Process design for bioconversion of cassava stalk through solid substrate fermentation

P Dewi¹, R Indrati², R Millati², Sardjono²
¹Biologi Department, Universitas Negeri Semarang, Indonesia
²Fakultas Teknologi Pertanian, Universitas Gadjah Mada, Indonesia

*Corresponding author: iwed_pramesti@mail.unnes.ac.id

Abstract. Cassava stalks are by products from cassava tuber production. Cassava stalks is lignocellulosic material (LM) which is composed by lignin, hemicelullose, and cellulose. Utilization of LM optimally will push a beneficial effort in producing sugar. The presence of lignin can limit the effort to convert cassava stalks into sugar. The lignin have to be reduced. Its introduced solid substrate fermentation (SSF) technique by using molds to optimize utilization of cassava stalks. Molds can produce enzyme for converting LM into sugar. There is no enough information of design process of solid state fermentation for cassava stalks bioconversion, starting from selection of suitable microorganism used, substrate suitability testing, lignin reducing pretreatment, design of fermentor, and the needed of aeration for optimizing molds growth. Based on research result, a process for bioconversion of cassava stalks into sugar through SSF techniques was defined as follow: Microorganism suitable used is Aspergillus niger, cassava stalks suitable as a growth media for mold as a physical support (DMC = 93, 76 %) and source of nutrient (C/N 19, 92), physical pretreatment by reduce the particle size was more suitable than alkaline pretreatment for lignin reduction, and the best fermentor is unforced aeration closed chamber at big capacity.

1. Introduction
Cassava stalks are produced during cassava (Manihot esculenta Crantz.) cultivation. These stalks can be used as a replanting material, but its just about 10 % of total stalks produced. The other stalks will be discharged or utilize as a source of fuel for traditional kitchen, especially in many villages of Indonesia.

Cassava stalks is lignocellulosic material, according to main components of the stalks. Lignocellulosic material mainly contains chemical compound of lignin, cellulose, and hemicelluloses [1] which are polymer. Lignin are heteropolymer, compose by monomer of aromatic groups and monosaccharides. Hemicellulose are heteropolymer too, composed by variate monosaccharide as monomer, whereas cellulose is homopolymer composed by glucose as a monomer.

Hydrolysis of cellulose and hemicellulose will produce abundant monosaccharides especially glucose [2]. Unfortunately, hydrolysis of cellulose and hemicellulose meet a constrain from the existence of lignin. Based on the structure of lignocellulose fibers, lignin is in the outermost layer of the fiber and the chemical structure of lignin is difficult to destroy chemically and biologically. Consequently, for optimal bioconversion of lignocellulosic material for example sugarcane bagasse...
[3], pineapple skin [4], apple pomace [5] to produce glucose (sugars) it needed to reduce lignin content.

Cassava stalks can be utilized optimally for producing sugars, especially glucose by hydrolyzed cellulose content through solid substrate fermentation (SSF) by using fungi (bioconversion). SSF is fermentation on solid growth medium with limited content of water [6]. SSF is predicted more suitable for growth of the fungi based on natural characters of fungi mycelium growth [7]. Fungi also can produce enzymes in a group of cellulases and hemicelluloses, so can grow on lignocellulosic material optimally [8].

The aims of this study is find out enough information of design process of solid state fermentation for cassava stalks bioconversion, starting from selection of suitable microorganism used, substrate suitability testing, lignin reducing pretreatment, design of fermentor, and evaluating needed of aeration for optimizing molds growth used in the bioconversion process.

2. Methods

2.1. Searching for suitable microorganism

The first step of this study was observed suitable microorganism which can grow on cassava stalk as a media. Cassava stalks were prepared as a growth media by chopped the stalks into coarse particles, drying at room temperature, and followed in a oven until water content around 14 %. The dried stalks grounded with disk mill to achieve powder particle size.

Powdered cassava stalks moistened with aquadest in a ratio 1:1 and sterilized in autoclave at 121°C for 15 minutes. During sterilization, cassava stalks placed in plastic bag and after sterilization transferred into plastic boxes with 30 mL capacity. The plastic boxes have been perforated at the bottom and edges.

2.2. Evaluate the fitness of cassava stalk as a medium

The suitability of cassava stalks as a medium for microorganism growth had been evaluated by analyze of chemical composition of the stalks. The result compares with microorganism growth requirement to the judgement of the suitability. Chemical composition analysis of cassava stalks consist of water content, dry matter content (DMC), carbon content, total nitrogen content, and also cellulose, hemicellulose, lignin, and amyylum content.

2.3. Identify the proper media-water ratio

Method to evaluate the proper ratio of media (cassava stalks powder) and water to ensure microorganism growth have been conducted with varying the amount of distilled water to media. Ratio media to distilled water were 1: 1 and 2: 1. These media sterilized in autoclave two time; each sterilization conducted for 15 minutes at 121 °C to ensure sterile condition. Sterilized media used as a solid substrate and inoculated with powder inoculum of mold. Reducing sugar obtained after incubation used as an indicator of mold growth.

2.4. Evaluate proper pre-treatment

An effort for reducing lignin content (pretreatment) consist of physcal and chemical pretreatments. Physical preteatment used was grounding the dried cassava stalks into powder size which was around 50-100 mesh [9]. Chemical pretreatment conducted using Ca(OH)₂ solution [10] for mase rate dried cassava stalks in water bath at 90 °C for 16 hours.

2.5. Evaluate the fitness of incubator model

In order to obtain proper model of fermentor it was designed 4 model of incubator, which were model A, B1, B2, dan B3, and then the growth of mold within the incubator used among various models compared. Model A was a rack covered with plastic, and humidity inside the rack served by warm distilled water in a plastic tray at the bottom of the rack. Model B was close chamber incubator with 3
variation, which are Model B1, B2, and B3. Schematic diagram of these models can be seen in Figure 1.

3. Results and Discussion

3.1. Searching for Suitable Microorganism

Result of step for searching suitable microorganism which can grow in a good condition on cassava stalks media shown in Figure 1. It can be seen from the figure that microorganism grown on a surface media was mold. *Aspergillus* sp. was the kind of mold, this genus is the best one.

![Figure 1. Incubator designed (1. Incubator model A: plastic rack covered with plastic; 2. Incubator model B1 and B3: closed chamber with unforced aeration in different capacity, 20 L and 55 L respectively; and 3. Incubator model B2: closed chamber with forced aeration)](image)

![Figure 1. Incubator designed (1. Incubator model A: plastic rack covered with plastic; 2. Incubator model B1 and B3: closed chamber with unforced aeration in different capacity, 20 L and 55 L respectively; and 3. Incubator model B2: closed chamber with forced aeration)](image)

![Figure 2. Media (cassava stalks had been powdered and moistened with distilled water in a ratio 1:1), without inoculum addition, shown molds growth (1. *Aspergillus flavus*, green colony; 2. *Aspergillus niger*, black colony)](image)

![Figure 2. Media (cassava stalks had been powdered and moistened with distilled water in a ratio 1:1), without inoculum addition, shown molds growth (1. *Aspergillus flavus*, green colony; 2. *Aspergillus niger*, black colony)](image)

Based on the result, it was indicated that suitable microorganism grown on cassava stalks media was mold, especially from the genus of *Aspergillus*. This result in accordance with the facts that in natural habitat, molds growing better in solid media with limited amount of water. Fungal mycelium can penetrate the media and grow elongated and branched.
3.2. Evaluation of the suitability of cassava stalks as a medium

Evaluation of the suitability of cassava stalks as microorganism growth media is based on the results of the analysis of chemical composition as seen in Table 1.

Table 1. Chemical analysis of Cassava Stalks

| No. | Parameter               | Value (Average ± SD) | Methods                        |
|-----|-------------------------|----------------------|--------------------------------|
| 1   | Water content, %        | 6.24 ± 0.21          | Gravimetric                     |
| 2   | Dry Matter Content, %   | 93.76 ± 0.21         | Calculation (100-WC)            |
| 3   | C/N                     | 19.92 ± 0.036        | C = 50 % DMC                    |
|     |                         |                      | N = Micro Kjeldahl              |
| 4   | Reducing sugar<sup>1</sup>, % (w/dw) | 6.31 ± 0.026     | DNSA                            |
| 5   | Cellulose content, % (d/dw) | 33.70 ± 1.075     | Van Soest                       |
| 6   | Hemicellulose content, % (d/dw) | 30.11 ± 0.25      | Van Soest                       |
| 7   | Lignin, % (d/dw)        | 27.04 ± 1.02         | Van Soest                       |
| 8   | Amylum content, % (d/dw) | 22.94 ± 0.76        | Acid hydrolysis                 |

Notes:  
<sup>1</sup> average of 2 times of measurements and standard deviation  
<sup>2</sup> after sterilization

It can be seen from Table 1, cassava stalks have high dry matter content (DMC). Its indicated that cassava stalks suitable as medium for molds growth through solid substrate fermentation and act as physical support for mycelium growth. According to the value of C/N ratio, cassava stalks has proper ratio of carbon and nitrogen content for molds grow. This value approaches the C/N ratio of synthetic media for mold/fungal growth (MEA = Malt Extract Agar) [11]. In other words, cassava stalks can both act as physical support and nutrients source of the mold, and proper used for SSF [12]

3.3. Identify the proper media-water ratio

![Reducing sugars from various media-water ratio](image)

Figure 3. Media (cassava powder in a 50-100 mesh particle size) which had been added water in a 1:1 ratio gave better reducing sugars content during 0-3 fermentation day rather than the other ratio (2:1)

Based on Fig.3, the ratio of media to water of 1:1 is proper ratio for mold growth, which indicated by higher reducing sugar content. At the ratio of 1:1, water served more abundant than in ratio of 2:1. SSF is fermentation with limited water content, but for the better mold growth they need enough water. In adequate supply of water, the mold will grow well, and produce reducing sugars through hydrolysis of cellulose and hemicellulose.
3.4. Evaluate proper pre-treatment

Result obtained from the step of evaluating proper pretreatment for reducing lignin content shown in Fig. 4.

![Graph showing effect of physical pretreatment on reducing sugar](image)

**Figure 4.** Reducing sugars content of various fermented media (A. Physically pre-treatment, fine and coarse particle size; B. Chemically pre-treatment/lime pre-treatment, Unpre-fine: fine particle without lime pre-treatment, Pre-fine: fine particle with lime pre-treatment)

From Figure 4, the highest reducing sugars content as a one of fermentation product was come from media pretreated physically into fine particle size. It can be explained that fine particle will be better media for mold growth [13] because fine size gives easier condition for mycelium to penetrate the media. In fine size media, they can reach media deeper, longer, make plenty branch, and hydrolyze the polymer more effective [14].

3.5. Evaluate the fitness of incubator model

The result in evaluation the fitness of incubator model for bioconversion of cassava stalks through SSF served in Table 2. From the table it can observe that model B3, which are close chamber without force aeration at 55 L capacity plastic container gave better fermentation products. The needed of aeration (oksigen supply) for the growth of mold over come from the air inside the box. Furthermore, close chamber incubator is able to give more sterile condition and more suitable environment condition [15], such as temperature and humidity as seen on Table 2.

| Parameters                  | Model A   | Model B.1 | Model B.2 | Model B.3 |
|-----------------------------|-----------|-----------|-----------|-----------|
| Fermentation Environment:   |           |           |           |           |
| - T (°C)                    | 27-28     | 29.0 – 30.7 | 29.9 – 31.0 | 27.9 – 29.9 |
| - RH (%)                    | 80-91     | 72.0 – 82.0 | 74.0 – 86.0 | 99        |
| - pH                        | NA        | 5.0 – 6.0  | 4.2 – 6.0  | NA        |
| - aw                        | NA        | 0.975 – 0.99 | 0.969 – 0.995 | 0.935 – 0.99 |
| - Headspace : Bed height    | NA        | 7.8 : 1    | 7.8 : 1    | 17 : 1    |

Table 2. The parameter matrix for environmental conditions and fermentation results of the four types of incubators
**Fermentation Products:**
- GS maks (10^4 μg/g media) NA 5.0 6.5 12.0
- RS maks (% w/w, dw) 4.43 * 0.32 0.3 3.7
- Spores number maks (10^10 spores/g media) NA 1.2 1.4 1.3
- Conversion of cellulose into sugars percentage (%)**

|       | 12.6 | 0.95 | 0.89 | 10.98 |

Note: NA = Not Available  
GS = glucosamine  
RS = reducing sugar  
Headspace = empty space in the incubator chamber at the top of the media  
Bed height = thickness of the substrate overlay on a fermentation tray or container  
* = derived from the growth of molds inoculum and other molds  
** = 67% conversion target

4. **Conclusion**

Process for bioconversion of cassava stalks through Solid Substrate Fermentation, should be designed as follow: a) Apply mold, especially *Aspergillus niger* as suitable microorganism. b) Added distilled water in a ratio 1:1 into cassava stalks powder. c) Apply physical pretreatment by milling into fine particle size (50-100 mesh), d) Using proper incubator model which are close chambers without forced aeration.

**References**

[1] Ali H K Q and Zulkali M M D 2011 *Croat. J. Food Technol. Biotechnol. Nutr.* 6 5  
[2] Brethauer S dan Studer, M H 2015 *Chimia* 69 572  
[3] Soni S K, Batra N, Bansal N, and Soni R 2010 *BioResources* 5 741  
[4] Kareem S, Akpan I, and Alebiowu O 2010 *Malays. J. Microbiol.* 6 161  
[5] Kumar A, and Jain V K 2008 *Afr. J. Biotechnol.* 7 644  
[6] Singhania RR, Kumar A, Soccol C R, and Pandey A 2009 *Biochem. Eng. J.* 44 13  
[7] Couto S and Sanroman M 2005 *J. Food Eng.* 76 291  
[8] Nahid P, Vossoughi M, Roostazad R, and Ahmadi M 2012 *IJE Transaction B: Application* 25 1  
[9] Guan J, Yang G, Yin H, Jia F, and Wang J 2014 *Am. J. Agric. For.* 2 1  
[10] Behera S, Arora R, Nandhagopal N, and Kumar S 2014 *Renew. Sustain. Energy Rev.* 36 91 106  
[11] Membrillo I, Sa’ nchez C, Meneses M, Favela E, and Loera O 2008 *Bioresource Technology* 99 7842  
[12] Bhargav S, Panda B P, Ali M., and Javed S 2008 *Chem. Biochem. Eng.* 22 49  
[13] Jiang J, Wang J, Zhang X, and Wolcott M 2016 *RSC Advances* 105 103026  
[14] Papagianni M, Nokes S E, and Filer K 2001 *Food Technol. Biotechnol.* 39 319  
[15] Ali H K Q and Zulkali M M D 2011 *Chem. Biochem. Eng.* 25 255