

AEROBIC WASTE SEWAGE SLUDGE BIOTREATMENT FOR ENHANCED ENVIRONMENTAL SAFETY

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Waste sewage sludge, an inevitable by-product of municipal sewage biotreatment, is a noxious and potentially hazardous slurry which needs effective treatment prior to its recycling to the natural environment as a soil conditioning agent. The most hazardous components in untreated waste sewage sludge are pathogens and toxic chemical residues.

The major problems affecting guaranteed irreversible inactivation of pathogenic agents are a failure to fully understand the mechanisms responsible for cell death and how enhanced cell death can be affected by sludge bioprocess operating conditions. In these contexts, the efficacies of thermophilic aerobic, thermophilic anaerobic and mesophilic anaerobic sludge biotreatment processes are discussed in this review paper. In order to achieve both effective and economic biotreatment performance, a combination of thermophilic aerobic pre-treatment for hygienization and mesophilic anaerobic treatment for stabilization is recommended.

Keywords: Sewage sludge, pathogens, thermophilic aerobic hygienization, mesophilic anaerobic stabilization.

INTRODUCTION

The discharge of large volume water-borne waste streams into the natural environment is a direct consequence of the introduction of unrestricted piped water distribution to individual premises and the introduction of the water-closet as the conventional means for sanitary waste removal from the mid-nineteenth century onwards. Two major types of aqueous waste streams exist; municipal sewage, comprising a highly complex mixture of domestic sewage, hospital and trade wastes, and industrial wastewater streams. The latter are sometimes less complex in composition and can be free from sanitary waste. Both types of stream can contain some storm water surface run-off with associated pollutants. Prior to the enactment and enforcement of effective water pollution designed to protect fresh surface, estuarine and coastal marine waters and groundwater reservoirs, variously polluted waste streams were discharged directly into the environment with negligible concern for either their ecological impact or their potentially adverse effects on human health. However, increasingly stringent environmental legislation and better enforcement has, in more recent decades and in the majority of industrialized countries, resulted in the widespread introduction of both municipal sewage and industrial wastewater treatment, most commonly by aerobic mesophilic activated sludge biotreatment plants. Unfortunately, this has created a further problem; waste sewage sludge treatment for either safe disposal or safe utilization in the environment. This review paper summarizes current knowledge concerning cell death during sewage sludge biotreatment processes.

Municipal sewage mirrors both the activities undertaken in and the general health of the municipality responsible for its production. Noxious components include pathogenic agents ranging from viruses, to a variety of micro and macroorganisms derived from both animal and human faecal matter. These include pathogenic bacteria, protozoa and flukes, and eggs of intestinal worms. Other hazards such as toxic organic chemicals, residues from hospitals, clinics, households and trade and industrial sources and toxic inorganic chemicals, most commonly from workshops and industrial activity are common. Should animal slaughterhouses (abattoirs), meat processing and/or animal waste rendering plants be discharging wastewater to sewer in any particular drainage area, the additional minuscule risk of prior release from specified waste material must also be assessed.

Many different variations in activated sludge plant design are employed. Inevitably, activated sludge biotreatment (secondary) stage plants are operated in conjunction with both mechanical (preliminary) and physical (primary) solids separation stage processes. The objectives of aerobic secondary treatment are the bio oxidation of both dissolved and finely divided biodegradable organic matter to produce a reduced mass of flocculated bacteria (sludge) and carbon dioxide, the nitrification of ammonium to nitrate, and where appropriately designed, complete denitrification of nitrate to elemental nitrogen. Some activated sludge plant designs also seek to eliminate phosphate by bioaccumulation mechanisms, rather than by its utilization in bacterial growth processes, while others employ chemical phosphate precipitation with various salts during biotreatment. The biomass (sludge) produced during secondary biotreatment is concentrated and either recycled to maintain bioprocess intensity or wasted. Secondary biotreatment is an unsteady state process partially due to diurnal changes in volumetric sewage flow and short term fluctuations in sewage component composition, but also as a result of the plant operating protocols employed that seek to minimize sludge production by empirical means. The effect of protozoan predation requires mention at this point, as it results in a reduction in overall sludge production and improved treated water clarity.

Waste sewage sludge is frequently considered to be flocculated microbial biomass and associated particulate solids that comprise the sludge wasted from secondary treatment, but in some operations, waste preliminary sludge and/or waste primary sludge are combined with it. Sewage treatment plants were introduced in order to reduce surface water pollution to levels where natural self-purification mechanisms could adequately cope with the residual pollutant loads discharged into any particular receiving water, although over reliance was frequently placed on the dilution of particular pollutants present in a discharge to less than detectable, apparently no effect, concentrations. Although it has been frequently claimed that sewage treatment focused on the maintenance of public health and environmental safety, the removal of pathogenic toxic and other noxious components from municipal sewage during treatment is essentially an incidental, rather than an intentional event. Some toxic chemicals, depending on their properties, can be sorbed onto particulate matter removed in primary physical treatment and, hence, even if biodegradable, will not be biodegraded prior to incorporation into waste sewage sludge. Some non-biodegradable toxic chemicals will also become secondary waste sludge components. Soluble non-biodegradable

toxic organic components that enter secondary treatment will, for the most part, pass through the system unaffected and become waste secondary sludge components. Pathogenic agents can be flocculated, becoming a component of waste secondary sludge, be biodegraded as particulate substrate and, hence, inactivated, or utilized as a food source by the protozoa present in secondary aeration tanks. Essentially, the majority of pathogenic agents present in raw sewage will be partitioned into waste sewage sludge, and become a potentially problematical component thereof. Hence, their ultimate fate depends on the efficacy of waste sewage sludge treatment and the prescribed means of waste sewage sludge utilization, most commonly either in agriculture or by application to green land now that neither marine dumping nor landfill are considered appropriate options. However, in spite of the risks frequently attributed to waste sewage sludge utilization for land fertilization and conditioning, the widespread use of such practices has resulted in little evidence that epidemics of infectious human diseases have been traced to the carryover of active pathogenic agents in waste sewage sludge, although it should be mentioned that the use of settle raw sewage for irrigating salad vegetable and soft fruit crops is a regular and widespread source of human enteric infection. Without doubt, the public perception of risk with respect to possible infection requires effective waste sewage sludge treatment prior to its recycling to the environment. Such treatment involves both stabilization and hygienization.

The two most critical criteria concerning secondary sewage sludge management are; minimization of the quantity of sludge produced per unit mass of pollutant oxidized (the sludge yield coefficient) and what, if any, processes are available for reliable and comprehensive sludge disinfection, at sensible overall cost, prior to either utilization or ultimate disposal. The first criterion is primarily concerned with the physiological state/ situation of the biomass mediating pollutant elimination from the settled sewage undergoing biotreatment. Until some 20 years ago, biotreatment processes were evaluated on the basis of an apparent lack of necessity to understand the physiological mechanisms governing process performance. One of the major difficulties of progressing beyond such a 'black box' type approach was the variable and complex nature of most aqueous waste streams, particularly the virtually exclusive use of 'lumped parameters', rather than individual pollutant concentrations, to describe waste stream composition and failure to understand applicable bioprocess kinetics, rate equations and, ultimately, bioprocess dynamics. In the case of activated

sludge processes, such problems began to be rectified by the introduction of realistic process modelling and simulation. Even so, the fundamental mechanism involved in process operation was thought to be biomass growth and the nebulous concept of endogenous metabolism was considered as the key process in reducing, even eliminating, biomass production. This occurred in spite of the fact that such a concept represented a trend that was inadequate in attaining anything approaching zero biomass (sludge) yield coefficients. The concept of endogenous metabolism is, in itself, a 'lumped parameter' comprising an array of potential mechanisms including, maintenance requirements, actual endogenous respiration, cell lysis, decay and subsequent 'cryptic' growth, cometabolism, fortuitous oxidation, predation (biovoracious activity) and physiological effects concerning biomass yield coefficient depression, particularly the effects of inhibitors as uncoupling agents during oxidative phosphorylation. However, at the end of the day, it might well be the physiology of non-growing (stationary phase) cells that should be the subject of primary investigation.

Disinfection involves two distinct types of overall activity; bactericidal mechanisms and bacteriostatic mechanisms. The former mechanisms can result in complete pathogen inactivation, although cases of recovery have been reported, while in the case of the latter mechanisms, removal of the active agent or physical condition employed, results in significant recovery of the pathogens present. Disinfection can be achieved by addition of noxious chemicals that frequently remain associated with the product undergoing treatment, or by physical effects, particularly elevated temperatures. In general, particulate matter, such as waste sewage sludge solids, exhibits adverse effects as far as disinfection performance is concerned, particularly in the case of chemical disinfectants, so elevated temperature treatment is the preferred technology as far as potential efficacy is concerned. Even so, process economics dictate that maximum benefit must be derived from the potential heat of combustion of the waste sewage sludge itself and that process heat recovery must be employed. However, the efficacy of waste sewage sludge disinfection (hygienization) depends on both the physiological state of the pathogens in the sludge undergoing treatment and the physiology of the process bacteria mediating the hygienization process.

PATHOGENIC AGENTS IN SEWAGE SLUDGE

In spite of the fact that the introduction of sewage treatment has frequently been credited with the control of epidemics of waterborne diseases, the primary event responsible for such control was the transportation of fecally contaminated waterborne waste to remote treatment or disposal sites via the sewer network. Traditional sewage treatment has erroneously been assumed to effectively deactivate pathogenic agents present in sewage but, generally speaking, few effects of appropriate intensity to cause deactivation have been identified, apart from the biologic activity of certain protozoans. However, what does occur during conventional sewage treatment is the partitioning of pathogenic agents into sewage sludge, rather than into the clarified effluent that is discharged from such plants. The fear of pathogenic agents in clarified effluent has, particularly in the USA, resulted in chlorination of clarified effluent, which although effective for pathogenic agent deactivation, also results in the formation of potentially carcinogenic organo-chlorine compounds as a result of chemical reaction between chlorine and dissolved residual humic matter present in clarified effluent and, hence, is an inappropriate technology.

In a relatively recent review, Dumontet et al. (2001) have discussed the pathogenic organisms present in both sewage and sewage sludge, with particular emphasis on stabilizing and sanitizing (hygienizing) sludge to be used in agriculture as a soil amendment (conditioning) product. The pathogenic agents potentially present in sewage sludge that are considered are viruses, bacteria, yeasts, fungi and zooparasites. The types of pathogens found in any particular sewage sludge depend on the state of the public health of the population producing the raw sewage and on the presence of hospitals, abattoirs, meat-processing factories and tanneries within the sewage catchment area. As far as survival is concerned, pathogens exhibit a range of strategies including spore and cyst formation. Even in those developed countries where high hygienic standards are generally maintained, the prevalence of pathogens in sewage is considerable and it is often food-borne pathogens that predominate. However, here it is inappropriate to discuss specific incidences of pathogenic agent contamination of sewage sludge, but necessary to detail as many as possible of the pathogens that have been isolated from sewage sludge.

Table 1 lists some of the pathogens that have been isolated from sewage sludge. Of course, no such list is fully comprehensive and further

additions, particularly with respect to newly emerging pathogens, will always be necessary. Two particular examples of such emerging pathogens that are worthy of mention are *Legionella pneumophila*, an opportunistic pathogenic bacterium and *Cryptosporidium parvum*, a protozoan parasite of man and other animals, that have been found in sewage and in sewage sludge. *Legionella* spp. are natural freshwater inhabitants that readily colonize artificial environments such as potable-water distribution systems and water cooling towers (James et al., 1999). They exhibit a high degree of environmental persistence. This is because of their ability to adapt to diverse ecological niches including growth as intracellular parasites of amoebae, as members of complex biofilm communities and as free-living planktonic cells, environmental conditions that seem to prepare them for starvation (stress) conditions and maintenance of their viable culturable state after long periods without growth. *Legionella* spp. are present in settled raw sewage used for irrigation in semi-arid regions (Fattal et al., 1986), so it can be anticipated that, when sewage is subjected to conventional biotreatment, such bacteria, if originally present in raw sewage, will partition, at least in part, into the sewage sludge produced. The second emerging pathogen mentioned above, the protozoan *Cryptosporidium parvum*, forms oocysts, containing infectious sporozoites, during its life cycle the gastrointestinal tract. The robust nature of the oocysts makes them resistant to deactivating stresses and confers the abilities to survive in inadequately treated sewage sludge and to contaminate crops, including salad vegetables, produced on agricultural land that has been subjected to amendment with such sewage sludge (Warnes and Keevil, 2003).

CELLULAR GROWTH AND DEATH

The bacteria primarily responsible for the bio oxidation of biodegradable pollutants during secondary activated sludge bio treatment processes function under conditions of relatively severe limitation, primarily as a result of high process intensities caused by biomass recycling and starvation during both bio oxidation and settling. Hence, growth occurs predominantly in the decelerating, stationary and declining (death) phases of the classical bacterial batch growth cycle. Until some twenty years ago, the death mechanisms of microbial cells were a virtual scientific taboo (Mason et al., 1986b).

Table 1. Partial list of pathogens that have been isolated from sewage sludge (Dumontet et al. 2001)

Viruses	Enteroviruses, including polio virus, coxsackievirus A and B, echovirus, adenovirus, parovirus, reovirus, hepatitis A, B and C viruses, rotavirus, astrovirus, calcicivirus, coronavirus, Northwalk agent and other small round viruses, and adeno-associated viruses; Polyomaviruses, including JC and BK
Bacteria	<i>Aeromonas</i> spp., <i>Arcobacter</i> spp., <i>Bacillus anthracis</i> , <i>Brucella</i> spp., <i>Campylobacter coli</i> , <i>Campylobacter fetus</i> , <i>Campylobacter jejuni</i> , <i>Clostridium botulinum</i> , <i>Clostridium perfringens</i> , <i>Escherichia coli</i> 0111:NM, 0157:H7 and 0184:H21, <i>Leptospira</i> spp., <i>Listeria monocytogenes</i> , <i>Mycobacterium</i> spp., <i>Pseudomonas aeruginosa</i> , <i>Salmonella</i> spp., <i>Shigella</i> spp., <i>Staphylococcus</i> (coagulase positive) strains, <i>Streptococcus</i> (beta-hemolyticus) strains, <i>Vibrio cholerae</i> , <i>Vibrio parahaemolyticus</i> , <i>Vibrio vulnificans</i> , and <i>Yersinia enterocolitica</i>
Opportunistic pathogens	<i>Citrobacter</i> spp., <i>Enterobacter</i> spp., <i>E. coli</i> , <i>Klebsiella</i> spp., <i>Proteus</i> spp., <i>Providencia</i> spp., and <i>Serratia</i> spp.
Yeasts	<i>Candida albicans</i> , <i>Candida guilliermondii</i> , <i>Candida krusei</i> , <i>Candida tropicalis</i> , <i>Cryptococcus neoformans</i> and <i>Trichosporon</i> spp.
Fungi	<i>Aspergillus</i> spp., <i>Geotrichum candidum</i> , <i>Epidermophyton</i> spp., <i>Phialophora richardsii</i> , <i>Trycophitum</i> spp.
Protozoa	<i>Cyclospora cayetanensis</i> , <i>Cryptosporidium parvum</i> , <i>Encephalitozoon intestinalis</i> , <i>Entamoeba histolytica</i> , <i>Giardia lamblia</i> , <i>Sarcocystis</i> spp., <i>Toxoplasma gondii</i> and <i>Vittaforma corneae</i>
Cestodes (tape worms)	<i>Diphyllobothrium latum</i> , <i>Echinococcus granulosus</i> , <i>Hymenolepis nana</i> , <i>Taenia saginata</i> and <i>Taenia solium</i>
Nematodes	<i>Ancylostoma duodenale</i> , <i>Ascaris lumbricoides</i> , <i>Necator americanus</i> , <i>Toxocara canis</i> , <i>Toxocara cati</i> , <i>Trichiurus trichiura</i>

While discussion of the various phases of bacterial growth during batch culture has been extensive, both the stationary phase and the phase of decline were largely ignored. Attempts to describe the overall batch growth curve for bacteria and other microorganisms in mathematical terms occurred throughout the twentieth century (Wanner and Egli, 1990). It is agreed that bacterial cultures exhibit a sequence of phases prior to reaching the stationary phase. First is the lag phase, where essentially acclimation to the physical and chemical characteristics of the growth environment occurs. It is followed by the accelerating phase, where cell size and macromolecular composition changes markedly, but replication does not occur. Then cultures enter the logarithmic (or exponential) growth phase, where unrestricted growth occurs, which is followed by the decelerating phase, where kinetic parameters, essentially either nutrient or substrate affinities, affect the specific growth rate coefficient.

Historically, growth curves were based on bacterial numbers, but since the 1950s, their basis has very largely changed to dry bacterial biomass or protein concentration. Numbers and mass rarely coincide during the accelerating and stationary phases. The logarithmic, decelerating and stationary phases of the typical sigmoidal growth curve that is regularly observed for heterotrophic bacterial growth on a single carbon energy substrate can be described by simple growth theory. A fundamental prerequisite of growth theory is that both the elemental and macromolecular compositions of the growing bacteria remain constant. Strictly speaking, this only occurs during the logarithmic growth phase and has been described as balanced growth.

The most widely used kinetic expression for the quantitative description of growth is the hyperbolic Monod relationship between the specific growth rate coefficient and the substrate concentration (Monod, 1935). The Monod relationship is based on Michaelis-Menten (saturation) type enzyme kinetics and makes the fundamental assumption that bacterial growth is controlled by the rate constant of the slowest enzymatic reaction involved in the overall growth process. Although such a master reaction approach has been increasingly challenged during the past 20 years, the Monod relationship remains dominant. Other kinetic relationships, e.g., Blackman kinetics (Dabes et al., 1973), have been used, but are largely disregarded by a majority of researchers. More recently, thermodynamic approaches have been introduced (Westerhoff et al., 1982; Tan et al., 1994). The fundamental problem with the Monod relationship is experimentally determining substrate concentrations in the

range where they impact on the value of the specific growth rate coefficient (Senn et al., 1994). In the overall batch growth curve, this situation applies throughout the decelerating growth phase.

Merchuk and Asenjo (1995) suggest that the Monod relationship is open to alternative interpretation and that analysis of limiting substrate mass transfer results in an essentially similar hyperbolic relationship. Further features of substrate and/or nutrient uptake by bacteria are the existence of strains with both high and low affinity enzyme uptake systems for and strains with multiple substrate and/or nutrient utilization pathways. However, in the context of the present review, it is more appropriate to consider the probable importance of stationary phase physiology where the several changing perceptions of more recent years were discussed by Kolter (1999). Unfortunately, the vast majority of recent studies concerning, particularly molecular level processes that occur under stationary (non-dividing) phase conditions involve laboratory strains of one bacterium, *E. coli*. While it is obviously questionable to generalize findings based exclusively on *E. coli* strains, it would also be unreasonable in the absence of more extensive experimental results, to ignore the implications that the numerous observations made, with *E. coli*, on the cessation of cell division.

The long-term survival of bacteria depends on their ability to establish protection against the various debilitating and lethal stresses to which they are either purposely or incidentally exposed. In the case of *E. coli*, understanding of the molecular basis underlying the induction of stress resistance has developed rapidly. Identification of the global regulators for the expression of genes required for the responses to various stresses and extended survival in the stationary phase has occurred. However, the fact that survival of vegetative bacterial cells occurs for years after the exhaustion of nutrients and substrates was established long ago (Steinhaus and Birkeland, 1939), although the fraction surviving after a year, compared to the apparently viable numbers present immediately after the cessation of growth, was between 0.017% and 0.67%, depending on species. This suggests that growth-arrested cells become moribund and that a resultant stochastic reduction in cell culturability occurs. Nystroem (1998) has suggested that the inability to replicate points towards two distinct mechanisms; either the irreversible loss of function of at least one cellular component that is essential for growth and replication as a direct result of a physical or chemical stress or the occurrence of programmed cell suicide. However, demonstrating bacterial cell death is essentially impossible unless complete cell lysis

occurs. In the case of bacteria, the association between cell lysis and starvation was first reported by Monod (1942), but this phenomenon has been very largely ignored in the interim. Clearly, it is essential to ensure repeated cycles of the death/lysis/cryptic growth sequence in biotreatment processes designed for pathogenic organism elimination from waste sewage sludge.

EFFECTIVE SEWAGE SLUDGE BIOTREATMENT

Conventional waste sewage sludge biotreatment processes involve mesophilic anaerobic digestion; a technology that is no longer considered satisfactory as far as complete pathogenic agent and toxic chemical elimination from the treated sludge is concerned. Even so, effective overall sludge stabilization, in terms of mineralization and obnoxious odour elimination is frequently achieved. A particularly attractive feature of anaerobic digestion is the production of biogas, a mixture of methane and carbon dioxide in the approximate ratio of 2:1, thus providing a fuel gas of general utility. However, the preferred temperature employed for mesophilic anaerobic digestion is usually 30 °C, at which the treatment cycle frequently exceeds 20 days. Under either temperate or cold climatic conditions, such process systems require heating, most commonly by burning the unrefined by-product biogas for provision of process heating. Although the environmental conditions pertaining during mesophilic anaerobic digestion processes are hostile as far as pathogenic agent survival is concerned, complete pathogen deactivation (elimination) cannot be guaranteed, as no single deactivation mechanism either dominates or has been optimized.

As far as digestion processes are concerned, it is elevated temperature that promotes deactivation of the pathogenic agents present. Hence, thermophilic anaerobic digestion would seem an appropriate alternative to mesophilic anaerobic digestion, provided that economic process heating could be provided from the biogas produced and that appropriate thermophilic process mediating cultures are available. It is this latter requirement that has yet to be achieved and in spite of the fact that thermophilic anaerobic digestion processes are reported to exist, such processes only function at thermotolerant temperatures, i.e., at a maximum of 55 °C, with a cycle length of 10 days (Iranpour et al., 2002). In order to achieve genuine thermophilic operating temperatures, i.e., in excess of 60 °C, it is necessary to resort to aerobic thermophilic digestion,

where appropriate energy economics can be attained with a combination auto thermal heating and effective process heat recovery. The irreversible damage (deactivation) of pathogenic bacteria by heat results from site-specific damage. In the case of *E. coli*, the pathogen indicator bacterium, such damage was summarized by Heitzer (1990) and subsequently cited in the context of sludge biotreatment by Mason *et al.* (1992). Clearly, in any process designed for the deactivation of mesophilic pathogenic organisms, operating temperatures must exceed 60 °C for a significant period of time and, hence, thermophilic bacteria must mediate effective processes.

Thermo tolerant bacteria are those species where the optimum temperature range for growth is 45 to 60 °C. thermophilic bacteria have a temperature optimum for growth between 60 °C and 72 °C; while caldophilic bacteria exhibit an optimum temperature range for growth in excess of 72 °C. Thermophilic bacteria are optimally adapted for growth at elevated temperatures rather than struggling to survive at such temperatures. With the possible exception of some *Legionella* spp., which may be thermophilic but are most probably thermo tolerant, thermophilic bacteria are non-pathogenic. Caldophilic bacteria are frequently fastidious with respect to their growth conditions and, thus, are generally unsuitable as process mediating cultures on process stability grounds.

Waste sludge bio-treatment process feed stocks from preliminary mechanical, primary physical and secondary biological sewage treatment processes are both highly complex and variable in their composition. In addition to biodegradable solids of microbial origin, they contain biodegradable and non-biodegradable solids of non-microbial origin, biodegradable and non-biodegradable soluble compounds, biodegradable and non-biodegradable immiscible compounds and biodegradable and non-biodegradable sorbed compounds. All four non-biodegradable compound categories are subject to negligible change during biotreatment, while the remaining five biodegradable compound categories are degraded by a variety of mechanisms. Biodegradable microbial solids include pathogenic agents present in the raw sewage prior to its treatment and non-pathogenic organisms, both present and produced in large quantities during secondary biotreatment processes. In spite of the fact that pathogenic agent destruction is a primary objective of waste sewage sludge treatment processes, the elucidation of mechanisms involved in microbial cell destruction has been largely ignored in the study of biodegradation. The two mechanisms by which

cells lyse, autolysis, a process involving the actions of the endo-enzymes of the cell, and exo-enzyme lysis, brought about by the action of enzymes produced by other microbes, exist. Even so, the question as to whether cell death occurs prior to or is coincident with cell lysis in biodegradation processes remains unanswered, although evidence suggests the latter (Mason *et al.*, 1986A). The most credible hypotheses concerning the primary process involved in cellular biodegradation is the bursting cell hypothesis proposed by Hamer and Mason (1987). Non-microbial biodegradable solids vary in form and in structure, but cellulose fibres most probably dominate and the biodegradation of cellulose is generally thought to follow the shrinking-site model of Humphrey *et al.* (1977). The biodegradation of many immiscible compounds frequently depends upon the availability of oxygen. Non-substituted hydrocarbons are, generally, only subject to aerobic biodegradation, so anaerobic digestion processes fail to remove them from the sludge undergoing biotreatment. Hydrocarbons can also be sorbed onto particulate matter, depending on their partition characteristics, as can a range of surface active agents such as toxic detergent residues commonly found in municipal sewage, which also require aerobiosis for their biodegradation (Brunner *et al.*, 1988). Such surfactant residue biodegradation markedly improves the settling and dewatering characteristics of treated sludge (Hamer and Zwiefelhofer, 1986).

Carbon energy growth substrates for aerobic chemoheterotrophic bacteria have been classified as either energy-excess or energy-deficient by Babel and Muller (1985). In the case of waste sewage sludge, the principal component is bacterial cells which, when reutilized as the carbon energy substrate for aerobic growth, represent an energy balanced substrate at maximum carbon incorporation efficiency. Although, such growth reduces the overall biomass concentration, it requires, in order to achieve the levels of biomass concentration reduction required for effective sludge stabilization, repeated cycling of the death/lysis/cryptic growth sequence (Hamer and Bryers, 1985).

PRACTICAL SLUDGE HYGIENIZATION TECHNOLOGY

While conventional mesophilic anaerobic waste sewage sludge biotreatment is a widely used and entirely effective process for sludge stabilization, it fails as far as effective sludge hygienization is concerned.

Accordingly, it is necessary to either introduce an alternative process that achieves both effective stabilization and effective hygienization or introduce a pre- or post-treatment stage that achieves effective hygienization when operating in conjunction with conventional stabilization. Thermophilic, in reality thermo tolerant, anaerobic sludge digestion processes have been proposed as alternatives to mesophilic processes using existing bioreactors and appropriate process mediating cultures. While the latter can, relatively easily be enriched, most existing sludge treatment bioreactors are of pre-stressed concrete construction and as such might not withstand prolonged operation at temperatures in excess of 50 °C, thus indicating a need to develop either effective, genuinely thermophilic, pre- or post-stabilization / hygienization processes or alternative simultaneous thermophilic stabilization / hygienization processes using novel bioreactor and/or process designs. The critical question with respect to such a choice is the relative cost effectiveness of alternative processes under location applicable fiscal conditions.

In the consideration of either pre- or post-conventional mesophilic process technologies, two fundamentally different approaches have been introduced and evaluated. The first of these technologies is pasteurization, an entirely physical process technology, which has been used as both a pre- or post-stabilization hygienization technology. In the former operating mode, pasteurization has proved to be entirely satisfactory from a technical viewpoint, but in the latter operating mode, failure to achieve complete sludge hygienization has been reported (Hamer and Zwiefelhofer, 1986). However, even operation in the former mode has proved economically unsatisfactory because of energy costs for process heating. As far as the employment of thermophilic aerobic biotreatment processes is concerned, both combined stabilization / hygienization as a single process stage and pre-treatment coupled with conventional mesophilic anaerobic process technology have been evaluated and employed.

For a techno-economic evaluation of thermophilic aerobic sludge biotreatment processes, it is necessary to examine the process kinetics, process dynamics, process efficacy and both capital and operating costs. In technical-scale plants operated in the semi-continuous (fill and draw) mode, as a means to avoid by-passing, much reduced operating cycle times are achieved compared with anaerobic digestion processes. However, operating costs are significantly higher in order to achieve comparable sludge stabilization, because of the energy required for both

mixing and aeration. In order to analyse the operation of thermophilic aerobic biotreatment processes, it is necessary to examine the operating patterns reported for the relatively few detailed investigations conducted under defined conditions in laboratory-scale bioreactors.

Particulate carbon energy substrates, such as intact microbial cells, present major problems with respect to their complete utilization (conversion) in microbially mediated digestion processes and solids solubilization is invariably the rate limiting step in such processes (Eastman and Ferguson, 1981). Unlike the structure of many particulate solids which, with the exception of void spaces, are essentially homogeneous throughout, microbial cells comprise a cell wall and membrane enclosing the soluble cytoplasm. It was proposed by Hamer and Mason (1987) that during cryptic growth, exo-enzyme attack on intact cells involves the puncturing and bursting of the cell membrane thereby releasing the soluble carbonaceous compound comprising the cytoplasm into the extra-cellular process environment, i.e., a bulk liquid phase lytic process. In the experimental studies conducted by Hamer and Mason (1987), semi-continuous operation at 60 °C, a cycle time of 3 days, partial oxygen limitation and a process feed comprising a concentrated suspension of bakers yeast cells were employed. The results showed, for the first time, the sequence of interrelated events that characterizes microbial cell biodegradation. Since yeast cell degradation is not necessarily totally similar to bacterial cell degradation, subsequent laboratory-scale experiments were conducted by Mason et al. (1992) using a concentrated suspension of the mildly pathogenic bacterium, *Klebsiella pneumoniae*. A semi-continuous cycle time of 2 days, oxygen limitation throughout and an operating temperature of 60 °C were employed. The results of these experiments indicated that a much-reduced time of only some 6 hours was required to achieve apparently complete cell lysis, compared with more than 24 hours in the case of the yeast feed. In contrast to the yeast feed results, simultaneous, rather than sequential, production and utilization of various short-chain carboxylic acids occurred within the reduced 2-day cycle of semi-continuous operation (Haener et al., 1994a). Because of the dynamic nature of the acetate and other carboxylic acid concentrations measured during bacterial cell biodegradation, Haener et al. (1994a) sought to differentiate the relative rates of the opposing trends by employing pulse additions of radio-labelled acetate, early and late, during process operating cycles that were extended to 2.5 days. In the case of an early pulse, 7 hours into the cycle, the radioactivity measured in the culture

supernatant immediately decreased at an essentially constant rate, while the total supernatant acetate concentration remained essentially constant for some 20 hours after the pulse. This confirmed simultaneous production and utilization during this part of the overall operating cycle. The subsequent apparent net removal of acetate after some 25 hours of the cycle suggests that reduced primary substrate availability had, by then, started to restrict acetate production. Further, the rate of utilization also begins to exceed the rate of loss of supernatant radioactivity, indicating that acetate is converted into other soluble apparently recalcitrant organic compounds that remain unutilized in the supernatant. This fact was confirmed by Haener *et al.* (1994b), who showed the molecular mass distribution pattern of the residual dissolved organic carbon comprised three distinct fractions; two extreme fractions with molecular masses of 2,000,000 and 2,000, respectively, and an intermediate fraction of molecular mass of 25,000. However, such apparently recalcitrant dissolved organic carbon comprises naturally occurring humic compounds that are of negligible environmental impact. Irrespective of the importance of these individual functional findings, the most important overall discovery that emerges from the above mentioned laboratory-scale studies is the fact that the highly active, heat generating, phase in the aerobic thermophilic digestion of bacterial cells is short in comparison with the time required for complete stabilization, thus confirming that such processes are best suited as pre-treatment stages for hygienization rather than as complete hygienization/stabilization systems. It is in such an operating mode that such processes have found considerable technical-scale application.

Hamer and Zwiefelhofer (1986) reported the detailed operation of a technical-scale hygienization facility, clearly demonstrating fully effective process performance in conjunction with mesophilic anaerobic stabilization. The pre-treatment plant, an UTB-Aerotherm system, was operated on a semi-continuous (fill and draw) basis as a means for preventing by-passing, with isolated batch process feed pre-heating from hot exiting hygienized product. The mean bioreactor residence time was 55 hours and the temperature was auto thermally maintained between 60 and 73 °C. The aerobic thermophilic pre-treatment was followed by mesophilic anaerobic stabilization at temperatures maintained between 34.5 and 37 °C and extended post-treatment treated sludge storage. Complete pathogen indicator organism and odour elimination were reported after such combined processing.

CONCLUSIONS

The research discussed in this review summarizes current knowledge concerning cell death as it applies to waste sewage sludge biotreatment processes. Clearly the knowledge base is both incomplete and fragmented as far as specific mechanisms and specific pathogenic agents are concerned, but in spite of such deficiencies, technically and economically successful bacterially mediated processes for hygienization and stabilization of waste sewage sludge have been developed. Such bioprocesses convert waste sludge into a valuable soil conditioner which is largely free from adverse environmental risk, certainly as far as pathogenic agent carryover is concerned. The most successful technology developed so far involves sequential thermophilic aerobic hygienization and mesophilic anaerobic stabilization. Although the treated sludge may contain low concentrations of potentially toxic inorganic chemical residues, sensible application of such sludge to agricultural land adds only similar quantities of such residues as do typical wet and dry precipitation. The ultimate solution for eliminating such residues from sewage sludge is removal at source, rather than removal by treatment.

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