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Review

Application of Thermophilic Aerobic Digestion (TAD) in the reprocessing and protein enrichment of agricultural wastes for animal feed use

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ABSTRACT

Agricultural and food industry wastes constitute significant proportions of world wide agricultural productivity. These wastes include fruit, vegetables, root / tuber, sugar industry and animal / livestock / fisheries wastes. They represent valuable biomass and potential solutions to problems of animal nutrition world-wide if appropriate technologies are deployed for their reprocessing into feed. In addition, reutilization of these wastes should help to address growing global demands for environmentally sustainable methods of production and pollution control. Various technologies are potentially available for the valorization of these wastes. In addition to conventional waste management, other processes that may be used include solid substrate fermentation, ensiling and high solid or slurry processes. In particular, the use of slurry processes in the form of Thermophilic Aerobic Digestion (TAD) is gaining prominence because of its potential advantages over conventional waste reprocessing technologies. In addition to capacity for self-heating, other advantages include capacity to achieve rapid, cost effective waste stabilization/pasteurization and protein enrichment of wastes for animal feed use. TAD is particularly suited for use with wastes generated as slurries, at high temperature or other high COD wastes and being considered for upgrading and recycling as animal feed supplement. Reprocessing of agricultural wastes by TAD has been shown to result in very significant protein accretion and effective conversion of mineral nitrogen supplement to high value feed grade protein for use in animal nutrition. The use of thermopiles in the process has significant safety benefits and may be optimized to enhance user confidence and acceptability.

KEYWORDS: Thermophilic Aerobic Digestion (TAD), animal feed supplement, protein enrichment; agricultural wastes

1. INTRODUCTION

Waste treatment by microbiological process is an age old process. However, scientific knowledge of the processes involved is relatively recent. Traditionally, methods of wastes treatment emphasized the removal of wastes from areas in which they were not wanted [1]. Hence, waste management processes were synonymous with waste disposal. These actions were inspired by man's desire to protect his immediate environment, and applied to both human and agricultural wastes. The early methods of waste treatment did not distinguish between wastes in terms of whether such wastes were reusable or not. Increase in the scale of the problems of environmental pollution, as well as changes in social attitude, have led to multidisciplinary approaches to the problems of waste management and pollution control. Increasing pressures on resources also mean that the vast quantities of organic materials that remain from human

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productive activities can no longer be seen solely as wastes that need to be disposed. In particular, ever-increasing world population, with the attendant food supply problems, as well as the lessons of the energy crisis of the 1970s, combined to force change in global attitude towards waste. Wastes, particularly organic and agricultural wastes, are progressively being seen as resources in the wrong location and form that should be recycled. Thus, terms such as biomass, reprocessing, recycling and reuse are progressively gaining currency in discussions on waste management, at the expense of such terms as refuse and disposal. Traditional subsistence agriculture led to the production of only limited agricultural wastes, which were generally disposed-of untreated to land at zero cost, or actually at a credit because of the use as farmyard manure and soil conditioner. Introduction of cheap inorganic fertiliser obviated much of the need for large scale use of farmyard manure. Also, increasing world population and industrialisation led to the introduction of intensive agriculture resulting in accumulation of large quantities of agricultural refuse in small land areas. Recent advances in the field of food technology, and the increasing demand for factory processed foods have led to considerable increase in the quantity of food industry/process waste. Also, the tendency to locate food processing factories close to farms has led to concentration of large volumes of agricultural refuse in limited land areas.

Conventional waste treatment processes are linked to very limited recycling of biomass as organic fertiliser, as a means of disposal rather than the target. The need to recycle waste is most relevant in the food and agricultural industries [2, 3], where biomass estimated to be in excess of 10^{17} tonnes are generated worldwide annually [4]. Refuse amounting to up to 40% of world agricultural yield is generated following diverse agricultural and food industry processes. These are very important energy-rich resources which, given appropriate technology may be reprocessed and recycled to more beneficial use than is currently possible. The key to successful processes of this nature is an economical "no loss" process, in which the cost of processing should be offset, at least in part, by the possibility of producing a valuable product, with the added benefit of stabilising the otherwise environmentally hazardous waste. Such processes should be satisfactory if they caused the overall cost of waste management to be even slightly less than the classical approach. The continued disposal of food industry refuse constitutes significant loss of agricultural productivity and calories which, if properly harnessed, can impact positively on worldwide food supply, animal production and global food security. Protein enrichment of refuse arising from agricultural productivity will increase the appeal of utilizing them in animal production and reduce the pressure on sources of calories that are of food value to humans. This should be particularly important in many parts of the world where animals and human compete for the same sources of protein and calories constraining to a large extent the production of animal protein. The use of low cost technologies such a Thermophilic Aerobic Digestion (TAD) for such processes will make them interesting to tropical developing countries where protein/energy malnutrition remain prevalent.

2. Thermophilic Aerobic Digestion (TAD)

TAD is a process in which the metabolic heat of growing microbial cells is conserved in a compact, insulated system, leading to the elevation of the temperature of the digesting mass to thermophilic range [5-9]. It arose as a modification of the activated sludge process. Given appropriate aeration, microbial action leads to production of sufficient heat to cause rise in temperatures, provided that heat loss is minimised by insulation of the digestion vessel. Achievable temperature may be manipulated by varying the level of available oxygen, and biodegradable organic matter [10-13].

Like all waste treatment processes, TAD is a mixed culture process. During start-up, a wide range of mesophilic and thermotolerant bacteria metabolise readily available waste components to generate heat. As the temperature increases, microbial succession and selection takes place until, at thermophilic temperatures, only a few species of micro-organisms remain active [9, 14]. Sensitive mesophiles (including most vegetative

and non spore-forming organisms) are inactivated in the process. Also, at thermophilic temperatures, hydrolysis of complex and otherwise recalcitrant molecules is enhanced. Thus, TAD resembles composting in many respects and has been described as liquid (slurry) composting [15].

The process is considered to be very flexible and versatile, for which reason it may be adaptable, not just to waste treatment, but also as a means of generating useful by-products from wastes. The high temperature of operation, and metabolic versatility of thermophiles, ensures that the process takes place very rapidly, and under conditions that may require less control than conventional bioprocessing [10, 16]. Of particular interest in this regard is the potential of TAD to achieve protein enrichment and reprocessing of agricultural waste for animal feed use [17]. Although thermophilic microbiology has a fairly long history, the application of thermophiles in biotechnology is only just beginning to attract attention. Consequently, TAD, which is relatively new, has received even less attention. A proper understanding of the process, and in particular the capabilities of the associated microflora, will help overcome potential problems, which may arise during full scale operation of this process for use in the protein enrichment of agricultural biomass.

2.1. Aeration/oxygenation in TAD

TAD uses the metabolic heat arising from microbial oxidation of organic materials to raise and maintain the digestion process at thermophilic temperatures. To achieve this, the metabolism of the microbial population has to be oxidative and rapid. Heat evolution is enhanced at thermophilic temperatures, particularly as uncoupling of metabolism leads to reduced cell yield, and dissipation of a greater amount of the energy content of the substrate as heat [10, 18]. The process requires efficient transfer of oxygen into solution, to be able to sustain the rapid metabolism of thermophiles [11, 19].

The problem of oxygen transfer has had profound influence on the economics, applicability, and in particular, on the development of this potentially versatile process. In the early stages, it was believed that thermophilic temperatures could only be achieved if pure oxygen [20], or oxygen enriched air was used. Thus aeration with pure oxygen was employed in pilot studies for thermophilic treatment of sewage sludge at the Ponthir Water Works [21]. Further studies have demonstrated that thermophilic temperatures can be achieved with air [21, 22]. And oxygenation efficiency greater than 10 % has been recommended as the minimum needed to maintain thermophilic temperatures [15]. The absence of nitrification at high temperatures by the dominant populations means that TAD has significantly lower oxygen requirement than aerobic mesophilic digestion [23, 24].

An additional problem associated with the aeration of TAD is the limited solubility of oxygen at elevated temperatures (50°C and above), as well as the high solids content often desired in such operations. However, in spite of the low solubility of oxygen, its molecular diffusivity increases with temperature [7]. This compensates for the low solubility, leading to comparable or better oxygen transfer at the temperatures of TAD [25]. Oxygen transfer at high temperature is also aided by the decrease in viscosity of the reaction medium with increase in temperature.

Various levels of aeration have been applied to TAD. These range from 0.5vvh (volume air per volume medium per hour) to 1.0vvm (volume air to volume medium per minute) with various level of heating and stabilization efficiency achieved [24, 26-33]. As the main cost of TAD is related to aeration, running it at microaerophilic rates should make it more economical [32-34]. However, a highly aerobic system will be desirable if TAD is to find a role in highly aerobic oxidations such as may be required in detoxification of aromatic and haloaromatic chemicals. Since heat generation in microbial systems is a function of oxygen consumption (at least to a point), it follows that highly aerobic systems will be more efficient in heat generation than less aerobic systems [11, 18, 35, 36]. A variety of aeration devices have been developed and used to achieve up to 20% aeration in TAD [37, 38]. An additional advantage of extensive aeration is in the evaporation of waste liquid, if this is desired to reduce the amount of waste available for disposal. Use of heat exchangers between digested and incoming sludge, particularly in cold climates is also believed to be beneficial [11, 38].

2.2. Operational temperature

There is no consensus on what constitutes optimal temperatures for the operation of TAD. Difficulties in setting temperature standards arise from the subjective and imprecise definition of thermophily [39], and also from the heterogeneity of microbial population that have been reported to be active in TAD, which result in a wide band of growth temperature optima. Thermophily applied to waste treatment has also been considered in relation to mesophilic treatment, or the differential between the reactor and feed temperatures. In temperate countries feed temperatures may vary from under 5°C to more than 20°C [31], while approaching 40°C in some tropical countries. Surucu *et al.* [6, 7] consider TAD to be a process that operates between 50° and 60°C. Matsch and Drnevich [40], Vismara [31] and Jewell [15] considered a process that operates in a range of 40°-50°C as TAD, while Kambhu and Andrew [5] and Frost *et al.* [26], defined TAD as a digestion which operates above 35°C, and up until temperature becomes the limiting factor.

Notwithstanding the seeming requirement for temperatures of up to 45°C and above, the relationship between operational temperature and stabilisation efficiency in TAD is not clear. This has led to the imposition of mesophilic digestion standards on TAD [31, 41]. In simulation studies, Kambhu and Andrew [5], considered that the highest reaction rate constant would be achieved at 55°C while decreasing to zero at 75°C. Carlson [34], reported increase in waste sludge degradation, as the reaction temperature increased to 57°C. Hawash *et al.* [24], also reported an increase in the rate of waste stabilisation with temperature, with the kinetic parameters of stabilisation nearly doubling with a 10°C increase in temperature within the permissible range. Tyagi *et al.* [42], reported increase in digestion efficiency between 45°and 55°C, followed by a gradual decline thereafter. Temperatures reported as the optima for TAD correspond to the optimum growth temperature found for a range of common aerobic thermopiles [39].

Sonnleitner and Fiechter [30, 43] consider extreme thermopiles (caldoactive organisms) fastidious with respect to their general (nutritional) requirements for growth, and hence poorly suited for application in TAD. Thus, only thermotolerant and moderately thermophilic organisms, classified by Hamer and Bryers [10] as those growing optimally at 40°-50°C and 50°-65°C respectively are expected to play roles in TAD. Thus TAD may be expected to operate efficiently in the range of 40°- 65°C. Although there is little experience with full-scale TAD, Kelly *et al.* [28], and Edginton and Clay [44] successfully operated pilot scale digesters within this range for over one year. Ponti *et al.* [45, 46] reported that TAD could be efficiently run at 65°C, particularly if the primary motive is to achieve waste pasteurisation. The need for pasteurisation is also the principle based upon which Messenger *et al.* [47, 48] Messenger and Ekama [49, 50] operated dual TAD-anaerobic mesophilic system at 65°C and above with efficient pasteurisation but poor waste stabilization.

In self heating systems, precise control of temperature is unlikely, and the emphasis will continue to be on a range which gives acceptable performance. The preferred temperature will vary with factors such as system design, type and organic loading of waste, pathogen content and the need for pasteurisation among others.

2.3. Effect of waste load on process heating

The nature of the waste determines the amount of biodegradable chemical oxygen demand (COD), which directly affects heat evolution. As the amount of biodegradable organic load increases, so does heat evolution, not least because the amount of ballast water present per unit mass of organic matter decreases.

Jewell and Kabrick [23], and Jewell [15], have related heat evolution to COD removal by the expression:

$$\Delta F = 3.5 \Delta COD$$

where ΔF is the heat released in kilocalories per litre and ΔCOD is the measured change in COD in gl^{-1}. One kilocalorie per litre is equal to 1°C change in the temperature.

The maximum temperature reached depends on the balance between heat loss and heat input.

This equillibrium state is facilitated by two prominent factors,

(a) as the biodegradable organic load increases the system soon becomes mass transfer limited, a situation accelerated by reduction in oxygen solubility with increase in waste load, and the rapid consumption of oxygen with proliferation of microbial populations, putting more pressure on the available oxygen.

(b) as the temperature of the digesting mass increases to 60°C and above, the number of viable thermopiles gradually starts to decline leading to decrease in organic matter removal, and consequently a decrease in heat production [40]. This self-regulation is similar to what obtains in composting.

Within the permissible range, an increase in temperature will be accompanied by an increase in digestion rate, following the classical van Hoff Arrhenius relationship, provided that care is taken to account for transition from growth of mesophiles to that of thermophiles if such temperature transition is involved. Hisset *et al.* [51] reported reduced respiration in piggery slurry aerated at 50°C when compared to that at 35-40°C. Yet reports from the laboratories of Sonnleitner indicate that respiration is greater at 60°C than at 50°C [45, 46]. Also, Surucu *et al.* [7] reported greater activity at 55° and 58°C than at 50°C. This supports the presence of transition temperature range between upper limits of thermotolerant mesophiles and the lower limits of facultative thermophiles; a range within which neither of the two groups of organisms is at a metabolic advantage [33]. This range does not take account of the behaviour of the more obligate thermophiles. As of now however, the choice of temperature seems to be a matter of trial and error, and varies widely with the process and the operator.

2.4. Effect of substrate load on process development

The solid content (biodegradable COD) required to maintain thermophilic temperatures in TAD is not well defined. In sewage sludge treatment, it has been recommended that high solids content is essential to achieve thermophilic temperature, hence the slurry nature of TAD [10]. However, as sewage sludge consists essentially of partially degradable organic matter, the solid content of sludge cannot be used as a direct index of biodegradable COD. Theoretically, since the principal source of energy of the process is the enthalpic content of the organic matter, the temperature reached in the reactor depends on the concentration of biodegradable material in the digesting waste [5, 23, 31, 52, 53].

In the absence of heat loss, the minimum theoretical concentration of biodegradable solids needed to produce a 50°C rise in temperature has been reported to be 3 g l^{-1} [31]. Jacob *et al.* [54] recommended a minimum solid content of 2.5 % for the attainment of thermophilic temperature in sewage sludge, while Jewell and Kabrick [23] recommend a solid content of 5 %, to be able to sustain thermophilic temperatures for long enough to achieve waste pasteurisation. Wastes with 2-6 % solids have been successfully treated at different thermophilic temperatures [21, 24, 28, 33, 42, 44].

3. Microbiology of TAD

There have been only a few studies on the microbiology of TAD which remains only poorly understood. Similarly, the possible biotechnological application of the process or of the potentially versatile micro-organisms has remained largely unexploited [55, 56]. Micro-organisms responsible for TAD develop from the proliferation of thermophiles and thermotolerant mesophiles present in the waste. These are autochthonous organisms whose growth would have been suppressed in the mesophilic or ambient temperature of the influent waste.

Factors that may influence the stability of microbial populations during the operation of TAD remain poorly understood. Sonnleitner and Fiechter [29, 30, 43] characterised at least 95 % of the organism responsible for TAD of sewage sludge as members of the genus *Bacillus* (of the *Bacillus stearothermophilus* group) with maximal growth temperatures in excess of 70°C. The balance of 5 % would have been similarly classified but for their inability to produce endospores in culture. The isolates showed very rapid growth rates (μ_m = 0.7 to 2.2 h^{-1}), but low final biomass yield (0.2 to 0.3g g^{-1}) when grown in carbohydrate medium in shake flask and exhibited rapid

adaptation at various retention times. During TAD of swine waste, Beaudet *et al.* [57], counted microbial populations at 55°C, varying from 10^4 to 10^7 ml^{-1} of waste. The organisms were identified as *Bacillus* spp., including *B. licheniformis*, and the population was shown to vary with the final pH of the digesting waste, which seemed to vary with the COD load similar to the result reported by Loll [58]. During TAD of sewage sludge, obligate (65°C) and facultative (55°C) thermophiles in excess of 10^6 and 10^8 ml^{-1} respectively were reported [59]. Surucu et al [7] and Malladi and Ingham [60] reported thermophilic aerobic spore-former population of up to 10^9 ml^{-1} during TAD of potato process waste water as did Ugwuanyi *et al* [61, 14]. Although temperature remains a major selector of active population type, a major difficulty encountered in comparing data from microbiological studies of TAD remains the differences in waste types, as well as other operational conditions employed in the process. It is obvious however, that a eurythermal population of thermotolerant and thermophilic organisms carry out TAD, with selection and succession responding to the local environment.

3.1. Physiology of thermophiles important in TAD

Thermophiles are organisms capable of growth at elevated temperatures. Those that grow over wide temperature ranges are known as eurythermal, while those with more restricted growth temperatures are stenothermal. Generally, thermophiles are considered as those organisms able to grow at 55°C and above. This range is selected for ecological reasons. While temperatures below 50°C are common on earth surfaces, associated with sun heated habitats, temperatures above 55°C are rare as biological/ natural habitats. Additionally, 60°C is the maximum for most eukaryotic life.

However, a temperature continuum exists within and between groups, making sharp delineation impracticable [62]. Suutari and Laakso [63] delineated thermophiles such that thermotolerant organisms are defined as those with T_{opt} of ≤45°C and T_{max} >45°C, and thermophiles as those with T_{opt} >50°C and T_{max} >60°C. They also defined as extreme thermophiles or caldoactive, those organisms with T_{opt} >65°C and T_{max} >90°C, and hyperthermophiles as the more exotic isolates able to grow at 100°C and above.

Thermophiles are mostly heterotrophic, and nutritionally versatile. They are capable of utilising a wide variety of organic carbon sources, including simple sugars, alcohols, organic acids and polysaccharides. Many are able to utilise more recalcitrant and exotic compounds such as phenols, cresols, benzoates and hydrocarbons [64]. They may grow prototrophically, or auxotrophically exhibiting a requirement for growth supplements such as vitamins, amino acids or complex organic mixtures [65]. Oxygen is important for the growth of aerobic thermophiles, but the low solubility of oxygen at high temperatures makes supply difficult during large scale cultivation, and may impose severe limitations on the attainable cell density [25]. They grow optimally in either acidic environment (pH 1.5-4.0) (thermo-acidophiles), or in neutral to moderately alkaline pH of 5.8 and above (neutrophiles). Thermophilic alkalophiles also exist although these seem to be more restricted in distribution.

3.1.1. Growth rate, yield and maintenance of thermophiles

Thermophiles are remarkable for their ability to grow rapidly at their optimum temperatures [66]. Several thermophiles with temperature optima between 55° and 70°C have generation times of 11 to 16 min as compared to 26 min for mesophilic *B. subtilis*. However, based on theoretical expectations from their high growth temperatures, it is believed that thermophiles do not grow efficiently when compared to mesophiles [29]. This may be due to an inability to sustain their growth rate, or due to low catalytic efficiency of some key thermophile enzymes [39]. They exhibit very short exponential growth phases, due perhaps to inadequate oxygen supply at high temperature, and cell density, particularly in batch culture [67, 68], or to enhanced susceptibility to toxic metabolites at high temperatures.

A yield of 65 g cells per mole glucose was reported for *B. stearothermophilus nondiastaticus* growing optimally at 55° to 58°C in batch conditions. This value is lower, than the range of 70 to 95 g per mole glucose reported for a variety of mesophilic aerobes [69]. Low yield of

thermophiles has also been reported during TAD of slaughterhouse effluent [66]. The low yield is believed to be due to the high maintenance requirement of thermophiles [65]. In continuous cultures however, thermophiles exhibit yields similar to mesophiles. Thus, *B. caldotenax* yielded 89 g per mole glucose at 65° and 70°C [68], while *B. acidocaldarius* gave 83.5 g per mole glucose at 51°C [70]. Similar variability in yield of thermophiles based on oxygen consumption has also been reported. For instance, *B. caldotenax* has a yield of 50 g per mole oxygen at 65° and 70°C compared to 56 g per mole at 30°C, and 28 g per mole at 35°C for *E. coli*, or 52 g per mole at 35°C for *Klebsiella aerogenes*, suggesting that thermophiles may be more efficient than mesophiles within their permissive temperature in continuous culture.

Some thermophiles, particularly *Bacillus* spp have high maintenance requirements, which have been postulated as the reason, in addition to high decay rate, for their low yield in batch culture [66]. For instance, *B. caldotenax* has a requirement of 4.1 and 20 mmMoles g^{-1} h^{-1} of glucose and oxygen respectively at 70°C and 3.8 and 20 mMoles respectively at 65°C. These figures are up to ten times the requirement of mesophiles. However, wide variations in maintenance requirements exist among thermophiles. It is envisaged that high maintenance requirements, coupled with high growth rates and low yield will lead to enhanced breakdown of organic materials, thus combining in TAD the low sludge production advantage of anaerobic digestion with the efficiency of aerobic processes. This is in addition to the accumulation of useful metabolic products at rates higher than would be expected of their mesophilic counterparts [43]. This could be the foundation for biotechnological exploitation of thermophiles in waste treatment and biotransformation.

3.1.2. Determinants of minimum and maximum growth temperature

All bacteria have characteristic minimum (T_{min}) and maximum (T_{max}) growth temperatures. The determinants and regulators of these limits are not clearly understood. However, the observations that heat sensitive mutations lead to a lowering of T_{max} but not T_{min} suggest that both are independent, single genome traits [65]. Cytoplasmic membranes of thermophilic eubacteria are classical lipid bilayer matrices, while those of the thermophilic archaea are covalently cross linked bilayer, structurally equivalent to an amphiphilic monolayer. However, membrane lipids of thermo-philes tend to have a greater abundance of high melting point (saturated long chain) fatty acids than their mesophilic counterparts [63]. These can be further adjusted, depending on growth temperature. Bacterial phospholipid bilayer membranes undergo a thermotrophic reversible transition between an ordered rigid gel (solid phase), and a fluid (liquid crystalline) phase. This transition involves melting of the hydrocarbon chains in the interior of the bilayer. The temperature at which this takes place depends on the melting point of the membrane fatty acids [71].

The heterogeneity of membrane lipids implies that, the phase change takes place over a range of temperature, rather than at a discrete point, with liquid crystal and solid gel phases existing simultaneously. Above the upper boundary, liquid crystals are obtained while solid gels exist below the lower boundary. Membrane bound proteins may, or may not have any effect on the phase transition temperature of membrane lipids [72, 73]. The quantity and quality of membrane lipids and proteins of thermophiles vary depending on growth temperature [63]. In *B. stearothermophilus* this manifests as a decrease in total lipid, and an increase in total membrane protein, whereas in the archaebacterium *T. aquaticus* the quantity of lipid increases with temperature. In *B. stearothermophilus* and *E. coli*, the existence of membranes in liquid crystalline phase is believed to be essential for performance of membrane functions. This implies a role for the membrane transition point in setting growth T_{min} and T_{max}.

Changes in conformation of essential enzymes, leading to loss or reduction of catalytic activity, or alteration of important regulatory characteristics, which may lead to arrest of growth below certain temperature (T_{min}), may also affect minimum temperature of thermophiles. Additionally, ribosomal assembly in thermophilic bacteria has been shown to be energy intensive and may thus contribute in setting a high minimum temperature for growth. As for T_{min}, determinants of T_{max} of thermophiles are not well understood. Early

suggestions were that the membrane lipid phase transition temperature may be important in setting the upper growth temperature. This position was prompted by observations in some thermopiles that an increase in growth temperature led to an increase in the melting point of membrane lipids. This position is contradicted however, by observations that the phase transition temperature may be varied by manipulating the fatty acid composition without affecting the T_{max}, while (in *B. stearothermophilus*) mutations which lowered T_{max} did not affect the lipid transition temperature [74]. In spite of these observations, membrane stability may be a major determinant of T_{max} in so far as cell death may be accelerated by heat damage to the membrane above its optimum temperature [68]. The fluidity of membrane lipid (transition phase) will therefore be an index of stability at a given temperature.

It is also believed that there is a limit to the gap that can exist between the transition temperature and T_{max} i.e. a limit to the level of fluidity of membranes in growing bacteria. Mutants of *E. coli* defective in lipid synthesis are unable to grow under conditions that led to the incorporation of very low melting point lipids. This may be because such incorporation led to production of membrane that was too fluid and unstable at the T_{opt}. A similar observation was made in the growth of heat sensitive *B. stearothermophius* [65]. This organism could not adjust its phase transition point beyond 41°C, and rapidly lost the ability to grow beyond 58°C, which became the $T_{opt,}$ T_{max} and upper limit for membrane stability. The wild type organism could adjust its phase transition point to 65°C, and so could grow at 72°C. The T_{max} of microbes may also be affected by temperature stability of various macromolecules, particularly enzymes [75].

3.1.3. Turn-over of macromolecules in thermophiles

It has been reported that proteins of thermophile are more stable than their mesophile counterparts, hence their low turnover at high temperatures [76]. The stability may be due their association with high concentrations of carbohydrates and ability to bind certain ions more strongly [77]. Increased stability of proteins has also been attributed to strategic substitutions in their amino acid sequence. Enhanced DNA stability has been attributed to a higher G+C content in thermophiles than in mesophiles. The G+C content of DNA of thermophiles has also been shown to reflect their growth temperature [75]. Enhancement of tRNA stability with growth temperature has been reported in *T. thermophilus* in which ribo-thymidylate, a normal component of tRNA is replaced with 5-methyl, 2-thiouridylate, and this increases with temperature. The higher growth and decay rate reported in thermophiles from TAD, than mesophiles indicate that there are great variability in stability and turnover among thermo-philes, compared to mesophiles [66].

3.2. Development, adaptation, stability and washout of thermophiles in TAD

Thermohilic populations responsible for TAD of sewage sludge develop from the proliferation of thermophiles present in the sewage [29, 30]. These thermophiles remain dormant in sludge during start up, while mesophiles, then thermotolerant mesophiles and facultative thermophiles metabolise easily utilisable waste components, building up the temperature until a thermophilic range is reached, when they begin to proliferate [59, 60]. This profile is similar to the composting process.

Although Sonnleitner and Fiechter [30] observed fluctuations in microbial populations of up to 5 log orders, washout of thermophiles was never observed. Over a two year observation of pilot scale continuous TAD, viable thermophilic population remained at or greater than 10^5 g^{-1} of sludge even when hydraulic retention time (HRT) was reduced to as low as 10 h, at aeration rate between 0.02 and 0.3 vvm. Populations of extreme thermophiles (growth at $\geq$ 60°C) were particularly well maintained. Similar stability was reported in the thermophilic biofilm responsible for the stabilisation of swine waste [57]. This has led to suggestions that TAD should be operated at the highest possible temperature, since that could result in substantial reduction in HRT without compromising efficiency of stabilisation and pasteurisation.

The persistence and stability of thermophiles in continuous TAD has been attributed to the selection of populations with very rapid growth

rates [29, 30, 39]. Unlike in (thermophilic) anaerobic digestion where the overall pace of the process is determined by the growth rate of the slowest growing populations in a synthrophic interaction, the fastest growing populations in TAD determine the stability and pace of digestion.

3.3. Enzymology of TAD

Development of enzyme activities during TAD has received limited attention, even though it is believed that these may be used as control parameters for the process [78]. Proteases were reported as the major extracellular activity present in sewage sludge during TAD [45, 46, 78, 79]. Bomio *et al.* [78], also detected low levels of lysozyme, and concluded that other macromolecules were either not present (hence did not induce activities) or passed out of the system with only minor modifications. However Burt *et al.* [59], measured considerable degradation of lipids in sludge during TAD and suggested that lipases produced during the process but may be easily degraded by proteases in the digesting mass. Grueninger *et al.* [80] had earlier suggested that proteases may be responsible for destruction of amylases produced by the *Bacillus* spp in TAD.

Micro-organisms associated with TAD are hydrolytic, and have been considered as a possible reservoir of organisms capable of producing thermostable, industrially useful enzymes [60, 80]. The elaboration of a variety of the enzymes is probably repressed in the presence of easily metabolisable waste components [30]. It is reasonable to expect that enzymatic activities associated with TAD will vary with the composition of waste.

3.4. Monitoring and control of Thermophilic Aerobic Digestion

In spite of the advantages of TAD, little has been done to achieve effective monitoring and control based on the biochemistry of the process [29, 30, 81, 82]. Consequently, control of the process is still subject of trial and error, with no TAD specific parameters. As a waste treatment process, control parameters have largely been based on those applied to activated sludge from which it evolved. Besides, much of the work carried out on the process have been limited to sewage sludge treatment (and hygeinisation), where classical control parameters have been conveniently, though inadequately applied [28].

Based on ‘degree-day’ control for activated sludge [41], Vismara [31] recommended that treatment may be considered satisfactory, if the product of temperature and time of operation equal or exceed 250. As a complete waste treatment process for sewage sludge, the EPA suggests that 40 % of volatile suspended solids of sludge should be removed, while maintaining a minimum dissolved oxygen content of 2 mg l^{-1} as a means of eliminating vector attraction potential of the waste [40]. Additionally, sludge to be disposed of to agricultural land should have been held at 55°C for at least three days. The UK Department of the Environment, recommends that sludge to be disposed of to agricultural land should have been held at 55°C for a minimum of four hours at a retention time of 7 days [26].

Matsch and Drnevich [40] suggested criteria based on the odour producing potential of waste, provided that the parameters can be related to an easily measurable variable such as OUR or redox potential. Other control parameters that have been applied to TAD include redox potential, [11], total/viable microbial count and count of thermophiles at 65°C, ATP level and dehydrogenase activity, as well as lipid content, and changes in pH [19, 83]. These are in addition to classical waste quality parameters such as organic acids, COD, BOD, TSS, and VSS. Although the latter parameters are acceptable where the process target is the safe disposal of wastes, such as in sewage sludge, they are inadequate where TAD is part of an integrated process, particularly during waste upgrading and recycling reactions.

Development of hydrolytic activities has been studied in a number of waste treatment processes as monitoring parameters, including landfill [84-88], anaerobic digestion [89-93] and in composting [94-97]. In these cases, different activities were considered reliable for monitoring process performance. This is understandable, as the degradation of polymers is known to be a rate controlling step in waste digestion [78, 98-102].

Protease has been reported as the predominant activity during TAD of sewage sludge [30, 78, 100]

though, it has not been used as a monitoring tool, nor has the profile of any other hydrolytic activities. Amylase, xylanase, cm-cellulase and protease activities have also been shown to be related with population development and stabilization of waste during TAD [32, 33] and the profiles of these activities have been suggested for use in the monitoring TAD. Measurement of hydrolytic activities is considered economical, easy to execute and interpret, and may be process and waste type specific and adaptable. Ultimately, the monitoring and control of TAD will take into consideration the source and type of waste, and the aim of the treatment process, as a waste stream that is being reprocessed for use in animal nutrition will require different control parameters from wastes meant for land application. Similarly, a stand alone TAD process will require different control parameters from one in which it is part of an integrated process, as either a pre- or a post-treatment, pasteurisation or hydrolytic process.

4. Applications of TAD

TAD arose as a response to the need to find a suitable, safe and environmentally friendly means of disposing excess sewage sludge to land, as an improvement on conventional activated sludge. This followed legislative restrictions on the disposal of sludge to sea, or of unpasteurised sludge to agricultural land [28]. It is understandable therefore, that virtually all early studies on TAD as well as more recent ones have focused on the use of this process in sewage sludge treatment and pasteurisation [24, 103-105]. TAD has also been used for the treatment of a variety of other waste types, particularly those of agricultural and food industry origin. It has been used as the sole treatment method for different high strength wastes such as swine slurry [57], slaughter house effluent [66], potato process waste [12, 13, 60], and dairy, brewery/distillery and other food industry wastes [58, 106-108], as well as spent liquor of pulp mills [109] oil mill waste [110] and piggery slurry [111].

Sonnleitner and Fiechter [30, 43], proposed that it may be used to treat virtually any kind of organic waste, provided that, for particularly recalcitrant situations, enough time is allowed for the adaptation of the process. It has also been proposed that the metabolic versatility of TAD associated thermophiles may be exploited for the reprocessing and protein enrichment of a variety of wastes for use in animal nutrition, and in the production of high value biochemicals [17, 53, 109, 112-114].

4.1. Pasteurisation of wastes by TAD

The recycling and reuse of a variety of wastes is tied to their microbiological and sanitary quality. Since conventional waste treatment processes, are accepted to be inefficient from a hygienic and epidemiological point of view, [115-122], one of the major attractions of TAD has been the potential for use of its high temperatures to achieve destruction of pathogenic microorganisms, protozoa, viruses and parasite eggs [100, 123-125].

Although various studies on the inactivation of waste associated pathogens have been reported during mesophilic waste treatment and in composts [126-131], few studies exist that describe the behaviour of pathogens and indicators in TAD [124]. Consequently, projections, often conflicting, have been made on the capacity of this process to achieve pathogen destruction, based on the known sensitivity of indicator organisms to thermophilic temperatures [45, 48]. Jewell and Kabrick [23], and Jewell [15], reported that maintaining digesting sludge at 50°C for 24 h destroys most pathogens including bacteria, viruses, protozoa and parasite (*Ascaris*) eggs, as does 60°C for 1 h. Carlson [34], reported that 24 h was required to completely inactivate enteric bacteria in sewage sludge at 57°C. Burt *et al.* [59] reported a reduction in populations of *E. coli* in sludge from 10^6-10^7 per gram to undetectable levels after digesting at 50°C, and a HRT of 1 day. Efficient destruction of helminth eggs and ova, as well as protozoan parasites, at different temperatures in TAD has also been reported [119-121, 132].

TAD achieves better pasteurisation than composting [126, 122, 133], probably due to the presence of high levels of solids in composts, which may afford pathogens some protection from thermal inactivation. Ponti *et al.* [45] applied TAD to achieve rapid inactivation of *E. coli* in sewage sludge at 55° and 57°C, and reported that waste solid considerably protected microbial cells from thermal destruction. Morgan and Gumson [21] reported that temperatures in excess of 50°C

were required to eliminate *E. coli* and *Salmonella* in a continuous flow TAD of sludge with retention time of 8 days. Murray *et al.* [134] reported efficient sludge pasteurisation even during periods of irregular feed, while Spaull and McCormack [135] employed TAD for the inactivation of plant pathogens and weed seeds with considerable success.

Though rapid destruction of mesophiles (*E. coli*) occurs between 54° and 62°C it has been suggested caution be exercised in the use of inactivation data obtained at (and around) 50°C, because this is the transition temperature between reversible and irreversible inactivation of *E. coli* [124]. Since the survival of a single pathogen is often enough to cause re-infection of treated sludge, it has been recommended that microbial death in waste treatment be monitored by plate count technique [136]. Recent studies have shown that in spite of projection of rapid destruction of mesophiles in composting, they survive for very long periods, and may even multiply in the process, at temperatures higher than what is likely to be employed in TAD [137]. Besides, various pathogens can be expected to respond differently to the effect of heat at different pH, DO and waste solid levels [120, 121, 124]. Although differences in design and assay procedures, make comparisons between inactivation data in TAD difficult, the Environmental Protection Agency (EPA) recommends that sludge intended for spreading on agricultural and pasture land should be held at 55°C for 3 days to achieve pasteurisation ('class A' status, [117]). The UK Department of the Environment (DoE) recommends exposure of sludge to a temperature of 55°C for at least 4 h, to reduce the number of viruses and pathogens to levels acceptable for disposal to agricultural land. Control of pathogens in wastes intended for land or sea disposal is the subject of several legislations in Europe and North America [117, 133, 137].

4.2. Detoxification of xenobiotics in TAD

The use of TAD for the destruction of xenobiotics and noxious chemicals such as synthetic polymers, aromatic and halogenated compounds that may contaminate recycleable waste biomass has received limited attention [138]. The potential for application of TAD in this area derives from the ability of aerobic thermophiles to rapidly degrade a variety of chemicals due to their enzymatic versatility [43, 64, 82, 139-142] and removal efficiency may only be constrained by the availability of the anthropogenic chemical [143]. Various enzymes such as oxygenases, ligninases and peroxidases, which are powerful and essential biocatalysts in xenobiotic degradation remain the preserve of aerobic organisms [144, 145]. In TAD, these may play significant roles in the detoxification of xenobiotics, and a variety of plant toxins, such as linamarin in cassava, and related plant cyanogenic glycosides, whose presence in plants limit their use [146], and will certainly increase its appeal as a waste management and recycling process.

Detoxification of cyanogenic glycosides associated with cassava and cassava process wastes using thermophiles from TAD of agricultural waste has been demonstrated [146]. Although, little information currently exists on the use of either this process or any other for detoxification of waste in full scale operations, it is expected that the high temperatures of TAD and selection of suitably adapted population could lead to the breakdown of the toxins and noxious chemicals in various waste streams.

4.3. Protein enrichment of agricultural wastes by TAD

Although in classical studies on the use of TAD, waste upgrade and reuse were not always the intended endpoints, it has been vigorously proposed that TAD, as the vehicle for the protein enrichment of waste, may be applied to waste intended for upgrading and recycling as (components of) animal feed, given also that several agricultural wastes are currently being studied for upgrading and recycling, particularly in solid state fermentations including by silage [147, 148]. The attraction for this application of TAD is also buoyed by the fact that many agricultural and food industry wastes are already being employed in this capacity [17, 112-114]. It is envisaged that carbohydrate rich wastes, high quality agricultural refuse and by-products, food industry wastes and other organic wastes may eventually be treated by this process, to achieve cost effective protein enrichment of zero cost

materials for animal feed use [8, 17, 109, 113]. The emphasis here is on the exploitation of the capacity of thermophilic populations to degrade carbohydrates and lipids (with loss of carbon as carbon dioxide) while accumulating nitrogen as microbial protein, [25, 77] besides the selective conservation of waste protein under appropriate (elevated temperature) digestion conditions where nitrification is unlikely to occur [53]. It has been reported that thermophilic bacteria including *Bacilus* spp accumulate high content of protein in biomass, and this may be basis for the selective application thermophiles in the protein enrichment of wastes for feed use [149].

Unfortunately, few studies exist that have studied the detailed application of TAD for the protein enrichment of agricultural or other wastes for animal feed use. The pattern of protein accumulation in TAD is influenced by the metabolism of intrinsic particulate and soluble waste proteins, the metabolism and disposition of microbial proteins, as well as the metabolism, particularly the conservation as microbial protein/biomass, of intrinsic and extrinsic other nitrogenous compounds including ammonia in the waste slurry. This is particularly so under conditions of high temperature such as was employed in this study [53, 61, 150, 151].

During digestion of a model waste (potato peel slurry), up to 131% increase protein content of waste was obtained following digestion at pH 7.0, 1.0vvm aeration and 55°C over a period of 7 days [61]. Similarly, during digestion of corn waste by select populations of thermophilic *Bacillus* spp up to 258% increase in crude protein was obtained under conditions in which mineral nitrogen supplementation was applied to the waste [150]. This has significant implications for the use of TAD in the protein enrichment of agricultural waste for use in animal nutrition. Amino acid analyses of the accumulated proteins indicate that the digested wastes compete favourably with Pekilo® for use as protein supplement in animal feed formulation [61, 150].

CONCLUDING REMARKS AND SAFETY CONSIDERATIONS

Protein enrichment of waste for use in animal nutrition offers opportunities for the economic reuse of abundant agricultural wastes and refuse. Low tech, low cost biotechnological processes such as TAD offers great opportunities for the reprocessing and reuse of abundant agricultural wastes (particularly those generated as slurries or at elevated temperatures) in animal nutrition. The use of thermophilic organisms (perhaps also with GRAS status) to effect the protein enrichment, biomass production and detoxification reactions will help improve confidence in the final products derived from this process, and help drive its development and application in the valorization of agricultural wastes.

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