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COMPARATIVE ENERGY YIELDS FROM THREE LIGNOCELLULOSIC WASTES

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Abstract— The saccharification of lignocellulosic substrates were studied using crude cellulase produced from *Streptomyces viridosporus* T7A. With the intention of producing sugar syrup, particularly glucose, from locally available materials, a simple procedure of enzymatic saccharification of Elephant grass (*Panicum maximum*), saw dust and *saccharum officinarum* (sugar cane) baggase lignocelluloses were studied. The lignocelluloses were pre-treated by autoclaving in 1.0% NaOH solution at 121°C (15Psi) for one hour. Saccharification of the deligaified substrates were examined using crude cellulase produced by the *Streptomyces spp.* Maximum enzyme activity was obtained with Elephant grass (3.8 unit ml^{-1}); followed by sugar cane baggases (3.5 unit ml^{-1}) and 1.5 unit ml^{-1} for saw dust. At 1.3 unit ml^{-1} enzyme activity, saccharifying efficiency for grass was 12.4%, 12.3% for sugar cane and 4.1% for saw dust. Corresponding glucose production varied with incubation time and type of substrate hydrolysed. Amount of glucose produced increased with incubation time for all the substrate while it varied with type of substrate.

1. INTRODUCTION

A seemingly endless list of potential sources of cellulose materials exist. Cellulose materials are the most abundant natural resources (renewable resources) available to man on earth. It comprises about 95% of earth's land based biomass[1].

Through photosynthesis, the single most important physico-biochemical process of the world on which existence of life on earth depends, solar energy is stored in plant tissues in form of carbohydrates. The oxidation of organic compounds releases stored energy to be utilised by organisms to drive essential metabolic processes. The general data regarding the amount of light energy received on the earth and its utilisation during photosynthesis is tentatively available[1].

Several possibilities exist for utilising the energy stored in plant residues, acid hydrolysis [2,3] or enzymatic hydrolysis [4,5] to sugars, biogas (methane) generation [6,7] and conversion to a highly digestible ruminant feed by chemical or mixed culture fermentation [8] techniques. In the first method, the rate of hydrolysis, yield and sugar recovered depend on the substrate source, but all products are available for both human and animal consumption as well for industrial raw materials. Methanogenesis can recover approximately 50% of the energy in waste fibres as methane, a readily usable gas. If all agricultural residues in the United Sates were fermented to methane, they would supply some 10% of the U.S. national energy needs [9].

The disadvantages of acid hydrolytic process which include additional heat requirement, corrosive proof equipment provide an excellent opportunity for the development of enzyme process for lignocellulose hydrolysis [5]. There has been considerable research activity in all the fields of enzymatic hydrolysis of lignocellulosic materials [10, 11, 12]. However, enzymatic hydrolysis of lignocellulosic wastes yields from each lignocellulosic substrate has been carried out. In particular, the ability of *Streptomyces viridosporus* T7A to produce cellulase capable of hydrolysing *Panicum maximum*, *Saccharum officinarum* baggase and saw dust to release glucose from these lignocellulosic wastes has not been adequately studied. In recognition of this and to compare the energy yield by the amount of glucose from these lignocellulosic wastes which consitute lignocellulosic wastes management disposal problems, the present study was initiated.

2. MATERIALS AND METHODS

2.1 Lignocellulosic wastes used

The lignocellulosic wastes used in this study were

i) *Panicum maximum* (Elephant Grass)
ii) *Saccharum officinarum* (Sugar cane) baggase
iii) Saw Dust

2.2 Pretreament of the Lignocellulosic wastes

The Lignocellulosic materials were treated as previously described [12].

2.3 Micro-organisms used

Streptomyces viridosporus T7A was used in this research. It was originally isolated from soil sample by Siden D.I. (M.S. Thesis, Department of Bacteriology and Biochemistry, University of Idaho Moscow, U.S.A.) and supplied by Prof. S. P. Antai, University of Calabar, Calabar, Nigeria. The organism was grown on yeast extract mineral salts Agar[15]. Stock culture was kept as slant either on Tryptone Yeast Extract Agar or Peptone Yeast Extract Agar (Oxoid).

2.4 Cellulase production and cellulase assay

The cellulase was produced according to the methods of Antai and Udotong [10,11] by growing the organism in mineral salts medium and assaying for cellulase production periodically. The crude culture filtrates methods of Hen *et al* [16] was used in the enzyme assay. It involved the determination of

Carboxymethyl cellulase saccharifying activity through the measurement of the amount of reducing sugar (as glucose) liberated from 1% carboxymethyl cellulose (CMC) solution 7LF (low viscosity, sigma).

2.5 Hydrolysis of the Lignocellulosic materials

To determine the susceptibility of the lignocellulose to the enzyme, 5 g (5%) of the pre-treated lignocellulose substrates were hydrolysed with 100 ml of phosphate buffered (pH 7.2) crude cellulase at 55°C with stirring. Samples (1.0 ml) were removed at 0.5, 1, 2, 4, 6, 8, 12, 24, 36, and 48 h and analysed for total reducing sugars [17]

2.6 Assay for glucose

Glucose was estimated using the Somogyi-Nelson method [18, 19] as previously described [4].

3. RESULTS

3.1 Cellulase production and assay

S. viridoisporus T7A were grown in mineral salt-cellulose broth. After 10 days incubation at 37°C, cellulase activities were detected. The activities were found to increase with incubation time. When incubated for 14 days, highest enzyme activity of 1.2 FPU ml^{-1} was obtained (Fig. 1). The enzyme activities were least (1.5 units/ml) when the organism was grown on saw dust and highest (3.8 units/ml) with elephant grass (Table 1.)

Table 1: Cellulase activity (I.U. ml-1) of crude cellulase produced by *S. viridosporus* T7A growing on lignocellulose substrates

Lignocellulose Substrate	CM-Cellulase Saccharifying Activity (units/ml)*
1. Saw dust	1.5
2. Sugar cane bagasse	3.5
3. Elephant grass	3.8

*One unit of carboxymethylecellulase (CMCase) is defined as the amount of enzyme releasing 1.0 mole of glucose from 1% carboxythylcellulose (CMC) in 1 hour at 55°C, pH 6.0.

3.1 Lignocellulose hydrolysis

Carbohydrate content was highest in grass (71.9%), followed by sugar cane bagasse (68.8%). It was least in saw dust (65.2%). Percentage of Klasson lignin was highest in saw dust (30.1%) while 25.2% and 19.4% were obtained in sugar cane bagasse and elephant grass, respectively (Table 2).

Table 2: Chemical composition (Lignin and carbohydrate content) of Lignocellulosic substrates

Substrate (i)	Klasson Lignin (%) (ii)	Carbohydrates (iii)
Saw dust	30.1	65.2
Sugar cane bagasse	25.2	68.8
Elephant grass	19.4	71.9

i) Fifty milligrams of each substrate was assayed

ii) Lignin content was determined by modified Klasson method [4]

iii) carbohydrate content of lignocellulosic substrates.

Table 3: Glucose production (mg/ml) as a result of 48 hours hydrolysis of lignocellulosic materials with *Streptomyces viridosporus* T7A.

Time (hrs)	Substrate: Sugar cane bagasse	Elephant grass	Saw dust
2	0.9	0.6	-
4	1.2	1.0	-
8	1.6	1.5	-
12	1.7	1.9	0.2
24	2.6	3.3	0.4
36	2.9	3.5	0.7
48	2.6	3.8	0.9

3.3 Rate of hydrolysis

Table 3 shows glucose production with time when the different lignocellulose substrates were hydrolysed. Highest hydrolysis was obtained after 48 hours. The rate of glucose production depended on time.

3.4 Degree of Saccharification

S. viridisporus T7A at 1.3 FP Unit ml^{-1} activity caused a 12.4% saccharification of grass, 12.3% of sugar cane and 4.1% saw dust. Saw dust showed the least susceptibility to the enzyme, and hence less glucose production (0.9).

4. DISCUSSION

The use of acid for the degradation of lignocellulosic materials into glucose has long been achieved as reported by many investigators [2, 4, 10, 11]. Due to corrosion problems, particularly with hydrochloric acid and sulphuric acid as well as potentially adverse environmental effects due to emissions from the acid hydrolysis processes, enzymatic hydrolysis of lignocellulose has been preferred [12, 20]. In view of the nature of lignocellulose, this hydrolysis however cannot be successfully achieved without the initial pre-treatment of the material since the major obstacle to enzymatic saccharification is the presence of lignin and the crystalline cellulose. Various methods of pre-treatment have been suggested [2] but most commended is the method of Toyama and Ogawa [21] used in this study. The combination of milling and autoclaving at 121°C (15 Psi - high pressure steaming) of material in a 1% NaOH solution for 1 hour used in this study has given an excellent pre-treatment of the lignocellulosic wastes.

Cellulase source is another essential factor in this study, as well as the process that can yield total hydrolysis of substrate. Cellulase is a complex enzyme containing chiefly endo-and exo-beta-glucanase plus cellubiase (beta-glucosidae)[12]

The carboxymethyl cellulase activity has been reported to have high saccharifying efficiency compared to other methods [12]. Table 1 indicates the carboxymethyl cellulase activity with the different lignocellulosic materials used in this study

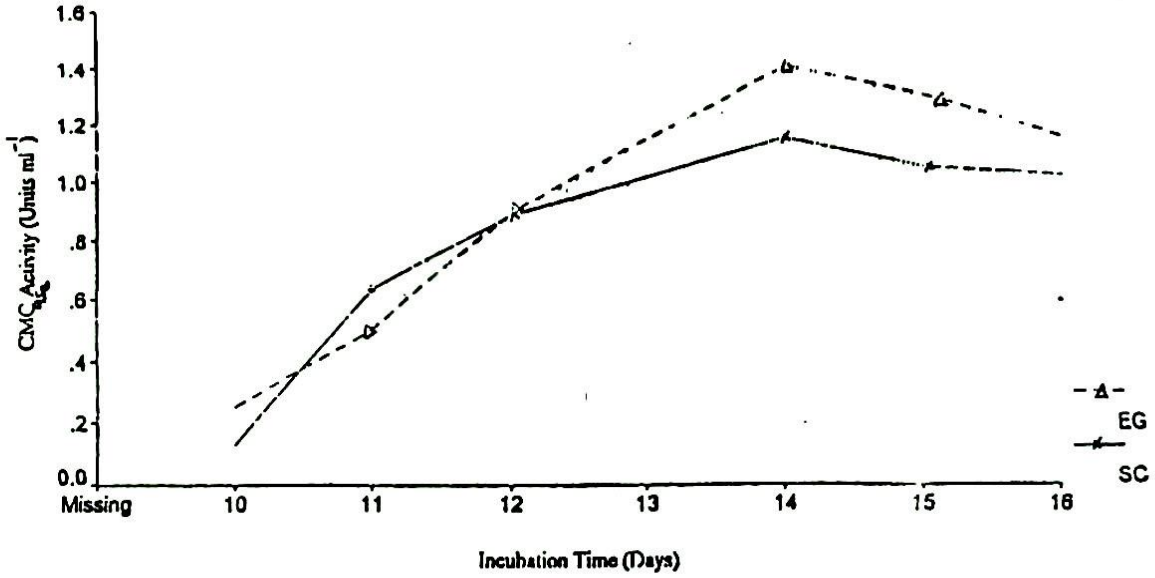

Fig. 1: Relationship between cellulase activity and incubation time of *S. viridosporus* T7A on pre-treated Elephant grass and sugar cane bagasse.

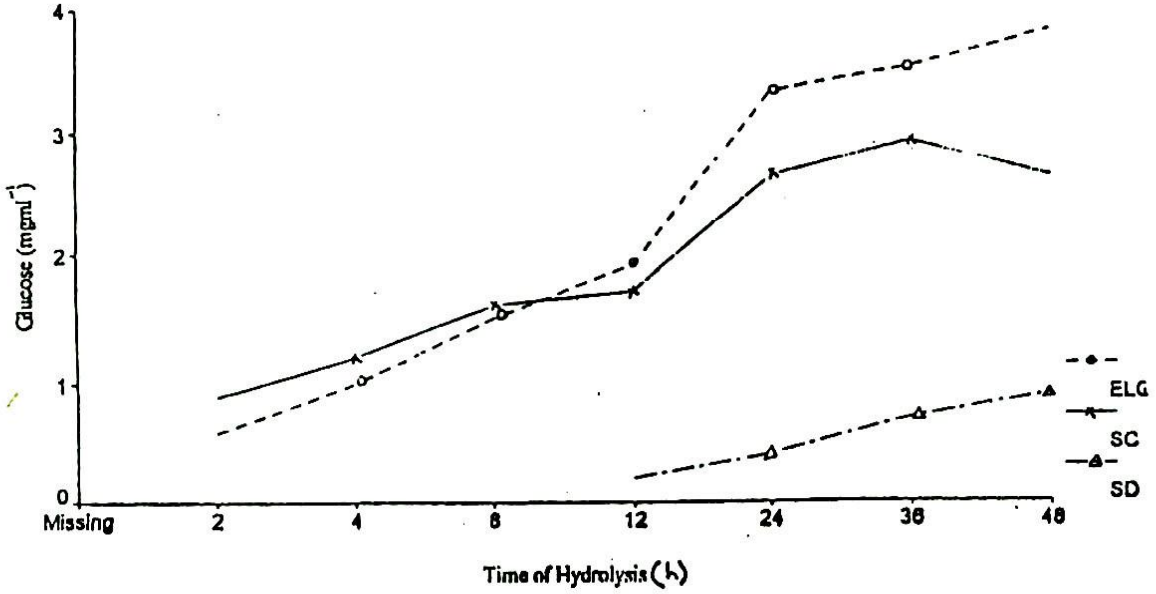

Fig. 2: Glucose produced during 48 hours hydrolysis of sugar cane bagasse (*Saccharum officinarum*) Elephant grass (*Panicum maximum*) and saw dust.

Saw dust recorded lowest value (1.5 unit/ml), an indication of low susceptibility of enzymes to saw dust components.

The chemical composition of the materials used, after pre-treatment is presented in Table 2. The percentages of lignin in saw dust was highest (30.1%), followed by sugar cane bagasse (20.2%) and then elephant grass (19.4%). This again gives possible explanation to low glucose value obtained from saw dust on Table 3 (low carbohydrate component and high lignin content). The saccharifying percentage for this cellulosic material (4.1%) is comparatively low because of the low susceptibility of the lignin in saw dust to enzymatic degradation. Sugar cane bagasse and elephant grass compared favourably in saccharifying percentages, glucose production and carbohydrates contents. Elephant grass seems the best among them with high potential for glucose production. However, sugar cane has been reported to be most susceptible to *Thermomonospora fusca* YX cellulase. With such report, studies on the hydrolysis of lignocellulosic materials is difficult to compare. The kind and concentration of enzyme and substrate, the condition of saccharification and the assays used for the determination could influence enzyme activities and reaction production according to Ericksson [22].

Cellulase is an inducible enzyme. Micro-organisms do not synthesise this enzyme unless cellulose is present in the environment [4, 5]. Thus, at initial stage of hydrolysis, (in the presence of large or optimal amount of cellulose substrate) the titre of cellulase increased. The rate of glucose production decreased with increased incubation time (Table 3, Fig 1). The enzyme activity relates to glucose production and this falls as incubation time progresses (corresponding with cellulose depletion). Glucose can be preferentially metabolised by micro-organisms and in large scale production, the combined action of induction and repression of cellulase may lead to low yields (and/or loss) of glucose obtainable from lignocellulosic hydrolysis [4]. This is why workers [3, 4] have suggested the use of catabolite repression resistant mutants in lignocellulosic hydrolysis. If genetically improved cellulytic organisms such as *Streptomyces spp* used in this study, is used, it will be free from catabolite repression. Hence cellulase induction can encourage large scale bioconversion of lignocellulosic materials, the largest \natural and renewable sources of raw materials on earth currently being discarded as wastes.

From this study, lignocelulosic wastes have the potential of being exploited not only as industrial raw materials but also as glucose energy base for man and animal feeds.

Glucose production from lignocellulosic waste can be unlimited source of energy. All living systems expend energy, not only for growth also to survive when not growing. This energy is ultimately derived from energy from the sunlight (solar energy) directly or indirectly through chemical transformation of organic molecules derived from photosynthesis [2, 3]. If this stored solar energy in lignocellulosic wastes can be adequately utilised through enzymatic hydrolysis and production of glucose, thus can help to harness the inexhaustible energy of the sun. A molecule of glucose is capable of yielding two molecules of ATP by fermentation. In aerobic respiration, ATP are produced from every molecule of glucose completely oxidised [2, 3]. In terms of the total free energy available from complete oxidation of glucose about 30% is recovered in aerobic pathway, the rest is released as heat In addition, glucose can be used to make solvents, plastics and other chemicals now made from petroleum. It can be converted into single cell protein or it can be fermented to a clean burning fuel such as ethanol [2].

5. CONCLUSION

The enzymatic conversion process of lignocellulosic substrates is one of the many options to be expected for harnessing solar energy stored in plants through photosynthesis. Conversion of only a fraction of the vast quantity of cellulose produced annually to such energy-rich storable material (glucose) which can be fermented for ethyl alcohol production can and undoubtedly will help us in sustaining industrial development and subsequently maintaining our way of life. With this we need not become subservient to any nation with regards to the supply of energy for our continued industrial progress and expansion. if we, through this know-how, ingenuity and dedication, are capable of developing practical and economic processes that can harness the inexhaustible energy of the sun, even in lignocellulosic wastes.

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