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# Enhanced production of biohydrogen from dairy waste activated sludge pre-treated using multi hydrolytic garbage enzyme complex and ultrasound-optimization

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ABSTRACT

Fermentative hydrogen produced from activated sludge has received much attention among the scientific community. In the present study, optimized multi hydrolytic garbage enzyme complex (OGEC) produced from pre-consumer organic waste was used to hydrolysis dairy waste activated sludge (DWAS). Statistical optimization tools were used to determine the optimal environmental conditions for OGEC-DWAS pre-treatment and the models were evaluated. The result showed the maximum suspended solid reduction and solubilized chemical oxygen demand of 25.77% and 28.68% respectively were obtained at optimum conditions viz pH 6, temperature 40 °C, agitation speed 210 RPM and duration of pre-treatment 30 h. Further, to improve the solubilization of OGEC-DWAS, the effect of ultrasound on OGEC pre-treated sludge was investigated and results showed that 25 min of sonication time with power density of 0.6 W/ml improves the solubilization of OGEC-DWAS. Simultaneously cumulative biohydrogen production using pretreated DWAS through batch anaerobic digestion was studied and concluded that the maximum biohydrogen can be produced from combined OGEC-Ultrasound pretreated DWAS compared with raw sludge. This study helps to achieve an eco-friendly sustainable methodology to utilize WAS for biohydrogen production.

## 1. Introduction

The Food and Agriculture Organization (FAO) has reported a 3.1% increase in world milk production from 765 million tons in 2013 to 789 million tons in 2014. India ranks first in milk production with 18.5% of world production by reaching yearly output of 146.3 million tones during 2014–15. Subsequently, the amount of wastewater generated and discharged from these industries has also increased enormously. Vourch et al. [1] reported that around 0.2–10 L of effluent generated per liter of processed milk. Due to strict environmental laws, these types of effluents need extensive treatment before being discharged into the natural water bodies. Generally, in dairy effluent treatment plants activated sludge process plays an important role in reducing the organic matter and produce excess waste activated sludge (WAS) which in turn need to be treated further before disposal. Campos et al. [2] stated that management of waste activated sludge contributes 60% of the wastewater treatment plant operating cost. Hence, many researchers are working to develop a cost effective, recycle or reuse technologies to utilize WAS effectively. Uma et al. [3] suggested that anaerobic digestion process is the only sustainable methodology to utilize solid and liquid waste to produce renewable fuel called biogas (Bio methane/Bio hydrogen). Anaerobic fermentation process can also address both a resource (energy) requirement and solid waste management problems. Biohydrogen through anaerobic digestion of organic waste has received more attention all over the world because it serves as eco-friendly sustainable energy source [4–6]. Anaerobic degradation can be achieved by several steps: hydrolysis, acidogenesis, acetogensis and methanogenesis. Among these, research is being mainly concentrated on hydrolysis and acidogenesis because these produce readily solubilized substances enriched with carbon [7,8]. The hydrolysis stage is the major rate limiting step and lengthens the degradation in anaerobic digestion of waste activated sludge [9,10]. Hence pre-treatment of WAS is required to increase the rate of degradation of organic matter in it and thus helps to enhance biogas production through anaerobic digestion process [11,12]. Hydrolytic enzyme plays an important role in hydrolysis of the complex organic matter in sludge [13,14]. Qi et al. [15] showed that the combined mixture of commercially available protease and α-amylase enzyme stimulate and increase the solubilisation rate of waste activated sludge in eco-friendly way. The cost of commercial enzyme used in treatment of WAS is higher and hence

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increase in the enzymatic pre-treatment process operating cost [16]. Recently in many developing countries, the production of enzyme and its mixture from cheap source become a major area of research.

In addition to industrial sludge, food and preconsumer organic waste become another global problem due to rapid growth of population. On a global scale, the generation of organic solid waste predicted to be increased up to 51% between 2005 and 2025 [17]. Managing and disposal of large quantity of organic solid waste from household, agricultural, commercial and industries in sustainable way become a public concern and globe environmental challenge [18]. Proper management of unwanted solid organic materials eliminates or reduces the adverse impacts on the human health, environment, supports economic development and improved quality of life [19]. A number of processes are implemented by government for effective management of solid waste. Among them, the solid organic waste reuse and recycle methods are the preferred options to prevent greenhouse gas emission, conserve resources, reduce the release of pollutants to natural environment, decrease the demand for waste treatment technology and reduce landfill space requirement [20]. Attention of recent investigation by researchers are directed towards processing of organic waste to produce value added product such as citric acid [21], enzymes [22,23], bioethanol [24] bio butanol, bio polymers [25], etc. In our earlier study garbage enzyme solution produced from different preconsumer organic waste was investigated [26]. The result from this study revealed that garbage enzyme from pineapple and citrus fruit waste has the higher potential for solubilisation of complex organic substance. Ultrasound is one of the best mechanical WAS pre-treatment process using sound waves that generate cavitation (disintegration). Researchers stated that ultrasound helps to enhance the disintegration of organic particulate matter and produce higher amount of biogas [27,28].

Till date no study was reported to optimize the pre-treatment of dairy waste activated sludge (DWAS) using multi hydrolytic garbage enzyme complex with or without ultrasound treatment to enhance the production of biohydrogen. Hence, the prime objectives of the present investigation are (i) To study the effect of quantity of optimized garbage enzyme complex on sludge solubilization, (ii) To determine the optimal conditions for garbage enzyme pretreatment process to improve the solubilization of DWAS (Dairy waste activate sludge) using statistical tools, (iii) To study effect of ultrasound sonication time and power on sludge solubilization, (iv) To prepare the of seed (anaerobic digested sludge) inoculum to improve the hydrogen producing microbes and (v) To determine the cumulative biohydrogen production using pretreated DWAS through batch anaerobic digestion.

## 2. Materials and methods

### 2.1. Preparation of optimized garbage enzyme complex (OGEC) from preconsumer waste

The pineapple and citrus peels were collected from fruit shop, Tiruchirappalli, India and stored in refrigerator at 4 °C for the production of garbage enzyme. 360 g of pineapple peels and 240 g of citrus peels were taken and mixed in a container. Subsequently the above mixture of waste was taken then mixed with 200 mL of molasses and 2000 mL of water in an anaerobic fermenter (3 L volume). The anaerobic fermentation of preconsumer organic waste was conducted at optimal conditions reported in our earlier studies [29] for 3 days. After three days of fermentation the reactor content was filtrated and centrifuged at 10,000 rpm for 30 min. The resultant crude mixture was used as OGEC solution for pre-treatment of DWAS. The characteristics of OGEC solution obtained after three days of fermentation were analysed and indicated in Table 1.

**Table 1**
Characteristic of optimized garbage enzyme solution.

| Parameters | Value |
|---|---|
| pH | 6.5 ± 1 |
| Protease | 78 ± 3 (Units/mL) |
| Amylase | 57 ± 7 (Units/mL) |
| Lipase | 43 ± 2 (Units/mL) |

### 2.2. Waste activated sludge (WAS) collection and characterization

The waste activated sludge samples were collected from the secondary clarifier tank of dairy wastewater treatment plant at Chennai in Tamil Nadu (India) and stored in refrigerator at 4 °C. The raw sludge characterisation was performed as per procedures in standard methods [30]. Carbohydrates in the WAS were determined using phenol sulphuric acid method and protein using Lowry assay procedure and the results are presented in Table 2.

**Table 2**
Characteristic of secondary clarifier waste activated sludge.

| Parameters | Value |
|---|---|
| pH | 6.5 ± 0.8 |
| Total solids | 12603 ± 7 mg/L |
| Suspended solids | 8960 ± 11 mg/L |
| Volatile solids | 6743 ± 17 mg/L |
| Total COD | 28708 ± 20 mg/L |
| Soluble COD | 1508 ± 13 mg/L |
| Soluble protein | 894 ± 4 mg/L |
| Soluble carbohydrates | 341 ± 6 mg/L |

### 2.3. Analytical methods for parameters determination

The effectiveness of sludge stabilization and hydrolysis process were determined using the parameters such as COD solubilisation and Suspended solid (SS) reduction. These parameters were analysed and calculated before and after pre-treatment process.

$$\text{Increased sCOD } \% = \frac{(After\ treatment\ SCOD - Before\ treatment\ SCOD)}{Before\ treatment\ SCOD} * 100 \quad (1)$$

$$\text{SS Reduction } \% = \frac{(Before\ treatment\ SS - After\ treatment\ SCOD)}{Before\ treatment\ SCOD} * 100 \quad (2)$$

The maximum sludge hydrolysis can be evaluated by determining the concentration of soluble protein and carbohydrates in WAS after pre-treatment process. Protein concentration was determined using Lowry-Folin method using BSA (Bovine serum albumin) as a standard. The absorbance of different concentration of standard and sample were determined at 620 nm using spectrophotomer [31]. A carbohydrate was measured by phenol sulphuric acid method using glucose as a standard. The absorbance of different concentration of standard and sample were determined at 490 nm using spectrophotomer [32].

### 2.4. Effect of quantity of optimized garbage enzyme complex on sludge solubilization

5 mL of optimized garbage enzyme complex and 50 mL of DWAS were taken in a container at the ratio of 5:50. The container was named as E5 (E-Experiment) and placed in orbit shaker to maintain the temperature (30 °C) with agitation of 100 RPM for 24 h. Similarly, different ratio 10:50, 15:50, 20:50, 25:50, 30:50, 35:50, 40:50, 45:50, 50:50 of optimized garbage enzyme to DWAS were mixed in E10, E15, E20, E25, E30, E35, E40, E45, E50 containers respectively and placed in orbit shakers to maintain the temperature (30 °C) with agitation of 100 RPM for 24 h. 5 mL of distilled water and 50 mL of raw DWAS were taken in a container and named as C (C-control). Control containers were also