Contents lists available at ScienceDirect

Process Safety and Environmental Protection

journal homepage: www.elsevier.com/locate/psep

Investigation of biocatalytic potential of garbage enzyme and its influence on stabilization of industrial waste activated sludge

C. Arun, P. Sivashanmugam*

Department of Chemical Engineering, National Institute of Technology, Tiruchirappalli 620015, Tamil Nadu, India

ABSTRACT

The decomposable waste thrown into the environment can be used to produce value added bio-product which in turn reduces the production of greenhouse gas. Garbage enzyme is one such value added product produced by fermentation of organic solid waste. In the present study enzyme activity and disinfectant potential of garbage enzyme was evaluated and its influence on reduction of total solids, suspended solids and pathogens in dairy waste activated sludge were studied. The result showed the garbage enzyme possesses protease, amylase and lipase activity and reduced 37.2% of total solids, 38.6% of suspended solids and 99% of pathogens in dairy waste activated sludge. This significant result may be helpful for researchers to compare the effectiveness of earth-friendly garbage enzyme treatment of industrial sludge with various physical and chemical pre-treatment methods to improve the biogas production from the sludge digestion unit.

Keywords: Decomposable waste; Fermentation; Value added product; Garbage enzyme; Disinfectant; Waste activated sludge

1. Introduction

All over the world food processing industries increased rapidly due to increasing human population and their food consumption. Such industries have one of the highest consumptions of water and the biggest producers of effluents; in addition, these industries generate a huge volume of sludge. Rapid increases in production of solid waste can cause health risks to human, animal and plant. Among many food processing industries, the dairy industry is responsible for the release of huge quantities of wastewater, approximately thousands of cubic metres/day (Abbasnejad et al., 2002). The relatively high concentrations of organic matter contained in dairy wastewater have been associated with number of pollution issues (Perle et al., 1995). Dairy industries produced waste effluents are normally treated by activated sludge (aeration) process, in turn, it produce large amount of waste activated sludge (WAS) from the secondary sedimentation tank. Dairy sludge can be particularly odorous because it is rich in poorly stabilized organic matter, low carbon to nitrogen ratio and it can cause nuisances during storage and land spreading. Hence, the waste sludge needs to be alleviated necessarily to reduce odour, organic content, and pathogen before disposing and further utilization. Sludge is disposed usually by landfilling and incineration. The disposal of dairy sludge by landfill is not good practice because its high nitrogen content creates the risk of nitrate contamination of groundwater. Incineration of dairy sludge is not an attractive option as it releases carbon and nitrogen oxides into the atmosphere. Recycling of sludge is the only alternate eco-friendly method to create a sustainable environment. In general recycling of dairy waste activated sludge is done by composting or by anaerobic digestion. These recycling of sludge could be enhanced by stabilization and by various pre-treatment methods (Beszédes et al., 2012; Uma Rani et al., 2012; Yang et al., 2013).

The sludge stabilization method aims to reduce organic ingredients and improves hygiene by reducing the pathogen in them. Stabilization can be achieved by a biological, chemical (lime addition) or thermal process. Chemical and thermal stabilization methods are expensive, producing operator handling problems and generate fatal air pollution. This requires the selection of the correct method focusing on efficient, environmentally safe treatment and disposal. According to Im et al. (2001) the biological methods are usually preferred over the physical and chemical methods in removing the majority of pollutants.

* *Corresponding author*. Tel.: +91 431 2503106; fax: +91 431 2500133.
E-mail addresses: lrcarun@gmail.com (C. Arun), psiva@nitt.edu (P. Sivashanmugam).
Received 24 May 2014; Received in revised form 4 September 2014; Accepted 15 October 2014
Available online 24 October 2014
http://dx.doi.org/10.1016/j.psep.2014.10.008

In biological method microbial hydrolytic enzymes play important role in dewatering and reduction of solids content of sludge by reducing the organic compounds, remove pathogenic organisms and odour. Thus the biological method improves the stability of sludge for further utilization or disposal (Godfrey and West, 1996; Ayol and Dentel, 2005; Roman et al., 2006). Direct addition of microorganisms for stabilization will contribute to a large amount of biomass, which increases the sludge volume; instead it can be reduced by adding enzyme directly, which is responsible for the degradation (Parmar et al., 2001b; Wawrzyńczyk et al., 2007). Parmar et al. (2001a) stated that treatment of sewage sludge with the addition of alkaline protease along with lipase and cellulase at 50 °C showed beneficial effects in pathogen reduction. Dean and Ward (1991) reported alkaline protease from *Bacillus* sp. is responsible for the lysis of *E. coli* cell wall. Researchers till now used commercial hydrolytic enzymes for sludge stabilization, but purchasing such enzyme for the treatment are not economical. So there is a need to find an alternative cheap source of enzymes available throughout the year. Enzymes are generally produced from animals, plants or microbes. Among them enzymes from plant source is relatively cheaper and have easier extraction and purification step.

Restaurants, vegetable markets, fruit markets and food processing industries produce decomposable waste such as fruits, vegetables and its peels, etc. in huge quantities. Management of these organic waste is currently a major issue all over the world. The disposal of these decomposable wastes either in the landfill or by composting produces greenhouse gases like methane and nitrous oxides. Hence the decomposable waste which is thrown into the environment can be used to produce value added bio-product which in turn reduces the production of greenhouse gas from it. One of such products was developed by researcher Dr. Rosukon from Thailand using organic solid waste in the year 2006 and named the solution obtained as garbage enzyme. This enzyme is a complex organic substance of protein chains (enzyme), organic acids and mineral salts produced easily by fermentation of waste fruits, vegetables or its peels, sugar (brown sugar or molasses sugar) and water. The garbage enzyme functions similarly to enzymes in achieving a high degree of degradation within a shorter time. Researchers suggested that this enzyme can function in four categories: decompose, compose, transforms and catalysis (Joean oon, 2008). It can be utilized as a low-cost alternative to improve wastewater treatment processes through the removal of impurities, harmful sludge and bacteria, which in turn promotes recycling of waste back into the earth (Bhavani Prakash, 2011). Nazim and Meera (2013) produced garbage enzyme for treatment of synthetic grey water using 5 and 10% garbage enzyme solution. They also characterized the environmental properties of garbage enzyme alone. Till now no work has been reported on characterization of biocatalytic property (i.e. enzyme property), antimicrobial and disinfectant property of garbage enzyme. And also, no work has been reported on using garbage enzyme in sludge stabilization process.

The prime objective of this study is to analyze the biocatalytic activity of garbage enzyme by changing the pH of garbage enzyme produced by fermentation of fruit peels, vegetable dregs, molasses and water. Successively antimicrobial potential of garbage enzymes on four major pathogenic microorganisms *Escherichia coli*, *Salmonella* sp., *Staphylococcus aureus*, *Candida albicans* were studied and the phenol coefficient of garbage enzyme were determined to examine the disinfectant potential. Subsequently the effect of garbage enzyme to stabilize the dairy industry waste activated sludge obtained from the milk processing unit was investigated.

2. Materials and methods

2.1. Production of garbage enzyme and characterization

Molasses from sugarcane processing industry, vegetables and fruit dregs from vegetable market and fruit shop respectively were collected. In this study tomato, cauliflower, pineapple, orange and mango dregs were taken and equal gram of each waste mixed, from that mixture 3 parts of waste were taken and mixed with 1 part of molasses and 10 parts of water in airtight containers (Joean oon, 2008). The container was placed in a cool, dry and well-ventilated area for complete degradation of organic matter; the fermentation was conducted for three months. After three months the solution was filtered and characteristics of pure garbage enzyme solution were analyzed.

The parameters like pH, total solids (TS), TDS (total dissolved solids), BOD (biological oxygen demand), COD (chemical oxygen demand) and MPN (Most probable number) were analyzed as per procedures in standard methods (APHA, 2005). Lowry protein assay (Lowry et al., 1951) was used for quantitative determination of protein concentration in garbage enzyme. In this tyrosine in protein was allowed to react with Folin's Ciocalteau reagent and the $CuSO_4$ solution to produce a blue colour with absorption maximum around 620 nm. The concentration of protein was estimated by referring to a standard curve obtained at the same time using a known concentration of bovine serum albumin.

2.2. Biocatalytic activity of garbage enzyme

The organic molecules like proteins, carbohydrates and lipids commonly found in larger quantity in sludge, can be degraded by enzyme protease, amylase and lipase respectively. Hence the protease, amylase and lipase activity in garbage enzyme were determined.

2.2.1. Proteolytic activity

Proteolytic activity was determined according to the method of Tsuchida et al. (1986) by using casein as a substrate. Casein Digestion Unit (CDU) is the amount of enzyme which produces 1 μg of tyrosine per minute in a 1% solution of casein. Casein (25 ml) was treated with an enzyme solution (3 ml) in 1 M sodium phosphate buffer for 15 min and the reaction was stopped by the addition of 5% trichloroacetic acid (TCA) (25 ml). The precipitated material from each reaction mixture was removed by centrifugation and the supernatant was assayed by Lowry's method (Lowry et al., 1951). The garbage enzyme obtained after three months of fermentation was filtered, centrifuged and stored in refrigerator. 5 numbers of 100 ml conical flasks were taken and to them 10 ml of garbage enzyme was added. Among them the pH was adjusted to 6, 7, 7.5, 8 using sodium phosphate buffer in four conical flasks and only one conical flask (pH 3.6) was not adjusted. An appropriate solution was prepared and absorbance values at 620 nm were measured with reference to the blank using spectrophotometer (Model: Spectroquant® Pharo 300 UV/VIS spectrophotometer, Make: Merck). The absorbance values were correlated to calculate the specific activity of the protease using casein as substrate. The specific activity is nothing but the activity of protease per milligram of protein per minute.

$$\text{Proteolytic activity} = (\text{Absorbance at } 620\,\text{nm}) \times (\text{protein concentration})^{-1} \times (\text{min}^{-1}) \quad (1)$$

2.2.2. Amylase activity

Amylase activity was determined by 3,5-dinitrosalicylic acid (DNS) method suggested by Miller (Bezerra et al., 2006). The garbage enzyme obtained after three months of fermentation was filtered, centrifuged and stored in refrigerator.

5 numbers of 100 ml conical flasks were taken and to them 10 ml of garbage enzyme was added. Among them the pH was adjusted to 6, 6.5, 7, 7.5, 8 using sodium phosphate buffer in four conical flasks and only one conical flask (pH 3.6) was not adjusted. 0.5 ml of 1% starch was incubated with 0.5 ml of garbage enzyme with 1 ml of phosphate buffer. The reaction mixture was incubated for 10 min. Reaction was stopped by the addition of 0.5 ml DNS colour reagent, incubated in a water bath for 30 min. The reaction mixer was cooled and 2.5 ml of distilled water was added. The absorbance was read at 540 nm with the help of a spectrophotometer against maltose as the standard. One unit of enzyme activity is estimated as the amount of enzyme which releases 1 μmol of reducing sugar as maltose per minute, under the assay conditions.

$$\text{Amylase acticity} = \frac{\text{mg of maltose released} \times \text{Dilution factor}}{\text{Volume of garbage enzyme}} \quad (2)$$

2.2.3. *Lipase activity*

The garbage enzyme obtained after three months of fermentation was filtered, centrifuged and stored in refrigerator. 5 numbers of 100 ml conical flasks were taken and to them 10 ml of garbage enzyme was added. Among them the pH was adjusted to 6, 6.5, 7, 7.5, 8 using sodium phosphate buffer in four conical flasks and only one conical flask (pH 3.6) was not adjusted. Lipase activity was determined by a titrimetric method (Pinsirodom and Parkin, 2001). 2.50 ml of ultra-pure water, 1 ml of Tris HCl buffer and 3 ml of olive oil were taken in blank and test conical flask and 1 ml of the garbage enzyme solution was added to test flask alone. Both the test and blank solution were mixed well and incubated at 37 °C for 15 min. After that 3 ml of 95% ethanol solution and 3–4 drops of thymolphthalein indicator were added to both test and blank solution. Both the test and blank solution were titrated with NaOH till the appearance of light blue colour. Under the assay conditions, one unit of lipase activity was estimated as the amount of enzyme which releases 1 μmol of fatty acids per minute.

$$\text{Lipase activity} = \frac{(\text{Volume of NaOH used for test} - \text{Volume of NaOH used for blank}) \times \text{Dilution factor}}{\text{Volume of garbage enzyme}} \quad (3)$$

2.3. *Medium preparation*

28.0 g nutrient agar (NA-Himedia) medium for bacteria and 39.0 g potato dextrose agar (PDA-Himedia) medium for fungi were taken in two different conical flask containing 1000 ml distilled water. The two conical flasks were heated and to dissolve the medium completely using magnetic heating plate. The conical flask with medium, petri plates are sterilized using autoclave at 15 lb pressures for 15 min. Few minutes later well mixed sterilized medium in conical flask was poured into sterile petri plates in the laminar flow hood to maintain sterile conditions and then petri plates were stored in the incubator.

2.4. *Antimicrobial assay*

The antimicrobial potential of garbage enzyme was investigated to confirm the pathogen killing/inhibiting property of garbage enzyme. Antimicrobial assay was done by disc diffusion method (NCCLS, 1993) using garbage enzymes (5, 10 and 15%). Petri plates were prepared by pouring 30 ml of NA or PDA medium for bacteria or fungi respectively. The test organism was inoculated on a solidified agar plate with the help of micropipette, spread and allowed to dry for 10 min. The surfaces of medium were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab was immersed into a standardized bacterial or fungi test suspension and used to evenly inoculate.

S. aureus (Gram positive), *E. coli* (Gram negative), *Salmonella typhi* (Gram negative) and *C. albicans* were used as test microorganisms in this study and these were obtained from the Microbiology Laboratory of the Thanjavur Medical College Hospital, Thanjavur, India.

Inoculums containing *E. coli*, *S. aureus* and *S. typhi* were spread on nutrient agar plates and *C. albicans* was spread on potato dextrose agar. In this process control standard used to compare the test solution was Chloramphenicol (25 mg/ml distilled water – 30 μl) for bacteria and Nystatin (25 mg/ml distilled water – 30 μl) for fungi.

Whattman filter paper (No: 1) was used to prepare discs approximately 6 mm in diameter, and these were placed in hot air for sterilization. After sterilization, the discs were loaded with control standards and 50 μl, 100 μl and 150 μl of garbage enzyme (5, 10 and 15% with pH adjusted to 7 and without adjusting the pH 3.6). Prepared disc was kept under refrigeration for 24 h. Using sterile forceps, the above prepared sterile filter papers (6 mm diameter) containing the garbage enzyme and standard were laid down on the surface of the inoculated agar plate. The plates were incubated at 37 °C for 24 h for the bacteria and at room temperature for 48 h for fungi strains. Each sample was tested in triplicate.

2.5. *Determination of phenol coefficient*

The phenol coefficient is the value obtained by dividing the highest dilution of the test solution (garbage enzyme) by the highest dilution of phenol that sterilizes the given culture of bacteria under standard conditions of time and temperature (Reybrouck, 1998; Gardner and Peel, 1991). In this test procedure the phenol was diluted from 1:100 to 1:120 and the garbage enzyme were diluted from 1:100 to 1:500. Their bactericidal activity was determined against *S. typhi* suspension. Subcultures were performed from both garbage enzyme solution and phenol at intervals of 2 min. The antimicrobial potential of garbage enzyme was determined on the basis of mean diameter of zone of inhibition around the disc in millimetres. The zones of inhibition of the test microorganisms in the presence of garbage enzyme were measured using a millimetre scale.

2.6. *Sludge sampling and characterization*

The waste activated sludge was obtained from a dairy processing plant at Trichy in Tamil Nadu (India). Samples were collected and stored in refrigerator at 4 °C. The characteristics of the raw sludge like pH, TS, volatile solids (VS), suspended solids (SS), COD and MPN were analyzed as per procedures in standard methods (APHA, 2005), total proteins

Table 1 – Characteristic of dairy waste activated sludge.

Parameters	Value
pH	6.8
Total solids	8753 mg/l
Volatile solids	5380 mg/l
Suspended solids	4843 mg/l
Total COD	21,321 mg/l
Soluble COD	520 mg/l
Total protein	810 mg/l
Carbohydrates	340 mg/l
MPN (CFU/100 ml)	9.4×10^7

by Lowry method and carbohydrates by the phenol sulphuric acid method were analyzed and tabulated in Table 1.

2.7. *Stabilization of sludge using garbage enzymes*

The sludge stabilization method aims to reduce organic ingredients and improves hygiene by reducing the pathogen in them. Solid reduction is the major parameter which reveals the stability of sludge and same is used widely for analysing the effectiveness of a sludge stabilization method (Uma Rani et al., 2012). Therefore the reduction of TS, SS and the pathogen are measured to identify the performance of garbage enzymes on industrial sludge.

Different concentration of garbage enzyme 5, 10, 15 and 20% were prepared and the pH of the solutions was adjusted to 7 with sodium phosphate buffer. 90 g of well mixed dairy waste activated sludge were taken in a beaker and 50 ml of garbage enzyme solution was added into the same beaker. After addition the beaker was incubated on an orbital shaker for 5 days at 37 °C (250 rpm). Simultaneously in another beaker 90 g of sludge and 50 ml of distilled water were added and kept in orbital shaker as a blank (untreated sludge). At regular time intervals (12 h) parameters like TS, TSS and MPN were estimated and the experiments were repeated to determine the consistency in the result determined.

3. Results and discussion

3.1. *Garbage enzyme composition*

Garbage enzyme was prepared using 3 parts of waste mixture, 1 part of molasses and 10 parts of water in airtight containers. After three months, the solution was filtered and separated from solid residues. The solution obtained was centrifuged for 30 min with 3000 rpm. The supernatant was separated and used as the garbage enzyme source. The characteristics of the garbage enzyme solution obtained after three months of fermentation were analyzed and is shown in Table 2.

During fermentation, carbohydrates were converted into volatile acids and in addition, organic acids present in waste material also leached out into fermented solution since the pH of garbage enzyme was acidic in nature.

Table 2 – Characteristic of garbage enzymes.

Parameters	Value
pH	3.6
TDS	1040 mg/l
BOD	79 mg/l
COD	158 mg/l
Total protein	42 mg/l
MPN (CFU/ml)	<3

Nazim and Meera (2013) produced garbage enzyme using the simple fermentation of fresh vegetable waste, brown sugar and water for two months and reported TDS as 1120 mg/l, BOD as 92.6 mg/l, and COD as 186 mg/l. In the present study fermentation was conducted for three months using molasses (waste product from sugar factory) instead of the jaggery (brown sugar). The result obtained showed that the value of TDS as 1040 mg/l, BOD as 79 mg/l and COD as 158 mg/l of the garbage enzyme solutions were reduced significantly when compared with results shown by Nazim and Meera (2013) because the microbes present in the molasses also helps to degrade the complex organic matter present in the organic waste.

3.2. *Determination of biocatalytic activity in garbage enzyme solution*

3.2.1. *Protease activity*

Proteolytic activity of garbage enzyme (pH 3.6, 6, 7, 7.5, 8) was determined using casein as a substrate and the result obtained is presented in Fig. 1a. From Fig. 1a it is observed that the protease specific activity is higher for garbage enzyme with pH 6.5 and lower for garbage enzyme with pH 3.6. In general the optimal pH for protease will be within the range pH 6–7. The catalytic ability of the protease is so tightly linked to the specific shape and chemical properties of its active site; alteration of normal ionic bonding patterns within the protein tends to reduce catalytic function. Because of this reason the protease activity of garbage enzyme is higher at pH 6.5 and lower at pH 3.6. This result indicates that to achieve higher proteolytic activity the pH of the garbage enzyme solution should be maintained between the ranges 6.5 and 7.

3.2.2. *Lipase activity*

A garbage enzyme with various pH (3.6, 6, 6.5, 7, 7.5, 8) were used to investigate lipase activity. Commonly the maximum lipase activity was obtained at pH 8 and decreased significantly when pH was increased from 8 to 12 (Shu et al., 2006). In the present study lipase activities were determined using olive oil as substrate and the analyzed result is shown in Fig. 1b. From Fig. 1b it is observed that the lipase activity is gradually increasing from pH 3.6 to 8, because the optimal pH range for most of the lipase falls within the range 7–10 (Shu et al., 2006). The observed result shows that the higher lipolytic activity of the garbage enzyme solution can be achieve by maintaining the pH between the ranges 7 and 8.

3.2.3. *Amylase activity*

Amylase activity was considerably decreased at low acidic as well as at high basic pH while studying the effect of pH on amylase activity (Smitha, 2010). In the present study a garbage enzyme with various pH (pH 3.6, 6, 6.5, 7, 7.5 and 8) were used to determine the amylase activities using starch as substrate. Fig. 1c reveals that the amylase activity is higher for garbage enzyme with pH 6.5 and lower for garbage enzyme with pH 3.6. This is because commonly the catalytic property of amylase was higher at pH range 6–7. The observed result signifies that to achieve higher amylase activity the pH of garbage enzyme should be maintained between 6 and 6.5.

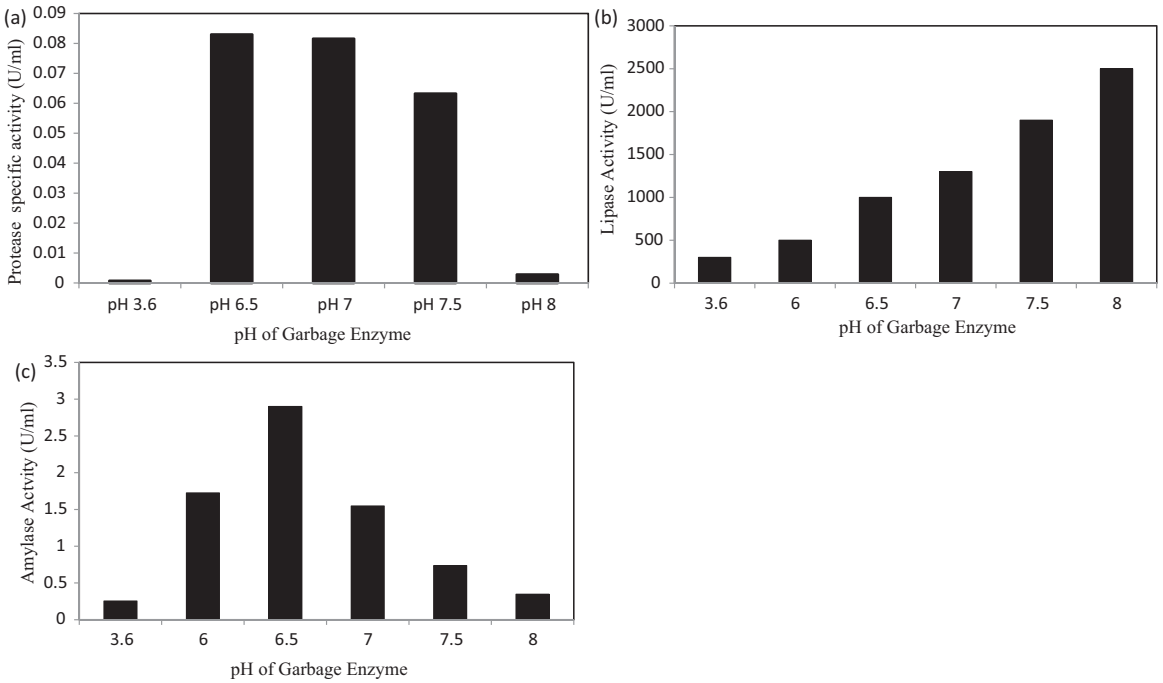

Fig. 1 – (a) Determination of protease activity present in garbage enzyme with different pH (3.6, 6.5, 7, 7.5, 8). Determination of (b) lipase and (c) amylase activity present in garbage enzyme with different pH (3.6, 6, 6.5, 7, 7.5, 8).

3.3. *Antimicrobial potential of garbage enzyme*

The antimicrobial potential of garbage enzyme for the four microorganisms was measured by the zone of inhibition around the disc placed on the inoculated agar plate. Chloramphenicol was used as the standard for bacteria and showed 17 mm, 18 mm and 13 mm zone of inhibition for *E. coli*, *S. aureus* and *S. typhi* respectively. Nystatin was used as the standard for fungi and showed 16 mm zone of inhibition for *C. albicans*.

The antimicrobial activity of garbage enzyme on bacteria and fungi are shown in Tables 3a and 3b respectively. When compared with positive control standards, the bacterial and fungal zone of inhibition for 150 μl of 15% garbage enzyme solution is higher. From Tables 3a and 3b it is observed that when using 150 μl of 15% garbage enzyme solution (pH 3.6), zone of inhibition for *E. coli*, *S. aureus*, *S. typhi* and *C. albicans* are 14, 12, 12 and 12 mm respectively and when using 150 μl of 15% garbage enzyme solution (pH 7), zone of inhibition for *E. coli*, *S. aureus*, *S. typhi* and *C. albicans* are 20, 18, 19 and 22 mm respectively. This observation clearly reveals that garbage enzyme adjusted to pH 7 has the highest power to reduce or inhibit the pathogen than with not adjusted (pH 3.6) because the acidic nature of garbage enzyme helps to extract extracellular enzymes from the organic waste materials into the solution during fermentation (Bhavani Prakash, 2011). These extracellular enzymes are likely responsible for the lytic action towards the pathogen like *E. coli*, *Salmonella* sp., etc. which are commonly found in waste activated sludge (Straub et al., 1993). The activity of such enzymes was suppressed when garbage enzyme solution was acidic in nature. When the pH of the garbage enzyme increased from 3.6 to 7 the activity of such extracellular enzymes present in garbage enzyme solution got increased which in turn enhances the antimicrobial activity (Puupponen-Pimiä et al., 2008). This observation confirms that garbage enzyme possesses pathogen killing/inhibiting property.

3.4. *Phenol coefficient of garbage enzymes*

In general disinfectants that are more effective than phenol have a coefficient greater than 1; those that are less effective have a coefficient less than 1. In this study the growth of test organisms in subculture plates were noted at intervals of 2, 4, 6, 8 and 10 min and tabulated in Table 4. The result showed that after 6 min exposure the test organism was killed completely by the garbage enzyme at a dilution of 1:400 and by phenol

Table 3a – Antimicrobial potential of garbage enzyme on bacteria.

Garbage enzyme conc. (μl)	Zone of inhibition (mm)																	
	E. coli						*S. aureus*						*S. typhi*					
	pH 3.6			pH 7			pH 3.6			pH 7			pH 3.6			pH 7		
	Garbage enzyme dilution																	
	5%	10%	15%	5%	10%	15%	5%	10%	15%	5%	10%	15%	5%	10%	15%	5%	10%	15%
50	6	9	11	7	11	18	–	7	10	9	12	16	–	5	7	8	10	13
100	6	10	12	9	13	18	5	8	10	10	15	16	–	8	10	10	12	13
150	8	12	14	9	16	20	8	11	12	10	18	19	–	8	12	10	14	15

Table 3b – Antimicrobial potential of garbage enzyme on fungi (*Candida albicans*).

Garbage enzyme conc. (μl)	Zone of inhibition (mm)					
	Candida albicans					
	pH 3.6			pH 7		
	Garbage enzyme dilution					
	5%	10%	15%	5%	10%	15%
50	–	–	10	8	12	19
100	–	8	12	8	15	19
150	–	11	12	10	18	22

Table 4 – Determining the phenol coefficient of garbage enzyme.

Disinfectant	Dilution	Growth of *Salmonella* subculture after exposure for				
		2 min	4 min	6 min	8 min	10 min
Garbage enzyme	1:100	G	G	G	G	NG
	1:200	G	G	G	NG	NG
	1:300	G	G	G	NG	NG
	1:400	G	G	NG	NG	NG
	1:500	G	G	G	G	G
Phenol	1:100	G	G	G	NG	NG
	1:105	G	G	G	NG	NG
	1:110	G	G	NG	NG	NG
	1:115	G	G	G	G	NG
	1:120	G	G	G	G	NG

G, growth; NG, no growth.

at a dilution of 1:110. Hence the phenol coefficient of garbage enzyme was calculated as follows

$$\text{Phenol coefficient of garbage enzyme} = \frac{1:400}{1:110} = 4 \quad \text{(approx.)}$$

Above result indicates that garbage enzyme can be diluted four times as much as phenol and still it possesses equivalent killing power of the test organism.

3.5. TS and SS removal

The removal percentage of TS and SS from sludge after treatment with garbage enzymes are presented in Figs. 2 and 3 respectively. From these figures it is observed that the TS and SS in sludge are decreased gradually with time when treated with 5, 10 and 20% but lower when compared with 15% diluted garbage enzyme solution. Also, it is observed that the sludge treated with 15% garbage enzyme collected after 120 h showed 37.2 and 38.6% reduction of TS and SS respectively.

3.6. MPN reduction

In general microbial counts of coliforms were used as indicators of pathogens. MPN methods are used commonly for detecting coliforms in sludge (Hass, 1989). The multiple tube decimal dilution procedure should be used for determining the MPN indices for both total and faecal coliforms. Straub et al. (1993) reported that densities of pathogen indicators in secondary sludge were detected as 10^7–10^8 CFU/g. MPN value

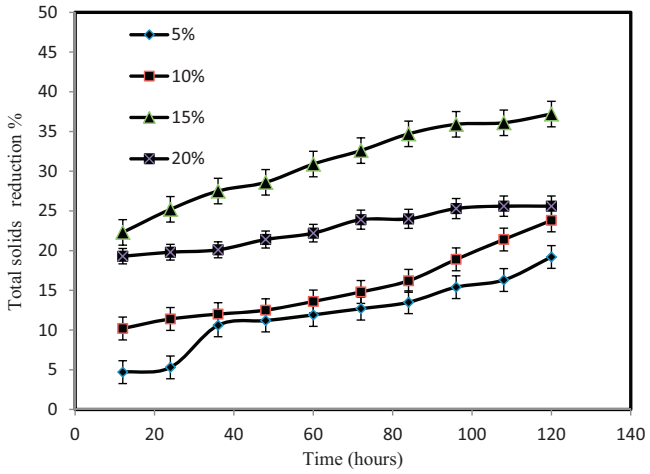

Fig. 2 – Reduction % of TS in waste activated sludge with time for different concentration of garbage enzyme (5%, 10%, 15% and 20%) for 5 days at 37 °C (250 rpm).

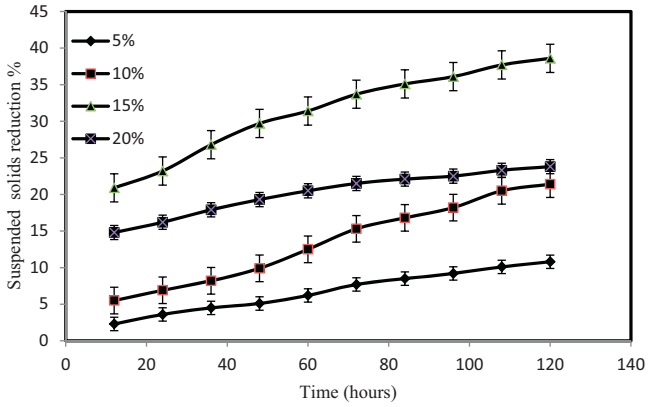

Fig. 3 – Reduction % of SS in waste activated sludge with time for different concentration of garbage enzyme (5%, 10%, 15% and 20%) for 5 days at 37 °C (250 rpm).

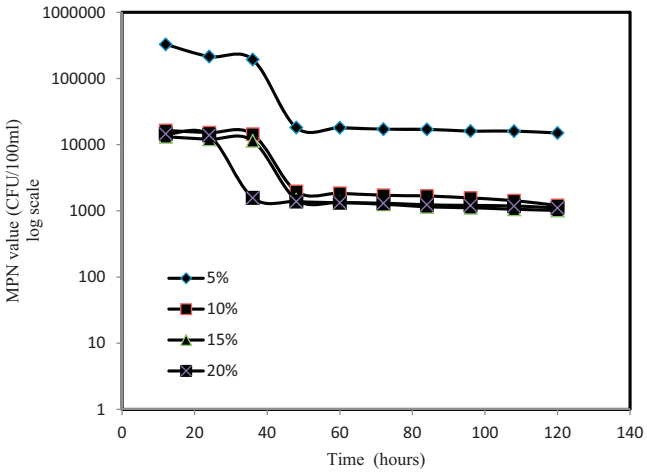

Fig. 4 – MPN value reduction in waste activated sludge with time for different concentration of garbage enzyme (5%, 10%, 15% and 20%) for 5 days at 37 °C (250 rpm).

reduction in time when treated with garbage enzyme is shown in Fig. 4.

The MPN value of raw sludge was observed to be 9.4×10^7 CFU/100 ml and from Fig. 4 it is observed that reduction of MPN value is up to 10^2 on a log scale, when the sludge was treated with 5, 10, 15 and 20% garbage enzyme solution. However 99% of pathogens were inhibited when the sludge was treated with 10, 15 and 20% garbage enzyme solution.

4. Conclusion

In the present study the garbage enzyme produced from decomposable organic solid waste were used to determine the biocatalytic activity and its influence on the stabilization of dairy waste activated sludge were investigated.

- Garbage enzyme produced by the fermentation was tested for biocatalytic activity. The result confirms that garbage enzyme possesses protease, lipase and amylase activity. As garbage enzyme possesses protease, amylase and lipase activity, it can be used to degrade proteins, carbohydrates and lipids in sludge. Hence this significant result may be helpful for researchers to compare the effectiveness of earth-friendly garbage enzyme pre-treatment of industrial sludge with various physical and chemical pre-treatment methods to improve the biogas production.
- Garbage enzyme produced by the fermentation was tested for antimicrobial activity. When compared with standards, higher antimicrobial activity of test microorganisms was observed when 150 μl of 15% garbage enzymes (pH 7 adjusted) was used. This confirms that garbage enzyme has pathogen killing/inhibiting property.
- Disinfectant potential of garbage enzyme was compared with phenol to determine the phenol coefficient. The result indicated that the garbage enzyme can be diluted four times as much as phenol, till attaining equivalent killing power for the test organism as that of phenol.

  The stability of the sludge is very important to enhance the recycling methods like composting and anaerobic digestion. In the present study an attempt has been made for the first time to predict the potential of garbage enzyme to increase the stability of dairy waste activated sludge obtained from the milk processing unit.
- Reduction of TS as 37.2% and SS as 38.6% were observed when sludge treated with 15% garbage enzyme solution. The result confirms that the garbage enzyme has the potential to reduce the organic content in the sludge.
- The MPN reduction percentage was found to be 99%, when sludge treated with 10, 15 and 20% garbage enzyme. Thus the result confirms that the garbage enzyme has the potential to kill/inhibit the pathogen in the sludge.

The results obtained in this study identified that garbage enzyme has both biocatalytic and pathogen inhibiting property. Therefore it has the potential to enhance the stability of sludge by removing the solids and suppressing the activity of microbes in the sludge.

References

Abbasnejad, H., Abbaspour, A., Soltan Mohammadzadeh, J.S., Dehghani, M., Vakilinia, S.H., 2002. Anaerobic treatment of dairy wastewater in a hybrid UASB and bio filter reactor. In: The 7th National Congress of Chemical Engineering, Faculty of Engineering, University of Tehran, Tehran, Iran.

American Public Health Association (APHA), 2005. Standard Methods for Examination of Water and Waste Water, 21st ed. APHA, Washington, DC.

Ayol, A., Dentel, S.K., 2005. Enzymatic treatment effects on dewaterability of anaerobically digested biosocial-II; laboratory characterization of drain ability and filterability. Process Biochem. 40 (7), 2435–2442.

Beszédes, S., Szabó, G., Géczi, G., 2012. Application of thermal and microwave pre-treatment for dairy waste water sludge. Ann. Fac. Eng. Hunedoara 3, 231–235.

Bezerra, R.P., Borba, F.K.S.L., Moreira, K.A., Lima-Filho, J.L., Porto, A.L.F., Chaves, A.C., 2006. Extraction of amylase from fermentation broth in poly(ethylene glycol) salt aqueous two-phase system. Braz. Arch. Biol. Technol. 49 (4), 547–555.

Bhavani Prakash, 2011. How to make and use garbage enzyme. http://www.ecowalkthetalk.com (accessed 17.12.13).

Dean, C.R., Ward, O.P., 1991. Nature of *Escherichia coli* cell lysis by culture supernatants of *Bacillus* species. Appl. Environ. Microbiol. 57, 1893–1898.

Gardner, J.F., Peel, M.M., 1991. Introduction to Sterilization and Disinfection Control, 2nd ed. Churchill Livingstone, Melbourne.

Godfrey, T., West, S., 1996. Industrial Enzymology. Stockton Press, New York.

Hass, C.N., 1989. Estimation of microbial densities from dilution count experiments. Appl. Environ. Microbiol. 55 (8), 1934–1942.

Im, J., Woo, H., Choi, M., Han, K., Kim, C., 2001. Simultaneous organic and nitrogen removal from municipal landfill leachate using an anaerobic–aerobic system. Water Res. 35, 2403–2410.

Joean oon, 2008. http://veg4planet.blogspot.in/2008/07/garbage-enzyme-anti-greenhouse-effect.html (accessed 17.10.12).

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Nazim, F., Meera, V., 2013. Treatment of synthetic grey water using 5% and 10% garbage enzyme solution. Bonfring Int. J. Ind. Eng. Manage. Sci. 3 (4), 111–117.

NCCLS – National Committee for Clinical Laboratory Standards, 1993. Performance Standards for Antimicrobial Disc Susceptibility Tests. NCCLS Publications, PA, M2-A5.

Parmar, N., Singh, A., Ward, O.P., 2001a. Characterization of the combined effects of enzyme, pH and temperature treatments for removal of pathogens from sewage sludge. World J. Microbiol. Biotechnol. 17, 169–172.

Parmar, N., Singh, A., Ward, O.P., 2001b. Enzyme treatment to reduce solids and improve settling of sewage sludge. J. Ind. Microbiol. Biotechnol. 26, 383–386.

Perle, M., Kimchie, S., Shelef, G., 1995. Some biochemical aspects of the anaerobic degradation of dairy wastewater. Water Res. 29, 1549–1554.

Pinsirodom, P., Parkin, K.L., 2001. Current Protocols in Food Analytical Chemistry, 3rd ed. John Wiley & sons, USA (inc. C3.1.1–C3.1.13).

Puupponen-Pimiä, R., Nohynek, L., Ammann, S., Oksman-Caldentey, K.M., Buchert, J., 2008. Enzyme-assisted processing increases antimicrobial and antioxidant activity of bilberry. J. Agric. Food Chem. 56, 681–688.

Reybrouck, G., 1998. The testing of disinfectant. Int. Biodeter. Biodegr. 41 (3–4), 269–272.

Roman, H.J., Burgess, J.E., Pletschke, B., 2006. Enzyme treatment to decrease solids and improve digestion of primary sewage sludge. Afr. J. Biotechnol. 5 (10), 963–967.

Shu, C.-H., Xu, C.-J., Lin, G.-C., 2006. Purification and partial characterization of a lipase from *Antrodia cinnamomea*. Process Biochem. 41, 734–738.

Smitha, R.B., 2010. Dual production of endotoxin and amylase from *Bacillus thuringiensis* subsp. *kurstaki* by fermentation and efficacy studies of endotoxin against eriophyid mite. http://shodhganga.inflibnet.ac.in/handle/10603/4017 (accessed 08.07.13).

Straub, T.M., Pepper, I.L., Gerba, C.P., 1993. Hazards from pathogenic micro-organisms in land-disposed sewage sludge. Rev. Environ. Contam. Toxicol. 132, 55–91.

Tsuchida, O., Yamagota, Y., Ishizuka, J., Arai, J., Yamada, J., Takeuchi, M., Ichishima, E., 1986. An alkaline protease of an alkalophilic *Bacillus* sp. Curr. Microbiol. 14, 7–12.

Uma Rani, R., Adish Kumar, S., Kaliappan, S., Yeom, I.-T., Rajesh Banu, J., 2012. Low temperature thermo-chemical pretreatment of dairy waste activated sludge for anaerobic digestion process. Bioresour. Technol. 103, 415–424.

Wawrzyńczyk, J., Recktenwald, M., Norrlöw, O., Dey Szwajcer, E., 2007. Solubilisation of sludge by combined chemical and enzymatic treatment. Afr. J. Biotechnol. 4 (17), 1994–1999.

Yang, Q., Yi, J., Luo, K., Jing, X., Li, X., Liu, Y., Zeng, G., 2013. Improving disintegration and acidification of waste activated sludge by combined alkaline and microwave pretreatment. Process Saf. Environ. Protect. 91, 521–526.