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

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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.

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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.