# Improving Biogas Production from Sewage Water by Using Enzymes

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#### Abstract

Biogas production rate from sewage water has been increased more than 80% by adding digestive enzymes to simulated and natural sewage water under different operational conditions. These enzymes have included pepsin, Ox bile extract, single and mixed with concentration of (0.25, 0.5, 1, 2, 4) mg/l and (20, 30, 40, 50, 60) mg/l as respectively. So the impact of addition of mixtures of enzymes (Lipase (L), Amylase (AM) and Protease (P)) at concentration shown in research, under temperature 20° and 37.5°C.pH 6.5-7.2, the duration of fermentation is 12 days. The amount of salinity is ranged between (1004 – 1474) micromhos/cm. The results showed that the addition of any digestive enzyme to sewage water increases biogas production at least10:20 % in short retention time. However, to get maximum biogas production, it is necessary to add at least two or three digestive enzymes simultaneously to sewage water. Up to two different digestive enzymes can increase biogas production from sewage water. For example, biogas production was increased 20% and 80% after using pepsin only and a mixture of pepsin and Ox bile extract respectively. The results also showed that the addition of digestive enzymes to the sewage water increases biogas production at 37.5°C over that at 20°C.

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Keywords: sewage water, biogas production, anaerobic, improving, enzymes.

## **1** INTRODUCTION

Recently, biogas production is welcomed world wide, where this production has both economical and environ-

mental benefits [1]. Commonly, wastes of humans and animals as well as biological wastes can be used in biogas production through anaerobic digestion process.

Generally, polymeric carbohydrates, proteins and lipids present in sewage water cannot be taken up by microscopic cells directly in the anaerobic process. Therefore, cellulose, amylase, protease, and lipase are little amounts of enzymes produced by microorganisms to breakdown and solubilize the macromolecular structures into soluble matter such as simple sugars, glycerol and fatty acids, amino acids to simplify transport through the cell film [2]. Direct microbial enzymatic hydrolysis of lignocellulose produces less than 20% of glucose from cellulosic fraction and biogas production usually don't exceed 60% of the theoretical value (475 L CH4/kgVS). Therefore, pretreatment of the substrate using enzymes is needed to make the holocelluloses more accessible to microbial attack and to improve hydrolysis [3].

Mayhew et al. [4, 5] and Cirne [6] have stated that an enhancement of biogas production was observed during anaerobic digestion. Further, enzymes enhanced microbial decomposition of active sludge and killing pathogens.

Prabhudessai [7] studied the effect of some enzymes (lipase and protease) separately on biogas production. Results confirmed that the increased ratio of biogas production related to the non enzyme case production is significant.

Eun et al. [8] stated that, the performance of enzymes mixtures is better than single enzymes.

Kim et al. [9] and Neves et al. [10] stated that the use of en-

zymes helps in the break down of hydrolyze cellulose, hemicellulases and cellobiase.

The using of the enzymes to the anaerobic treatment and the biogas production is very sensitive. Through the study presented by both Rintala and Ahring that there was evidence indicating that the additions of enzymes directly to the biogas reactors do not have a large amount of influence in production [11].

Miah et al. have studied the effect of adding the protease activity of the Geobacillus sp. train AT1, during thermophilic digestion 65°C. Their results showed that the enhancement of biogas production reached a 210% [12].

Warthmann et al. [13] studied the effect of using twenty five different enzymes separately. They found that the enzyme treatment reduced the amount of sludge and the increased output of biogas production. Also found that, treatments with enzymes improve the anaerobic decomposition of biomass residues that resulting from energy crops process. It is indicated that anaerobic treatment needs high concentration of enzymes, so that it was unlikely to be economically. Some of enzymes increased biogas production by around 10% with green and grass silage in batch tests. These enzymes also increased methane ratio of the gasses concentration in the gas produced in the first seven days.

Sonakya et al. increased biogas production (7–14%) using some enzymes (protease,  $\alpha$ -amylase and cellulose) for treatment of wheat grains [14].

Enzymatic pretreatment significantly increased total biogas production about 30%; no significant differences were observed with different concentration of enzymes [15].

Suarez Quinones et al. [16] studied the effect of added enzymes under conditions 3 hours, pH 5 to 6, 40 °C and at a concentration of 0.04 g enzyme ODM. Feed stocks were used the following: rye grain silage, maize silage, grass silage, feed residues and solid cattle manure. The largest increases in biogas production from feed residues and solid cattle manure 50% and 100%, respectively; rye grain silage 15 % increase and maize silage 30 % increase. Also the results obtained show a clear increase in methane production after enzyme application for solid cattle manure by 5 % to 10 %.

From the above literature, it is clear that, there is a very few results related to the addition of enzymes for the treatment of the sewage water. Therefore, the main objective of the present work is to improve the biogas production from sewage water treatment through the addition of some digestive enzymes and estimating the optimum operating conditions.

## 2 METHODS AND MATERIALS

The experiments are made in sanitary laboratory engineering civil department, Faculty of engineering. The time of study continued twelve months from 25/10/2014 to 25/10/2015. The samples used in the experiments are simulated and natural sewage. Natural sewage has been taken from sewer network, Mansoura city. Simulated sewage water is prepared from molasses, urea and nutrients [17]. Samples were prepared by mixing 1 ml of melissa cane sugar, 1 ml milk fresh beef, 1 g/l yeast, 1 g/l flour, 2.5 mg/l phosphorus resulting from analysis of fertilizer super omnidirectional phosphate and 2 mg/l nitrogen resulting from the decomposition of urea 46% nitrogen.

## **3 BIOGAS MEASUREMENT**

Biogas production is measured from the experimental reactor by mechanical flow of liquid using fixed test tube of 250 ml capacity upturned in a fiber beaker capacity 7 liter. The beaker is filled with water to a certain height, as well as test tube full by rarefaction of air. To measure the gas in the reactor passing through the connector, the reactor and the bottom of the mouth of inverted test tube are connected using rubber tube. Biogas produced was collected in the graduated cylinder connected with a water reservoir which allowed volumetric biogas measurements at atmospheric pressure. This measure was stated by some researchers as [7, 18].

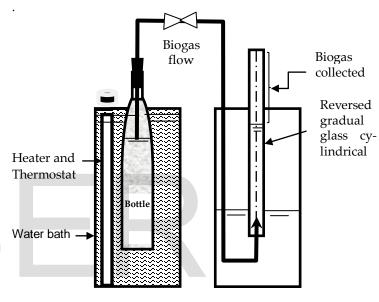
# **5 EXPERIMENT SETUP AND PROCEDURE**

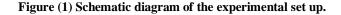
Anaerobic fermentation of experiment has been done by using of laboratory brewers size 2 liters with connecting of these components by rubber tubes sealed into stopcock. These brewers have been put in a thermal bath under temperature of 20° and 37.5°C. The system has been operated at each of these temperatures in one batch and one system feed.Then measuring the amount of gas emitted by using of the displacement method of the liquid at specific time intervals. Results have been corrected, using the general equation for gases. In this study, pH was controlled constant pH 6.5 - 7.2 during biogas formed.

These experiments are conducted in the laboratory as follows:

**1)** Preparation of the required enzymes and chemical materials also set up the required weight of material to

wastewater simulation, **2**) Mixing these materials with each other, **3**) The addition of the required quantity of water in reactors, **4**) The samples are placed in a large water bath or incubator, **5**) The experimental data were recorded daily during the last 12 days of each experimental period, **6**) Biogas production after 6 and 12 day was evaluated, **7**) The results obtained are corrected using the general law of gases ideal. The programs excel is used in the statistical analysis





# **6 RESULTS AND DISCUSSION**

This section includes the effect of single enzymes and mixture at different enzymes addition on biogas production.

#### **6.1**SIINGLE ENZYMES RESULTS

This item displays the results of enzymes that have been used individually to improve the amount of biogas production, including enzymes pepsin and ox bile extract.

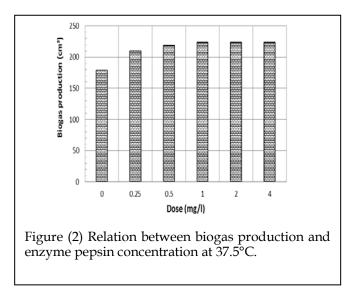


Figure (2) shows the relation between enzyme pepsin concentration and biogas production at concentrations varying from 0.25 mg/l to 4mg/l. Biogas production slightly increases with concentration. Biogas production is 210 cm3 at concentration of 0.25mg/l and attains maximum value of 224 cm3 at concentration of 2 mg/l. Then take a constant value with the increase of concentration. The increase in productivity is low.

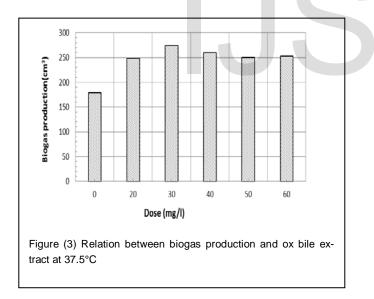


Figure (3) shows the relation between ox bile extract concentration and biogas production at concentration varying from 20 mg/l to 60 mg/l. Biogas production sharply increases with the concentration up to a maximum value of 248.6 cm3 at a concentration of 30 mg/l. Then biogas production takes nearly a constant value with concentration. This could be attributed to inhibition effect of higher concentration on activity of bacteria.

# 6.2 Mixed enzymes results

This item discuses the effect of mixed enzymes in improv-

ing biogas production.

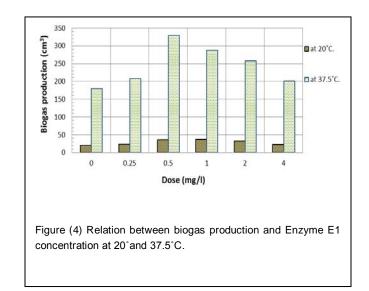


Figure (4) shows the relation between biogas production and the concentration of mixed enzymes E1 at two levels of temperature 20° and 37.5°C enzyme E1 consisted of 30mg/l ox bile extract and  $\Delta$ pepsin. Generally, biogas production is higher at higher temperature. However, the highest biogas production is observed under the concentration of 0.5 mg/l. After that, the production deceases, this may be attributed to the inhibition effect of the enzymes on the decomposing bacteria and the faster decomposition of simply decomposition organic material.

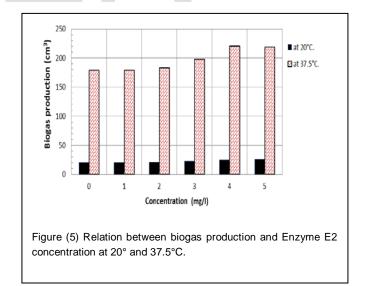


Figure (5) shows the relation between biogas production and E2 concentration. Enzymes (E2) with simulated sewage water at temperature 20° and 37.5°C. Enzymes E2 consists of 0.125 Lipase (L), 0.09375 Amylase (AM) and 0.00468 Protease (P) and its multiplication by 1, 2, 3, 4 and five. Biogas production slightly increases with E2 concentration even at the first concentration there is no any increase. Biogas production attains, its maximum.

mum value (220.034 cm3) at 4\*E2 concentration and then decreases with further increases in E2 concentration. Where 4\*E2 is (0.5mg/l (L), 0.375 mg/l (AM), 0.01872 mg/l (P)).

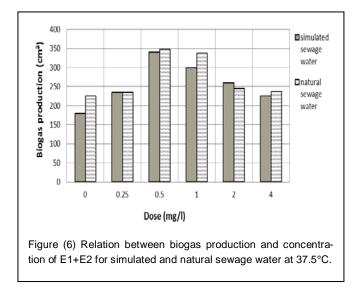


Figure (6) illustrates biogas production from both simulated and natural sewage water under combination of group enzymes (E1+ E2) at 37.5°C. The figure shows no significant differences in biogas production between simulated and natural sewage water. Biogas production increases with concentration until it reaches a maximum value of 340.86cm3 at a concentration of 0.5 mg/l and then decreases with further increase in concentration.

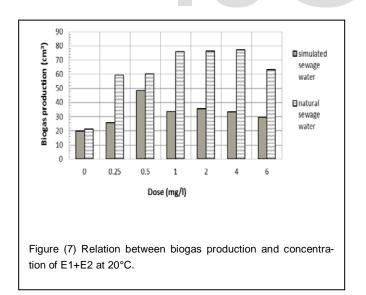


Figure (7) shows the relation between biogas productions for concentration of enzyme pepsin varying from 0.25 mg/l to 6 mg/l at temperature of 20°C. The figure shows that biogas production increases with concentration for both simulated and natural sewage water until it reaches a maximum value of 328.25cm3 at a concentration of pepsin (1) mg/l and then slightly decreases with further increase in pepsin concentration.

## Conclusion

1. Biogas production was increased by 80 % and 54 % using additions of enzymes mixture to simulated and natural sew-age respectively at 37.5°C compared to the conventional conditions.

2. It did not record a significant increase during the processing enzyme pepsin arrived to 24%, but with the use of ox bile extract have noticed a new increase in productivity reached to 53%.

3. Mixing of enzyme pepsin with ox bile extract gives an increase of 80% in biogas production.

4. Maximum and minimum biogas production is observed at 37.5 ° and 20°C respectively.

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