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RESEARCH ARTICLE

ACTI-ZYME BIOCHEMICAL PROPERTIES: POTENTIAL FOR USE IN ANAEROBIC SEWAGE TREATMENT CO-GENERATING BIOGAS

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ABSTRACT

The biochemical properties for Acti-zyme, a biocatalyst were characterized for potential use in anaerobic sewage treatment with the aim of producing biogas. Sterile Acti-zyme media containing peptone water was plated on the MacConkey Agar, Starch Agar, Kliger Agar, Urease Agar, Sulphide Indole and Motility Agar at 37.5°C for 24 hours, at a pH of 7±0.2. Acti-zyme was found to be immotile and contained several enzymes that have different applications in sewage treatment. These included catalase which detoxified harmful substances, protease which broke down the proteins as well as amylase which broke down the polysaccharides available in sewage. However, Acti-zyme did not contain urease; an ammonium catalyzing enzyme. Acti-zyme did not to promote H_2S production which is a contaminant in biogas production. Acti-zyme also did not contain pathogenic Enterobacteriaceae such as *E. Coli* and *Salmonella*. The identified Acti-zyme biochemical properties make it useful in sewage treatment co-producing biogas.

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INTRODUCTION

Acti-zyme, a biocatalyst has been used for vast applications such as wastewater treatment, drains cleaning and odor elimination since 50 years ago (Tshuma, 2010). Acti-zyme has been reported to treat wastewater either aerobically or an aerobically, reducing wastewater contaminants properties such as total nitrogen, total phosphorous, nitrates, ammonia, biochemical oxygen demand and total suspended solids by >40%. Acti-zyme also increases dissolved oxygen by > 100% in treated wastewater promoting aquatic life (Tshuma, 2010). The use of Acti-zyme like any biological catalyst under anaerobic conditions has the potential to favor biogas production (Tshuma, 2010; Reali *et al*., 2001). On the other hand sewage, a form of wastewater is being generated every day and there is need for sustainable and economical ways of treating this sewage; harnessing biogas being one of them (Malik and Bhart, 2009). Biogas is mainly composed of methane and can be used to meet electricity demands as well as other energy requirements of the sewage plants (Neczaj *et al*., 2013). Acti-zyme therefore poses an economic and environmentally friendly way of treating sewage utilizing its biochemical properties. Although Acti-zyme has been used for over 5 decades in one way or the other, its biochemical characteristics were not investigated especially its potential and suitability to be used in sewage treatment for biogas production. This study focused on biochemical analysis of Acti-zyme so that its use in sewage treatment as a biological technique can be validated. The biochemical analyses will provide an understanding of the sewage pollutants Acti-zyme targets during its bio activity in biological treatment.

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MATERIALS

Acti-zyme was obtained from AusTech, an Acti-zyme company based in Australia in September 2013. Acti-zyme was in pellets form and was stored at room temperature and only became active when it was introduced to a media. Peptone water was used as the sterile media for the MacConkey Agar, Starch Agar, Kliger Agar, Urease Agar, Sulphide Indole and Motility Agar. An Inco Therm Labotec incubator was used for incubation. Pure agars were obtained from Sun firm Distributors, a chemicals supplying company.

METHODS

Eight biochemical tests were performed on the Acti-zyme. Acti-zyme was inoculated in a vial containing sterile media containing peptone water. The vial was incubated at 37.5°C for a period of 24 hours. The media was characterized by a pH of 7±0.2. After the 24 hour period, the sample was plated on the

following media: MacConkey Agar, Starch Agar, Kliger Agar, Urease Agar, Sulphide Indole and Motility Agar. Growth on the various agars was noted in terms of size of colonies, colour and odour. Afterwards various biochemical tests such as Catalase test, Mannitol test, Urease test, Kligler test, Indole test and Motility test were performed in order to determine the properties of Acti-zyme especially for application in sewage treatment and biogas production (Schreckenberger and Blazzevic, 1974; Vashist *et al*., 2013).

RESULTS AND DISCUSSION

Catalase Test

This test is used to determine if bacterium contains catalase (Schreckenberger and Blazzevic, 1974; Vashist *et al*., 2013). Effervescence will indicate a positive result showing that the Acti-zyme contains the enzyme catalase. Rapid effervescence was observed when Acti-zyme was added to the catalase media. This indicated the presence of the biocatalyst component catalase which breaks down hazardous compounds and facilitates detoxification according to Reaction 1. Catalase optimally works at pH of 7 (Yumoto *et al*., 2000), which was an indication that sewage treatment and detoxification of contaminants will optimally take place at this pH when Acti-zyme is employed.

$$2H_2O_2 \xrightarrow{Catalase} 2H_2O + O_2 \qquad \text{.....1}$$

Motility Test

The motility test is used to determine if a bacterium is motile through the identification of *Flagella* (Schreckenberger and Blazzevic, 1974; Vashist *et al*., 2013). The motility test showed that no *Flagellum* was present in Acti-zyme. This implied that Acti-zyme was immotile hence it was not capable of moving freely which makes agitation necessary during sewage treatment and biogas production. Acti-zyme was negative for *Entrobacteriaceae*, a family of pathogens which include *Salmonella, E. Coli, Shigella* and *Enterobacter. Entrobacteriaceae* are gram negative bacteria. This indicated that the application of Acti-zyme in sewage treatment will not promote further pathogenic growth as the inoculant will not provide additional nutrients. In addition; immotility promotes Acti-zyme stability in sewage especially at increased loading in as far as the enzyme technology is concerned (Nisha *et al*., 2012).

Gelatine Liquefaction test

The Gelatine Liquefaction test is used to determine if a bacterium contains the enzyme galatinase which breaks down gelatine, a form of protein through galatinase (Schreckenberger and Blazzevic, 1974; Vashist *et al*., 2013). Galatinase breaks down proteins to polypeptides then to amino acids. Acti-zyme tested positive for the Gelatine Liquefaction test through hydrolysis using a nutrient gelatine medium on which a positive test results in a liquid medium on Acti-zyme addition. This indicated that Acti-zyme contained the enzyme protease which can break down proteins in accordance to Reaction 2. Protease hydrolyses amino acid sequence by its bio catalytic activity in hydrolysis amino acid esters, amides and peptides bonds optimally at pH ranges of 6-11 (Cheu *et al*., 2004). Furthermore, this meant Acti-zyme contains gram positive aerobic and anaerobic bacteria such as *Bacillus Anthracis, B. Cereus, B. Subtilis, Clostridium Perfringens* and *C. Tetani*. The anaerobic bacteria will promote biogas production. In addition, protease containing substances also favour high quality methane production due to improved digestion (Prabhudessai *et al*., 2014).

$$Protein + H_2O \xrightarrow{Gelatinase} Polypetides + H_2O \xrightarrow{Gelatinase} Amino\ acids \qquad \text{.....2}$$

Methyl Red and Voges Proskaeur test

The Methyl Red and Voges Proskaeur test is used to determine if a bacterium produces mixed acids from glucose (Schreckenberger and Blazzevic, 1974; Vashist *et al*., 2013). These mixed acids have a tendency of lowering the substance pH to 5. Acti-zyme was negative for the Methyl Red and Voges Proskaeur test. This further reconfirmed that *Enterobacteriaceae* is not present in Acti-zyme in relation to the motility test. *Enterobacteriaceae* favours hydrogen production rather than methane production during sewage treatment hence its absence in Acti-zyme makes it attractive for quality biogas production (Nath *et al*., 2006). Furthermore the negativity of the Methyl Red and Voges Proskaeur test indicated that no mixed acids were formed during use of Acti-zyme. Mixed acids may result in acidic sewage effluent pH causing harm to aquatic life. In addition, acidic pH does not favour biogas production. pH of around 7 is ideal for biogas production.

Starch hydrolysis test

The starch hydrolysis test shows the ability of a bacterium to breakdown starch using amylase (Schreckenberger and Blazzevic, 1974; Vashist *et al*., 2013). Acti-zyme broke down starch into constituent sugars such as maltose and glucose which can easily be metabolized due to the presence of amylase (Reaction 3). The test confirmed the presence of *B. Subtilis* which can endure extreme environmental conditions and the absence of *E. Coli*. Amylase hydrolyses polysaccharides into smaller units during sewage treatment making its presence in Acti-zyme critical (Faccin *et al*., 2014). Amylase optimally functions at a pH of 6-7 (Obah, 2005).

$$Starch \xrightarrow{Amylase} Maltose + Glucose + Dextrin \qquad \text{.....3}$$

This result indicated that Acti-zyme can treat sewage that is highly contaminated due to its resistivity to harsh conditions.
Tryptophan Hydrolysis test (*Indole test*)

The Tryptophan Hydrolysis (Indole Test) shows the ability of a bacterium to break down Tryptophan using the enzyme tryptophanase (Schreckenberger and Blazzevic, 1974; Vashist *et al*., 2013). This test can be used to differentiate between *Enterobacter* and *E. Coli*. The Tryptophan hydrolysis test (Indole test) was found to be negative in Acti-zyme. The result indicated that Acti-zyme contains *Enterobacter* but does not have *E. Coli*. In addition, this means Acti-zyme does not promote the growth and reproduction of already existing pathogens in sewage such as *E. Coli* and *Salmonella*.

Urea test

The Urea test shows the ability of the bacterium to break down Urea using the enzyme urease to ammonia and carbon dioxide

(Schreckenberger and Blazzevic, 1974; Vashist *et al*., 2013). Furthermore, it shows the presence of urease positive *Proteus* from other *Enterobacteriaceae* (Schreckenberger and Blazzevic, 1974; Vashist *et al*., 2013). Acti-zyme did not test positive for urease. This was another indication of the absence of *Enterobacteriacae b*acteria. The absence of urease is good for biogas production in sewage treatment. The presence of urease would have resulted in the release of ammonia gas which in-turn increases the pH of the sewage away from the optimum, to acidic conditions, hence reducing the amount of biogas produced.

Hydrogen Sulphide production test

The Hydrogen Sulphide production shows the ability of the bacterium to produce hydrogen sulphide gas by checking for the enzyme thiosulfate reductase (Schreckenberger and Blazzevic, 1974; Vashist *et al*., 2013). Acti-zyme did not promote the production of H_2S gas on testing; this revealed that the enzyme thiosulfate reductase was absent. The presence of thiosulfate reductase is known to impair methane production during anaerobic digestion (Madden *et al*., 2014). Therefore its absence in Acti-zyme is good for quality biogas production. Also, since H_2S is a known greenhouse gas, is malodorous, is inhibitory to methanogenesis and can corrode sewage plants equipment's. Acti-zyme's capability of not promoting H_2S production makes the use of Acti-zyme environmentally attractive in sewage treatment.

Conclusion

Acti-zyme was found to be an immotile biocatalyst. In addition, it contained several enzymes that can be used in sewage treatment such as catalase which has a detoxifying effect, protease which breaks down proteins in sewage and amylase which breaks down the complex sugars to simple sugars in sewage. However, Acti-zyme did not produce urease which promotes ammonia production which has a potential to cause eutrophication. From the biochemical tests, Acti-zyme does not promote hydrogen sulfide production meaning that the biogas produced will be of good quality and will not need to go through further treatment processes. Lastly Acti-zyme does not contain *Enterobacteriaceae* especially *E. Coli* and *Salmonella* which are pathogenic and are found in sewage. Therefore, Acti-zyme usage is not associated with health hazards and can efficiently promote biogas production in anaerobic sewage treatment.

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