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Production of Fermentable Sugars from Organosolv Pretreated Cassava Peels

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Abstract

Cassava peels are rich in lignocellulolytic materials which are not readily amenable to enzymatic hydrolysis; hence, there is a need for a suitable pretreatment method that will support enzymatic hydrolysis. This study was designed to investigate lignocellulolytic organisms that would effectively support the bioconversion of organosolv pretreated cassava peels to fermentable sugars. Decaying cassava peels were collected into sterile bottles and microorganisms isolated, characterized and screened for lignocellulolytic enzymes production. Optimum temperature, pH and nutrient sources for enzyme production were determined. Organosolv pretreatment was carried out using methanol with varied concentration of catalyst (0.01 - 3 M), reaction time (15 - 60 min) and substrate size. Crude enzymes (cellulase and xylanase) from the isolates were added to the pretreated peels and bioconversion was monitored by measuring the concentration of reducing sugar and calculating the percentage peel hydrolysis. The fermentable sugars produced were quantified using gas chromatography. *Pseudomonas fluorescens* and *Aspergillus terreus* were isolated. *P. fluorescens* produces 2.8 u/mL of crude enzymes optimally at 50˚C and pH 8 while *A. terreus* produces 3.4 u/mL optimally at 40˚C, pH 6. Both isolates utilizes CarboxyMethylCellulose (CMC) and yeast extract as their best carbon and nitrogen sources. Highest percentage of peel hydrolysis was 67% for *P. fluorescens* at 0.01 M and 0.05 M for *A. terreus* (94%). Highest concentration of fermentable sugar was produced by *A. terreus* crude enzyme (331.79 mg/L glucose, 45.3 mg/L rhamnose and 46.52 mg/L xylose). *P. fluorescens* and *A. terreus* effectively supported the bioconversion of organosolv pretreated cassava peels to fermentable sugars.

Keywords

Cassava Peels, Lignocellulose Bioconversion, Organosolv Pretreatment, Fermentable Sugars

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1. Introduction

Cassava peels are the main by-product from processing tuberous roots of cassava for human consumption. Peels are regarded as wastes and are usually discarded and allowed to rot and are generally classified as an agricultural biomass or lignocellulolytic biomass. Cassava peels have been evaluated as a feedstuff for animals [1], for the production of biogas, biofuels and ethanol and also as substrates for microbial protein enrichment [2]. The lignocellulolytic properties of cassava peels enhance its usefulness as a source of fermentable sugars after bioconversion. The peel is made up of cellulose, hemicelluloses and lignin but the structures of these materials are complex with recalcitrant and heterogeneous characteristics and are highly resistant to enzymatic hydrolysis; thus there is a need to pretreat the peels thereby making the enzymes amenable to the sugars embedded in the cellulose and hemicelluloses component of the peel [3].

Pretreatment is an important step for the bioconversion of lignocellulosic biomass. An effective pretreatment disrupts cell wall physical barriers as well as cellulose crystallinity and association with lignin so that hydrolytic enzymes can access the biomass macrostructure [4]. Many methods have been developed for pretreating lignocellulosic biomass but only few are promising for industrial purposes [5].

Some of the methods used for pretreating lignocellulolytic biomass are acid hydrolysis, alkaline hydrolysis, hydrothermal, AFEX, among others.

The organosolvation method is a promising pretreatment strategy, and it has attracted much attention and demonstrated the potential for utilization in lignocellulosic pretreatment [6]. The method involves the process of extracting lignin from lignocellulosic biomass with organic solvents or their aqueous solutions [6]. Common solvents used for the process include ethanol, methanol, acetone and ethylene glycol [7]. Also, organic acids such as oxalic, acetylsalicylic, and salicylic acids can be used as catalysts in the organosolvation process [8]. Temperatures used for the process can be as high as 200˚C, but lower temperatures are desirable depending on the type of biomass and the use of a catalyst [9].

The aim of this study is to obtain fermentable sugars from organosolv treated cassava peels using hydrolysis of microbial enzymes obtained from lignocellulolytic microorganisms.

2. Materials and Methods

2.1. Preparation of Substrate

Cassava peels were obtained from the processing sites and they were thoroughly sorted to remove dirts and dried in an oven at 80˚C for 24 hours. These substrates were used for the experimental work.

2.2. Microorganisms

Pseudomonas fluorescens SC1 and *Aspergillus terreus* SC9 used in this study were obtained from isolation of decaying cassava peels in the laboratory using nutrient agar and malt extract agar respectively. After obtaining the isolates, morphological and biochemical characterisation was done for the purpose of identification. The organisms were maintained on slant and kept at 4˚C for further use.

2.3. Organosolv Pretreatment of Cassava Peels

Cassava peel (2%) was suspended in 100 mL of methanol with varying concentrations of sodium acetate (0.01 M to 3 M) as catalyst. After treatment the solid residues were collected and washed under running tap water for 10minutes prior to enzymatic hydrolysis [10].

2.4. Preparation of Crude Enzyme Solution

Pseudomonas fluorescens and *Aspergillus terreus* was cultivated in 100 mL of basal medium with the following composition KH_2PO_4 10 g/L $(NH_4)_2SO_4$ 10.5 g/L, $MgSO_4 \cdot 7H_2O$ 0.33 g/L, $CaCl_2$ 0.5 g/L, $ZnSO_4 \cdot 7H_2O$ 0.004 g/L, $FeSO_4 \cdot H_2O$ 0.0013 g/L, $MnSO_4 \cdot H_2O$ 0.004 g/L, $CaCl_2 \cdot 6H_2O$—0.0067 g/L peptone 0.4 g/L with 1% carboxylmethylcellulose powder (CMC). The medium was inoculated with 5 mL of *Pseudomonas fluorescens* culture and 1×10^5 spores/cells of *Aspergillus terreus* respectively. After growth, the suspension was centrifuged at 10,000 rpm for 10 min at 4˚C using refrigerated centrifuge (IEC centre, MP4R model).

2.5. Enzymatic Hydrolysis of Cassava Peels

Pretreated cassava peels (1.5%) was suspended in basal medium (1 g/L yeast extract, 1 g/L $MgSO_4 \cdot 7H_2O$, 1 g/L $(NH_4)_2SO_4$, 0.5 g/L KH_2PO_4. Crude enzymes obtained from *Aspergillus terreus* and *Pseudomonas fluorescens* was used for the hydrolysis. The enzymatic hydrolysis was studied to determine the optimal condition by varying catalyst concentration (0.01 M to 3 M), size of substrate, reaction time (15 - 60 min). The culture was incubated in a GALLENKAMP rotary shaker at 50 rpm for 48 hr at 30˚C. The percentage peel hydrolysis was calculated by applying the method of Onilude [11]:

$$\%\ \text{peel hydrolysis} = \frac{\text{reducing sugars produced by growth} - \text{reducing sugar in content}}{\text{reducing sugar in control}} \times 100.$$

Amount of reducing sugar produced was determined using the dinitrosalytic acid (DNSA) method [12]. Fermentable sugars concentration was measured using gas chromatography (HP 6890) using a BPX70 column and a flame ionisation detector [13]. All determinations were replicated three times.

3. Results

3.1. Identification of Isolates

Pseudomonas fluorescens was a Gram negative, rod shaped organisms with raised elevation, rough edges and cream colour on nutrient agar plate. It was oxidase positive, catalase positive and does not ferment glucose.

Aspergillus terreus was identified based on observable characters on the culture plates and under the microscope. It has a columnar head with a yellowish brown reverse. The colony diameter is 2.0 to 2.5 cm and the conidia is globose and in long chains making a compact column of uniform diameter.

3.2. Optimal Growth Conditions for Crude Enzyme Production

Pseudomonas fluorescens produced highest concentration of crude enzymes optimally at 40˚C, pH 8 using carboxylmethylcellulose (CMC) and yeast extract as its best choice of carbon and nitrogen source respectively, while *Aspergillus terreus* produce optimally at 40˚C, pH 6 and also utilizing CMC as its carbon source and yeast extract as its nitrogen source. Other optimal growth condition *P. fluorescens* produces 2.5 mg/mL of enzyme while *A. terreus* produce 3.4 mg/mL (**Table 1**).

3.3. Effect of Reaction Time

Pseudomonas fluorescens was able to hydrolyse the pretreated peel for the production of fermentable sugar optimally after 30 minutes exposure (78%) while *Aspergillus terreus* had 98.8% peel hydrolysis 45 minutes of exposure (**Table 2**).

3.4. Peel Hydrolysis

Pseudomonas fluorescens crude enzymes inoculated on organosolv treated cassava peels produced highest percentage of peel hydrolysis at 0.01 M concentration of catalyst (66.6%), for *Aspergillus terreus*, highest percentage peel hydrolysis was produced at 0.05 M concentration (94.4%) (**Table 3**). Milled organosolv treated cassava peels were better hydrolysed by isolates enzymes achieving 78% and 94% of peel hydrolysis and optimum percentage peel hydrolysis was achieved at 45 minutes reaction time for both isolates (75% and 98%) respectively (**Table 4**). After optimization of the various conditions for the bioconversion of organosolv treated cassava peels, further analysis was done using gas chromatography.

Pseudomonas fluorescens inoculated on organosolv treated cassava peels produced 246.01 mg/L glucose, 31.96 mg/L rhamnose and 39.11 mg/L of xylose while organosolv treated cassava peels inoculated with *A. terreus* crude enzyme produces 331.79 mg/L of glucose, 45.39 mg/L rhamnose and 46.52 mg/L xylose (**Table 5**).

4. Discussion

Isolating *Pseudomonas fluorescens* and *Aspergillus terreus* from decaying part of cassava peels obtained was in agreement with the work of Hoorman [14] and Gupta and Mukergi [15] both stated that these organisms are

Table 1. Optimal growth condition for enzyme production.

	pH	Temperature ˚C	Nitrogen source	Carbon source
Pseudomonas fluorescens	8	40	Yeast extract	Carboxymethylcellulose
Aspergillus terreus	6	40	Yeast extract	Carboxymethylcellulose

Table 2. Effect of reaction time on 0.05 M organosolv pretreated cassava peel hydrolysed by *Pseudomonas fluorescens* and *Aspergillus terreus*.

27˚C	**15 min**	**30 min**	**45 min**	**60 min**
Pseudomonas fluorescens	65.5	78	75	67
Aspergillus terreus	65.5	78	98.8	77

Table 3. Percentage peel hydrolysis of *Pseudomonas fluorescens* and *Aspergillus terreus* on different concentrations of Organosolv pretreated cassava peels.

Concentration	*Pseudomonas fluorescens*	*Aspergillus terreus*
0.01 M	50 ± 2.6	44.4 ± 3.4
0.05 M	66.6 ± 2.2	94.4 ± 3.7
0.1 M	55.5 ± 2.3	66.6 ± 3.8
0.2 M	55.5 ± 3.1	50 ± 3.2
0.25 M	50 ± 2.6	61.1 ± 3.7

Table 4. Effect of sample size on reducing sugar production by *Pseudomonas fluorescens* and *Aspergillus terreus* (%).

	Pseudomonas fluorescens	*Aspergillus terreus*
Milled cassava peel	78	94
Unmilled cassava peel	67	92

Table 5. Production of fermentable sugars from organosolv treated cassava peels.

mg/L	**HMF**	**RIBOSE**	**XYLOSE**	**ARABINOSE**	**RHAMNOSE**	**FRUCTOSE**	**GLUCOSE**	**MALTOSE**	**LACTOSE**	**SUCROSE**
0.05M	_	6.3	2.71	4.16	9.51	1.21	47.6	5.68	5.1	9.8
0.05MB	1.71	8.57	39.11	4.5	31.96	1.21	246.01	5.68	5.12	1.51
0.05MF	3.64	6.98	46.52	5.83	45.39	1.21	331.79	5.68	5.1	9.69

Keys: M—organosolv pretreated peels alone; MB—organosolv pretreated peels inoculated with *Pseudomonas fluorescen*; MF—organosolv pretreated peels inoculated with *Aspergillus terreus.*

natural inhabitants of the soil and decaying litters. Converting cassava peels into value-added products like fermentable sugars provides a potential alternative for treatment and disposal of the cassava peels. There are various type of pretreatment used in converting lignocelluloses materials but the type of method used or chosen must have a significant effect on the yield of reducing sugar.

Organosolv pretreatment method involves the use of an organic solvent like methanol with the addition of a catalyst. This method effectively removes the lignin of the lignocelluloses biomass before enzymatic hydrolysis. In addition to lignin removal, hemicelluloses hydrolysis occur leading to improve enzymatic digestibility of the cellulose fraction [7]. The crude enzymes of *Pseudomonas fluorescens* and *Aspergillus terreus* used for this research were able to hydrolyse the organosolv pretreated cassava peels for maximum reducing sugar production at reduced temperature and also at low concentration of catalyst usage making the method to be highly cost effective and efficient for fermentable sugar production. Ghose *et al.* [9] and Sun and Cheng [16] confirms in their researches that lower temperature is sufficiently favourable for the production of high yield of fermentable sugars using organosolv method prior to enzymatic hydrolysis.

Furthermore, taking into consideration the size of the cassava peels, it was observed that milled cassava peels prior to pretreatment and enzymatic hydrolysis teach to increase in the accessibility of the microbial enzymes thus bringing about efficient hydrolysis. Mohammed *et al*. [17] reported that milling of lignocellulolytic biomass improves susceptibility of the cassava peel to enzymatic hydrolysis because the particle size and degree of crys-

tallinity of the cassava peels has been reduced.

The predominant fermentable sugars produced after enzymatic hydrolysis of organosolv treated cassava peels is glucose followed by xylose and rhamnose. This was in agreement with the findings of Patiwat and Chalerm [18] who states that among these hydrolytic products, glucose is normally the most abundant, followed by xylose and other lower concentration sugars. During fermentable sugar production, the concentration of by-products like 5-hydroxylmethyl furfural (5-HMF) produced was greatly insignificant in organosolv pretreated peels, this will make the fermentable sugars produced to be suitable for further processing especially for biofuels production.

Combining enzymatic hydrolysis with organosolv pretreatment of the cassava peel leads to an increase in the level of fermentable sugars produced. Lin and Tunaka [19] explained that one of the advantages of enzymatic hydrolysis was the reduced number of side reactions that accompanies the process and also because of the higher conversion yield compared to using only chemical pretreatment.

5. Conclusion

Crude enzymes of *Aspergillus terreus* produced higher concentration of fermentable sugar than *Pseudomonas fluorescens* with minimal by products formation. Therefore, combination of enzymatic hydrolysis and organosolv pretreatment technique are promising techniques for the production of fermentable sugars from cassava peels.

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