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Effect of temperature and changes in medium pH on enzymatic hydrolysis of β(1-4) glycosidic bond in orange mesocarp

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ABSTRACT

This study evaluates Orange mesocarp as a feed stock for production of glucose syrup using Trichoderma. reesei. The material was crushed to 100 – 150 µm*, 200 – 250* µm *and 300 – 425µm particle sizes and fed into a bioreactor where delignification was effected.Enzymatic hydrolysis of the substrates was carried out at different temperatures, 30^{o}C, 37^{o}C, 40^{o}C, 50^{o}C and 55^{o}C and the glucose produced at different temperature was analysed using Randox glucose kit and colorimeter. Medium of different pH was used for the enzymatic hydrolysis of orange mesocarp within the range of 3.0 – 6.0 using sodium acetate buffer. The optimal pH for the hydrolysis was evaluated by analysing glucose concentration produced at different pH level (Wen et al., 2004).Glucose concentration of 2.1mmol L^{-1}, 2.3mmol L^{-1}, 1.7mmol L^{-1}, 2.3mmol L^{-1} and 2.3mmol L^{-1} were obtained as the maximum glucose concentrations at temperatures 30^{o}C, 37^{o}C, 40^{o}C, 50^{o}C and 55^{o}C respectively. This indicates that the optimum temperature is 37^{o}C and denaturation begins at a reaction time of 20 hours at temperature of 55^{o}C. Optimum pH of 4.5 was observed for best enzyme activity.*

Key words: Orange Mesocarp, Glucose, *Trichodermareesei*

INTRODUCTION

Orange mesocarp, a waste product from citrus processing factories and farmers, is partly used for animal feed. However, a larger part of the citrus waste produced, (about 66 million tonnes) annually [1,2] is still being discarded to nature, causing environmental problem.

Orange mesocarp contains various carbohydrate polymers. Particularly, its cellulose content ranges between 30-60% [2,3] which makes it an interesting choice for production of metabolites such as fermentable sugars and ethanol by appropriate micro-organisms. Researchers have successfully converted many cellulosic materials such as saw dust, solid waste, crop residues, cotton stalks e.t.c [2,4,5,6,7,8] to more valuable products such as fermentable sugars.

Many fungi are capable of producing extracellular enzymes that can degrade cellulose. They are Trichodermareesei, T. viride, Penicillumfuniculosum, Fusariumsalani and so on. Bacterial species such as cellulomanas along with Clostridium thermocellum can also produce Cellulases [5].

Efficient enzymatic degradation of insoluble polysaccharides often requires a tight interaction between the enzymes and their substrates. In the case of Cellulose degradation, many cellulases are known to bind to crystalline and/ or amorphous cellulose via cellulose binding domain (CBDs) which are distinct from the catalytic domains [8].

pH is an important parameter in the production of enzyme by *T. reesei* |(Denison, 2000). Earlier reports indicated that a rather normal pH (7.0) is essential for good production of xylanases by *T. reesei*. Rut C-30 on cellulose- and xylan-based growth media, although growth (broth viscosity) was evidently better at pH 4.0 than at pH 7.0. Meanwhile, good production of cellulases was found at low pH (4.0) [9]. A neutral pH (7.0) was found essential for high Xylanase production by *Trichodermalongibrachiatum* in cellulose medium [10].

The goal of this research is to study the enzymatic hydrolysis of orange mesocarp by T. reesei and to carry out the effect of temperature and changes in medium pH on glucose concentration produced from orange mesocarp.

MATERIALS AND METHODS

Orange mesocarp were collected, ground into particle sizes (P_1, P_2, P_3). These particle sizes were pretreated by three distinct pretreatment agents (SAC); sodium hydroxide, ammonia and calcium hydroxide. Pretreatment breaks down the lignin, allowing access to the cellulose and hemicelluloses. This increases porosity and reduces cellulose fibrecrystalinity (tightly packed crystallites) [8]. The hemicelluloses and cellulose are also separated from each other allowing for more efficient access by hydrolysis enzyme. Applying the method used by Yakubu*et al*., (2001), 4g of orange mesocarp (OMP) was weighed, and pre-treated at varying pretreatment conditions (0.1M, 0.2M, 0.3M and 0.4M) Sodium hydroxide (NaOH) and calcium hydroxide $Ca(OH)_2$ at 100^oC and time (15, 20, 25 and 30mins) in different runs.

Additionally, the ammonia steeping method too was employed to delignify the orange mesocarp. The delignifiedmesocarp was treated with 0.3M HCl acid at 100^0C for 1 hour to remove hemicelluloses. The pre-treated cellulose was washed with de – ionized water to remove residual acid. All samples were dried in an oven at 50^oC for 48 hours and kept in the laboratory stock for further use.

In a typical run,the temperature of the water bath (sharmond model) was set at 37^0c, a hundred milliliters (100ml)of 0.1M sodium acetate buffer solution (pH 4.5) was poured into an Erlenmeyer flask fitted with stimrig mechanism. 0.1g of isolated cellulose enzyme and 2.0g of pre-treated orange mesocarp of different particle sizes were added. 40 micro litres of each sample were withdrawn every 4hrs within 58 – 72hrs reaction time for analysis. The glucose concentration in the sample was determined by using Randox glucose kit and colorimeter (Model WPA, 5001, USA) at 540nm according to Lee and Fan, (1982) and Lee, (1992; 2002). Each run was repeated three times and the average was taken to assume accuracy. In separate runs, the effect of temperature and changes in the medium pH on extent of hydrolysis was studied.

Enzymatic hydrolysis of the substrates was carried out at different temperatures, 30^oc, 37^oc, 40^oc, 50^oc and 55^oc and the glucose produced at different temperature was analysed using Randox glucose kit and colorimeter. Medium of different pH was used for the enzymatic hydrolysis of orange mesocarp within the range of 3.0 – 6.0 using sodium acetate buffer. The optimal pH for the hydrolysis was evaluated by analysing glucose concentration produced at different pH level [11].

RESULTS AND DISCUSSION

Effect of Temperature

The effect of temperature on glucose concentration from the hydrolysis process is shown in Figure 1. Temperatures of 30^oC, 37^oC, 40^oC, 50^oC and 55^oC were studied and glucose concentration of 2.1mmol L^{-1}, 2.3mmol L^{-1}, 1.7mmol L^{-1}, 2.3mmol L^{-1} and 2.3mmol L^{-1} were obtained as the maximum glucose concentrations produced respectively. This indicates that the optimum temperature is 37^oC and denaturation begins at a reaction time of 20 hours at temperature of 55^oC.

Table 1: Effect of Temperature on glucose concentration

Time /hr	Temperature ^{0}C				
	30^{0}C	37^{0}C	40^{0}C	50^{0}C	55^{0}C
	Glucose Concentration mmol/L				
0	1.2804	1.4938	1.0715	1.4938	1.2805
4	1.7072	1.9206	1.2805	1.9206	1.4938
8	1.9206	2.134	1.4938	2.134	1.7072
10	1.9206	2.134	1.4938	2.134	1.7072
24	2.134	2.3474	1.7072	2.134	2.3474
28	2.134	2.3474	1.7072	2.3474	1.7072
30	2.134	2.3474	1.7072	2.3474	1.7072
34	2.134	2.3474	1.7072	2.3474	1.7072
48	2.134	2.3474	1.7072	2.3474	1.7072
52	2.134	2.3474	1.7072	2.3474	1.7072
58	2.134	2.3474	1.7072	2.3474	1.7072

Isolated cellulose enzyme=0.1g
Substrate=2.0g
pH=4.5
λ=540nm
size=P1-P2

Aderemi*et al*., (2008) also reported an optimum glucose yield at 50^{o}c on the hydrolysis of rice straw. Tengbo*et al*., (2001) also reported an optimum glucose yield at 50^{o}C on the hydrolysis of softwood. The hydrolysis of animal lignocellulosics by Wen *et al*., (2004) was found to have an optimum glucose concentration at 50^{o}C. This is also in agreement with the results obtained from the hydrolysis of skop (fibre waste) and cellolignin reported by Castellanos *et al*., (1995) which showed optimum glucose concentration at 50^{o}C. From the result in figure 1, glucose concentration was at a maximum (2.3mmol L^{-1}) in the 24th hour at 37, 50 and 55^{o}c and decreased later in 55^{o}c due probably to enzyme denaturation.

Effect of pH

The rate of an enzyme reaction is strongly influenced by the pH of the reaction solution both in vivo and in vitro. The effect of pH in the hydrolysis of orange mesocarp-cellulose as studied is shown in Table 2. The result reveals that the pH value of 4.5 gives the optimum concentration of glucose with a mean value of 2.1340 as against 1.8818 of pH 5.5. Aderemi*et al*., (2008) gave optimum glucose yield at pH 4.5 – 5.0; while Tengbor*et al*., (2001) gave optimum glucose yield as pH 4.8.

Table 2: Effect of Medium pH

Time/hr	pH/ Glucose Concentration mmol/L					
	3.0	3.5	4.5	5.0	5.5	6.0
0	1.7072	1.2804	1.4938	1.4938	1.7072	1.4938
4	1.9206	1.4938	1.9206	1.7072	1.9206	1.7072
8	2.134	1.9206	1.9206	1.7072	1.9206	1.9206
10	2.134	1.9206	1.9206	1.7072	2.134	1.9206
24	2.134	1.9206	2.134	1.4938	2.3474	2.134
28	1.7072	1.7072	2.3474	1.2804	2.134	1.7072
30	1.7072	1.2804	2.3474	1.2804	1.7072	1.4938
34	1.7072	1.2804	2.3474	1.2804	1.7072	1.4938
48	1.7072	1.2804	2.3474	1.2804	1.7072	1.4938
52	1.7072	1.2804	2.3474	1.2804	1.7072	1.4938
58	1.7072	1.2804	2.3474	1.2804	1.7072	1.4938

Isolated cellulase enzyme=0.1g
Substrate=2.0g
Temp=37^{0}C
λ=540nm
size=P1-P2

CONCLUSION

This study reveals that optimum temperature value for enzymatic activity is 37^{o}C and optimum pH of 4.5 was observed for best enzyme activity.

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