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ENVIRONMENTAL FACTORS AFFECTING THE BIOMETHANIZATION PROCESS

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Abstract: The paper describes the most important factors controlling the process of methanogenesis in the biomethanization technology. It discusses the operational regimes of temperature as well as pH, C/N ratio, the necessity for micronutrients and sensitivity to a number of toxic compounds. Components with an inhibitory effect are characterized as biostatic compounds (ammonia, VFAs, hydrogen sulfide and salinity-inducing substances) and biocidal substances (such as surfactants and pharmaceuticals). The threshold limits of the compounds in question securing the system against disturbances are introduced, as well as the measures counteracting inhibition. Some ways of overcoming the negative impact of environmental factors on the system are presented, including co-fermentation, supplementation of nutrients, removal of ammonia and hydrogen sulfide by different methods and acclimatization of methanogens to inhibitory substances.

Keywords: Biomethanization, nutrition requirements, temperature operating regimes, biocidal inhibition, biostatic inhibition

INTRODUCTION

Anaerobic digestion, also called biomethanization or biogasification, is defined as a biomass conversion process without external electron acceptors (Grady et al., 1999). It has been applied to the degradation of different types of waste such as sewage sludge, the organic fraction from municipal solid waste, animal manure, slaughterhouse waste, waste from food industry, plant and animal fat production, pulp and paper industry and others. As a result, the production of both an energy-rich biogas and a digested medium is achieved. The residue from biomethanization is characterized by a low percent of dry matter (< 20%, usually 4-10%) in the case of wet processes, or by a higher share of total solids in the suspension (> 20%), if the process is dry. The composition of the digest depends strongly on the character of the waste degraded. The second final product, i.e. biogas, consists of methane and carbon dioxide, and the relative composition of the gases reflects the composition of the waste degraded, similarly to the residue.

Anaerobic digestion is often termed a "structured process", including four basic stages: hydrolysis, acidogenesis (i.e. fermentation), acetogenesis and methanogenesis. Methanogenesis is only the biological part of the ecosystem which includes chemical and physical parameters that can be referred to as environmental factors. Some of them, such as temperature, availability of substrates and nutrients as well as the presence or absence of toxic and inhibitory compounds, are generally recognized to be the most important (Mata-Alvarez, 2003).

The present paper describes the impact of environmental factors on the methane production efficiency as well as indicates some technological solutions leading to the stabilization of the biomethanization process. The proposed solutions comprise co-fermentation, addition of chemical substances either to eliminate excess amount of ammonia and hydrogen sulfide or to improve nutrient balance in the system, and finally acclimatization of methanogenic consortia for low pH environment, high ammonia concentrations or other potentially inhibitory substances. Directions of future research in this regard are also indicated.

Temperature

Temperature is an essential variable in controlling the rate of biochemical and enzymatic reactions within cells, causing increased growth rates (Westermann et al., 1989). Temperature has also a strong influence on physicochemical parameters related to mass transfer rates, solubility and phase distribution. Diffusivity and solubility of solids increases with temperature, thus the gas–liquid transfer rate is higher and some particulates such as fats may be melted or emulsified, increasing solids availability. On the contrary, viscosity decreases with increasing temperature, which promotes easier mixing and pump transport, thus improving both mass and gas-liquid transfer.

Biomethanization occurs over a wide range of temperatures divided into the following microbial operating regimes: psychrophilic - typically ambient conditions (0-20^{0}C) (Safley and Westerman, 1992, Kashyap et al., 2003), mesophilic (25-40^{0}C) and thermophilic (45-60^{0}C) (Angelidaki and Sanders, 2004). An optimal temperature value is recognized to be around 35^{0}C for mesophilic digestion and 55^{0}C for thermophilic one (De Baere, 2000). The achievement of stable operating conditions requires not only maintaining the temperature at a proper level, but also keeping it within close limits during the overall process. Such an approach allows the methanogens to survive and grow in spite of their sensitivity to sudden thermal fluctuations (Garba, 1996).

The main advantage of the thermophilic regime compared to the mesophilic process is the destruction of pathogens. It is particularly important in the case of sewage sludge and municipal wastes because of a significant sanitation of the biomass. Thermophilic conditions improve sanitary effects and minimize the risk of spreading pathogens (Lund et al. 1995), however an adequate sanitation time has to be controlled and maintained between addition of undigested feed and removal of digested medium. Other advantages of thermophilic regime comprise an improved solid-liquid separation, an increased destruction rate of organic solids, a better degradation of log-chain fatty acids and less biomass formation, e.g. lower growth yield of thermophilic bacteria due to their increased decay rate (Vogelaar et al., 2003) which can be achieved with regard to reduction of hydraulic retention time (HRT) in the digesters. The thermophilic process is however more sensitive to environmental changes than the mesophilic one (Kim et al., 2002, Kim et al., 2006). Some imbalances may occur particularly in hyperthermophilic conditions (60-75^{0}C). They can result in the chronically excessive volatile fatty acid (VFA) production of the acidogenesis phase, affecting unstable methanogenesis (because of high propionate concentrations), poor supernatant quality, larger risk of ammonia inhibition and lower than expected levels of gas production, especially for highly biodegradable waste (Scherer et al., 2000). Another important effect connected with temperature increase results from a lower solubility of gases and a higher inhibition of ammonia or sulfides. When the temperature is increasing, more CO_2 is transferred to the gas phase so its contribution of biogas is higher (because of relatively low methane solubility). Moreover, gas contains more water vapor (Batstone et al., 2002). Angelidaki and Ahring (1994) reported that reduction in temperature from 55^{0}C to 46^{0}C for digestion process of the waste with high ammonia load resulted in an increase of biogas yield and improvement of process stability due to diminished VFA concentration.

pH and VSAs

Methanogenesis occurs at an optimum pH value between 7 and 8. Some substrates submitted to methanogenesis lead spontaneously to an alkaline steady state (pH > 8). On the contrary, others lead spontaneously to a steady acidic state (pH,6,5). It is generally reported that with pH values declining below 6.0 there are disturbances such as lower methane production and problems resulting from biomass retention (Brummeler et al., 1985, Speece, 1996). However, recent research findings seem to contradict the currently accepted levels of pH for effective biomethanization (Jain and Mattiasson, 1998, Kim et al., 2004, Taconi et al., 2007). Researchers have investigated the ability of some methanogens to tolerate acidic environment. The results show that the microorganisms in question may adapt to or even prefer a more acidic pH than generally accepted. Jain and Mattiasson (1998) obtained variable acidic conditions by step-wise decreasing pH values from 7.0 to 4.0, using a 0.5 pH interval in the system seeded with activated sludge. Initially, low methane yields were observed due to a much longer lag- phase compared with typical systems operating at a neutral pH (particularly when pH drops not gradually but directly from 7.0 to 4.5 or 4.0). However, after some acclimatization researches attained the efficiency of methane production of 67%, 37% and 34%, respectively at pH of 5.0, 4.5 and 4.0, compared with the results of biomethanization at neutral pH. The contents of methane in biogas ranged from 55% to 65%. When pH surpassed 5.0, methane production efficiencies of above 75% were achieved (Jain and Mattiasson, 1998).

Kim et al. (2004) supposed the hydrogen-utilizing methanogens (autotrophic methanogens reducing CO_2 with electrons derived from the oxidation of H_2) to be more tolerant to acidic conditions than other methanogens (heterotrophic methanogens converting acetate, formate or other organic compounds). Researchers notified that methanogenic activity at a pH of 4.5, observed in a semi-continuous reactor for the production of hydrogen, originated from the hydrogen-utilizing methanogens, whereas the acetoclastic methanogenic activity was inhibited. Consenquently it was suggested that the pH in the reactor could influence the anaerobic conversion pathway rather than cause the inhibition of methanogens. Results recently published by Taconi et al. (2007) seem to confirm such a theory. The investigation refers to the anaerobic digestion of a synthetic acetic acid wastewater inoculated with digested sludge from municipal wastewater treatment plant. Experiments were conducted in batch reactors at acidic (4.5) and neutral (7.0) initial system conditions. It was shown that a mixed culture of methanogens had the ability to acclimatize and tolerate a much lower pH than a neutral one, which was confirmed by the production of 70% of the total methane yield with an average pH system rising from 4.5 to 6.79 as a result of the conversion of acetic acid substrate. Moreover, a noticeable influence of decreasing an initial pH from 7.0 to 4.5 was observed, affecting a higher methane yield and an increased total gas yield of 30% and 80%, respectively. Additionally, the methane yield at a low initial pH was 30% above the value stoichiometrically expected from methanogenic degradation of acetic acid. Such an increase was probably attributed to a higher amount of carbon dioxide in the aqueous phase as well as the combined effect of autotrophic and heterotrophic methanogens converting substrates to methane on different pathways. In the system examined the autotrophic methanogens preferred a more acidic pH range, while the heterotrophic methanogens prevailed gas production at a neutral pH. Considering that a lower initial pH reduces the amount of carbon dioxide dissolved as bicarbonates in the liquid phase, a two-fold effect on biogas production is suggested: (1) more carbon dioxide remaining in the gas phase increases the total gas yield; and (2) the aqueous carbon dioxide in the liquid phase is converted to methane by autotrophic methanogens (Taconi et al., 2007). A system that combines both the heterotrophic conversion of acetic acid and autotrophic conversion of CO_2/H_2 seems to be especially promising for improving the quality and amount of the biogas.

In order to maintain pH within the typically recommended range of 6.8-7.0 (Speece, 1996) some technical measures should be taken. A drastic reduction in pH level resulting from acidification could be overcome by combining waste originating from different sources in a suitable ratio (for instance cattle dung and OSMW) to achieve a well buffered environment (Sharma, 2002). Such an activity reflects a co-digestion approach which secures the system against a negative impact of pH, using as a feedstock a mixture of acidifying and non-acidifying biomass substrates to eliminate the threat of acidification. Another way to attain the required pH level is to add alkalinity in the form of sodium carbonate, soda ash, caustic soda or soda bicarbonate to industrial acidic waste (Speece, 1996) and feed the reactor at an optimum loading rate with waste from other sources, thus protecting the system from an excessive amount of intermediary compounds such as VFAs.

Volatile fatty acids, such as propionate and butyrate produced during acidogenesis, are normally found in anaerobic digestion processes as intermediary compounds. It is generally assumed that VFA inhibition of the aceticlastic phase results from their accumulation in the system and a subsequent decrease in pH value. However, several experiments have shown that VFAs are themselves toxic. Concentration of VFAs should not exceed 2000 mg/dm^3, particularly in the case of acetic acid (Yadvika et al., 2004). Boone and Xum (1987) reported that propionic acid concentration over 3000 mg/dm^3 caused failure of the anaerobic digestion system. Accumulation of acetate results from an organic compound overload, biostatic inhibition or other stress, and leads to a drop in pH. Both a low pH level and the acetate stored in the system cause inhibition of acetogens and hydrogenotrophs. In this case the system is fully inhibited (pH normally below 5), which means that the overall COD is converted to acids, whereas methanogenesis does not occur. However, there is some waste with a high ammonia load (household waste, animal slurry), for which the decrease of pH below 7 is usually not observed due to the high buffering capacity of ammonia. Such systems with interactions between free ammonia, volatile fatty acids and pH operate in an "inhibited steady state". This means that the digestion process is running stable, thus a complete failure of the system is not observed, however methane production yield is low (Angelidaki and Ahring, 1993, Angelidaki et al., 2006).

Nutrients

Nutritional requirements refer to macro-nutrients, such as carbon, nitrogen, phosphorus, potassium and sulfur, and some micro-nutrients, including calcium, magnesium, sodium, cobalt, iron, nickel, copper, molybdenum, selenium, tungsten and zinc (Kayhanian and Rich, 1995, Kim et al., 2002, Mata-Alvarez, 2003).

Carbon, known as the primary source of energy and an essential base for building microbial cell material, is generally not a limiting nutrient, particularly for carbon-rich organic substrates. Instead, there is a need to calculate the values of specified ratios such as C/N, C/P and C/K, because nitrogen and phosphorus are necessarily required for microbial synthesis of proteins and nucleic acids, whereas potassium increases cell wall permeability. An average ratio of around COD/N/P = 600/7/1 was recommended for substrates feeding anaerobic digester (Mata-Alvarez, 2003). Henze and Harremoes (1983) reported values for COD/N ratio between 400/7 in high-load systems (dry systems) and 1000/7 in low-load systems (wet systems). Exceeding the required levels of C/N ratio may indicate nitrogen deficiency, whereas falling short of the range can couple with inhibitory ammonia effect. The problem of ammonia deficiency could be overcome by addition of ammonia-rich waste to the system fed with waste from different sources.

Nutrients (of organic and inorganic character) have to be present in the feedstock of the digester in the adequate ratios and concentrations, while the operating conditions of the digester influence the quantitative requirements (Mata-Alvarez, 2003). Moreover, the availability of the nutrients should be confirmed since there are some cases in which the nutrient contents in the waste seems to be sufficient so nothing indicates their shortage in the system, and yet a disturbance reflecting the lack in bioavailability of nutrients appears. It should be noted that deficiency of nutrients can result in a growth limitation of methanogens and an unstable substrate bioconversion, thus it may cause a failure of the anaerobic digestion system (Kayhanian and Rich, 1995). However, in the case of their excessive amounts related to certain threshold levels an inhibitory effect can also be observed. The main functions and required concentrations of micro-nutrients are presented in Table 1.

Experimental studies indicate the need of nutrient supplementation, particularly with regard to such micro-nutrients as Co, Fe, Mo, Ni and Se, which stimulate anaerobic digestion of waste from different sources (Kayhanian and Rich, 1995). The concentrations of the nutrients added into anaerobic digester should be adequate for various substrates (Table 1). Considering the biodegradable organic fraction of municipal solid waste it is especially difficult to determine an optimal nutrient content in the feedstock since this type of waste is heterogeneous. In order to fulfill nutrient requirements in such a situation the demand to experimentally assess the nutrient characteristic and deficiencies has to be satisfied.

Table 1. Functions and characteristic concentrations of micro-nutrients in anaerobic digestion

Nutrient	Concentration required	Inhibition level	Functions	References
Ca	100-200 mg/dm^3	2,5-4,5 g/dm^3 (strong inhibition at 8 g/dm^3)	cellular cation, enzyme cofactor, biomass granulation, precipitation of carbonate and phosphate	Operation Manual of Municipal Wastewater Treatment Plants (OMMWTP), 1990 Yu et al., 2001 van Langerak et al., 1998
Mg	75-150 mg/dm^3	1,0-1,5 g/dm^3 (strong inhibition at 3,0 g/dm^3)	enzyme cofactor, stimulation of single cell production	OMMWTP, 1990 Schmidt and Ahring, 1993
Na	100-200 mg/dm^3	3,5 – 5,5 g/dm^3 (strong inhibition at 8 g/dm^3)	role in the ATP formation	OMMWTP, 1990 Takashima and Speece, 1990
Co	1,7 – 8,3 mg/kg* (dairy manure) 0,05-0,19 (biomass)		enzyme component in acetate forming process	Takashima and Speece, 1990
Fe	6,9-34,4 mg/kg* (dairy manure) 0-0,39 mg/kg (biomass)		enzyme activation, large reduction capacity, precipitation of sulfide, promotion of extracellular polymer secretion	Takashima and Speece, 1990
Ni	4,2-6,2 mg/kg* (dairy manure) 0,11-0,25 mg/kg for biomass		activation of methanogenesis co-factor 430	Takashima and Speece, 1990 Kayhanian and Rich, 1995
Se	0,062 mg/kg* (biomass)		intensification of fatty acid conversion	Takashima and Speece, 1990
Mo	0,16-0,3 mg/kg* (biomass)		enzyme component, inhibition of sulfate reducing bacteria, limitation of sulfide formation	Takashima and Speece, 1990
W			enzyme component, probable enhancement of CO_2 and H_2 metabolism	Takashima and Speece, 1990
Cu			enzyme component	Takashima and Speece, 1990
Zn			enzyme component	Takashima and Speece, 1990

*different stimulatory levels for various substrates

Following this, it seems to be profitable, especially for the high-load systems (dry systems with total solids about 24-30%), to mix two or three organic wastes from different sources to prepare sufficient feedstock for the biomethanization process. Kayhanian and Rich (1995) proposed a supplementation of the biodegradable organic fraction with the combined addition of nutrient-rich organic waste to eliminate nutritional deficiencies. The addition of thickened activated sludge from the wastewater treatment plant and of dairy manure to the biodegradable organic fraction with a dry basis ratio of 7:1:0,5 (biodegradable organic fraction/manure/activated sludge, respectively) was recognized as sufficient to fulfill nutritional demands. It was proved that the proposed approach increased the biogas production rate significantly (by about 30%) and enhanced the process stability of the thermophilic regime system. The optimal nutrient concentrations calculated for high-solids thermophilic digestion are presented in table 2.

Table 2. The optimal nutrient characteristics of mixed feedstock for high-load thermophilic digestion (Kayhanian and Rich, 1995).

Nutrient	Unit	Value (dry basis)	
		Range	Average
C/N*	-	20 – 30	25
C/P*	-	120 - 150	180
C/K*	-	45 - 100	65
Co	ppm	< 1-5	2
Fe	ppm	100 - 5000	1000
Ni	ppm	5 – 20	10
Se	ppm	< 0,05	0,03
Mo	ppm	< 1 – 5	2
W	ppm	< 1	0,1

*C/N, C/P and C/K ratios are based on biodegradable organic carbon and total nitrogen, phosphorus and potassium.

Kim et al. (2002) stated that nutrients bioavailability had a significant role in the anaerobic digestion process both in mesophilic and thermophilic systems. Supplementing inorganic nutrients (a coctail of Ca, Fe, Co and Ni, considered to be stimulating nutrients) resulted in a rapid decrease of VFA concentration in all reactors, except the non-mixed reactor, as well as in an increase of gas production accompanied by a pH reduction. Such a case was not normally observed due to the alkalinity production during methanogenesis. The results obtained by the authors (Kim et al, 2002) seem to be explained by the hypothesis that supplementation of the above-mentioned nutrients increases both the rate of acidogenesis and methanogenesis. Stimulated acidogenesis produced more VFA and H^+ ions. As a result, methanogenesis, which was also stimulated, removed more VFA, but did not produce enough alkalinity to neutralize H^+ ions, so a pH reduction took place.

Inhibitory factors

Inhibition generally means the blocking or limiting of the activity of microbial metabolism. The restriction of biological processes reflects a sensitive response of anaerobes to changes of such environmental factors as pH, temperature and the levels of substances exceeding threshold limits (Sung and Liu, 2003). The concentration of other substances should also be taken into account due to its synergistic or antagonistic effect on different toxicants (Mata-Alvarez, 2003). The arrest of transformations can be caused by a high concentration of the inhibitory substrate or product (Mosche and Jordening, 1999) with a toxic or inhibitory effect. Speece (1996) divided the two inhibition processes into toxicity with an adverse effect on microbial metabolism and inhibition with an impairment of bacterial function. Batstone et al. (2002) further clarified these terms as biocidal inhibition, biostatic inhibition and end-product inhibition.

Biostatic inhibition

Biostatic inhibition is considered to be a non-reactive reversible toxicity affecting the restriction of bacterial function. The inhibitory compound does not render functional component, but rather disrupts intracellular homeostasis due to the changes of pH, redox potential or salts concentration and leads, as a consequence, to anabolism limitation. The most frequent biostatic substances are VFAs, free ammonia and hydrogen sulphur. The undissociated compounds reach the inner part of the cell, so free acid and free ammonia are particularly threatening (Hashimoto, 1986, Angelidaki and Ahring, 1993). Salinity, pH changes and some xenobiotics can also cause biostatic inhibition. Biostatic inhibition is also caused by drops in thermodynamic yield resulting from product accumulation. Batstone et al. (2002) call it end-product inhibition which results from decreasing free energy available from catabolism, thus accumulation of the product is observed. The most common example of such an inhibition is the hydrogen inhibition of acetogens, though a high concentration of acetate can also inhibit the microorganisms in question. The VFAs, such as propionate and butyrate produced during acidogenesis, are converted to acetate and H_2 by obligate hydrogen producing acetogens (OHPA), whereas hydrogen consuming methanogens are involved in conversion of H_2 and CO_2 to methane. Importantly, only a narrow range of H_2 concentration between 10^{-4} to 10^{-6} atm is favorable both for H_2 producers and its consumers. This is a severe limitation which makes such a syntrophic reaction thermodynamically possible only at low H_2 concentration. Diffusion of dissolved H_2 from the microorganisms producing it to the consortia consuming it is the main mechanism for transfer and therefore close microbial proximity favors low H_2 concentrations (Schmidt and Ahring, 1993). Hydrogen is the most important end product, since its production exceeds stoichiometrically the production of other compounds, e.g. acetate. According to Mosche and Jordening (1999) product inhibition is best described with the model of competitive inhibition. Thus the inhibition can be expected to occur at an acetate/propionate-ratio which is in the range of normal operating conditions of digesters.

Ammonia

Nowadays, animal manure is used as the main feedstock in agricultural biogasification systems and the high ammonia concentration resulting from its hydrolysis is considered to be an essential technological problem. Ammonia is formed as a product of the hydrolysis and further acidogenesis of protein-rich organic materials. It is generally believed that ammonia concentrations below 200 mg/dm^3 are beneficial for anaerobic consortia since nitrogen is an essential nutrient (Liu and Sung, 2002). Both animal waste, such as cattle waste (Hashimoto and Roman, 1986), swine manure (Hansen et al., 1998), poultry waste (Salminen and Rintala, 2002) and some food industry waste often have a total ammonia concentration higher than 4 g N/dm^3, thus providing the anaerobic systems with a significant amount of ammonia. Unionised ammonia, unlike ammonia ions, can easily diffuse across the cell membrane (Kadam and Boone, 1996), so it is particularly threatening in relation to microbial community. The problem of ammonia inhibition is complex since there are some factors, such as pH, solubility, temperature and acclimation, influencing the ammonia threshold levels as well as the waste digestion results (Angelidaki and Ahring, 1993, Kayhanian, 1999, Sung and Liu, 2003). Ammonium and hydrogen ions prevail in the aqueous phase at lower pH values, whereas other forms, such as free ammonia in solution, ammonia in the gas phase and hydroxyl ions, become dominant at higher pH levels. According to this, the inhibitory effect depends both on total ammonia nitrogen concentration and a given pH value. Moreover, higher temperature causes an intensification of biostatic inhibition subsequent to high ammonia content, since the concentration of unionized ammonia rises with temperature. As a consequence, the thermophilic regime promotes ammonia-sourced inhibition in comparison to mesophilic conditions (Angelidaki and Ahring, 1994). Furthermore, higher pH values imply greater sensitivity of methanogenesis to ammonia (Koster, 1986), resulting in an increase of free ammonia concentration in the system.

Free ammonia inhibits aceticlastic methanogenesis at initial concentrations of about 0,1-1,1 g N/dm^3 (De Baere et al., 1984, Hashimoto, 1986, Angelidaki and Ahring, 1993, Hansen et al., 1998), however inhibition of the hydrogenotrophic methanogens occurs at higher concentrations, exceeding 1,2 g N/dm^3 (Hansen et al., 1998). It can be assumed that aceticlastic methanogens constitute the rate-limiting factor during anaerobic digestion of the ammonia-rich swine manure (Hansen et al, 1998), since approximately 70% of the methane is produced by the microbial community mentioned (Ahring et al., 1995). Methanogens may adapt to ammonia concentrations several times higher than the initial threshold level, obviously after a certain period of acclimation. A study on the thermophilic degradation of cattle manure reported that free ammonia above 700 mg/dm^3 influenced a poor digestion efficiency at a pH of 7,4-7,9 (Angelidaki and Ahring, 1994). Another experiment carried on the thermophilic regime showed a 50% inhibition of methanogenesis at pH of 7,6, resulting from the presence of free ammonia at concentrations of 560-568 mg/dm^3 (Gallert and Winter, 1997). Hansen et al. (1999) found that thermophilic anerobic degradation of swine manure was possible even at an ammonia content (NH_4^++NH_3) of 6 g N/dm^3, free ammonia concentration of 1,6g N/dm^3 and a concentration of VFAs as high as 11,5 g acetate/dm^3, however the obtained methane yield was low and amounted to 67 ml CH_4/g of volatile solids. Lu et al. (2008) stated that ammonia strongly inhibited protein acidogenesis at such a high concentration as 16 g N/dm^3, however the contents of under 8 g N/dm^3 slightly reduced the efficiency of the acidogenic phase. The study examined the mesophilic acidogenesis of protein-rich organic waste at a neutral pH. It was also shown that ammonia inhibited the methabolic pathways differently, influencing the schemes of acidogenic product distribution.

Recent studies have focused on inhibition effects of ammonia in terms of total ammonia nitrogen under various pH levels as well as on acclimatization conditions of thermophilic aceticlastic methanogens (Sung and Liu, 2003). Modeling based on the results of batch anaerobic toxicity evaluations indicated lower sensitivity of acclimated aceticlastic methanogens to increasing concentration of total ammonia nitrogen. Although acclimation caused a decrease in the specific methanogenic activity, it promoted a higher methanogen tolerance of various levels of pH and ammonia concentrations. An overall inhibition was observed in the range of 8-13 g/dm^3 of total ammonia nitrogen concentrations, depending on acclimatization conditions and pH level.

The possible manners of minimizing negative ammonia influence on the anaerobic digestion process are proposed by several researches. Krylova et al. (1997) found that phosphorite ore could restrict ammonia inhibition probably by either an increase of the buffering capacity resulting from methanogen immobilization on the mineral grains or by exchanging ammonium ions for cations such as Ca, Mg or K. Liao et al. (1995) reported air stripping to be a promising solution in diminishing ammonia influence on the digestion process by removing ammonia from waste before feeding it to anaerobic reactor. Another approach focused on the conversion of ammonia to struvite by adding stoichiometric amounts of magnesium and ortophosphate to obtain insoluble precipitation product (Maekawa et al., 1995, Demeestere et al., 2001). Recently, a new trend for avoiding ammonia inhibition and achieving improved methane production has led to the use of different kinds of inorganic additives, such as zeolite and clay, during biomethanation process. Angelidaki and Ahring (1993) investigated bentonite clay, Milan et al. (2001) tested a natural zeolite, which consists of clinoptylolite, modernite, montmorillonite and others, while Hansen et al.(1999) examined usefulness of glauconite for these purposes.

A comprehensive study on the effect of inorganic additives on the methane production regarding the anaerobic digestion of ammonia-rich waste was presented by Tada et al. (2005). The researchers examined four zeolites (modernite, clinoptylolite, zeolite 3A and zeolite 4A), a clay mineral (vermiculite) and manganese oxides (hollandite, birnessite) to establish their impact on the anaerobic digestion efficiency of ammonia-rich waste (4,5 g N/dm^3). It was proved that the addition of most mentioned materials significantly decreased NH_4^+ content of the digested sewage sludge and the same level of decrease was achieved. However, it did not refer to H-type zeolite 3A and birnessite, for which slightly diminished values of ammonia concentration was observed. On the other hand the amount of methane produced was enhanced only in the case of natural modernite (addition of 5% or 10% w/w organic waste) and it reached the level approximately three times higher than the control probe without additives. Natural modernite released calcium ions which increased the methane production. Thus natural modernite, due to its synergistic effect on Ca^{2+} supply and NH_4^+ removal, enhanced methane production from ammonia-rich sludge (Tada et al., 2005). Angelidaki et al. (2006) proposed another approach to reduce free ammonia inhibition during thermophilic anaerobic digestion of the source-sorted, protein-rich organic fraction of municipal solid waste. The authors found that feedstock dilution (of 1:4 ratio) with recirculated process water after NH_3 stripping led to avoiding a potential NH_3-sourced inhibition and showed a good performance of the biomethanization process with methane yield of 0,43 m^3 CH_4/kg VS (when the load of volatile solids amounted to 11,4 g VS/d and retention time was 15 days).

Hydrogen sulfide

Sulfur requirements for the anaerobic digestion process are quite complex, because methanogens may use only certain forms of sulfur and the sulfate form promotes inhibition due to sulfate reduction. However, sulfides have been reported to stimulate the growth of various methanogens.

Sulfates are common constituents of many industrial wastewaters (O'Flaherty et al., 1998a). In anaerobic reactors sulfate reducing bacteria (SRB) convert them to sulfides. Inhibition caused by hydrogen sulfide is, like in the case of ammonia, significantly dependent on the environmental conditions (mainly alkalinity and pH). Hydrogen sulfide can be formed as a product of fermentation of the sludges originated from breweries, wine production industry and distilleries. Some types of waste (particularly protein-rich swine manure) contain, in addition to a high sulfate concentration, a high ammonia amount (Hansen et al., 1999). Others, such as distillery waste, have no "ammonia problem", whereas due to a high concentration of sulfate (6-11 g/dm^3) their digestion still remains a significant challenge (Gadre, 1982 za Ranade et al., 1999). Sulfate is used as electron acceptor of the sulfate reducing bacteria (Petersen and Ahring, 1992, Hao et al., 1996). Moreover, methane production is less favorable energetically than sulfate reduction, which leaves methanogens outcompeted by sulfate reducing bacteria in relation to substrates such as acetate and H_2/CO_2 (Hao et al., 1996) and finally results in conversion of sulfates to sulfides (S^{2-} + HS^- + H_2S). It was reported that sulfides inhibited methanogenesis at concentrations around 50 mg S^{2-}/dm^3, whereas strong inhibition was observed at the level exceeding 150 to 200 mg S^{2-}/dm^3 (Karhadkar et al., 1987). However, Hansen et al. (1999) found that even a small amount of sulfide as 23 mg S_2^-/dm^3 could increase ammonia inhibition at concentration of 4,6 g N/dm^3, which led to approximately 40% decrease of methane production. The batch experiments showed that sulfide was able to intensify inhibition of the methanogenesis in the ammonia–rich systems due to the combined effect of the two inhibitors influencing each other. Furthermore, it was proved that a reduction of the sulfide concentration could counteract an inhibition.

The decrease of sulfide concentration may include hydrogen sulfide gas production and precipitation of sulfides by heavy metals (present as nutrients in the system or providing it with supplements). Increasing the bacterial activity and gas production rates allows to stripping some sulfides from solution (Kayhanian and Rich, 1995). Another possible way to accomplish the minimization of the sulfide content is adsorption onto activated carbon (at concentrations equal to 2,5% w/w or higher) or precipitation as ferrous sulfide by addition of $FeCl_2$ (4,4 mM) (Hansen et al., 1999). In order to diminish an amount of sulfide in the anaerobic digesters a selective inhibition of sulfate reducing bacteria was also considered. Several researchers tested the usefulness of molybdate (MoO_4^{2-}) at doses of 2,5-3,0 mM as a selective inhibitor of sulfate reduction, and thus sulfide production (Isa and Anderson, 2005, Ranade et al., 1999). Although it was shown that sulfate reducing bacteria were severely inhibited both by single and continuous dosing of 3 mM molybdate, the effect was not persistent and declined after a few days (Ranade et al., 1999). Moreover, acetoclastic methanogens were also inhibited, thus the methanogenic activity decreased significantly. Isa and Anderson (2005) reported molybdate at dose of 2,5 mM as unsuitable to selective inhibition, considering both its bacteriocidal influence on the methane producing bacteria and its bacteriostatic impact on sulfate reducing bacteria.

Salinity

The presence of a high sodium or chloride concentration is considered as another inhibitory factor for the digestion process. Salinity inhibition refers mainly to the wastewater and waste from agro-food (particularly fish-farm), tannery, petroleum and leather industries. Kugelman and McCarty (1965) stated that strong inhibition of methanogenesis occurred, when a sodium concentration exceeded 10 g/dm^3. However, in the recent studies it has been proved that the adaptation of an active methanogenic biomass was possible even at a high salinity level when a suitable adapting strategy was applied (Omil et al., 1995).

The performance of the reactors was highly dependent on the nature of the substrates, the antagonistic effects of other ions at adequate concentrations as well as the nature and progressive adaptation of the microbial community to high salinity (Feijoo et al., 1995). Lefebvre et al. (2007) reported a similar level of inhibition (approximately 90% of the specific methanogenic activity) both for the lower NaCl concentration of 10 g/dm^3 and the higher concentration of 60 g/dm^3 with different substrates such as distillery vinasse and ethanol, respectively. Moreover, a similar level of inhibition (reflecting in 50% of the methanogenic activity) was attained for two operational strategies: a continuous-exposure system with a sodium content higher than 20 g/dm^3 and a shock-exposure one at concentration in the range of 6-13 g/dm^3 (Lefebvre and Moletta, 2006).

Biocidal inhibition

Biocidal inhibition means reactive toxicity which has an adverse or sometimes lethal influence on microbial community. The toxic compound reacts (normally irreversibly) with a functional component of a microbial cell, making it non-functional. There are several substances classified as biocidal with regard to some or all anaerobes (especially to methanogenic bacteria). The most typical are xenobiotics (solvents, some surfactants, pesticides, phenols), but there are other compounds like detergents, drugs, dyes and cyanide, which have a similar impact. Moreover, substances containing nitrocompounds, amines, sulphur, halogens, hydroxyl, ketones and other functional groups should be included in the biocidal compounds (Mata-Alvarez, 2003).

Different wastes contain their specific organic contaminants at various levels. Linear alkylbenzene sulphonates (LAS) used in household detergents, nonylphenols (NP), nonylphenol ethoxylates (NPEO), polycyclic aromatic hydrocarbons (PAH) and phtalates have recently been identified as major anthropogenic organic contaminants in sewage sludge. Organic industrial waste from different sources, as well as manure and organic household waste, may contain hazardous components which, even in small amounts, can show an adverse effect on the ecosystem, e.g. farmland amended with sewage sludge. Special attention should be paid to those organic components which may have a potential for acute toxicity, mutagenesis, carcinogenesis, teratogenesis or posses estrogenic effects (Angelidaki et al., 2000).

Surfactants

Linear alkylbenzene sulphonates (LAS) are the most widely used synthetic anionic surfactants, whose concentrations in the primary sewage sludge are high due to their widespread use as domestic detergents and to a strong sorption on the digested sludge (Ying, 2006). Other compounds, such as polycyclic aromatic hydrocarbons (PAH) originating from industrial processes, have a tendency to adsorb on the primary sludge supplying the digesters. On the other hand, dehydrogenated tallow dimethyl ammonium chloride (DTDMAC) is considered to be the most widely used active ingredient in fabric softeners.

Most surfactants are susceptible to an anaerobic degradation although some of them have been found to be persistent under the conditions in question and have an inhibitory effect particularly on the methanogenic microorganisms. The following substances can be included in the non-degradable or poorly-degradable group: anionic surfactants such as linear alkylbenzene sulphonates (LAS) and secondary alkane sulphonates (SAS), nonionic surfactants such as fatty acid esters (FES) and alkylphenol ethoxylates (NPEO), and the cationic surfactant dehydrogenated tallow dimethyl ammonium chloride (DTDMAC) (Garcia et al., 1999, Angelidaki et al., 2000, Ying, 2006). It was shown that the inhibition caused by surfactants had a biocidal nature and was not affected by inactivation of extracellular hydrolytic enzymes (Feltkenhauer, 2004).

The LAS concentrations ranging from 5 to 15 g/kg dry weight have been observed in anaerobically degraded sewage sludge (Jensen, 1999), however in some cases even a concentration of 30 g/kg dry weight has caused any inhibition (Ying, 2006). The LAS toxicity can be diminished by their sorption on anaerobic sludge, which reduces the available fraction of the surfactant in liquid phase. It was shown by Garçia et al. (2006) that LAS toxicity was closely dependent on its fraction in the aqueous phase, moreover the surfactant content on sludge was related to the total amount of calcium and magnesium extractable ions. The presence of divalent cations probably promoted the association of LAS with anaerobic sludge, decreasing their bioavailability and inhibitory effect on the biogas production (Garcia et al., 2006). Gavala and Ahring (2002) examined an inhibitory effect of LAS on the acetogenesis and methanogenesis. It was shown that LAS inhibited acetogenesis from propionate and methanogenesis from acetate and hydrogen. The results indicated propionate-utilizing bacteria to be more sensitive to the presence of LAS than acetoclastic methanogens. The upper allowable biomass-specific LAS concentration was established at the level of 14 mg LAS/g volatile suspended solids (Gavala and Ahring, 2002). Similarly, Mosche and Meyer (2002) reported that the concentrations of LAS such as 14 mg/dm^3 and 27 mg/dm^3 caused a 50% immediate inhibition of acetate and propionate degradation, respectively. Moreover, the decay rates of acetate and propionate degradation activity were increased by a factor of 10 for each 12 mg/dm^3 and 23 mg/dm^3 of surfactant, respectively.

Alkylphenol ethoxylates (NPEO) are evidently less biodegradable than LAS and only partial conversion of NPEO molecules takes place, probably due to the recalcitrance of the intermediates forming as a result of the degradation (Brunner et al., 1988). Other compounds, such as polycyclic aromatic hydrocarbons (PAH), are also included in poorly degradable substances. In sewage sludge high contents of these contaminants of up to 2000 mg/kg of dry mass were found (Bodzek et al., 1997). Moreover, an accumulation of such compounds was observed in the digested sludge, resulting in poor degradation ability of PAH under anaerobic conditions. Angelidaki et al. (2000) reported anaerobic sludge to reveal a great potential for biological degradation of LAS, PAH and NPEO providing that a proper choice of the inoculum is made, ensuring a richness of microorganisms capable of transforming the recalcitrant xenobiotics.

Dehydrogenated tallow dimethyl ammonium chloride (DTDMAC) has been found in digested sludge at such a high concentrations as 5870 mg/kg (Ying, 2006). The cationic surfactant in question containing a quaternary ammonium (e.g. R_4N^+; where R = alkyl chain and N = quaternary nitrogen) has a strong biocidal nature (Baleux and Caumette, 1977). Moreover, no degradation of DTDMAC is observed in anaerobic screening tests (Garcia et al., 2000) and its presence in the digesters at high concentrations can cause a strong inhibition of the anaerobic microorganisms.

Pharmaceuticals

Pharmaceuticals, among them antibiotics, are present in municipal sewage as a result of their common use in human medical care. Agents in question have a tendency to adsorb both to the primary sludge and excess activated sludge, thus by entering the anaerobic digesters they may inhibit biomethanization process (Fountoulakis et al., 2004, Gartiser et al., 2007). It was recognized that some pharmaceuticals cause a mild or moderate inhibition, reflected particularly in a decrease of the activity of acetoclastic methanogens. However, there are a few antibiotics which may cause strong or even complete inhibition of acetoclastic methanogenic bacteria and, as a result, disturb or damage the biogas production. Sanz et al. (1996) investigated 15 antimicrobial agents with different specificities and modes of action. The results showed that the examined compounds could be divided into three groups. The agents belonging to the first one, such as macrolide erythromycin, seem to have no inhibitory effect on the biogas production. However, antibiotics classified into the second category (especially aminoglycosides), which interfere with cell wall synthesis, RNA polymerase activity and protein synthesis, partially inhibit the biomethanization, decreasing methane production by limiting the activity of propionic-acid- and butyric-acid-degrading bacteria. Finally, the third group of antibiotics can be distinguished. It includes extremely powerful agents, among them protein synthesis inhibitors such as chlorotetracycline with toxicity expressed by the value of IC_{50} 40 mg/dm^3 (i.e. the concentration at which bioactivity is 50% of the control probe) and chloramphenicol characterized by the value of IC_{50} 15-20 mg/dm^3. The majority of the antibiotics tested have been active only on the acetogenic bacteria. However, chloramphenicol and chlorotetracycline are found to completely inhibit the acetoclastic methanogenic bacteria. Gartiser et al. (2007) used ISO standards 13641 (2003) and 11734 (1995) for assessing the anaerobic inhibition of 16 and the anaerobic biodegradability of 9 antibiotics, respectively. It was recognized that most antibiotics showed only moderate inhibition effects after a 7-day incubation period, with EC_{50} values (i.e. concentration having 50% of effect compared to control) between 24 mg/dm^3 and more than 1000 mg/dm^3 (equal to mg/g dry weight). In contrast, metronidazol was decisively toxic to anaerobic bacteria with the value EC_{50} of 0,7 mg/dm^3. In the anaerobic biodegradability test only benzylpenicillin showed some ultimate biodegradation after 60 days, whereas most antibiotics had an inhibitory effect on the digesting sludge (Gartiser et al., 2007).

CONCLUSIONS

Some technological solutions leading to the stabilization of the biomethanization process are suggested. They include co-fermentation, investigated recently in various configurations with regard to the sewage sludge, OFMSW, animal manure and other wastes, addition of chemical substances either to eliminate excess amount of ammonia or hydrogen sulfide or to improve nutrient balance in the system, and finally acclimatization of methanogenic consortia for low pH environment, high ammonia concentrations or other potentially inhibitory substances.

Considering the general problems related to increased methane production, co-digestion now seems to be an especially promising solution. It leads to an increase in the biogas yields and the loads of biodegradable organic matter, improves the nutrient balance, dilutes potential toxic compounds and allows detoxification based on the co-metabolism process (possible removal of some xenobiotics), giving a good quality fertilizer.

Another solution consists in supplementing such nutrients as Ca, Fe, Co and Ni, increasing the rate of acidogenesis and methanogenesis and effectively of gas production.

Addition of chosen substances may restrict ammonia or hydrogen sulfide inhibition. Phosphorite ore immobilizes methanogens on the mineral grains or exchanges ammonium ions for divalent cations. Inorganic additives, such as natural zeolite (consisting of clinoptylolite, modernite, montmorillonite), bentonite clay and glauconite are also suitable for ammonia removal. To enhance methane production from ammonia-rich sludge, natural modernite should be used due to its synergistic effect on Ca^{2+} supply and NH_4^+ removal. Air stripping and conversion of ammonia to struvite also restrict ammonia inhibition. On the other hand, precipitation of sulfides by heavy metals or $FeCl_2$ addition, adsorption onto activated carbon and selective inhibition of sulfate reducing bacteria all decrease sulfide concentration.

The most interesting direction of future research is an examination of the system that combines both the heterotrophic conversion of acetic acid and the autotrophic conversion of CO_2/H_2. A mixed culture of methanogens, which have the ability to acclimatize and tolerate a much lower pH than the neutral one, have to be engaged. Such an approach seems to be especially promising for improving the quality and amount of the biogas.

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