Determination of yeast co-culture ratio and stirring for optimization of bioethanol content of garlic (*Allium sativum*) peels and corn (*Zea mays* L.) cob

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Determination of yeast co-culture ratio and stirring for optimization of bioethanol content of garlic (Allium sativum) peels and corn (Zea mays L.) cob

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Abstract. Bioethanol as a source of energy from biomass of garlic peels and corn (Zea mays L.) cobs waste come into one alternative to reduce the dependence of fossil fuels and the increasingly severe environmental damage. The aims at the research were bioethanol producing with optimum level of garlic (Allium sativum) peels and corn cob revealed from yeast co culture and stirring ratio and interaction between both. Fermentation performed during 3 days with various yeast co culture of yeast tape and bread yeast with stirring ratio and control (without stirring). Data analyzed on each type of waste source by design of factorial treatment (4x2) and Randomized Block Design (RBD ) with 4 repetitions. As the first factor is the ratio of yeast tape co-culture and bread yeast consisting of 4 levels, that is 7.5%:7.5%; 10%:5%; 15%:5% and 20%:5%. A second factor is done stirring and without stirring. Comparison of the ethanol production percentage performed between the raw materials source. The research can be concluded that the most optimal of ethanol content of garlic peels are 7.428 ±0.072% on interaction ratio of tape yeast 15% and bread yeast 5% with stirring. The most optimum ethanol product of corn cobs is 8.19% produced at ratio of 10% yeast tape and 5% yeast bread with stirring.

Keywords: bioethanol, garlic peels, corn (Zea mays L.) cobs, yeast, stirring

1. Introduction
Fuel energy requirements derived from fossil exploration continue to increase in line with increasing industrial and economic growth. This can be a major problem, when the state has not been able to reduce dependence on fossil fuels or fuel oil while the reserves of these energy sources are increasingly limited [1]. It is time for the use of renewable energy sources of the form of biofuels or increased biofuels, replacing the fossil fuels that are dwindling as in Indonesia Presidential Instruction No. 1/2006 and Indonesia Presidential Regulation No. 5/2006 on national energy policy. One of the liquid vegetable fuel is a substitute for gasoline named bioethanol.

From the last several years, the worldwide economic and environmental pollution issues there has been increasing research interest in the value of bio-sourced lignocellulosic biomass. Agro-industrial biomass comprised of lignocellulosic waste is an inexpensive, renewable, abundant and provides a unique natural resource for large-scale and cost-effective bio-energy collection. In this background green biotechnology present a promising approach to convert most of the solid agricultural wastes particularly lignocellulosic materials into liquid bio based energy-fuels [2]. The use of much cheaper
and widely available feedstock, lignocellulosic materials from agricultural and forestry industry wastes needs to be further developed.

One of the most widely found agricultural waste is garlic peels and corn cobs. Garlic peels are an industrial waste and agriculture that has not been utilized maximally, especially in its potential as an energy source alternative. An investigation into the composition of garlic peels showed the presence of proteins, lipids, lignin, mannitol, pectin and polysaccharides. Garlic peels are rich in pectin (27%), combined rhamnose (11.42%) and galactose (5.6%) [3]. Production garlic Indonesia in 2016 according to data sourced from Directorate General of Horticulture is amounted to 21.15 thousand tons [4]. According to the data also only 71% of garlic can be consumed. This means that about 29% is the waste of garlic products. Based on the percentage, the waste of garlic product would be 6.1335 thousand tons, garlic peels included. Research on garlic peels content that could be used as raw material for bioethanol performed by [5], where there was 26.58% of total carbohydrate, 18.62% cellulose and 0.4% protein.

The content of carbohydrates from garlic peels could be hydrolyzed into glucose, when it is fermented, becoming ethanol. The corn cobs are preferred over other agricultural by-products due to its composition which is easily converted to bioethanol. A viable alternative to bio-fuel can be pursued by utilization of corn cobs due to suitable lignocellulosic waste for bioethanol production of chemical pre-treatments. The lignocellulosic bio mass comprises of cellulose, hemicellulose and lignin. Now-a-day the demand of bioethanol has increased considerably because of its use as a gasohol in addition to the other application for industries which need production of alcohols on large scale. According to [6], the advantageous utilization of co-cultures instead of single cultivations included the production of bulk chemicals, enzymes, food additives, antimicrobial substances and microbial fuel cells. Co-culture fermentations may result in increased yields, improved control of product qualities and the possibility of utilizing cheaper substrates. The use of co-culture techniques in the fermentation process to produce bioethanol proved to provide better results than the use of pure culture of \( S. \) cerevisiae and single yeast tape culture [7].

Based on the above scientific explanation, the research aims were bioethanol producing with optimum level of garlic peels and corn (\( Zea \) mays \( L. \)) cobs revealed from yeast co-culture and stirring ratio and interaction between both.

### 2. Materials and method

#### 2.1. Materials

The garlic peels is obtained from a nut factory in Pati, corn (\( Zea \) mays \( L. \)) cobss, molasses, tape yeast bought from the market in Salatiga, and yeast bread bought from a bakery in Salatiga. Chemicals used include NaOH p.a (Merck), HCl p.a (Merck), \( \text{H}_2\text{SO}_4 \), Nutrient Broth, glucose standard, DNS reagents, and KNa Tartrate. The tools used include drying cabinet, TomY SS-240 autoclave, Autonics TC45 incubator, magnetic stirrer, Optizen 2120 UV-Vis spectrophotometer, 1 set of distillation equipment, alcohol meter.

#### 2.2. Methods

##### 2.2.1. Delignification ([8] modified)

The garlic peels powder was delignification with 15% NaOH (1:10) (b/v) in 121°C autoclave for 15 minutes, while the corn cobs powder delignification using 0.25 M NaOH (1: 8) (w / v), then rinsed until the neutral pH is then dried in a 50°C drying cabinet for 24 hours.

##### 2.2.2. Cellulose hydrolysis ([9] modified)

Delignified garlic peels powder was hydrolyzed with 4N HCl (1:10) (b/v) and corn cobs powder with 15% \( \text{H}_2\text{SO}_4 \)(1:10) (w / v) in reflux at a temperature of 100°C for 120 minutes.
2.2.3. Bioethanol fermentation

Hydrolyzed substrate was neutralized to pH 4.6 then added molasses and aquadest with ratio of substrate: molasses: aquadest (6: 2: 2), then solution fermented with ratio of yeast tape: bread yeast (% v/v) 7.5: 7.5; 10: 5; 15: 5; and 20: 5. Yeast tape is given first then after 24 hours bread yeast added and fermentation continued up to 72 hours. All ratios are treated with stirring and without stirring.

2.2.4. Bioethanol distillation

The solution was distilled at 78.5°C for ± 4 hours until no drips and ethanol content were measured with alcohol meter.

2.3 Data Analyse

Data analyzed on each type of waste source by design of factorial treatment (4x2) and Randomized Block Design (RBD ) with 4 repetitions. As the first factor is the ratio of yeast tape co-culture and bread yeast consisting of 4 levels, that is 7.5%:7.5%; 10%:5%; 15%:5% and 20%:5%. A second factor is done stirring and without stirring.

3. Result and discussion

3.1. The effect of various yeast co-culture ratios on ethanol content

The mean ethanol content (in % ± SE) in the various yeast ratios of tape and bread yeasts ranged from 4.690 ± 0.545% to 6.894 ± 0.272% (Table 1).

| Tape yeast : Bread yeast (%) | Average ± SE | W = 0.143 |
|-----------------------------|-------------|-----------|
| 7.5 : 7.5                   | 4.768 ± 0.145 | (a) |
| 10 : 5                      | 5.715 ± 0.222 | (b) |
| 15 : 5                      | 6.894 ± 0.272 | (c) |
| 20 : 5                      | 4.690 ± 0.545 | (a) |

Note: *W = HSD 5%
* The numbers followed by the same letter indicate that the treatments were not significantly different, while the numbers followed by the unequal letters showed different treatments. This description also applies to the next Table.

Table 1 shows that tape yeast up to 15% in the co-culture ratio increases in the ethanol content. The optimal level is produced by 15% yeast tape ratio and 5% yeast bread is 6.894 ± 0.272%. This value is lower when compared with research conducted by [7] with cassava base, 5% yeast tape ratio and 5% yeast bread produce 11% ethanol. The decrease in ethanol contents after a 15% tape yeast ratio due to glucose on the fermented substrate is no longer sufficient to convert to ethanol.

Average of ethanol content (% ± SE) from corn cobs ranged from 5.113 ± 0.221 to 7.510 ± 0.345 (Table 2).

| Tape yeast : Bread yeast (%) | Average ± SE | W = 0.097 |
|-----------------------------|-------------|-----------|
| 7.5 : 7.5                   | 6.199 ± 0.234 | (b) |
| 10 : 5                      | 7.510 ± 0.345 | (c) |
| 15 : 5                      | 6.255 ± 0.320 | (b) |
| 20 : 5                      | 5.113 ± 0.221 | (a) |

Note: *W = HSD 5%
Table 2 shows the same phenomenon, showing that the addition of tape yeast increases to the ethanol content and the addition of bread yeast does not affect the ethanol content of the corn at 15% yeast tape ratio and 5% yeast yeast.

3.2. The influence of stirring on bioethanol product
The average ethanol content of Garlic peels with stirring ranged from 5.157 ± 0.383% to 5.950 ± 0.431% (Table 3). Purify levels of bioethanol (in % ± SE) in various yeast ratios of tape and bread yeasts ranged from 4.690 ± 0.545% to 6.894 ± 0.272%.

| Table 3. The average of bioetanol content of garlic peels among various yeast ratio |
|-------------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                    | Tape Yeast : Bread Yeast (% ± SE)                     |
|                                    | 7.5 : 7.5      | 10 : 5          | 15 : 5          | 20 : 5          |
| Average ± SE                       | 4.768 ± 0.145  | 5.715 ± 0.222   | 6.894 ± 0.272   | 4.690 ± 0.545   |
| W = 0.143                          | (a)            | (b)            | (c)            | (a)            |

Average ethanol content of corn cobs fermentation ranges of 5.113 ± 0.221 up to 7.510 ± 0.345 (Table 4).

| Table 4. Average ethanol levels corn cobs among various yeast ratio |
|-------------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                    | Tape yeast : Bread yeast (% ± SE)                     |
|                                    | 7.5 : 7.5      | 10 : 5          | 15 : 5          | 20 : 5          |
| Average ± SE                       | 6.199 ± 0.234  | 7.510 ± 0.345   | 6.255 ± 0.320   | 5.113 ± 0.221   |
| W = 0.097                          | (b)            | (c)            | (b)            | (a)            |

Table 4 shows that the addition of tape yeast increases the ethanol content and the addition of bread yeast does not affect the ethanol content. Ethanol content increased at 10% yeast tape ratio and 5% yeast bread then decreased at 15% yeast tape ratio and 5% yeast bread.

3.3. Interaction between various yeast co-culture ratios and stirring on bioethanol product
The average garlic peels content of the interaction of the various yeast co-culture and stirring ratios ranged from 4.188 ± 0.157% to 7.428 ± 0.072% (Table 5 and Table 6). Table 5 shows that the ethanol content between ratios will increase from the addition of tape yeast to 15%, then decrease to the addition of tape yeast 20%. Furthermore, the highest ethanol content was obtained with 15% yeast tape ratio and 5% yeast yeast in stirring of 7.428 ± 0.072% Decrease in ethanol levels over 15% tape yeast is likely due to the availability of fermented glucose that is no longer sufficient to convert to ethanol. Stirring serves to help cell and substrate contact, keep the microorganisms from settling down and flatten the temperature so that the yeast can work more optimally [9].
Table 5. Content of ethanol of garlic peels based on interaction between yeast ratio and stirring

| Tape yeast : Bread yeast | Stirred | Without stirring |
|-------------------------|---------|-----------------|
| W = 0.151               |         |                 |
| Ratio (% ± SE) Tape : Bread yeast | 7.5:7.5 | 7.5:7.5 | 15:05 | 20:05 | 7.5:7.5 | 10:05 | 15:05 | 20:05 |
|                         | 5.045   | 6.135           | 7.428 | 5.193 | 4.490   | 5.045 | 6.360 | 4.188 |
| ± ± ± ± ± ± ± ± ± ± ± ± |
| (a) (b) (c) (d) (e) (f) (g) (h) (i) (j) (k) (l) (m) (n) (o) (p) (q) (r) (s) (t) (u) (v) (w) (x) (y) (z) |

Table 6. Ethanol content of corn cobs based on interaction between yeast ratio and stirring

| Tape yeast : Bread yeast | Stirred | Without stirring |
|-------------------------|---------|-----------------|
| W = 0.103               |         |                 |
| Ratio (% ± SE) Tape : Bread yeast | 7.5:7.5 | 7.5:7.5 | 15:05 | 20:05 | 7.5:7.5 | 10:05 | 15:05 | 20:05 |
|                         | 6.658   | 8.190           | 6.878 | 5.543 | 5.740   | 6.830 | 5.633 | 4.683 |
| ± ± ± ± ± ± ± ± ± ± ± ± |
| (b) (d) (c) (a) (b) (c) (a) (b) (c) (a) |

Figure 1 illustrates the linkage of added yeast ratio and the effect of stirring on the production of ethanol. The highest production of ethanol was obtained from garlic peels fermentation (KBP) by stirring and ratio of the yeast tape (15): bread yeast (5). Different results are shown by the fermentation of the stirred corn cobs, the highest ethanol product actually produced by the ratio of tape yeast (10): bread yeast (5).
4. Conclusion

The research can be concluded that the most optimal of ethanol content of garlic peels are 7.428 ±0.072% on interaction ratio of tape yeast 15% and bread yeast 5% with stirring. The most optimum ethanol product of corn cobs is 8.19% produced at ratio of 10% yeast tape and 5% yeast bread with stirring.

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References
[1] Zou C, Qun Z., Guosheng Z and Bo X. 2016 Natural Gas Industry B. 3(1) 1.
[2] Anwar Z., Gulfraz M and Muhammad I 2015 Journal of Radiation Research and Applied Sciences. 7(2) 163.
[3] Abdel-Fattah A F and Edrees M 1972 Journal of the Science of Food and Agriculture. 23(7) 871.
[4] Pusat Data dan Sistem Informasi Pertanian, 2017. Buletin Konsumsi Pangan Semester 2.
[5] Sugave D, 2014. Characterization Of Garlic Peels And Its Evaluation As Biomaterial. Rourkela.
[6] Bader J, Mast-Gerlach E, Popović M K, Bajpai R and Stahl U 2010 J. Appl. Microbiol. 109(2) 371.
[7] Arnata I W and Anggraeni A A M D 2013 J. Agrointek 7 (1).
[8] Sukumaran R.K, Singhania RR, Mathew G.M and Pandey A 2009 Renewable Energy. 34 (2) 21.
[9] Kurniawan S, Juhanda, S. Syamsudin, R and Lukman M.A 2011 J. STU, ISSN: 1693.
[10] Hossain A B M S, Boyce A N, Salleh A and Chandran S 2010 African Journal of Agricultural Research 5(14) 1851