Screening of Effective Bacteria for Cassava waste and Paper Sludge degradation

Miss Chanat Wongsiwasakul
Department of Soil Science Faculty of Agriculture at Kamphaeng Saen
Kasetsart University Kamphaeng Saen Campus
## Paper industry

<table>
<thead>
<tr>
<th>Industry (factory)</th>
<th>small</th>
<th>medium</th>
<th>large</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper and paper product</td>
<td>51</td>
<td>43</td>
<td>3</td>
</tr>
</tbody>
</table>
Activated sludge process

http://www.ewisa.co.za/misc/WasteWater/defaultas1.htm
Belt press

https://spanish.alibaba.com
Paper sludge

In paper sludge, group OH on cellulose chain cause bond with H in water molecule (Jirawatcharch, 2014).

Cassava waste

In cassava waste, the average of protein, lipid, fiber and starch is 2.37, 0.39, 13.99 and 50.19% respectively (Kosoom, 2009).
Amylase, any member of a class of enzymes that catalyze the hydrolysis (splitting of a compound by addition of a water molecule) of starch into smaller carbohydrate molecules such as maltose.

Cellulase is the enzyme that hydrolyze β-1,4 linkages in cellulose chains. It was disaccharide that assembly by 2 unit of glucose.
Yuan et al. (2012) screening and identification of cellulase-producing strain of *Fusarium Oxysporum*. Use CMC-Na to test width of clear zone, and use DNS to test the cellulase activity.

Choubane et al. (2016) screening and phenotypic diversity of amylase producing rhizospheric bacteria from some north african plants. The result showed that *Caratonia ciliqua* and *Ficus carica* gave the best amylase production.
OBJECTIVE

- To screening of effective bacteria for cassava waste and paper sludge degradation

- To produce inoculum of bacteria for cassava waste and paper sludge degradation
MATERIALS

Higher-Speed Bench top Microcentrifuge

Spectrophotometer

Laminar Flow

Electrophoresis and power supply
shaker incubator

water bath

Autoclave

Transilluminator
METHODS

Cassava waste

AS E85  Decanter E85

Paper sludge

ETP2  ETP2A
**METHODS**

- Distilled water 90 ml
- 10 g sample

Cassava waste (AS E85, Decanter E85) → Starch agar

Paper Sludge (ETP2, ETP2A) → Carboxymethyl Cellulose
**METHODS**

Cassava waste → Starch agar 7 days → Iodine solution

Paper Sludge → CMC 0.1% congo red (15 minute) +

To wash by NaCl 2-3 times
Nutrient broth 100 ml
24 hours

OD = 0.6 at 600 nm

CMC
Add Decanter E85, AS E85, ETP2, ETP2A 1 g to replace for Carboxymethyl Cellulose

Shake for 7 days

The absorbance was measured at 540 nm
Figure 1  Standard curve of glucose concentration

\[ y = 0.0037x + 0.0583 \]

\[ R^2 = 0.9907 \]
**METHODS**

Genomic DNA extraction from bacteria for identification

- Nutrient broth 24 hours
- Centrifuged at 8,000 rpm
- Precipitate
- NucleoSpin tissue
- DNA
- -20°C
- Transilluminator
- Electrophoresis
Inoculum production

Culture of each isolate nutrient broth for 24 hours

Spread plate

Count colony

Plot graph

Fly ash 10 g + Peat 10 g
Figure 2  The colony of starch degrading bacteria on starch agar;
a= SD1, b= SD2, c=SA1
Figure 3  The colony of cellulose degrading bacteria on CMC medium; a= CE1, b= CE2
**RESULT**

Figure 4  The clear zone of bacteria on starch agar and CMC medium a= bacteria from cassava waste , b= bacteria from paper sludge
### RESULT

Table 1. The average of colony width, clear zone and ratio of clear zone width per colony width on starch agar

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Colony (cm.)</th>
<th>Clear zone (cm.)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD1</td>
<td>0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SD2</td>
<td>0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SA1</td>
<td>1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>F-test</td>
<td>**</td>
<td>**</td>
<td>*</td>
</tr>
<tr>
<td>CV (%)</td>
<td>18.741</td>
<td>15.809</td>
<td>33.124</td>
</tr>
</tbody>
</table>

** : Statistical difference at P<0.01  
*  : Statistical difference at P<0.05
Table 2. The average of colony width, clear zone and ratio of clear zone width per colony width on CMC medium

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Colony (cm.)</th>
<th>Clear zone (cm.)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE1</td>
<td>0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.8</td>
</tr>
<tr>
<td>CE2</td>
<td>1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.2</td>
</tr>
<tr>
<td>F-test</td>
<td>**</td>
<td>**</td>
<td>ns</td>
</tr>
<tr>
<td>CV (%)</td>
<td>17.765</td>
<td>5.371</td>
<td>35.872</td>
</tr>
</tbody>
</table>

** : Statistical difference at P<0.01
ns : No statistical difference
Figure 5  Reducing sugar releasing in CMC medium by using Decanter E85 as substrate
RESULT

Figure 6 Reducing sugar releasing in CMC medium by using AS E85 as substrate
Figure 7 Reducing sugar releasing in CMC medium by using ETP2 as substrate
Figure 8  Reducing sugar releasing of reducing sugar in CMC medium by using ETP2A as substrate
Figure 9  Gram stain of bacteria degraded cassava waste and paper sludge. a = SD2 and b = CE1
Table 3  Identification of bacteria degraded cassava waste and paper sludge

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Species</th>
<th>Identification (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD2</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>99</td>
</tr>
<tr>
<td>CE1</td>
<td><em>Staphylococcus Kloosii</em></td>
<td>99</td>
</tr>
<tr>
<td>SS4</td>
<td><em>Bacillus megaterium</em></td>
<td>99</td>
</tr>
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</table>
**Figure 9** Standard curve of microorganism at different OD.

The graph shows a linear relationship between the logarithm of colony-forming units (LOG CFU) and optical density (OD). The equation of the line is given by $y = 2.8571x + 5.7714$ with a high coefficient of determination $R^2 = 0.9368$. The data points on the graph represent the relationship at different OD values.
CONCLUSION

The result showed that bacterium SD2 was the ratio of clear zone width per colony width as 5.7, was the high reducing-sugar production as 678.228 µmol/ml, and degraded cassava residue these bacterium was identified as *Klebsiella pneumoniae*. However, it is not suitable to produce inoculum for composting.

In term of bacterium CE1, it was the ratio of clear zone width per colony width and maximum reducing-sugar production were 4.8 and 689.740 µmol/ml respectively. These bacterium was identified as *Staphylococcus kloosii* then to produce inoculum by it can be applied to make compost from paper sludge.
THANK YOU