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Effect of particle size on the hydrolysis of β(1-4) glycosidic bond in orange mesocarp (*Citrus sinensis*) by trichoderma reesei for glucose production

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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. The orange mesocarp, enzyme, and buffer were both incubated at 37^{0}C together in different substrate particle sizes. The samples were agitated at intervals. Samples were drawn for glucose analysis using a 1ml cuvette. The result shows that as the particle size range reduces from (300-425) µm through (100-150) µm, the glucose concentration increased from 1.06mmol/L to 3.4mmol/L.*

Key words: Orange Mesocarp, Glucose, *Trichoderma reesei*

INTRODUCTION

Sweet orange (Citrus Sinensis) production in Nigeria is significant, with heavy direct consumption due primarily to few and small capacity processing industries which convert the fruits to juice, concentrates and canned fruit. Nigeria produces 3% (1.98million) tones of fresh citrus in the world, and Africa produces 5.6% (3.741million) tonnes of varieties of citrus fruits of which Nigeria contributes 3,240,000 tonnes [4,8]. 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 [9,10] is still being discarded to nature, causing environmental problem.

Orange mesocarp contains various carbohydrate polymers. Particularly, its cellulose content ranges between 30-60% [7,10], which makes it an interesting choice for production of metabolites such as fermentable sugars and ethanol by appropriate micro-organisms. As it is the case of most existing biological fermentation process, the major setbacks in the development of cellulosic conversion technology are primarily the complex nature of the cellulose structure and it's resistance to degradation, due to hydrogen bonding. Other difficulties include the limited number of commercially available enzymes capable of digesting cellulose and the high enzyme loading requirement, coupled with the long period usually needed to attain an appreciable conversion level [1,6]. In other to address these problems, researchers have alternatively used soluble cellulose, filter paper, saw dust and animal solid wastes, obtaining impressive results [5,12].

Trichoderma reesei is a mesophilic and filamentous fungus. It is an anamorph of the fungus *Hypocrea jecorina*. *T. reesei* has the capacity to secret large amount of cellulolytic enzymes (cellulases and hemicellulases). Microbial cellulases have industrial application in the conversion of cellulose, a major component of plant biomass, to glucose.

Recent advancement in the biochemistry of cellulose enzymology: the mechanism of cellulose hydrolysis, strain improvement, molecular cloning and process engineering are bringing *T. reesei* cellulases closer to being a commercially viable route to cellulose hydrolysis. Major advances have been made in the isolation of Trichoderm mutants [2].

Novozyme reported studies on enzymatic hydrolysis of cellulose using *Trichoderma reesei* cellulase. Also enzyme and chelating agents in cotton pretreatment has been reported by Emilia *et al*.,(2001). Waag et al., (2005) studied the efficient cellulose production from corn straw by *Trichoderma reseei*. Aderemi et al., (2008) studied the kinetics of glucose production from rice straw by *Aspergillus niger*. Little information, however, exist in literature concerning the effect of particle size on hydrolysis of glycosidic bond of orange mesocarp. It is therefore the goal of this research to study the conversion of a chemically treated orange mesocarp to glucose using cell of *Trichoderm reesei*.

MATERIALS AND METHODS

Orange mesocarp were collected, ground into particle sizes (P_1, P_2, P_3).The particle sizes of the orange mesocarp was selected by passing them through different sieve sizes. 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 fibre crystalinity (tightly packed crystallites) (McMillan, 1994). The hemicelluloses and cellulose are also separated from each other allowing for more efficient access by hydrolysis enzyme. Applying the method used by Yakubu etal, (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.

In a typical run 4g of OMP was weighed into a 250ml conical flask and 20ml of 0.1M NaOH was added. This was heated in a hot plate with stirrer model (MR 3000 1k) at 100^oC for 15munites. Then the pretreated orange mesocarp was washed free of base with de-ionized water. Then all samples were dried in an oven model M250-VF at 50^0C for 48hrs. The samples were then kept in white polyethylene bags in the laboratory stock for further use.

Additionally, the ammonia steeping method too was employed to delignify the orange mesocarp. The delignified mesocarp 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.

The treated particle size ranges (100-150µm, 200-250µm, and 300-425µm) alongside the untreated ones of the same size range was then hydrolysed and analysed for glucose production (Wen et al., 2004). The orange mesocarp (substrate), enzyme, and buffer were both incubated at 37^0C together in different substrate particle sizes. The samples were agitated at intervals and after 4 hours interval, up to 72 hours, samples were drawn for glucose analysis using a 1ml cuvette. Into the 1ml cuvette, 40µl of sample and 400 µl of glucose reagent was added and absorbance was taken at 540nm using a Jenway Colorimeter (Model 6051). A control (substrate and buffer) otherwise called spectro-zero was used to set the colorimeter at zero absorbance. The linear glucose standard was used to translate the absorbance values into glucose concentration equivalence. Glucose concentration from the absorbance values was calculated. Then the effect of particle size was studied.

RESULTS AND DISCUSSION

Particle size ranges of 100 – 150 µm; 200-250 µm and 300 – 425 µm were studied. The corresponding result of this study is presented in Tables 1,2 and 3.

From the results, it shows that as the particle size range reduces from (300-425) µm through (100-150) µm, the glucose concentration increased from 1.06mmol/L to 3.4mmol/L. This may be due to the following reasons:

- Increase in surface area available for enzyme attack [1].
- Increase in activity of the enzymes.
- Increase in the accessibility of glycosidic bond sites to the cellulose complex [7].

- Availability of cellulose in the medium.
- Elimination of air-liquid interface by covering the reactor.
- There is reduced enzyme deactivation due to covering the reactor, avoiding the enzyme surface interaction and by reduced shaking of the reactor.

Table 1: Effect of Particle size in $Ca(OH)_2$ Treated Mesocarp

Time/hr	P1	P2	P3	U1	U2	U3
0	0.2134	1.4938	0.8536	0.4268	0.8538	0.4268
4	1.2804	1.9206	2	1.067	1.0715	0.6402
8	3.201	1.9206	2.314	1.4938	1.7072	1.067
10	3.4144	1.9206	2.3474	1.7072	1.9206	1.2804
24	3.201	2.134	2.134	1.7072	1.9206	1.7072
28	3.201	2.3474	2.134	1.7072	1.9206	1.7072
30	3.201	2.3474	2.134	1.4938	2.134	1.7072
32	3.201	2.3474	2.134	1.4938	2.134	1.7072
34	3.201	2.3474	2.134	1.4938	2.134	1.7072
48	3.201	2.3474	2.134	1.4938	2.134	1.7072
52	3.201	2.3474	2.134	1.4938	2.134	1.7072
58	3.201	2.3474	2.134	1.4938	2.134	1.7072
72	3.201	2.3474	2.134	1.4938	2.134	1.7072

Isolated cellulase enzyme=0.1g
Substrate=2.0g
pH=4.5
Temp=37^{0}C
λ=540nm
size=P1-P2
U1, P1 = 100-150µm
U2, P2= 200-250 µm
U3,P3= 300-425 µm
U= Untreated Orange Mesocarp
P= Treated Orange Mesocarp

Table 2: Effect of Particle size in Ammonia Treated Mesocarp

Time /H	P1	P2	P3
0	0.4268	0	0.2134
4	2.3474	1.2804	1.2804
8	2.7742	1.9206	1.9206
10	2.5608	2.3474	2.134
24	2.3474	1.4938	1.067
28	1.9206	1.4938	1.067
30	1.9206	1.4938	1.067
32	1.9206	1.4938	1.067
34	1.9206	1.4938	1.067
48	1.9206	1.4938	1.067
52	1.9206	1.4938	1.067
58	1.9206	1.4938	1.067
72	1.9206	1.4938	1.067

Isolated cellulase enzyme=0.1g
Substrate=2.0g
pH=4.5
Temp=37^{0}C
λ=540nm
size=P1-P2
P1 = 100-150µm
P2= 200-250 µm
P3= 300-425 µm

Low glucose production after optimum value may probably be as a result of conversion of formed sugar into other products [5,13]. This is in agreement with the work reported by Aderemi *et al* (2008) on the hydrolysis of rice straw for glucose production, where it was observed that as the particle size was reduced from 425 to 75µm the glucose concentration rose from 3.2mg/dl to 5.0mg/dl.

Table 3: Effect of Particle size in Sodium Treated Mesocarp

Time /H	P1	P2	P3
0	0.6402	0.2134	1.2804
4	1.4938	1.4938	1.4938
8	1.9206	1.7072	1.4938
10	1.9206	1.7072	1.4938
24	1.9206	1.7072	1.7072
28	1.9206	1.4938	1.7072
30	1.7072	1.4938	1.9206
32	1.7072	1.4938	1.9206
34	1.7072	1.4938	1.9206
48	1.7072	1.4938	1.9206
52	1.7072	1.4938	1.9206
58	1.7072	1.4938	1.9206
72	1.7072	1.4938	1.9206

Isolated cellulase enzyme=0.1g
Substrate=2.0g
pH=4.5
Temp=37^{0}C
λ=540nm
size=P1-P2
P1 = 100-150μm
P2= 200-250 μm
P3= 300-425 μm

The results show that, for all the three particle size ranges, 100-150, 200-250 and 300-425 micrometer, the 100-150 micrometer particle size yielded the highest glucose concentration due to increased surface area.

CONCLUSION

The study clearly reveals that particle size of the substrate (Orange mesocarp) affects the concentration of glucose produced from it. As the particles size range reduces, the glucose concentration increases.

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