

African Journal of Biotechnology Vol. 5 (10), pp. 963-967, 16 May 2006
Available online at <http://www.academicjournals.org/AJB>
ISSN 1684-5315 © 2006 Academic Journals

Full Length Research Paper

Enzyme treatment to decrease solids and improve digestion of primary sewage sludge

H.J. Roman, J.E. Burgess and B.I. Pletschke*

Department of Biochemistry, Microbiology and Biotechnology, Rhodes University, PO Box 94, Grahamstown, 6140, South Africa.

Accepted 29 March, 2006

The aim of anaerobic digestion of primary sewage sludge is to convert the carbonaceous material contained in the solids into methane and carbon dioxide. The products of digestion are therefore gases, stabilised sludge solids which are subsequently dewatered and disposed of, and sludge liquor which is generally further treated. This investigation assessed the impact of addition of hydrolytic enzymes to anaerobic digesters. Cellulase and pronase E were added singly and in combination, and it was found that the mixture of the two enzymes resulted in an 80% reduction in solids (cf. 20% in the control), 93% removal of particulate chemical oxygen demand (COD) (59% in the control) and 97% total COD removal (vs. 63%). The total suspended solids (TSS) concentration was reduced by 80%, from 25 g/l to 5 g/l. Single enzymes had little or no impact on sludge solubilisation, and final COD and TSS, but all of the enzyme additions were seen to decrease the production of volatile fatty acids (VFAs). Since accumulation of VFAs can lead to digester failure, it was concluded that the enzyme additives enhanced digester performance in terms of degradation of COD, reduction in sludge solids remaining after digestion and improved digester stability owing to the stable prevailing pH. The results indicate that enzyme addition at full scale could be expected to lead to greater methane yields, lower strength sludge liquors and a significant reduction in the requirements for and costs of digested sludge dewatering and disposal.

Key words: Biosolids, sludge management, sewage sludge, sludge production, dewatering, disintegration.

INTRODUCTION

Wastewater treatment has developed slowly from the use of simple sewage farms to more sophisticated processes, such as activated sludge. However, this change has brought about the production of an increasing volume of sludge, and with changes in legislation with respect to increased removal of carbon and nutrients from water, greater volumes of sludge are being generated in the attempt to produce cleaner wastewater treatment works (WWTW) effluents. For example, the European Union

(EU) currently operates over 40 300 WWTW, producing approx. 6.5 million tonnes of dry solids (DS) per annum. With the introduction of the EU Urban Waste Water Treatment Directive, which imposed certain minimum wastewater treatment standards according to sensitivity of receiving waters, sludge production in the EU had risen to more than 10 million tonnes DS per annum by the end of 2005.

All of this sludge requires treatment. Settled sludges are usually stabilised and thickened by anaerobic digestion prior to their disposal. During digestion, anaerobic bacteria break down organic compounds, converting them through a complex set of processes to methane and carbon dioxide. The possible final disposal

*Corresponding author. E-mail: B.Pletschke@ru.ac.za.

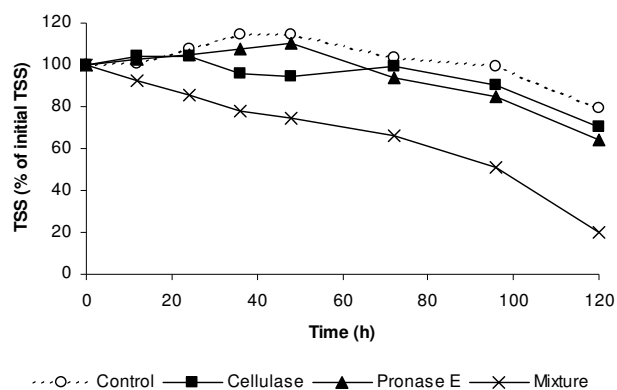


Figure 1. Total suspended solids measured in well mixed digesting sludge with and without enzyme addition (y-axis error bars omitted for figure clarity).

options for digested sludge include use in agriculture, land reclamation and forestry, recycling through composting and minor uses such as building materials, fuels, incineration and disposal to landfill. Local and national geographical, agronomic, economic and sociopolitical factors have some influence on the disposal options available. In recent years, the disposal of sludge has become more, rather than less difficult, and this has been exacerbated by a considerable increase in waste and environmental legislation. The methods of sludge disposal currently create processing, transport and disposal costs of up to 65% of the total cost of operating a WWTW (Lui, 2003). Sludge management is thus often regarded as a major problem of water pollution control technology. The cost of sludge disposal is usually calculated per unit volume, hence a reduction in the volume which must be disposed of can represent a significant cost saving. Sludge production throughout the world is ever increasing, while the environmental quality requirements for sludge are becoming increasingly stringent, disposal outlets are decreasing and economic pressures require low-cost solutions to sludge disposal problems.

As with all wastes, the hierarchy of actions reads "reduce, reuse, recycle". While significant effort has been spent on the reuse and recycle routes, less research has been carried out on the reduction of sludge volume generated. A recent review of this field is Ramakrishna and Viraraghavan (2005), and an excellent historical review is Low and Chase (1999). Briefly, attention has been paid to reduction at the sludge source by the use of metabolic uncouplers in secondary wastewater treatment processes (Ye and Li, 2005), enhancing cell lysis (Yasui et al., 1996), biomass grazing by predatory organisms (Lapinski and Tunnacliffe, 2003), application of bacteriophages (Withey et al., 2005) and increasing the energetic requirements of cell maintenance (Hamoda and

Al-attar, 1995). These methods all address the reduction of the biomass component of the DS. However, since primary sludge accounts for 60% of the sludge produced at a WWTW, and 70-80% of the primary DS is organic matter which is not biomass, another approach is to attempt to decrease the mass of the organic solids which are not readily broken down by anaerobic digester bacteria. To this end, the objective of this study was to investigate the effects of commercially available enzymes in solubilising the organic, bacterially undegraded component of digested sludge solids. The enzymes used were cellulase, pronase E, and a combination of both. These enzymes were chosen to target the undegraded toilet tissue paper, protein and dietary fibre (mainly cellulose) components of primary sludge DS.

MATERIALS AND METHODS

Anaerobic sludge was obtained from the methanogenic digesters of Grahamstown municipal WWTW in Eastern Cape, South Africa.

For enzyme treatment experiments, 500 ml aliquots of well mixed sludge were placed in 1000 ml Erlenmeyer flasks. To standardise the reactors, the total suspended solids (TSS) concentration of the initial sludge was measured and the same amount of TSS used in each flask as far as possible. Fluka brand cellulase from *Aspergillus* sp. (CAS number 9012-54-8) and pronase E (protease from *Streptomyces griseus*, CAS number 9036-06-0) were both obtained from Sigma-Aldrich Ltd. After addition of 0.03% of enzyme solution (150 μ l), flasks were incubated on a rotary shaker (200 rpm) at 40°C for 120 h. Control flasks of sludge without any enzyme, but with 150 μ l deionised water added to keep the volumes constant were run in parallel with each experiment. Samples were taken at the following times: 0, 12, 24, 36, 48, 72, 96, 120 h. The samples were analysed to determine their TSS to check for solids reduction, pH, total volatile fatty acids (VFAs) to assess the progress of the degradation, and total, soluble and particulate COD (COD_T, COD_S and COD_P) to determine the partitioning of the organic material present.

The concentrations of TSS and VFAs were measured according to Standard Methods (APHA et al., 1999). The COD determinations were carried out using a spectrophotometric test kit (Merck Spectroquant test 14679) which is analogous to Standard Method number 5220D. The pH was measured using a portable electrode (CyberScan 2500, Eutech Instruments, Singapore).

RESULTS AND DISCUSSION

Total suspended solids reduction

The TSS in all of the digesters was reduced during the digestion process. Figure 1 shows that the sludge treated with a mixture of cellulase and pronase E displayed an 80% reduction in solids, the TSS of the sludges treated with pronase E and cellulase were reduced by 36% and 29%, respectively, and the control sludge TSS was reduced by 20% after 5 days' digestion. Paired two sample t-tests performed for mean values at 95% confidence limits showed that all three enzyme

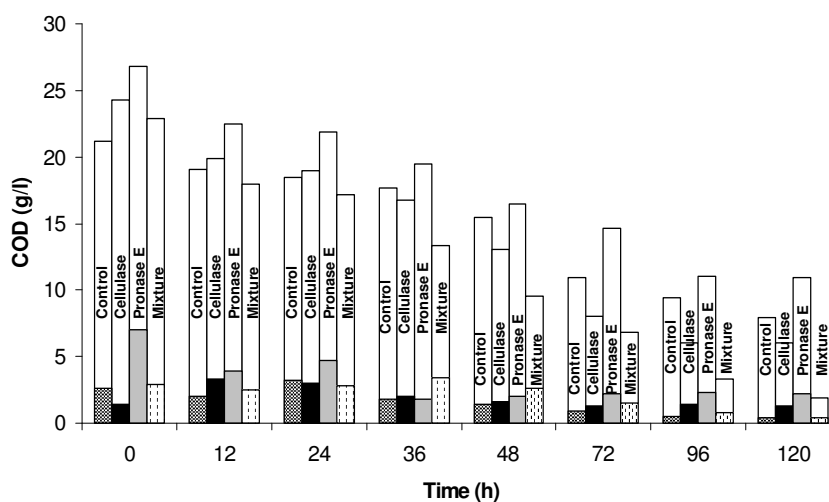


Figure 2. Total bar height indicates COD_T. White upper sections indicate COD_P and shaded lower areas indicate COD_S.

treatments resulted in statistically significant greater TSS reduction than was observed in the control ($t=10.10$ for control vs. cellulase; $t=5.95$ for control vs. pronase E and $t=3.08$ for control vs. mixture). Paired t -tests also showed that the TSS reduction obtained using the enzyme mixture was significantly higher than using either enzyme alone ($t=2.36$ for cellulase vs. mixture and $t=2.01$ for pronase E vs. mixture).

The TSS reduction figures are comparable with those reported in the literature. Parmar et al. (2001) found that addition of a mixture of protease, lipase and hemicellulase to anaerobically digesting sludge resulted in 29% reduction in TSS in 96 h. The TSS reduction observed in sludge with the enzyme mixture indicated enhanced solubilisation of solids, as the reduction figure (80% solids removal with HRT = 5 d) was higher than in the control (20%, same conditions), and higher than data in the literature (e.g. 13% solids removal with HRT = 40 d (Bolzonella et al., 2005)). Ayol (2005) also tested the addition of a commercially available additive named Enviro-Zyme 216, which contained a mixture of micro-organisms and enzymes including lipase, protease and hydrolytic enzymes, to municipal sludge, and observed between 14 and 45% greater solids. The results presented here demonstrate that enzyme addition improved solids reduction.

The significance of the results arises because anaerobic digestion generally consists of four stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The biochemical processes generally require long sludge retention times and show low removal efficiencies of organic carbon. The first stage (hydrolysis) is the rate-limiting step in anaerobic digestion (Watson et al., 2004).

To minimise the effect of this rate-limiting step, enzyme treatment can accelerate the solubilisation of the sludge solids, thus improving anaerobic digestion (Tiehm et al., 2001). Breakdown of large, organic particulate matter into smaller particles increases the surface area available for contact with the bacteria responsible for degradation. In addition, disruption of the floc structure of the sludge itself releases associated dissolved organic material entrapped in the floc matrix and renders it bioavailable, thus enhancing the utilisation of the organic matter as the substrate for the anaerobic bacteria. Physical disintegration of sludge solids in order to reduce particle size has also been shown to improve the digestion process, as indicated by increased biogas production (Onyeché et al., 2002) and COD removal. Solids reduction therefore increases the yield of methane, decreases the residual COD in sludge liquor and reduces the volume of solids requiring dewatering and disposal.

COD

The concentrations of COD_S and COD_T were measured and used to calculate COD_P. Figure 2 illustrates that the reductions seen in the TSS values were echoed in the COD_T concentrations. However, the control sludge achieved 59% COD_P removal, while the sludges treated with cellulase and pronase E only attained 45 and 21% removal of COD_P, respectively. This was not expected. Conversely, sludge treated with the enzyme mixture showed 93% COD_P removal. The COD_S removal was also improved by the addition of the enzyme mixture, but not by the addition of single enzymes. As the levels of

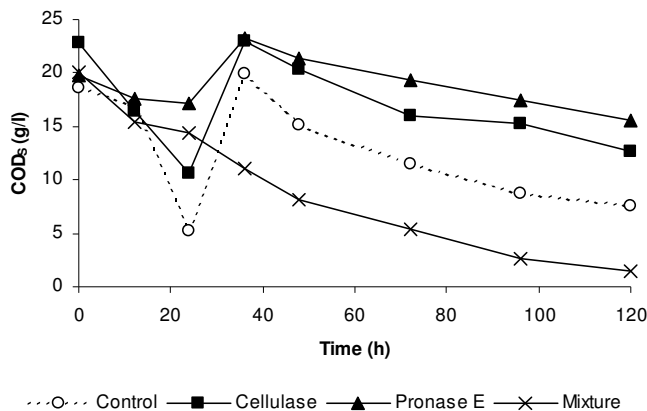


Figure 3. Initial increases in COD_S followed by subsequent decreases were indicative of rapid solubilisation of COD_P .

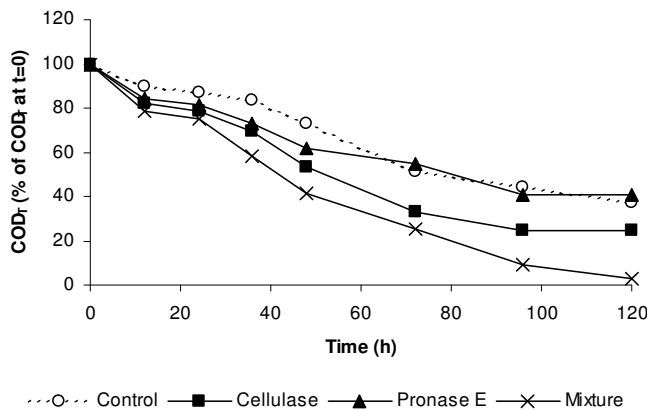


Figure 4. Decreasing COD_T during digestion. Data normalised against each digester's starting concentration of COD_T .

COD_S increased and then decreased, and COD_P concentration decreased, the efficiency of anaerobic digestion was also expected to improve.

Initial increases in COD_S (Figure 3) indicated that organic particles were being solubilised. Subsequent decreases in COD_T (Figure 4) and COD_S demonstrated that the digestion process and degradation of the organic material present was improved by the mixture of enzymes, whereas the single enzyme additives did not perform significantly better than the control, as indicated by the initial and final concentrations of the COD fractions in each digester (Table 1).

Previous authors have reported similar increases in COD_S ; Scheidat et al. (1997) observed 15–40% increases in COD_S effected by the addition of cellulases, peptidases and carbohydrases to sludge digested at 38°C. The pattern of increased followed by decreased COD_S and decreased final COD_T suggested that the complex substances in the sludge were first solubilised

into readily biodegradable dissolved substances, which were then converted into methane. Thus, the solubilisation of sludge by hydrolytic enzymes plays an important role in enhancing anaerobic digestion and the mineralisation of organic substances. This theory would be supported by the observation of increases in the amounts of VFA produced.

Acid production and pH

The reduction in TSS during digestion is approximately equal to the total amount of VFA converted from the volatile solids. Dissolved solids are produced from the hydrolysis of TSS, and the VFA is converted to methane gas. Reduction in the TSS can be expressed as the sum of the residual VFA and the methane gas produced from the anaerobic digester. Therefore, the hydrolysis of particulate organics has a significant influence on the production of VFAs.

However, the final pH of the sludges was found to be raised from 7.62 in the control to 7.69, 7.82 and 8.22 by the addition of cellulase, pronase E and mixed enzymes, respectively. This was linked to a reduction in the concentration of VFAs accumulated in the sludge (Figure 5). This trend contradicted the expectations that greater bioavailability of soluble organic substances would result in higher VFA concentrations. The data also contradict results reported by Park et al. (2005), who found that enhanced anaerobic digestion resulted in higher VFA production. However, the VFAs produced in digestion are generally utilised by the methanogenic bacteria, so it is possible that a portion of the VFAs produced were utilised and did not remain available for measurement. Song et al. (2004) also reported that VFA concentrations decreased in line with COD_S during mesophilic sludge digestion and stoichiometrically linked their VFA concentrations to an increase in methane production.

Measurements of gas production would be recommended for future study to enable the same comparison to be made here. Reductions in VFA concentrations prevailing in the sludge are an operational advantage, as methanogenesis consumes VFAs. Moreover, many anaerobic digesters suffer from long term VFA accumulation which eventually decrease the pH to less than 6.5 and inhibit complete stabilisation of the solids.

Conclusion

In general, the trends of stable prevailing pH, low VFA concentrations and high TSS removal observed here indicate improved performance through the symbiotic relationship between acid producers and consumers. This involves close microbial consortia proximity to

Table 1. Concentrations of COD fractions at the beginning and end of digestion.

Enzyme(s)	[COD _r] (g/l)		[COD _s] (g/l)		[COD _p] (g/l)	
	t = 0 h	t = 120 h	t = 0 h	t = 120 h	t = 0 h	t = 120 h
Control	21.22	7.91	2.650	0.38	18.57	7.60
Cellulase	24.23	6.00	1.40	1.26	22.83	12.66
Pronase E	26.80	10.90	7.05	2.25	19.75	15.54
Mixture	22.90	0.70	2.88	0.44	20.03	1.50

enhance interspecies hydrogen transfer, even at low H⁺ concentrations, improving the thermodynamic performance of digestion. Removal of the rate-limiting effects of hydrolysis allowed the digesters to display enhanced TSS and COD removal and improved operational conditions (prevailing pH and VFAs). The significance of the results lies in their demonstration of the potential of a biochemical means of reducing the volumes of sludge solids to be dewatered/dried and disposed of (and hence disposal costs in terms of finance and land area), increasing the production of methane which can be used in combined heat and power plants as a renewable energy source and the overall improved transfer of carbonaceous material remaining in both the solid and aqueous phases (where it requires further treatment) to the gaseous phase, where it represents a resource.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to the National Research Foundation of South Africa.

REFERENCES

- American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF) (1999). Standard Methods for the Examination of Water and Wastewater. 20th edn, Washington D.C., USA. ISBN: 0875532357.
- Ayol A (2005). Enzymatic treatment effects on dewaterability of anaerobically digested biosolids-I: performance evaluations. *Proc. Biochem.* 40(7): 2427-2434.
- Bolzonella D, Pavan P, Battistoni P, Cecchi F (2005). Mesophilic anaerobic digestion of waste activated sludge: influence of the solid retention time in the wastewater treatment process. *Proc. Biochem.* 40(3-4): 1453-1460.
- Hamoda MF and Al-attar IMS (1995). Effects of high sodium chloride concentrations on activated sludge treatment. *Water Sci. Technol.* 31(9): 61-72.
- Lapinski J, Tunnacliffe A (2003). Reduction of suspended biomass in municipal wastewater using bdelloid rotifers. *Water Res.* 37: 2027-2034.
- Low EW, Chase HA (1999). Reducing production of excess biomass during wastewater treatment. *Water Res.* 33: 1119-1132.
- Lui Y (2003). Chemically reduced excess sludge production in the activated sludge process. *Chemosphere* 50: 1-7.
- Onyeche TI, Schläfer O, Bormann H, Schröder C, Sievers M (2002). Ultrasonic cell disruption of stabilised sludge with subsequent anaerobic digestion. *Ultrasonics* 40: 31-35.
- Park C, Lee C, Kim S, Chen Y, Chase HA (2005). Upgrading of anaerobic digestion by incorporating two different hydrolysis processes. *J. Biosci. Bioeng.* 100(2): 164-167.
- Parmar N, Singh A, Ward OP (2001). Enzyme treatment to reduce solids and improve settling of sewage sludge. *J. Ind. Microbiol. Biotechnol* 26: 383-386.
- Ramakrishna DM, Viraraghavan T (2005). Strategies for sludge minimization in activated sludge process - a review. *Fresenius Environ. Bull.* 14(1): 2-12.
- Scheidat D, Kasche V, Sekoulov I (1997). Solubilisation of primary sludge by addition of hydrolytic enzymes. *Technische Universität Hamburg-Harburg. Biotechnologie, Biotransformation und Biosensorik.*
- Song YC, Kwon SJ, Woo JH (2004). Mesophilic and thermophilic temperature co-phase anaerobic digestion compared with single-stage mesophilic- and thermophilic digestion of sewage sludge. *Water Res.* 38: 1653-1662.
- Tiehm A, Nickel K, Zellhorn M, Neis U (2001) Ultrasonic waste activated sludge disintegration for improving anaerobic stabilization, *Water Res.* 35: 2003-2009.
- Watson SD, Akhurst T, Whiteley CG, Rose PD, Pletschke BI (2004). Primary sludge floc degradation is accelerated under biosulphidogenic conditions: enzymological aspects. *Enzyme Microbial Technol.* 34(6): 595-602.
- Withy S, Cartmell E, Avery LM, Stephenson T (2005). Bacteriophages - potential for application in wastewater treatment processes. *Sci. Tot. Environ.* 339(1-3): 1-18.
- Yasui H, Nakamura K, Sakuma S, Iwasaki M and Sakai Y (1996). A full-scale operation of a novel activated sludge process without excess sludge production. *Water Sci. Technol.* 34(3): 395-404.
- Ye FX, Li Y (2005). Reduction of excess sludge production by 3,3',4', 5-tetrachlorosalicylanilide in an activated sludge process. *Appl. Microbiol. Biotechnol.* 67(2): 269-274.