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Enzyme treatment to reduce solids and improve settling of sewage sludge

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The effect of microbial enzymes in reducing the disposable solid content of sludge was investigated. A mixture of industrial cellulase, protease, and lipase, in equal proportion by weight, reduced total suspended solids (TSS) by 30–50% and improved settling of solids. An increase in solid reduction was observed with increasing enzyme concentration. The effect of combinations of enzyme treatments indicated that two-enzyme combinations of protease and cellulase produced better solid reduction than individual enzymes and that lipase further augmented this effect. Among the individual enzymes, protease produced a more settleable sludge as compared to cellulase and lipase. Adjustment of the pH of the enzymatically treated sludge to the acidic range (pH 2–4) further improved solid reduction, and adjustment to the alkaline range (pH 10–12) improved settleability. *Journal of Industrial Microbiology & Biotechnology* (2001) **26,** 383–386.

Keywords: enzyme treatment; solids reduction; sewage sludge; sludge settling; protease

Introduction

Annual biosolid production amounts to 20,000 dry tonnes per million people. In Europe and North America, approximately 40% of the total is applied to land as fertilizer. Most of the remaining 60% is disposed of in landfills or by incineration. The costs of disposal and the negative environmental impacts of the latter disposal methods have motivated a search for methods that reduce the overall solid content of sewage sludge requiring disposal.

Sludges produced from primary and secondary settling tanks as a result of aerobic and/or anaerobic digestion processes typically have a solid content of 0.5–5% [8]. Because these sludges have thixotrophic properties, they do not settle easily and are difficult to dewater. Sewage sludge is generally thickened using chemical flocculents and conditioners and mechanically dewatered using screw and belt presses, centrifuges, and other devices. Thus, in evaluating new methods for reduction of sludge solids or volume, the impact on sludge settleability and filterability is of great interest.

The solid component of sludge consists mainly of organic and inorganic material, having an approximate ratio of 60:40 [11]. Protein makes up the largest organic component of sewage sludge, representing approximately 50%. Carbohydrate and humic substances account for up to 20%. Bacterial biomass, which develops during the sewage digestion process, is a major solid component of sludge. The composition of soluble microbial products (SMP) in wastewater treatment systems reflects the microbial biomass composition, including proteins, carbohydrates, and lipids from which they derive [18].

Evidence from composting experiments indicates that 50% of sludge solids is biodegradable [5]. This 50% can probably be equated to the pool of organic compounds, known as SMP released from biomass during growth/metabolism/decay of microorganisms in wastewater treatment systems [1]. This material includes lipids, proteins and enzymes, carbohydrates, and nucleic acids. Factors affecting production of SMP include starvation of bacteria, substrate-accelerated death, response to environmental stress, extreme temperatures, and osmotic shock. Extracellular enzymes are produced during situations of microbial stress.

The objective of this study was to investigate the effects of microbial enzymes in reducing the disposable solid content of sludge. The intent is to recycle the solubilized material back to the sewage treatment plant for biodegradation. The principal rationale for using proteases and lipases relates to the high levels of protein and lipids in sewage sludge. Commercial cellulases, hemicellulases, and amylases were tested to address any element of the carbohydrate fraction from undegraded paper or plant material carried over into the sludge and because these commercial carbohydrases contain contaminant enzymes that may attack microbial cells.

Materials and methods

Enzymes

Alcalase and Esperase were obtained from Novo Nordisk (Franklinton, NC); Econase CEP, Lipase EX, Alkaline protease L-FG, and Septizyme from Enzyme Development Corporation (New York, NY); Fungal protease and Sumizyme from Bayer Corp. (Elkhart, ID); biohemicellulase from Biocon (Cork, Ireland), and Lipase VII from Sigma (St. Louis, MO).

Sewage sludge

Sewage sludge samples were obtained from the anaerobic digesters of the municipal wastewater treatment plant in Waterloo, Ontario, Canada.

Enzymatic degradation studies

For enzymatic treatment of sludge, 95-ml aliquots of well-mixed sludge samples were placed in 250-ml Erlenmeyer flasks. After addition of the desired concentration of enzyme solution (5 ml),

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Table 1 Effect of enzyme treatment on solid reduction of sewage sludge

Enzymes	Final pH	TSS (%)	Percentage solid reduction
None	7.9	2.9	6.1
Enzyme mixture (0.1%)	8.3	2.2	29.0
Yeast extract (0.1%)	8.9	2.3	25.8
Peptone (0.1%)	9.0	2.2	29.0

Initial solids (TSS), 3.1%; enzyme mixture of fungal protease, yeast lipase VII, Sumizyme (cellulase), and biohemicellulase, 0.025% each; initial pH, 7.0; temperature, 40°C; shaking, 200 rpm.

flasks were incubated on a rotary shaker (200 rpm) at 40°C for 96 h. Control flasks, containing 95 ml sludge and 5 ml distilled water, were run in parallel during each experiment. At regular time intervals, total suspended solids (TSS) measurements and settleability tests of enzyme-treated and untreated sludge were carried out to determine the effect of the enzyme treatment. Each experiment was conducted in duplicate and experiments were repeated to determine consistency in the results obtained.

Analytical methods

To determine TSS, a well-mixed sample (20 ml) was centrifuged at 7000×*g* for 10 min. After discarding the supernatant, the residue was washed with 20 ml of water and centrifuged again for 10 min. Solids were transferred to a pre-weighed aluminum dish and dried overnight at about 100°C.

Settleability was determined by placing 100 ml of sludge sample in a measuring cylinder to settle at room temperature for 2 h. The volume of settled solids was recorded and presented as milliliters of settled solids per 100 ml total sample.

Total carbohydrate was determined using Anthrone reagent [20]. Protein and lipid contents were determined following the methods of Lowry *et al.* [13] and Bligh and Dyer [2], respectively. Ammonia-N and nitrate-N were analyzed by the standard methods for wastewater analysis [9].

A known amount of sample in a pre-weighed silica crucible was heated at 600°C for 4 h to determine ash content [9]. Metals were analyzed by atomic adsorption spectrophotometry at Analytical Services Laboratory, University of Guelph, Guelph, Ontario.

Table 2 Chemical compositional changes of sewage sludge due to enzyme treatment

Components	Treatment time (h)		
	0	48	96
TSS (%)	3.1	2.4	2.1
Percentage TSS reduction	–	22.6	32.3
Protein (%)	9.9	8.8	3.8
Percentage protein reduction	–	11.1	61.6
Lipids (%)	12.0	7.7	7.2
Percentage lipid reduction	–	35.8	40.0
Carbohydrates (%)	27.3	26.3	19.1
Percentage carbohydrate reduction	–	3.7	30.0
Total organic matter (%)	57.3	48.8	31.5
Percentage organic matter reduction	–	14.8	45.0
Ash content (%)	42.7	51.2	68.5
Percentage ash increase	–	16.6	37.7

Initial solids (TSS), 3.1%; enzyme mixture of fungal protease, yeast lipase VII, Sumizyme (cellulase), and biohemicellulase, 0.025% each; initial pH, 7.0; temperature, 40°C; shaking, 200 rpm.

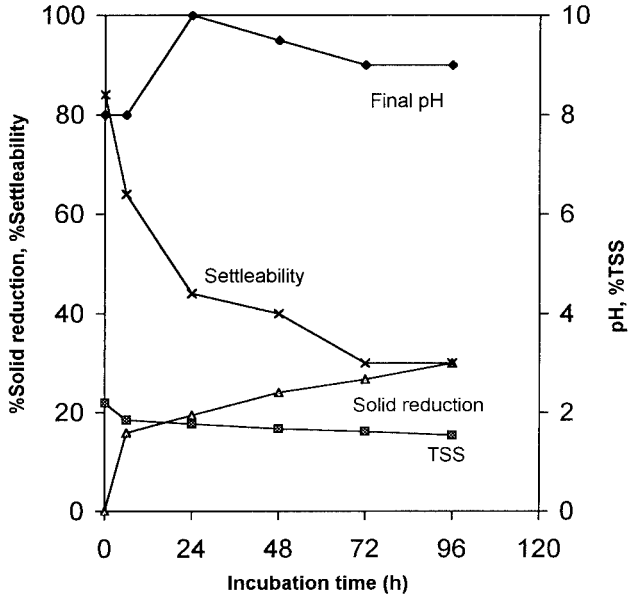

Figure 1 Enzymatic degradation of sludge solids as a function of time. Initial solids (TSS), 2.2%; mixture of Econase CEP, Lipase EX, and Protease L-FG, 0.03%; initial pH, 8.0; temperature, 40°C; shaking, 200 rpm.

Results

In preliminary experiments, sewage sludge (3.1% solids) was treated with a combination of commercial protease, lipase, cellulase, and hemicellulase by incubation at 40°C on an orbital shaker for 96 h. The results are shown in Table 1. Percentage solid reduction in the enzyme-treated sample was 29% compared to a reduction of 6.1% in the control. An analysis of the organic and inorganic compositional changes in the sludge as a result of enzyme treatment is provided in Table 2. At time zero, the sludge consisted of 57.3% organic matter and 42.7% ash. Chemical compositional changes as a result of digestion with a combination of enzymes showed a reduction in sludge organic matter of 45%. The most dramatic reduction was observed in the sludge protein component, amounting to 61.6%. Lipid and carbohydrate reductions were 40% and 30%, respectively.

The time course of solid reduction was monitored over a 96-h incubation period (Figure 1). A dramatic reduction in solids was

Table 3 Effect of treatment with individual enzymes on solid reduction and settleability of sewage sludge

Enzymes	Final pH	TSS (%)	Percentage solid reduction	Settleability (ml/100 ml)
None	8.6	2.6	7.1	80
Alkaline protease L-FG	9.5	2.1	25.0	35
Econase CEP	8.0	1.9	32.1	55
Lipase EX	7.0	2.2	21.4	50

Initial solids (TSS), 2.8%; enzyme concentration, 0.03%; initial pH, 8.0; temperature, 40°C; incubation, 96 h; shaking, 200 rpm.

Table 4 Effect of enzyme concentration on solid reduction and settleability of sludge

Enzyme concentration (%)	TSS (%)	Percentage solid reduction	Settleability (ml/100 ml)
No enzyme	2.3	8.0	80
Protease L-FG (0.03%)	1.7	32.0	32
Protease L-FG (0.02%)	1.8	28.0	42
Protease L-FG (0.01%)	1.9	24.0	44
Esperase (0.05%)	1.7	32.0	34
Esperase (0.03%)	1.8	28.2	40
Esperase (0.02%)	1.8	28.0	38
Esperase (0.01%)	1.9	24.0	38
Alcalase (0.03%)	1.9	24.0	34
Alcalase (0.02%)	1.8	28.0	32
Alcalase (0.01%)	2.0	20.0	38
Cellulase (0.06%)	1.8	28.0	68
Cellulase (0.03%)	1.8	28.0	54
Lipase EX (0.06%)	1.9	24.0	64
Lipase EX (0.03%)	2.0	20.0	84

Initial solids (TSS), 2.5%; initial pH, 7.45; temperature, 40°C; shaking, 200 rpm; incubation, 96 h.

observed in the first 6 h, after which reduction continued at a slower rate. The reducing volumes of settled solids indicate that the treatment improved the settleability of sludge solids.

When sewage sludge was treated with individual enzyme preparations (Table 3), solid reductions of 21.4%, 25%, and 32.1% were observed for lipase, cellulase, and alkaline protease, respectively. However, protease had the greatest impact in improving sludge settleability.

The effect of different concentrations of individual enzymes on solid reduction and settleability is presented in Table 4. Slight increases in solid reduction were observed with increasing enzyme concentrations. Even at the lowest treatment rate, proteases had a significant effect on settleability.

Two-enzyme combinations of protease and cellulase produced better solid reduction than individual enzymes and lipase further augmented this effect (Table 5). Again, protease-containing combinations exhibited the greatest degree of solid settleability.

Since the protein content of sludge is altered the most during enzyme digestion and since pH has such a dramatic effect on both protein and enzyme conformation, the effect of enzyme digestion on sludge settleability and solid reduction was evaluated at different pHs. Enzymes were tested in the regions of their known pH optima. The results are presented in Table 6. In almost all cases, the pH of the sludge increased during the digestion period. In general, settleability was poor at low pHs and best at high pHs. The most dramatic reductions in solid content were observed at initial pH values of 3–6, with cellulase, fungal protease, and lipase (46–51%), and at an initial pH of 9 with the alkaline protease, Alcalase. In the latter case, the high solid reduction was accompanied by excellent settleability.

Table 5 Sewage sludge treatment with combinations of enzymes

Enzymes (0.03%)	Final pH	TSS (%)	Percentage olid reduction	Settleability (ml/100 ml)
No enzyme	8.6	2.6	7.1	80
Econase CEP+ Lipase EX	8.5	2.1	25.0	45
Protease L-FG+ Econase CEP	9.5	1.8	35.7	35
Protease L-FG+ Lipase EX	8.5	2.0	28.6	35
Econase CEP+ Lipase EX+ Protease L-FG	9.5	1.7	39.3	35

Initial solids (TSS), 2.8%; enzyme concentration, 0.03% each; initial pH, 8.0; temperature, 40°C, shaking, 200 rpm; incubation, 96h.

Table 6 Effect of enzyme treatment at acidic and alkaline pH on settleability and solid reduction

Enzyme treatment	Initial pH	Final pH	Settleability (ml/100 ml)	TSS (%)	Percentage solid reduction
Original sludge	6.9	–	–	3.9	–
Control (no adjustments)	6.9	8.8	80	3.1	20.5
Sumizyme (cellulase)	3	3.3	84	2.7	30.8
	4	7.8	80	2.8	28.2
	5	7.5	88	2.9	25.6
	6	7.9	92	2.9	25.6
Fungal protease	3	3.4	90	2.7	30.8
	4	6.7	60	2.7	30.8
	5	7.3	82	2.7	30.8
	6	8.3	80	2.9	25.6
Lipase EX	3	3.1	90	2.5	35.9
	4	7.2	74	2.8	28.2
	5	7.4	92	2.8	28.2
	6	8.2	80	2.8	28.2
Sumizyme+ Fungal Protease+ Lipase EX	3	3.6	76	1.9	51.3
	4	5.6	56	2.1	46.2
	5	7.8	94	2.1	46.2
	6	8.2	78	2.0	48.7
Septizyme	3	3.7	90	2.6	33.3
	4	6.1	86	3.0	23.1
	5	7.4	84	2.7	30.8
	6	8.1	70	2.7	30.8
Original sludge	7.8	–	–	2.6	–
Control (no adjustments)	7.8	8.4	56	2.0	23.1
Protease L-FG	7	8.5	44	1.8	30.8
	8	8.2	52	1.6	38.5
	9	9.3	34	1.7	34.6
	9.5	9.5	30	1.7	34.6
	10	9.5	22	1.7	34.6
Esperase	7	8.2	42	1.8	30.8
	8	8.4	40	1.8	30.8
	9	8.9	30	1.7	34.6
	9.5	9.3	26	1.6	38.5
	10	9.5	20	1.6	38.5
Alcalase	7	8.2	42	1.9	26.9
	8	8.3	40	1.9	26.9
	9	9.0	22	1.2	53.8
	9.5	9.2	20	1.6	38.5
	10	9.5	20	1.5	42.3

pH of the sludge was adjusted with 6 N HCl/6 N NaOH and then treated with different enzymes (0.03%) at 40°C for 96 h.

Discussion

These results illustrate the potential to use enzymes to reduce 50% of sewage sludge solids, and the resulting sludge manifested

improved settleability properties. Given that organic matter of sewage sludge solids amounts to approximately 60%, the enzyme treatment has the potential to reduce about 80% of the organic fraction of biosolids.

The greatest percentage reduction among organic components is of proteins. This is consistent with the observation that proteases are most effective in reducing sludge solids and in improving settling. Based on enzyme treatments, it was concluded that polysaccharides, lipopolysaccharides, and protein were important in determining rheological characteristics of sludges [6]. Endogenous peptidases associated with the exocellular polymeric substances (EPS) matrix [7] are likely to be responsible for the weakening of flocs in stored sludges. The characteristic capsular matrix or extracellular fibrils around cells of floc-forming bacteria from activated sludge is susceptible to attack by protease and cellulase and deflocculation was observed after these treatments [12]. Proteases are important in the natural lysis of *Bacillus thuringiensis* subsp. *israelensis* cells during spore formation and release [15].

The removal of protein is thought to weaken the floc, which explains the role of proteases in solid settling [10]. Activated sludge flocs are formed by bacteria, EPS, and inorganic material, driven by the aggregating and adhesion mechanisms between bacterial surface structures [21]. The EPS, which play a key role in binding floc components together, contain carbohydrate and protein as major components together with some lipid, nucleic acids, and humic substance [14]. The main forces involved in these binding functions are van der Waals, hydrophobic, and electrostatic interactions, which are mediated by cationic polymer bridging [19,21]. These surface components are important in determining the characteristic rheological properties of sewage sludge.

Changes in sludge biomass and EPS composition occur during anaerobic storage of sludge. A reduction in the sludge organic fraction is accompanied by degradation of EPS protein and carbohydrate contents [14]. The dominance of protein and its ability to bind flocs and form different matrices is emphasized. Enzymes have been localized in the sludge floc matrix [7]. A large portion of sludge enzyme activity is immobilized by adsorption in the EPS matrix to the extent that exo-enzymes have been considered as an integral part of the EPS matrix. Peptidase was the most dominant enzyme in the matrix [7].

Added enzymes and a biocide destroyed the filamentous bacteria in process water [16]. Proteases attack the sheath surrounding filamentous bacteria. Mixtures of enzymes, including protease, amylase, cellulase, and lipase have been proposed as a means of cleaning sewer lines [8]. Cellulase has been proposed for removal of microbial slime in wastewater treatment plants [19]. Proteases from *Bacillus* species played a major role in lysis of *Escherichia coli* cells [4] and protease and β-1,3-glucanase were important in yeast cell lysis [17]. Bacterial enzyme mixtures have been proposed for reduction of sludge [3,16]. Although some of the above investigations provided promising results in wastewater treatment, the viability of application of the extracellular enzyme blends as a large-scale treatment option was not clear.

The reductions in biosolids of up to 50% observed through use of proteases is consistent with the observation that up to 50% of sewage sludge solids can be biodegraded through composting [5]. The potential benefits related to settleability in laboratory tests need to be confirmed in large-scale biosolid dewatering operations. Industrial enzymes have found their most effective application in the food processing and detergent industries. If we assume an overall enzyme treatment cost of US$3 per cubic meter of 3% biosolids, this corresponds to US$100 per dry ton, which is somewhat high. Part of the cost reduction can be achieved by further optimization of the enzyme step. The ultimate potential for use of enzymes in biosolid management will be driven by the costs of the enzyme and the ability of enzyme producers to tailor the costs to comply with economic constraints of the water processing industry.

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