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Review Article

Anaerobic Treatment of Organic Waste for Methane Production under Psychrophilic Conditions

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Abstract

The anaerobically controlled biological degradation process is an innovative waste-recycling method to treat bio-wastes by comprehensive coordinated measures of microbes in order to significantly recover clean energy and organic fertilizer for managing versatile waste complexities. Biogas generation drops off drastically at the temperate conditions due to failure of mesophilic microorganisms to tolerate psychrophilic temperatures. As anaerobic microbes are very sensitive to temperature, decrease in temperature not only influences the composition, structure and activities of the microbial communities, but also alters the bio-chemical degradation steps of organic substances. The decrease in the population, growth and activity of microbial consortia increases the solids retention time twice to thrice, compared to the mesophilic and thermophilic anaerobic digestions and process instability. The low temperature adapted inoculum can enhance the startup and digestion operation as it may contain psychrophiles and mesophilic bacteria acclimatized on psychrophilic temperatures. The Instorage Psychrophilic Anaerobic Digestion (ISPAD) might be feasible for treating organic wastes due to its simplicity in temperate conditions. The aim of this study is to review psychrophilic anaerobic digestion treating organic wastes for biogas yield at the low temperature conditions.

Keywords: Biogas; Cold adapted inoculums; ISPAD; Psychrophilic anaerobic digestion

Introduction

The biomethanantion of organic waste is a long-standing and well-recognized technique. It is extensively used for biogas production and organic matter removal. Besides renewable energy recovery, biological degradation of organic matter provides many benefits including reclaiming nutrient rich fertilizer, reduction of odours and removal of pathogens (Chynoweth, 1996; Nohra *et al.*, 2003; Li *et al.*, 2011). This process has successfully been used since a long time for mesophilic (20−45°C) and thermophilic (45−60°C) environments (Borja *et al*., 2002) but the use of psychrophilic (<20°C) anaerobic digestion (PAD) is scarce (Dhaked *et al*., 2010). The reason for not broadly using the anaerobic digestion process at low temperatures is mainly due to the conviction that PAD was economically infeasible (Lettinga *et al*., 2001). Despite this, the most of the parts of the world have low-ambient temperatures. As waste generation is a natural consequence of human life, many wastes are discharged at temperate conditions. Moreover, disposal of wastes including animal wastes, farm wastes, municipal wastes, kitchen wastes and human waste at the hilly and high altitudes is a serious problem as untreated wastes are the sources of aesthetic irritation, contamination, toxicity, pollution, and diseases (Dhaked *et al*., 2010).

In winters, the temperature in the hilly and mountainous regions falls extremely low, and thus the production of biogas decreases drastically while the energy requirements in those areas are very high. The failure or low production of methane from biogas plants is reported due to intolerant capacity of mesophilic bacteria and archea under psychrophilic environments (Kashyap *et al*., 2003). Though such wastes contain high amount of biodegradable compounds, it is great challenge to stabilize economically because a large quantity of energy is needed to operate mesophilic or thermophilic bioreactor at optimal operational temperature for high biogas production rate (Kashyap *et al*., 2003). It does not mean necessarily psychrophilic anaerobic digestion cannot significantly degrade organic wastes to produce biogas. Low temperature anaerobic digestion would be smart substitute for the treatment of organic wastes, which are discharged under psychrophilic temperature ranges (Lettinga *et al*., 2001). In this paper, the literatures about psychrophilic anaerobic digestion have been reviewed. The cold adapted inoculum and ISPAD for biogas yield at the low temperature conditions have also been discussed.

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Biomethanation Process

Anaerobic digestion process is complex with a number of sequential and parallel steps that are carried out by different types of microbes in an oxygen-free environment to produce biogas, which mainly consists of 50-70% methane as an energy source, and 30-40% carbon dioxide along with other gases in small sum (Batstone *et al.*, 2002). This conversion includes four biochemical steps, which are hydrolysis, acidogenesis, acetogenesis and methanogenesis (Jha *et al.*, 2011; Veeken *et al.*, 2000). However, different bacterial species are responsible for different steps, microbes of each step depends on others for nutrient requirements, growth and survival. Anaerobic digestion begins with hydrolytic bacteria that hydrolyze multifaceted organic matters including carbohydrates, proteins, lipids and fats into uncomplicated monomeric carbohydrates, amino acids, sugars and long chain fatty acids by extra cellular enzymes (Jha *et al.*, 2011). Extremely low rate of hydrolysis is one of the bottlenecks for biological treatment of organic wastes under psychrophilic conditions. The monomeric compounds can easily be converted into volatile fatty acids, hydrogen, carbon dioxide and other minor products by fermentative microorganisms. In comparison to other volatile fatty acids, propionate is hard to degrade as well as extremely toxic for the anaerobic digestion process if it is accumulated (Dhaked *et al.*, 2010). Butyric acid performs as a vital intermediate product and is degraded preferentially over propionate (Nozhevnikova *et al.*, 2000). The organic acids are further converted into acetate, carbon dioxide and hydrogen by acetogenic bacteria. These products are considered as the direct substrates for methanogens in order to produce methane (Gerardi, 2003).

The superior activities, greater development and better growth rate of homoacetogenic bacteria under psychrophilic conditions are key benefits of this process. In addition, homoacetogens effectively participate with fermenting bacteria and hydrogenotrophic methanogenic archaea for general substrates (Kotsyurbenko, 2005). Acetotrophic methanogens convert acetate into methane as energy resource and carbon dioxide. The formation of acetate and acetoclastic methanogenesis is regarded as the major pathway in an anaerobic digestion process in order to produce methane from organic matter (Lokshina and Vavilin, 1999; Kotsyurbenko, 2005). Under lower temperature ranges and high concentration of acetate, acetoclastic methanogenesis is treated as a rate limiting step for methane fermentation process (Nozhevnikova *et al.*, 2007). From fig. 1, it is clear that the temperature affects both the rate of bio-methanation and the pathways for methane production by shifting the activities and profusion of individual anaerobic microbes (Kotsyurbenko *et al.*, 2007). Under low temperature ranges, H_2/CO_2 is transformed into methane in two steps. First, the acetate is produced. Then, methane is produced from acetate. The facts of the psychrophilic temperature bio-degradation pathways for different substrates and the composition of the anaerobic microbial communities under diverse methanogenic conditions are still not well-known.

Methane Production

Biogas production is temperature dependent (Nozhevnikova *et al.*, 2001). It increases linearly from 0°C to 20°C (Sutter and Wellinger, 1985) and attains most favorable under mesophilic environments from 32–38°C while the optimum temperature range is 50–55°C in case of thermophilic anaerobic digestion process. In psychrophilic environments, bio-chemical reactions carry on extremely slow compared to under the mesophilic and thermophilic temperature ranges (Chynoweth *et al.*, 1999) as higher energy is required to be proceeded the bio-chemical reactions for degradation of organic polymers into methane under psychrophilic temperature ranges (Lettinga *et al.*, 2001). The decrease in the population, growth and activity of microbial consortia increases the solids retention time twice to thrice, compared to the mesophilic anaerobic digestion and process instability. Low temperature causes deleterious effect on anaerobic digestion because of relatively longer generation time of bacterial populations and lower biochemical activity, resulting in the decrease of biogas yield and digester failure (Singh *et al*., 1999). The researches on low temperature anaerobic digestion for methane production are mainly concentrated on low temperature acclimatized mesophiles, which are psychrotrophs, not exact psychrophiles (Kashyap *et al.*, 2003). The activities of psychrophiles have not been reported at mesophilic conditions. Therefore, the most of the literatures have suggested to increase the operational temperature of the bioreactors up to mesophilic ranges for enhancing biogas production (Zeeman *et al.*, 1988; Lettinga *et al.*, 2001; Connaughton *et al.*, 2006a,b; Dhaked *et al.*, 2010). There are several proposed methods to increase the digester temperature. They are: i. heating of digesters and/or feed slurry using solar thermal heater or gas generated partly; ii. Insulating the digesters; iii. Integration of a greenhouse; iv. Construction of bioreactors below buildings such that heat can be transferred from barn to the digesters and so on (Lettinga *et al.*, 2001; Sutter and Wellinger, 1985; Zeeman *et al.*, 1988). However, the above methods seem smart; they are generally suffered from techno-economical barriers (Kashyap *et al.*, 2003). The energy required to heat the process makes it uneconomical in temperate climates.

Psychrophilic Anaerobic Digesters

The major challenge for biogas technology is its acceptability in cold regions in the winter season for potential energy recovery and sustainable waste management because the population and activity of anaerobes decrease substantially at the low temperature. With the extension of retention time and diminishing

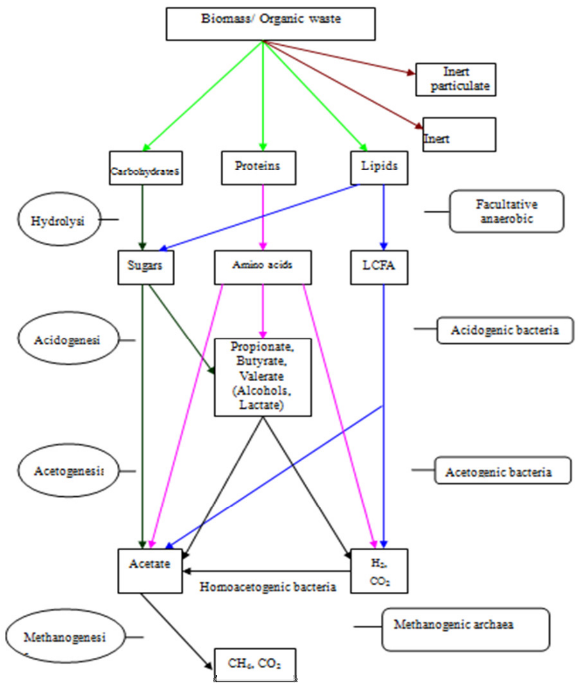

Fig. 1: Main steps and pathways of anaerobic digestion (Modified from Batstone *et al.*, 2002 and Kotsyurbenko *et al.*, 2007)

loading rate, psychrophilic anaerobic digesters can successfully degrade organic matters for reasonable biogas production (Sutter and Wellinger, 1985; Lettinga *et al.*, 2001). Rieradevall *et al.* (1983) used psychrophilic anaerobic bioreactors to treat swine manure at the hydraulic retention time of 100 days and observed that per cubic meter of the digesters produced 0.03 to 0.09 m^3 of biogas. Chandler *et al.* (1983) have observed that 0.66–0.92 m^3/m^2/day of biogas with 70% methane could be collected from a lagoon in California while the manure temperature was maintained in between 10 and 11°C at the hydraulic retention time of 50 days. Yu and Gu (1996) presented that anaerobic digestion at low ambient temperature is steady and is as competent as the digesters operating under mesophilic or thermophilic temperature ranges. A diminution in pathogenic microorganisms was also noted during anaerobic digestion at low ambient temperature (Côté *et al.*, 2006).

Psychrophilic Anaerobic Digesters for Waste Water Treatment

Low operating temperature may lead to slow-down of bacteria growth that the decrease degradation rate of substrates. Two measures could be adopted to improve the removal rate of psychrophilic wastewater treatment. First, the effect of a reduced temperature on system performances could be compensated by the high biomass population. Thus, the decrease hydraulic retention time could achieve it. Second, under relatively low average temperature of wastewater, heating of wastewater would be necessary.

As at low temperature conditions, anaerobic digestion of waste water has not been successful, a number of crucial enhancements are required to the design of traditional bioreactors (Dhaked *et al.*, 2010). Moreover, it is well-known that the viscosity of liquids including wastewater becomes more at low ambient temperatures. As a result, a large amount of energy is needed for blending and thus sludge bed reactors happen hard to be mixed, especially when the rates of biogas production are low (Lettinga *et al.*, 2001). As biomethanation happens in two-stages (Kashyap *et al.*, 2003), the division of the stages in high rate bioreactors provides superior prospects than single stage bio-digester and can enhance the overall anaerobic digestion process with the adequate biogas production (van Lier *et al.*, 1997). Lettinga *et al.* (2001) reported that the expanded granular sludge bed bioreactor are considered to be a viable system at psychrophilic temperature ranges in order to treat wastewater anaerobically. In their investigations using two laboratory-scale psychrophilic expanded granular sludge bed bioreactors, which were seeded with mesophilic methanogenic granular sludge and fed with a mixture of VFAs, in series in order to treat wastewater at temperature ranges from 3 to 8°C, comparatively a large amount of propionate were observed in the effluent of the first stage, but propionate was proficiently degraded in the second stage. Actually, a low hydrogen partial pressure and a low acetate concentration were beneficial for propionate oxidation. Lettinga *et al.* (2001) also found that a two-step system having either an anaerobic up flow sludge bed bioreactor combined with an expanded granular sludge bed bioreactor or an anaerobic filter combined with an anaerobic hybrid reactor was successful for biological treatment of sewage at 13°C with a total chemical oxygen demand (COD) removal efficiency of 50% and 70%, respectively. In addition, Connaughton *et al.* (2006b) have observed that considering biogas yields and chemical oxygen demand removal efficiencies, psychrophilic expanded granular sludge bed bioreactor was comparable with mesophilic expanded granular sludge bed bioreactor.

Cold Adapted Inoculum

The main factors to be considered during the startup period for smooth operation are as follows: i. mass and quality of inoculum, ii. adaptation or tolerance of anaerobic microorganisms, iii. rate of growth of micro-organisms, iv. hydraulic characteristics of bioreactor and so on (Chynoweth *et al.*, 1999). The inoculum should have high methanogenic activities; otherwise the mass of inoculum is required to be enlarged. The psychrophilic anaerobic digestion appears to have been carried out by mesophilic

Table 1: Operational characteristics of PAD using cold adapted inoculums

Substrates	Inoculum	Reactor	Temp.(°C)	RT (d)	Gas yield	References
Night soil	Night soil	Batch	10-30	25		Singh *et al.*, 1999
Swine manure	Natural biota	Batch	10	190	0.334 m^3 CH_4/kg CODin	Karumanchi, 2009
Wastewater	Cattle manure	Upflow anaerobic sludge blanket	15		0.25 $m^3CH_4/kgCOD_r$	Akila and Chandra, 2007
Cow dung	Cow dung	Batch	15	84	0.174 $m^3CH_4/kgVS_r$	Jha, 2012

RT: Retention Time, d: days

bacteria acclimatized to low ambient temperatures (Kashyap *et al.*, 2003). It is beneficial to inoculate a digester by means of psychrophilic temperature adapted inoculum to accelerate start up and achieve stable biogas production (Sutter and Wellinger, 1985; Zeeman *et al.*, 1988). Table 1 shows the operation characteristics of psychrophilic anaerobic digestion using cold adapted inoculum. Singh *et al.* (1999) reported that anaerobic digestion of night soil could be carried out at 10°C using a cold adapted inoculum. Singh *et al.* (1999) has also studied the effect of temperature fluctuation of night soil using 10°C adapted inoculum. No significant deleterious effect was observed during initial shock of one week but repeated exposures reduced the counts of hydrogenotrophic methanogens. The cold adapted inoculum makes the treatment of waste water at 15°C is feasible with 90- 95% COD removal (Akila and Chandra, 2007).

The subsistence of psychrophilic anaerobic bacteria and consequent methane yield in natural environments was reported by many researchers (Nozhevnikova *et al.*, 2001; Kotsyurbenko, 2005; Nozhevnikova *et al.*, 2007). Natural inocula contain psychrophiles and make the digestion feasible at low temperature. Karumanchi (2009) has presented that the inocula from natural biota could assist to decrease the startup period needed to commence psychrophilic anaerobic digestion processes. However, it should be beneficial if the initial startup should be performed with cold adapted natural inoculation materials, such inoculation materials are hardly ever accessible.

Jha (2012) has presented that the psychrophilic anaerobic digestion has the potential to become an economical and easy-to-use process to treat cow dung for methane production at low-ambient temperatures with the use of cold adapted inoculum. The inoculums sampled from psychrophilic and mesophilic environments were introduced and their effects on psychrophilic anaerobic digestion of cow dung for methane production at 15°C were investigated in single-stage batch reactors for 84 days. The results showed that the specific methane yield and volatile solids removal in the fermentation system inoculated with psychrotroph flora had been enhanced by 28.28% and 28.60%, respectively, than those in the system inoculated with mesophilic flora. Furthermore, the startup and performance of the process had been improved. Most of detected microbial communities such as *Clostridiaceae bacterium*, *Lactobacillus coleohominis* and *Prevotella* were mesophilic bacteria, acclimated at psychrophilic temperature, indicating that mesophilic bacteria could adapt the low temperature and the adaptation rate was increased with the digestion time. The specific methane yield was greatest when the psychrophilic anaerobic fermentation process was inoculated with a weight of 50% of the substrate, among the systems with psychrophilic inocula of 30%, 50% and 70%. An increment in amount of the psychrophilic inoculum considerably boosted the digestion efficiency and consequently resulted in the enhancement of the methane yield and organic materials removal efficiency but its larger mass failed to produce higher quantity of biogas. Compared to mesophilic and thermophilic anaerobic digestion processes, psychrophilic anaerobic digestion produced lower biogas and methane yields, and organic material removal efficiency but higher methane content was detected in the biogas yielded from low temperature anaerobic digestion of cow dung (Jha, 2012).

In-storage Psychrophilic Anaerobic Digestion

In Storage Psychrophilic Anaerobic Digestion (ISPAD) has originally been initiated in Canadian farmhouses to treat manures at low ambient temperature under anaerobically controlled environments. In fact, it was developed as a resolution for manure management, in which system the manure is stored up to long term under psychrophilic conditions (Wellinger and Kaufmann, 1982). Manure methanization by ISPAD is occurred in covered dung storage space or tanks under air-tight conditions; once the anaerobic microbial communities in the dung has been acclimatized to the low ambient temperature (Nohra *et al.*, 2003). ISPAD was actually developed based on reports of spontaneous methane production from stored manure and trials of psychrophilic digestion conceived to reduce the cost of technology for agricultural applications. ISPAD is comparatively steady having need of some degree of technical supervision. It needs feeding through siphons in order to make air-tight and preserving 20% of the mass, while it is unfilled, to seed the next inward manure. Carrying out psychrophilic anaerobic digestion in storage entails revealing the microorganisms to psychrophilic temperature ranges, which has a vital effect to accelerate the rate of biological treatment processes. It can influence the microbial inhabitants in two ways: by instantaneous alteration in activity rate, and by a variation over longer time, in the composition of the inhabitants themselves. Microorganisms can adjust to low ambient temperatures under anaerobically controlled environments in case of the hydraulic retention time is double of a mesophilic anaerobic treatment and the microorganisms are well adapted during the start up processes (van Lier *et al*., 1997). King *et al.* (2011) treated swine manure using ISPAD and found that psychrophilic anaerobic treatment process can convert 24% of the manure organic matter in the term of volatile solids into methane (0.27 m^3/kgVS) without accumulation of intermediate products. The microbial communities observed in the unprocessed wastewaters or manures, are mesophilic in origin entailing that shortly once setting up ISPAD, at least one year psychrophilic acclimation time is needed (Zeeman *et al*., 1988; King *et al*., 2011). Unlike mesophilic and thermophilic anaerobic digestion processes, low temperature anaerobic digestion limit biogas ammonia levels, regardless of the crash of proteins (King *et al.*, 2011). ISPAD effluent can offer a higher fertilizer value during land application, as compared to manure of similar age stored in an open tank because anaerobic digestion preserves nitrogen (Nohra *et al.*, 2003). This technology is very useful for developing countries. The requirements of the large space and long retention time are the main disadvantages of ISPAD and can limit the applications.

Conclusion

The low temperature adapted inoculum can enhance the startup and digestion operation as it may contain psychrophilic and mesophilic bacteria acclimatized on psychrophilic temperatures. The ISPAD may be feasible to treat organic wastes at temperate conditions in developing countries due to its low cost and simple in design.

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