogas process have their limitations, just like all other living organisms, so it is a matter of pushing them just enough and at just the right pace. Here, the operator must proceed carefully and not change too much at once. Otherwise the interaction between the microorganisms is disrupted and the digestion process stops. Many of the active organisms are sensitive to large and rapid changes. However, if they just have time, they can often adapt to the most extreme conditions. With the right technology it is possible to go a long way and in this chapter some parameters are discussed that are critically important in controlling the biogas process and the activity of the microbes in it.

2.1 Start-up of a Biogas Process

The biogas process and the organisms that are active during the decomposition process occur naturally in our environment. Examples of environments where biogas is produced naturally are wetlands, lake sediments, rice paddies and in the stomachs of ruminants. Therefore, when a man-made biogas reactor is started up, it is possible to start with something like cow manure as the so-called inoculant. In principle, the rumen of a cow works like a miniature biogas reactor: all of the organisms necessary for methane production are found here. The temperature in the rumen is also higher (+39°C) than in sediment, and the organisms that live in the rumen are adapted to a temperature in the mesophilic range, a temperature range that is suitable for biogas production in man-made biogas reactors. The microorganisms in cow manure will find in a biogas reactor the environment to which they are accustomed.

When a biogas reactor is started up, microorganisms from the inoculant need time to adjust to the substrate that the specific biogas plant is going to treat. In a biogas plant, both the substrate and the environment will differ from the original environment and it is important for the organisms to adapt to enable a stable process. During this adjustment period, the organisms in the inoculant that are best able to survive in the new environment will grow and become established. Microorganisms that are added via the new substrate may also play a role in the process. The more the environment from which the inoculant is taken differs from the environment in the digestion tank, the longer the start-up period will be. To achieve a quick and reliable start-up of the process, it is best if a microbial community is established already from the beginning, based on adaptation to a similar substrate. One way to achieve this is to start the process using digestion tank contents from an already operating process that uses a similar substrate.

Figure 1. A biogas reactor can be started up using manure from cows or the contents of a digestion tank from an already operating biogas process. Photo Anna Schnürer

2.2 Process Design

The process takes place in a closed tank that is free from oxygen. This is very important because methane-producing microorganisms cannot tolerate oxygen (see Chapter 1). However, some of the microorganisms in the process are facultatives that can use oxygen for their metabolism. This means that a small amount of oxygen can penetrate into the tank without stopping the process from functioning. However, an oxygen leak allows facultative, non-methane-producing organisms to use organic matter for their growth, which means that a smaller proportion of the carbon is converted to methane (see Chapter 1 in the section on oxygen).

The digestion tank, or reactor as it is sometimes called, can be built out of steel or concrete and can also be equipped with heating pipes and insulation material for good heat retention. The biogas formed is collected from the top of the container, while the substrate is usually pumped into the process. The digestion residue is removed by pumping it through an overflow pipe for further storage or to return it to the process (Edström and Nordberg 2004). A more detailed description of different types of processes and models can be found in, among other publications, Gerardi (2003), A Guide to Anaerobic Digestion (2005) and Biogas from manure and waste products - Swedish case studies (2008).

The initial treatment of substrate differs at different plants, depending on the material to be digested. Sometimes one or more pre-treatment steps are involved, for example to increase the digestibility of waste that is hard to break down, such as crop residues or packaged food, or to thicken materials with a small content of dry solids. It is also common to mix and/or dilute the ingoing substrate in a reception tank/substrate tank. For material of animal origin, such as waste from a slaughterhouse, food waste and manure, digestion is preceded by a sanitation stage, which usually involves heating the material to 70°C for one hour (see Chapter 3).

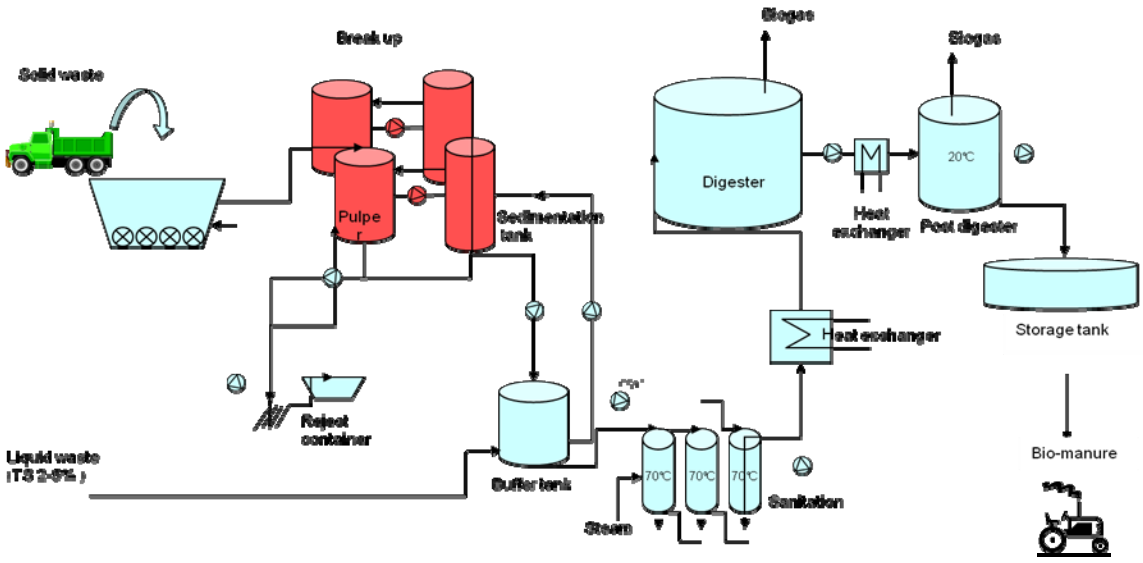

Figure 2. Schematic diagram of a biogas plant. The design may vary in different plants. Modified from Niklas Leksell

Continuous or Batch Digestion

The process may be operated either continuously or in batches depending on the substrate being digested (Fannin and Biljetina 1984, Nyns 1986, Verstraete et al 1996, Lettinga 2005, Sakar et al 2009). With continuous digestion, new material is continuously pumped into the digestion tank, creating a very smooth inflow of raw material, and hence also a smooth production of gas. It is possible to do this for substrates in liquid form that have a dry solids content of less than 5%, such as municipal and industrial waste water. Anaerobic digestion of this type of substrate primarily takes place abroad, but there are also examples of digestion of liquid substrate in Sweden, for example in industrial processes (Biogas from manure and waste products - Swedish case studies 2008). Material in the form of sludge with a dry solids content that is between 5% and 15%, such as slurry and sewage sludge, can also be added to the process more or less continuously. This is known as semi-continuous anaerobic digestion, where material is pumped in 1-8 times per day. In the case of solids with a dry solids concentration above 20%-25%, such as crop residues and food waste, it is common to add new material less frequently and in larger portions. By adding liquid, the solid materials can be rendered to a form that can be pumped, making it possible to continuously add matter to the process. In addition to being more practical for the operator, this is also advantageous for the microorganisms because they get a more uniform supply of the substrate. This helps the interaction between various groups of microorganisms in the breakdown chain and reduces the risk that microorganisms will become overloaded due to the addition of a

large quantity of substrate at one time. In this manner, the steady addition of substrates can make a higher total load possible.

In contrast, in batch digestion all the material is digested at once and the material remains undisturbed in the same place throughout the entire digestion process. No new material is added nor is any digestion residue removed during the process. Methane production is generally highest at the beginning and then decreases over time. When the material is digested, the entire container is emptied of its contents and a new batch of substrate is added. An example of batch digestion, when waste is treated in the same place for a long time, is in landfills. Batch digestion is also common in connection with biogas production for individual households, which is particularly common in Asian countries. Batch digestion is advantageous from a microbiological point of view because the organisms have plenty of time to break down the organic matter. Also, the organisms do not get washed out of the system. However, sometimes it can be difficult to achieve a high and even digestion rate, particularly if the substrate has a high content of dry solids (Kreuger and Björnsson 2006, Nordberg and Nordberg 2007).

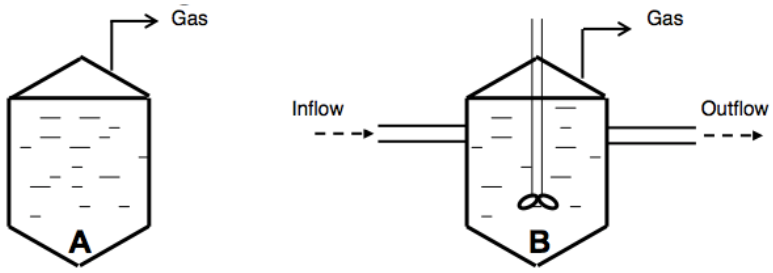

Figure 3. Schematic sketch of the batch (A) and continuous digestion (B). Modified based on Nyns 1986.

Digestion in One or Two Stages

The simplest model for biogas production is to use a single digestion tank for the entire process, so-called one-step digestion. With one-step digestion, all stages in the microbial breakdown process, i.e. hydrolysis, fermentation, anaerobic oxidation and methane production take place at the same time and in the same place. It is common for one-step digestion to take place in total mixed processes. A common type of biogas reactor is the Continuously Stirred Tank Reactor (CSTR). The substrate is completely mixed by various mixers. It is often used in one-stage processes for treating sludge, food waste, manure, etc. Sometimes some of the residues/process liquids are returned to the process. This increases the retention time of material (see below) and helps more microorganisms to remain in the process (Nordberg et al 2007).

An alternative to a single-stage process is to divide the process into two parts, called two-stage digestion. In two-stage digestion, the first step is to load raw material into a digestion tank where the process is focused on hydrolysis and fermentation. This primarily results in acid formation, but a certain amount of biogas is normally also produced, because it is difficult to completely divide the process. Then the digestate or the process liquid from this process is separated and

added to another digestion tank that is specially adapted for methanogenesis (Pohland and Gosh 1971). This type of process may be appropriate when a substrate contains material that is easy to break down and the hydrolysis stage is fast (see Energy crops/crop residue in Chapter 3).

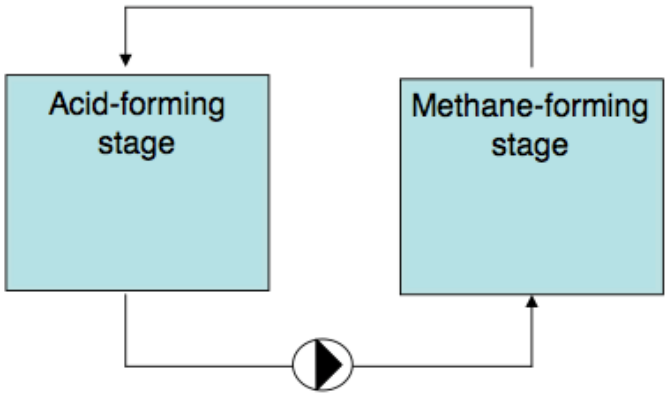

Figure 4. Schematic diagram of a two-stage process

The second stage can, for example, be designed as an anaerobic filter, that is, a digestion tank with built-in carrier material that will help to retain the microorganisms and establish contacts between the methane producers and the organisms that carry out anaerobic oxidation. This division of the process often results in fast and efficient formation of biogas in the second stage, with methane concentrations of up to 85% (Colleran et al 1982, Verrier et al 1987). Carrier material can, for example, be made of plastic or glass, but other materials such as straw or sisal fibres have also been found to work (Held et al 2002, Anderson and Björnsson 2002, Mshandete et al 2008). A plug flow is usually applied to anaerobic filters: i.e. liquid to be treated is allowed to sieve through the support material in a steady stream from one end to the other. Models exist both for input from the top, allowing a downward flow through the filter, and input from the bottom for an upward flow of liquid. Two-stage digestion can also be performed with two full-mixing digestion tanks linked in a series (Pohland and Gosh 1971).

Dry Digestion

Currently, the dominant method of decomposing organic matter into biogas is to use slurry-based processes with a relatively low content of dry solids (2%-15%), known as wet digestion, where mass is transported by pumping. Dry digestion is an alternative for the digestion of materials with a high content of solids such as solid waste, manure, crop residues and bioenergy crops (Nordberg and Nordberg 2007). In this case, the material does not need to be diluted with liquid. Instead, the digestion is adapted to a high solids content (between about 20% and 35%). The technology is being used in Germany, where it is common for dry digestion to take place in batch processes with new materials being loaded about once a month. In order to achieve a process with a high degree of decomposition, the right type of microorganisms need to be present. Digested materials are often mixed into fresh material or the material is gradually inoculated by circulating process fluid through the dry digestion bed.

There are several advantages to this technology. The relatively small amounts of liquids circulated require smaller pipe and pump dimensions and lower electricity consumption than wet digestion. Storage and transport of substrate and digestion residue is more efficient because the water content is lower. In addition, problems with foaming are avoided. When dry batch digestion takes place in several modules, loading and removal can be done in sequence, one after the other, so that a relatively even gas removal can take place over time. If a disturbance occurs, methane production from the individual batch will be reduced, but it does not necessarily mean that gas production from the entire process stops. This means that a disturbance in a batch dry digestion plant has less serious consequences than a disturbance in a wet digestion plant. However, it is important to keep the water content from getting too low. A water content of at least 65%, i.e. a maximum of 35% dry solids, is usually referred to as the limit for maintaining good microbial activity (Jewell et al 1981).

2.3 Important Operating Parameters

This section describes some important operating parameters and their impact on the biogas process. Chapter 5 deals with monitoring, and also describes how measuring and recording these parameters takes place in the biogas process and how different values can be calculated.

Temperature

Temperature is a very important factor to take into consideration during anaerobic digestion. In the presence of oxygen, heat is released by the breakdown of the organic matter, causing aerated compost to heat up. In an oxygen-free (anaerobic) biogas process, only very small amounts of energy are released in the form of heat. Instead, most of the energy released by cellular respiration binds directly to the final product, methane. Therefore, this product will be energy rich, while the process itself is not significantly heated up. For the microorganisms to grow best, and thus also form a lot of biogas, an external heat supply is required.

Temperatures normally used for digestion in the biogas process are around 37°C (mesophilic) or 55°C (thermophilic). Microorganisms grow best at these temperatures in the mesophilic and thermophilic ranges. However, examples of the digestion of bioenergy crops shows that it may be possible to achieve a stable process throughout the entire 35°C-50°C range (Lindorfer et al 2008). Sometimes some of the energy in the methane gas is used to heat the process. It may also be necessary to heat some of the incoming substrate before it is fed into the digestion tank, especially during cold winter months. The facilities that sanitised the substrate at 70°C before digestion may need to cool the material down before it is pumped into the digestion tank. The general rule for mesophilic and thermophilic digestion temperature is that once the temperature is set, it should be kept constant and not vary more than +/- 0.5°C to achieve the best results (VAV P42 1981). Small temperature fluctuations (max. +/- 2-3°C) can be tolerated, especially if the process is otherwise stable with respect to such things as alkalinity (personal communication Halina Rybczynski, Kalmar Biogas AB). Methane producers are generally more sensitive to temperature fluctuations than other microorganisms in the process. A steady temperature in the digestion tank

is most easily achieved by using some form of agitation. It is also important that the digestion tank has sufficient thermal insulation (Svahn 2006).

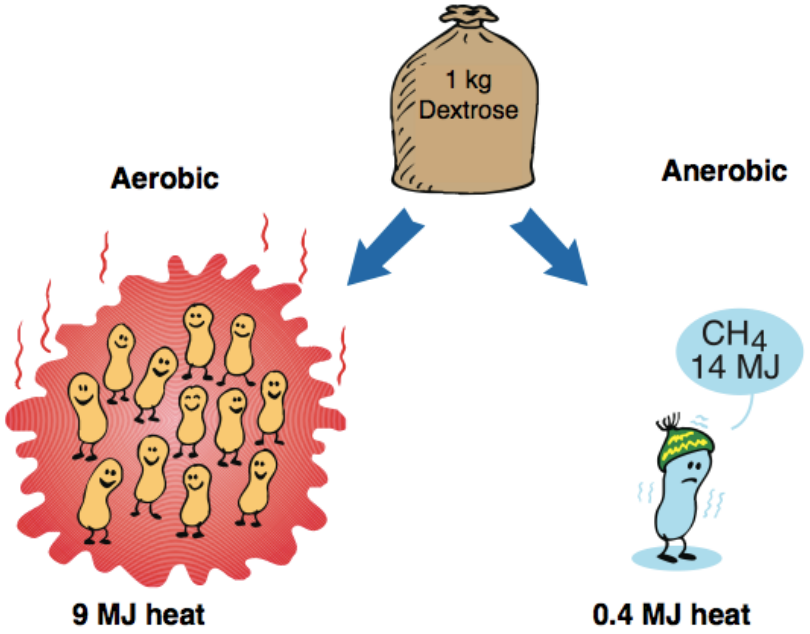

Figure 5. Aerobic and anaerobic energy production. Aerated compost produces a large amount of heat, while the energy produced by anaerobic digestion primarily binds to methane. Kim Gutekunst, JTI.

Mesophilic Digestion

The mesophilic range lies between about 25°C and 40°C, but biogas production can only be maintained if temperatures do not drop below about 32°C (Gerardi 2003). It is primarily the methane producers that grow more slowly at lower temperatures. The optimal temperature for mesophilic methane producers is around 35°C-37°C. If the temperature falls below the optimum temperature, fermenting organisms that are less sensitive to temperature fluctuations continue to produce various fatty acids and alcohols. Because the methane producers are no longer as active, they cannot digest of all of the fermentation products that are formed. Therefore, these accumulate rapidly with the result that the pH drops and the process stops.

Thermophilic Digestion

At temperatures between 40°C and 50°C, the mesophilic methane producers are inactivated and at about 42°C most mesophiles die, although thermotolerant microorganisms survive (Gerardi 2003, Madigan and Martinko 2006, Wagner and Wiegel 2008). Studies have shown that approximately 10% of the microbial flora in a mesophilic process can consist of thermophilic species (Chen 1983). The temperature may, however, reach about 50°C before thermophiles take over at full strength. The thermophilic range for the biogas process is between 50°C and 60°C, and the working temperature of biogas plants that use thermophilic digestion is usually between 50°C and 55°C. Heat causes the microorganisms to be 25%-50% more active than in mesophilic digestion (Gerardi 2003).

What temperature should be selected?

Mesophilic or thermophilic: which process is preferable? Generally, the process is faster at higher temperatures. The heat makes the microorganisms work faster, provided that the species being added are adapted to this climate. More of the materials is broken down in less time and the digestion tank volume can be reduced compared to if the same amount of material were digested at a lower temperature (Duran and Speece 1997, Edström and Nordberg 2004). A higher temperature may also increase the availability of certain organic compounds because solubility generally increases with increasing temperature. As a result of increased solubility, the viscosity of certain materials may be lower in thermophilic conditions, which facilitates mixing (van Lier 1995, Ryan 2008). Another advantage of thermophilic digestion is that the high temperature provides natural sanitation of the material; undesired pathogenic microorganisms such as Salmonella are destroyed more efficiently at higher temperatures (Sahlström 2003). The time that the material is exposed to the high temperature is critical for whether it is possible to achieve adequate sanitation.

However, with thermophilic digestion, the need for heating the digestion tank and the substrates is greater in comparison with mesophilic digestion. On the other hand, digestion plants that sanitise the incoming substrate at 70°C, have a higher heat surplus that creates more of a need to cool down the substrate before loading the mesophilic process, in comparison with the thermophilic process.

Thermophilic conditions can also make the process more sensitive to disturbances (Zinder 1986, Ryan 2008). This is partly due to the microorganism's optimal temperature being close to the maximum temperature at which many microorganisms die or become inactivated (Madigan and Martinko 2006). Raising the temperature a few degrees can lead to a process disturbance. Lowering the temperature a few degrees may not disturb the process as much, but even this may lead to an imbalance between fermentation and methane formation. Another explanation for the ease with which the thermophilic process can be disturbed, is that because of the higher decomposition rate, it responds to toxic elements more quickly. For example, ammonia inhibition occurs more quickly, in strict chemical terms, at a higher digestion temperature, because more ammonia is released when the temperature rises. Ammonia is in equilibrium with the water-soluble form, ammonium, which is harmless for the microorganisms. When the temperature rises, this equilibrium steadily shifts towards gaseous ammonia (Aylward and Findlay 1994, see also Chapter 3). Experience from some Swedish plants shows that digestion at lower thermophilic temperatures (around 50°C-51°C) can have a positive effect on the digestion of nitrogen-rich material (personal communication, Pernilla Bratt, Municipality of Skövde).

In general, fewer species of microorganisms are present and active in thermophilic, compared to mesophilic digestion (Levén et al 2007). Thus, the mesophilic process often involves a greater diversity of organisms and can therefore be more stable and better equipped to adapt to changes. A greater diversity of organisms may also be the explanation for why mesophilic processes may have a better degree of decomposition of some organic impurities than thermophilic processes

(Levén and Schnürer 2005, Levén et al 2005). The total number of active microorganisms can be as large in a thermophilic as in a mesophilic process (Nordberg et al 1999). The microbial biomass formed per amount of substrate is slightly lower for thermophilic compared to mesophilic microorganisms (van Lier 1995, Duran and Speece 1997), which can result in a smaller amount of excess sludge produced by the thermophilic process (Zinder 1986).

Temperature range	Temperature (°C)
Psychrophilic	4-25
Mesophilic	25-40
Thermophilic	50-60
Hypertermophilic	> 65

Table 1. Temperature range for methane producers. Anaerobic digestion works best in the mesophilic and thermophilic ranges (based on Gerardi 2003, Edström and Nordberg 2004)

Modified Temperature

Each biogas process develops its own microbial flora that is adapted to the prevailing conditions. The microbes that are present interact and develop an 'organism-community' that functions and is unique to the environment. Of course, it takes some time for such a flora to be established, but it also takes time for it to change and adapt to new conditions. This is especially the case regarding microorganism responses to temperature fluctuations. A mesophilic process can be adapted to thermophilic temperatures, but such adaptation takes time. The thermophilic organisms, which are often present in small amounts (about 10% of the total flora, Chen 1983), have to manage to grow, while many of the formerly active mesophilic species are gradually eliminated or inactivated by the high temperature. An increase of about one degree per day can be a guideline for adjusting a process to higher temperatures, although there are examples described in the literature where a higher rate of temperature increase has also worked (Dinsdale et al 1997, Pender 2000, Philpott 2001). With all changes it is important to start from a stable process, for example, with respect to alkalinity (see below under Alkalinity and pH).

The substrate and process type can also have an impact on how the process handles temperature increases from a mesophilic to a thermophilic environment. In a laboratory experiment with digestion of sewage sludge, Bouskova et al (2005) found that an instantaneous increase in temperature from 37°C to 55°C initially caused a powerful disturbance. However, the process recovered fully after 30 days, whereas when the corresponding increase was carried out in stages

it took 70 days before the process adapted to thermophilic conditions. However, based on the digestion of energy crops, Lindorfer et al (2008) suggest that small incremental increases in temperature that allow the microorganisms to adapt to higher temperatures are probably best.

It may be more difficult to change from thermophilic to mesophilic digestion temperatures while maintaining biogas production. There are often very few mesophilic species present in a thermophilic process, because the high temperatures knock them out. Therefore, if the temperature is lowered, no specialists would be found in the mesophilic range that could grow and work optimally at the lower temperatures. Thermophilic species can survive temperature decreases, but they will then work more slowly, because conditions are not optimal.

Loading

Biological decomposition of organic matter occurs continuously in a biogas process. If no new material is added, the process gradually stops. Loading is a term that indicates how much new material is added to the process per unit of time. It is usually referred to as organic loading or organic loading rate (OLR). In this case it is important to know the dry solids (DS) and organic matter (VS [volatile solids]) content in the substrate in order to give the biogas process the right loading rate. Dry solids are the material that remains when all of the water is dried off, while VS indicates the organic part of the dry solids.

The load should be tailored to the active microbial flora. It is common to start a new process with a low loading, for example, 0.5 kg organic matter per m^3 digestion tank/day, and then successively increase it as the microorganisms grow (Angelidaki et al 2006).

Sometimes it may take several months before the desired load is reached. This can largely be explained by the slow growth of the anaerobic microorganisms. For example, as previously mentioned (Chapter 1) methane-producing microorganisms have a doubling time of several days. If a large amount of substrate is suddenly added at the start of a process, there are simply too few microorganisms to be able to absorb this quantity of "food". An excess of undecomposed material, such as different fatty acids, builds up. This, in turn, results in a reduction in pH and the creation of an imbalance in the entire decomposition chain. The process is no longer stable.

Adaptation to Substrate and Temperature

The rate at which the load can be increased, and by how much, depends in part on the substrate. Substrates consisting of easily digestible materials, such as process water from the food industry, that are high in sugar or starch, are easier for microorganisms to digest, while fibre-rich plant material is less palatable and requires a longer period of adjustment. On the other hand, easily digestible materials cause problems with the accumulation of fatty acids because degradation is too fast (see Chapter 3). It may also be necessary to add nitrogen-rich substrate, such as protein-rich offal, in small portions (i.e. at a low loading), among other things, to avoid the formation of large amounts of ammonia and hydrogen sulphide. Another prerequisite for rapid adaptation is that the substrate does not contain substances that are toxic to the microorganisms, such as heavy metals or various organic contaminants (see Chapter 4). Here the operator has to proceed by trial

and error, that is, increase the loading successively and continuously to ensure that the process responds to the increased loading with a corresponding increase in methane production whilst maintaining stability in the form of neutral pH values, etc. (See Chapter 5 for appropriate monitoring parameters). Only then is it possible to increase the load another step.

After the start-up phase, a well-functioning thermophilic biogas process can generally be loaded with more organic matter, on the order of 4-5 kg VS/ m3 digestion tank/day, than a mesophilic process that normally has a load of about 2-3 kg VS / m3 digestion tank/day. Even higher loads may be possible. During thermophilic dry digestion in laboratory experiments with sorghum and cellulose, Richards et al (1991) achieved a load of 24 kg VS/m3 digestion tank/day, which is among the highest loads reported in the literature. In other laboratory tests, efficient thermophilic digestion of food waste was achieved with a load of 13.5 kg VS/m3 digestion tank and a retention time of 10 days. In corresponding experiments with mesophilic digestion of food waste, a load of 6 kg VS /m3 digestion tank at a retention time of 20 days was achieved (Figure 6; Nordberg et al 1999). However, the addition of de-foamer was necessary for the mesophilic process. The potential loading depends on the nature of the material and the microorganisms that are active in the specific process (see Chapter 3). With water-rich substrates, with a low concentration of VS, it may be difficult to achieve sufficiently high loading using the available digestion tank volume, while keeping it economically justifiable. For example, this might apply to municipal and industrial sewage sludge that has the characteristics of a liquid, with a dry solids content of only 3%-6%. Dehydrating (thickening) of the substrate may become necessary to increase the proportion of organic material before it is added to the process.

When the process reaches the desired load, it is important to continue with the same input pattern. The organic loading rate should be kept as constant as possible over time and not vary more than 10%-15% per week. It is also desirable not to vary the composition of the incoming substrate too much because the microorganisms in the process adapt to the input material. Generally, it is easier for microorganisms to handle new material a little at a time, and it is therefore advantageous if the substrate can be added to the process in small portions evenly distributed over time. If the substrate has a high water content, such as sewage sludge or slurry, it is easiest to achieve this by pumping the materials in more or less continuously during the process (about 20 times/day). On the other hand, it may be harder to achieve uniform loading during several days with substrates of high dry solids content, such as plant material or food waste. Sometimes it may be advantageous to dilute "dry" substrates to facilitate loading, for example by adding slurry or liquid that is returned from the process.

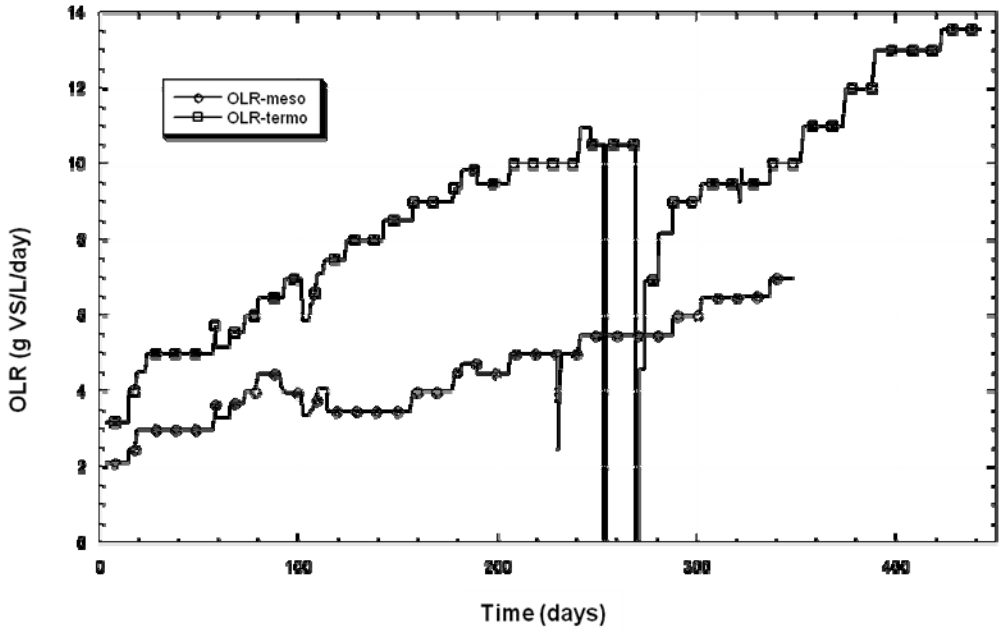

Figure 6. Successively increasing organic load (OLR) to mesophilic (37°C) and thermophilic (55°C) biogas processes respectively at laboratory scale (volume 45 liters). The processes were fed with source-separated food waste and lasted 348 and 442 days respectively. The temporary disturbance in the thermophilic process was caused by a crack in the wall of the digestion tank that made it necessary to put the material in a new digestion tank. Nordberg et al 1999.

Retention Time

Retention time is defined as the time it takes to replace all of the material (entire volume) in the digestion tank. In a biogas process, hydrocarbons in solid form are converted to methane gas and carbon dioxide gas. Thus, the amount of solid material is continuously reduced, i.e. the content of organic matter decreases during the process. When new carbon-containing substrate is added to the process, this will also be converted to gas. Typically, however, more substrate is added than is completely decomposed between each addition of substrate. By removing part of the contents of the digestion tank at regular intervals, a constant volume is maintained in the process. The volume of solid material being added is sometimes greater than the volume of solid material removal, because some is removed as gas during the process. The volume of added and removed material is also regulated by the amount of liquid added. The removed material is partly composed of water, including dissolved salts, and organic matter that has been digested in the chamber for shorter or longer periods of time, known as digestion residue. This residue also contains biomass, i.e. microorganisms grown during the process. The removed material, e.g. water, dissolved salts, digestion residue and biomass, is often termed ‘digestate’.

Hydraulic and Particulate Retention Time

Retention time is usually referred to as hydraulic retention time (HRT), and for the biogas process it is usually between about 10 and 25 days, but can also be longer. Sometimes the retention time of the particulate material, or solids retention time (SRT), in the process is listed instead. In many cases, HRT and SRT are equal, but in a digestion tank in which part of the residues are returned to the process, SRT becomes longer than HRT. This may occur, for example, during digestion of industrial sewage sludge, where added material has a high water content and where the recirculation of digested, thickened sludge, including biomass, allows a longer time for the microorganisms to break down the incoming organic matter.

The length of the retention time needed, depends partly on the composition of the substrate and the digestion temperature. Microorganisms generally manage to decompose a substrate rich in sugar and starch, which is easily broken down, in a short time. An example is industrial waste water that only contains soluble organic matter. In this case, no hydrolysis is necessary, which allows for a relatively short HRT. On the other hand, microorganisms may need significantly more time to effectively attack and break down fibre-rich and cellulose-rich plant matter. For such material, it is often hydrolysis and not methanogenesis that limits the rate of decomposition. In Germany, among other places, retention times of up to 50-100 days are used to ensure stable operation and satisfactory digestion of energy crops (Ergebnisse des Biogas Messprogramms 2005).

Degree of Digestion

More time in the digestion tank can often lead to more methane being extracted from the substrate, because of the increased contact time between the microorganisms and the substrate. The degree of digestion is defined as the percentage of the organic material broken down and converted into biogas during a specific period of time. Generally, batch processes have a higher degree of digestion than continuous digestion. In a batch process, the degree of digestion can theoretically be 100%. However, it is normally not economically or practically possible to extract all the methane from a given substrate. In batch digestion, biogas production is normally greatest at the start of the process. Later, less biogas is formed over time. The degree of digestion also varies with the substrate. Readily biodegradable substrates, such as the liquid from pressed sugar beets, can have a degree of digestion of more than 90%, while only a little more than 60% of a high-fibre grass crop is degraded during the corresponding period (Edström and Nordberg 2004).

It is common practice to pump the residue into a store for digestion residue, where the decomposition of organic matter and biogas production can continue over a long period of time. Generally, the lower the degree of digestion in the actual digestion tank, the greater is the potential for methane production in this post-storage stage. It is always important that this subsequent digestion takes place in covered containers to prevent the methane gas and other environmentally harmful gases from leaking into the atmosphere (see Chapter 6).

Raw material	Degradation ratio (% of VS)
Cattle manure	35
Pig manure	46
Forage crops	64
Sugar beets	93
Fruit and vegetable waste	91

Table 2. Approximate degrees of digestion/decomposition of a few different substrates (based on Edström and Nordberg 2004).

Load and Temperature Effect

Retention time and loading rate should be controlled and adjusted in relation to one another to achieve maximum gas yield. Generally, high loads need longer retention times. If a process with a short retention time is heavily loaded, there is a risk that the degree of digestion of the material becomes too low. Temperature is also an important control on the retention time. In a mesophilic process, the retention time should be at least 15 days and usually longer, while a thermophilic process can manage digestion of the material more quickly, perhaps in 10 days (Nordberg et al 1999). Frequently, however, the thermophilic process also operates with a slightly longer retention time (at least 12 days) to ensure stable operation (Kim et al 2006).

Microorganisms can be retained in the process

If the retention time is much too short, there is a great risk that the microorganisms will not manage to grow at the rate at which material is removed from the process. As mentioned earlier (Chapter 1), the dominant methane producers in a biogas process, often have doubling times of up to 12 days. This means that the retention time for the material in the digestion tank can seldom be shorter than this. In other cases, the microorganisms are simply rinsed out in such large numbers that the populations do not have time to recover until the next time digested matter is removed. Thickening the sludge before digestion increases the dry solids content and the concentration of active microorganisms in the digestion tank. This method is often used in Swedish waste water treatment plants (VAV P42 1981).

Microorganisms can also be retained using various technical solutions. As previously mentioned, different carrier materials can be used, as is done for example, in anaerobic filters and in AFBR

(Anaerobic Fluidised Bed Reactors), (Kumar et al 2008). The microorganisms attach to the carrier material which makes it easier to retain them in the digestion tank. This means that the solid retention time (SRT) in the process is increased in proportion to the hydraulic retention time (HRT). The longer retention time of particles also enables microorganisms to adapt to salts, ammonia, sulphides, and other substances that might otherwise be toxic at high concentrations.

Figure 7. The plastic rings can be used as carrier material in anaerobic filters. Photo: Åsa Jarvis.

Other types of biogas reactors that can be used when the microorganisms need to be kept in the process include UASB (upflow anaerobic sludge blanket) or EGSB (expanded granular sludge bed). In these processes, microorganisms are allowed to accumulate and grow in clumps (aggregates). Despite the high inflow of substrate, the microorganisms can remain in the digestion tank. New material is pumped with such force that it provides sufficient mixing to create contact between the microorganisms and substrate (Lettinga et al 1980, Kumar et al 2008). Processes with retained biomass are often used to treat industrial waste water that contains a high portion of dissolved organic matter (Digman and Kim 2008).

Mixing

Digestion tanks should be equipped with agitators (mechanical agitators or pumps) to mix the substrate. Mixing facilitates contact between the microorganisms, the substrate and nutrients and provides a uniform temperature throughout the process. It is particularly important for hydrolytic microorganisms to make good contact with the various molecules that they should digest and that their enzymes can be distributed across a large surface area within the substrate. Mixing also prevents material from accumulating on the bottom of the digestion tank and reduces the risk of foaming.

Mixing also facilitates the important contact and transfer of hydrogen between methane producers and the organisms that carry out anaerobic oxidation. However, mixing ought not to be too strong. Often these microorganisms grow in tight clumps, called aggregates, which facilitates their close cooperation and thus the transfer of hydrogen. Gentle mixing benefits the formation of aggregates and prevents methane producers from being washed out in the liquid. Continuous mixing avoids sedimentation and utilises the existing digestion tank volume in the best manner. Substrates with high dry solids content are generally harder to mix than more liquid materials. Mixing in the substrate tank is also important to avoid sedimentation and thus uneven loading of the digestion tank.

Alkalinity and pH

Biogas processes usually run best at neutral pH values or slightly above neutral (pH between 7.0 and 8.5). Maintaining neutral and stable pH values requires that the process has a relatively high and constant alkalinity. Alkalinity is a measure of the amount of alkaline (basic) substances in the biogas process. The higher the alkalinity, the greater the buffer capacity in the process, which in turn promotes a stable pH value. Alkalinity consists primarily of bicarbonate ions that are in equilibrium with carbon dioxide (Equation 1). Carbon dioxide and carbonate ions also contribute to alkalinity. Decomposition of nitrogen-rich substrate with high proportions of proteins and amino acids can increase alkalinity, because the ammonia released can react with dissolved carbon dioxide to form ammonium bicarbonate.

Equation 1. Carbon dioxide is in equilibrium with carbonic acid and carbonates (Gerardi 2003)

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+$$

CO_2 = carbon dioxide
H_2CO_3 = carbonic acid
HCO_3^- = bicarbonate
CO_3^{2-} = carbonate

Alkalinity of a biogas process can be measured both as total alkalinity (TA), and as bicarbonate alkalinity (BA). BA for stable processes usually varies in the range 3,000 - 15,000 mg HCO_3/ litre. Often, digestion of various organic materials such as crops and slaughterhouse waste, results in higher alkalinity than in processes in which only sewage sludge is treated (VAV P42 1981, Nordberg et al 2007, personal communication Halina Rybczynski, Kamlar Biogas AB).

Low alkalinity may be due to the acid production process being too high in relation to the capacity to produce methane. This is especially common at start-up, during overload and temperature fluctuations, or if the microorganisms are subject to toxic substances that inhibit their activity. On the other hand, alkalinity that is too high can lead to the release of ammonia and inhibit methane producers. The critical limit for this may vary between different processes depending on the degree to which microorganisms in the process adapt to high ammonia concentrations. If the alkalinity of a biogas process is not stable, this should be considered a warning sign for pH

changes and the cause should be determined. A low pH only says that the disturbance has already had an effect, because a sharp decrease only occurs when most of the alkalinity is consumed. This means that it is often easier to temporarily adjust the pH in a biogas process than to permanently alter the alkalinity. Alkalinity and pH can be adjusted in the biogas process by adding various stabilising agents, see Chapter 5.

CHECK YOUR KNOWLEDGE

- What is the difference between a continuous process and a batch process?
- When might it be of interest to have a two-stage process?
- Is it possible to go from mesophilic to thermophilic digestion temperatures?
- What does organic loading rate mean?
- What is the degree of digestion?
- Why do processes that operate at thermophilic digestion temperatures often have shorter retention times than processes that operate at lower temperatures?
- In some process types, SRT is higher than HRT. How is this possible?
- What is alkalinity a measure of?

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3. SUBSTRATES

The material added to a biogas process is substrate (food) for the microbes and its properties have a major influence on process stability and efficiency. Substrate composition is important both for the amount of gas formed and the quality of the gas. The composition ultimately also affects the quality of the digestion residue (digestate), both in terms of plant nutrient content and potential contamination (metals, organic compounds, disease-causing organisms, etc). Choosing the right material gives you the opportunity to influence the outcome of the process, maximise energy output and produce biofertilizer of good quality.

3.1 Substrates for biogas production

Many different types of organic material can potentially be used for biogas production, probably many more than those used today. The main source of organic material for biogas production in Sweden today is sludge from municipal wastewater treatment plants. Other common substrates for biogas production in co-digestion plants include slaughterhouse waste, waste from the food and feed industries, source-sorted food waste and manure. Examples of other materials which are also treated in these facilities include waste from grease traps, fryer fat, wastes from the dairy and pharmaceutical industries, grass silage, and distillation waste (residues from ethanol production). In the future, different crops and waste from the agricultural sector are also likely to become important substrates for biogas production. Other less common materials that are currently being evaluated for biogas production include algae, grass, feathers and woody biomass (e.g. willow). Total biogas production today corresponds to an energy output of about 1.3 TWh/year, but the theoretical potential energy production from domestic wastes, excluding forest waste, is considered to be around 15 TWh/year (Nordberg 2006, Linné et al 2008).

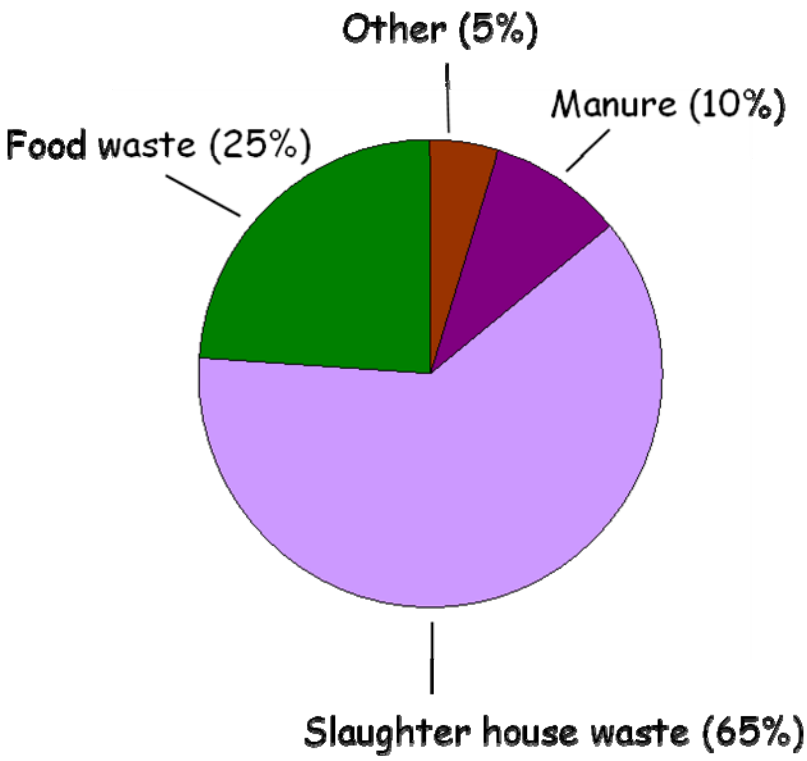

Figure 1. The proportion of biogas production from different substrates at the Swedish co-digestion plants (sewage sludge not included). Nordberg 2006

3.2 How to choose a substrate for a biogas process

Many different organic materials can be decomposed to biogas in a digestion chamber (Gunaseelan 1997). Some materials are more appropriate than others, and some general guidelines can be applied. However, process parameters such as load, temperature and retention time have a great influence on how efficiently a given substrate is broken down. How well a particular material works in a biogas process can also depend on what pre-treatment is applied and whether it is the sole substrate or if it is co-digested with other materials. The presence of toxic substances or lignin, which is not at all broken down in a biogas process, also plays a role. Below follows a discussion of the importance of substrate composition for the microorganisms and gas production, and how to evaluate materials to be used for biogas production.

3.3 The importance of substrates for microorganisms and gas production

The composition of a substrate is very important for the microorganisms in the biogas process and thus also for process stability and gas production. The substrate must meet the nutritional requirements of the microorganisms, in terms of energy sources and various components needed to build new cells (Chapter 1). The substrate also needs to include various components needed for the activity of microbial enzyme systems, such as trace elements and vitamins. In the case of

decomposition of organic material in a biogas process, the ratio of carbon to nitrogen (C/N ratio) is also considered to be of great importance. It is important that the ratio is not too low, in other words, that there is not too much nitrogen relative to carbon. If so, the process can easily suffer from ammonia inhibition (see below under Protein-rich materials). The ratio should also not be too high, since the bacteria in the process may then experience nitrogen deficiency (Yen and Brune 2007). It is hard to say exactly what ratio is optimal because it varies with different substrates and also with the process conditions.

Several factors affect the optimum C/N ratio for such a process:

1. If the substrate is limited by factors other than the amount of carbon or nitrogen, for example, low levels of phosphorus and trace elements. This can have an effect on the function of the process which becomes more important than the C/N ratio (Handreichung 2004, Speece 1984).
2. Process decomposition efficiency. If the degree of decomposition in the process, (i.e. the proportion of the organic material that is converted to methane), is low, a smaller portion of nitrogen is released as ammonia compared to a process with a high degree of decomposition. Such a process "handles" a substrate with a low C/N ratio better than a process with more efficient degradation (personal communication, Pernilla Bratt, Municipality of Skövde).
3. The composition of the substrate, (i.e. which components are actually responsible for the C/N ratio). Long-chain carbon compounds, such as cellulose, are broken down slowly, and the risk of acidification of the process is significantly lower than where most of the carbon is glucose, which degrades very quickly. Some of the carbon can also occur in the form of lignin, which in its intact form does not decompose at all during the process (Gunaseelan 2007)

The values of C/N ratio reported in the literature that work well in biogas processes vary between 10 and 30, with an optimum between 15 and 25 (Speece 1984, Nyns 1986 Handreichung 2004, Yadvika et al 2004, Hansson and Christenssen 2005, Yen and Brune 2007, Liu et al 2008). The C/N ratio has a significant impact on fatty acid production and also which fatty acids are formed (Liu et al 2008). An increasing C/N ratio (within the range 10-30) increases the formation of fatty acids in the process (Callaghan et al 2002, Yen and Brune 2007, Liu et al 2008). If levels are not too high, this can also stimulate methanogenesis (Callahgan et al 2002, Sosnowski et al 2003, Yen and Brune 2007).

Material	C/N- ratio
Cattle manure-liquid	6-20

Chicken manure	3-10
Swine manure-liquid	5
Straw	50-150
Grass	12-26
Potatoes	35-60
Sugar beet/beet foliage	35-46/14
Cereals	16-40
Fruits and vegetables	7-35
Mixed food waste	15-32
Slaughterhouse waste-soft tissue	4
Slaughterhouse waste –guts	22-37
Food waste	3-17
Distillation waste	8

Table 1. C/N ratio of some materials that can be used as a substrate for biogas production (Kang 1993, Eklind et al 1997, Bernersson et al 1999, Hadders et al 2001, Murto et al 2004, Gunaseelan 2007, Cirne et al 2007, Parawira et al 2008, Lethomäki 2008a, Carlsson and Uldal 2009). The ratio can vary slightly depending on the origin/culture of a given material.

It is also preferable to use a substrate that is not too diluted, that is, contains too much water in relation to the amount of organic substrate. If the material is too diluted, and contains too little organic matter, the risk is that microorganisms are washed out in a continuous process. This is because their growth rate is low. The preferred water content depends on the type of process used. A highly diluted material can be treated by various techniques to retain the microorganisms, for example, using a carrier material or adding back biomass (Fannin and Biljetina 1984, Barber

and Stuckey 1999, Mahmoud et al 2003, Liao et al 2006). A good outline for a continuous process, which is generally used for more solid waste, is a dry solids value (DS) of 7-10% (Gunalseelan 1997, Svärd and Jansen 2003, Yadvika et al 2004, Nordberg 2006). The dry solids content of the sludge that is digested in sewage treatment plants is usually somewhat lower, around 4-6% (Svärd and Jansen 2003). Another factor of importance is the bioavailability of the substrate to the organisms. Chopping up the material increases its availability to microorganisms, which can speed up the gas formation process and provide a higher yield.

3.4 Substrate composition

The various components of organic material have different energy contents and therefore generate varying amounts of gas and gas of variable methane content. Since the microorganisms that are active during anaerobic decomposition use very small amounts of energy for their own growth, the majority of the available energy from the substrate becomes methane. Table 2 shows approximate biogas volumes and methane contents that can be formed from carbohydrates, protein and fat. Using these values for a mixed material, it is possible to make a theoretical calculation of the amount of gas that can be formed.

	~ Biogas formed (m3/kg VS)	Biogas composition: CH_4: CO_2 (%)
Carbohydrates	0.38	50:50
Fat	1.0	70:30
Protein	0.53	60:40

Table 2. Theoretical quantity and composition of biogas formed from carbohydrate, fat and protein (Berglund and Börjesson 2003)

An example calculation

Question. What is the amount of biogas that it is theoretically possible to get from 1 tonne of food waste with the following composition?

Parameters	Food waste
Wet weight	1000 kg
DS (dry solids)	33% of wet weight

VS (volatile solids)	90% of dry solids
Fat	19% of volatile solids
Protein	20% of volatile solids
Carbohydrate	61% of volatile solids

Answer: In the calculation, it is important to note that the microorganisms can only utilise the organic fraction, i.e. VS (volatile solids), which therefore forms the basis for biogas production.

Fat: 1000 kg (wet weight) x 0.33 (% dry weight of wet weight) x 0.9 (% VS of DS) x 0.19 (% fat by VS) x 1.0 (m^3 biogas/kg fat) = 56 m^3 of biogas per tonne food waste
Protein:1000 x 0.33 x 0.9 x 0.20 x 0.53 = 31 m^3biogas per tonne food waste
Carbohydrate: 1000 x 0.33 x 0.9 x 0.61 x 0.38 = 69 m^3biogas per tonne food waste
Total biogas: 56 + 31 + 69 = 156 m^3
Total methane: (56 x 0.70) + (31 x 0.60) + (69 x 0.5) = ~ 92 m^3

In making such calculatiions, it is important to note that theory and practice are not always entirely consistent. Since there are many factors affecting the final degree of degradation and hence the amount of biogas, there are several reasons for discrepancies between theory and practice. One reason is that some of the energy available in the substrate is used for production of new biomass, that is, new microorganisms, and therefore not used to produce methane. Another reason is that not all materials that go into a continuous process will be digested. This is not a sign of a poorly functioning process, but simply due to the continuous loading and removal. When the biogas process is functioning properly, the degree of decomposition is usually between about 50 and 70%. Some of the organic material does not decompose at all in a biogas process, such as lignin. This means that even if the process works very well, the amount of gas obtained will never match the theoretical amount calculated. On the other hand, in a batch process, virtually all the biodegradable organic material generates biogas.

Another reason for the discrepancy between theory and practice is that different sugars, proteins and fats have different compositions and structures, and these may vary in energy content. Thus the amount of gas produced can also vary with, for example, the different types of sugar. It is possible to make more accurate calculations by taking into account the contents of carbon, oxygen, nitrogen and hydrogen in the material to be digested (Berglund and Börjesson 2003, Möller et al 2004, Gunaseelan 2007). Some of the organic material (e.g. lignin) is also unavailable to the microorganisms and does not decompose to any significant degree. In addition, excessive concentrations of individual components may reduce methane production. For example, a high proportion of protein, which in theory produces a lot of gas, can generate significantly smaller

amounts of biogas than expected due to ammonia inhibition of the methane-producing microorganisms.

Another way to evaluate a substrate, which gives slightly more accurate results, is to perform batch or continuous experiments in the laboratory. In batch digestion experiments, the methane formation potential of a substrate is evaluated and calculated (see Chapter 7). Such experiments make it possible to better assess the value of a given material for biogas production. Table 3 shows the approximate methane yield potential of various substrates, determined by batch digestion experiments at mesophilic temperatures.

Substrate	Approximate methane yield (CH_4 m^3/tonne VS)
Food waste Fruit and vegetable waste	400-600 200-500
Manure from cattle, pigs or chickens	100-300
Slaughterhouse waste	700
Cereals	300-400
Sugar beets	300-800
Silage grass	350-390
Grass	200-400
Straw	100-320
Municipal sludge	160-350
Distillation waste	300-400

Table 3. Specific gas production for various potential substrates in biogas production. The values given are approximate (Bolin et al 1988, Gunaseelan 1997, 2004, 2007, Möller et al 2004, Davidsson et al 2006, Leksell 2005, Stenströmmer Moglia 2007, 2008, Åkerlund 2008, Lethomäki et al 2008a, b, Demetriades 2008) . More values are given in Linné et al (2008) and Carlsson and Uldal (2009).

Specific gas production may vary between different tests on the same type of substrate. This variation is due to the fact that the inoculum (i.e. the microorganisms that are performing the decomposition) varies between different sites and in its ability to decompose a specific substrate. This variation may also be due to substrate characteristics, temperature of digestion and the pre-treatment used. Food waste, for example, does not always have the same composition. It varies from place to place and also depends on what time of year it is collected. The composition of a crop varies according to where and under what conditions it is grown (soil type, climate, etc.) and also when it is harvested (Gunaseelan 1997, Kreuger et al 2007, Anon et al 2007, Lethomäki et al 2008a). In addition, storage conditions and storage time of crops also affect gas yield (Pakarinen et al 2008, Anon et al 2007, Pakarinen et al 2008). Poor storage can, for example, allow decomposing microorganisms to use a fraction of the available energy in the crop during storage and thereby reduce the gas potential (Haraldsson 2008).

Co-digestion of different substrates

Generally, co-digestion of different materials gives better performance (Nordberg et al 1997, van Lier et al 2001, Ahring 2003, Yadvika et al 2004, Alvarez and Lidén 2008). Co-digestion often produces more gas than expected on the basis of gas production from the individual substrates (Table 4). The explanation for this is that a complex material is more likely to include all the components that are important for microbial growth. A mixture can, for example, provide better availability of trace elements or a more optimal C/N ratio. In addition, substrates that are complex and not too uniform promote the growth of several types of microorganisms in the digester. A continuous process which is fed for a long time with a substrate that is too uniform, for example, only a sugar-rich material, may find it difficult to digest proteins and fats. Most of the organisms capable of breaking down fat and protein would already have been washed out of the process.

A variety of substrates is therefore desirable, as it increases the likelihood of a stable and robust process. If a diverse microbial community is allowed to develop by decomposing many different types of components, the process will be better able to handle large future variations in substrate composition. Co-digestion also improves the chances of the process to "cope with" substrates containing toxic (poisonous) components. If initially there are many different microorganisms which fulfill the same function, for example of degrading sugars, the process will continue to perform well, even if one or more of these are eliminated due to toxic effects. As long as some survive, the process will function well. Finally, co-digestion can also improve the technical conditions, for example by making it easier to pump and stir the material to be degraded. (Nordberg et al 1997).

Mixture (% VS)		Methane yield
Beet foliage	Potato waste	m^3/kg VS/day

100	-	2.1
-	100	2.5
33	67	3.9

Table 4. The methane exchange from co-digestion of potato waste and sugar beet foliage (Parawira et al 2008). The ratio of C/N in the potato waste was 35 and 14 for the beet foliage.

In order to achieve a stable digestion process with a mixture of substrates, it is desirable if the mixing takes place under controlled conditions in a substrate tank. It is important to know the composition of the material to get a suitable mix of different components and provide a constant supply of substrate to the microorganisms. For example, it is possible to verify that the load is fairly constant by regularly analyzing the VS of the different substrates, or of the finished substrate mixture.

3.5 Pre-treatment

Commonly, the material is pre-treated before it enters the biogas process. There are many reasons for such pre-treatment (Mata-Alvarez et al 2000, Tsao 1987):

1. To kill pathogenic microorganisms, i.e. sanitation.
2. To remove materials that cannot be degraded and/or that disrupt the process. This pre-treatment may involve tearing up and removing the plastic bags that are not broken down in the process or removing sand or cutlery from food waste that wear down grinders and shredders and sink to the bottom of the digester. This pre-treatment is more important from a technical perspective and will not be discussed further in this guide.
3. To concentrate the organic material content, i.e. thickening.
4. To increase the availability of organic matter, namely a reduction of particle size or increasing the solubility.

Sanitation

It is important to kill (sanitise) any pathogens in order to avoid contamination from handling substrates and digested materials. The most common method of sanitizing substrates in biogas plants is heating them to 70° C for one hour, a treatment which is required for low-risk animal waste (Category 3) under the EU Regulation EEC 1774/2002. Alternative methods that provide a corresponding level of sanitation to this so-called 'pasteurisation' method are, as of 2007, also allowed (see Chapter 6). The pre-condition is that the method used reduces concentrations of bacteria by 1 000 000 times and heat-resistant viruses by 1000 times. The presence of pathogens,

or pathogenic microorganisms, in the substrate does not usually affect the outcome of the biogas process. However, the presence of pathogens in the substrate can influence the quality and therefore the usefulness of the digestion residue, which is why this subject is explained in more detail in Chapter 6.

Thickening

The dry solids content of the substrate can be increased by allowing the material to pass through a press or screw. This is desirable because it reduces the volumetric load on the digester. The freed-up volume may instead be used to increase the organic load and thus increase the gas yield. A disadvantage of removing some water is that there is a risk that certain essential nutrients, such as salts, will be lost. Some organic material can also be dissolved in water and this will also be lost as a substrate for gas production. Dehydration can also lead to increased wear on the grinders, mixers, etc., because non-organic materials such as gravel also become concentrated.

Reduction of particle size/increased solubility

There are many different pre-treatments applied to the substrate for the biogas process to increase its availability for decomposition. The most common is mechanical disruption using a mill, blender, screw, or rotating knives. Disintegration can also be achieved by thermal, chemical or biological means using steam explosion, heat treatment, the addition of acids/bases, ultrasound, electroporation, hydrolytic enzymes, etc. (Tsao 1987, Alvarez 2008, Yadvika et al 2004, Medes et al 2005, Bourgrier et al 2006, 2008, Davidsson and Jansen 2006, Davidsson et al 2007, Bochmann et al 2007, 2007 Dewil, Dåverhög and Balmer 2008, Wawrzyn'czyk 2007, Xie et al 2007, Stenströmmer Moglia 2008, Demetriades 2008). By combining methods, it may also be possible to achieve a higher degree of disintegration. The method that produces the best results depends on the substrate's chemical composition and structure.

Why does pre-treatment result in increased gas production? Well, pre-treatment is positive for microorganisms, since decomposition often increases solubility and hence the availability of the organic material. Grinding also means that the total surface area of the material increases. Many microorganisms, especially those that are active in the initial hydrolytic step, prefer to attach to the material that they are degrading. The smaller the particles, the higher the total surface area that they can attach to. By attaching themselves, the organisms can secrete enzymes and more or less simultaneously absorb the resulting catalytic products into the cell. In this way, they get a competitive advantage over organisms that cannot attach to the organic matter, and the degradation rate increases.

According to EU Regulation EC 208/2006, the proposed maximum particle size for adequate digestion is 12 mm. Several studies also show a clear correlation between particle size and methane yield, and for maximum digestion, particle size should preferably be just a few mm or less (Figure 2; Mshandete et al 2006, Yadvika et al 2004, Angelidaki and Ahring 2000, Tsao 1987, Hills and Nakamo 1984). However, if the particle size is too small, this can cause problems at large-scale plants by clogging the equipment.

It is important to remember that pre-treatment does not necessarily increase the potential gas yield, i.e. the total amount of biogas that can be extracted from a certain material, even if the initial digestion stage is faster. The organisms in the biogas process have a unique ability to break down many different types of compounds. Often, it is "only" a question of giving them enough time. However, the decomposition rate may be very important for the economic performance of a biogas plant. If digestion is faster, it means that the retention time at the plant may be decreased without risking a reduction in gas yield.

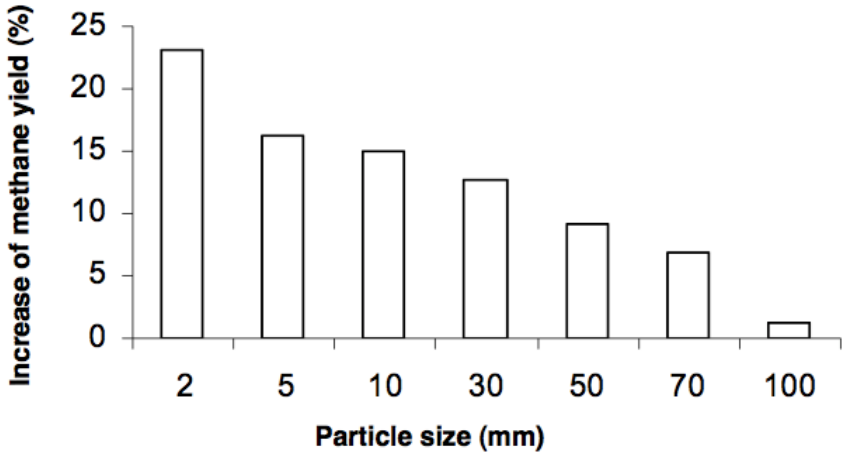

Figure 2. Effect of particle size on methane yield of sisal fibre (Modified from Mshandete et al 2006). The increase in methane recovery is in comparison with the untreated material.

3.6 The importance of different substrate components for the process

Different components in the substrate, as mentioned above, can provide varying amounts of gas because of differences in energy content. The components can also influence the process in other ways. Some general information is given below on anaerobic digestion of materials with a high content of protein, carbohydrate or fat.

Protein-rich materials

Many organic wastes contain proteins, which, just like fat, are rich in energy and produce a relatively high amount of methane in the biogas. Examples of materials that are rich in protein are slaughterhouse waste, swine and chicken manure and stillage from the ethanol industry. Other materials such as food waste also contain proteins, but in smaller quantities. Proteins consist of long chains of amino acids. There are 20 different amino acids in proteins, and the composition of the chains varies. Common to all amino acids is that they have amine groups (-NH_2). In a biogas process, proteins are first converted to individual amino acids or peptides (short chains of amino acids) during hydrolysis.

In the next step, fermentation, the amino acids are broken down and amine groups are released as ammonia (NH_3) or ammonium (NH_4^+). Ammonia and ammonium are in equilibrium with

each other. Which of these dominates depends strongly on the prevailing pH and temperature. At high concentrations, ammonia (not ammonium) can kill many organisms. In the biogas process, methane-producing microorganisms are the first to become inhibited when the concentration of ammonia begins to increase (Warren 1962, Sprott and Patel 1986, Hashimoto 1986, Schnürer and Nordberg 2008). This inhibition results in process instability (see Chapter 8).

Why does ammonia inhibit methane production? The reason for inhibition is not fully known, but it has been suggested that it may be due to the fact that ammonia, which is an uncharged compound, can enter the cell. In the cell, ammonia is converted to ammonium, a reaction that "consumes" hydrogen ions. When hydrogen ions disappear, the cell must compensate for this in some way, otherwise the pH of the cell will change. To keep the pH constant, the methanogen pumps in hydrogen ions from the environment while pumping out potassium ions. This results in a lack of potassium within the cell. Different methane producers have different initial amounts of potassium in the cell, which means that they are inhibited at different ammonia concentrations. In general, methane producers that use acetate have a lower potassium content than methane producers that use hydrogen as an energy source and are therefore inhibited at lower ammonia concentrations (Sprott and Patel 1986).

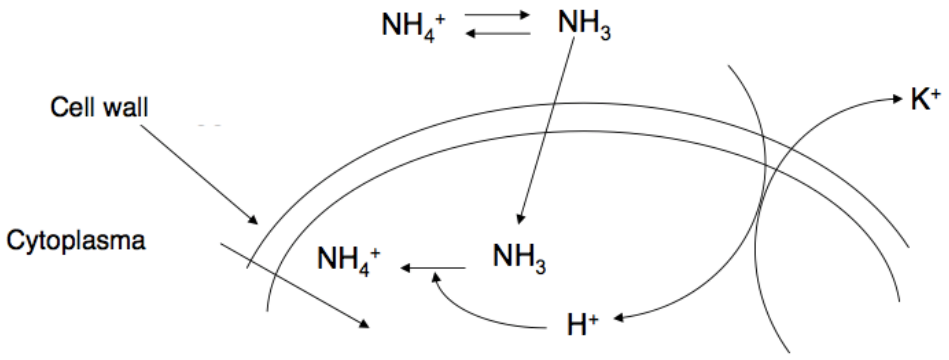

Figure 3. Effect of ammonia on methane formation (hypothesis proposed by Sprott and Patel 1986)

Different values have been found for the concentration of ammonia/ammonium that cause inhibition. Often 2-3 g NH_4^+-N per litre is given as a threshold value (Hasimoto 1986, Van Velsen 1981). However, there are many examples of processes that can handle higher levels and there are also reports of inhibition at lower concentrations (van Velsen 1981, Koster and Lettinga 1984, 1988, Hashimoto 1986, Hansen et al 1998). There are several reasons for this variation. A significant factor is whether pure cultures of methane producers or complex biogas processes are studied. The type of methane producer studied (i.e. whether they use acetate or hydrogen) is also of importance. Another crucial factor is whether the microorganisms have been able to slowly adapt, or if the ammonia content increases rapidly. A rapid increase in ammonia concentration generally results in inhibition at lower levels than slow rates of increase and adaptation. Finally, in many cases, ammonium rather than ammonia concentrations are stated. Since only ammonia

causes inhibition, these values are somewhat misleading, and it is therefore difficult to compare values between different biogas facilities. Two plants with the same ammonium concentrations may have very different levels of ammonia and hence different degrees of inhibition. The balance between ammonium and ammonia shifts to a higher proportion of ammonia with increasing pH and temperature. This means for instance that thermophilic biogas processes can be inhibited at an earlier stage than mesophilic processes (see Chapter 2).

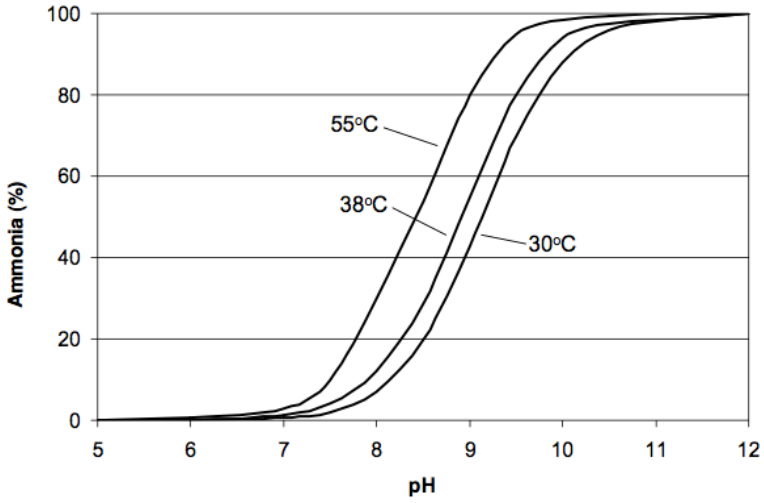

Figure 4. The distribution between ammonia and ammonium, depending on pH and temperature. Image modified from Fricke et al (2007).

As mentioned above, biogas processes can operate at much higher ammonia/ammonium contents than the critical threshold values would suggest is possible. This is because the microorganisms adapt to the higher concentrations. Adaptation to increasing ammonia concentrations is partly due to a change in the methane formation pathway from acetate (Schnürer and Nordberg 2008). As described in Chapter 1, methane is usually formed from acetate by the activity of acetotrophic methane producers. At high concentrations of ammonia, methane is instead formed from acetate via syntrophic acetate oxidation (see Chapter 1 Alternate Methane Production Pathway from Acetate). The development of this digestion pathway, which occurs between 128-330 mg per litre of ammonia, is a result of the selective inhibition of methane producers that use acetate.

Syntrophic acetate oxidation has been shown to occur in several mesophilic biogas processes with high concentrations of ammonia, but it is currently unclear what happens to the microorganisms at increasing ammonia concentrations in biogas processes running at thermophilic temperatures (Schnürer et al 1999, Karakashev 2006, Schnürer and Nordberg 2008). Syntrophic acetate oxidation has been described for thermophilic systems, but there is still no proven link with the ammonia concentration (Hattori 2008). Studies in mesophilic laboratory reactors showed instability problems (foaming) when the shift occurred, problems which were resolved when the new pathway became well established. The organisms that are active during syntrophic acetate oxidation grow more slowly than the methane producers that use acetate (Schnürer et al 1994).

This means that a biogas process with syntrophic acetate oxidation as the main methane formation pathway for acetate may require a relatively long retention time (more than 30 days).

Carbohydrate-rich materials

Carbohydrates are a common name for various sugars, including simple sugars such as glucose, disaccharides (two sugar units joined together such as in sugar cane), or chains of sugars (polysaccharides). The group of polysaccharides includes cellulose, hemicellulose, starch and glycogen. Plant-derived materials are typical carbohydrate-rich substrates.

Since carbohydrates are very different in their nature, they are digested at different rates in the biogas process. Simple sugars and disaccharides are broken down easily and very quickly. This may seem good, but it can lead to instability problems due to increasing contents of fatty acids (Gunaseelan 1997, Lee 1999, Bouallagui et al 2004). Hydrolysis and fermentation occur very rapidly for substrates containing high contents of these sugars. However, methane producing microbes are slow-growing and this becomes a process bottleneck because they are important to drive the degradation of fatty acids (see Chapter 1 Anaerobic Oxidations). Methane producers become the bottleneck since they cannot force the degradation of the fatty acids at the rate at which they are formed, which causes them to accumulate. Because of the accumulation of fatty acids, and because carbohydrate-rich materials tend to have poor buffering capacity, there is a risk of process problems due to decreasing alkalinity (Demirel and Scherer 2008).

Materials of high sugar content should be mixed with another material containing less digestable compounds and preferably more nitrogen in order to achieve a balanced process (Murto 2004, Parawira et al 2004, 2008, Kaparaju and Rintala 2005). This is to ensure that the initial stages of the process are not too fast. An alternative is to use a two-step process, where the acid formation and methane formation steps are separated (Bouallagui et al 2004, Parawira et al 2008). Examples of materials that are rich in rapidly degradable sugar compounds include pure sugar solutions, fruits, potatoes and sugar beets.

Polysaccharides are composed of various sugars, and they are common in plant-derived materials. Polysaccharides generally have a relatively low solubility, and their composition and structure varies. Therefore, they are also degraded at very different rates in a biogas process. Starch is the commonest polysaccharide in major dietary items such as potatoes, rice and pasta. It consists of straight or branched chains of glucose and is digested relatively easily in the biogas process. Too much material which is rich in starch can lead to similar problems as with simple sugars, that is to say that the process goes "sour".

Cellulose is the most common organic compound on earth, and therefore represents a large potential for biogas production. However, it is much more difficult to degrade. Cellulose is an important component in the cell walls of plants and consists of long chains of the sugar glucose, an average of approximately 5000 glucose/chain. In the cell wall, a number of parallel chains of cellulose bind to each other to form microfibrils. Because of this complex structure, cellulose is not soluble and therefore difficult to digest.

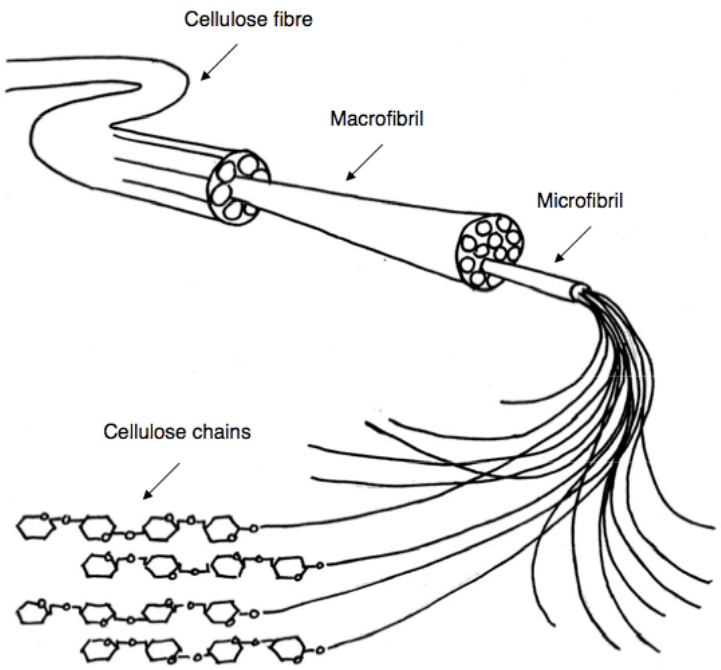

Figure 5. The structure of a microfibril

In plant matter, the cellulose is linked together with both hemicellulose and lignin, which makes it even less available to microbial degradation (Gunaseelan 1997, Zhang et al 2007). Lignin, which is an aromatic compound with a very complex structure, does not decompose at all in the biogas process. Hemicellulose is composed of several different sugars, not only glucose, and the exact composition varies depending on its origin (i.e. different plants have different hemicelluloses). Hemicellulose also consists of branched polysaccharides, which reduces its degradability. Because of the complex structures of cellulose and hemicellulose, and the fact that they also are bound to each other, hydrolysis is the step that slows the rate of degradation of plant material (Gunaseelan 1997, Zhang et al 2007). The enzymes secreted by the hydrolysing microorganisms have difficulty "accessing" the structure, and the hydrolysis step is therefore slow.

In the case of cellulose-rich materials such as straw or silage, pre-treatment determines the rate of hydrolysis, and thus by extension, the rate of production of gas. Accessibility and digestibility can be improved by disrupting the material. The smaller the particle size, the better the accessibility (see above). Research has also shown that chemical pre-treatment, which breaks up the crystalline structure of cellulose, can increase the rate of degradation and provide a higher yield (Gunaseelan 1997, Liu et al 2002, Yadvika et al 2004, Stenströmmer Moglia 2008, Demetriades 2008; Fig. 6) . Similarly, pre-treatment with various cellulases (enzymes) helps speed up decomposition (Bochman et al 2007). However, the microorganisms in the biogas process are themselves able to degrade cellulose, given enough time.

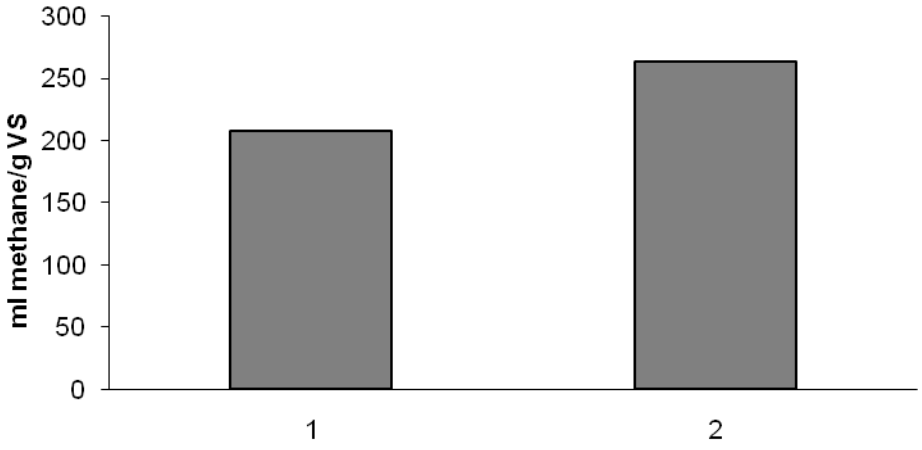

Figure 6. Methane production potential of straw, (1) untreated and (2) treated with steam explosion. Modified from Demetriades 2008. The potential is determined in batch digestion experiments performed at 37oC.

Fatty materials

Typical fatty materials that are currently used in biogas processes are slaughterhouse waste, grease trap waste, waste from the dairy industry and various oils, such as fryer oils (Li et al 2005, Demirel et al 2005, Cavaleiro et al 2008). Like protein-rich material, fat is very energy-rich and can produce a lot of gas with a high content of methane. However, fat may also cause problems with process instability (Pereira et al 2004, Fernandez et al 2005). Fats consist mainly of fatty acids and glycerol, and vary with respect to the composition of the fatty acids. They are usually classified as either saturated, monounsaturated or polyunsaturated fats. Saturated fat is found in meat and dairy products, polyunsaturated fats, for example, are found in fish and corn oil, and monounsaturated fats are found in vegetable oils and in nuts. Saturated fat has a higher melting point than unsaturated fat, making it less available for biodegradation. Pre-treatment with heat may increase the digestability of these fats.

Triglycerides (neutral fats) are the commonest type of fat. They are readily hydrolyzed in a biogas reactor into long chain fatty acids (LCFA) and glycerol. There are different types of LCFA, but they share a common trait in that they all contain more than 18 carbon atoms. Common long chain fatty acids include stearic acid, palmitic acid, oleic acid and linoleic acid. Glycerol is rapidly converted into biogas while the degradation of LCFA is more complicated. Degradation of these acids requires, just like short fatty acids, the presence of a methane producer that uses hydrogen, i.e. decomposition occurs in syntrophy (Sousa et al 2007, see Chapter 1). This means that this type of fatty acid is also easily accumulated during a process disturbance. A further complication is the fact that several LCFA's at high concentrations have an inhibitory effect on many different organism groups in the biogas process, including the methane producers (Koster and Kramer 1987, Angelidaki and Ahring 1992, Lalman and Bagley 2001, Chen et al 2008). Oleic acid and

stearic acid have been shown to affect methane producers at concentrations around 0.2-0.5 grams per litre (Angelidaki and Ahring 1992).

Moreover, just as for ammonia inhibition, the methane producers that use acetate are more sensitive than those that use hydrogen. Thus, this inhibition results in a less efficient process (less biogas) and sometimes leads to instability problems. The inhibition of LCFA is partially reversible, i.e. when the concentrations decrease below toxic levels, the process can recover (Pereira et al 2004, Cirne et al 2007). The period before digestion starts (the lag-phase) can be relatively long (days - weeks), and since cells bind to fat, there may be a risk of rinsing out biomass (Pereira et al 2004). Some studies also show that adaption to high fat levels is possible if growth occurs slowly, and if fat is added in repeated pulses (Cavaleiro et al 2008).

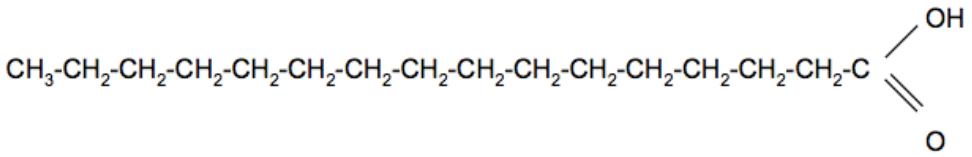

Figure 7. Palmitic acid, a long chain fatty acid (LCFA), which inhibits the formation of methane, but also may provide high yields of methane, if the concentration is not too high.

Another aspect is that the long chain fatty acids have surface-active properties and therefore readily form foam if concentrations become too high. A survey recently carried out at 13 co-digestion plants showed a clear link between the percentage of fat in the input material and the frequency of foaming (Albertsson 2007). It was also common for slaughterhouse waste or grease-trap waste to foam in both the tanker truck delivering the material to the plant and the substrate mixing tank. The problem was greatest in the summer months when temperatures were relatively high. The reason for this is that the hydrolysis of fat started before it went into the digester, and this process was accelerated when the temperature was high. During hydrolysis, LCFA's were released, resulting in foaming. When this material is added, the reactor becomes overloaded with high concentrations of fatty acids, which also causes foaming problems. If fatty acids are released slowly during the digestion of fats in the actual biogas process, and if excessive concentrations are not reached, there is less risk of instability than if the process is instantaneously loaded with high contents of LCFA's. There are also technological solutions to foaming problems (see Chapter 8).

3.7 Important information about various substrates

The list of different substrates that are or could be used in a biogas process is very long. This section describes a few important types of material commonly used today, or that may become important in the near future, with an emphasis on their availability to the microorganisms in the biogas process and potential limiting factors. Chemical characterization and/or experiments at the laboratory scale are recommended in order to predict the potential and/or limitations of an "unknown" material intended for digestion.

Stillage and other sulphate-containing substrates

Stillage (a distillation waste product from ethanol production) is not a very common substrate in Sweden, but its use will probably increase in the future. Today, only a few mesophilic processes use this substrate. By linking the production of bioethanol and biogas, energy efficiency can be increased, which of course is interesting (Börjesson and Mattiasson 2007). Stillage can work well as a substrate for a biogas plant, but as the sole substrate, there is some risk that the ammonia concentration becomes too high. Only sugar is consumed during ethanol production, which is usually carried out by the addition of yeast. This makes the waste product rich in protein, and the stillage can lead to processing problems due to ammonia inhibition (see above under the heading of protein and Chapter 8). It is therefore very important to monitor ammonia concentrations if stillage is used as a substrate in a biogas process. The process can benefit if the stillage is mixed with a more carbohydrate-rich material.

If stillage is to be used as a substrate for a biogas process, it is also important to consider the ethanol production facility that supplies the waste. In ethanol production, the sugar substrate, which in Sweden is mainly wheat, but also may include other crops or cellulose-rich residues, must be pre-treated to make it available for the yeast. This pre-treatment can be done in slightly different ways, but usually involves heat treatment in the presence of sulphuric acid (H_2SO_4), (Hahn-Hägerdahl et al 2006). Sulphuric acid is sometimes also added to regulate pH during ethanol production. Traces of acid in the stillage result in relatively high concentrations of sulphate (SO_4^{2-}). This stimulates the growth of sulphate reducers, which usually means that the sulphate reducers out-compete the methane producers (Dar et al 2008, Chen et al 2008, Chapter 1). The consequence is a reduction in biogas production and increased production of hydrogen sulphide (H_2S). A high concentration of hydrogen sulphide is not desirable since it results in poorer gas quality and also inhibits the microorganisms in the biogas process. In addition, hydrogen sulphide is very corrosive and can cause problems in pipelines and tanks (Hasnaian and Anderson 2005). Sulphate and other sulphur compounds are also used in the production of pulp, which explains why residues from the pulp industry can lead to similar problems.

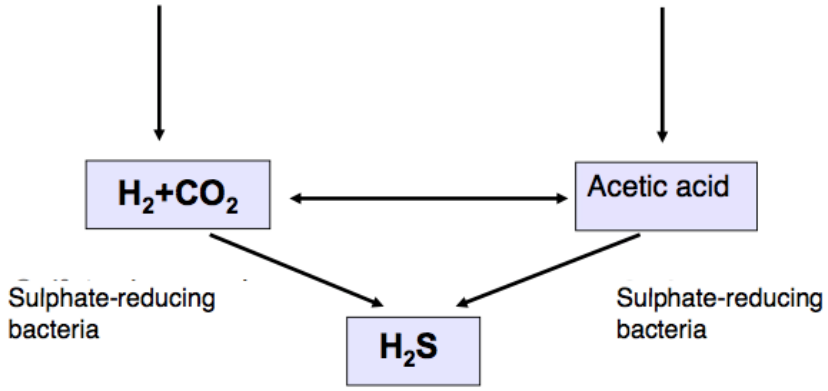

Figure 8. Sulphate-reducing bacteria compete with the methane producers for its substrates, acetate and hydrogen. The activity of the sulphate reducers leads to the formation of hydrogen sulphide.

Degradation of the lignocellulose structure during pre-treatment prior to ethanol production also releases furfurals, small phenolic compounds that can cause inhibition of methane producers, especially acetate-using methane producers (Torry-Smith 2003, Olguin-Lora et al 2003).

Food waste

Food waste is commonly used for biogas production. Several biogas processes, both at mesophilic and thermophilic temperatures, use food waste from households, food industries, restaurants, etc. with good results. The composition of food waste is usually very diverse, and because it contains proteins, fats, carbohydrates and various trace elements, it has the potential to function very well in a biogas process (Gunaseelan 1997). However, it is important that the mixture of the waste is varied, i.e. does not contain too much meat waste in relation to vegetable and fruit wastes. If the waste contains too much protein, problems can arise with ammonia inhibition (Fricke et al 2007, Akunna et al 2007). Similarly, too much fat or sugar can cause problems (see above). A recent study showed that food waste, which contained a lot of fried food residues, could only be digested under stable conditions after the addition of various trace elements (Climenhaga and Banks 2008). Chemical characterisation of a food waste from households in Uppsala, however, showed that the composition of protein, carbohydrate and fat was beneficial for biogas production (C/N ratio was 15; Eklind et al 1997). The material contained some pesticide residues, probably from fruit peels, but concentrations were low, so they were unlikely to pose a problem for either the process or the use of the digestate as fertilizer (Nilsson 2000). This food waste was used as the sole substrate for a continuous anaerobic digestion experiment at the laboratory scale at both mesophilic and thermophilic temperatures for a period of 8 years (Schnürer and Schnürer 2006, Levén 2006).

Manure

The composition of manure from different animals varies, and therefore manure will also vary in its suitability as a substrate for biogas processes (Möller et al 2004). Manure can be classified into solid and liquid manure (or slurry) depending on the dry solids content. Solid manure typically has a higher carbon content and dry solids content (27-70%) than liquid manure, since it includes straw and hay in addition to the faeces (Nordberg 2006). Liquid manure is more accessible for digestion, as it contains more nitrogen and has a dry solids content of 5-10%. In general, manure from cattle yields less gas than manure from pigs and poultry (see Table 3, Möller et al 2004). This is because a lot of the organic material available in the feed has already been digested and converted into methane in the stomachs of ruminants (i.e. cattle). If the manure is digested along with other types of materials, such as food waste or forage crops, the gas yield can increase (see above under Co-digestion of different substrates; Lethomäki et al 2007). Manure from cattle can also sometimes have a stabilising effect on an unstable biogas process, since addition of manure results in the inoculation of more microorganisms as well as nutrients. In addition, a dilution may reduce the concentrations of inhibitory components such as ammonia or volatile fatty acids.

Digestion of manure also provides many environmental benefits, including reduced emissions of methane from manure storage facilities (Börjesson and Mattiasson 2007, Biogas Syd 2008).

Manure from pigs and chickens contains more protein than manure from cattle, which can lead to problems with ammonia inhibition if these materials are digested without including materials containing more carbohydrates (Van Velsen 1981, Chynoweth et al 1999, Möller et al 2004, Hansson and Christensson 2005, Litorell and Persson 2007).

Figure 9. Biogas can be produced from cow manure. Photo Åsa Jarvis

Bioenergy crops/crop residues

Many different crops and plant materials can be used for biogas production, such as corn, grain, sugar beets, potatoes, fruit, grass, silage, etc. (Hansson et al 2007, Demirel and Scherer 2008, Lehtomäki et al 2007, 2008 a, b, Anon et al 2007). As mentioned earlier, the methane yield of energy crops is affected by the storage process, site properties and time of harvest, since these factors affect the chemical composition of the crop and hence the ability of the microorganisms to use plants as substrates for their growth (Gunaseelan 1997, Kreuger et al 2007, Anon et al 2007, Lethomäki 2008a).

Bioenergy crops often have a relatively high content of dry solids (10-50%). Thus, in wet digestion processes, it is appropriate to dilute (co-digest) this material with substrates with a higher water content or alternatively to recirculate the process fluid (Lehtomäki 2008a, Parawira et al 2008). Dilution with other material may also be appropriate, since many plant materials contain relatively low concentrations of trace elements, which can limit the degradation process. The microorganisms, and thus the biogas process, may not operate at maximum efficiency at low concentrations of trace elements. A deficiency in trace elements can be avoided partly by co-

digestion, but also through the addition of a solution of trace elements. Additives with trace elements are, for example, used in German on-farm plants which primarily digest crops (personal communication, Ralf Winterberg, Elbe Energy). Many bioenergy crops also have a high C/N ratio and mixing with more nitrogen-rich material can achieve optimum process conditions. Co-digestion of energy crops with, for example, manure has been shown to generate a 16-65% increase in methane recovery (Lethomäki et al 2008b).

Figure 10. Silage grass crops can be used for biogas production. Photo Anna Schnürer.

Bioenergy crops with high contents of cellulose, hemicellulose and lignin (e.g. straw) are degraded slowly in a biogas process due to their complex structure. In order to maximize the digestion rate of cellulose-rich materials, it is beneficial to chop it up and/or apply pre-treatment to break up the complex structure of cellulose and make it more accessible for digestion. Treatments that have proven to be favourable for gas yield include, for example, treatment with bleach, hydrogen peroxide or heat (Stenströmmer Moglia 2008, Yadvika et al 2004).

For bioenergy crops containing more readily degradable carbohydrates such as fruit, potatoes and sugar beets, the issue becomes the reverse. With these crops as substrates for the biogas process, hydrolysis occurs rather quickly and there is a clear risk of accumulation of acids with a lowered pH as a result (Bouallagui et al 2004, Parawira et al 2008). Different strategies can be adopted to prevent acidification during digestion of carbohydrate-rich materials. One obvious approach is to co-digest with more nitrogen-rich materials (Lethomäki et al 2008b, Parawira et al 2008, 2004). Another strategy is to add buffering agents and extra nitrogen to the process. A recent study showed that it was possible to degrade sugar beets (without foliage) as the sole substrate by regular additions of different buffering agents, nitrogen and trace elements (Demirel and Scherer 2008). Finally, another strategy is to use a two step process (see Chapter 2), possibly together with co-digestion. There are several examples in the literature that show an increase in methane yield after separating the acid-forming steps and the methane formation step (Lethomäki et al

2008b, Parawira et al 2008, Bouallagui et al 2004). In such a process, it is possible to "protect" the methane-producing organisms from low pH by regulating the input to this step. Recycling the process fluid between the two reactors reduces the need for external fluids and also promotes degradation in the first reactor (Parawira et al 2008).

Bioenergy crop residues can also cause self-heating of the process. A recently published scientific article showed that about half of all (20 of 41) biogas plants in Austria, which digested bioenergy crops, showed an increase in process temperature (Lindorfer et al 2007, 2008). Instead of the planned process temperature of between 35-39 °C, they were 42-49°C. Calculations with data from these plants showed that the temperature increase was between 0.15-0.5 ° C per day. The reason for heating was considered to be the release of energy during the breakdown of carbohydrates, which could happen if the substrate has a high energy density, that is, contains a high percentage of starch, and at a relatively high load. This unwanted increase in temperature sometimes resulted in a disturbance of the process, with increasing fatty acid concentrations as a result. The response of the microorganisms and the process, however, varied between different plants (Lindorfer et al 2008).

Slaughterhouse waste

As previously mentioned, slaughterhouse waste contains high contents of fats and proteins, which are very energy-rich and have the potential to generate a lot of biogas. However, excessive fat and protein contents lead to increased concentrations of ammonia, volatile fatty acids and LCFA's, which can lead to process breakdowns (Cuetos et al 2008, Salminen and Rintala 2002, see above, The importance of different substrate components). It is therefore difficult to use slaughterhouse waste as the sole substrate, especially at thermophilic temperatures, because the proportion of ammonia in relation to ammonium can easily become too high. Slaughterhouse wastes have a high C/N ratio, but with co-digestion, the likelihood of a stable process operation is significantly improved (Cuetos et al 2008, Salminen and Rintala 2002, Rosenwinkel and Meyer 1999). Co-digestion with manure, sewage sludge and food waste, which improves, among other things, the C/N ratio, have all been shown to lead to more stable processes. An alternative to co-digestion is to apply a two-step digestion process (Wang and Banks 2003).

Sewage sludge

At present, sludges from various stages of sewage treatment account for the largest single source of biogas production in Sweden. The sludge can contain different chemical compounds, which may inhibit the microorganisms in the process, such as metals and organic pollutants. It may also have a relatively low content of organic matter (3-4%). Although a large amount of biogas is produced by anaerobic digestion of sewage sludge, some of the organic matter may remain in the residual sludge, i.e. the digestion process has a relatively low efficiency in this case. This may be due to several factors. The retention time may be too short to allow time for the microorganisms to degrade the material, or the process may be inefficient due to the presence of inhibitory substances. In addition, the organic matter in the sludge is often too complex for the microbial hydrolysing enzymes to effectively "break up" the material.

Pre-treatment of sludge has been shown to have a positive effect by, for example, reducing the foaming rate. Foaming can be caused by several factors (see also Chapter 8), but a common cause in digestion tanks at wastewater treatment plants is the presence of the organisms Microtrix parvicella and/or Nocardia. Both of these organisms can cause foaming in the active sludge process (i.e. the purification step that has access to oxygen), but can also remain in the system and cause problems in the digester as well. Microtrix survive in the anaerobic digester and can potentially even grow in this environment. The cell walls of this organism are hydrophobic, i.e. they bind to fats. They also like to bind to each other and with organic material and this 'flocculation' makes the sludge difficult to break down and increases the risk of foaming. Pre-treatment to break up the sludge structure makes it more accessible for degradation and also reduces the risk of problems with foaming.

Several wastewater treatment plants are currently working on various projects designed to optimise and increase biogas production in the digesters. Different types of urban waste, food waste, sludge from grease traps and fruit and vegetable waste, for example, have been shown to stimulate sludge degradation and produce a higher methane yield than could be expected by digesting the waste separately (Jansen et al 2004, Leksell 2005). Different pre-treatments and combinations of pre-treatments have also been shown to increase gas production by making the sludge more available for digestion. One study has shown that the direct addition of enzymes to the digestion chamber can increase gas yield (Davidsson et al 2007).

3.8 Odour

Unpleasant odours can arise around biogas plants (Rönnols and Jonerholm 2007) due to various compounds formed when microorganisms degrade organic matter. Examples of substances that cause unpleasant odours are sulphides, mercaptans (sulphur compounds), amines and indoles (nitrogen compounds) as well as organic acids and aldehydes (Higgins et al 2008). Which of these odourous compounds are formed depends on the composition of the organic material, which microorganisms are present in the digester and the management of the process. Although the choice of material and correct process management can minimize odour problems, odourous compounds are always formed to some extent, regardless of material and operations, as these are a natural part of the decomposition process which generates biogas. However, there are various techniques to reduce and minimise odours in and around biogas plants. For example, processes generating odours can be built indoors and outgoing air can be treated with biological filters. More examples of technical solutions are listed in the report by Röönlos and Jonerholm (2007).

It is clear that both the output of the biogas process and its stability is strongly influenced by the characteristics of the substrates. It is therefore imperative to ensure the availability and delivery of appropriate substrates when planning a biogas plant. Depending on the type of material, it can also be important to closely examine the type of pre-treatment to be used, since this also has a significant impact on the final gas yield.

CHECK YOUR KNOWLEDGE

- Which materials generate the most biogas, fat, sugar or protein?
- Why is it a good idea to pre-treat a substrate that is to be used in a biogas process?
- What are the typical problems you may have with a material that is high in fat, carbohydrates or protein?
- What is the significance of the C/N ratio for biogas process efficiency and stability?
- Under what conditions is the inhibition of ammonium/ammonia strongest? Why?
- Why is there a risk of low methane yield when the substrate contains high concentrations of sulphate?
- Why does cattle manure have a lower methane yield than pig manure?
- Why can it be difficult to digest energy crops or slaughterhouse waste individually?
- What is it that smells in a biogas process?

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4 TOXICITY

Many substances may inhibit a biogas process, and it is the methane producers which are usually the most sensitive and first become disturbed. Inhibiting substances can enter the process either by poorly sorted or contaminated material or can be formed during the degradation of an initially non-inhibiting substance. The effect of a toxic substance may vary, and the process may respond in different ways, depending on factors such as the concentration of inhibitory substances, retention time, temperature, pH, concentration and type of microorganisms present, other inhibiting substances, etc. If the digested residue is used as fertilizer, traces of various toxic substances may also adversely affect soil microorganisms.

4.1 Inhibition levels

There is considerable variation in the concentrations of various substances that have been reported to lead to inhibition or a stop in the process. These variations result from the fact that the degree of inhibition is affected by many factors, such as (Speece 1987, Chen et al 2008):

1. antagonism, i.e. when the presence of several inhibitory substances collectively produce a lower degree of inhibition than each of the individual components.
2. synergism, i.e. when the presence of several inhibitory substances collectively produce a higher degree of inhibition than each of the individual components.
3. complex formation, i.e. when the inhibitory substance binds to different or similar structures and become "invisible" to the microorganisms.
4. adaptation/acclimatisation, i.e. when the microorganisms adapt to the toxic environment and can once again begin to grow. This adjustment may be due to several factors, such as cells learning to break down the inhibitory substance, thus decreasing the concentration of the substance, or the microorganisms mobilising a defence system, such as altering the composition of the cell to make it more resistant.

The organisms in the process can sometimes recover after a disturbance, but sometimes the inhibition is irreversible, that is, the microorganisms cannot recover from the inhibitory effects, even if the inhibitory substance disappears. The process must then be restarted and/or new "fresh" microorganisms must be added. Alternatively, the organisms may be "only" inhibited and then after an adjustment period they can recover (acclimatisation). In these cases, it is common to talk about a lag period, which refers to the period when the organisms stop growing or grow less well due to the inhibitory effects. The lag phase can also represent a period during which growth occurs of microorganisms that are equipped to cope with the presence of an inhibitory component. These organisms may initially represent a very small group but their numbers and importance may increase when the inhibitory component is introduced to the process.

In order not to risk a complete breakdown of the process during this lag period, it is important to extend the retention time or decrease the load in a continuous process, otherwise there is a clear risk that the microorganisms will be washed out. It would also be important to try to reduce the concentration of the inhibitory substance, unless aclimatisation to increasing concentrations of a inhibiting substance is actually the objective. The levels of inhibitory substances can be reduced by altering the composition of the substrate so that it contains less of the inhibitory substance or components that release inhibitory substances during decomposition. To avoid problems with long adjustment periods, it can sometimes be advantageous to start a process, or replenish an already existing process, with material from another biogas plant with pre-adapted organisms.

4.2 Inhibiting substances

Many substances may inhibit the biogas process. The following table lists some major groups of components that are known to inhibit the process (Chen et al 2008). Below is a more detailed

description of some important groups of substances that may inhibit a biogas process combined with the approximate inhibiting concentration for each substance.

NH_3
Cations (Na^+, K^+, Ca^{2+}, Mg^{2+})
Alternative electron acceptor (SO_4^{2-}, NO_3^-)
Compounds containing a benzene ring, such as phenol, toluene, benzene and xylene
Cyanides (compounds with-CN group)
Heavy metals
Detergents such as lauryl sulphate
Hydrogen sulphide
Solvents
Disinfectants
Long chain fatty acids (LCFA)
Formaldehyde
Chlorinating hydrocarbons (chloroform, carbon tetrachloride, methylene chloride, trichloromethane, etc.)
Organic nitrogen compounds, such as acrylonitrile
Antibiotics, such as monensin
Lignin derivatives (furfural)

Table 1. Substances that may inhibit the biogas process

Ammonia

Ammonia is released during decomposition of protein-rich materials and primarily inhibits the methane-producing organisms. Ammonia (NH_3) is in equilibrium with the ammonium ion (NH_4^+). The predominating form depends on the process temperature and pH as well as some other factors. The inhibitory form is ammonia and inhibition can occur at concentrations as low as 30 mg per litre of ammonia, but there are reports of processes that can handle much higher levels (~ 100 mg NH_3 per litre; Fricke et al 2007). Usually the process is monitored by analysis of ammonium-nitrogen (NH_4^+-N), which is a composite measurement of ammonia and ammonium. Inhibiting concentrations of ammonium-nitrogen (NH_4^+-N) are reported between 1.5 and 14 g/L (Calli et al 2005, Chen et al 2008, Fricke et al 2007). Levels around and above 3 g/L have been reported to frequently cause inhibition independent of pH (Calli et al 2005). A thorough explanation of ammonia toxicity is given in Chapter 3 Protein-rich materials.

Cations

Cations are necessary for the microorganisms, but tend to be inhibitory to the process at high concentrations. High salt concentrations lead to a collapse of the bacterial cell as it attempts to "dilute" ambient concentrations by pumping out the water (Figure 1). Some microorganisms can to some extent adapt to high salt concentrations by forming so-called osmolytes, compounds that stabilise the cell in the presence of high salt concentrations. Some studies have also shown that the addition of osmolytes (glycine, betaine and choline) may increase the methane yield in processes treating food waste with high sodium content (Oh et al 2008). There is a high start-concentration of salt in various industrial wastes, such as waste from fish and food, but cations can also be released during the decomposition of other organic substrates. Cations are also linked to various alkaline components (sodium hydroxide (NaOH), sodium carbonate ($NaCO_3$)), which are sometimes added to increase pH and alkalinity in the digester (Chen et al 2008).The optimal concentration for methane producers is reported to be around 100-200 mg per litre for sodium, 400 mg per litre for potassium, 200 mg per litre for calcium and 720 mg per litre for magnesium (Chen et al 2008). Inhibition has been shown with salt concentrations around 1500 mg per litre, but concentrations up to 8000 mg per litre can work, if a gradual adjustment is allowed to take place. The concentration that produces inhibition may vary depending on what substrate is being digested, which is probably related to the fact that different organisms grow in different substrates and that they in turn have varying abilities to cope with high salt concentrations (Lefebvre et al 2007). The presence of cations may also have a positive effect on the process because they have been shown to reduce the impact of ammonia inhibition, i.e. antagonism. (Chen et al 2008).

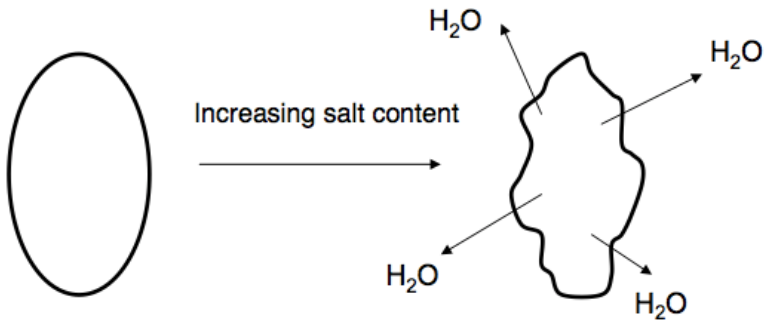

Figure 1. High salinity makes the bacteria "lose" water

Alternative electron acceptors

These are compounds that cause the flow of electrons in a biogas process to be steered away from methane production and instead increase the activity of competing microorganisms, such as sulphate-reducing and nitrate-reducing microorganisms (see Chapter 1). These organisms are in competition with both methane producers and fermentative bacteria for their substrate, and usually win this competition. The result is decreased production of biogas and instead increased formation of hydrogen sulphide or various nitrogen-rich gas compounds (nitrogen, nitrous oxide). High levels of sulphate, for example, can be found in residues from distillaries and the pulp and paper industry. For more information about materials containing sulphates, see chapter 3. Nitrate (NO3-) can be found in various types of wastewater, for example from the pharmaceutical industry (Rodriguez-Martinez et al 2005).

It is difficult to specify exact concentrations at which methane producers are inhibited. Several factors may be relevant (Isa et al 1986, Shabir et al 2008, Dar et al 2008). The effect of sulphate, for example, is strongly linked to the ratio between COD (Chemical Oxygen Demand) and sulphate, where COD is a measure of the amount of organic matter available for decomposition. One study has shown that when the ratio of COD/SO_4^{2-} in the medium was over 2.7, methane producers dominated (the acetate users), but when the ratio fell below 1.7, the sulphate reducing bacteria became more competitive, with decreased amounts of biogas as a result (Dar et al 2008). Lower substrate concentrations in relation to the amount of sulphate are therefore positive for sulphate reducers. The digestion temperature has also been shown to affect the outcome of the competition between sulphate-reducing bacteria and methane producers, where methane producers seem to have an advantage at thermophilic digestion temperatures (Pender et al 2004). In the case of nitrate, one study showed that concentrations above 62 mg/L resulted in an increase in denitrification activity in relation to both sulphate reduction and methane formation (Rodriguez-Martinez et al 2005). The importance of the COD/NO_3^--N ratio has also been demonstrated by Sonza and Atalay (2005). Ratios between 2 and 3.7 were shown to be optimal for denitrification.

Organic pollutants

Organic pollutants are found in crop residues and various industrial and food wastes (Chen et al 2008). Thus, many organic pollutants can end up in a biogas reactor, which can have adverse effects on the microorganisms in the process (Nilsson 2000, Engwall and Schnürer 2002, Olsman et al 2002, Levén et al 2005, Levén 2006, Chen et al 2008, Elefsiniotis and Li 2008). Threshold concentrations for inhibition depend on several factors, such as type of compound, exposure time, processing temperature and loading frequency (Chen et al 2008). In general, many organic pollutants are poorly soluble in water and instead bind tightly to organic material. These pollutants can also bind to, and damage, the bacterial cell membrane, which leads to the cell no longer being able to perform its metabolism. Growth of microorganisms can, as a result, be strongly inhibited and the organisms may even die. After a period of adaptation, some organic pollutants can be broken down by the microorganisms in a biogas process. In general, the microorganisms in the biogas process are able to degrade many organic compounds (Alexander 1994, Baker and Herson 1999, Zhang and Bennett 2005). Some compounds are broken down even more efficiently in an anaerobic process than in the presence of oxygen. This means that biogas processes are sometimes also used to treat various types of industrial wastewater (Farhadian et al 2008) and are not primarily used for the production of methane.

The capacity of the process to degrade various organic pollutants is strongly influenced by the conditions under which the process is run, and obviously also by the concentration of the compound. A compound that at high concentrations appears to have an inhibitory effect, can be easily degraded if it appears at lower concentrations (Chen et al 2008). In general, the mesophilic process has a greater biodegradation capacity since there are many different microorganisms that thrive in this temperature range compared to a higher temperature (Levén and Schnürer 2005, Levén et al 2005). Sometimes the retention time can also be a decisive factor, because many organic pollutants are degraded very slowly. Contaminants that are known to cause problems in biogas processes include benzene compounds (phenol, xylene, benzene, toluene), phthalates (plasticisers in plastics), halogenated aliphatics (chloroform, carbon tetrachloride, trichloroethane) and halogenated aromatics (e.g. chlorophenols such as PCP), compounds with benzene rings (phenol, toluene, xylene, etc.) and various nitrous compounds (nitrophenol, aminophenol, amines, etc.; Chen et al 2008).

Many inhibitory compounds enter the process with contaminated waste, but some groups are also naturally present in some substrates that are of interest for biogas production. This group includes various types of phenols such as phenol, cresols, tannins, toluene and other aromatic structures. Phenols and toluene can be found in pig manure (Loughrin et al 2006). Different phenol derivatives and tannins are common structures in plant material and can be released during decomposition of this material. Thermal pre-treatment of plant material and the decomposition of lignin can lead to the formation of so-called furfurals (Figure 2). These may inhibit the biogas process (Rivard and Grohman 1991). Biogas production from food waste has also been shown to lead to the accumulation of various aromatic acids, some of which showed an inhibitory effect on the biogas process (Hecht and Griehl 2008). At low concentrations, all of these compounds can be degraded in biogas processes if the process conditions are optimal.

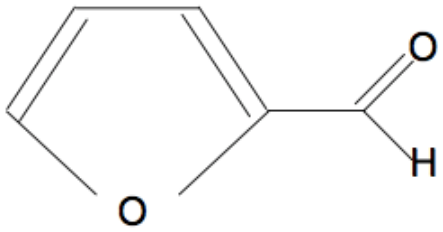

Figure 2. Furfural, a compound that can inhibit the biogas process.

Heavy metals

Heavy metals may be present at relatively high concentrations in industrial waste, and sometimes in municipal sludge. Metals are not degraded, so there is a risk that they accumulate to toxic levels in the reactor. This group includes toxic metals such as lead, mercury, cadmium and uranium, but also vital metals such as iron, zinc, copper, chromium, manganese, molybdenum, nickel and selenium. Metals appear to have an inhibitory effect because they interfere with the organisms' enzyme system by binding to different groups of these enzymes. Low concentrations of certain heavy metals are necessary for microbial activity. Cobalt, molybdenum and nickel are heavy metals which are important for the activity of methane producers and their enzymes. As discussed earlier in Chapter 3, certain substrates (some bioenergy crops) have low concentrations of metals and deficiencies may thus occur.

It is difficult to say what concentration of heavy metals causes inhibition and which metals are toxic because many of the results reported in the literature vary considerably (Chen et al 2008). The range is wide in terms of threshold concentrations that result in inhibition by the various heavy metals, but they are in the order of 100 milligrams per litre (Chen et al 2008). Some metals, such as iron, however, are relatively non-toxic and may appear in the process in the order of several hundred grams per litre without causing any problems. Some reports also indicate that the process can avoid disruption even at high concentrations of more toxic metals. A likely explanation for this is that metals bind to different organic compounds (so-called chelates) in the process, or form precipitates such as sulphides. This results in the metals becoming "invisible" to the microorganisms and thus the process is not inhibited. Examples of compounds that have been shown to bind metals are bentonite and citrate (Chen et al 2008). The level at which inhibition occurs is also affected by the fact that different metals together can have both synergistic and antagonistic effects. For example, nickel has been shown to have an antagonistic effect (i.e. reduces the inhibitory effects) to cadmium and zinc. However, the same metal led to an increase in the inhibitory effect of copper, molybdenum and cobalt (Chen et al 2008).

Long chain fatty acids

Long chain fatty acids (LCFA's) such as stearic acid, palmitic acid, oleic acid and linoleic acid and others are released during digestion of fatty materials and may have a strong inhibitory effect on both methane producers and other microorganisms (see Chapter 3). The fatty acids bind to the

cell membrane, leading to the cell membrane no longer being able to perform important functions, such as protecting the cell and transportation of materials in and out of the cell (Chen et al 2008). Thermophilic organisms have been reported to be more sensitive to long chain fatty acids than mesophilic organisms. LCFA's can have an acute toxic effect and have also been shown to cause permanent inhibition of methane producers (Chen et al 2008). Other studies show that LCFA's can be broken down and that the process can recover, but recovery times may be long (Cirne et al 2008, Chen et al 2008). The toxicity appears to be more dependent on the physical characteristics of the sludge (size distribution and surface area) than the compound's biological nature. Biodigestion has been shown to be possible at concentrations up to 5 g COD-LCFA/g VS (Pereira et al 2002). It has been shown that the digestion of fats can be stimulated by the addition of hydrolysing enzymes (lipases, Cirne et al 2008, Rigo et al 2008). However, if the concentration of lipases was too high, this led to a stronger inhibition of the process, probably due to the release of LCFA's.

Antibiotics

Methane producers are generally less sensitive to antibiotics than are bacteria. This is because they have a different type of cell wall. However, the biogas process can be disturbed by the fact that, for example, fermentative or acetic acid-forming bacteria are inhibited, as this prevents them from producing substrate for the methane producers. If acetic acid-producers are inhibited, various fatty acids can accumulate, and the process may then be disrupted due to a decrease in pH. Certain antibiotics also directly inhibit methane producers, such as chloramphenicol and chlortetracycline, which has been shown in tests to cause a 50% decrease in methane production at 20 and 40 mg/L (Sanz et al 1996). Thiamphenicol has also been shown to have an inhibitory effect on methane generation, resulting in approximately 60% reduction at 80 mg/L (Lallai et al 2002). Different antibiotics may be found in manure if the livestock have been treated for an infection. Sometimes animals receive antibiotics directly in their feed to stimulate their growth. This group of antibiotics includes, for example, monensin and rumensin, both of which have a strong inhibitory effect on the biogas process (Sanz et al 1996, Zitomer et al 2007). Antibiotics can, of course, also be present in the sludge from sewage treatment plants. Several antibiotics have been shown to have an inhibitory effect on the biogas process at sewage treatment plants, but the process has also been shown to degrade some antibiotics (Gartiser et al 2007).

Detergents

Detergents are compounds used to lower surface tension and can be found in the sludge of sewage treatment plants (Garcia et al 2006). Several of these may inhibit the biogas process (Garcia et al 2000, Gavala and Ahring 2002). One of the most common detergents is the surfactant LAS (linear alkylbenzenes sulphonates). LAS inhibits both bacteria and methane producers and is only slowly degraded in a biogas process (Hariklia and Ahring 2002). The degree of inhibition depends on the concentration in the biomass and an upper limit of 14 mg LAS/g VS has been proposed.

Sulphides

Several different active microbial groups in a biogas process can be inhibited by hydrogen sulphide. As usual, the methane producers belong to one of the more sensitive groups. Hydrogen

sulphide (H_2S) is in equilibrium with the hydrosulphide ion (HS^-) and this equilibrium is shifted towards H_2S (which is the toxic component) with decreasing pH (less than 7). This means that the degree of inhibition increases with decreasing pH. Levels reported to be toxic are 50-400 mg/l H_2S (Chen et al 2008). Sulphide ions (S^{2-}) can also bind to different metals to form precipitates. This may mean that microorganisms lack access to certain metals that are necessary for their growth and activity (Hasnaian and Anderson 2004). Hydrogen sulphide is formed not only from sulphate by the activity of sulphate-reducing bacteria, but also by fermentation of amino acids containing sulphur, such as cysteine and methionine. With addition of ferric chloride ($FeCl_2$ or $FeCl_3$) directly to the biogas process, it is possible to "get rid of" some of sulphide as iron sulphide. This method is applied by several Swedish biogas plants (Lanz 2008).

Processing problems due to inhibitory substances can often be avoided by exercising caution and carefully selecting the substrate as well as by managing the process in a way that allows for adequate biodigestibility of potentially inhibitory organic compounds. Sometimes it can be difficult to know whether a substrate contains inhibitory substances or not and whether the pollutants can be degraded. In order to evaluate an unknown substrate in this respect (without compromising operations), it may be worth carrying out batch or continuous digestion experiments in the laboratory or making so-called activity measurements (see Chapter 7; Rozzi and Remigi 2004).

CHECK YOUR KNOWLEDGE

- What are the various factors affecting the inhibitory effect of a compound in the biogas process?
- Why can salts inhibit the biogas process?
- Which groups of pollutants can be broken down in a biogas process?
- Are all heavy metals dangerous for the biogas process?
- Do antibiotics inhibit methane producers?
- How can one evaluate the "toxicity" of a particular substrate?

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5. Monitoring

It is important to carefully monitor the biogas process. This makes it possible to detect problems in a timely manner and catch them before things have gone so far that the process deteriorates. Some microorganisms, such as methane producers, are extremely sensitive and may stop growing and/or are washed out of the process if they do not thrive. For example, the process temperature must be closely monitored because anaerobic microorganisms are very sensitive to temperature fluctuations. Alkalinity and pH, the concentration of fatty acids and ammonium, and the carbon dioxide and methane contents of the gas are other important parameters that should be followed throughout the process. This chapter describes different ways to measure and monitor the biogas process and its microorganisms. Chapter 2 provides a description of the relevance of the various parameters for the microorganisms.

5.1 Monitoring Methods

The biogas process is dynamic and requires regular supervision. In addition to the fact that pumps, mixers, gas collecting facilities, etc. must work and need to be checked, the process itself and the microbial activity must also be monitored. In addition, it is useful to monitor the substrate tanks (incoming substrate) and the digestate tanks (outgoing digestion residue), since a certain amount of microbiological activity also takes place there. For example, the substrate tank can be kept at a low temperature in order to prevent the decomposition process from starting and causing problems related to low pH and foaming, or the digestate tank can be covered to prevent unintentional emissions of methane and nitrous oxide (see Chapter 6).

Daily routines for monitoring the biogas process should be set up, and it is important to make sure that the staff is well-trained. It is useful to draw up a schedule for monitoring, to ensure that certain parameters, such as alkalinity, pH, temperature, ammonium nitrogen, fatty acids and gas flow are monitored on a daily or weekly basis, while other parameters can be measured with a somewhat longer time interval between samplings. The ease and speed of analysis using existing sampling techniques and laboratory equipment is often a determining factor for the type of analysis to be performed and the frequency of sampling. There are several quick measurement methods available today. Technological advances are also taking place, and new methods are being designed to facilitate a more complete and continuous monitoring of the process.

The sampling procedure at the site is very important. The microbiological process can be examined in several places, and appropriate sampling locations can be within the process (for example, at the inlets and outlets of the digester), from the substrate or sanitation tanks, and from the post-digestion container. It is important that the sample be as representative as possible and that it be taken in the same way every time. It is usually best to sample at the time of mixing and pumping of the material, since otherwise there is a risk that the material is stratified and not sufficiently mixed if the sample is taken from an unstirred process. Sampling can be done at shorter time intervals if imbalance or problems are suspected.

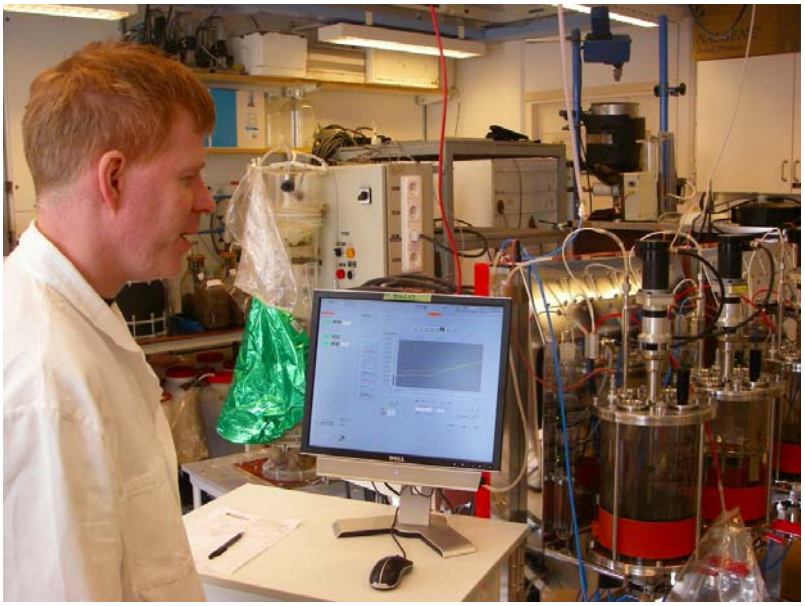

Figure 1. Close monitoring and control of the biogas process is important. Photo: Åsa Jarvis.

Loading and retention time

A constant decomposition rate during the biogas process requires a uniform substrate loading rate. The retention time determines the time available for digestion and thus the amount of substrate degraded. From a microbiological point of view, both of these parameters are crucial for the efficiency of the process. The microorganisms thrive best with a uniform inflow of substrate over time and they also need a certain amount of time to break down the substrate to a

sufficiently high degree. The load and retention time are therefore controlled relative to one another. Uniform loading of the digester can be ensured by analyzing the substrate mixture in the tank, for example with respect to the contents of dry solids (DS) and volatile solids (VS), and by mixing the substrate thoroughly in the tank. It is sometimes difficult to know exactly what organic loading rate is suitable, and a trial and error approach may often be necessary, especially at start-up and when a new substrate is introduced. Usually the process starts at a relatively low load, for example, 0.5 kg VS per m^3 digester volume and per day and is then gradually increased.

One way to find out how the process responds to a change in loading rate is to determine the specific methane production, that is, the amount of methane produced per amount of organic matter fed every day (m_3 CH4 per kg VS and day). Another way to evaluate how the process handles an increase in loading is to look at the degree of digestion (see Chapter 2) of the organic material, i.e., the proportion of the organic material degraded during the retention time in question. The formula below calculates the degree of digestion:

$$\text{Degree of digestion (\%)} = ((\ DS_{in} \times VS_{in} - DS_{out} \times VS_{out}\ )/(DS_{in} \times VS_{in})) \times 100$$

Retention time is regulated based on several different factors, such as process temperature and the characteristics of the substrate. In general, slowly digestible material such as cellulose-rich plant materials need a longer retention time than is required for more easily degradable materials such as food waste (see Chapter 3). Thermophilic processes can often be run at a slightly lower retention times than corresponding mesophilic processes, since microbiological activity increases with increased temperature. Normally, a mesophilic process requires a retention time of at least 15 days, while a thermophilic process may function well at 12 days (Kim et al 2006). Most co-digestion plants in Sweden, however, work at much longer retention times; about 25-30 days is common, but both longer and shorter periods are also used. For digestion of materials with a high water content, such as industrial process water, digested sludge can be reintroduced into the process to retain the microorganisms that might otherwise be washed out. In this case, the solids retention time (SRT) is increased in comparison with the hydraulic retention time (HRT). In this type of process, the retention time (HRT) can be only a few days.

A numeric example:
Calculate a) the retention time and b) organic loading rate for the following process:

Digester volume = 2500 m^3
Feeding of substrate into the digester = 75 m^3 per day
Dry solids (DS) content of substrate = 10% of wet weight
Volatile solids (VS) content of substrate = 90% of dry solids

a) The retention time is 2500 m^3/75 m^3 per day = 33 days[1]

b) The organic loading rate is (75,000 litres[2] per day) x 0.1 (% dry matter of wet weight) x 0.9 (% volatile solids of dry solids))/2500 m^3 (digester volume) = 2.7 kg volatile solids/m^3 digester and per day

[1] Approximate value; may fluctuate due to gas release; see under Retention Time, Chapter 2
[2] The substrate is assumed to have a weight per volume (density) of 1 kg/litre

Determining organic content

The organic matter content is commonly determined from analyses of VS (volatile solids). In the course of VS analysis, the dry solids (DS) content of the material is determined first by removing all the water at 105° C. The organic matter (VS) in the material can then be calculated after the dried fraction is heated to a much high temperature (550° C). VS is calculated as the amount of dry solids minus the amount of residual ash and is the part of the material that is biodegradable. This is sometimes referred to as loss on ignition, while the remaining ash residue is called residue after ignition (Standard Methods 1995).

When determining DS and VS, it is important to understand that high contents of volatile fatty acids (VFA) in the source material can produce misleading results, since they may volatilize from the material when it is first heated and thus give DS and VS values that are too low. This in turn can produce incorrect estimates of biogas production and the degree of digestion, both of which depend on VS. One way to avoid this is to increase the pH of the DS and VS samples before drying, but this method is not yet fully developed. The results of DS and VS-analyses of samples with high fatty acid contents should therefore be interpreted with caution (personal communication Åke Nordberg, JTI).

The organic matter content can also be determined by other methods, such as by COD (Chemical Oxygen Demand) and BOD (Biological Oxygen Demand, Standard Methods 1995) analysis. COD is a general measure of the amount of soluble organic compounds and can give an indication of the amount of soluble carbon compounds in the process that may give rise to methane. COD, i.e., the equivalent amount of oxygen that would be needed to oxidise all soluble organic compounds in the liquid phase, can be determined by using an oxidising agent, such as dichromate.

Temperature

The temperature of the biogas process should be monitored continuously, since a constant temperature is desirable to maintain stable microbial activity (see Chapter 2). To measure the process temperature one or more probes/thermometers can be installed directly in the reactor, and the measured values should preferably read and recorded digitally on a display. For example, a penetration probe can be installed into the digester, and can be removed without any of the contents escaping. Most biogas plants now monitor the temperature continuously via online instruments and have automatic alarms if the temperature deviates from the normal range. In this context, it is important that the thermometers are calibrated at regular intervals.

Alkalinity

Alkalinity is a measure of the buffering capacity of the process and the amount of alkaline or base ions in the biogas process. Alkalinity provides information about the state of the buffering capacity within the process and the amount of fatty acids that can accumulate in the process before the pH starts to decrease. Thus, high alkalinity allows a certain imbalance in the interaction between microorganisms and an increase in fatty acids without the process being affected by a low pH (Gerardi 2003).

To determine alkalinity, samples are taken from the digester and the alkaline ion content (bicarbonate, carbonate, carbon dioxide, etc.) is determined by titration with acid, which is added until the pH is brought down to a certain value. There is a distinction between bicarbonate alkalinity (BA) and total alkalinity (TA); BA indicates bicarbonate content, while TA is a measure of the total amount of alkaline ions.

BA can be determined by titrating to pH 5.75 with 0.05 M hydrochloric acid using the following formula (VAV P54 1984):

BA = 381 x (amount of hydrochloric acid in ml) = mg HCO_3^-/l

Or:

BA = a x M x 61 x f x k

a = ml hydrochloric acid
M = molarity of hydrochloric acid
f = dilution factor
k = 1.25 (correction factor for making adjustments due to the fact that only 80% of the salts participate in the reaction)
HCO_3^- = bicarbonate

Furthermore, TA can be determined by titration to pH 4.0 with 0.05 M hydrochloric acid and is calculated according to the same formulas as above, changing BA to TA.

Other units for BA and TA are also used, for example, milliequivalents per litre and mg calcium carbonate per litre. The following relationship applies to conversions:

1 milliequivalent per litre = 50 mg calcium carbonate per litre = 61 mg of bicarbonate per litre

By determining the value of BA, problems can be detected at an earlier stage than if only TA is measured (Brovko and Chen 1977, VAV P42 1981). In stable processes, BA can vary within a relatively wide range, 3000 - 15000 mg HCO_3/L, while the TA-value usually ranges between about 5000 and 20000 mg HCO_3/L. If the concentration of fatty acids is low (<300 mg acetic acid per litre), which is common for digesting sludge in sewage treatment plants, the BA is only a few

percent less than the TA value. Co-digestion plants often have higher contents of fatty acids (more than 1000 mg/L is common) and here the BA and TA values differ more. In the event of a faulty operation, a high proportion of volatile fatty acids results in very low or negative BA values. Alkalinity should be measured at least once a week; more frequent measurements are recommended following changes in operation and substrate composition.

One way to assess process stability is to look at the volatile fatty acids (VFA) to alkalinity ratio. Three critical values are suggested for VFA/TA (VAV P42 1981):

<0.3 Stable process
0.3 to 0.5 Some instability
> 0.5 Marked instability

At a VFA/TA ratio greater than 1.0, the risk of a sharp decline in gas production and foaming is high. Problems can arise even when the ratio is below 1, especially in times of rapid changes (personal communication, Pernilla Bratt Skövde municipality).

pH

The pH of the process can be measured using a pH meter with an electrode dipped in a small amount of slurry/process liquid collected from the digester. It is important to analyse the liquid immediately upon sampling, since the release of dissolved carbon dioxide may change the pH. There are also probes that can be installed directly in the digester for measuring pH online. In this way the pH, which is typically between pH 7 and 8.5 in a biogas process, can be monitored on a daily basis. It is also recommended to measure pH and alkalinity in the substrate tank (the storage tank for incoming substrate) to ensure that the contents do not become too acidic due to high fatty acid production.

Addition of stabilisers

Carbonates and bicarbonates combined with sodium or potassium, calcium carbonate (lime) and ammonia can be added to stabilise the alkalinity and increase the pH in biogas processes. Bicarbonates are preferable since the methane-producing microorganisms require bicarbonate ions in their environment (Capri and Marais 1975). Normally, sodium carbonate, sodium bicarbonate, potassium carbonate or potassium bicarbonate are chosen, which are difficult to overdose. Other alkaline substances, such as lime, ammonia and lye can be easily overdosed resulting in excessively high pH and, occasionally, in a temporary negative pressure in the digester. The addition of lime can rapidly increase the pH, but it does not contribute significantly to increased alkalinity in the process. Ammonia should be used with caution because it is toxic to microorganisms if it is not quickly converted to the soluble ammonium form (see Chapter 3).

If the alkalinity is too high, it can be adjusted by adding, for example, ferric chloride or citrate (Gerardi 2003). Generally, all these chemicals should be added to the process gradually and in reasonable amounts. Otherwise, pH, alkalinity or ionic strength may change excessively, which can cause, among other things, problems with foaming in the reactor. The exact amount of

buffering substances that must be added to alter the alkalinity may vary between different biogas processes and is dependent on several factors, such as the bicarbonate content, temperature, pH, fatty acid concentration, and ammonia content, etc. (Capri and Marais 1975). Therefore, it can be difficult to calculate exactly what should be added to adjust the alkalinity. It is preferable to add smaller doses repeatedly and test between additions to see how the process responds. Examples of how various stabilisers may be added in order to increase alkalinity are provided in Capri and Marais (1975) and VAV P42 (1981).

Gas Quantity

Gas production is a very important measure of the process status. Reduced gas production or production rates that do not "correspond to" the load of new substrate are signs that the process is not functioning optimally. The relationship between the quantity of gas produced and the amount of organic matter supplied also provides a measure of the efficiency of the process. A normal biogas process yields biogas in the order of magnitude of 1-3 m^3 per m^3 digester volume and day, depending on the substrate digested. The plant must therefore be equipped to collect this amount of gas every day. It is appropriate to connect a device for measuring the quantity of produced biogas to the gas collection system. Various types of flow metres can be used for this purpose.

The amount of biogas is usually specified in normal cubic metres (Nm^3), i.e. the gas volume at 0°C and atmospheric pressure (absolute pressure 1.01325 bars). It is important in this context to convert the output of the instrument to normal pressure as the gas volume changes with pressure and temperature. The following formula can be used to determine the amount of gas in Nm^3:

$Nm^3 = G \times (1.01325 + P) / 1.01325 \times 273.15 \text{ degrees Kelvin} / (273.15 + T)$

G = quantity of gas in m^3
P = positive gas pressure in bars
T = gas temperature in degrees Celsius

Instruments are available that record gas production directly in Nm^3. Gas production can be expressed, for example, as the biogas volume produced per digester volume and day (volumetric gas production).

Gas composition

The gas composition is another important measure of the process status. A smaller percentage of methane, and thus an increased proportion of carbon dioxide, suggests that methane production is inhibited. This can be regarded as an indication that there is a problem in the process. All gases contained in the biogas are produced during the decomposition of various organic substances by microorganisms (see Chapters 1 and 3). Raw biogas is composed mainly of methane (45-85%) and carbon dioxide (15-45%), with other gases in small quantities (hydrogen sulphide, ammonia, and nitrogen). Most often, biogas is also saturated with water vapour (Basic data on biogas 2007).

The gas composition can be determined by allowing the generated biogas to pass continuously through an analyser instrument. Another way is to collect separate samples from the gas phase for subsequent analysis. This method is often used when the process is studied in the laboratory (see Chapter 7). Several different methods of analysis can be applied. A graduated fermentation tube known as Einhorn's saccharometer is one quick method of determining carbon dioxide concentrations. This contains a strong solution (7M) of lye into which a known amount of gas sample is injected. Carbon dioxide dissolves in the lye, while the methane forms a gas bubble in the tube. The carbon dioxide content can then be determined by reading the total volume of gas and relating this to the injected volume. With this method, it is important to be aware of the fact that sudden changes in pH can release the bicarbonate, which is dissolved in the contents of the digester, as carbon dioxide. The measured carbon dioxide content then becomes higher than would be expected based on the current biogas production.

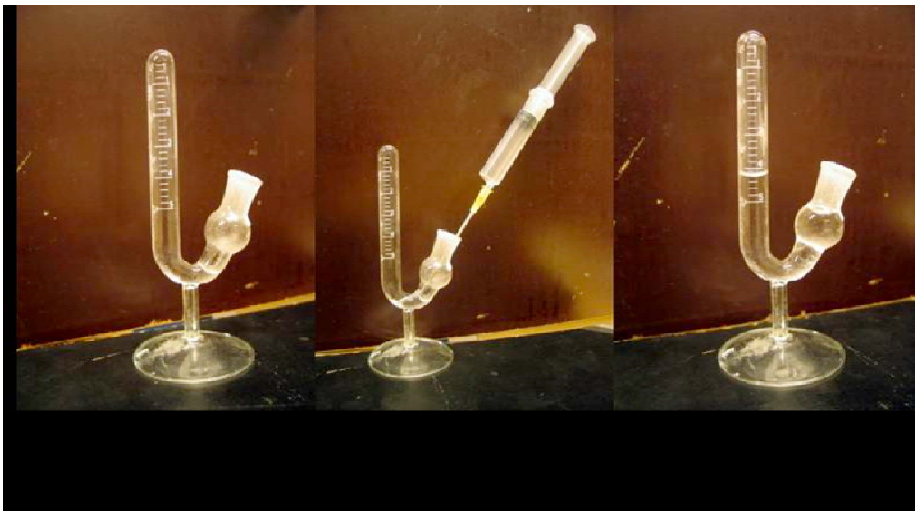

Figure 2. Fermentation tube (Einhorn's saccharometer). Photo Anna Schnürer.

Methane and other gases in biogas such as hydrogen sulphide and hydrogen are usually analysed by gas chromatography. Most often, hydrogen occurs in very small quantities, but it may be useful to be aware of its concentration, because even a small increase in hydrogen concentration means that the interaction between anaerobic oxidation and methane formation is not working properly. The analysis of hydrogen is rather expensive because it requires a special detector (mercury) and so is generally not performed routinely. Online instruments that provide measurements of the contents of several components in the biogas, such as methane, hydrogen sulphide, carbon dioxide, etc., are now being developed for full-scale processes.

Ammonium/ammonia

Amino acids are produced during the hydrolysis of protein-rich materials; when these are fermented, ammonium and ammonia are formed. Of these two compounds, ammonia inhibits methane formation (see Chapter 3). Most biogas plants in Sweden analyse total Kjeldahl nitrogen

(TKN) and/or ammonium-nitrogen (NH_4^+-N).The analysis of ammonium results in an aggregate measurement of both ammonia and ammonium, as ammonia is converted to ammonium during the analysis. However, since it is ammonia that is the inhibiting component, it is interesting to calculate the ammonia content instead. This can be calculated by applying the current pH and temperature (T, °C) values to the following formulas (Calli et al 2005):

NH_3 (g/L) = NH_4^+-N (g/L) / $(1 + 10^{(pKa - pH)})$

pKa = 0.09018 + 2729.92 / (T +273.15)

pKa = dissociation constant for ammonium (NH_4^+)

Fatty acids

Hydrolysis and fermentation produce a range of different fatty acids. These are further degraded in anaerobic oxidation processes and ultimately converted into methane and carbon dioxide. Acids will accumulate rapidly in the process if the degradation of fatty acids fails, for example, if methane formation is inhibited. Fatty acids can also accumulate in the event of overloading. In the case of overloading, the increasing concentration of fatty acids is not due to inhibition, but to the fact that the hydrolysing and fermenting bacteria grow faster than the methane producers that are unable to oxidise fatty acids at the same rate as they are formed. This, in turn, results in lower pH levels and the process becomes unstable. An increased concentration of fatty acids is therefore an important indicator of problems in the process. It may be useful to analyse the fatty acid content as early as in the substrate tank. If hydrolysis has proceeded too far in the substrate tank, this can cause problems with overloading in the subsequent digestion process.

A distinction is made between short volatile fatty acids (VFA) and long-chain fatty acids (LCFA). The short fatty acids such as acetic, propionic and butyric acids, are analysed in samples taken out of the process at regular intervals, preferably at least once a week and more often if possible. Measurements of the propionic acid content are particularly useful. An increase in the propionic acid content is often a clear indication that the interaction between fermentation/anaerobic oxidation and methane formation is not functioning optimally. The analysis can, for example, be done by gas chromatography (GC) or liquid chromatography (HPLC). Aggregated analyses can be made using different off-the shelf rapid tests. Long-chain fatty acids such as stearic acid, palmitic acid, and oleic acid can also be analysed by GC (Sousa et al 2007). Long-chain fatty acids are formed earlier in the anaerobic breakdown chain than short fatty acids. This means that the analysis of LCFA may indicate a problem at an earlier stage than the analysis of short fatty acids.

Other tests that can provide information on the process

Increasing contents of aromatics may be an early sign of a problem (Hecht and Griehl 2009). Decomposition of aromatic structures in a biogas process often requires the involvement of methane producers that use hydrogen (Harwood et al 1999); inhibition of these organisms will therefore result in inefficient breakdown of aromatics.

It is also important to monitor components in the substrate, not only to load the process correctly but also to watch for substances that are potentially toxic to the microorganisms, such as organic pollutants and heavy metals. The presence of such compounds may also have implications for the usefulness of digester residues as fertilizers. The carbon to nitrogen ratio (C/N) is another important measure of substrate digestibility (see Chapter 3). The total amount of C and N can be determined, for example, by dry combustion (Eklind et al 1997). Total nitrogen may be determined by the Kjeldahl method (Standard Methods 1995).

5.2 Summary

A biogas process can be monitored using a range of analyses. However, the number of analyses is often limited due to time and cost constraints. Table 1 indicates the tests that can be considered as a minimum required to maintain good control of the process and to indicate if there is a problem. More tests may be needed, for example when changes are made in the process operation. Some parameters should be monitored daily, such as temperature, gas production and gas composition in relation to the amount of incomingsubstrate. Others may be monitored weekly or twice weekly, for example, short fatty acids (VFA) and alkalinity. Access to a reliable and well-equipped analytical laboratory is an advantage. It is also very important to set up fixed procedures for checking and monitoring the process and that staff is well-trained in all operational procedures and in the analyses that need to be performed.

Indicator	Reduction	Increase
Biogas Production	X	
Methane content in the biogas	X	
Alkalinity	X	
pH	X	
Concentration of fatty acids		X
Carbon dioxide content in the biogas		X

Table 1. Key indicators of problems in the biogas processes (as per Gerardi 2003)

The values of monitored parameters may vary between different biogas processes due to differences in substrates, process temperatures, adaptation times, etc. Generally, as long as the measured values remain relatively constant during a certain period, the individual process is most likely stable. For all digestion processes, the variation of measured parameters in time yields more valuable information than absolute individual values. This becomes particularly important when changes take place, for example during start-up, when the substrate is changed, or the load is increased. In such cases, the monitoring must be increased temporarily to make sure that the process is stable in spite of the change.

Moderate variations in, for example, pH, quantity of gas, gas composition etc., commonly occur even within normal and stable processes. It is therefore important to learn as much as you can about your process, since that makes it easier to detect deviations of a more serious nature (for example, sustained upward or downward trends) and also to take timely corrective action.

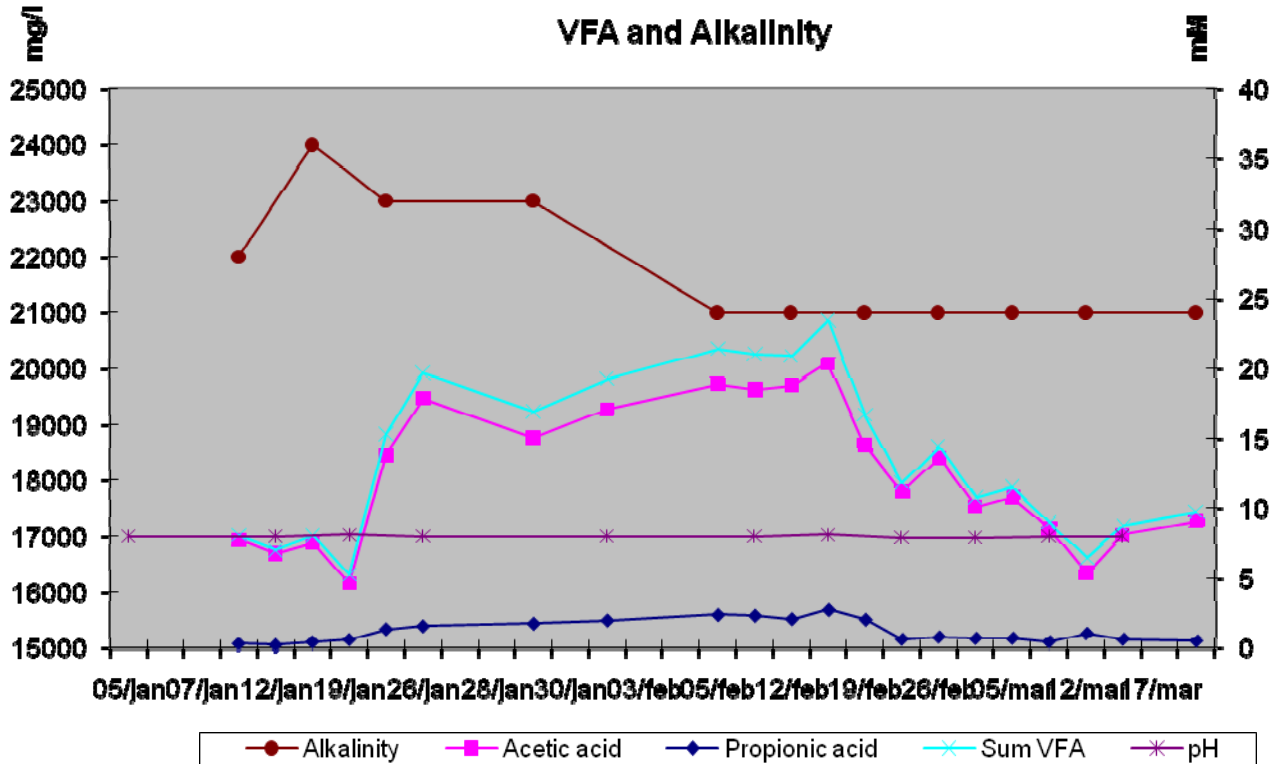

Figure 3. Examples of natural variations in an otherwise stable biogas process. Alkalinity is expressed in milligrams per litre HCO3 (left y-axis) while concentrations of acetic acid, propionic acid and total volatile fatty acids (VFA) are given in mM (right y-axis). pH varied between 7.9 and 8.1 averaging 8.0 over the entire period.

CHECK YOUR KNOWLEDGE

- Why is it important to monitor a biogas process?

- Why isn't there always a drop in pH when fatty acids accumulate?
- Why is it important to have a balanced loading rate and what actually is the loading rate?
- What is the difference between BA and TA?
- Is it possible to adjust the alkalinity of a biogas process?
- Why is it important to analyse both the quantity and the composition of the gas formed?
- Are all chemical values (e.g., pH, fatty acids) constant in a stable process?

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6. The digested residual product - biofertilizer

The degradation of organic material in a biogas process produces biogas in addition to a residue (the digestate) which, if it is of good quality, can be used as fertilizer. The mineral nutrition available in the organic material (substrate) is released and concentrated in the digested end product. If digestion is performed with relatively "pure" substrates such as manure, food waste separated at source, and plant material, the residue can be used as fertilizer (biofertilizer) in food production. This product should not be confused with the residue, known as sludge, obtained from the digestion of sludge at wastewater treatment plants. Because of its content of metals and/or organic pollutants, digested sludge is not always appropriate for application on agricultural land. The quality and nutritional content of digestate is influenced by several factors, including the type of substrate, pretreatment method, process conditions (temperature, retention time, etc.), post-digestion, and storage. This section provides an overview of digestate as a fertilizer with a focus on the microorganisms in the substrate, in the process, in the digestate itself, and in the soil. More detailed information about the use of digestate with respect to application techniques, regulations, etc., is available through the Avfall Sverige website, www.avfallsverige.se (in Swedish).

6.1 Function and use as fertilizer

In Sweden about 200,000 tonnes of digestate is produced per year and about 90% of this is used as a fertilizer on arable land (www.avfallsverige.se). Compared to compost, the use of digestate is a relatively new phenomenon and there is still a need for technological development and research. Digestate clearly works very well as a fertilizer and can give similar or even better crop yields as mineral fertilizers (Avfall Sverige 2005, Odlare 2005, Baky et al 2006, Johansson 2008). It also has positive effects on the soil chemical status, soil structure, and microorganism communities (Odlare et al 2008).

Liquid digestate has a solids content of 2-7%, approximately the same solids content as liquid manure, and can also be spread using the same techniques and the same equipment as used for liquid manure (Avfall Sverige 2005). At some biogas plants, digestate is divided into a solid and a liquid part. In such cases, the liquid portion contains more nutrients, while the solid part contains more humus precursors. Liquid digestate is usually spread by trailing hose spreaders or by shallow injection. Solid digestate is spread as ordinary solid manure (Baky et al 2006). Digestate is normally spread from planting until the crop is about 20 cm (8 inches) tall. One advantage of spreading fertilizers in a growing crop is that the land can support heavy vehicles better, and that the nutrients are incorporated during a period when the plants have the greatest nutrient needs (Berg 2000). Farmers who use digestate are providing mostly positive testimonials. According to them, digestate gives a better nitrogen effect than liquid manure and it also has better characteristics with respect to smell, pathogens and spreadability (Avfall Sverige 2005).

Figure 1. Spreading of digestion residues with a trailing hose spreader. Photo: Lena Rhode.

6.2 Plant nutrient value

During the microbial breakdown of organic material in a biogas process, various minerals are released. Digestate contains N (nitrogen), P (phosphorus), K (potassium) and Mg (magnesium) in plant-available form. Digestate also contains various trace elements necessary for plants. Plant nutrient value, i.e. the concentration of the different elements, varies between digestate from different biogas plants, and depends largely on the substrate used in the biogas process and on how the process is run (Table 1). A major benefit of using digestate as fertilizer is that it contains a high proportion of ammonium nitrogen (NH_4^+-N), which can be directly taken up by plants.

Since not all organic material is converted to biogas during the anaerobic digestion process, digestate also usually includes a certain amount of organic carbon and nitrogen. Part of this fraction is further broken down in the soil and this, in the long-term, results in the release of more plant nutrients. The organic fraction also has a general stimulating effect on biological activity in the soil, which is beneficial for the plants. However, digestate may sometimes contain a slightly lower amount of phosphorus (P), and this nutrient may need to be added as a supplement to avoid phosphorus deficiency in the soil in the long-run, when using digestate.

	DS content (%)	Tot-N (kg/m ^{3})	NH_4^+-N (kg/m ^{3})	P (kg/m ^{3})	K (kg/m ^{3})
Digestate 1[a]	5.0	7.1	5.3	0.80	1.0
Digestate 2[b]	1.6	3.6	2.6	0.20	1.1

Digestate 3[c]	4.8	5.7	4.3	0.38	2.0
Digestate (Mean)[d]	3.8	4.5	3.2	0.40	1.2
Cattle manure (mean)[e]	9.8	3.9	1.8	0.80	4.0
Swine Manure (Mean)[e]	8.8	5.1	3.3	1.9	3.0

a included substrates: manure 10%, slaughterhouse waste 75%, waste from food industry 5%

b included substrates: household and restaurant source-separated waste

c includeds substrates: manure 61%, 17% abattoir waste, food waste 2%, fat 11%, waste from food industry 9%

d average of seven certified biogas plants in 2005

e plant nutrient content of individual samples may vary 17-35%

Table 1. Plant nutrients in manure from pigs and cattle, and digestate (liquid), average and produced by digestion of substrates with different compositions (Avfall Sverige 2005, Baky et al 2006).

Since the digestion residue contains large amounts of water (93-98%), transport is expensive. In addition, because of the high water content, there is some risk of compacting (packing) of the soil when digestate is used (Avfall Sverige 2005). To mitigate these problems, a high content of NH_4^+-N is advantageous because this allows the application of smaller volumes. Digestate (liquid) that is to be used as a fertilizer should contain at least 2 kg per tonnes of ammonium nitrogen and 3-4 kg/tonnes of total nitrogen (Baky et al 2006). As mentioned earlier (Chapter 3), the content of nitrogen in the digestate may be increased by increasing the protein content in the substrate that is loaded into the biogas process. It is then important to note that excessive amounts of protein can also result in process-related problems due to ammonia inhibition of the methane-producing microorganisms. Of course, the content of other nutrients in the biofertilizer can also to some extent be controlled by the composition of the input material. Another way to reduce the volume of digestion residue that must be transported for land application is to dehydrate the digestate. Dehydration provides a nitrogen-rich liquid phase and a solid phase of high phosphorus content. Solid digestate, however, generally gives lower crop yields than liquid digestate, probably due to a higher content of slower-acting organic nitrogen (Baky et al 2006). Another problem whith dehydration is that losses of nitrogen in the form of ammonia may occur (up to 90% of the nitrogen may be lost, Rivard et al 1995).

6.3 Effects on the soil

The quality of any given soil is determined by physical (porosity, texture, moisture), chemical (moisture, pH), and biological parameters (number and activity of organisms living in the soil). For several reasons, digestate improves soil quality, unless it contains chemical contaminants that are toxic to soil organisms. For example, organic matter derived from digestate increases the buffering capacity of the soil and retains water and air in the soil profile. Introduction of digestate also affects the microorganisms in the soil in a positive way. The majority of soil microorganisms are known to be heterotrophic, which means that they use organic carbon compounds as carbon and energy sources for growth. Addition of organic materials with digestate therefore results in a general stimulation of microbial growth (Odlare et al 2008, Johansson 2008). Microorganisms play a key role in soil fertility since they mineralise organic matter and thus release various plant nutrients. Microorganisms facilitate plant nutrient uptake, form polysaccharides that stimulate the formation of stable soil aggregates, and also protect plants from disease attack. In addition, the content of mineral nitrogen (ammonium) in digestate provides essential nutrients to plants which results in good plant growth, which in turn increases the carbon content in soil due to root secretion (Svensson et al 2004). This carbon, in turn, stimulates the growth of various microorganisms.

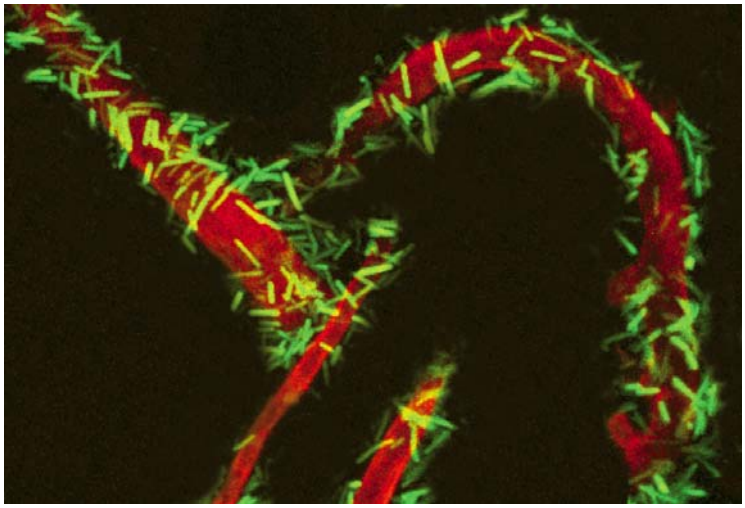

Figure 2. Microorganisms in the soil are important for the plant. Photo: Veronica Arthurson.

The use of organic fertilizers can result in emissions of ammonia (NH_3) and greenhouse gases such as nitrous oxide (N_2O) and methane (CH_4) Rodhe et al 2006, Flessa and Beese 2000). Ammonia is released primarily from the digestate, while nitrous oxide and methane are formed as a result of increased microbial activity in the soil. However, emissions of greenhouse gases are caused not only by the use of digestate from biogas systems, but also by use of raw manure as fertilizer. Ammonia can also be released when spreading mineral fertilizers. Ammonia may be released from digestate both during storage (see below) and during application. The amount of ammonia lost is strongly affected by the spreading method. Surface spreading generally results in

greater nitrogen losses than shallow injection (Rhode et al 2006). Shallow injection may increase the risk of formation of greenhouse gases (Rodhe et al 2006, Flessa and Beese 2000). Ammonia can be used by ammonium-oxidising microorganisms in the soil which can result in the formation of nitrous oxide (Enwall 2008). Microbial breakdown of organic matter in the soil also results in methane emissions. Covered shallow injection reduces these emissions in comparison with open injection (Rhode et al 2006). In addition to the spreading technology, the soil type also exerts a very important control on emissions. Sandy soils, for example, may have higher emissions than clay soils (Jarecki et al 2008). Guidelines are available for how to apply fertilizer to minimize leaching of nutrients to groundwater and ammonia losses to the atmosphere. A summary of these can be found in Lindström (2008).

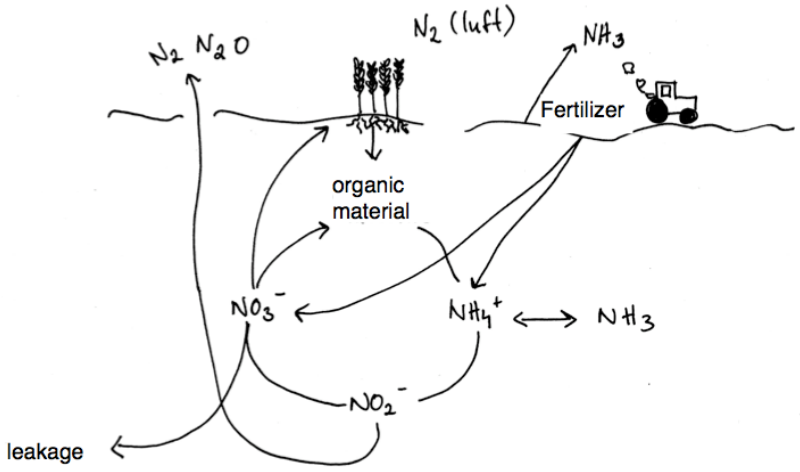

Figure 3. Nitrogen cycle in the soil. Modified after Nyberg 2006

6.4 Quality and Certification

If the digester residue is to be used as a fertilizer, it is essential that it is of good quality. In Sweden, a voluntary certification system (SPCR 120) has been introduced to ensure digestate quality. The certification system includes quality documentation of the entire chain from raw material (substrate) to the final product (digestate). The approved digestate can be marked with a quality certification symbol (Figure 4).

Figure 4. Certification Symbol

The declaration of contents of certified digestate include the content and concentration of plant nutrients, heavy metals, visible pollutants, seeds and plant parts, as well as the content of organic matter. The hygienic quality must also be known. A complete sampling program for certification can be obtained from the Technical Research Institute of Sweden (SP). SP is an independent certification agency for this system and is also the authority that issues the certificate. In order to keep the certification regulations as current as possible, they are updated regularly and a current version can be downloaded from SP's website (http://www.sp.se).

6.5 Contamination

Depending on the substrate used, different chemical contaminants can sometimes be present in the digester residues. At high concentrations, they can adversely affect the activity of the microorganisms in the soil. Since microbial activity is very important for long-term soil fertility, inhibition may eventually lead to a reduced crop yield. It is also important to ensure that digestate does not contain pathogens that can damage crops or people/animals. Hygienic quality is further discussed in the "Hygiene" section below.

Chemical pollutants

Sludge from digestion processes at sewage treatment plants can have a relatively high metal content, and sometimes also contains organic pollutants. Therefore, it may not always be possible to use such digestion residues in the same way as other digestates. Digestion residues produced from "cleaner" waste, such as food waste, manure or crops, have significantly lower contents of various contaminants. Normally the presence of such compounds at such low concentrations does not pose problems for the use of digestate as fertilizer. Contaminants that have been shown to occur in digestate are, for example, residues of biocides, phenols and PCBs (Nilsson, 2000, Engwall and Schnürer 2002, Olsman et al 2002, Levén et al 2005, Levén 2006). These chemicals usually do not originate from poorly sorted materials, but are rather present in the organic material or are formed during decomposition in the biogas process. Some chemicals can also end up in the material through atmospheric deposition as a result of its use elsewhere in the world. So far, no studies have shown that these compounds would pose any obstacle to the use of digestate

as fertilizers. The concentrations are low, often below the limits of what is allowed in food, and it has been shown that a number of these compounds are degraded either in the biogas process or in the soil (Nilsson 2000, Levén et al 2006). However, to avoid problems with soil fertility in the long run, it is important to control and minimise the content of these compounds. This is presently done by ensuring the quality of the substrate added to the biogas process.

It is also important to minimise the contents of various organic contaminants in the added material to ensure that the biogas process is efficient and stable. Some chemicals have an inhibitory effect on the microorganisms in the biogas process and can cause problems in the process (see Chapter 4). An efficient biogas process is a good starting point for achieving a more efficient degradation of these compounds. A variety of organic pollutants can be degraded in the digestion process if conditions are right, i.e. the concentrations of contaminants are below inhibition thresholds and all other process parameters are maintained at appropriate levels.

It is difficult to provide general guidelines for process parameters, since their impact may vary for different pollutants. One factor that may have a significant impact on the degradation of some compounds is process temperature (Nilsson 2000, Engwall and Schnürer 2002, Levén and Schnürer 2005, Levén et al 2005, Olsman et al 2007). Higher temperatures generally promote higher solubility, which can be advantageous since it increases the availability of the compound, but it can also be disadvantageous since the inhibitory effect of a particular compound may be more pronounced. Theoretically, factors such as pH and retention time should also have an impact on decomposition. pH is sometimes crucial for the structure of a compound, i.e. whether or not it has an attached hydrogen ion. The structure of a particular compound may also be of importance with respect to its availability and toxicity (Lagas 1988, Sercu et al 2005). The retention time can be important for certain complex molecules. To degrade these complex compounds may require the activity of various different organisms and several steps, including slow-growing syntrophic colonies of organisms (see Chapter 1).

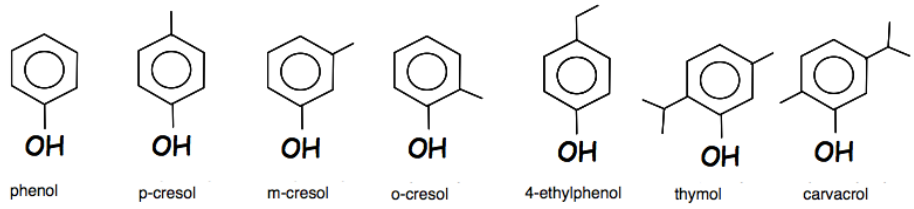

Figure 5. Phenols -an example of organic compounds that are present in pig manure and can therefore also be found in digestate.

6.6 Hygiene

It is important to ensure that the digestate does not contain harmful microorganisms (pathogens), so that it does not pose a risk of spreading infection. Biological materials used as substrates in a biogas process could theoretically include pathogenic bacteria, viruses and fungi as

well as parasites and prions (Deportés et al 1995, Gale and Stanfield 2001, Sahlström 2006, Schnürer and Schnürer 2006, Huang et al 2007, Haraldsson 2008, Zetterström 2008). The risk of these organisms being present in digestate varies depending on the substrate supplied to the biogas process, how the process is controlled and the sanitation method used (Smith et al 2005, Albihn and Vinnerås 2007, Ottoson et al 2008, Sahlström et al 2008). Pathogenic microorganisms in biological materials can come from diseased animals or humans or from infected individuals who are not sick themselves. Pathogens can be present in urine and faeces, but also in tissues. Some pathogens attack only plants and can therefore be found in plant-derived materials such as crops or agricultural residues. Many pathogens are host-specific, but some (known as zoonoses) can be transmitted between humans and animals. These pose a higher risk of disease outbreaks that are more extensive and harder to control. Generally speaking, however, the animal health situation is satisfactory in Sweden with respect to various infectious diseases, and the risk of spreading disease is considered to be relatively small.

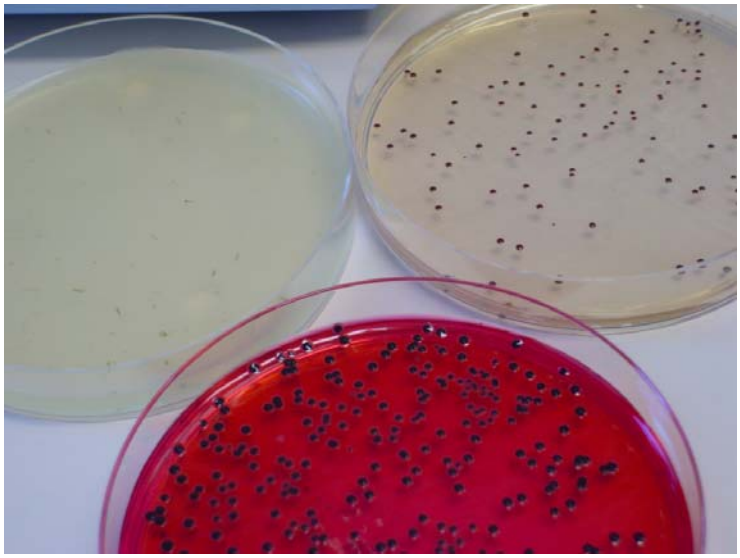

Figure 6. Culture plates with colonies of Salmonella (red plate), enterococci (top right) and plaque from bacteriophages, i.e., viruses that infect bacteria. Photo: Josefine Elving.

Risk of Infection

The greatest risk of spreading human/animal pathogens is posed by animal by-products (ABP) and manure, both of which can contain microorganisms that can cause infection. Animal by-products are divided into three categories depending on the risk of infection, which must be treated in different ways (Table 2). ABP Category 3, and manure (Category 2), may be used in a biogas plant for the production of biogas if the material is sterilised/disinfected (i.e. the pathogenic microorganisms are killed with an appropriate method, Table 2). The standard method of sterilisation today is exposure to 70 ° C for one hour (pasteurisation), but alternative methods with the same effect as this treatment are allowed according to Regulation (EC) 208/2006 (Ottoson et al 2008). Heating to 70° C for one hour has been shown to kill many different types of pathogenic microorganisms (Benedixen 1999).

ABP Category	Examples of materials	Proposed Handling
1	Organs of animals suspected of being infected by prions	Destruction, for example by burning
2.	Manure, ABP not included under Category 1 or 3.	Sterilisation at 133 ° C for 20 min, 3 bar (there are exceptions for manure)
3.	Waste from animals approved for consumption and food waste (excluding food waste as per EC Regulation 1774/2002)	Pasteurisation at 70 ° C, for 60 min, followed by stabilisation or alternative sanitisation method according to Regulation (EC) No. 2008/2006

Table 2. Animal byproducts (ABP), risk category according to the regulation EC1774/2002.

Sanitation is currently not required for treatment of crops or other plant matter. Sanitisation is not required either for biogas production from manure and subsequent use of digestate at the farm of origin. However, if digestate is transported and used at several different farms, the same regulations apply as for animal products, because the risk of contamination increases markedly.

Even non-pathogenic organisms may pose problems in connection with biogas production from various organic wastes. The degradation of the material has often already started before it reaches the biogas plant, hence, there is a risk of aerosolization (spreading in the air) of different types of microorganisms during the handling of the waste at the plant. For example, several fungi have been found to grow in organic waste (Schnürer and Schnürer 2006). Fungi form so-called spores that can be easily spread in the air in the form of aerosols (Schnürer and Schnürer 2006). These organisms usually do not cause infection in healthy individuals, but may pose a risk to people with weak or impaired immune systems. Aerosols of airborne microorganisms can also cause respiratory problems and allergies in healthy individuals and it is therefore imperative to ensure good ventilation at biogas plants.

The topic of infection by plant pathogens is still relatively poorly studied. A few studies of plant pathogenic fungi have been carried out (see Section "Organisms that survive pasteurisation"), but so far no study has been published on survival of pathogenic plant viruses in biogas processes.

Indicator Organisms/Evaluation of Hygiene

Since it is impractical and expensive to analyse all possible pathogens that may exist in a given waste, contrasting so-called indicator organisms are analysed to evaluate the effect of a certain

sanitisation method. Indicator organisms are usually non-pathogenic microorganisms that have characteristics similar to the pathogens of interest, which can be analysed using simple, fast and cheap methods (Bitton 1999). Different indicator organisms are used to study the risk of the presence of different pathogenic organisms. Salmonella and Esherichia coli (E. coli) or Enterococci are typically analysed to assess the effects of sanitisation at biogas plants. These indicator organisms are expected to reflect the characteristics of enteropathogenic microorganisms and the presence of these microorganisms indicates fecal contamination. The analysis is usually done after both the sanitisation stage and in the digestion residues.

Studies on digestion plants have revealed the presence of both Salmonella and Escherichia coli in sewage sludge from different wastewater treatment plants (Sahlström et al 2004, Sahlström 2006). Since there are currently no requirements for sanitisation at sewage treatment plants, the presence of these organisms is not surprising. If sludge is to be used as a fertilizer, it is therefore necessary to introduce a sanitisation step here as well. The presence of enterococci has also been shown in various types of digestate, both from sewage treatment plants and from co-digestion plants (Bagge et al 2005). Since different enterococci can be part of a digestion tank's normal flora, the presence of these organisms need not indicate fecal contamination or an increased risk of infection.

Alternative Sanitisation Methods

The sanitisation method should reduce the levels of Salmonella and Enterococci by 100,000 times and viruses 1,000 times. If the sanitisation method is chemical, it must also be possible to detect a reduction of parasite levels by 100 times. An example of an alternative sanitisation method is to only rely on thermophilic digestion. The higher digestion temperature kills many pathogenic organisms (Sahlström 2006, Wagner et al 2008). Anaerobic digestion at mesophilic temperatures may also kill various pathogens, but the method is far less effective than digestion at higher temperatures. If thermophilic anaerobic digestion is used as the sole sanitisation method, it is important to bear in mind that digestion in continuous processes involves adding the material to the digestion tank and removing it from the digestion tank at regular intervals, sometimes at short intervals. In order to achieve a satisfactory reduction of pathogens, it is necessary that a certain period of time must pass between each substrate addition to the process, so that all the material is exposed to the high temperature for a guaranteed minimum amount of time. How long the interval of time needs to be depends on the digestion temperature. A 10-hour interval has been established for digestion at 52° C (Norin 2007).

Another interesting and promising sanitisation method is digestion at high ammonia contents. Recent research showed a satisfactory reduction of Salmonella and Enterococci during mesophilic digestion at high ammonia contents obtained from digestion of a protein-rich material (Ottoson et al 2008). Further research on this method is necessary before a full assessment of its value as an alternative sanitisation method can be made. Norin (2007) discusses other methods and their efficacy, so this subject is not discussed in further detail in this section.

6.7 Organisms that Survive Pasteurisation

As mentioned earlier, pasteurisation of organic matter results in a satisfactory reduction of various microorganisms. Some organisms, however, survive the treatment. This group includes various spore-forming microorganisms. Spores represent a survival stage formed by some organisms as a response to adverse environmental factors, such as poor access to food or high temperature. Spores have strong cell walls and are often highly resistant, i.e. they can survive in a "tough" environment such as high temperature or poor nutritional conditions. Subsequently, when conditions become more favourable, the spores become actively growing organisms. This group includes bacteria in the genus Clostridium, Bacillus, and various fungi. This means that even if the material fed to the biogas process has been pasteurized, there is still a risk that the digestion residues contain these groups of organisms.

Clostridium

Studies of various pathogenic bacteria in the genus Clostridium show that these organisms survive the digestion process if they are present in the substrate (Chauret et al 1999, Aitken et al 2005, Bagge et al 2005). Survival rates vary between different species. For example, Clostridium chauvoei, which causes blackleg, does not like the environment in the digester, and its numbers are reduced considerably with time in a biogas process. Clostridium septicum and Clostridium sordelii, however, do very well (personal communication, Elisabeth Bagge, SVA). Infection by C. septicum causes edema and C. sordelli causes wound infections in animals. Although a reduction could take place, the likelihood of finding organisms from the genus Clostridium in the digestate is very high. This is partly because many survive the process well and partly because the time between loads into the digestion tank is not long enough to allow a complete kill. Also, many species within the genus Clostridium are part of the normal flora of the digestion tank. Many of these organisms are not pathogens and do not increase the risk of infection when the digestate is used.

Bacteria in the genus Clostridium are also common in animal manure and are also naturally found in relatively high numbers in soils (Gyles and Thoen 1993, del Mar Gamboa et al 2005, Songer and Post 2005). Spores from, for example, both Clostridium botulinum (which causes botulism) and Clostridium tetani (which causes tetanus) are already present in the soil, so a fertilization with digestate will not likely pose any increased risk of disease caused by these organisms.

Certain types of Clostridia, which may exist in organic waste and in the anaerobic digestion process are not, as mentioned above, pathogenic organisms, but are still involved in the debate regarding the risks of using digestate. An example of such an organism is Clostridium tyrobutyricum, which is a recognized problem organism in cheese processing (Klinj et al 1995). High levels of this organism in the soil can lead to direct contamination of cow udders or contamination of animal feed produced from the soil. The organism then survives in the gastrointestinal tract of the cow and finds its way into the manure, which can also contaminate the cow udder. If this organism finds its way into the milk, problems in cheesemaking are caused partly because it forms gas (producing big holes in the cheese) and partly because it produces

butyric acid (giving a bad taste). There are some indications that the grass (silage) produced from soil repeatedly fertilized with liquid manure contains higher levels of this bacterium (Rammer and Lingvall 1997, Johansson 2008). However, this organism is also naturally present in the soil and there is so far no indication that the use of digestate would mean an increase in these problems associated with cheesemaking.

Fungi

Fungi also form spores and can survive the pasteurisation stage (Schnürer and Schnürer 2006). Few fungi are human pathogens and therefore they do not pose a great risk of infection for humans. However, aerosols of fungal spores can cause problems like respiratory irritation and allergies if the amount of fungal spores is high around a biogas plant or in connection with waste or digestate management (Bunger et al 2000). However, many plant pathogenic fungi may enter the digestion process if infected crops are used as substrate. Studies of several common plant pathogens show that they are very quickly killed in the biogas process (Zetterström 2008, Haraldsson 2008). However, if these organisms enter into the process and if the loading frequency is high, there is a risk that some of these organisms may survive. A secondary digestion step or storage of the digestate for two to seven days is, however, sufficient to kill all the investigated fungi completely (Zetterström 2008, Haraldsson 2008, Karin Jacobsson SLU, personal communication). However, it is difficult to fully evaluate the risks of spreading plant pathogens, since several plant pathogenic fungi are hard to cultivate in the laboratory.

Figure 7. Cladosporium cladosporoides, a storage pathogen that does not thrive in the biogas process. Photo: Karin Zetterström (2008).

6.8 Post-digestion and Storage

It is important to mix digestate well during storage. If it is not mixed well enough, there is a risk of sedimentation of organic material in the storage tank. This may even cause nutrients that are primarily found in organic form, such as phosphorus, to sink to the bottom (Baky et al 2006). It is important to cover the digester residue (digestate) storage tank, to prevent gaseous emissions of ammonia, nitrous oxide and methane. Sometimes a floating crust is formed that may limit these emissions, but normally it is necessary to use some type of cover material, such as cut-up straw (Hansson and Christensson 2005). To avoid undesirable emissions of methane, nitrous oxide and

ammonia, digestate should be stored and transported in a way such that microbiological activity is minimized. Temperature is an important factor, since activity generally increases with increasing temperature. Storage and transport of digestion residues during hot summer days can therefore lead to greater microbial activity than handling in winter. The risk of re-contamination by potential pathogens must also be kept in mind during storage and transport. A study by Bagge et al (2005) showed that both E. coli and Enterococci content increased during storage, which was assumed to be due either to the fact that the storage tank was not clean or that the same vehicle had been used for transporting digestate and raw liquid manure.

6.9 Digestate as fertilizer - Environmental benefits

There are several environmental advantages to using digestate compared to mineral fertilizer. The most obvious is that it reduces the use of fossil fuels and recycles nutrients to the soil. Production and transport of mineral fertilizers is an energy-intensive process that also results in emissions of nitrous oxide, a very powerful greenhouse gas. Nutrient recycling from cities to the countryside is necessary because otherwise we face depletion of soil fertility in the long run. Plants take up nutrients and when they are harvested they are lost from the soil. If these nutrients are not replaced, the supply is eventually depleted and the production capacity of the soil decreases.

It is also advantageous to digest manure and spread the end-product on the fields instead of using the undigested manure as fertilizer. The availability of plant nutrients in raw manure is different depending on the animal species, but generally the nutrients are not in plant-available forms and cannot be directly absorbed by the roots of the plants. As a result, there is an increased risk of the nutrients leaching from the soil and reaching groundwater and streams where they can cause eutrophication. During digestion of manure, a large part of the organically-bound nitrogen is converted (mineralized) to ammonium nitrogen, which is more easily absorbed by plants. Using the end product (digestate), after anaerobic digestion of the manure, therefore results in a reduced risk of leaching of nitrogen from agricultural land (Hansson and Christensson 2005).

Anaerobic digestion of manure before its use as a fertilizer also reduces the risk of emissions of methane and nitrous oxide. Compared to carbon dioxide, these gases are about 20 and 300 times more potent in terms of the greenhouse effect (Börjesson and Mattiasson 2007). Anaerobic digestion reduces the risk of emission of these gases because the organic matter in manure is converted to methane during storage. Unless this gas is collected in a controlled manner, as is the case during anaerobic digestion, it will leak freely out into the atmosphere. The microorganisms that are responsible for this conversion come from the digestive tract of animals and are naturally present in manure. Organic matter in manure can be used by microorganisms in the soil as their carbon and energy source and as a result of this utilization, nitrous oxide, among other gases, is formed (see above). Anaerobic digestion of manure is also beneficial since it reduces both the number of pathogenic microorganisms and the concentration of malodorous components in the manure.

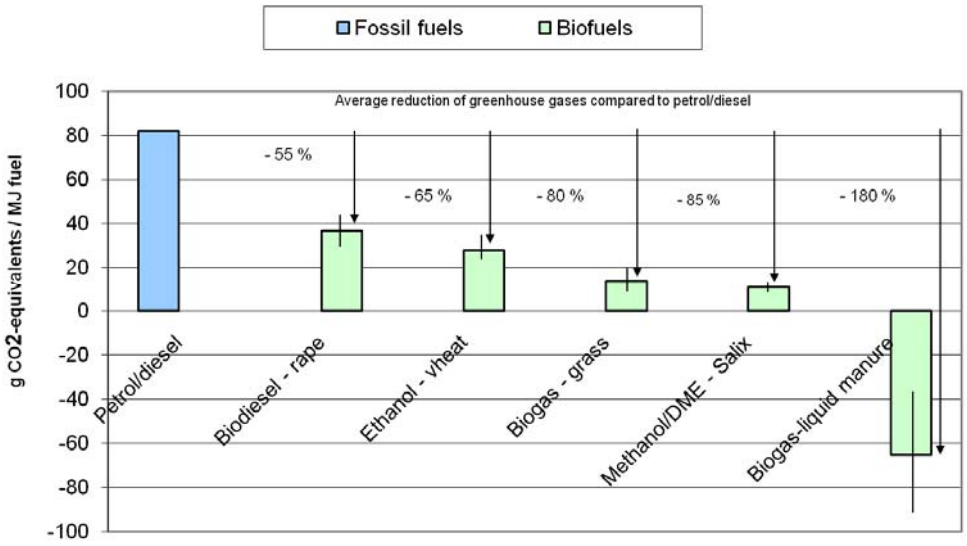

Figure 8. Anaerobic digestion of manure results in a negative carbon dioxide effect when biogas is used as fuel. The calculations are based on farms in southern Sweden with average arable land and facilities that run on biofuels with economical allocation of secondary products. Pål Börjesson, LTH.

6.10 Transportation of digestate

If digestate is transported by trucks that are also used for transportation of manure or other waste materials, it is essential that the vehicles are kept clean to avoid cross-contamination. A biogas plant is obliged to ensure that transport vehicles are clean both inside and outside, and that they do not become dirty again after cleaning. A study of vehicles from various plants shows that it is difficult to clean them between the transport of manure and of the digested residue (Ekvall 2005). No matter what cleaning method was used, there were places around taps, mixers, etc. where microorganisms were hiding. An alternative is to use different vehicles for transport in- and out of the plant, or to have separate tanks for substrates and digestate.

One means of distribution, used by the NSR biogas plant in Helsingborg, is to pump digestate through buried pipelines out to cropland in the surrounding area where it is used as a fertilizer. In addition to reducing the number of heavy and energy-intensive truck loads, this also prevents digestate from coming into contact with incoming shipments of non-sanitised substrate.

Figure 9. Cleaning the deck of a truck used for transportation of digestate. Photo Mikael Andersson.

6.11 Digested Sludge as Fertilizer

Digested sludge from sewage treatment plants is now used mainly as cover material for landfills and for embankment material for road construction as well as soil for golf courses, etc. Digested sludge is often treated prior to its final use through such means as composting and/or adding various materials like sand, sawdust or bark. A certification system for using digested sludge as fertilizer on agricultural land has been developed by Svenskt Vatten (www.svensktvatten.se). It was developed in close consultation with stakeholders, including the agriculture and food industry, grocery stores, various public agencies and consumer organizations. This certification system originated in the project 'Pure Plant Nutrients from Wastewater' (ReVAQ; www.revaq.se), which was a project implemented at Swedish treatment plants that aimed to improve the quality of sludge and its usefulness as a fertilizer. Among other things, great emphasis is placed on upstream documentation, i.e. cooperation with industries and households that deliver their wastewater to treatment plants to detect and eliminate pollution sources.

CHECK YOUR KNOWLEDGE

- Does digestate provide the same fertilizer effect as mineral fertilizers?
- How does digestate impact the microbiological and physiological properties of soil?
- Do all digestates have the same nutrient content?
- What are the advantages of digesting manure before using it as fertilizer?
- How is the quality of digestate controlled?
- Can sludge be used as biofertilizer?
- Are all pathogenic bacteria destroyed during pasteurisation?

- Can the same vehicle be used for delivery of substrates to the biogas plant and for the retrieval of digestate?

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7. Research and Development

The constantly increasing demand for biogas as an environmentally friendly fuel implies an increasing demand for biogas plants to be efficient and to produce biogas with a high methane content. Malfunctions can be very expensive so stable biogas processes are highly desirable. However, the biogas process represents a complex interplay between many different microbial groups, each with its specific requirements for nutrition and environment. For the process to function optimally, all steps in the conversion of the substrate material to methane and carbon dioxide must be active and synchronised. More knowledge is needed about the participating microorganisms and the factors that control their activity. A better understanding of these microbiological processes makes it easier to adapt the technology and control the process. The goal is always to get maximum production from the microorganisms under given conditions.

7.1 Important Research Areas

Important ongoing research areas in biological processes include adaptation of microorganisms to high ammonia and salt content, microbial trace element requirements, cellulose splitting, (i.e. stimulation and acceleration of cellulose hydrolysis) and avoidance of foaming. The introduction of new substrates and substrate mixtures also means that more knowledge is required on topics such as pre-treatment, sanitisation, retention time and process loads.

The use of digestion residues, including both sewage sludge and digestate, is another area where more research and development work is needed. For biofertilizer, this work has progressed very well, with good quality control and an established certification system. However, more work is needed to ensure the quality of the end products of digestion at sewage treatment plants. Long-term work at operational biogas plants is aimed at generating a greater acceptance and

distribution of digester residues in agriculture. This is important, because in the long run the goal must be to fully recycle nutrients. A better understanding is needed of the digestion residue, with respect to its nutrient content and potential contamination, microbial activity and its physical suitability for efficient land application, etc. Another important topic is how the residue (digestate as well as digested sludge) should be handled before land application. Sometimes it is divided into different fertilizer products with contrasting water and nutrient contents. Dehydration is a key issue, since it affects transportation costs. The more water that is left in the residue, the greater is the final volume to be transported to the user. Dehydration also makes it possible to separate water soluble nitrogen, i.e. ammonium, from the liquid and return it to the fertilizer product.

New methods for monitoring the biogas process need to be developed. Since the biological process is sensitive to problems, it is important to detect any changes quickly and at an early process stage, so that damage can be avoided. Today, part of the sampling at biogas plants is still done manually, sometimes using time-consuming testing. Research is underway to develop new methods and improve existing technologies in order to facilitate sampling and analysis. One of the goals is to develop a continuous method for following the biogas process, for example, by adapting online measurement methods for registration of alkalinity, pH, gas flow, gas quality, fatty acid levels, etc. This will give staff a better basis for decision-making and will enable early detection of process problems, so that they can be fixed in a timely manner.

It is important in this context that the methods are adapted to the particular environment that will be sampled. The biogas process is a tough environment where, for example corrosion will quickly wear down unsuitable detectors and measurement devices.

Systems analyses of the entire biogas chain from handling of various wastes and cultivation of bioenergy crops to the final distribution of biogas and land application of digestate should also be carried out to enable comparisons between biogas and other available biofuels. It is essential that the biogas process is optimised, for example, with respect to energy consumption and substrate degradation efficiency, in order to maximise the environmental benefits of biogas as a biofuel. Sanitation and pre-treatment of the substrate must be energy-efficient and effective and digested residues must be stored in such a way that methane and nitrous oxide emissions are minimised. With the help of systems analyses, energy inputs and environmental benefits of different production chains can be calculated and compared to aid the design of future biogas systems.

7.2 Methods for Studying the Biogas Process

Studies of individual microorganisms or groups of organisms in the laboratory can provide us with much information about the underlying microbial mechanisms of anaerobic digestion. However, it is also important to do experiments with samples directly from the biogas process, because this environment usually differs from the environment to which the microorganisms are exposed in the laboratory.

Research on the biogas process is often done at the laboratory scale, where biogas reactors are studied in miniature, but these laboratory analyses are commonly linked to biogas plants at pilot and full scales. For example, inoculant (contents of an active digestion tank) or digester residue is often taken from full-scale biogas plants for further study in the laboratory. One common method is the batch test, i.e., batch-by-batch digestion, to examine how much biogas can be produced from a certain substrate (Hansen et al 2004, Demetriades 2009).

Batch Digestion Experiment

Start-up

The microorganisms that degrade the organic substrate in the digestion test are collected from a well-functioning biogas process. A sample from the reactor contents, the so-called inoculant, contains all the microorganisms needed to degrade different types of organic matter. It is most appropriate to take material from a system fed with a mixed substrate to obtain microorganism communities with a broad ability to decompose organic matter. If a substrate is to be evaluated for use in a specific facility, an inoculant from this particular biogas process should be preferred. To get active microorganisms in the inoculant, it is important to let the reactor contents flow for a while before the sample is collected. Otherwise, there is a risk that material is collected which has been stagnant in the piping, so that the microorganisms may be less active due to unfavourable environmental conditions.

Figure 1. Collection of inoculant for the batch digestion test. Photo Anna Schnürer.

The inoculant is collected in containers that are subsequently closed and preferably connected to a gas bag (or similar) to collect the gas that is formed and equalize the pressure in the container. The microorganisms will continue to form gas from the organic material remaining in the inoculants for some time, so a positive pressure can easily develop in the container. Since not all organic material is broken down in a continuous process, there is always a certain amount left when the sample is taken. Before the digestion test is started, it is important that this remaining organic material is broken down and that gas production in the inoculant has subsided. Otherwise it is difficult to differentiate between gas production from the inoculant and that from the substrate to which the inoculant has been added. The time it takes for the gas production to subside depends on the character of the inoculant and the temperature of the container. If the container is kept at the same temperature as the initial process, it may be approximately 4-7 days before the tests can start.

The inoculant is then loaded into smaller bottles (250 ml - 1 liter) together with the organic substrate that is to be tested. Nitrogen gas flows through the bottles during the loading of the inoculant and substrate into the bottles. This is to protect the microorganisms from exposure to excessive levels of oxygen, to which they are very sensitive. The substrate should be ground in a mixer or chopped up, before it is loaded into the bottles. Grinding ensures that the microorganisms have a greater surface area to attach to and as a result the rate of breakdown is increased. It is also important that an appropriate amount of substrate is added. It should be sufficient for the gas production to be measured, but not so much as to cause overload. A suitable load is 3 g and 5 g VS per litre of inoculant (= organic load) for tests at mesophilic and thermophilic temperatures, respectively. It has been found that an important factor when starting the batch tests is the relationship between the amount of organic material in the inoculant relative to the added substrate. A ratio of 2:1 between the VS content of the inoculants and the substrate has been proposed as a minimum to obtain the maximum methane production potential (Hashimoto 1989, Neves et al 2004). Since the variation between bottles is sometimes fairly high, it is important that each susbtrate is analysed in at least 3-5 replicate bottles to enable statistical analysis of the results.

The bottles are closed and the test is started as soon as they have been placed at 37° C or 55° C, or at whatever temperature the large-scale process in question is operated. The bottles can be gently shaken during the test to ensure effective decomposition. Shaking is not strictly necessary, but it accelerates the gas production process. Shaking, like mixing in a large-scale continuous process, improves the contact between microorganisms and the organic material to be degraded. However, it is important that the shaking is not too vigorous, since this may break up clusters of microorganisms that are essential for efficient decomposition. An appropriate mixing rate is when the entire contents of the bottle are slowly moving.

Figure 2. Batch digestion test. The bottles are sitting on an oscillating table. Photo: Anna Schnürer.

Determining the gas production potential

In order to evaluate and calculate the gas production potential of the added substrate, the gas output is monitored, both with respect to the total volume of biogas and the concentrations of methane and carbon dioxide in the gas through time. The gas may be analysed directly using an analytical instrument or gas samples can be collected for subsequent separate analyses.

One way to follow the gas production is by means of pressure measurement. If the test takes place in closed bottles without continuous gas collection, a positive pressure is quickly formed in the gaseous phase. This pressure increase can be used to determine the amount of gas produced via a connection to a pressure gauge. The measured pressure can then be converted to the amount of gas produced over a specific time period. After recording the pressure, the excess gas is let out, whereupon the newly formed biogas again builds up a positive pressure in the bottle. Pressure is measured at regular intervals and is converted to the gas produced in milliliters. The gas composition is then determined, usually by gas chromatography, in separate samples that should be collected in connection with pressure measurement.

When the gas production has subsided, the specific gas production is calculated, i.e., the total amount of methane formed per added amount of organic matter (VS). An example of a gas production curve is shown in Figure 3. In order to compare biogas potential from different tests conducted at different digestion temperatures, the biogas potential is usually indicated in normal cubic metres (Nm^3) of produced methane, that is, the volume of gas at 0° C and atmospheric pressure (see Chapter 5).

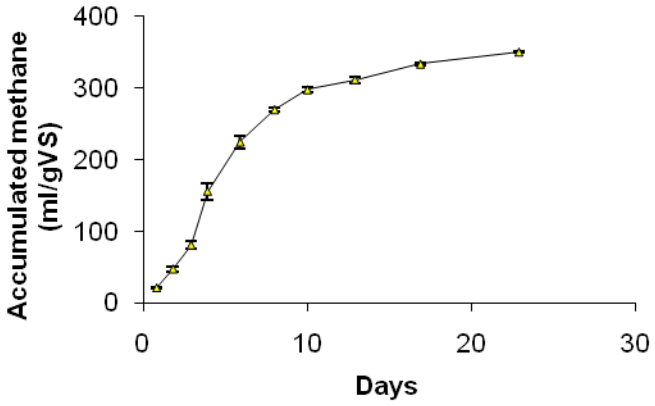

Figure 3. Amount of methane formed (ml/g VS), with standard deviation from three bottles, as a function of time in a batch digestion experiment with silage as substrate (Stenströmmer Moglia, 2007).

Continuous Experiments

Batch experiments provide a measure of the maximum methane formation potential and rate, but not the amount of methane that can be realistically obtained from a given amount of substrate in a continuous large-scale process. In a continuous process, decomposition is almost never complete; a certain portion of material leaves the process entirely or is only partly decomposed. It can also be difficult to account for inhibitory effects in batch experiments. If the substrate contains highly inhibitory compounds, this will probably be seen as a reduction in methane formation potential even in a batch experiment. However, less inhibiting effects are more difficult to detect in the limited time the batch experiment is conducted. Potential limitations related to a certain substrate, such as a low content of trace elements or a high proportion of protein, are also difficult to detect in batch experiments.

A continuous full-scale process can be scaled-down to the laboratory in order to fully evaluate the potential of a substrate or mixtures of substrates in a specific facility. A continuous system is also better suited for studies of co-digestion effects. Positive effects of improved nutritional compositions will also be difficult to see during short-term batch experiment runs. Earlier experiments have shown that it is possible to scale down a system and get the same process results as in a full-scale system (Leksell 2005). The advantage of such a 'down-scaling' is that it is possible to evaluate different substrates and changes in process parameters (temperature, load, retention time, etc.) without compromising the day-to-day operation of a large-scale system. When the evaluation is completed, it is then possible to test new substrates, or other operational changes under safer conditions at full-scale.

There are many different well functioning systems that can be used for continuous experiments, from a simple glass container that is fed manually and heated in a water bath, to more or less automated reactors. The system used is less important, as long as the reactors are maintained and

monitored in a similar way as the large-scale ones. The volumes are typically in the range of 3 to 50 liters. For accurate evaluation, it is an advantage if several reactors (at least 2) can be run in parallel during the experiments. Laboratory-scale evaluation of different substrates in both batch and continuous experiments can be contracted out to various consulting companies (see below).

Figure 4. Continuous biogas reactors at laboratory scale (8 L). Photo: Anna Schnürer.

Studies of Microorganisms

Several different methods can be used to study microorganisms in the biogas process. A relatively simple way is to prepare small slides of the processing liquid/slurry and study them under a microscope. Methane producers can be distinguished from other microorganisms thanks to their unique cell structure. Because methane producers belong to a special group of microorganisms, the Archaea, they have unique components in their cell membranes. One of these, cofactor F420, emits a bluish fluorescent light when illuminated by ultraviolet (UV) light (see Figure 6, Chapter 1). Methane producers can thus be distinguished from other microorganisms using a microscope equipped with a UV lamp, as they fluoresce with a green-blue colour (Cheeseman et al 1972, Delafontaine et al 1979, Gorris et al 1988). It is often difficult to discern individual microorganisms in slurry that also contains many other particulates, but this is not a problem for methane producers studied under UV light. It is possible to get a sense of whether the population is stable over time, or if it changes, by viewing methane producers repeatedly under a microscope. Changes may occur if the composition of the substrate changes. If the same substrate is used, any change may be an indication of some type of problem.

Another method based on microscope technology is FISH (Fluorescence In Situ Hybridization). Samples from the digester content are fixed and marked with fluorescent probes and examined

later under a microscope. Probes can be made to adhere to certain groups of microorganisms or can even be species-specific. This allows studies of all types of microorganisms, not just those methane producers that have natural fluorescence (Allison 2007, Fernandez et al 2008).

Microorganisms can also be cultivated and concentrated in specific nutrient solutions in the laboratory. In this way, it is possible to obtain pure cultures of the organisms that may need to be further studied. Isolated organisms can then be used to increase understanding and knowledge about the biogas process, for example, by microscope studies or through different activity tests. Isolated organisms can also be used to study, for example, the effects of inhibiting substances or to explore nutritional requirements for optimal digestion. Since the microorganisms of the biogas process are anaerobes, specific methods are needed for this work (Hungate 1973, Zehnder et al 1980, Schnürer et al 1996). Cultivation must be done in a completely oxygen-free environment, and the equipment must be adjusted accordingly (Figure 5). In order to further reduce the oxygen content, various reducing agents must also be added to the nutrient solutions.

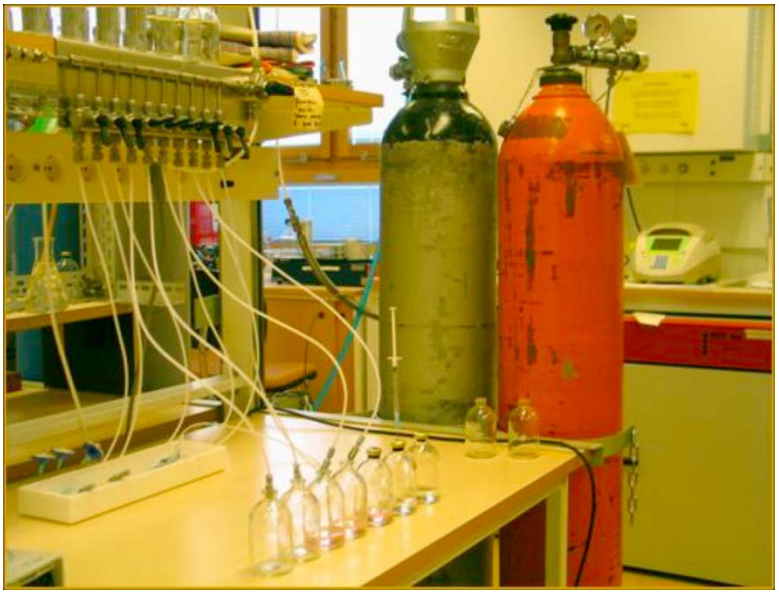

Figure 5. Cultivation of methane producers in sealed serum bottles. The air in the bottles is replaced with a gas mixture of nitrogen gas and carbon dioxide by means of the gas equipment in the background. Photo: Anna Schnürer

One method that has become increasingly common in recent years is to analyse DNA from various microorganisms in samples extracted from the biogas process (Ng et al 1994, Levén et al 2007, Collin et al 2006, Hatamoto et al 2008). By using different types of molecular techniques such as PCR, DGGE and TRFLP, extracted DNA can be studied in detail (Allison 2007). Molecular methods have long been used, for example, in medical research, and it opens new possibilities for studies of microorganisms in the digester environment. By using these methods, it is possible to get a better understanding of the population of a given system and also to study the changes occurring in the population as a response to changes in substrate composition or other process

parameters. Such knowledge will be important in the future, to understand the connections between population composition and process function.

Besides trying to detect and identify different microorganisms in the biogas process it is also of interest to study their activity. Bottle tests are often used for this purpose, where inoculant from a digester or one or more microorganisms can be studied under controlled conditions (van den Berg et al 1974, Dolfing and Bloemen 1985, Jarvis 1996). For example, the ability of methane producers enriched from a particular process to grow with acetic acid/acetate as sole substrates can be investigated by incubating them in a bottle with a nutrient solution containing acetic acid and then measuring the methane formation rate over time. Such bottle experiments are also suitable for investigating how a particular inoculant or microorganism reacts to different toxins added to the culture medium (Owen et al 1979, Shelton and Tiedje 1984, Urra et al 2008). Digestion pathways for different substrates and substances can be studied in detail using isotope methods, for example, by adding ^{14}C or ^{13}C-labeled substrate to inoculant and then recording how the labeled carbon is distributed to various digestion products (Jeris and McCarty 1965; Zehnder et al. 1979, Schnürer et al 1994, 1996, Levén and Schnürer, 2005).

NIR

Near-infrared spectroscopy (NIR) can also be used to study the biogas process (Hansson et al 2002, 2003, Holm-Nielsen et al 2007, 2008). This technique is currently used in the chemical industry, for example, to measure the quality of grains. The method consists of a probe installed in the digester. The probe emits a spectrum of beams at different wavelengths, which are either absorbed or reflected by the material. The result is a pattern that is unique to the material and related to its characteristics, such as the content of various volatile fatty acids. Preliminary tests have shown that the NIR pattern can be correlated with the concentration of propionate, which is a very important indicator of problems in the process. The NIR technique can also be applied to various substrate mixtures to determine the organic matter content. The organic matter content can then be used to calculate the correct loading rate for the process.

Microbiological soil tests

The effect of various fertilizers, including digestate, on the microbial activity in the soil can be studied with various microbiological soil tests (Torstensson et al 1998, Pell et al 2005). Such tests make it possible both to analyse overall microbial activity (respiration test) and the activity of specific groups such as denitrifying bacteria (PDA test) and ammonium oxidising bacteria (PAO test). Most tests are designed to study the effect of various fertilizers, but also for analyzing the effects of certain metals and organic pollutants on the microbial health of the soil (Pell et al 1998, Enwall et al 2006, Levén et al 2005, Odlare et al 2008). So far, most reports concentrate on the short-term effects of digestate, and currently it is unclear what the effect on the soil will be after repeated applications of different fertilizers for many years. However, all these reports suggest that digestate works well as a fertilizer.

Figure 6. Metreing digestate for a soil microorganism test. Photo: Mikael Pell.

System analysis
It can sometimes be difficult to interpret all information obtained from various measurements and to draw the right conclusions regarding the way the process is to be controlled. A project is therefore ongoing to develop computer models that can be used to get a better overview and interpretation of the various biogas process parameters. An example of such a model under development is known as the ADM1 model (Anaerobic Digestion Model no 1, Batstone et al 2002), which simulates digestion, microbial activity and inhibition (Rozzi and Remigi 2004).

7.3 Ongoing research and development

Research and development of biogas processes is currently ongoing in many parts of the country. Research is being conducted by universities and research institutes as well as biogas plants and by various consulting firms. Development is also taking place through the various regional collaborative efforts that have been established in Sweden:

Biogas West, www.biogasvast.se
Biogas South, www.biogassyd.se
Biogas East, www.biogasost.se
Biogas North, www.biofuelregion.se

More such regional cooperation projects are being established. Listed below are universities, colleges and other organisations where research and development of biogas processes is being

conducted, and some examples of current research. More information about the research taking place in this subject can be found at the Avfall Sverige website, www.avfallsverige.se.

For a complete list of current biogas plants in Sweden and consulting companies where research and development is performed in the field of biogas, see the report "Biogas from manure and waste products - Swedish case studies" that can be downloaded from the Energigas Sverige website (www.energigas.se).

Avfall Sverige (AS), www.avfallsverige.se
Pre-treatment of food waste
Voluntary efforts to detect methane leaks in biogas plants
Certification of digestion residues (digestate) in cooperation with the Swedish Technical Research Institute SP (www.sp.se)

Borås College (www.hb.se)
Pre-treatment of cellulose-rich materials
Biogas production from textile waste

Swedish Institute for Agricultural and Environmental Engineering (JTI), www.jti.se
Agroptigas: digestion of crops, cooperation with Svensk Växtkraft in Västerås, Sweco, Stockholm Gas.
Investigations into different substrates for biogas production, farm biogas, land application of digestion residue (digestate), potential yield improvements, digestion at sewage treatment plants
Bioenergy portal: a national Web site that collects information and knowledge about digestion of agricultural crops, among other things (www.bioenergiportalen.se)

Linköping University (LIU), www.liu.se
Process improvements with various substrate mixtures, appropriate loading rates, micronutrients, population studies of microorganisms

Luleå Technical University (LTU), www.ltu.se
Pretreatment, biogas upgrading, biogas for producing electricity and heat

Lund Institute of Technology (LTH), www.lth.se
Low-temperature digestion, farm biogas, gas purification
System studies, energy efficiency, and environmental benefits of different biofuels
Crops4Biogas: interdisciplinary research on biogas from energy crops.

Mälardalen University (MDH), www.mdh.se
Energy systems, use of digestate, biogas production from crops

Swedish Gas Center (SGC), www.sgc.se

Coordinates Swedish efforts in research, development and demonstration within the energy gas sector. Activities funded by the Swedish Energy Agency and the gas industry are divided into the following areas: biogas technology, gaseous fuels, distribution and storage, gasification and methanation, environmental technology and energy gas utilization.

Swedish University of Agricultural Sciences (SLU), www.slu.se
Breakdown of organic environmental pollutants, adaptation of the process to high ammonia levels
Evaluation of the effects of digestion residues (digestate) on soil fertility
Population Analysis and isolation of various organisms from the biogas process
MicroDrivE: digestion of stillage from ethanol production, biogas from cellulose-rich materials (MicroDrivE.slu.se)
Agrobiogas: EU projects with focus on farm-based biogas production (www.agrobiogas.eu)

National Veterinary Institute (SVA), www.sva.se
Hygiene in digestion residues (digestate) and gas pipelines
Sanitation before, during and after digestion
Alternative sanitation methods

Waste Refinery, www.wasterefinery.se
Center for waste and recycling issues

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8. Common Problems & Solutions

As shown in the previous chapters, it is important that microbiological processes in the digester are in balance. A stable biogas process with high methane production can be achieved only when the interactions between the various microbial groups involved are working properly. There is a great risk that the process will deteriorate or even stop, if these interactions are disrupted for any reason. With the help of technology, it is possible to adapt the environment to the capacity of the microbes and their requirements for nutrients, temperature, pH, etc. This chapter discusses some problems that may arise during digestion and their underlying microbiological causes. Some ways to avoid and/or resolve problems are also discussed. The chapter summarizes what has been discussed in more detail in previous chapters.

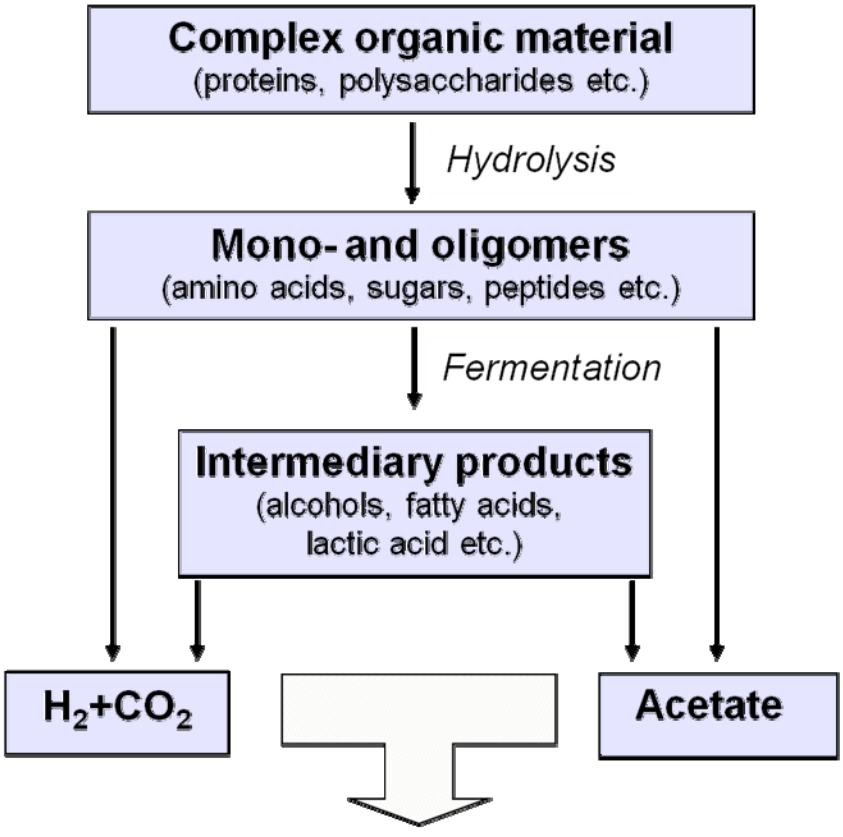
8.1 What happens in a process malfunction?

It is difficult to provide a simple unique answer to this question. Many microorganisms are involved in the digestion process, each with its own specific requirements for nutrition and environment and they also respond differently to various disturbances. Some groups of organisms, however, have specific abilities that give them a more prominent role and importance for the functionof biogas processes and in some situations, its malfunctioning. These include methane producers, responsible for the final step in the process. Hydrolysing microorganisms are another important group, accounting for the initial breakdown of cellulose-rich materials into smaller, more manageable substances. Generally, all interactions between microbial communities must work for the process to run all the way to the formation of the final product, methane.

Methane producers

Because of their slow growth and sensitivity, methane producers are often directly or indirectly involved in process malfunctions. What happens when these organisms become inhibited or killed? Because the methane-producing microorganisms utilize several of the end-products of previous breakdown steps, inhibition of this group of organisms may result in a complete process failure. Inhibition of methane producers initially results in an accumulation of the substrate which these organisms exploit, i.e. acetic acid (acetate), hydrogen, and carbon dioxide. The inhibition of methane producers can be detected early by a decrease in gas production and a change in gas composition towards a higher percentage of carbon dioxide. The proportion of hydrogen in the gas also increases, but this requires more complex analytical instruments to detect than the increase of carbon dioxide.

An increase in hydrogen concentration in the process fluid also inhibits the organisms that carry out anaerobic oxidation. The outcome is an accumulation of substances such as volatile fatty acids (VFA), long-chain fatty acids (LCFA), various aromatics, and others. Inhibition of the hydrogen-consuming methane producers can therefore be detected at an early stage as an increased concentration of fatty acids. For this reason, analysis of fatty acid concentration is an excellent tool to detect any disturbance of the biogas process. The accumulation of fatty acids results in a gradual decrease in pH, which also affects other microorganisms than those that are initially inhibited. If the pH drops drastically, this could result in a very sharp, and sometimes irreversible, inhibition of the whole biogas process. The rate at which pH declines depends on the buffering capacity of the process (see Chapter 2 under the heading Alkalinity and pH). Some processes may have a stable pH over a relatively long period of time even if the concentration of fatty acids is steadily increasing. When the buffering capacity is exhausted, however, the pH drops quickly. Processes with an initially low buffering capacity respond faster with a decrease in pH soon after an increase in the level of fatty acids.



Accumulation of intermediary products, acetate, H_2 and CO_2

Figure 1. Inhibition of methane producers results in accumulation of fatty acids, carbon dioxide and hydrogen.

Degradation of cellulose

Hydrolysis is an important starting point for subsequent decomposition, and if this step does not function properly, it affects the entire process. The prominent feature of this step is the formation of various enzymes that degrade the substrate into smaller parts, which can then be used by a variety of microorganisms for growth. Without these enzymes, which are produced by bacteria in the first stage of the biogas process, degradation cannot continue. Hydrolysis of various components proceeds at different rates and efficiencies, and this can have a major impact on both the rate of the process and the final gas yield. Generally, cellulose-rich material is difficult to degrade because of its complexity. Thus, a high proportion of cellulose material in the substrate leads to a bottleneck in the first step and the process becomes slow and inefficient.

8.2 Typical problems

Increasing levels of fatty acids

As mentioned above, fatty acids (both VFA and LCFA) accumulate if the anaerobic oxidation reactions do not work. This may be due to direct interference with organisms that perform this step or, more commonly, to the methane producers not being able to consume hydrogen at a sufficient rate. The reasons for this may include:

- The presence of inhibitory substances such as ammonia (see Chapter 4)
- Temperature or pH changes (causing a lower growth rate)
- Overload, namely the substrate for the methane producers is formed faster than it is consumed (see below).

Increasing levels of ammonium/ammonia

Ammonium/ammonia is released during the decomposition of proteins in the biogas process. If the proportion of protein in the substrate is high relative to the other material (low C/N ratio), there is a risk of a gradual increase of ammonium/ammonia content. Ammonia inhibits many organisms in the process, especially methane producers. This inhibitory effect makes the methane formation step proceed slowly and it is often followed by an accumulation of fatty acids. It is important to remember here that the analysis of ammonium nitrogen, which provides an aggregated value of ammonium and ammonia, only shows part of the truth, as only ammonia is inhibitory. The ammonia concentration increases in relation to ammonium with increasing pH and temperature. This is the reason why thermophilic processes often exhibit inhibition symptoms earlier than mesophilic systems, despite the same ammonium nitrogen content.

pH decrease/increase

A change in pH is usually caused by increasing contents of fatty acids formed during substrate decomposition. An increase in fatty acids may occur either because of an overload or because the activity of methane producers is inhibited. An increase in pH is often associated with an increase in ammonia content during the degradation of protein-rich material. Ammonia is released, which

is a strong base. Changes in pH may also occur if the substrate is highly acidic or alkaline. The rate at which a change in pH occurs is strongly related to the buffering capacity (alkalinity) of the process. A process with good buffering capacity can cope with relatively high levels of fatty acids before any change occurs.

Overload

Microorganisms will not manage to degrade the substrate sufficiently quickly in a biogas process that is fed with too much material. There may be too few organisms or they grow too slowly. This may give rise to different symptoms depending on the nature of the substrate, i.e. whether it is high in sugar, fats, or proteins.

Sugar

The initial decomposition steps proceed relatively rapidly for substrates containing high sugar contents and large amounts of fatty acids are quickly formed. In contrast, degradation of fatty acids proceeds slowly because the fatty acid oxidising organisms must cooperate with the slow-growing hydrogen-consuming methane producers. As fatty acids are formed faster than they are consumed, a high sugar load results in an accumulation of fatty acids and this is eventually followed by a decrease in pH.

Protein

The rate of protein decomposition varies, but ammonium/ammonia is formed irrespective of the rate. Ammonia inhibits many organisms in the process, but the methane-producing organisms are disturbed the most. The rate of methane formation then decreases, and this is followed by an increase in the contents of fatty acids and eventually by a decrease in pH. The ammonia content increases in relation to ammonium with increasing pH and temperature, so different processes can exhibit a broad range with respect to the load that can be allowed before an overload occurs.

Fat

Decomposition of fats leads to the formation of long-chain fatty acids (LCFA). LCFA's may adversely affect the process, partly because they can have an inhibitory effect on the methane-producing microorganisms and partly because they are detergents. An excessive load of fats can cause both an accumulation of volatile fatty acids (VFA) and foaming problems. It is worth noting that the hydrolysis of fats sometimes starts as early as in the transport vehicle or in the substrate tank, especially in warm weather. Thus, there is a risk that the biogas process is fed with material which contains a rather high amount of fatty acids from the outset. LCFA's can be degraded in the process, but this is slow and in many cases can only occur if the concentration of acids is not too high. High load and high concentrations of LCFA therefore results in a greater risk of overloading symptoms, including foaming.

Uneven load

The microorganisms in the process thrive best with a constant organic load and if growth matches the rate of substrate addition. If the load is uneven, there is a risk of overload or of failing to maximize the capacity of the organisms to degrade substrate. Problems can arise when

fluctuations occur even if the load is within a range that can be considered a normal load. This is because a reduction in the load will not only result in reduced gas production, but also in a decrease in the total amount of organisms in the process, since the food is insufficient to maintain a full population. If the load is low and then suddenly becomes much higher, an insufficient number of microorganisms will be present to handle the food, even if the final load is not abnormally high. As a result, the process will exhibit symptoms of overload, such as increasing contents of fatty acids. The capacity of a process to handle load fluctuations will vary, depending on factors such as retention time, temperature, and initial load level. An interruption or missed load for a couple of days often causes no problem, but if the load has been reduced during a longer period, it is advisable to gradually increase the load again to avoid problems.

Foaming/floating crust

Foaming in biogas reactors may be due to several factors. One reason may be poor mixing in combination with a high proportion of poorly digestible materials in the substrate such as lignin or plastic. This can result in a "crust" being formed, with materials that float above the surface of the liquid in the digester. This makes it difficult for the produced gas to escape, which may cause the entire "crust" to rise. Another reason could be high contents of long-chain fatty acids (LCFA), which have chemical properties that result in foaming. LCFA's are formed during breakdown of fat-rich materials.

Low/uneven gas production

Low/uneven gas production could be due to several factors such as:

- poor gas production potential in the substrate (low energy content, high content of hard-to-digest components, lack of trace elements, too coarse material, etc.)
- uneven load
- presence of inhibitory substances
- low degree of digestion
- fluctuations in temperature

A well-functioning biogas process always has some fluctuation in gas production and this variation is not in itself a sign that the process has problems. There may be several reasons for production rates that are smaller than expected from theory, but probably the degree of digestion of the material is low, which may be because the retention time is too short, the material contains a high proportion of poorly digestible material or the substrate consists of large aggregates with insufficient surface area for the microorganisms to exploit. If gas production suddenly drops even though the same substrate is being used, this may be a sign that the load is uneven or that some toxic substance has accumulated to levels that inhibit the microorganisms.

A change in gas production may also occur in the event of a change in the input material if the gas production potential of the new substrate is different. If the new material contains inhibitory substances, gas production may decline. In this context, it should be pointed out that the measured gas production may sometimes change due to temporary changes in pH, since pH

affects the amount of carbon dioxide which is dissolved in the process fluid. If pH increases, more carbon dioxide can be dissolved in the liquid and this may affect the methane content in the gas.

A change in gas production may also occur if the process experiences changes in temperature. This is because temperature affects the microbial growth rate and hence gas production. Worth noting in this discussion about gas production potential is that even if gas production is even, the concentrations of carbon dioxide and methane may change. As mentioned earlier, inhibition of methane producers may result in an increased percentage of carbon dioxide in the gas. This may become apparent before the total gas production changes.

Temperature Increase/Decrease

Temperature fluctuations are most often due to technical problems, but the underlying cause may also be biological. The breakdown of certain materials (crops) may result in heat production in the biogas process and, hence in problems caused by temperature fluctuations. Many microorganisms are inhibited if temperature fluctuations in the process are too large. For example, a reduction in temperature results in slow growth of the methane producers and a risk that they are gradually washed out of the system and are thus not able to effectively degrade fatty acids. This can result in an accumulation of decomposition products, followed by problems with instability. This problem will be especially severe if the temperature fluctuates back and forth because the organisms will not have time to adjust.

8.3 Corrective Measures

It is difficult to give general recommendations on measures to deal with specific symptoms. The reasons for symptoms of process malfunction may vary from one process to another, since each biogas process has its own specific community of microorganisms and operates under specific conditions. However, some general guidelines can be given to avoid malfunctions with a microbiological cause:

- constant and controlled organic load
- constant process temperature
- good mixing, both in the substrate tank and the digester
- small substrate particle size
- monitor the substrate composition, i.e. C/N ratio, organic matter content, inhibiting substances, etc.
- careful monitoring of the process, i.e. regular analysis of fatty acids (VFA), pH, alkalinity, gas production/gas composition, ammonium content, temperature, etc.

If the process becomes unstable, it is important to urgently try to investigate what is causing the problem. In the long run it is better to completely eliminate the source of the problem than to try to avoid the symptoms temporarily. Below are some brief tips on steps that can be taken in the event of various types of problems. Note that these are suggestions for actions that can be taken

and are not "recipes" for universal solutions. All biogas processes are unique and may respond differently to different measures.

Accumulation of Ammonium and/or Fatty Acids

It is important to reduce the load, that is, reduce the input of organic matter or extend the retention period if fatty acids have accumulated in the process. This gives the process a chance to recover. Also, try to investigate the nature of the problem. When the load is reduced, fewer acids are formed and the "work load" of the microorganisms also decreases. Sometimes a total load stop may be necessary, to reverse the trend of increasing acid levels and to allow the concentration to decrease. There may be several reasons for the increasing levels of acids, such as overload or inhibition of methane producers.

Some materials, especially those with high sugar contents, have a higher risk of problems with acid formation than others. It is advisable to digest sugar-rich materials together with a more nitrogen-rich substrate, or to use a two-step process (see Chapter 2) in order to avoid acid formation.

An increase in nitrogen content often goes hand in hand with an increase in the content of fatty acids, since ammonia, which is released during decomposition of nitrogen-rich materials, inhibits methane producers. If the ammonium nitrogen content in the process is allowed to gradually and slowly increase, microorganisms can adapt. However, there is a limit to the concentrations they can tolerate, and when fatty acids begin to accumulate, this is a sign that the limit has been reached. There are various strategies available to reduce the concentration of ammonium nitrogen. An appropriate action could be to reduce the proportion of protein-rich material that goes into the process (co-digestion with materials of low nitrogen content). In this case, less ammonium is released and eventually the ammonium content will also decrease in the process.

Another strategy might be to extend the retention time to the point where the reduction in digestion rate that occurs as a result of inhibition does not adversely affect the process. Another, perhaps more drastic, approach may be to do the opposite, namely to reduce the retention time. This reduces the degree of digestion of the material and smaller amounts of ammonium are released. The low degree of conversion of the material means that the total gas potential of the material is not being fully utilized. Experience also shows that the risk of ammonia inhibition in thermophilic processes can be reduced by using a lower process temperature, i.e. approximately 50-51° C. If the problem with ammonium is acute, that is to say that the process is disturbed to the degree that gas production and perhaps also the pH has dropped to low levels, it may be necessary to dilute the reactor contents. This can be done by adding water, manure, or digester content from another facility. If manure or material from another site is used, it will also act as a supply of new "fresh" microorganisms and this may possibly shorten the recovery period.

Decreasing pH

Decreasing pH is an indication that the process has produced acids in such quantities that the buffering capacity of the process has been exhausted. This problem can be temporarily solved by

adding buffering agents (see Chapter 5). A better and more long-term strategy is to try to reduce acid formation (see above).

Foaming/Floating Crust

Problems with temporary foaming can be reduced or corrected by the addition of anti-foaming agents. If foaming occurs more frequently and is a major problem, it is better to rectify the source of the problem. Perhaps foaming is caused by high fat content in the substrate. If so, digestion together with another less fat-rich material can be the solution. Another solution could be to either reduce the organic load and/or increase the loading frequency (whilst maintaining the load). Thus, the process is loaded with a smaller amount of material on each occasion. LCFA degradation, which is the source of the foam problem when using fatty materials, will be more effective if a small amount is added more frequently than a larger amount added during one load. Problems with a floating crust can sometimes be resolved either by mixing more efficiently, by improving pre-treatment (reducing particle size by improved grinding), or by reducing the amount of lignin in the initial substrate.

Figure 2. Process failure at laboratory scale. Photo: Åsa Jarvis.

Process Failure

If the process is subjected to large and rapid changes (higher or lower) in pH or temperature, microorganisms may be killed to such an extent that it is no longer possible to restart the process. This may also happen if the microorganisms are exposed to high concentrations of inhibiting substances. To restart gas production within a reasonable time frame, it is best to obtain new

inoculant material from another process. This material can be used to replace all or part of the reactor contents.

8.4 Concluding remarks

As mentioned at the beginning of this guide, the biogas process is a natural biological process that requires co-operation between different microorganisms and groups of microorganisms to function properly. It can be likened to the process that occurs when grass and feed concentrates are broken down in the stomach of a cow. Just as the cow needs care and access to balanced and nutritious feed, the biogas process also needs careful treatment and supervision to function properly. Thus, the best results will be only obtained when an understanding of the biological processes informs and guides applications of biogas technology.

Glossary

Acetate = CH_3COO^-, the anion of acetic acid (CH_3COOH).

Acetogen = acetate-producing microorganism.

Acetotroph = microorganism using acetate (acetic acid) as a substrate. One example is the acetic-acid-splitting microorganisms that form methane and carbon dioxide from acetate.

Alkalinity = measure of the amount of alkaline (basic) substances. Bicarbonate, carbonate and carbon dioxide are examples of substances that contribute to alkalinity in a biogas process.

Anaerobic = oxygen-free.

Anaerobic oxidation = degradation step between fermentation and methane formation. Intermediate products such as alcohols and fatty acids are broken down in this step to hydrogen, carbon dioxide, and acetate.

Anaerobic filter = digester with built-in support material that helps retain the microorganisms. Can be used for methane formation in the second step of a two-step digestion process.

Archaea = a group of microorganisms with unique properties that have developed in parallel with bacteria and fungi. Methane-producing microorganisms belong to the group Archaea.

Batch digestion = material is digested without any material added or withdrawn during the process.

Biogas = the gas, consisting mostly of carbon dioxide and methane, which is produced when organic materials break down in an oxygen-free environment (anaerobic digestion).

COD = Chemical Oxygen Demand, a general measure of the amount of soluble organic compounds.

CSTR = Continuously Stirred Tank Reactor, that is, a biogas reactor in which the materials are mixed using an agitator.

Co-digestion = digestion of multiple substrates simultaneously. Often provides a higher methane yield than in the case where each material is digested separately.

Continuous digestion = new material (substrate) is pumped continuously into the digester with a steady flow during the day. This is feasible for liquid substrates (DS-content below 5%), while sludge-like substrates with higher DS levels are often pumped in portions over the day. This is known as semi-continuous digestion.

Degree of digestion = indicates, as a percentage, how much of the organic material has been broken down and converted into biogas during a certain time period.

Digestate = residue from biogas systems that digest relatively uncontaminated waste such as manure, source-separated food waste, waste from the food industry, agricultural crops, etc.

Digestion residue = liquid or sludge-like product that is formed after digestion and contains water, non-degraded material, nutrients and microorganisms (biomass).

Digested sludge = digestion residue formed after digestion of sewage sludge at wastewater treatment facilities.

Dry digestion = digestion at high DS levels (20 - 35%), often occurs in the form of batch digestion.

DS = dry solids, what is left when a material is dried. Usually stated as a percentage of wet weight.

Electron Acceptor = molecule that receives electrons during respiration or fermentation through which energy can be extracted. In aerobic respiration, oxygen is the final electron acceptor, while other organic (e.g. pyruvate) or inorganic (e.g. nitrate, sulphate or carbon dioxide) molecules are used as electron acceptors for fermentation and anaerobic respiration.

Fermentation = the second degradation step of the biogas process in which sugars, amino acids etc. are broken down under oxygen-free conditions to various fermentation products, such as alcohols, fatty acids, carbon dioxide, and hydrogen.

Floating crust = may form when undegraded materials accumulate and float above the liquid surface in the digester or the residue storage tank.

Foaming = when the presence of surfactants lowers the surface tension. Examples of surfactants are long-chain fatty acids that are formed during breakdown of fat.

Gas production potential= Amount of biogas in Nm^3 produced per unit weight of organic material. Nm^3 = normal cubic metre, volume under normal conditions, i.e. 0° C and atmospheric pressure (1.01325 bars).

Hydrogenotroph = hydrogen-consuming organism, for example methane producers that form methane from hydrogen and carbon dioxide.

Hydrolysis = the first degradation step in the biogas process in which large organic molecules (proteins, sugars, fats) are broken down into smaller components.

IHT = Inter-species Hydrogen Transfer, the transfer of hydrogen between different species of microorganisms. In the biogas process, this is done between organisms that carry out anaerobic oxidations (such as formation of acetate and hydrogen from propionate) and methane producers. These microorganisms live in syntrophy with each other.

LCFA = Long Chain Fatty Acids. Formed during the hydrolysis of fats.

Load = usually stated as organic load or organic loading rate (OLR). Describes how much organic material is introduced into the process per digester volume and day.

Mesophilic temperature = within the range of about 25° C - 40° C. Mesophilic biogas processes typically run at a temperature of about 35° to 37° C.

Methane = CH_4, the simplest hydrocarbon, an odorless gas of high energy value (9.81 kWh/Nm^3).

Methanogen = methane-producing microorganism.

Methane yield = amount of methane in Nm^3 formed per unit weight of organic matter load. Nm^3 = normal cubic metre, volume at normal conditions, i.e. 0° C and atmospheric pressure (1.01325 bar).

NIR = Near Infrared spectroscopy, a method which provides a comprehensive analysis of a mixture of different substances. Can be used to study the biogas process, such as its content of volatile fatty acids or the amount of organic material.

Pathogen = Disease-causing organisms; may be bacteria, virus or parasites.

Propionate = $CH_3CH_2COO^-$, anion of propionic acid (CH_3CH_2COOH).

Retention time = time that the substrate is in the digester. Frequently referred to as hydraulic retention time (HRT) and describes the time it takes to replace all the material in the digester. Sometimes the retention time is instead given as the residence time for the particulate material in the digester, solids retention time (SRT).

Sanitation = heat treatment/pasteurisation to reduce the number of pathogens in the substrate. In general, sanitation is performed at 70° C for one hour before digestion.

Specific methane production = the quantity of methane produced per quantity of organic matter input (m^3 CH_4 per kg VS per day)

Substrate = organic material suitable for digestion.

Support material = material, usually plastic, which can be added to the digester to retain microorganisms.

Syntrophy = collaboration between two organisms where both benefit from the cooperation. An example of syntrophy in the biogas process is the transfer of hydrogen (IHT) between microorganisms that carry out anaerobic oxidation and methane producers.

Syntrophic acetate oxidation = SAO, alternative methane formation pathway from acetate, where acetate is broken down first to hydrogen and carbon dioxide by non-methane-producing bacteria. These products are then used by another microorganism, a hydrogenotrophic methane producer, to produce biogas.

Thermophilic temperatures = temperatures above 40° C. Thermophilic biogas processes typically run at temperatures around 50° - 55° C.

Two-step process = the biogas production process is divided into an acid-forming and a methane-producing part, where the stages can be optimised separately, usually in two separate digesters.

UASB Upflow Anaerobic Sludge Blanket = digester that allows microorganisms to accumulate and grow in clusters (aggregates). New material is pumped into a strong upward flow, which provides sufficient mixing to create contact between microorganisms and substrate. Used, for example, in facilities for digestion of sewage.

VFA = volatile fatty acids.

VS = volatile solids, organic content, i.e. dry weight minus ash. Usually stated as a percentage of DS. Sometimes referred to as loss on combustion.

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