Studies on the Combination of Some Selected Microorganisms for Biodegradation of Cassava Peel Wastes (*Manihot Esculenta*)

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Abstract: Cassava peel has a lot of nutrients which can be utilized as fertilizer in agriculture. However, high cellulose contents may cause difficulty for organic digestion and slow down the nutrient absorption for plants. This study aims to reach the microorganisms or their combination for biodegrading the cassava peel waste. The initial result shows that after 21 days the glucose concentration in all samples was the highest during experiment times. The reducing glucose produced in mg/mL by the selected bacteria was 9.32, higher than the selected fungus of 1.57 mg/mL. The percentage degradation of cassava peel weight was the highest for fungi which was 52% of weight, respectively. Meanwhile, bacteria only degraded about 31.6% after 4 weeks. However, the combination between bacteria and fungi provides the highest glucose concentration, 19.29 mg/mL and digests 62% of the mass cassava in experiment. This study suggests that biodegradation of agro-wastes in the environment to reduce biomass waste pollution is very promising.

Key words: Biodegradation, bacteria, cassava peel wastes, fungi.

1. Introduction

Cassava (*Manihot esculenta*) is one of the most important crops in many tropical countries, including Vietnam. Cassava area in Vietnam reached 560,000 ha in 2014, which nearly doubled the figure in 2000 [1]. The constant expansion has changed cassava role in Vietnam from food crops to industrial crops.

After the process of starch production, the plant has generated a huge amount of solid waste such as cassava bagasse and cassava peel. Cassava bagasse is available for many applications, including reuse as feed livestock or mushroom growing substrate. However, cassava peel in Vietnam haven't been used or recycled much; the current treatment is often discarding or landfill.

Landfill method is not only a waste of valuable materials but also affects environment due to bad odor emitted from untreated cassava peel after fermentation. Furthermore, it requires a large disposal area because peel occupies with 20-35% of the total weight of tuber. The increasing of annual amount of peel waste and their accumulation are a major source of pollution.

The problem of pollution from the industrial, agriculture or other sectors of the economy growing, and the number of research that aim to improve the use of waste also occur more conversion of cassava peel by the organism isolation means that these waste could be used as raw materials in industries for valuable added product. This will reduce wastage and improve economic profit. Although it is economically and technologically possible, emphasis should be put on the utilization of waste materials for recycling and upgrading purposes.

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On the other hand, from the agriculture perspective, cassava peel waste utilization as organic fertilizer would be very useful while chemical fertilizer prices are increasing. Some researches show that organic amendments could be used for enhancing soil fertility, planting nutrient status, saving cost of secondary- and micro-nutrients requires for obtaining good yields and leads to less environmental pollution. Consequently, it was proved to have significant increase in yield application on the rice crop [2, 3]. Thus, based on the demand for organic fertilizers, the overall aim of this study is on the treatment and the utility of cassava peel waste into organic fertilizer. In order to reuse cassava peel waste as an organic fertilizer, the priority is to degrade the cellulose component in cassava peel.

Some studies have shown that cellulase enzymes from microorganisms such as fungi, bacteria have cellulose-degradation ability to cause significant changes in the nutritional composition of cassava peel waste like increasing glucose, nitrogen [4]. Beside, cellulase enzymes also help reducing the breakdown time of cellulose in environment [5].

Cassava Starch Factory in Vietnam is facing a difficulty due to the significant increasing of the discarded cassava peel waste volume, which generates bad odor and increases risk of disease outbreaks. Biodegradation of agricultural waste by microorganisms is an acceptable method which results in useful end product and reduces environmental pollution.

Thus, the objective of the study is how to biodegradable cassava peel waste into organic fertilizer using biological method. This study is the next step after the isolation and selection of the best microorganism strains, and the authors will test the breakdown of cellulose by cellulase enzyme secretion activity of each selected strains of microorganisms on the cassava peel and evaluate combined capabilities of microorganisms to break down cassava peel in the shortest possible time.

2. Materials and Methods

2.1 Materials

2.1.1 Substrate

Cassava peel was taken from Hung Duy 2 Cassava Starch Plant in Chau Thanh District, Tay Ninh Province. They were cut into pieces, which were crushed and sifted through a filter 0.154 mm. The substrates were then separately at room temperature in different foam boxes for further use.

2.1.2 Culture Media

All culture media (Gauses, Vinogradski, Czapek-dox and Carboxyl Methyl Cellulose (CMC) Agar) were prepared in accordance with the manufacturers' specification, homogenized and thereafter sterilized by autoclaving at 121 °C for 15 mins. Streptomycin (0.1%) and Penicillin (0.1%) were added to the Czapek-dox media to prevent bacterial contamination.

2.2 Methods

2.2.1 Microorganisms Isolation and Identification

The microbial strains were isolated from cassava peel waste on culture medium Gauses, Vinogradski, Czapek-dox for actinomycetes, bacteria, fungi. After the isolation and selection, the authors identified four strains of microorganisms with the best activity to perform in this study. The identifications were done at Nam Khoa laboratory by using PCR amplify and sequence the 16S rRNA and 28S rRNA, and then by using BLAST to search the database and identifying the species that mostly matched their sequence results for the genes.

2.2.2 Biodegradation of Substrates

Biodegradation was proceeded by weighing 1 g crushed cassava peel added 150 mL liquid medium corresponding to the strains of microorganisms isolated in 250 mL flask, incubated at 28 ± 2 °C within 28 days. Each flask sterilized at 121 °C for 15 minutes, and then was allowed to cool and added 0.1 µL liquid biomass of pair microorganisms (each flask 2 strains). Beside, the control flasks were done but

adding media without microorganisms. The evaluation of biodegradable by filtering sediment (cassava peel) and dried in an oven at 103-105 °C for 1 hour, 1 hour in the extraction hood and then weighed to determine the amount of cassava peel after biodegradation. The experiments were evaluated based on a cycle of 7 days/time and repeated 3 times ($10 \times 3 = 30$ erlen for a week, 120 erlen for total 4 weeks).

2.2.3 Analytical Method (Enzyme assay)

Qualitative assessment by the color changes when adding lugol (iodine) and CMC's biodegradable strains were compared based on the extent of ring diameter filaments and resolution on the substrates CMC environment.

Reducing sugar content was determined by Dinitrosalicylic Acid (DNS) colorimeter method of Miller (1959). A reaction mixture composed of 0.2 mL of crude enzyme solution was added to 1.8 mL of 0.5% CMC in 50 mM sodium phosphate buffered (pH 7). The mixture was incubated at 37 °C in a shaking water bath for 30 min. The reaction was terminated by adding 3 mL of DNS reagent. The mixture was boiled for 5 min and the optical density of samples was measured at 575 nm against a blank containing all the reagents minus the crude enzyme. The process of culturing microorganisms generated cellulase enzymes which accelerated the degradation of cellulose to glucose.

Amylase enzyme is quantified by method of Heinkel (1956) and protease enzyme is quantified by method of Anson (1938).

3. Results

3.1 Physical and Chemical Activity of the Selected Microorganisms

41 strains of microorganisms capable of cellulose

degradation were isolated from samples of cassava peel waste. Four strains of microorganisms were selected through the evaluation process activated by qualitative method for resolution on the culture medium with carbon source, Carboxyl Methyl Cellulose (CMC). Testing process showed that fertility cellulase enzyme was better than other enzymes. They are identified as results that bacteria named *Corynebacterium Alkanolyticum* (C); *Bacillus amyloliquefaciens* (B) and fungi named *Aspergillus niger* (N), *Aspergillus fumigatus* (F). Table 1 showed that the order of cellulase is $F > C > B > N$.

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According to other researches in Vietnam, enzyme activity of fungi is 30.19 IU/mL [7], and bacteria is 31.31 IU/mL [8]. In some foreign documents, the figure for bacteria is 39.50 IU/mL/min [9] and fungi is 40.05 IU/mL/min [10]. Thus, the research results from cassava peel isolated relatively high compared with other researches.

3.2 Assessment the Change of pH and Glucose

Fig. 2 shows that pH values decrease when concentrations of reducing sugar increase, which is in accordance with study of Michael et al. [4]. At the third week (after 21 days), concentration of glucose in most of samples were reached to the highest; after that it decreased. The results indicated that 21 days (about 3 weeks) could be the best time for degradable process. *Bacillus* seems to like low pH around 4 when the fungi prefer neutral pH, varying from 7 to 8.

Table 1 Test the fertility cellulase, amylase and protease anzymes (IU/g).

Microorganisms	Cellulase	Amylase	Protease
<i>Aspergillus Fumigatus</i> (F)	33.92	2.75	3.1
<i>Aspergillus Niger</i> (N)	29.93	1.16	2.95
<i>Bacillus Amyloliquefaciens</i> (B)	35.49	3.39	2.33
<i>Corynebacterium Alkanolyticum</i> (C)	35.82	2.22	1.07

Fig. 1 Continuous streak (left) and biodegradable round (right) of microorganisms (with B-21 mm, C-20 mm, F-24 mm, N-21 mm).

Fig. 2 Trends of pH and glucose concentration for 28 days vs tested organisms.

(A-Results of independent strains, B-Results of combined strains), B = *Bacillus amyloliquefaciens*, C = *Corynebacterium Alkanolyticum*, F = *Aspergillus fumigatus*, N = *Aspergillus Niger*, BC = *Bacillus* and *Coryne*, BF = *Bacillus* and *Fumigatus*, CF = *Coryne* and *Fumigatus*, BN = *Bacillus* and *Niger*, CN = *Coryne* and *Niger*, NF = *Fungi* Combination.

Strain B *Bacillus amyloliquefaciens* gained the highest amount of reducing glucose, 9.32 ± 0.05 mg/mL. In terms of the combined sample, sample BF,

combination of *Bacillus amyloliquefaciens* and *Aspergillus fumigatus* produced the highest amount of glucose up to 19.29 ± 0.05 mg/mL.

There are also two good combinations between microorganisms, **CN**, **CF** with glucose concentration reached $17.36 \pm 0.0\text{mg/mL}$ and 14.80 mg/mL , respectively. Thereby it was found that the combination of microorganisms can improve the efficiency of cassava peel biodegradation.

3.3 Assessment the Biodegradable Cassava Peel of Selected Microorganisms

In order to assess the biodegradation effectiveness of the selected microorganism, the study has examined the mass of the cassava peel decreased after four-week experiments. The biomass loss is calculated based on the difference of dry weights of experimented cassava peel before and after 4 weeks of experiment. Fig. 3 shows the decline of cassava peel weight each week. The result indicated that the bio-degradation of *Fumigatus* was the best which the cassava peel mass could be reduced approximate 52% (at the begin of experiment, the weight was 1g and after 4 weeks the remained weight was $0.48 \pm 0.06 \text{ g}$) and *Bacillus* was the worst of the selected microorganism which bio-degradation was about 31.6% (the remained cassava peel weight was $0.68 \pm 0.05 \text{ g}$). The biodegradation of *Corynebacterium* was 49.6% (which was $0.5 \pm 0.04 \text{ g}$) and *Niger* was about 34% (which was $0.66 \pm 0.07 \text{ g}$). The bacterial species used in this study were able to degrade the cassava peel. They were not as effective as the fungi species because fungal mycelia were known to penetrate the substrate

for nutrients uptake while bacteria is founded growing on the surface of the waste material. This complied with the work of Nigam and Singh [11].

The result of the combination of the selected microorganism for biodegradation of cassava peel wastes was promising. The combination of **B** and **F** gave the best result which the degradable cassava peel weight could be degraded approximated 62% (the rest of cassava peel weigh was about $0.37 \pm 0.05 \text{ g}$). The biodegradation of **BC**, **CF** and **CN** were good, 55%, 57% and 54%, respectively. This indicates that the combination of the selected microorganism can have better degradation rate. However, some combination had worse degradation than individual microorganism. For example, the biodegradation effectiveness of **BN** and **NF** were approximated 30% of the cassava peel weigh, the reason that the microorganism could be inhibited and declined of the degradation of the cassava peel wastes. Abarca demonstrated *Niger* has been reported to produce potent *mycotoxins* called *ochratoxins*. Therefore, this microorganism can be inhibited the biodegradation of cassava peel wastes.

4. Conclusion

The results of this present study show that among 10 strains of microorganisms of cassava peel biodegradation experiment, 4 single strains and 6 twin-strains (combined 2 microorganisms) have been selected. The combination strains function are much

Fig. 3 Trends of mass of cassava peel during experiment.
(A-Results of independent strains, B-Results of combined strains).

better than a single strain. Experimental results showed that combining 2 fungi strains will inhibit each other and therefore have lower efficiency than bacillus combination with fungi (about 30%). Four pairs provide better cassava peel degradation rate are BF, BC, CN, CF with biodegradation percentage of 62%, 55%, 57%, 54% and reducing sugar created of 19.29; 13.57; 17.36; 14.80 mg/mL, respectively. This would be a premise for next studies like testing the decomposition of cassava peel in shaking culture condition and testing the effects of ambient temperature, and finally set a pattern for aerobic decomposition cassava peel with the ultimate goal of reusing cassava peel as an organic fertilizer in the shortest possible time. Although the research is still undergoing different stages, its benefits of economy and environment are promising.

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