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Physiology and performance of thermophilic microorganisms in sewage sludge treatment processes

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Introduction

A combined treatment of domestic and industrial liquid wastes is preferably used in most industrialized countries. The primary objective of wastewater treatment plants is a high degree of water purification. But relatively little attention is payed to the primary byproduct, sewage sludge. The economical value of sewage sludge is among the lowest of all materials available on earth, but sludge will undoubtedly continue to be produced in very large quantities in the future. Its effective treatment will be one of the most important problems to solve.

In Switzerland, 95% of the population are connected to one of the more than 1000 wastewater treatment plants. The production of wet sewage sludge is 4′000′000 m^3 a^{-1} or 250′000 t of dry matter a^{-1}, corresponding to 0.1 kg of dry matter d^{-1} person^{-1}. This high specific quantity of sewage sludge is typical for industrialized countries and is expected to increase in the future, particularly in those countries where wastewater treatment is still expanding (personal communications BUWAL (= Environmental Protection Agency of CH) 1990).

The sludge produced in Switzerland is presently used in agriculture (as a substitute for synthetic fertilizers), deposited in landfill sites, or incinerated. The amount recycled in agriculture is presently 30 to 50% of the total production; the percentage varies from region to region. About 40 to 50% of the sludge produced are deposited in landfills and 10 to 30% are incinerated.

The use of sewage sludge in agriculture is declining: at the beginning of the 1980s, the amount used was 50% of the total sludge production. Realistic estimates predict that this quantity will reduce to 20% or 50′000 of dry matter t a^{-1} in the next years (Schweizer 1988). Extended or prolonged use of sewage sludge as fertilizer is possible only if waste sludge can be processed in a way to render appropriate bacteriological, chemical and physical properties.

Biological processes for the treatment of the sewage sludge have been implemented in 75% of all wastewater treatment plants in Switzerland. Until now, the most popular biological treatment techniques established were either mesophilic anaerobic stabilization in large plants or *aerobic* cold stabilization (uncontrolled process: just aeration, no elevation of temperature) in small plants. The *aerobic thermophilic* process represents a relatively new technology: it can easily be integrated in the traditional treatment sequence or it can substitute for the anaerobic process. In any way, the so treated sewage sludge becomes hygienized and its physical chemical quality is improved. Table 1 summarizes the types of sludge bioprocessing used in Switzerland (BUWAL 1990). The 20 plants exploiting an aerobic thermophilic sludge treatment step (ATS) constructed in Switzerland during the 1980s do not reflect just improved microbiological understanding or technological innovation, on the contrary, this was caused by legislation (Klärschlammverordnung, Federal Department of Interior 1981) prescribing that 100 *Enterobacteriaceae* per g of sludge must not be exceeded for use in agriculture. Further, this sludge must also be free of parasitic worm eggs and viruses.

Between 1975 and 1985, the development of the aerobic thermophilic sludge treatment technology was characterized by rapid progression to practice without the development of a sound basic scientific background (i.e.: trial and error instead of deterministic design based on knowledge of physiology and/or population dynamics). The majority of the studies carried out improved performance of sludge treatment in existing plants. However, virtually no attempt was made to examine the physiology of the microorganisms involved in aerobic thermophilic treatment using the only realistic substrate – sewage sludge itself.

A biotechnological approach to the study of sludge treatment involves investigations of microorganisms as biocatalysts in the process environment. However, the currently proposed process for sludge biotreatment differs from most biotechnological processes in the following ways:

- application of mixed populations (non sterile)
- complex and very ill-defined substrates
- largely varying substrate feeds (qualitatively and quantitatively)
- irrelevant commercial value of the final product
- non-profit basis for process operation
- low technology

Aerobic bioprocesses for the treatment of sewage sludge at high temperatures offer many advantages. The benefits of the technology allow the product to be safely used as an agricultural fertilizer. When disposal is the favoured option, the quality of the treated sludge can definitely be improved. Advantages are summarized as follows:

- biodegradation of normally biodegradable organic components at high rates
- biodegradation of recalcitrant compounds
- improvement of the physical characteristics of the sludge
- stability and reliability of the process due to the uniform microflora employed (selectivity pressure of the temperature)
- sanitization of the sludge (inactivation of all potentially pathogenic organisms and viruses)

The sanitization of sludge can also be achieved using non-biological thermal technology, by physical processes such as irradiation or chemical procedures such as the addition of lime. However, such processes do not incorporate the full range of benefits that can be gained with bioprocessing.

The treatment of the sewage sludge with thermophilic microorganisms represents an example of a low technology bioprocess (Bergquist et al. 1987). The non-profit nature of wastewater treatment industry generally inhibits innovation, but legislation can give the necessary positive impulse to improve the *status quo*.

Definition and choice of appropriate objectives for a process must be the starting point for all experimental work. This should be done on the basis of both theoretical and practical considerations, e.g. by evaluating different scenarios. It is very important to use such a dual approach in the wastewater treatment industry, where different interest groups interact. A range of different goals possibly influence the development of sludge treatment processes. Chemically, the maximal reduction of organic matter is a primary objective. Physically, dewaterability and heat production are most important goals, but also retention time is a central factor. Consideration of biological factors indicates four different goals: the hygienization of the sludge is the cardinal factor to be achieved in an aerobic thermophilic treatment. At the same time, high biocatalytic activity and low biomass yield are desired. When the aerobic thermophilic treatment is

Table 1. Total number and percentage of wastewater treatment plants with integrated bioprocesses for sewage sludge treatment. Both, producing and projected plants are included reflecting the situation in Switzerland in 1990.

Sludge treatment	Number of plants	Percentage
Anaerobic mesophilic	575	75%
Aerobic cold	139	18%
Sanitization	52 = 23 + 29*	7%
Total	766	100%

Anaerobic mesophilic = classical method to stabilize sludge, T approximately 35° C.
Aerobic cold = uncontrolled process: just aeration, no elevation of temperature.
Sanitization* = ATS treatment (23 plants) and thermal inactivation (29 plants).

considered as a pretreatment, a further goal is an 'improvement' of the substrate sludge for the next process step.

The treatment of sewage sludge with aerobic thermophilic microorganisms is a genuine practical application of thermophilic process microbes. Most development work has been done in Germany, Switzerland, Sweden, UK, Republic of South Africa and USA. Unfortunately, quantitative data about physiology, kinetics and population dynamics that would permit process development and design are generally missing.

Methodology

Several different bioreactors were used in our own investigations: optimization works were carried out in a compact loop bioreactor of 7 l total volume. Studies on inactivation of pathogens and experiments exploiting the fed batch cultivation technique were carried out in a 50 l compact loop reactor (COLOR): these reactors were equipped with mechanical foam breakers (Hess 1988). A pilot scale bioreactor (4000 l total volume) was a commercial design but modified during the experimental phase. The characteristic properties of these reactors are summarized in Table 2. The pilot scale reactor is shown in Fig. 1.

During all experiments, the following variables were measured on-line: fluxes of liquid and gas phases, temperature, pH, pO_2, pCO_2, redox potential, and exhaust gas composition. To our knowledge, this is the first time that an extensive on line measurement of sewage sludge populations has been performed. Detailed description of microorganisms, media, off-line analyses, and sewage sludge can be found elsewhere (Bomio 1990).

Results and discussion

Thermophilic populations and their dynamics

The characterization of representative sets of process microorganisms from aerobic thermophilic sludge treatment plants according to their microbiological and biochemical properties resulted in the classification of such isolates as *Bacillus stearothermophilus* populations. This was based on comparisons with references strains and held true for at least 95% of all strains, while 5% of the strains could not be safely identified as *Bacillus* only because sporulation could not be induced. All isolates showed extremely high specific growth rates (μ_{max} in the order of 2 h^{-1}) and generally low yield coefficients ($Y_{x/s}$ between 0.2 and 0.3 g g^{-1}; Sonnleitner & Fiechter 1983c).

These thermophilic populations respond with a fast reaction to changes of the substrate composition when cultivated in defined media. The lag phases after different fluctuations in substrates composition vary between 1 and 5 h, depending on the nature of the new substrate. A similar response was observed in pilot scale: high amylase activity (with an optimum at 70° C) was observed to appear rapidly during experiments, when the feed sludge contained some starch (e.g. potato residues from

Table 2. Characteristics of reactors used for studies on lab and pilot scale according to Bomio 1990 (COLOR = compact loop reactor).

		7 l Color	50 l Color	Pilot scale reactor
Total volume	[m^3]	0.007	0.050	4
Working volume	[m^3]	0.004	0.014	3
Height	[m]	0.030	0.792	2.68
Diameter	[m]	0.015	0.261	1.38
Max. aeration	[vvm]	1.5	1.5	0.1
Power input		impeller	impeller	circulation pump (loop)
Heat exchange		reactor jacket	reactor jacket	external loop
Foam breaker		external	internal	internal
Insulation		none	none	10 cm Alufloc

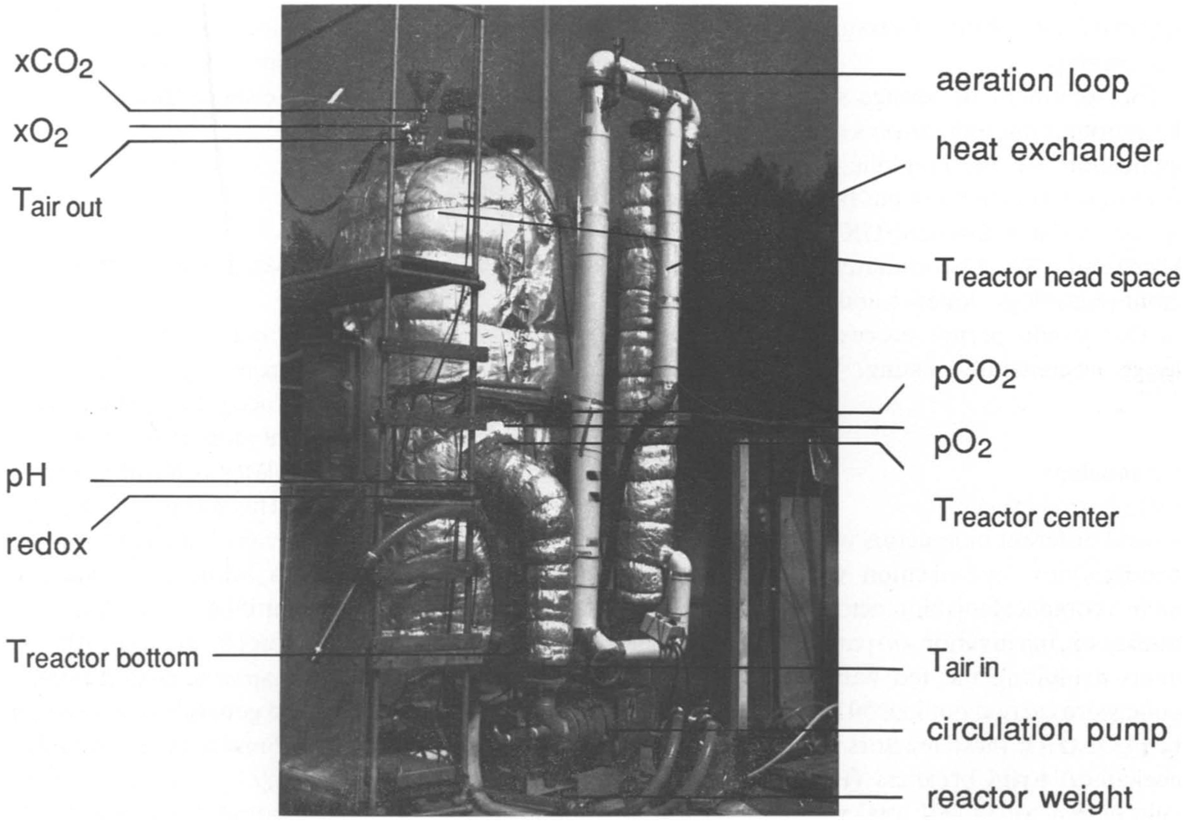

Fig. 1. The pilot scale bioreactor and measurement equipment: several temperature (T) sensors, sensors for partial pressures (pO_2 and pCO_2), redox potential and exhaust gas composition (xO_2 and xCO_2).

food industry; Bomio, 1990). Although the presence of starch in raw sludge is rare, these results show that the thermophilic populations are able to react immediately and efficiently to changing (chemical) environmental conditions, as illustrated in Fig. 2.

A similar rapid dynamic response of the process microflora was reported upon glucose addition to raw sewage sludge during continuous cultivation: the production of acetic and other volatile fatty acids was found to be induced without delay (Baier 1987). This situation is generally not observed in raw sewage sludge: either oxygen limited or unlimited sludge treatment processes at different scales show that the acidification of the sewage sludge is very unlikely (i.e. the exception rather than the rule).

Baier et al. (1986) have compiled some other characteristic population dynamic aspects of isolated thermophilic process bacteria grown in the lab over many hundreds of generations. The following pheno- and genotypic variations of the original strains were found significant:

Observed difference	Origin/effect
genetics	loss of plasmids
physiology	restricted exoenzyme formation
kinetics	different kinetic behavior
morphology	altered cell wall structure
phenotype	cell shape, culture appearance
metabolic control	several inhibition effects

Biocatalytic activities

Biomass

Microbial growth can be easily determined in clear culture media by measuring the increase of cell mass (biomass). This is essential for the evaluation of both biomass yield coefficients and specific growth rates. But in sewage sludge, which contains particulate matter, this direct method cannot be applied. Hence, it is necessary to exploit any indirect methodology that can be correlated with biomass formation or activity. Several methods are available for the determination of biomass on the basis of a chemical index (White et al. 1979). Indirect variables considered and the evaluation of five methods are summarized in Table 3. Of greatest interest for evaluation are the applicability in real sewage sludge, good correlation with biomass concentration, reproducibility and simplicity of the analytical procedure.

The determination of ATP proposed as biomass indicator (Holm-Hansen 1973; Droste & Sanchez 1983) is not found satisfactory: instability of the ATP extract and pronounced variations of ATP concentrations depending on growth phase and substrate being metabolized (King & White 1977) are the restricting factors.

The cell plate count method, as proposed for mesophilic aerobic sludge samples (Droste & Sanchez 1983), can give satisfactory results but it is laborious, very time consuming and difficult to apply for (mixed) thermophilic populations. The general disadvantage of the method is the inherent underestimation of the 'true' cell number concentration. The existence of cell aggregates in sludge causes a misinterpretation of raw data because the assumption – a colony derives from 1 single cell – is obviously not true.

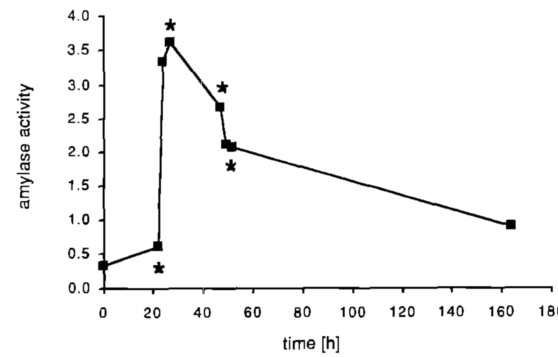

Fig. 2. Time course for amylase activity during fed batch cultivations in the 4'000 l pilot scale bioreactor. The mean hydraulic retention time was 14 h, volume changes every 2.5 h (*). Starch was present in the lot of sludge replaced at time 20 h, and amylase activity increased rapidly showing the short adaptation time of the thermophiles to new environmental conditions. Following volume changes after activity maximum showed a characteristic wash out behavior: the subsequent lots of sludge changed did no longer contain starch.

The overall dehydrogenase activity reflects the rate of oxygen consumption under strictly aerobic metabolic conditions (Lopez et al. 1986). After method optimization, dehydrogenase activity is found to be proportional to biomass concentration when the oxidative metabolism is not limited.

DNA is used to estimate microbial biomass in activated sludge processes (Thomanetz 1982). The determination of DNA as a biomass indicator is excellent for samples from defined media cultures but extremely unpracticable from sludge when interference from the complex matrix become dominant.

Phospholipids, also an indirect estimate for microbial mass, shows two important restrictive factors when applied to determine growth of aerobic thermophilic process organisms. These are the presence of high concentrations of lipids in the raw sludge and the metabolism of the phospholipids by thermophilic organisms. Therefore, the method was not found very successful.

The evaluation for following microbial growth in/on sewage sludge is: total dehydrogenase activity can be most reliably used as an indirect measure for biomass. However, it reflects the cells activity rather than their mass concentration.

Limiting factors

Growth is generally under the control of extracellular chemical factors such as available carbon energy source(s), nutrients, trace elements and growth factors. Sewage sludge is both, ill-defined and highly variable with respect to concentration and availability. Experiments designed to identify the dynamics of suitable carbon sources and limiting factors for thermophilic bacteria during the treatment process, however, suffer from analytical difficulties.

The nature of limitations responsible for the decrease of both, the oxygen uptake rate and the total dehydrogenase activity during limited growth were determined by pulsing different carbon energy substrates, salts, trace elements and vitamins to the cultures. Figure 3 shows a typical response to a carbon substrate pulse: the increase of the oxygen uptake rate and the simultaneous decrease of the oxygen partial pressure in the sludge immediately after pulsing fructose shows that a carbon limitation had been released.

The pulse experiments done at the end of fed batch cultures clearly show that biodegradable carbon energy source availability is the only limitation there and salts, trace elements and vitamins are not limiting. Neither is oxygen limiting because pO_2 was always $\geq 80\%$ of air saturation during these pulse experiments (lab scale studies with raw sludge, Bomio et al. 1989).

Activities in sewage sludge and pure cultures

The above cited experiments indicate permanent proteolytic activity because growth was observed immediately after pulses of either casein, gelatine and soya protein. This could be confirmed by measuring the total activity of proteolytic enzymes. Isolated mixed sub-populations of thermophilic process bacteria grew on semi-synthetic media with soya protein as the sole carbon source (Bomio et al. 1989). Further extended pulse experiments showed that extracellular proteases are the only polymer degrading enzymes produced during growth of the process microflora on sewage sludge. Other non-proteinaceous polymers generally present in sewage sludge represent potential substrates for thermophiles, but are normally not hydrolyzed by the process populations. The activities tested are summarized in Table 4.

The proteolytic activity was found to correlate with the increase of the oxygen uptake rate during both, batch and repetitive batch growth of aerobic thermophilic microorganisms on sewage sludge. This indicates a tightly growth associated production of proteolytic enzymes (Fig. 4) which also correlates with an increase in free ammonium concentration in the culture.

The respiratory quotient (RQ value) in sewage sludge cultivations had an average value of 0.82 during both exponential and oxygen limited growth

Table 3. Comparison of indirect methods for biomass determination.

Method	Applicability in defined media	Applicability in sewage sludge	Validity	Reproducibility	Analytical ease	Overall evaluation
ATP	–	–	–	+	–	–
cell count	++	++	+	–	–	=
dehydrogenase	++	+	+	+	+	++
DNA	++	=	+	+	+	+
phospholipids	+	+	–	+	+	+

++ : very good
+ : good
= : satisfactory
– : not satisfactory

phase; this is typical for oxidative metabolism of proteins (Lentner 1981). This value then rises to 1.1 in the carbon limited phase indicating changes in the overall metabolism. Control cultivations performed on a semi-synthetic media with the same inoculum but with glucose as sole carbon source showed an average RQ of 1.05 which is typical for fully oxidative growth on carbohydrates.

The above referenced findings (RQ values, proteolytic activity, the pulse experiments with protein substrates and the growth associated production of free ammonium) strongly indicate that the thermophilic populations use proteinaceous material as their preferred carbon source.

Determinations of lipase, cellulase and pectinase activities in ATS processes failed; negative results were obtained with both the original and modified analytical methods, i.e. after specific adaptations to the complex medium sludge.

Cultivations on synthetic medium showed that the proteolytic activity is not cell wall bound: high activity was found in the cell free supernatant. On the other hand, activities determined in the supernatant of cultivations on sewage sludge as substrate were comparably low. The conclusion that the enzymes are excreted into the medium but immediately absorbed by particles in the sludge, is indicated by the behavior shown in Fig. 5: during an initial lag phase, there is no *de novo* formation of proteases. However, in this phase, the sludge contains a significant amount of proteolytic enzymes.

Table 4. Extracellular enzymatic activities in sewage sludge evaluated after pulses of polymers and determination of the respective enzymatic activities.

Enzymes	Increase of OUR after a pulse experiment	Enzymatic activity found
Protease	+	+
Amylase	–	*
Cellulase	–	–
Keratinase	–	–
Lipase	–	–
Pectinase	–	–

+ : always found
– : never found
* : occasionally found

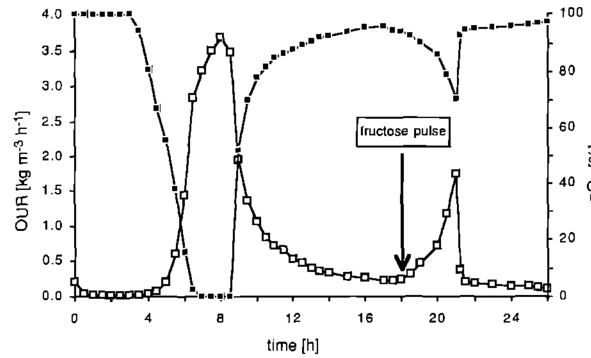

Fig. 3. Oxygen uptake rate (□) and oxygen partial pressure pO_2 (■) during a fed batch cultivation at 65° C, 1500 min^{-1}, 1 vvm and pH 7 in a lab scale reactor: fructose pulse experiment in the carbon limited growth phase. The exponential increase of the OUR immediately after the pulse shows that the fructose pulsed released a (carbon) limitation.

After the lag phase, thermophilic populations begin to grow on sludge with an RQ of 0.81. The proteolytic activity measured in vitro at 80° C and pH 7 rises rapidly, indicating that thermophilic proteases are now formed and also excreted into the medium. These proteases show little activity at 65° C (Sundaram 1988). Activity measured at this temperature does not increase during the growth phase. The hydrolysis of proteinaceous materials with subsequent metabolism of amino acids (obviously oxidative desamination) causes ammonium to be released into the medium. The proteolytic activity originating from the thermophiles has an optimum around 80° C, but at this temperature, raw sludge proteases are not active. Therefore, microbiological activity and process performance can be effectively followed by measuring proteolytic activity at 80° C (Bomio et al. 1989).

Cryptic growth

Some authors have suggested the existence of either antibiotic or 'antagonistic' effects of thermophilic populations on potentially pathogenic microorganisms (Nebiker 1981, Hammel 1983). However, inactivation experiments showed no significant influence of thermophiles on the inactivation of potential pathogens added to raw sludge. Experimental determination of possible antimicrobial substances, using the MIC (Minimal Inhibitory Concentration) test (Hamilton-Miller 1977; Kres-

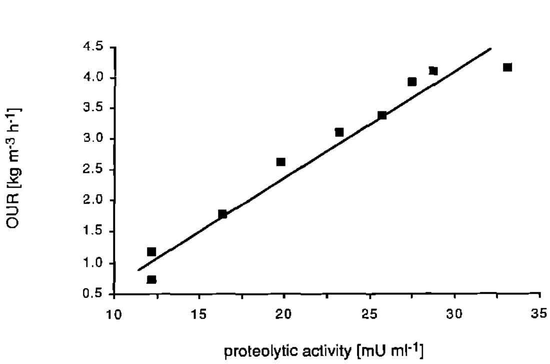

Fig. 4. Linear correlation between proteolytic activity and oxygen uptake rate during a batch cultivation on sewage sludge at 2500 min^{-1}, 65° C, 0.8 vvm and pH 7 in a lab scale reactor. Correlation coefficient r = 0.935. The activity was determined relative to the calibration standard proteinase K (Sigma P-0390, type XI) measured at 80° C. The correlation indicates a growth associated production of extracellular protease by the process microbes.

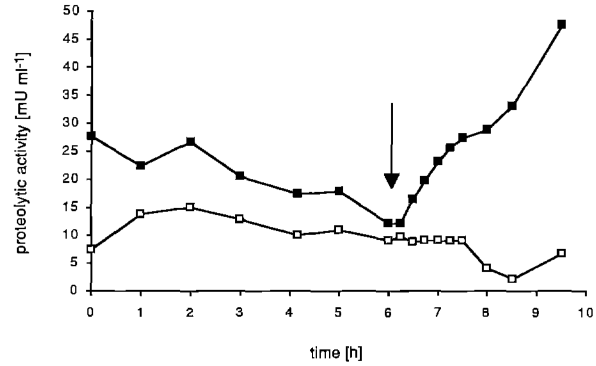

Fig. 5. Proteolytic activity in the sludge (■) and supernatant (□) measured at 80° C and pH 7 during a sludge cultivation at 2500 min^{-1}, 65° C, 0.8 vvm and pH 7 in a lab scale reactor. The activity is given in terms of proteinase K standards. The arrow shows the end of the lag phase. The activity measured in the supernatant does not increase after the lag phase, indicating that the proteolytic enzymes are absorbed to sludge particles.

ken & Wiedmann 1984), conclusively confirmed the absence of such effects.

A non-selectively enriched population of strictly mesophilic microorganisms isolated from sewage sludge, *Escherichia coli* and *Staphylococcus aureus* were cultivated on Isosensitest Broth. Different amounts of raw and aerobic thermophilic (= treated) sludge were added to these cultures. Some results are shown in Fig. 6; similar results were obtained with *E. coli* and *S. aureus*. Growth of the mesophilic microorganisms was enhanced by the addition of sludge (both raw and treated) and not inhibited. The enhancement is linearly correlated with the amount added and significantly greater for aerobic thermophilic than for the raw sludge. This typical response rules out any production of antimicrobial agents during aerobic thermophilic sludge treatment. Its means that some substrates have been made 'more accessible' during thermophilic aerobic treatment of sludge (ATS); even more, the described ATS-process behaves really as a pre-treatment and is not a full stabilization. Comparable results were obtained with thermophilic populations isolated from sewage sludge at temperatures of 45° C (Wassen 1975).

Thermophilic populations isolated from sewage sludge were shown to be able to grow in semi-synthetic media utilizing intact yeast cells as sole substrate (Mason et al. 1986, Hamer and Mason 1987). The formation of a lytic enzyme by *Bacillus stearothermophilus* cultures after induction by mitomycin C has also been described (Walker & Campbell 1966). These observations stimulated the search for lysozyme excreted by mixed populations of *B. stearothermophilus* strains grown on sewage sludge. Unfortunately, lytic activity was found generally to be extremely low in sewage sludge (Bomio et al. 1989). These experiments suggest that inactivation of pathogenic microorganism is a predominantly thermal process and synergistic effects originating from the thermophilic process microbes are negligible.

Inactivation of pathogenic microorganisms

In the early 1970s, inactivation of potentially pathogenic organisms in sewage sludge became an important goal. This resulted in the introduction of different competing technologies. After disastrous experience with pasteurization units installed after the anaerobic digestion step, ATS or pasteurization processes were introduced as pre-treatment subprocesses. But only a few kinetic data for microbially mediated inactivation processes are yet available.

Enterobacteriaceae are widely used as indicator organisms for 'potentially pathogens'. They can be

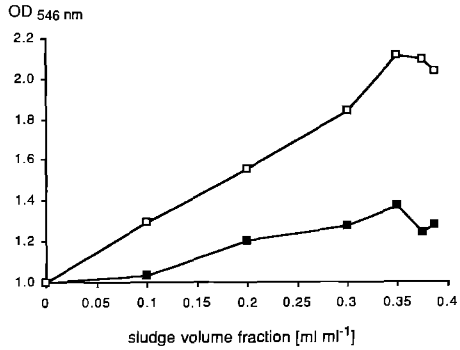

Fig. 6. Relative absorption at 546 nm of a non-selectively enriched culture of mesophiles after 18 h of incubation at 37° C with different fractions of either raw (■) or aerobic thermophilic sludge (□) added to the cultures. The positive and constant slopes obtained indicate that there are no inhibitory effects in both raw and ATS sludge. Treated sludge accelerated the growth of potential pathogens even better (greater slope) demonstrating that this ATS process is operated as a *pre*-treatment step prior to anaerobic digestion and not as a single stabilization process.

selectively determined in homogeneous aqueous suspension after plating on VRBD-agar (Violet Red Bile Dextrose). This classical technique (established in food or environmental analysis) fails when used to analyse sludge samples. Many oxidase positive or slowly growing species occur and make a sound quantitative analysis impossible. This uncertainty has of course forensic consequences; it would be wise to consider the use of other indicator strains which can be easily and reliably quantified for routine hygienic analyses.

Process development

Objective and constraints

Definition of objectives and constraints is one of the most critical points during development of a process for the treatment of sewage sludge. Table 5 summarizes such an evaluation. The evaluation of the variables that are of significance for process performance is crucial. Simultaneous multivariable optimization should be employed because the interdependencies of process variables and parameters are not known a priori for the system sewage sludge (missing background in physiology and population dynamics).

Fed batch cultivations carried out at different temperatures, but with all other variables (pH, stirrer speed, aeration rate and inoculum preparation) kept constant, revealed the effect of temperature on the process performance. This can be monitored by the total oxygen uptake rate, which is proportional to the heat generated by the growing culture (Birou et al. 1987). There was only a minor dependence of the total oxygen uptake rate on temperature found in the range 64 to 68° C. This result confirms well the choice for the process temperature already used in technical-scale plants operating (in Switzerland 65° C). An identical result was obtained for spontaneous thermophilic composting of sewage sludge with poplar sawdust (Viel et al. 1987).

Temperatures lower than 60° C are not considered further because of the decreasing efficiency of sanitization (Feachem et al. 1983; Sonnleitner & Fiechter 1983a, b). Spontaneous population development at temperatures > 70° C occurs only after a considerable lag; at 74° C this extends to 36 hours. This is, of course, inappropriate for a process targeting to auto-generate the necessary heat for the hygienization effect. Growth was observed in processes operated up to 76° C and this result is in agreement with other work on *B. stearothermophilus* (Gibson & Gordon 1975; Baier 1987).

The effect of pH can be examined during cultures with (at pH = 7) and without pH control. In fact, the integral oxygen uptake rates for the two culture types are similar. The thermophilic populations present in the sludge do not show any activity during cultivation at pH values less than 6. The pH of the uncontrolled cultures normally increases to final values of approximately 8.8. These results indicate that the pH value does not have a significant influence on the response function for the process provided the pH value remains in the permissive window between > 6 and 8.8. It is therefore extremely important that the pH value of the raw sludge is known: when processes are operated without direct pH control, it will be necessary to assure that the feed of fresh sludge does not acidify the process by constrainting the feed rate (≈ in-

Table 5. Objectives and constraints for sewage sludge treatment processes.

Process objectives	Process constraints
Robust	continuous substrate supply
Rapid	feed volume and concentration variable
Low tech (minimal costs)	low cost
Reliable	expected load with toxic components
Flexible	variable substrate composition
Performance quantification	product quality must satisfy legal requirements

direct pH control). A pH value below 6 does not allow effective aerobic thermophilic treatment.

The aerobic thermophilic process involves spontaneous reaction under batch conditions. *B. stearothermophilus* strains are ubiquitous components of raw sludge at concentrations of approx. 10^4 colony-forming units (c.f.u.) ml^{-1}. They develop spontaneously when temperature and pH meet the physiological requirements described. The availability (preparation) of a constant active inoculum is of fundamental importance for spontaneous processes in order to achieve some reproducibility and to minimize unproductive lag times. Operating the process in the repetitive (fed) batch mode circumvents this problem. During experiments with this technique, major differences were observed with respect to the duration of the lag phase in the various cultivations. The lag phases are summarized in Table 6.

Not all of these cultures were oxygen limited, i.e., no plateau at the maximum Oxygen Uptake Rate (OUR_{max}) was attained, and they became carbon limited. In this case, the hydraulic retention time had a fundamental influence on the process efficiency. With frequent volume changes (every 3.5 h), part of the treated sludge is replaced with new feed close to the time where the maximum oxygen uptake rate occurs. Although the microorganisms have only a short lag phase on the new medium (1.8 h), such an operating procedure produces sludge that is not totally biodegraded; the organisms are never starved and, therefore, do not significantly tend to sporulate. When feeding is carried out less frequently (every 10.5 h), the process is more stable and shows a good reproducibility with respect to the oxygen uptake rate although the lag phase is longer (3.5 h).

This longer lag phase is related to partial sporulation of the process microflora during the carbon limited phase (Fig. 7). Partially responsible was also the temperature shock which, under these conditions, is greater than when volume changes occur every 3.5 or 7 h; heat exchange between treated and raw sludge was not possible in the experimental facility used (Bomio 1990). According to the trajectories of dipicolinic acid, a typical spore component, and the spore counts (determined on germination agar with valine as the spore-germination agent as described by Foerster 1983) sporulation occured immediately after the metabolically active phase in the carbon limited growth phase (in the example in Fig. 7: after 9 h cultivation time). Sporulation exerts a significant influence on the process performance.

The rates of reduction of the Chemical Oxygen Demand (COD) decreased as the volume change frequency decreased because of the longer lag phases. A fed-batch process with frequent volume changes is most effective with respect to the rate of destroying biodegradable organic matter. The cul-

Table 6. Dependence of the lag phases on the repetitive batch cycling conditions.

Time for volume changes [h]	3.5	7	10.5
Length of average lag phase [h]	1.8 ± 3.9	2.0 ± 1.3	3.5 ± 0.8

tivation time between volume changes during a fed batch process influences also microbiological heat production: frequent volume changes allow a greater heat production. This indicates strongly that a continuously operated process should be most efficient. However, continuous operation is said to be not acceptable; the argumentation is based on hygienic aspects and the fact that hydraulic short circuiting cannot be ruled out.

The heat produced by the metabolic activity of aerobic microorganisms (growth associated biogenic heat) is proportional to the oxygen uptake (Birou et al. 1987). The conversion factor is reported to be 440 kJ mol^{-1} oxygen independent of both, organisms and media. Now, the heat production rate was definitely lower in sludge cultivations with greater volume changes. This is a consequence of the more extended lag phase where no heat production occurs. The microbiological heat yield coefficient was essentially constant during cultivations at different scales and was found to be around 15 MJ kg^{-1} COD removal (see also McCarty 1965; Cummings & Jewell 1977; Breitenbücher 1983; Jewell & Kabrick 1980; Loll 1974; Kapp 1986).

Optimization

According to the definitions of objective functions, optimization experiments were carried out using a two-dimensional field of variables. This involved variation of the stirrer speed and the aeration rate because these two variables had a significant influence on process performance and on the chosen response function. Further, they are the only variables permitting the operator to vary with a considerable degree of freedom. The optimization according to the function that identifies the reduction of organic matter as a partial objective was repeated with different sludge batches in order to evaluate the practicability and reproducibility of the simplex method with a complex substrate such as sewage sludge (Fig. 8). A very sharp optimum was found in a laboratory scale reactor near a stirrer speed of 1500 min^{-1} with an air flow rate = 360 Nl h^{-1} (i.e. 1.5 vol/$vol^{-1} \cdot min^{-1}$). The reproducibility of the simplex method was excellent and the first optimum was nicely reproduced in spite of beginning

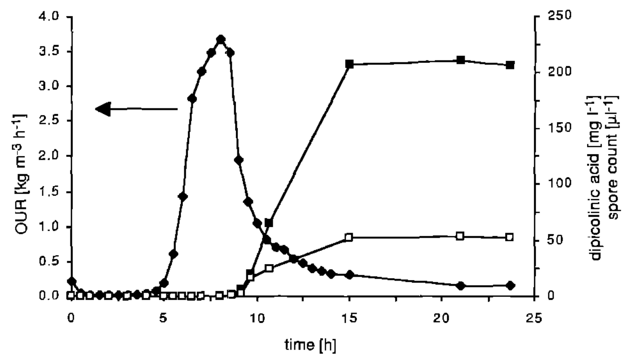

Fig. 7. Oxygen uptake rate (◆), spore count (□) and dipicolinic acid concentration (■) in sewage sludge during aerobic thermophilic sludge treatment in a lab scale bioreactor at 65° C, pH 7, stirrer speed 1500 min^{-1} and 1 vvm. In the carbon limited phase, sporulation of the thermophiles is observable by both spore count and by the detection of dipicolinic acid, a typical spore component.

the optimization with a different substrate and with considerably different starting simplex vertices (Bomio 1990). The optimum found seems to be independent of sludge composition.

The influence of the aeration rate was always positive; increased aeration resulted in increased response functions due to greater oxygen transfer rates. However, the mechanical power input (i.e. stirrer speed) did not have the same influence on process efficiency. Indeed, a too high stirrer speed sharply depressed the response function. *B. stearothermophilus* populations are not known to be shear sensitive, so the explanation of this event is not likely to be found in a damage of the single cells. The microbiological activity was found to be largely due to the metabolism of the particulate fraction. The degradation mechanisms correlate with intensive contact between microorganisms and the insoluble substrates, as demonstrated for cryptic growth (Hamer & Mason 1987). Most probably, excessively high mechanical power input (stirrer speed) does not allow an adequate contact between biomass and particulate substrates to be maintained or even destroys the aggregates and, hence, results in a drastic reduction of process efficiency. In general, the optimization results obtained with the simplex algorithm confirm the physiological knowledge about these populations

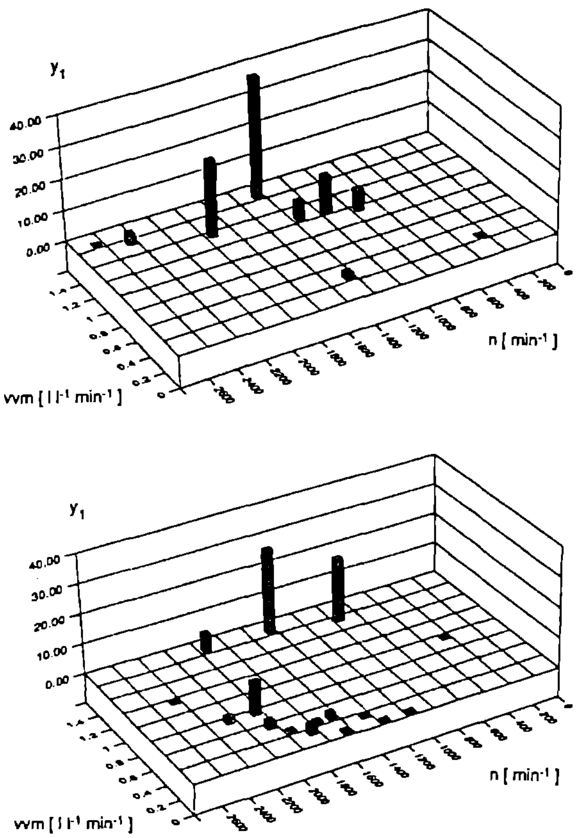

Fig. 8. Process optimization with the simplex algorithm; 2 different experimental series are shown. The maximum found is independent of both the raw sludge used and the starting vertices of the simplex. y_1 is an objective function consisting of: maximization of biogenic heat formation and COD removal, and minimization of biomass formation and process time; n is the stirrer speed in a 7 l COLOR; from Bomio 1990.

(i.e.: high potential for oxygen uptake and rapid growth).

Conclusions

The development of an efficient process for the treatment of waste sewage sludge involves definition and quantification of objective functions. In the case of a microbiological process in the wastewater treatment industry, this step requires synergistic interactions between scientific authorities, political and legislative authorities, and plant constructors. Reported results and unclear objectives attest that the synergistic effect is far from being fruitful.

The performance of aerobic thermophilic sludge treatment can be improved basing on augmented physiological knowledge about population dynamics in correlation with metabolism of different substrates in sewage sludge, i.e., studies of the biomass substrate relations must be intensified.

Aerobic thermophilic sludge treatment plants operating at 65° C take advantage of maximal thermophilic microbial respiratory activity and associated heat production. Temperatures less than 60° C are of little interest because of low hygienization efficiency. Temperatures greater than 70° C are too restricted with respect to microbial resources (the breadth of spectrum of organisms decreases rapidly with increasing temperature). The pH value is not a relevant factor for process efficiency provided it is not less than 6.8 because the permissive window is broad; pH regulation during the treatment process is not likely to be necessary, however, pH values as low as 5 . . . 6 often occur in untreated waste sludge. This must be either fed directly at a sufficiently low rate (in order not to acidify the process) or fed only after increasing pH by appropriate chemicals; the first solution is to be preferred. It is clear that the measurement of pH is an important process indicator.

Proteolytic activity is the dominating extracellular enzymatic activity found generally associated with the growth of thermophilic process microbes; it was always detected during an effective treatment. It obviously has an activity optimum at 80° C. The proteases present in the raw sludge were found to be completely inactive at this temperature. Therefore, it is possible to quantify the performance of a treatment unit by measuring the proteolytic activity at 80° C.

Extracellular proteases are the enzymes that are permanently active during normal operating conditions, but the presence of other easily degradable polymers in sludge, e.g. starch, rapidly induces the production of the respective degrading enzymes, in this example amylases.

Actively respiring thermophilic biomass can be quantified only with indirect methods such as total dehydrogenase activity. Such measurements allow the minimization of microbial growth during process development and optimization. Decreases in activity during batch and fed batch cultivations were found to be a consequence of oxygen or carbon limitation.

The heat inactivation of potentially pathogenic microorganisms is a pure temperature effect with no synergistic effects from the thermophilic populations. Neither antimicrobial agents nor lysozyme could be detected in sludge undergoing treatment. When potentially pathogenic microorganisms were added to ATS-processes their inactivation was slowed down compared with the death kinetics in pure aqueous medium; obviously, sludge components act protectively.

A great advantage of the aerobic thermophilic process over a purely thermal one (i.e. using microorganisms to generate heat) is the enhancement of sludge settling characteristics. This extra benefit is due to a decrease of small particles and an increase of particles larger than 30 μm during the process.

The repetitive (fed) batch cultivation technique is essential for the reproducibility of the process. Process harvest and feed patterns influence sludge biodegradation. Sporulation of thermophilic biomass is disadvantageous for the process because it results in long lag phases without significant microbial activity and must, therefore, be suppressed. Sporulation can be avoided by frequent volume changes, but this affects the level of stabilization achieved, i.e. high rates can so be maintained but the turn over is less than 100%. The yield coefficient – heat produced per COD removed – can be expected to be always close to 15 MJ kg^{-1}.

Continuing increases in sludge production in the future and increasing awareness of environmental problems might well stimulate research and development of technical scale sludge treatment processes using thermophilic microorganisms. Should this occur, the presented overview may give practical and scientific background knowledge for the design and construction of sludge treatment processes.

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