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Preliminary Cellulase catalyzed digestion experiment using different cellulose substrates

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ABSTRACT

The use of enzymes for digestion process provides a cleaner option when compared to conventional chemical methods. However there is need to improve the efficiency of these unit operations involving bioprocess by way of process intensification techniques. In this project commercially available enzyme is used for the digestion process. Three different cellulosic substrates, namely, rice husk, bagasse and cotton were used for the degradation by two different concentrations of commercially available Cellulase. Two different approaches were employed for this purpose. In one of the experiment was conducted on fresh samples and in the other it was conducted on the samples which were incubated overnight. Various standard process parameters such as, enzyme concentration, time, temperature and pH were followed in this enzyme assisted digestion process. The results indicated that cellulase enzyme based digestion system gave results when the samples were incubated for longer hours. Although more work is proposed to know the yield patterns, the present work clearly indicates that it is a novel and eco-friendly procedure.

Key words: Cellulase, Digestion, Bagasse, Rice husk, Cotton.

INTRODUCTION

In recent years, enzyme aided bioprocess of digestion provides potential alternative to chemical based processing. In fact there is a need for developing viable, cleaner bioprocess techniques for extraction because of the growing environmental concern in chemical processing industry, using many of the chemicals to extract the product which possess environmental problems. Consequently extraction process has been given wide attention for development of chemical free process in product making. One of the enzymes useful in degrading cell wall in order to bring out the cell products is cellulase enzyme. This enzyme was used for digestion process. Several eco-friendly extraction systems have been studied earlier for their potential benefits and review on the subject matter have been available in the literature. Enzyme based applications are useful but also involved diffusion and can break the cell wall in the material. There is need to improve extraction system more effectively by way of enzymatic methods

CELLULASE:
In the present paper, the use of enzyme in digestion process has been studied in order to harvest the benefit of enzyme for intensification of bio process of digestion, presently named enzyme assisted technology. Cellulase enzyme can be isolated from fungi namely, *Aspergillus niger*. This enzyme is also commercially available. The present study used commercially available Cellulase enzyme for digestion efficiency under particular concentration, time, temperature and pH.

Cellulase refers to a suite of enzymes produced chiefly by fungi, bacteria, and protozoans that catalyze cellulolysis (i.e. the hydrolysis of cellulose). However, there are also cellulases produced by a few other types of organisms, such as some termites and the microbial intestinal symbionts of other termites. Several different kinds of cellulases are known, which differ structurally and mechanistically.

COTTON:
Cotton is a soft, fluffy staple fiber that grows in a boll, or protective capsule, around the seeds of cotton plants of the genus *Gossypium*. The fiber is almost pure cellulose. Under natural conditions, the cotton bolls will tend to increase the dispersion of the seeds. Cotton fiber represent the purest natural form of cellulose, containing more than 90% of this polysaccharide.

RICE HUSK
Rice hulls (or rice husks) are the hard protecting coverings of grains of rice. In addition to protecting rice during the growing season, rice hulls can be put to use as building material, fertilizer, insulation material or fuel. Rice husk was collected from rice mill near Chennai, India. It was sun dried for more than a week to remove the moisture content from the material.

BAGASSE
Bagasse is the fibrous matter that remains after sugarcane or sorghum stalks are crushed to extract their juice. It is currently used as a biofuel and in the manufacture of pulp and building materials.

The high moisture content of bagasse, typically 40 to 50%, is detrimental to its use as a fuel. In general, bagasse is stored prior to further processing. For electricity production, it is stored under moist conditions, and the mild exothermic reaction that results from the degradation of residual sugars dries the bagasse pile slightly. For paper and pulp production, it is normally stored wet in order to assist in removal of the short pith fibers, which impede the papermaking process, as well as to remove any remaining sugar. Bagasse was collected from juice shop Chennai. It was dried well at sunlight more than two weeks to remove the moisture content of from the material.

The aim of the present work is to understand the efficacy of commercial cellulase enzyme on the digestion of cellulosic materials like rice husk, bagasse and cotton at different incubation conditions. Cellulose, the major constituent of all plant materials forms about half to one third of plant tissues and is constantly replenished by photosynthesis. In particular cellulose is the main constituent of higher plants, including sugarcane bagasse and rice husk, attempt has been made to analysis structural changes in cotton fibre accrued during bio polishing using cellulose obtained *Trichoderma reesei*. Cellulase hydrolysis results in weight loss of the samples, which in turn result in the splitting of fibres and removal of surface irregultarries of the fibre. Raw cotton fibres were used as the substrate and were used to eliminate influence of chemical pre treatment on enzymatic hydrolysis. Lignocellulose is one of the cheapest complex organic carbon that exists in nature in abundance in the form of plant biomass. Cellulose, hemicelluloses and lignin are the three major constituents of lignocellulosic substrates [1]. Rice straw is a main agricultural by products in many countries in which rice is the major crop [2]. Being considered as a renewable resource, rice can be converted to biofuels through the application of biotechnology. An environmentally friendly method of rice straw disposal save energy by conversion of these agricultural wastes into value added products [3]. Cellulases are currently regarded as the third largest volume of industrial enzymes [4]. Cellulytic enzymes have a wide range of applications in industry including biomass hydrolysis for the production of biofuels.

It is estimated that approximately 20% of the 1 billion US dollars of the world's sale of industrial enzymes consists of cellulases, hemicellulases and pectinases. Since the production of cellulase enzyme is a major process and economically viable, much work has been done on the production of cellulases from lignocellulosics. The bioconversion of various complex cellulosic waste materials such as baggase, Corncob saw dust rice husk have been reported [5, 6, 7, 8, 9]. The crystallinity and lignification limit the accessibility and susceptibility of cellulose to cellulolytic enzymes and other hydrolytic agents [10]. Therefore pretreatment of these materials is necessary to increase the rate of hydrolysis of cellulose to fermentable sugars [11].

Pretreatment of cellulose opens up the structure and removes secondary interaction between glucose chains and make more accessible to cellulase producing microorganisms [12]. The application of enzymes in the textile industry is becoming increasingly popular because of the mild conditions of temperature and pH that are required and the capability of enzymes of replacing harsh organic chemicals. Also important is that wastewater from

enzymatic treatments is readily biodegradable and, accordingly, does not pose any environmental hazard [13]. At present, the typical applications of enzymes for treatment of cotton can be summarized as desizing, scouring bleaching cleanup, bio-finishing, bio-stoning, garment laundering and dyeing [14, 15, 16, 17]. Enzymatic processing of cotton, like any wet processing system, involves mass transfer from the processing liquid medium across the surface of the textile substrate.

MATERIALS AND METHODS

Experimental set-up

The digestion process was performed using orbital shaker incubator in a glass vessel (220 rpm). Hot air Oven was used for drying the sample. Experiments were carried out at 37°C and pH 5.5 with gentle agitation.

Purified cellulase enzyme was purchased from Sisco Research Laborites Pvt. Ltd., Mumbai, India. Distilled water was used in all the digestion experiments whenever required. Specification of enzymes are cellulase extra pure (0348215) (9012-54-8) extracted from *Aspergillus niger*, activity-22,000 CMC units/gm.

PROCESS DETAILS

Dried Bagasse, rice husk and commercial cotton material were collected from varies place at Chennai, India. The materials were cut in uniform size. The enzyme was prepared in two different concentrations for this extraction process. Solution (A) consisted of(0.1 mg) of enzyme and the volume was made up to 50ml by adding distilled water in the 50ml SMF. Solution (B) was prepared by adding (0.2 mg) enzyme in 50 ml distilled water. 5 grams of dried materials were taken into conical flasks. 5 ml of Solution A was taken and 45ml of distilled water was added. Similarly 5ml of solution (B) was taken into another conical flask containing 5 gms of sample and 45ml of distilled water was added. 5 ml of 50 m Mol. Sodium citrate buffer were added to maintain, pH 5.5. 5 ml of Solution A was into each flask and were gently agitated for few minutes for proper mixing of enzyme solution and the buffer solution. These conical flasks were kept in orbital shaker incubator at 220(rpm) for eight hours. Every two hours 5ml of sample was taken and tested for reducing sugar for 8 hours.

The second experiment was done using the same solution A & B with the difference that the material were mixed with 10ml of enzyme solutions and incubated overnight. 95ml of distilled water were added to the conical flasks and agitated in shaker incubator for next 8 hours and analysed for reducing sugar activity at every 2 hours. After eight hours the left over extract was filtered and taken and centrifuge at 5000 rpm for 15 min and the supernatant were stored in 4 degree centigrade till it was analysed for reducing sugars. The pellet was stored and used for the presence of polysaccharides.

Polysaccharide was assayed using a method described by Updegraff, 1969 [18].

Methodology for Assay of Polysaccharide in the pellet

A liquid of 3ml of acetic/nitric reagent was added to a known amount (0.5g or 1gm) of the sample in a test tube and mixed in a vortex mixer and placed in a vortex mixer in a water bath at 100°C for 30 min, cooled and centrifuged for 15-20 min and the supernatant discarded. Then the pellet was washed with distilled water.10ml of 67%H_2SO_4 was added and was allowed to stand for 1hrs.1ml of above solution was diluted to 100ml. To 1ml of this diluted solution 10ml of Anthrone reagent was added and mixed well. The tubes were kept in a boiling water bath for 10 min, cooled and colour was measured at 620nm using colorimeter. The values are given in Tables 1-4. All the O.D values were measured at 620nm.

ASSAY FOR POLYSACCHARIDE IN SUPERNATANTS:

1ml of sample was taken in test tube and 1ml of iodine reagent was added and the appearance of blue colour was a confirmation of polysaccharides in supernatant sample.

Assay for reducing Sugars in the supernantants

i. 2ml ml of the supernatant solution was taken, equal amount of Benedict's reagent is added and heated in a boiling water bath for few minutes. A reddish brown precipitate was formed indicating the presence of Reducing sugar in supernatant.

ii. 2ml of the supernatant solution was taken, equal amount of Fehling's reagent is added and heated in a boiling water bath for few minutes. Reddish orange precipitate was formed indicating presence of reducing sugars.

iii. 1ml of sample was taken in test tube and 1ml of iodine reagent was added to it. The appearance of blue colour is confirmation of polysaccharides in supernatant sample.

RESULTS AND DISCUSSION

Table 1: At enzyme Conc. 0.1mg/50 ml (of fresh samples)

Fresh Materials	O.D values
Husk	0.09
Cotton	0.12
Bagasse	0.10

Table: 2 At Enzyme Conc. 0.1 mg/50 ml. (Incubated at overnight samples)

Overnight Materials	O.D values
Husk	0.12
Cotton	0.14
Bagasse	0.11

Table: 3 At enzyme Conc. 0.2mg/50 ml. (of fresh samples)

Fresh Materials	O.D values
Husk	0.12
Cotton	0.14
Bagasse	0.11

Table: 4 At enzyme Conc. 0.2mg/50 ml. (Incubated overnight samples)

Overnight Materials	O.D values
Husk	0.12
Cotton	0.14
Bagasse	0.11

From the above results it was inferred that at both concentrations of enzyme(0.1 and 0.2 mg/50 ml) the O.D values for the presence of polysaccharides in the pellet did not vary perceptibly. It was also observed that polysaccharides were present in the supernatants also.

Reducing sugars were present in the supernatants at both the concentrations of the enzymes.

CONCLUSION

It was concluded from the above experiments that at different cellulose concentrations and at particular pH and temperature, cellulose was degraded from all the three samples namely, rice husk, bagasse and cotton, which were taken as samples for cellulose. Further work is in progress to ascertain the quantitative values of the degradation of cellulose. This knowledge will help in suggesting the efficacy of the use of enzyme cellulase in digestion of cellulose for different sources as compared to the other industrial processes that involve chemicals for cellulose digestion. If the efficacy proves to be better than the use of chemicals for the process it can reduce the impact of pollution caused by the traditional use chemicals and prove the importance of using enzymes for degradation of cellulose, which is ecofriendly and cheap.

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