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ScienceDirect
Bioresource Technology 99 (2008) 4044–4064
BIORESOURCE TECHNOLOGY

Review

Inhibition of anaerobic digestion process: A review

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Received 5 April 2005; received in revised form 25 January 2007; accepted 25 January 2007
Available online 30 March 2007

Abstract

Anaerobic digestion is an attractive waste treatment practice in which both pollution control and energy recovery can be achieved. Many agricultural and industrial wastes are ideal candidates for anaerobic digestion because they contain high levels of easily biodegradable materials. Problems such as low methane yield and process instability are often encountered in anaerobic digestion, preventing this technique from being widely applied. A wide variety of inhibitory substances are the primary cause of anaerobic digester upset or failure since they are present in substantial concentrations in wastes. Considerable research efforts have been made to identify the mechanism and the controlling factors of inhibition. This review provides a detailed summary of the research conducted on the inhibition of anaerobic processes. The inhibitors commonly present in anaerobic digesters include ammonia, sulfide, light metal ions, heavy metals, and organics. Due to the difference in anaerobic inocula, waste composition, and experimental methods and conditions, literature results on inhibition caused by specific toxicants vary widely. Co-digestion with other waste, adaptation of microorganisms to inhibitory substances, and incorporation of methods to remove or counteract toxicants before anaerobic digestion can significantly improve the waste treatment efficiency.

Keywords: Anaerobic digestion; Agricultural wastes; Industrial wastes; Inhibition; Municipal wastes

1. Introduction

Anaerobic digestion involves the degradation and stabilization of organic materials under anaerobic conditions by microbial organisms and leads to the formation of biogas (a mixture of carbon dioxide and methane, a renewable energy source) and microbial biomass (Kelleher et al., 2000). Anaerobic treatment provides a method of reducing pollution from agricultural and industrial operations while at the same time offsetting the operations' usage of fossil fuels. As one of the most efficient waste and wastewater treatment technologies, anaerobic digestion has been widely used for the treatment of municipal sludge and limited application in the treatment of organic industrial wastes including fruit and vegetable processing wastes, packinghouse wastes, and agricultural wastes (Parkin and Miller, 1983). Anaerobic digestion offers numerous significant advantages, such as low sludge production, low energy requirement, and possible energy recovery (Ghosh and Pohland, 1974; van Staikenburg, 1997). Compared to mesophilic digestion, thermophilic anaerobic digestion has additional benefits including a high degree of waste stabilization, more thorough destruction of viral and bacterial pathogens, and improved post-treatment sludge dewatering (Lo et al., 1985). In spite of these benefits, however, poor operational stability still prevents anaerobic digestion from being widely commercialized (Dupla et al., 2004).

In anaerobic digestion, the acid forming and the methane forming microorganisms differ widely in terms of physiology, nutritional needs, growth kinetics, and sensitivity to environmental conditions (Pohland and Ghosh, 1971). Failure to maintain the balance between these two groups of microorganisms is the primary cause of reactor instability (Demirel and Yenigün, 2002). Inhibitory substances are often found to be the leading cause of anaerobic reactor upset and failure since they are present in substantial concentrations in wastewaters and sludges. A wide variety of

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0960-8524/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2007.01.057

Nomenclature

CSTR	continuously stirred tank reactor
FA	free ammonia
HRT	hydraulic retention time
IC_{50}, IC_{90}, IC_{100}	the toxicant concentration that causes 50%, 90%, and 100% reduction in cumulative methane production, respectively, over a fixed period of exposure time
LCFAs	long chain fatty acids
MPB	methane producing bacteria
SRB	sulfate reducing bacteria
TAN	total ammonia nitrogen
VFAs	volatile fatty acids
UASB	upflow anaerobic sludge blanket reactor

substances have been reported to be inhibitory to the anaerobic digestion processes. A material may be judged inhibitory when it causes an adverse shift in the microbial population or inhibition of bacterial growth. Inhibition is usually indicated by a decrease of the steady-state rate of methane gas production and accumulation of organic acids (Kroeker et al., 1979).

The aim of this review is to present a detailed comparative summary of the previous and current research on the inhibition of anaerobic processes by various inorganic and organic substances, focusing on: (1) mechanisms of inhibition, (2) factors affecting inhibition, and (3) common operating problems encountered in waste treatment processes.

2. Inhibitors

Literature on anaerobic digestion shows considerable variation in the inhibition/toxicity levels reported for most substances. The major reason for these variations is the complexity of the anaerobic digestion process where mechanisms such as antagonism, synergism, acclimation, and complexing could significantly affect the phenomenon of inhibition.

2.1. Ammonia

Ammonia is produced by the biological degradation of the nitrogenous matter, mostly in the form of proteins and urea (Kayhanian, 1999). The quantity of ammonia that will be generated from an anaerobic biodegradation of organic substrate can be estimated using the following stoichiometric relationship (Tchobanoglous et al., 1993):

$$C_aH_bO_cN_d + \frac{4a-b-2c+3d}{4}H_2O \rightarrow \frac{4a+b-2c-3d}{8}CH_4 + \frac{4a-b+2c+3d}{8}CO_2 + dNH_3 \quad (1)$$

Several mechanisms for ammonia inhibition have been proposed, such as a change in the intracellular pH, increase of maintenance energy requirement, and inhibition of a specific enzyme reaction (Whittmann et al., 1995). Ammonium ion (NH_4^+) and free ammonia (FA) (NH_3) are the two principal forms of inorganic ammonia nitrogen in aqueous solution. FA has been suggested to be the main cause of inhibition since it is freely membrane-permeable (Kroeker et al., 1979; de Baere et al., 1984). The hydrophobic ammonia molecule may diffuse passively into the cell, causing proton imbalance, and/or potassium deficiency (Sprott and Patel, 1986; Gallert et al., 1998).

Among the four types of anaerobic microorganisms, the methanogens are the least tolerant and the most likely to cease growth due to ammonia inhibition (Kayhanian, 1994). As ammonia concentrations were increased in the range of 4051–5734 mg NH_3–N L^{-1}, acidogenic populations in the granular sludge were hardly affected while the methanogenic population lost 56.5% of its activity (Koster and Lettinga, 1988). There is conflicting information in the literature about the sensitivity of aceticlastic and hydrogenotrophic methanogens. Some research based on the comparison of methane production and growth rate indicated that the inhibitory effect was in general stronger for the aceticlastic than for the hydrogenotrophic methanogens (Koster and Lettinga, 1984; Zeeman et al., 1985; Sprott and Patel, 1986; Bhattacharya and Parkin, 1989; Robbins et al., 1989; Angelidaki and Ahring, 1993; Borja et al., 1996a), while others observed the relatively high resistance of acetate consuming methanogens to high total ammonia nitrogen (TAN) levels as compared to hydrogen utilizing methanogens (Zeeman et al., 1985; Wiegant and Zeeman, 1986). Among the methanogenic strains commonly isolated from sludge digesters, i.e. *Methanospirillum hungatei*, *Methanosarcina barkeri*, *Methanobacterium thermoautotrophicum*, and *Methanobacterium formicicum*, *Methanospirillum hungatei* was the most sensitive, being inhibited at 4.2 g/L; the other three strains tested were resistant to ammonia levels higher than 10 g/L (Jarrell et al., 1987).

2.1.1. Factors controlling ammonia inhibition

2.1.1.1. Concentration. It is generally believed that ammonia concentrations below 200 mg/L are beneficial to anaerobic process since nitrogen is an essential nutrient for anaerobic microorganisms (Liu and Sung, 2002). A wide range of inhibiting ammonia concentrations has been reported in the literature, with the inhibitory TAN concentration that caused a 50% reduction in methane production ranging from 1.7 to 14 g/L (Kroeker et al., 1979; van Vel-

sen, 1979; Braun et al., 1981; Parkin and Miller, 1983; de Baere et al., 1984; Zeeman et al., 1985; Hashimoto, 1986; Jarrell et al., 1987; Koster and Lettinga, 1988; Bhattacharya and Parkin, 1989; Hendriksen and Ahring, 1991; Angelidaki and Ahring, 1993; Angelidaki and Ahring, 1994; Soubes et al., 1994; Kayhanian, 1994; Borja et al., 1996b; Boardman and McVeigh, 1997; Gallert and Winter, 1997; Guerrero et al., 1997; Krylova et al., 1997; Poggi-Varaldo et al., 1997; Chamy et al., 1998; Gallert et al., 1998; Hansen et al., 1998; Bujoczek et al., 2000; Sung and Liu, 2003). The significant difference in inhibiting ammonia concentration can be attributed to the differences in substrates and inocula, environmental conditions (temperature, pH), and acclimation periods (van Velsen et al., 1979; de Baere et al., 1984; Hashimoto, 1986; Angelidaki and Ahring, 1994).

2.1.1.2. pH. During treatment of waste containing high concentrations of TAN, pH affects the growth of microorganisms as well as the composition of TAN (Kroeker et al., 1979; Hashimoto, 1983, 1984; Hansen et al., 1999). Since the FA form of ammonia has been suggested to be the actual toxic agent, an increase in pH would result in increased toxicity (Borja et al., 1996b) because of the shift to a higher FA to ionized (NH_4^+) ammonia ratio at higher pH. Process instability due to ammonia often results in volatile fatty acids (VFAs) accumulation, which again leads to a decrease in pH and thereby declining concentration of FA. The interaction between FA, VFAs and pH may lead to an ''inhibited steady state'', a condition where the process is running stably but with a lower methane yield (Angelidaki and Ahring, 1993; Angelidaki et al., 1993).

Control of pH within the growth optimum of microorganisms may reduce ammonia toxicity (Bhattacharya and Parkin, 1989). Acidification of crab wastewater has been reported to enhance UASB reactor performance, as indicated by the lower effluent COD concentration (Boardman and McVeigh, 1997). Reducing pH from 7.5 to 7.0 during thermophilic anaerobic digestion of cow manure also increased the methane production by four times (Zeeman et al., 1985). During anaerobic digestion of liquid piggery manure (pH 8), VFAs accumulated to 316 mg/L. Adjustment of pH to 7.4 led to reutilization of VFAs and lowered VFAs concentrations to 20 mg/L. The better performance at pH 7.4 has been attributed to the relief of ammonia-induced inhibition at low pH (Braun et al., 1981). It should also be noted that both methanogenic and acidogenic microorganisms have their optimal pH. Failing to maintain pH within an appropriate range could cause reactor failure although ammonia is at a safe level (Kroeker et al., 1979).

2.1.1.3. Temperature. Both microbial growth rates and FA concentration are affected by temperature change. An increased process temperature in general has a positive effect on the metabolic rate of the microorganisms but also results in a higher concentration of FA. Several authors have found that anaerobic fermentation of wastes with a high concentration of ammonia was more easily inhibited and less stable at thermophilic temperatures than at mesophilic temperatures (Braun et al., 1981; Parkin and Miller, 1983). Thermophilic digestion at 50 °C of cow manure with TAN above 3 g/L was found to be very difficult (Hashimoto, 1983). A decrease in operating temperature from 60 °C to 37 °C in anaerobic digesters with a high ammonia concentration provided relief from inhibition caused by FA, as indicated by an increase in biogas yield (Angelidaki and Ahring, 1994; Hansen et al., 1999). Contrary to these findings, Gallert and Winter (1997) studied the anaerobic digestion of organic wastes and reported that methane production was inhibited 50% by 0.22 g/L FA at 37 °C and by 0.69 g/L FA at 55 °C, indicating that thermophilic flora tolerated at least twice as much FA as compared to mesophilic flora.

2.1.1.4. Presence of other ions. Certain ions such as Na^+, Ca^{2+}, and Mg^{2+} were found to be antagonistic to ammonia inhibition, a phenomenon in which the toxicity of one ion is decreased by the presence of other ion(s) (McCarty and McKinney, 1961; Braun et al., 1981; Hendriksen and Ahring, 1991). Ammonia and sodium showed mutual antagonism, a situation where each ion can antagonize the toxicity produced by another ion. While 0.15 M ammonia reduced the methane production from acetic acid by 20%, addition of 0.002–0.05 M Na^+ produced 5% more methane compared to that from the control (a sample without addition of inhibitor). Combination of Na^+ and K^+ or Na^+ and Mg^{2+} resulted in around 10% increase in methane yield compared to that produced by Na^+ alone (Kugelman and McCarty, 1964). The addition of 10% (w/v) phosphorite ore was also reported to stimulate biogas generation from poultry manure when NH_4^+Cl was as high as 30 g/L (Krylova et al., 1997). This stimulation effect of phosphorite can be partially attributed to the immobilization of the biomass on mineral particles, which prevented biomass washout from the reactor. Alleviation of ammonia inhibition was also thought to be partially due to the antagonistic effect provided by minerals in the phosphorite ore (K^+, Ca^{2+}, Mg^{2+}). However, inhibition caused by more than 50 g/L of NH_4^+Cl was irreversible and could not be eliminated by addition of phosphorite (Krylova et al., 1997).

2.1.1.5. Acclimation. Acclimation is another factor that can influence the degree of ammonia inhibition. One of the first reports dealing with adaptation of methanogens to ammonia by exposing them to slowly increasing concentrations was the sludge digestion study of Melbinger and Donnellon (1971). At present, adaptation of methanogens to a wide variety of potentially inhibitory substances has been reported (Parkin and Miller, 1983; Speece, 1983; Speece and Parkin, 1983). The adaptation may be the result of internal changes in the predominant species of methanogens, or of a shift in the methanogenic population (Zeeman et al., 1985).

Once adapted, the microorganisms can retain viability at concentrations far exceeding the initial inhibitory concentrations (Kroeker et al., 1979; Parkin and Miller, 1983; Bhattacharya and Parkin, 1989; Angelidaki and Ahring, 1993). Koster and Lettinga (1988) reported that while unacclimated methanogens failed to produce methane at 1.9–2 g N/L, they produced methane at 11 g N/L after adaptation. Hashimoto (1986) observed that ammonia inhibition began at about 2.5 g/L and 4 g/L for unacclimated and acclimated thermophilic methanogens, respectively. Successful operation of anaerobic filters has been achieved at 6 g/L and 7.8 g/L after adaptation (Parkin et al., 1983; de Baere et al., 1984). Parkin and Miller (1983) reported that levels as high as 8–9 g/L of TAN could be tolerated with no significant decrease in methane production after acclimation. The experiments clearly demonstrated the possibility of obtaining stable digestion of manure with ammonia concentrations exceeding 5 g N/L after an initial adaptation period. However, the methane yield was lower than that for reactors with a lower ammonia load (Koster and Lettinga, 1988; Borja et al., 1996a).

2.1.2. Methods to counteract ammonia inhibition

To remove ammonia from the substrate, two physical–chemical methods can be utilized: air stripping and chemical precipitation. Both have been proven to be technically feasible at high ammonia concentrations and in a complex wastewater matrix (Kabdasli et al., 2000). A common approach to ammonia inhibition relies on dilution of the manure to a total solid level of 0.5–3.0%. However, the resulting increase in waste volume that must be processed makes this method economically unattractive (Callaghan et al., 1999).

Various types of inhibition can be counteracted by increasing the biomass retention in the reactor. It was found that the methane yield in a CSTR could be increased by switching off the stirrer half an hour before and after substrate addition. This operation increased biomass retention due to improved sedimentation resulting in an effluent with a reduced concentration of biomass solids. This method, where particles within the reactor were allowed to settle, was promising since it was easy and economical to achieve (Hansen et al., 1998). Immobilizing the microorganisms with different types of inert material (clay, activated carbon, zeolite) has been demonstrated to reduce inhibition of the biogas process and make the process more stable (Angelidaki et al., 1990; Nakhla et al., 1990; Borja et al., 1993; Hanaki et al., 1994; Hansen et al., 1998). Addition of ionic exchangers or adsorbants which can remove inhibitors mitigates the ammonia inhibition (Borja et al., 1996a). Natural zeolite and glauconite show high selectivity for ammonium ion and can be used as an ionic exchanger for ammonia (Borja et al., 1996a; Hansen et al., 1998). When treating swine manure, addition of activated carbon at concentrations equal to 2.5% (w/w) or higher or $FeCl_2$ removed most of the sulfide in solution. Although activated carbon did not adsorb ammonia, it reduced inhibition of ammonia by removing sulfide, which otherwise would act synergistically with ammonia (Hansen et al., 1999). Addition of antagonistic cations such as Mg^{2+} or Ca^{2+} stabilizes anaerobic degradation (McCarty and McKinney, 1961). The positive effect of zeolite on the anaerobic process could partially be attributed to the presence of cations such as Ca^{2+} and Na^{+} that have been shown to counteract the inhibitory effect of ammonia (Borja et al., 1996a).

2.2. Sulfide

Sulfate is a common constituent of many industrial wastewaters (O'Flaherty et al., 1998a). In anaerobic reactors, sulfate is reduced to sulfide by the sulfate reducing bacteria (SRB) (Koster et al., 1986; Hilton and Oleszkiewicz, 1988). Sulfate reduction is performed by two major groups of SRB including incomplete oxidizers, which reduce compounds such as lactate to acetate and CO_2, and complete oxidizers, which completely convert acetate to CO_2 and HCO_3^-. Two stages of inhibition exist as a result of sulfate reduction. Primary inhibition is due to competition for common organic and inorganic substrates from SRB, which suppresses methane production (Harada et al., 1994). Secondary inhibition results from the toxicity of sulfide to various bacteria groups (Anderson et al., 1982; Oude Elferink et al., 1994; Colleran et al., 1995; Colleran et al., 1998).

2.2.1. Competition of SRB and other anaerobes

SRB are very diverse in terms of their metabolic pathways (Oude Elferink et al., 1994). Compounds which can be completely or partially degraded by SRB include branched-chain and long chain fatty acids, ethanol and other alcohols, organic acids, and aromatic compounds (Oude Elferink et al., 1994). Laanbroek et al. (1984) ranked the affinity of SRB for reduced substrates in the order of H_2 > propionate > other organic electron donors. Because of the variety in substrate utilization exhibited by SRB, they compete with several different types of microorganisms involved in anaerobic digestion. SRB may compete with methanogens, acetogens, or fermentative microorganisms for available acetate, H_2, propionate, and butyrate in anaerobic systems (McCartney and Oleszkiewicz, 1993; Colleran et al., 1995).

The outcome of the competition between SRB and other anaerobic microorganisms determines the concentration of sulfide in the reactor system. Sulfide is toxic to methanogens as well as to the SRB themselves (Winfrey and Zeikus, 1977; Karhadkar et al., 1987; McCartney and Oleszkiewicz, 1991; Reis et al., 1992; Okabe et al., 1995). Thus the concentration of sulfide and the susceptibility of anaerobes to sulfide feed back into the competition between SRB and other anaerobes.

2.2.1.1. Competition between SRB and hydrolytic and acidogenic bacteria. SRB do not degrade natural biopolymers such as starch, glycogen, protein, or lipids and thus

depend on the activity of other organisms for providing them with degradation products (Hansen, 1993). Consequently, competition does not occur in the hydrolysis stage. Although a few strains of SRB have been shown to utilize sugars and amino acids as substrate (Klemps et al., 1985; Min and Zinder, 1990), vigourous growth of SRB on typical acedogenic substrates is not common (Hansen, 1993). It is generally agreed that SRB cannot effectively compete against the fast-growing fermentative microorganisms involved in monomer degradation (Postgate, 1984). O'Flaherty et al. (1999) conducted tests to detect SRB in an anaerobic digester fed with glucose and lactose. No change of their degradation rates was detected upon addition of sulfate, indicating that SRB species did not play any substantial role in the degradation of glucose and lactose.

2.2.1.2. Competition between SRB and acetogens. From a purely thermodynamic and kinetic standpoint, SRB should out-compete other anaerobes for substrate (Oude Elferink et al., 1994; Colleran et al., 1995; O'Flaherty et al., 1998a). In practice, however, factors such as COD/SO_4^{2-} ratio, the relative population of SRB and other anaerobes, and the sensitivity of SRB and other anaerobes to sulfide toxicity influence the competition. As a result, the literature on anaerobic digestion of sulfate-containing wastewaters is highly complex and often contradictory.

Propionate is a key intermediate in anaerobic digestion and a substrate for all SRB. Degradation of propionate by SRB involves an incomplete conversion to acetate (O'Flaherty et al., 1998a). SRB show a higher affinity for propionate and faster growth rates than the propionate-utilizing syntrophic species (Parkin et al., 1990; Uberoi and Bhattacharya, 1995; Omil et al., 1996a). The K_s and μ_{max} values for SRB were 0.15 d^{-1} and 23 mg/L in full scale anaerobic digester while K_s and μ_{max} values for syntrophic bacteria were 0.05 d^{-1} and 34 mg/L, respectively (O'Flaherty et al., 1997, 1998b). As a result, sulfidogenic oxidation of propionate should be favored over the syntrophic route (Colleran et al., 1995). Several studies using various anaerobic systems and sludges have confirmed the importance of SRB in the degradation of propionate, indicating that sufidogenic oxidation is the key degradation pathway of this substrate (Mulder, 1984; Ukei et al., 1988; Qatibi et al., 1990; Hepner et al., 1992; Colleran et al., 1994, 1998; O'Flaherty et al., 1997, 1998a).

Butyrate and ethanol are also important fermentation intermediates in anaerobic digestion. Butyrate was utilized exclusively by SRB in a UASB reactor fed mixed VFAs and sucrose ($COD/SO_4^{2-} = 0.5$) (Visser et al., 1993). In another hybrid reactor, both sulfidogenic and methanogenic anaerobes were present at a COD/SO_4^{2-} ratio of 3 and 5.6, indicating that competition occurred among SRB and other anaerobes for butyrate and ethanol (Colleran et al., 1998; O'Flaherty et al., 1998a). The effective competition of non-SRB was attributed to the lower affinity of SRB for butyrate and ethanol (Laanbroek et al., 1984; Overmeire et al., 1994).

2.2.1.3. Competition between SRB and hydrogenotrophic methanogens. From thermodynamic and substrate affinity considerations, H_2-oxidizing SRB should effectively outcompete hydrogenotrophic methanogens under the conditions prevailing in anaerobic digesters (Zinder, 1993). This view was supported by data reported previously indicating that in reactors treating sulfate-containing wastewaters, H_2 oxidation is almost exclusively catalyzed by SRB (Rinzema and Lettinga, 1988; Visser et al., 1993; Alphenaar et al., 1993; Harada et al., 1994; Uberoi and Bhattacharya, 1995; Omil et al., 1996a; Colleran et al., 1998; O'Flaherty et al., 1999). Methanogenesis appeared to occur simultaneously with sulfate-reduction, but methanogens could not compete for H_2 with the SRB (Oremland and Taylor, 1978). The predominance of SRB in H_2 utilization has been related to the more favorable kinetic parameters for SRB. Hydrogenotrophic SRB have a lower hydrogen threshold concentration than hydrogenotrophic methanogens (Oude Elferink et al., 1994; Colleran et al., 1995). Temperature has been reported to impact the outcome of the competition between SRB and hydrogenotrophic methane producing bacteria (MPB). SRB were dominant at mesophilic condition (37 °C), while MPB outcompeted SRB at thermophilic conditions (55 °C). An explanation for this difference was not offered (Colleran and Pender, 2002).

2.2.2. Competition between SRB and aceticlastic methanogens

Literature data on the outcome of competition between SRB and MPB for acetate are contradictory, with some authors reporting successful competition of SRB (Rinzema and Lettinga, 1988; Alphenaar et al., 1993; Stucki et al., 1993; Gupta et al., 1994), whereas others reported dominance of MPB (Rinzema et al., 1988; Isa et al., 1986a,b; Visser et al., 1993; Omil et al., 1996a; Oude Elferink et al., 1994; Colleran et al., 1998; O'Flaherty et al., 1998a; De Smul et al., 1999; Colleran and Pender, 2002).

Various mechanisms have been proposed to explain the observed discrepancies. Choi and Rim (1991) attributed the outcome of the competition to the COD/SO_4^{2-} ratio. Aceticlastic MPB predominated when the COD/SO_4^{2-} was above 2.7; SRB predominated when this ratio was below 1.7. Active competition occurred between these ratios. O'Flaherty et al. (1998b) correlated the performance of MPB and SRB to the different growth properties at different pH values. Oude Elferink et al. (1994) observed that the initial population of SRB played a role in the competition between SRB and MPB. They calculated that by starting with a ratio of aceticlastic MPB/SRB of 10^4:1 and with a biomass retention time in the reactor of 0.02 d^{-1}, it would take one year before the number of SRB equaled that of the MPB in the reactor.

Isa et al. (1986a,b) attributed the successful competition of MPB to their superior attachment capabilities. In fixed-film reactors, better attachment of microorganisms can effectively prevent biomass washout (Omil et al., 1996a).

Colleran and Pender (2002) concluded that aceticlastic methanogens predominated because SRB have a lower affinity for acetate than for other substrates. Under sulfate-limiting conditions, acetate was believed to be the least favored substrate for sulfate reduction (Uberoi and Bhattacharya, 1995). However, the dominance of SRB in acetate degradation was attributed to the kinetic advantages of SRB over MPB (Rinzema and Lettinga, 1988; Gupta et al., 1994; Harada et al., 1994). Alphenaar et al. (1993) attributed the higher extent of organic removal by SRB to the long HRT used in the UASB/CSTR reactor, which led to the washout of the dispersed growing MPB.

2.2.3. Sulfide inhibition towards different trophic groups

There is considerable confusion in the literature with respect to the nature of sulfide toxicity and the effect of different sulfides on microorganisms. Tursman and Cork (1988) reported that H_2S was the toxic form of sulfide since it can diffuse into the cell membrane. Once inside the cytoplasm, H_2S may be inhibitory by denaturing native proteins through the formation of sulfide and disulfide cross-links between polypeptide chains (Conn et al., 1987), interfering with the various coenzyme sulfide linkages, and interfering with the assimilatory metabolism of sulfur (Vogels et al., 1988). This theory was supported by the studies of Speece (1983). By contrast, McCartney and Oleszkiewicz (1991) observed that sulfide toxicity increased with increasing pH. Other studies on sulfide inhibition indicated that more than one inhibition threshold might be present under different conditions. Koster et al. (1986) observed a high correlation between the unionized sulfide concentration and the maximum specific aceticlastic methanogenic activity in the pH range of 6.4–7.2. At pH 7.8–8.0, total sulfide concentration dictated the degree of inhibition. O'Flaherty et al. (1998b) observed that sulfide inhibition for all of the groups of bacteria was related to the unionized sulfide concentration in the pH range of 6.8–7.2 and total sulfide concentrations above pH 7.2. Hilton and Oleszkiewicz (1990) showed that inhibition of SRB and MPB was correlated with the total sulfide and unionized sulfide concentration, respectively.

There is also considerable discrepancy in the literature with respect to the levels of sulfide which can cause inhibition to various trophic groups and which steps of the anaerobic transformation are most adversely affected by sulfide. Much of the data reported in the literature was obtained by adding sulfide to a system rather than by feeding sulfate. Thus, the interaction between SRB and non-SRB was not considered (Parkin et al., 1990). In addition, information on pH was rarely included, making it impossible to draw reliable conclusions on the inhibition concentrations. The inhibitory sulfide levels reported in the literature were in the range of 100–800 mg/L dissolved sulfide or approximately 50–400 mg/L undissociated H_2S (Parkin et al., 1990). Fermentative microorganisms which are responsible for the breakdown of monomers into smaller products were less affected by sulfide toxicity than SRB or MPB (McCartney and Oleszkiewicz, 1991; Maillacheruvu et al., 1993). Acetogens were found to be less susceptible to sulfide inhibition than MPB; toxicity thresholds for acetogens were comparable with those of the SRB (O'Flaherty et al., 1998b).

Sulfur is a required nutrient for methanogens (O'Flaherty et al., 1999). It has been shown that the sulfur content of methanogens was higher than in other groups of microorganisms generally found in anaerobic systems (Speece, 1983). The optimal level of sulfur reported in the literature varies from 1 to 25 mg S/L (Scherer and Sahm, 1981). The levels reported in the literature for inhibition of MPB also vary, with IC_{50} values of 50–125 mg H_2S/L at pH 7–8 for suspended sludge and 250 mg H_2S/L and 90 mg H_2S/L at pH 6.4–7.2 and pH 7.8–8.0, respectively (Parkin et al., 1983; Koster et al., 1986; Oleskiewicz et al., 1989; McCartney and Oleszkiewicz, 1993; Maillacheruvu et al., 1993; O'Flaherty et al., 1998a).

2.2.4. Sulfate/sulfide toxicity control

Several processes can be applied to promote the removal of dissolved sulfate. One method to prevent sulfide toxicity is to dilute the wastewater stream, although in general this approach is considered undesirable because of the increase in the total volume of wastewater that must be treated. An alternative way to reduce the sulfide concentration in an anaerobic treatment system is by incorporating a sulfide removal step in the overall process. Sulfide removal techniques include physico-chemical techniques (stripping), chemical reactions (coagulation, oxidation, precipitation), or biological conversions (partial oxidation to elemental sulfur) (Oude Elferink et al., 1994; Song et al., 2001). Adaptation of the MPB to free H_2S, particularly in reactors with fixed biomass, could increase the tolerance of MPB to sulfide. Isa et al. (1986a) reported that acclimated aceticlastic and hydrogenotrophic MPB were only slightly inhibited at more than 1000 mg/L free H_2S.

2.3. Light metals ions (Na, K, Mg, Ca, and Al)

Salt toxicity has been studied in the biological field for several decades. High salt levels cause bacterial cells to dehydrate due to osmotic pressure (de Baere et al., 1984; Yerkes et al., 1997). Although the cations of salts in solution must always be associated with the anions, the toxicity of salts was found to be predominantly determined by the cation (McCarty and McKinney, 1961). The light metal ions including sodium, potassium, calcium, and magnesium are present in the influent of anaerobic digesters. They may be released by the breakdown of organic matter (such as biomass), or added as pH adjustment chemicals (Grady et al., 1999). They are required for microbial growth and, consequently, affect specific growth rate like any other nutrient. While moderate concentrations stimulate microbial growth, excessive amounts slow down the growth, and even higher concentrations can cause severe inhibition or toxicity (Soto et al., 1993a).

2.3.1. Aluminum

Information in the literature about the effect of aluminum on anaerobic digestion is minimal. The mechanism of aluminum inhibition was reported to be due to its competition with iron and manganese or to its adhesion to the microbial cell membrane or wall, which may affect microbial growth (Cabirol et al., 2003). Both acetogenic and methanogenic microorganisms were inhibited by the addition of $Al(OH)_3$. After exposed to 1000 mg/L $Al(OH)_3$ for 59 days, the specific activity of methanogenic and acetogenic microorganisms decreased by 50% and 72%, respectively (Cabirol et al., 2003). Jackson-Moss and Duncan (1991) reported that 2,500 mg/L Al^{3+} could be tolerated by anaerobes after acclimation.

2.3.2. Calcium

Calcium is known to be essential for the growth of certain strains of methanogens (Murray and Zinder, 1985). It is also important in the formation of microbial aggregates (Thiele et al., 1990; Huang and Pinder, 1995). Excessive amounts of calcium lead to precipitation of carbonate and phosphate, which may result in (i) scaling of reactors and pipes, (ii) scaling of biomass and reduced specific methanogenic activity, (iii) loss of buffer capacity and essential nutrients for anaerobic degradation (Keenan et al., 1993; El-Mamouni et al., 1995; van Langerak et al., 1998).

Very little is known about the toxicity of Ca^{2+} in the anaerobic system. Jackson-Moss et al. (1989) observed that Ca^{2+} concentrations of up to 7000 mg/L had no inhibitory effect on anaerobic digestion. A large proportion of the Ca^{2+} passed through the digester and was present in the effluent. Kugelman and McCarty (1964) reported a much lower toxicity threshold. They showed that the optimum Ca^{2+} concentration for methanation of acetic acid was 200 mg/L. Ca^{2+} was moderately inhibitory at a concentration of 2500–4000 mg/L, but was strongly inhibitory at a concentration of 8000 mg/L. Addition of calcium can have a positive impact on reactors in which retention of biomass is desired. Addition of Ca^{2+} increased the accumulation of biofilm when Ca^{2+} concentration in the feed was below 120 mg/L. For Ca^{2+} concentrations higher than 120 mg/L, an accumulation of minerals and a decrease in water content in the biofilm caused an inhibition of cellular metabolism (Huang and Pinder, 1995). Similarly in UASB reactors, low Ca^{2+} concentrations from 100 to 200 mg/L were reported to be beneficial for sludge granulation (Cail and Barford, 1985; Mahoney et al., 1987; Yu et al., 2001), whereas high Ca^{2+} concentrations (greater than 300 mg/L) were reported to be detrimental (Hulshoff Pol et al., 1983; Thiele et al., 1990; Yu et al., 2001).

Calcium carbonate precipitation could also impact biomass activity. Calcium carbonate precipitation is dependent on the Ca^{2+} concentration and on the COD removal efficiency (van Langerak et al., 1998). The impact of precipitate on the biomass activity is complex. Highly scaled biomass is less active because of mass transfer limitations. However, active biomass could be formed in thin biofilms on the surface of the precipitates. The overall activity of the biomass would be the average of the two effects (van Langerak et al., 1998).

2.3.3. Magnesium

The optimal Mg^{2+} concentration was reported to be 720 mg/L for the anaerobic bacterium *Methanosarcina thermophila* TM1 and a *Methanosarcinae*-dominated UASB reactor (Ahring et al., 1991; Schmidt and Ahring, 1993). Cultures could be adapted to 300 mM Mg^{2+} without a change in growth rate, but growth ceased at 400 mg/L Mg^{2+} (Schmidt and Ahring, 1993). Magnesium ions at high concentrations have been shown to stimulate the production of single cells (Harris, 1987; Xun et al., 1988; Schmidt and Ahring, 1993). The high sensitivity of single cells to lysis is an important factor in the loss of aceticlastic activity in anaerobic reactors.

2.3.4. Potassium

Maintenance of high levels of potassium in culture media or in a digester is undesirable since pure culture studies have shown that high levels of extracellular potassium (1.0 M) lead to a passive influx of potassium ions that neutralize the membrane potential (Jarrell et al., 1984). In addition, potassium is one of the best extractants for metals bound to the exchangeable sites in sludge. Ilangovan and Noyola (1993) observed the increase of micronutrients (Cu^{2+}, Zn^{2+}, Ni^{2+}, Mo^{2+}, Co^{2+}) in a UASB reactor treating molasses stillage containing a high concentration of potassium. The removal of the essential micronutrients from active sludge was believed to be responsible for the low activity of anaerobic methanogenic population.

The toxic effect of potassium is rarely referenced in the literature. Low concentrations of potassium (less than 400 mg/L) were observed to cause an enhancement in performance in both the thermophilic and mesophilic ranges while at higher concentrations there was an inhibitory effect that was more pronounced in the thermophilic temperature range. Slug feed studies, in which the concentration of the cation was suddenly increased in actively fermenting cultures, were conducted to determine the toxicity of individual cations (Kugelman and McCarty, 1964). It was observed that 0.15 M K^+ caused 50% inhibition of acetate-utilizing methanogens. A series of studies have shown that K^+ inhibits the thermophilic digestion of simulated coffee wastes (Fernandez and Forster, 1993, 1994; Shi and Forster, 1994). Information about the sensitivity of different groups of microorganisms to potassium is conflicting. The results of batch tests using acetate as the carbon source showed that the gas production from both the control and samples with elevated K^+ were identical, indicating that the inhibition could be at the acidogenic stage (Fernandez and Forster, 1994). Mouneimne et al. (2003) investigated the biotoxicity of potassium using acetate and glucose as substrates and anaerobic sludge as inoculum. The IC_{50} for acetate-utilizing microorganisms was

found to be 0.74 mol/L. However, the degradation rates of glucose were virtually unaffected by potassium, indicating that the acetate-utilizing microorganisms exhibited a greater sensitivity to the toxic effects of cations than the acid-forming ones.

Sodium, magnesium, and ammonium were observed to mitigate potassium toxicity, with sodium producing the best results. Combinations of cations produce antagonism superior to that of single cations. The best results were obtained for combinations of sodium and calcium, and combinations of sodium, calcium and ammonia (Kugelman and McCarty, 1964).

2.3.5. Sodium

Wastewaters with high concentrations of sodium are produced in the food processing industry (Soto et al., 1991). Mesophilic and thermophilic anaerobic filters treating effluents from a mussel cooking factory were compared. The mesophilic reactor exhibited better performance than the thermophilic reactor, which was attributed to the more rapid adaptation of mesophilic sludges to the high salinity of the wastewater (Soto et al., 1991; Soto et al., 1992). In comparing VFA-degrading bacteria, sodium was more toxic to propionic acid-utilizing microorganisms than to acetic acid-utilizing ones (Soto et al., 1993b). This result was in agreement with the findings of Liu and Boone (1991), who found the NaCl toxicity decreased in the order of lignocellulose-degrading > acetate-utilizing > propionate-utilizing > H_2/CO_2-utilizing organisms.

2.3.5.1. Concentration. At low concentrations, sodium is essential for methanogens, probably because of its role in the formation of adenosine triphosphate or in the oxidation of NADH (Dimroth and Thomer, 1989). McCarty (1964) reported sodium concentrations in the range of 100–200 mg/L to be beneficial for the growth of mesophilic anaerobes. According to Kugelman and Chin (1971), the optimal sodium concentration for mesophilic aceticlastic methanogens in waste treatment processes was 230 mg Na^+/L. The optimal growth conditions for mesophilic hydrogenotrophic methanogens reportedly occurred at 350 mg Na^+/L (Patel and Roth, 1977). At high concentrations, sodium could readily affect the activity of microorganisms and interfere with their metabolism (Kugelman and McCarty, 1964; Rinzema et al., 1988; Gourdon et al., 1989; Balsleve-Olsen et al., 1990; Mendéz et al., 1995). The level of inhibition depends on the concentration of sodium ions. An early study reported sodium concentrations ranging from 3500 to 5500 mg/L to be moderately and 8000 mg/L to be strongly inhibitory to methanogens at mesophilic temperatures (McCarty, 1964). The IC_{50} for sodium inhibition has been reported to be 5.6–53 g/L, depending on the adaptation period, antagonistic/synergistic effects, substrate, and reactor configuration (Patel and Roth, 1977; Rinzema et al., 1988; Liu and Boone, 1991; Soto et al., 1993b; Feijoo et al., 1995; Omil et al., 1995a,b; Aspé et al., 1997; Kim et al., 2000; Vallero et al., 2002; Chen et al., 2003; Vallero et al., 2003a,b).

2.3.5.2. Acclimation. Acclimation of methanogens to high concentrations of sodium over prolonged periods of time could increase the tolerance and shorten the lag phase before methane production begins (de Baere et al., 1984; Feijoo et al., 1995; Omil et al., 1995a,b, 1996b; Chen et al., 2003). The tolerance is related to the Na^+ concentration the methanogens acclimated to and the time of exposure. The IC_{100} of methanogens increased from 12.7 to 22.8 g/L when the methanogens were acclimated to 4.1 and 12.0 g/L of Na^+, respectively (Chen et al., 2003). Inoculum showed increased tolerance for sodium as the time of acclimation increases. Mendéz et al. (1995) reported that IC_{90} of inocula was 12.0 g/L when sludge was taken from anaerobic reactor after one day of acclimation and greater than 17.0 g/L when the acclimation period was 719 days. Anaerobic filter sludge that treating high salinity wastewater for 2 years also exhibited better performance than sludge that had been sampled from a central activity digester employed for wastewater treatment for 1 year (Feijoo et al., 1995). Application of experimental results obtained from a batch reactor to a continuous reactor often overestimated the sensitivity of the microorganisms. This may be attributable to the sudden change in sodium levels that microorganisms were exposed to in batch testing, giving them minimum time to adapt. In continuous testing, sodium concentration was often increased gradually, allowing sufficient time for the microorganisms to adapt (Feijoo et al., 1995).

Contrary to the findings in the previous section, Rinzema et al. (1988) found no adaptation of *Methanothrix* sp. to high sodium concentrations after 12 weeks. Similarly, when treating methanol in a sulfate-reducing reactor, stepwise increases in NaCl could not increase the tolerance of SRB to sodium, indicating that the adaptation of thermophilic, sulfidogenic methanol-degrading bacteria to a high NaCl environment was unlikely to occur (Vallero et al., 2002, 2003a,b).

2.3.5.3. Antagonistic/synergistic effects. Microorganisms accumulate cations and/or low-molecular-weight organic compounds, known as compatible solutes, when the extracellular solute concentration exceeds that of the cell cytoplasm (Lai and Gunsalus, 1992). The role of compatible solutes in osmoregulation was recognized and the antagonistic effect of cations and betaine towards sodium has been investigated. Kugelman and McCarty (1964) showed that a combination of potassium and calcium significantly increased the antagonism over that achieved by potassium alone. An anaerobic toxicity assay of sludge from mussel-processing wastewater also confirmed that when using the effluent of the anaerobic filter as assay medium, the tolerance to sodium was highly increased compared to distilled water (Soto et al., 1993b). This effect was attributed to the antagonism exerted by the presence of sea salts, probably

K^+, Mg^{2+}, and Ca^{2+}. Information in the literature about the effect of Mg^{2+} is conflicting. Ahring et al. (1991) reported an antagonistic effect of Mg^{2+} for Na^+. The inhibition by Na^+ was directly related to the Mg^{2+} concentration. When the Mg^{2+} was 0.05 mM or less, 0.35 M Na^+ completely inhibited growth. More Na^+ was required for inhibition at higher Mg^{2+} concentrations. However, beyond 0.01 M/L, Mg^{2+} was reported to start showing a synergistic effect towards Na^+ (Kugelman and McCarty, 1964). Ca^{2+} and NH_4^+ also showed synergistic effects towards Na^+ (Kugelman and McCarty, 1964). The antagonistic effect of the compatible solute betaine ($(CH_3)_3N^+CH_2COO^-$) towards sodium toxicity was investigated by Yerkes et al. (1997). Bacteria subjected to salinity stress have been shown to accumulate betaine in proportion to the salinity of the medium (Poukomailian and Booth, 1992). Concentrations of betaine as low as 1 mM have been shown to be effective in reducing the toxicity of high concentrations of sodium by reducing acclimation or lag time, increasing substrate uptake rate, and increasing gas production (Yerkes et al., 1997).

2.4. Heavy metals

Heavy metals can be present in significant concentrations in municipal sewage and sludge. The heavy metals identified to be of particular concern include chromium, iron, cobalt, copper, zinc, cadmium, and nickel (Jin et al., 1998). A distinguishing feature of heavy metals is that, unlike many other toxic substances, they are not biodegradable and can accumulate to potentially toxic concentrations (Sterritt and Lester, 1980). In one extensive study of anaerobic digester performance, it was found that heavy metal toxicity is one of the major causes of digester upset or failure (Swanwick et al., 1969). The toxic effect of heavy metals is attributed to disruption of enzyme function and structure by binding of the metals with thiol and other groups on protein molecules or by replacing naturally occurring metals in enzyme prosthetic groups (Vallee and Ulner, 1972).

2.4.1. Factors controlling heavy metal inhibition

Many heavy metals are part of the essential enzymes that drive numerous anaerobic reactions. Analysis of ten methanogenic strains showed the following order of heavy metal composition in the cell: $Fe \gg Zn \geqslant Ni > Co = Mo > Cu$ (Takashima and Speece, 1989). Whether heavy metals would be stimulatory or inhibitory to anaerobic microorganisms is determined by the total metal concentration, chemical forms of the metals, and process-related factors such as pH and redox potential (Mosey et al., 1971; Lin and Chen, 1999; Zayed and Winter, 2000). It is generally believed that acidogens are more resistant to heavy metal toxicity than methanogens (Zayed and Winter, 2000). However, Hickey et al. (1989) have speculated that some trophic group(s) or organisms within the anaerobic consortia in digesters might be more severely inhibited by a pulsed addition of heavy metals than the methanogenic populations.

2.4.1.1. Chemical forms of heavy metal. Because of the complexity of the anaerobic system, heavy metals may be involved in many physico-chemical processes including (1) precipitation as sulfide (except Cr), carbonate and hydroxides (Lawrence and McCarty, 1965; Mosey et al., 1971), (2) sorption to the solid fraction, either biomass or inert particulate matter (Shen et al., 1993; Shin et al., 1997), and (3) formation of complexes in solution with intermediates and product compounds produced during digestion (Hayes and Theis, 1978; Hickey et al., 1989; Callander and Barford, 1983a,b). Among these metal forms, only metals in soluble, free form are toxic to the microorganisms (Lawrence and McCarty, 1965; Mosey and Hughes, 1975; Oleszkiewicz and Sharma, 1990). Several studies have confirmed that the heavy metal toxicity correlated better to the metal's free ionic concentration (determined through a combination of dialysis and ion exchange) than to its total concentration (Bhattacharya and Safferman, 1989; Bhattacharya et al., 1995a,b). In previous reports, the various physico-chemical forms of a particular heavy metal were rarely distinguished due to the complex interactions between the heavy metals and anaerobic sludge and/or lack of analytical techniques for separating metal species (Gould and Genetelli, 1978; Hayes and Theis, 1978; Oleszkiewicz and Sharma, 1990; Zayed and Winter, 2000). This is one factor that explains the wide variation in reported toxic concentrations of heavy metals.

2.4.1.2. Concentrations. In addition to physico-chemical form, differences in substrate, bacteria genre, and environmental factors also explain the wide variation (from several to several hundreds of mg/L) in both the reported dosages of heavy metals and their relative toxicity (Lawrence and McCarty, 1965; Hickey et al., 1989; Bhattacharya et al., 1995a; Jin et al., 1998; Lin and Chen, 1999; Zayed and Winter, 2000). Moreover, the operating solids level significantly impacts the heavy metal toxicity in anaerobic digesters by providing protection from metal inhibition. It has been suggested that inhibition due to heavy metals would be more comparable if metal dosage was expressed as milligram of metal per gram of volatile solids (Hickey et al., 1989). Unfortunately, most of the literature only reported the inhibition concentration values in mg/L, which makes the comparison of inhibition concentrations more difficult. Heavy metal concentrations that caused 50% inhibition of methanogenesis during whey methanation indicated that toxicity decreased in the order of $Cu > Zn > Ni$. Similar results were obtained by Lin (1992, 1993) and Lin and Chen (1999). This is, however, not surprising since Zn and Ni are components of several enzymes in anaerobic microorganisms (Nies, 1999). The relative sensitivity of acidogenesis and methanogenesis to heavy metals is $Cu > Zn > Cr > Cd > Ni > Pb$ and $Cd > Cu > Cr > Zn > Pb > Ni$, respectively (Lin, 1992, 1993). The relative

toxicity of four metals to the anaerobic digestion of sewage sludge was reported to be Cr > Ni > Cu > Zn (Wong and Cheung, 1995).

2.4.1.3. Antagonistic and synergistic effects. Industrial wastewaters or sludges generally contain many kinds of heavy metals which cause synergistic or antagonistic effects on anaerobic digestion. The level of inhibition is determined by the species and the ratio of the individual components. Although toxicity of most mixed heavy metals such as Cr–Cd, Cr–Pb, Cr–Cd–Pb, and Zn–Cu–Ni was synergistic (Lin, 1992), some of the metal mixtures showed antagonistic inhibition (Lin, 1993). In a variety of aerobic, facultative and anaerobic studies reviewed by Babich and Stotzky (1983), Ni was shown to act synergistically in Ni–Cu, Ni–Mo–Co, and Ni–Hg systems; antagonistically in Ni–Cd, Ni–Zn systems. Ahring and Westermann (1985) found that Ni decreased the toxicity of Cd and Cu.

2.4.2. Detoxification of heavy metals

The most important methods for mitigating heavy metal toxicity are precipitation, sorption and chelation by organic and inorganic ligands (Oleszkiewicz and Sharma, 1990). Sulfide has been the main agent used to precipitate heavy metals. Reactor recovery from 20 mg/L of copper exposure was observed when sulfide was added after copper exposure. The addition of sulfide before copper exposure can significantly shorten the time required for recovery (Jin et al., 1998; Zayed and Winter, 2000). However, caution must be exercised since excess sulfide can also be an important inhibitor to methanogens (Anderson et al., 1982). Excessive quantities of sulfide might be minimized by adding ferrous sulfate, which has the highest solubility of all toxic heavy metals. Heavy metals would combine with the sulfide in FeS, releasing Fe^{2+}, which is relatively non-toxic up to several hundred mg/L. Presence of a solid phase in the reactor provides protection for anaerobic microorganisms from heavy metal inhibition (Jarrell et al., 1987). The protection effect is proportional to the surface area or the amount of solids. The mechanism is believed to be chemisorption (Gould and Genetelli, 1984; Alibhai et al., 1985). The affinity of sludge for heavy metals has been proposed as (in decreasing order): Cu > Cd > Zn > Ni (Gould and Genetelli, 1984). Similarly, sorption of heavy metals to activated carbon, kaolin, bentonite, diatomite and waste materials such as compost and cellulose pulp waste can also mitigate inhibition (Ulmanu et al., 2003). Chelation by organic ligands has been well documented for several metals. Babich and Stotzky (1983) have shown a decrease in nickel toxicity by EDTA, PDA, NTA, aspartate, and citrate, in that order.

Exposure of microorganisms to heavy metals is known to activate a wide variety of intracellular detoxification strategies (Gadd and Griffiths, 1978). The intracellular defense systems include biologically mediated precipitation or chelation of metal ions at the cell surface (Wood and Wang, 1983), biomethylation (Summers, 1986), exocytosis (expulsion of metals after their chemical inactivation inside the cell) (Silver and Phung, 1996) and plasmid-mediated resistance (Wood and Wang, 1983).

2.5. Organics

A wide range of organic compounds can inhibit anaerobic processes. Organic chemicals which are poorly soluble in water or adsorbed to the surfaces of sludge solids may accumulate to high levels in anaerobic digesters. The accumulation of apolar pollutants in bacterial membranes causes the membrane to swell and leak, disrupting ion gradients and eventually causing cell lysis (Heipieper et al., 1994; Sikkema et al., 1994).

Organic compounds which have been reported to be toxic to the anaerobic processes include alkyl benzenes (Yang and Speece, 1986; Renard et al., 1993), halogenated benzenes (van Beelen and van Vlaardingen, 1994), nitrobenzenes (Bhattacharya et al., 1996), phenol and alkyl phenols (Sierra-Alvarez and Lettinga, 1991a; Soto et al., 1991; Fang et al., 1995), halogenated phenols (Shin and Kwon, 1998), nitrophenols (Borja et al., 1997; Uberoi and Bhattacharya, 1997a; McCue et al., 2003), alkanes (Mormile and Suflita, 1996), halogenated aliphatics (Stuckey et al., 1980; Boucquey et al., 1995), alcohols (Dimirer and Speece, 1998), halogenated alcohols (Blum and Speece, 1991), aldehydes (Gonzales-Gil et al., 2002), ethers (Playne and Smith, 1983; Hayward and Lau, 1989), ketones (Playne and Smith, 1983; Hayward and Lau, 1989), acrylates, carboxylic acids, amines, nitriles, amides (Blum and Speece, 1991; Stergar et al., 2003), and pyridine and its derivatives (Liu et al., 1998). Moreover, some LCFAs (Koster and Cramer, 1987), surfactants, and detergents were also reported to adversely impact anaerobic digestion (Madsen and Rasmussen, 1996; Gavala and Ahring, 2002).

The inhibition concentration ranges vary widely for specific toxicants. The parameters that affect the toxicity of organic compounds include toxicant concentration, biomass concentration, toxicant exposure time, cell age, feeding pattern, acclimation, and temperature (Yang and Speece, 1986). At lower concentrations, biodegradation of some toxicants can prevent inhibition; higher concentrations of toxicants generally lead to significant inhibition of anaerobic processes (Anthony and Breimhurst, 1981; O'Connor and Young, 1989). With higher biomass concentration, reactors exhibit greater process stability in the presence of toxic shocks (Uberoi and Bhattacharya, 1997a). At equal solids concentrations, younger cultures were proved to be more robust and resistant to toxicants than older cultures (Yang and Speece, 1986). Inhibition has been usually quantified by determining the IC_{50} concentration. Because of a lack of consistency in exposure times between studies, which varied from 30 min (Dutka et al., 1983) to 285 h (Johnson and Young, 1983), the inhibition concentrations can vary significantly.

As with other inhibitory substances, microbial acclimation is an important parameter in assessing the inhibitory

effects of organic substances. Four interrelated mechanisms by which adaptation can occur have been suggested: (1) enrichment of organisms which can degrade the toxic compounds, (2) induction of specific enzymes for the degradation, (3) genetic engineering and (4) exhaustion of preferential substrates before switching to the xenobiotic substrate, i.e. a diauxic pattern (Spain et al., 1980; Spain and van Veld, 1983; van der Meer, 1994). Acclimation of anaerobic microorganisms both increases their tolerance to the toxicants shock and enhances toxicant biodegradability (Stuckey et al., 1980; Wu et al., 1993).

2.5.1. Chlorophenols

Chlorophenols include monochlorophenols (CPs), dichlorophenols (DCPs), trichlorophenols (TCP), tetrachlorophenols (TeCPs), and pentachlorophenol (PCP). Chlorophenols are toxic to many organisms by disrupting the proton gradient across membranes and interfering with energy transduction of cells. The effect of aromatic compounds on membrane processes has been reviewed by Sikkema et al. (1995).

The relative toxicity of chlorophenols has been investigated by many researchers and the results are somewhat contradictory. Among different isomer series, PCP was the most toxic to acidogens and methanogens. Approximately 0.5–10 mg/L PCP caused inhibition to acidogenic and methanogenic populations (Bauer and Capone, 1985; Godsy et al., 1986; Blum and Speece, 1991; Patel et al., 1991; Uberoi and Bhattacharya, 1997b; Piringer and Bhattacharya, 1999). Sierra-Alverez and Lettinga (1991b) have described the relationship between the increase in the number of chloro-substituents in the aromatic benzene rings and their toxicity to methanogenic processes. This theory was supported by the finding of Jin and Bhattacharya (1996) that TCPs were more toxic than DCPs and CPs. However, other studies found no correlation between the toxicity of DCPs and TCPs and the number of chloro-substituents (Blum and Speece, 1991). Within individual isomer series, toxicity due to DCPs and TCPs to both propionate and acetate degradation was dependent on the substitution position of chlorine atoms on the benzene ring. Different orders of relative toxicity have been reported (Jin and Bhattacharya, 1996; Uberoi and Bhattacharya, 1997b). The chlorine position on CPs did not significantly effect toxicity to either propionate or acetate degradation (Kim et al., 1994; Uberoi and Bhattacharya, 1997b). Davies-Venn et al. (1992), however, found that the toxicity of CPs to aceticlastic methanogenesis increased as the substituted chlorine group changed from the *ortho* to the *meta* to the *para* position on the benzene ring.

In relation to physico-chemical properties, previous studies with aromatic compounds indicated that those structural characteristics that decrease polarity increase toxicity (Kamlet et al., 1986; Patel et al., 1991). Compounds of greater hydrophobicity accumulate more efficiently in membranes, causing a greater disturbance to the membrane structure (Heipieper et al., 1994; Sikkema et al., 1994). A high correlation of the methanogenic toxicity to the $\log P$ (logarithm of the octano-water partition coefficient) was obtained for chloro-substituted benzenes and phenols (Sierra-Alverez and Lettinga, 1991b; Ennik-Maarsen et al., 1998). The relative hydrophobicity also provides an explanation for the relative toxicity of nitrophenols and hydroxyphenols (Wang et al., 1991).

Literature data on the tolerance of different important subpopulations in methanogenic sludges to the chlorophenols are also conflicting. This variation could be attributed to variations in microbial specific growth rates and/or the physiological state, which may affect the tolerance to an inhibitory compound as well as the accumulation of organic compounds in the cell (Ennik-Maarsen et al., 1998). Colleran et al. (1992) reported that aceticlastic methanogens, butyrate oxidizers and ethanol oxidizers, were similarly sensitive to halogenated aromatics, while hydrogenotrophs were less sensitive (Kim et al., 1996). In other studies, propionate degraders were found to be the most sensitive to chlorophenols (Johnson and Young, 1983; Wu et al., 1993; Jin and Bhattacharya, 1996), whereas Kim et al. (1994) concluded that ethanol degraders were not as sensitive as aceticlastic methanogens towards chnorophenols and chloroanilines. Degradation of initially inhibitory compounds was observed for 2,4-DCP, 2,3,6-TCP, 2,3,5-TCP (Uberoi and Bhattacharya, 1997b), 4-CP, and 2,4,6-TCP (Fantroussi et al., 1998) after acclimation. Acclimation was related to the concentration of substrates and their chemical structures (Linkfield et al., 1989).

2.5.2. Halogenated aliphatics

Most of the halogenated aliphatics are strong inhibitors of methanogenesis. In general, the brominated compounds were more inhibitory to methanogens than their chlorinated analogs (Belay and Daniels, 1987). Renard et al. (1993) measured the toxicity of a mixture of polychlorinated organic compounds including C_4Cl_6, C_2Cl_6, C_2Cl_4, etc. and found that 50% inhibition of methanogenesis started at 3.3 mg/L. Complete inhibition of methanogenesis occurred at 100 mg/L. Methanogenic toxicity data for other chlorinated aliphatic hydrocarbons are lacking (Stuckey et al., 1980; Anthony and Breimhurst, 1981). However, the methanogenic toxicity of chloroform, the most widely used chloroaliphatics, has been studied extensively. Among six chlorinated hydrocarbon solvents including carbon tetrachloride and 1,1,1-trichloroethane, chloroform was found to be the most toxic to the anaerobic digestion of sewage sludge (Swanwick and Foulkes, 1971). A concentration of 0.01 mg/L or more of chloroform in the sewage was likely to have an adverse effect on sludge digestion (Stickley, 1970). The IC_{50} concentration for this compound has been reported to range from 0.15 mg/L in unacclimated methanogenic consortia to 50 mg/L in acclimated consortia (Anthony and Breimhurst, 1981; Parkin and Speece, 1983; Salenieks and Henry, 1986; Yang and Speece, 1986; Hickey et al., 1987; In et al., 1992). During anaerobic degradation of chloroform, reactive and toxic intermediates

were formed, which partly contributed to its strong toxicity (van Beelen and van Vlaardingen, 1994).

Unlike chloroaromatics, no relationship between the number of chloro-substituents and toxicity could be identified for chloroaliphatics. Polarity is an important factor, but it is insufficient for the prediction of the toxicity of the different compounds (Sanz et al., 1997). It was reported that tri- and tetrachloride derivatives of methane and ethane were more toxic than dichlorinated compounds. Perchlorinated derivatives of ethane and ethene were scarcely inhibitory at concentrations near their maximum water solubility (Sanz et al., 1994). Compared to their saturated counterparts, unsaturated chloroaliphatics were less toxic (Chou et al., 1978; Sanz et al., 1997).

Acclimation of methanogenic consortium to polychlorinated aliphatic compounds is possible. Yang and Speece (1986) found that an anaerobic culture was able to acclimate to the presence of chloroform while fermenting acetate to methane. Inhibition of unacclimated cultures by chloroform was noted at 0.5 mg/L, but with acclimation 15 mg/L could be tolerated. Filho et al. (1992) reported that fermentative microorganisms acclimate more quickly than hydrogenotrophic ones, followed by H_2 producing acetogens and aceticlastic methanogens.

2.5.3. N-substituted aromatics

N-substituted aromatics are reactive toxicants including nitrobenzenes, nitrophenols, aminophenols, aromatic amines, etc. (Blum and Speece, 1991). Reactive toxicity is caused by specific chemical interactions with enzymes or interference with metabolic pathways (Balderston and Payne, 1976).

Nitroaromatics are very toxic compounds to methanogens, with IC_{50} values generally ranging from 0.014 to 0.12 mM (Johnson and Young, 1983; Donlon et al., 1995; Bhattacharya et al., 1996). Aromatic amines, in contrast, are less inhibitory; the IC_{50} values were between 3.2 and 67 mM, perhaps due to their lower hydrophobicity (Razo-Flores et al., 1997). Nitroanilines were found to be the most toxic among N-substituted aromatics, partly because of their high chemical reactivity (Razo-Flores et al., 1997).

Increasing the number of nitro groups beyond one had limited effect in increasing the toxicity of nitrobenzenes. The addition of an extra amino group to aminophenol resulted in more toxic compounds, while the addition of an amino group to aniline resulted in less toxic phenylenediamines (Donlon et al., 1995). However, the combination of nitro and amino groups, e.g. nitroanilines, was found to be the most toxic substitute pattern (Donlon et al., 1995). Toxicity due to the mononitrophenols also depends on the substitution position. Among the nitrophenols studied, the toxicity increased in the order of 4-nitrophenol > 2-nitrophenol > 3-nitrophenol for an acetate enrichment culture (Haghighi-Podeh et al., 1995; Haghighi-Podeh and Bhattacharya, 1996). Similar results were observed for acetate and propionate enrichment culture (Uberoi and Bhattacharya, 1997a). Another study found that *para*-nitrophenol was more toxic than *meta*-nitrophenol and the *ortho*-nitrophenol was the least toxic to methanogens (Tseng and Yang, 1994).

Acclimation of anaerobes to N-substituted aromatics decreases their toxicity and therefore enhances biodegradation. 4-Nitrophenol and 2,4-dinitrophenol can be degraded at concentrations up to 200 mg/L after sufficient acclimation. The lag time before biodegradation started increased with increasing toxicant concentration (O'Connor and Young, 1989).

2.5.4. LCFAs

Treatment of fatty materials by anaerobic digestion is often hampered because of the inhibitory effect of LCFAs. LCFAs have been reported to be inhibitory at low concentrations for gram-positive but not gram-negative microorganisms (Kabara et al., 1977). Methanogens can be inhibited by LCFAs due to their cell wall, which resembles that of gram-positive bacteria (Zeikus, 1977). LCFAs show acute toxicity towards anaerobic consortium by adsorption onto the cell wall/membrane, interference with the transport or protective function (Rinzema et al., 1994). In addition, sorption of a light layer of LCFAs to biomass leads to the flotation of sludge and consequent sludge washout (Rinzema et al., 1989). In UASB reactors, granular sludge flotation sometimes occurred at concentrations far below the toxicity limit (Hwu et al., 1998).

Oleic acid was almost as inhibitory as lauric acid, which exhibited I_{50} of 4.3 mM. Cyprylic acid was only slightly inhibitory (Koster and Cramer, 1987). Koster and Cramer (1987) also observed enhanced toxicity of capric acid and myristic acid when lauric acid was present. LCFA toxicity varied with the type of anaerobic sludges and was more correlated to the sludges' physical characteristics (specific surface area and size distribution) than to their biological characteristics. Suspended and flocculent sludges, which have a higher specific surface area, suffered much greater inhibition than did granular sludge (Hwu et al., 1996). Thermophiles have been reported to be more sensitive to LCFAs than mesophiles, possibly due to the different composition of cell membranes (Hwu and Lettinga, 1997).

Biodegradation of LCFAs has been reported in both mesophilic and thermophilic environments (Hanaki et al., 1981; Angelidaki and Ahring, 1992). It has been suggested that LCFAs exerted a bactericidal effect and no adaptation of methanogens occured (Hanaki et al., 1981; Koster and Cramer, 1987; Angelidaki and Ahring, 1992). However, recent studies based on the degradation of oleic acid in an anaerobic fixed-bed reactor showed that acclimation improved the resistance of the biofilm to the presence of oleate and improved the biodegradation capacity compared to the biofilm formed in the absence of lipids (Alves et al., 2001a,b). Addition of calcium has been shown to reduce LCFA inhibition, probably because of the formation of insoluble salts (Hanaki et al., 1981; Angelidaki

and Ahring, 1990). However, calcium addition cannot solve the problem of sludge flotation (Alves et al., 2001a,b).

2.5.5. Lignins and lignin related compounds

Lignin derivatives with aldehyde groups or apolar substituents are highly toxic to methanogens. The aromatic carboxylic acids, however, were only mildly toxic. Op den Camp et al. (1988) tested the toxicity of several lignin model phenolic acids to the anaerobic degradation of cellulose and observed that the acids only caused inhibition of methane production at very high concentrations. Benjamin et al. (1984) evaluated the methanogenic toxicity of various lignin derived monomers present in kraft condensates and found that eugenol, with an apolar side chain, was more toxic than its counterpart guaiacol which lacks the side chain.

3. Engineering significance

3.1. Agricultural wastes

Animal waste includes voided waste from livestock and poultry, wastewater, feedlot runoff, silage juices, bedding, and feed. These wastes are a substantial contributor to non-point source pollution and can affect wetland habitats and contaminate drinking water sources. Animal waste often has very high total ammonia nitrogen concentrations due to the presence of ammonia as well as protein and urea that readily release ammonia upon anaerobic treatment (Zeeman et al., 1985; Krylova et al., 1997; Hansen et al., 1998). Consequently, the principal instability associated with the anaerobic digestion of animal waste is ammonia inhibition (Zeeman et al., 1985; Hashimoto, 1986; Kayhanian, 1994). Sudden increases in ammonia concentration in the feedstock are unusual (Hobson, 1991). However, feed slurry that has been stored for some time in the animal house often contains high concentration of ammonia released from decomposition of organic nitrogen. Shock loading of this feed slurry can cause inhibition of anaerobic digesters (Hobson, 1991). In addition to ammonia, swine manure also contains a high sulfate concentration derived from a protein-rich diet. The inhibition caused by ammonia and by sulfide influences each other (Hansen et al., 1999). Feed additives (antibiotics, chemotherapeutics) for improving food utilization and disinfectants for preventing infectious diseases have been widely used in intensive animal production (Hilpert et al., 1984). In most cases, these compounds are in very low concentrations (less than 30 ppm) in the waste and are generally not inhibitory (Hobson, 1991). However, some synthetic chemotherapeutics such as Olaquindox may be strongly inhibitory even at 1 mg/L (Hilpert et al., 1984). This concentration may be reached in practice and special treatments such as predilution may be needed before anaerobic digestion (Varel and Hashimoto, 1981; Hilpert et al., 1984; Poels et al., 1984).

Crop residues represent another fraction of agricultural waste. Substantial quantities of unused stalks, straws, and bark are produced from a variety of crops, which could be used for energy generation (Kalra and Panwar, 1986). Crop residues typically contain a high lignocellulosic content. Problems such as low gas yield during anaerobic digestion of these materials are usually associated with a high C/N ratio or high lignin content. In addition, the inhibition caused by pesticide and herbicide residues would affect digestion process kinetics (Khalil et al., 1991; Chakraborty et al., 2002). Certain plants generate resin extracts which protect them from biological damage. These extracts may be inhibitory to the digestion process (Speece, 1987). Pretreatments such as acid or base hydrolysis are often employed before anaerobic digestion to increase biogas yield. However, byproducts formed in the pretreatment (fufural, hydroxymethyl fufural, formic acid, and levulinic acid) are potential inhibitors of anaerobic digestion. Microorganisms may eventually adapt and/or degrade these byproducts, but process kinetics could be affected (Speece, 1987).

3.2. Municipal wastes

More than 181.4 million metric tons of municipal solid wastes are produced in the United States annually (US EPA, 2000) with up to 60% organic material (de Laclos et al., 1997). Due to the increase of source and on-site recycling and improved refuse-handling equipment, substantial fractions of the discarded paper fraction, metals, and glass are being recycled, resulting in the production of more organic-rich and less biotoxic biowaste (Ghosh et al., 2000). Because of its value as a potential renewable energy source and high biodegradability, there is a growing interest in anaerobic digestion of the biowaste. The principal instability associated with the anaerobic digestion of biowaste is ammonia inhibition due to the degradation of protein-containing materials (Kayhanian, 1994; Gallert and Winter, 1997; Gallert et al., 1998). The composition of the organic matter depends greatly on the source of the organic fraction. It was found that the digestion process deteriorated with increasing levels of ammonia-N, with total process cessation at a COD/N ratio of 50 (Poggi-Varaldo et al., 1997, 1998). Two practical methods, dilution of digester contents with water and adjustment of feedstock C/N, have been tested successfully to mitigate ammonia inhibition (Kayhanian, 1999). In general, for solid wastes with a C/N ratio above 20, the ammonia inhibition effect can be compensated by dilution with water which lowers the concentration of potential inhibitors.

Sludge production is an integral part of the domestic sewage treatment process. Due to their resistance to biodegradation, heavy metals present in the raw sewage often accumulate in the sludge to potentially toxic concentrations. Petrasek and Kugelman (1983) found that most metals were concentrated 10–30-fold in the sludges produced in conventional wastewater treatment plants. Anaerobic digestion is the most typical method for stabilization of sludge prior to ultimate disposal in many countries

(Hobson et al., 1981) and is usually the most sensitive process to heavy metal toxicity (Lester et al., 1983). The effects on digestion of chemical treatment of sludge were investigated by Gossett et al. (1978). Alum, ferric chloride and organic flocculent were reported to reduce: (1) gas production, (2) the methane content in the biogas, (3) COD removal, and (4) volatile suspended solids removal. A similar effect was observed for sewage emulsifiers such as alkyl benzene sulphonate (ABS).

3.3. Industrial wastes

3.3.1. Food industry wastes

The food industries that could benefit from anaerobic treatment include fruit and vegetable canning, edible oil refining, dairy production, seafood processing, meat processing, starch and sugar production, brewing, and fermentation. Wastes from food processing are high in organic matter and are therefore ideal for anaerobic digestion. However, the application of this technique may be hindered by the presence of various inhibitors. Seafood processing wastewaters contain high concentrations of different cations and anions, mainly Na^+, Cl^-, and SO_4^{2-} (Feijoo et al., 1995). The sodium concentration in these wastewaters may reach that of sea water (approximately 12 g/L) (Rinzema et al., 1988). Hypersaline wastewaters are also generated in vegetable, vegetable oil, and dairy processing industries. Dairy wastewaters are rich in fats, proteins and carbohydrates (Rico et al., 1991). Wastewaters from the dairy industry are usually generated in an intermittent way, and the flow and characteristics of wastewaters change from one factory to another depending on the type of systems and the methods of operation (Rico et al., 1991). It has been reported that the intermediates of fat degradation, glycerol and LCFAs, were below inhibitory concentrations. However, ammonia produced from the degradation of milk proteins was 62.2 mg/L, close to the inhibition level of the anaerobic digestion process (Vidal et al., 2000).

Meat processing wastes are substantially different from other food industry wastes. They are very strong wastes containing grease, blood, faeces, and recalcitrant organic matter such as straw and hair. During anaerobic digestion, protein and lipids degradation leads to the accumulation of ammonia and LCFAs, which are important inhibitors of the anaerobic microorganisms (Salminen and Rintala, 1999). The wastes also frequently include high concentrations of biocides and disinfectants such as hypochlorite (Tritt, 1992). The difficult nature of these wastes could be overcome by co-digestion, which could be advantageous due to an improved C/N ratio and dilution of the inhibitory compounds (Tritt, 1992).

3.3.2. Paper and pulp industry wastes

The pulp and paper industry has several high strength waste streams that are of concern from an environmental standpoint. Since the pulp produced corresponds to only 40–45% of the original weight of the wood, the effluents exhibit high COD concentrations (Ali and Sreekrishnan, 2001), which together with effluent's warm temperature (the waste is typically around 35 °C), makes anaerobic digestion a favorable waste treatment technique. Satisfactory digestion of paper mill effluent has been reported (Rintala et al., 1991). The most common inhibitors to the anaerobic digestion process include sulfide, tannins, resin acids, LCFAs, and halogenated compounds (Ali and Sreekrishnan, 2001). Sulfate is primarily produced in pulping operations using the sulfite process (Thompson et al., 2001). Sulfide removal can be achieved by sulfur bacteria which convert the sulfide ions to elemental sulfur (Buisman et al., 1991). Chen and Horan (1998) reported the use of a two-stage anaerobic-aerobic approach to remove COD and sulfate from newsprint mill wastewater. Tannins were found to contribute up to 50% of the COD of the debarking process wastewater. They are known to exhibit methanogenic toxicity proportional to the extent of polymerization (Field et al., 1988). Naturally occurred resin acids and LCFAs in the wood and bark could be transferred to process waters during pulping operations. They have been shown to inhibit methanogens, especially the aceticlastic methanogens (Hanaki et al., 1981; Koster and Cramer, 1987). Halogenated compounds are produced in the bleaching process. Their toxicity to anaerobes has been well documented. Most of the organic inhibitors are biodegradable to a certain extent (Ali and Sreekrishnan, 2001). However, knowledge of the possible contaminants present in the wastewater, their origins, and their degree of toxicity is essential to successful anaerobic treatment.

3.3.3. Textile industrial wastes

The main sources of wastewater generated by the textile industry originate from the washing (or scouring) and bleaching of natural fibers and from the dyeing and finishing steps. Given the great variety of fibers, dyes, process aids, and finishing products in use, these processes generate wastewaters of great chemical complexity (Vandevivere et al., 1998). Several laboratory-scale investigations have illustrated the potential of sequential anaerobic/aerobic biotreatment steps for textile wastewaters. Other studies have shown, however, that methanogenesis, and hence COD removal, is easily inhibited by textile effluents (Athanasopoulos, 1992). The components of textile wastewater that could be potential inhibitors are dye, dyeing auxiliaries (polyacrylates, phosphonates), surfactants (alkyl phenol ethoxylates), adsorbable organic halogens (chloroform), and heavy metals (Feitkenhauer, 2004; Lee and Pavlostathis, 2004).

3.3.4. Petrochemical refineries wastes

Anaerobic digestion could also be of use in petrochemical refineries. It has been found that after prolonged acclimation, aldehydes, acids, alcohols, and esters could be used for methane production (Chou et al., 1978). The presence of hydroxyl groups and an increasing carbon chain reduced

the toxicity of compounds to the digester microflora. Acclimation to aromatic ring and double-bond compounds was also possible. Chou et al. (1978) concluded that digestion of petrochemical wastes would not only result in a saving of energy over aerobic processes but would also produce methane on a scale for use as a fuel.

4. Conclusion

Anaerobic digestion is an efficient waste treatment technology that harnesses natural anaerobic decomposition to reduce waste volume and generate biogas at the same time. It has been widely applied to the treatment of waste from agricultural and industrial operations. Depending on the origin, the waste stream may contain inhibitory or even toxic substances such as ammonia, sulfide, heavy metals, and organics. Accumulation of these substances may cause reactor upset, as indicated by reduced biogas production and/or biogas methane content, and possible reactor failure. Due to the difference in anaerobic microorganisms, waste composition, and experimental methods and conditions, results from previous investigations on inhibition of anaerobic processes vary substantially. Obtaining information on waste components is necessary for successful application of anaerobic digestion. It has been suggested that co-digestion with other waste, adaptation of microorganisms to inhibitory substances, and incorporation of methods to remove or counteract toxicants before anaerobic digestion can significantly improve the waste treatment efficiency.

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