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REVIEW PAPER

Microbial Degradation of Organic Wastes at Low Temperatures

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ABSTRACT

Microbial degradation of organic wastes mainly comprising animal and human wastes, is drastically reduced at extreme low temperatures. For the biodegradation of these wastes, technological inputs are required from disciplines like microbiology, biochemistry, molecular biology, digester modelling and heat transfer at extreme low temperature climates. Various steps in the process of biodegradation have to be studied to formulate an effective organic waste disposal method. Anaerobic digestion of organic wastes is preferred over aerobic waste treatment method, since it yields biogas as a by-product, which in turn can be utilised for heating the digester contents to increase its efficiency. Furthermore, one of the possibilities that can be explored is the utilisation of high rate anaerobic digesters which maintain temperature by means of artificial heating. It is either met by non-conventional energy sources, such as solar and wind energy, or by expending liquid fuels. In addition, insulation of the digester with polymeric materials and immobilisation of slow growing bacterial population may enhance the digester performance to a great extent. In spite of several developments, inoculum adaptation is considered to be one of the essential steps for low temperature anaerobic digestion to obtain methane as a by-product. With advancements in recombinant DNA technology, it may be possible to increase the efficiency of various microbial population that take part in the anaerobic digestion. However, till date, the options available for low temperature biodegradation are digester insulation, inoculum adaptation, and use of high rate/second-generation digesters.

1. INTRODUCTION

In recent decades the threat due to pollution has become a matter of serious concern. As the quantity of untreated sewage is enormous, it accumulates in the vicinity of human dwellings, leading to uncontrolled decomposition of organic content. Disposal of human waste at high altitudes and snowbound areas is an ever growing problem. It is more aggrevated in the Himalayan ranges of India, where no proper treatment is in practice.

In Antarctica, similar problem is being encountered by the expedition teams due to strict international enforcement laws on the disposal of night-soil and other wastes that pollute the pristine habitat of Antarctica. The conventional physicochemical methods, such as incineration, physical handling, isotopic destruction of pathogens and chemical treatment of nightsoil suffer from one or the other drawbacks. Chemicals are known to reduce pathogens with very little effect on the total organic content of the waste. Because of these constraints, microbial degradation is an efficient method for the disposal of nightsoil. As evident, biodegradation offers a rapid, effective and convenient means of degrading the nightsoil in an eco-friendly manner[1,2].

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The aim of the present review is to give state-of-the-art in biodegradation with emphasis on microbiology, process optimisation and integration. In addition, modern approaches in the field of low temperature anaerobic digestion and other important disciplines to upgrade the waste treatment have been discussed.

2. BIODEGRADATION/WASTE TREATMENT

2.1 Definition

Biodegradation is defined as the transformation of organic compounds by microorganisms into microbial biomass and other simpler compounds, ultimately releasing water, CO_2 and CH_4 into the atmosphere, whereas bioremediation is defined as the use of microbial activities (biological processes) to clean up environmental pollutants. Biodegradation includes various methods of sewage treatment and disposal of toxic pollutants present in soil, water and landfills, principally by the intervention of microorganisms. Biodegradation is drastically reduced at extreme low temperatures due to decreased growth rate of the microorganisms. Earlier studies were conducted on anaerobic digestion of night-soil at low temperatures[3,4]. The fermentation of night-soil at 10 °C was carried out using an adapted inoculum from cow-dung digesters.

2.2 Objectives

Waste treatment methods were considered as the best management practices as stipulated by the 1987 Amendments to the Federal Water Pollution Control Act, when the system was designed to take care of the following parameters and components:

(a) Reduction of waste volume and its mass is a matter of primary concern in waste treatment process. Also, treatment is required to increase the settling tendency of the polluting organic particles by microbial growth and flocculation.

(b) Removal of pathogenic microorganisms

(c) Production of stabilised effluent or humus with an appropriate $C{:}N$ ratio and low biochemical oxygen demand (BOD) range

(d) Denitrification of NO_3^- to nitrogen and minimisation of NO_3^- seepage (percolation) into ground water as it is a highly water soluble component contributed by sewage water. Potable water for human consumption containing NO_3^- levels above 10 mg/l are considered unfit, and at the same time levels above 100 mg/l are also not suitable for live stock consumption.

(e) Control over the PO_4^{-3} discharge into surface waters to avoid eutrophication. Because the PO_4^{-3} concentration as low as 0.0001 mg/l is known to be associated with eutrophication of surface waters.

(f) Control over the escape of reduced gaseous components, such as CH_4 and H_2S or volatile fatty acids responsible for foul smelling at the treatment site[5].

3. TYPES OF BIODEGRADATIONS

3.1 Aerobic Biodegradation of Organic Wastes

The principle organic substances found in any domestic waste and in some of the animal wastes are proteins (40-60 per cent), carbohydrates (25-50 per cent), fats and oils (10 per cent). In most biological sewage treatment systems, the principle biochemical reactions that occur during the active waste stabilisation[6] process are:

(a) Hydrolysis

(b) Oxidation

(c) Cell synthesis/biomass generation

(d) Endogenous respiration.

Hydrolytic reactions supply building blocks for biomass generation and substrate for cellular oxidation. Oxidation is carried out to obtain energy for the synthesis of new bacterial biomass. The rate of molecular synthesis and growth rate of bacteria is, therefore, a function of the ability of microorganisms to degrade the substrate. During endogenous respiration, the microorganisms begin to consume their own protoplasm due to depletion of nutrients in the medium. This phenomenon causes cell death and lysis. As a result > 5-80 per cent of the cell constituents are oxidised[7].

3.2 Biochemistry of Methanogenesis

The metabolic activity of anaerobes yields CO_2, H_2O, H_2S, CH_4, NH_4^+ and nitrogen. According to Zinder[8], the naerobic bacteria of digesters have been grouped into:

(a) Hydrolytic bacteria

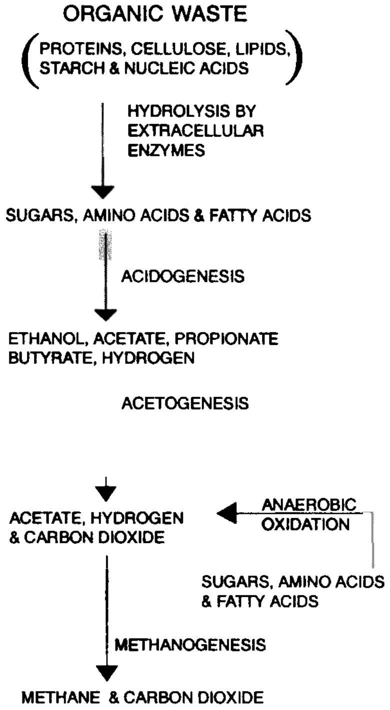
(b) Fermentative bacteria

(c) Acetogenic bacteria

(d) CO_2-reducing methanogens (lithotrophic methanogens)

(e) Acetoclastic methanogens.

The obligate anaerobes which convert organic acids to CH_4 and CO_2 (methanogens) are highly sensitive to low temperature and drop in p*H* levels. Microbial reactions that are expected to affect the p*H* in the anaerobic digesters include acetogenesis, methanogenesis, autotrophic carbon fixation as in the case of homoacetogens, such as *Acetobacter woodii* and denitrification[9]. While the facultative anaerobes are least affected by temperature, they produce fatty acids uninterruptedly, causing fatty acid accumulation. Higher volatile fatty acid levels, in turn, may suppress the methanogens, leading to the digester souring and failure (scheme 1). It has been noticed that the microbial growth and CH_4 production is very slow at sub-ambient temperature (< 35 °C) and the rate of methanogenesis is highly dependent on p*H* and temperature. The oxidation products of anaerobic acid-forming bacteria are CO_2 and CH_3COO^-. However, long chain fatty acids are sometimes produced as a means of scavenging surplus hydrogen. When conditions are favourable, syntrophic bacteria oxidise higher acids to hydrogen and CH_3COO^- to provide substrate for methanogens. The Gibbs' free energy and metabolic heat of these fermentative reactions are very small and many of these occur near equilibrium conditions and are readily reversible. The bacteria are capable of trapping the limited quantity of energy evolved by the biochemical reactions[10]. Even though the reactions occurring in anaerobic digester are exothermic, a large fraction of the energy of the system is entrapped in gaseous products. Hence, the energy available for bacterial growth is very small. Therefore, the theory of high rate anaerobic digesters was borne in mind for bacterial retention and buildup of large amounts of bacterial biomass using mechanical devices or modifications in the digester designing.



Scheme 1. Steps involved in anaerobic digestion (methanogenesis) of organic waste.

3.3 Anaerobic Treatment of Organic Wastes

Anaerobic digestion is one of the potential renewable energy sources and has assumed much importance in the context of night-soil disposal at mesophilic and psychrophilic temperature ranges. The rationale behind the use of anaerobic digestion is that the biogas produced at low temperatures can be used as fuel for heating the digester contents, so as to increase the digester efficiency at extreme low temperatures. Earlier, low temperature anaerobic digestion studies were conducted using domestic sewage sludge, cow-dung, industrial wastes and various types of animal wastes. However, studies involving night-soil are limited and most of these were carried out at mesophilic temperatures[11]. In our Laboratory, the anaerobic fermentation of night-soil was carried out at psychrophilic temperatures using the adapted slurry as inoculum[4]. Slurry from cow-dung digesters operating in hilly regions (*Palampur*, HP) was collected and adapted to night-soil and to 10 °C with a decrement of 2 °C at a 3-month interval. Biogas production was studied using 25 l digesters at 10 °C with semi-continuous feeding for 20-40 days hydraulic retention time (HRT). Digester operating for 25 days HRT produced 69.7 l of biogas/kg volatile solids/day with 73 per cent CH_4. Also, it was observed that lower HRT caused digester souring whereas higher HRT was not economical at low temperatures in as much as the digester volume should increase proportionately. To reduce the volatile fatty acid buildup in the digester as a result of low ambient temperature or sludge overloading, addition of alternate electron acceptor, such as SO_4^- was found beneficial. At 20 mm concentration, the compound enhanced the utilisation of higher fatty acids, such as propionate, butyrate and valerate without effect on CH_3COO^- consumption. Also, 10-fold increase in SO_4^--reducing bacterial counts and 2-fold increase in biogas production was noticed[12]. Furthermore, it was noticed that one per cent $CaCO_3$ enhanced the utilisation of volatile solids without accumulation of volatile fatty acids in comparison to control digester[13].

3.4 Pathogens Inactivation

The treatment of night-soil is not only required to reduce organic pollution but also to inactivate pathogens responsible for various gastrointestinal disorders. Pathogen destruction during sewage treatment is a highly temperature-dependent phenomena, hence the effect of aerobic and anaerobic treatment at temperature range 5-37 °C was studied on the inactivation of *Escherichia coli, Salmonella typhii* and *Staphylococcus aureus*[14]. It was found that *Escherichia coli* was most sensitive to aerobic treatment and T_{90} value increased to 5.7 days at 5 °C as against 4.6 days at 20 °C. Similarly, anaerobic treatment displayed better ability to inactivate all other pathogens and *Salmonella typhii* was found to be the most susceptible organism. It was observed that the cumulative effect of several factors, namely, temperature and metabolic products (volatile fatty acids and sulphide) of digester microflora play a major role in the elimination of pathogens. The maximum reduction of pathogens (99.99 per cent) occurred when the digester was functioning at 10 °C and for 25 days HRT.

3.5 Digesters at Low Temperatures

A limited study was carried out on anaerobic digestion in Korea where the winter temperature often falls to – 15 °C for about five months in a year. Insulation of the digester was attempted by covering it with rice hull. The digester temperature was around + 8 °C, while the ambient temperature was – 12 °C. The digester temperature around 15 °C was attained by erecting vinyl sheets over the digester. It was also observed that an increase in temperature by about 5-10 °C enhanced the gas production at least 10-fold. It was also demonstrated that fixed-dome digesters, completely buried under the ground, were known to be beneficial. Theoretical calculations show that the installation of digesters (5 m deep) are beneficial because the geothermal energy is expected to help in the maintenance of digester temperature[15] at around 15 °C.

3.6 Inoculum Adaptation

Several investigators also indicated the importance of adapted inoculum for efficient treatment at psychrophilic temperatures. Kettunen and Rintala[16] studied the effect of adapted inoculum on methanogenesis between 5-29 °C. The utilisation of CH_3COO^-, hydrogen and CO_2 in CH_4 formation was noticed even at 5 °C. The enhancement in methanogenic activity using adapted biomass (20 °C for 4 months) was 7 and 5-fold more at 11 °C and 22 °C, respectively.

3.7 Lagoons for the Treatment of Wastes

Oxidation ponds, facultative ponds and anaerobic lagoons are highly suitable for the treatment of wastes even at chilling temperatures of winter months. It is one of the most widely used methods of waste-water treatment at low temperatures in Canada[17]. There are about 1000 waste-water lagoons in Canada for the treatment of urban waste. Remarkably, lagoons are suitable to a wide range of climatic conditions and these are being used in regions ranging from tropics to arctic. Such oxidation ponds have been evaluated for their performance. Alaska ponds of USA, the Lubrinch ponds of Russia and Qiqihar ponds of China exemplify their wide range of applications. In all these ponds, drastic reduction in biodegradation rate was observed during winter months of the year. Depending on their nature, the ponds have been classified mainly into the following categories:

3.7.1 *Aerobic Ponds*

The aerobic ponds depend on surface aerators for aeration of the sewage. Thus, the process is basically similar to activated sludge process and thus known to function between 5-30 °C.

3.7.2 *Anaerobic Ponds*

The anaerobic ponds are mainly used for the treatment of high strength organic waste solids. To conserve microbial heat and to create anaerobic conditions, the ponds should be constructed more than 2 m deep and care must be taken to maintain anaerobiosis throughout the depth. These are designed to function at slightly higher temperatures (6-50 °C) in comparison to the aerobic ponds. The low temperature effects can be reduced either by increasing the depth of the lagoons or by decreasing the surface area. Furthermore, low temperature effects can be reduced by resorting to different modes of pond operation (in series or in parallel). By using lagoons, BOD reductions up to 60-70 per cent have been observed within 30-200 days. Anaerobic ponds are mainly used for pre-treatment of high strength waste water. The above-mentioned waste treatment methods are widely practised at low temperatures because there is no dearth of energy for mixing and pumping of sewage. Remarkably, these ponds require large area of land for construction, making them unsuitable in high altitude regions.

4. ROLE OF PSYCHROPHILIC & PSYCHROTROPHIC BACTERIA

Recently, it has been proposed that bacterial strains that grow optimally at low temperatures (< 25 °C) can be used to augment biodegradation at temperatures as low as 5-10 °C. Studies reported in the literature were mainly concentrated on the identification and characterisation of naturally occurring cold-active microorganisms and their enzymes. A few available reports highlight the degradation and detoxification of pollutants at very low temperatures[18]. Substantial decomposition of organic wastes is known to occur in the polar soils at < 5 °C. Lindeboom[19], *et al.* indicated that the microbial mineralisation of Penguin guano at temperatures as low as 5 °C.

Morita[20] defined psychrophilic bacteria as those that grow optimally at < 15 °C with an upper growth temperature of 20 °C. However, bacteria that grow optimally between 15-25 °C are known as psychrotrophs. These psychrophilic bacteria can also grow sub-optimally between 0-15 °C.

5. LOW TEMPERATURE BIODEGRADATION IN NATURE

Cold-active bacteria are abundant at sub-zero temperatures in permafrost soil of arctic and antarctic regions, as well as, in other snowbound high altitude glacier regions[21]. In the oceanic environment, biodegradation of various polymeric materials is mainly attributed to physiologically divergent population of bacteria. The organic polymeric matter originates from both animal and plant activities[22]. Cold-active bacteria are known to be more beneficial in aerobic degradation of organic matter under low temperatures than mesophiles[23]. Aerobic bacteria are highly suitable in the engineered biological waste treatment systems. The same also applies to the problem of night-soil disposal at high altitudes where energy supplies are limited.

For biodegradation of organic matter and secretion of hydrolytic enzymes, such as proteases, amylases, lipases, cellulases, nucleases, phosphatase, and β-galactosidase are essential and the presence of these enzymes is well documented in cold-active bacteria[23,24]. Since, most of the organic nitrogen and carbon released into the natural habitats and polluted sites is present in the form of high molecular weight proteins, nucleic acids, starch and cellulose, these are hydrolysed by extracellular hydrolytic enzymes, prior to microbial utilisation. In marine habitat, the water column bacteria mainly consists of psychrophilic *Vibrio* species whose optimum growth temperature[25] ranges from 5-20 °C.

6. PSYCHROPHILIC ANAEROBES

In addition, cold-active anaerobic microorganisms, such as acetogens and methanogens play an important role in the anaerobic degradation and CH_4 formation in a variety of ecosystems, ranging from deep granite rock aquifers, forest soils to deep lake sediments and Tundra soil. Antarctic methanogen, *Methanococcoides burtonii*, isolated from Ace lake was able to grow at 1.7 °C, utilising only methanol (CH_3OH) and methylamines[26] (CH_3NH_2). Methanogens and homoacetogens that could grow at psychrophilic temperatures have not been reported till date.

7. RECOMMENDATIONS FOR EFFECTIVE WASTE TREATMENT

7.1 Biosupplements & Bioaugmentation

In recent years, much attention has been paid to identify and define ways to use microorganisms and their degradative capabilities to enhance the biodegradation. Addition of specific microorganisms to increase biodegradation is known as bioaugmentation and the bacterial culture itself is termed as biosupplement. The biological treatment processes are intended to bring together the microbial cultures and organic matter to enable the microorganisms to utilise organic components as nutrient source under controlled conditions[27]. During biodegradation process, organic matter is converted into CO_2, H_2O, NH_3^+, NO_3^- and SO_4^{-2} and the whole process is carried out usually by a mixed microbial population than by a single species of bacterium[28,29]. In this context, a number of approaches have been developed for obtaining highly efficient, defined bacterial cultures. In general, batch enrichment and chemostat selection methods were often used for the isolation and characterisation of new bacterial strains with better efficiency. These methods also select the bacteria with enhanced environmental fitness and survivability. The widely known superbugs are microorganisms that can degrade a wide variety of pollutants at much faster rates than the bacterial strains which are available under natural conditions. Some of the methods used to develop or select the superbugs are: (i) adaptation (ii) mutation (iii) recombinant DNA technique, and (iv) screening of natural bacteria for their enhanced biodegradative property.

Superbugs are usually confined to bioreactor system for waste treatment under controlled conditions. A limitation of superbugs is their inability to establish a niche in the environment and their low survival rate under natural conditions. Several studies have suggested that non-indigenous bacterial population were competetively eliminated by naturally selected indigenous microbial population. However, addition of large quantities of initial microbial supplement with periodic maintenance doses was suggested as a remedy to overcome the competition. Bacterial supplements are increasingly being marketed as an effective and economical means to enhance biological waste treatment[30,31]. It has been suggested that *in situ* biodegradation of pollutants at different temperatures can be tackled with appropriate microorganism cultures[32]. In some instances, polluted sites are located at ambient temperature that coincides with the optimum growth temperatures of cold-active bacteria[33]. A limited number of investigations have highlighted the importance of cold-active bacteria in the bioremediation of soil, water and marine conditions[18,34].

7.2 Immobilisation & Process Intensification

Immobilisation of microorganisms on carrier matrices has attained importance for enhancing biodegradation under aerobic and anaerobic conditions. The high rate anaerobic reactors, such as fixed beds (packed beds), expanded beds, and fluidised beds are simple to construct and can be operated at low energy levels.

7.2.1 *High Rate Anaerobic Digestion*

Modern anaerobic digesters operated with mixing and heating to maintain the digester temperature near 35 °C are classified as high rate anaerobic digesters. In most cases, mixing is provided by recycling the head space gases through the mixed liquor (digester slurry) or by means of mechanically-driven propeller with draft tubes. The incorporation of heat exchange equipment is a prerequisite and in most of the cases, a simple internal heating coil is sufficient. Recently, many anaerobic digesters have been developed. The high rate digesters work with short retention periods and maximum treatment efficiencies along with higher biogas yields. High operating efficiencies can be achieved by recirculation of CO_2 for mixing the contents.

The principle of high rate anaerobic reactor is based on phenomena, such as biomass retention by means of settling, attached growth on solids and by recirculation of biomass. Immobilisation of bacterial biomass in the digester, as bacterial aggregates or in the form of biofilms, allows efficient interspecies mass transfer with discrimination between HRT and mean solids retention time (MSRT). Reactors of this kind have the ability to retain the slow growing microorganisms. Besides, the system helps overcoming limitations of substrate and product diffusion from bulk liquid to biofilm and the formation of granules with enhanced biomass activity due to adaptation and growth. Based on these, the up-flow anaerobic sludge blanket (UASB) reactor was designed for the formation of flocs and granules. The reactor system consists of a gas/solid/liquid separation device for efficient separation of the above three components. It exemplifies the importance of biomass retention to be highly desirable, particularly, in the case of obligate hydrogen producing acetogenic bacteria. Moreover, thermodynamically, acetogenesis is not feasible unless the concentration of one of the products, i.e., hydrogen, is maintained at an extremely low level. Solids retention time (SRT) in anaerobic digesters is largely dependent on the process temperature. Therefore at lower temperatures, higher SRTs are required for the removal of organic matter to the desired level.

7.2.2 *Digester Start-Up*

SRT (θ_c^m) is about two days at 35 °C but it increases to 10 days at 20 °C. The simple anaerobic digester can be classified as suspended growth system, where θ_c denotes MSRT.

$$\theta_c = \frac{X}{\Delta x / \Delta t}$$

where X is the total microbial biomass in a reactor, and $\Delta x/\Delta t$ is the total quantity of solids withdrawn daily. θ_c^m is the minimum value of θ_c at which digester function is normal. At MSRT less than θ_c^m, slow growing anaerobes are removed at a faster rate than their growth rate causing washout of the microbial biomass. θ_c^d is the design value of θ_c which is sometimes as high as 4-20 times higher than θ_c^m. Therefore, the ratio θ_c^d/θ_c^m denotes the safety factor of the digester. The safety factor is required to be adjusted to the variable nature of feed and climatic temperature fluctuations and other parameters that may perturb the microbial growth. The presence of biomass on suspended carriers facilitates the high settling of sludge and have a high biomass concentration, leading to low volume reactors (high loading rates). The formation of bacterial biofilm on suspended carriers or any other matrix is possible only at short liquid residence time. At longer liquid residence time $D < U_{max}$, the suspended undesirable microorganisms are retained in the digester and biofilm formation is hampered by methanogens because of suspended fast growing facultative anaerobes can out-compete the slow growing microorganisms for biofilm formation. At very short liquid residence time ($D > U_{max}$), a biofilm of methanogens and other complementary anaerobes can develop in the reactor without much competition and the suspended microorganisms are eliminated by washout. Hence, shortest possible liquid residence time has been recommended for biofilm formation on carrier matrices.

7.2.3 *Matrices for Immobilisation*

The high rate anaerobic reactors, such as fixed beds (packed beds) and expanded beds are simple to construct and can be operated at low energy levels. Fluidised bed was stated to be an efficient anaerobic treatment system with high loading rates and short liquid residence time[35]. Because small size particles are used in the fluidised bed (0.2-0.6mm in diameter), the superficial area of bed material is enormous for bacterial biofilm formation. In trickling filters, a superficial area of 800 m^2/m^3, 206 m^2/m^3 in anaerobic filters and 3000 m^2/m^3 in fluidised bed can be attained[36]. Most often, these types of reactors are recommended for the treatment of low strength wastewaters. The effect of fluctuating temperature and the introduction of toxic compounds has almost negligible effect on the attached growth processes. Because the changes that occur in the bulk liquid are delayed to reach microorganisms embedded within biofilms and granules of high density[34]. According to earlier reports, the physiology and morphology of *Methanothrix* is mainly responsible for biofilm and granular sludge formation in second-generation type of anaerobic digesters. Mass transfer of hydrogen between two bacterial population is nearly 200 times higher in aggregated biomass than in dispersed growth.

For immobilisation of anaerobic microbial population in the digester, 20 different matrices in 100 ml serum vials were evaluated and amongst them, it was found that coconut coir, polyurethane foam, polystyrene pads and glass wool were suitable. These selected matrices were further evaluated for digestion of nightsoil in 2 l digesters at 10 °C and 5-20 HRT. Rubberised coir (25 g/l) was found to be of significant potential and it increased biogas production by two to three-fold. Immobilisation of bacteria on coir was confirmed by scanning electron microscope and fluorescent microscope. This method of immobilisation can be, particularly, useful for biodegradation of night-soil at low temperatures where the retention of bacterial biomass is an important attribute.

7.3 Merits of Anaerobic Digestion

The CH_4 produced in anaerobic digestion can be useful to heat the digester and with suitable mechanical device, it can be used to mix the slurry. Addition of chemical in relatively small quantities may be required for satisfactory ultimate disposal. Low biomass yields of anaerobic bacteria result in low sludge production and low amounts of inorganic nutrients are required for bacterial growth. Thus, wastes that are not properly balanced with nitrogen and phosphorous containing compounds can also be digested. No oxygen is required unlike aerobic treatments and energy consumption is comparatively low than aerobic treatments. The by-product CH_4 is a valuable renewable energy resource. Removal of pathogenic microorganisms is effective, but heating of the digester is required at low temperatures and the capital costs of anaerobic digesters could be high. Cost-effectiveness can be achieved by treatment of high strength wastes.

8. POSSIBLE MEASURES FOR PROCESS OPTIMISATION

(a) Improvements in digester designing and fabrication.

(b) Development and selection of polymeric material and metals to be used for digester construction. The material should facilitate temperature maintenance through insulation, at higher side.

(c) Heating by solar energy or by spending liquid fuel is indispensable for the maintenance of digester temperature. Attempts are being made to construct green houses and instal wind mills around the digester to utilise these sources for heating up the digester contents. Automation as an interface between the energy sources and temperature control in the digester is essential to maintain digester performance at steadystate.

(d) Fixed-dome digesters are more suitable for under ground installation.

(e) Immobilisation of anaerobic microbial populations on matrices is essential to protect them from environmental perturbations and also to enhance their residence time. Screening and identification of suitable matrices which are easily available and transportable to hilly terrains are preferred.

(f) Adaptation of inoculum to temperatures as low as 5-10 °C and the temperature maintenance around the ranges is a prerequisite using either biogas or any other non-conventional energy source. Adaptation is a long-term programme required for the accumulation of psychrotrophic and psychrophilic bacteria by autoselection and spontaneous mutations.

(g) Mixing of the digester contents may enhance the digester efficiency. It can be achieved by recycling the head space gas or by mechanical stirrers.

(h) Anaerobic-aerobic mode of treatment is suitable for night-soil disposal, since the effluent from anaerobic digester still may contain NH_3 (120 mg/l), pH (130 mg/l), and residual COD between 50 and 300 mg/l. The cold-active bacteria can be useful for the removal of volatile fatty acids, H_2S, NO_3^-, and PO_4^{-3}.

9. RECOMBINANT DNA TECHNIQUE

With the advent of recombinant DNA technique and recent developments in molecular biology, it has become possibile to degrade various xenobiotic compounds. Enzymes which take part in biodegradation of organic compounds can be constitutively expressed by modifying the regulatory gene sequences by recombinant DNA technique. In most cases, the enzyme production was a limiting factor for optimum biodegradation. Fusion of structural genes, downstream of strong promoter present in another operon, may provide a suitable method for increasing gene expression in heterologous hosts. Similarly, temperature-sensitive promoters (cold-promoters) can be used for high level gene expression, specifically at low temperatures. The presence of cold-promoter has been delineated recently in antarctic bacterial strains. The development of recombinant DNA techniques to the strain improvement of methanogens has been hampered due to lack of suitable vectors for cloning genes.

The presence of plasmid pME2001 has been described in a methanogen, *Methanobacterium thermoautotrophicum*[37]. However, with the development of new cloning vectors, it would be possible in the near-future to come out with efficient strains of methanogens adapted to low temperatures.

CONCLUSION

It can be concluded that adapted inoculum slurry or mixed microbial consortium still appears to be an important modality for enhancing biodegradation of organic wastes at extreme low temperatures. The efficiency of anaerobic digesters can further be augmented with minimum heating, utilising non-conventional energy sources or by designing and installating high rate digesters. However, the proposition at the moment is that substantial improvements in the methanogenic ability of bacteria can be brought by recombinant DNA techniques and this can be achieved only by a thorough study of the anaerobic bacteria at molecular level.

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