Screening of Effective Bacteria for Cassava waste and Paper Sludge degradation

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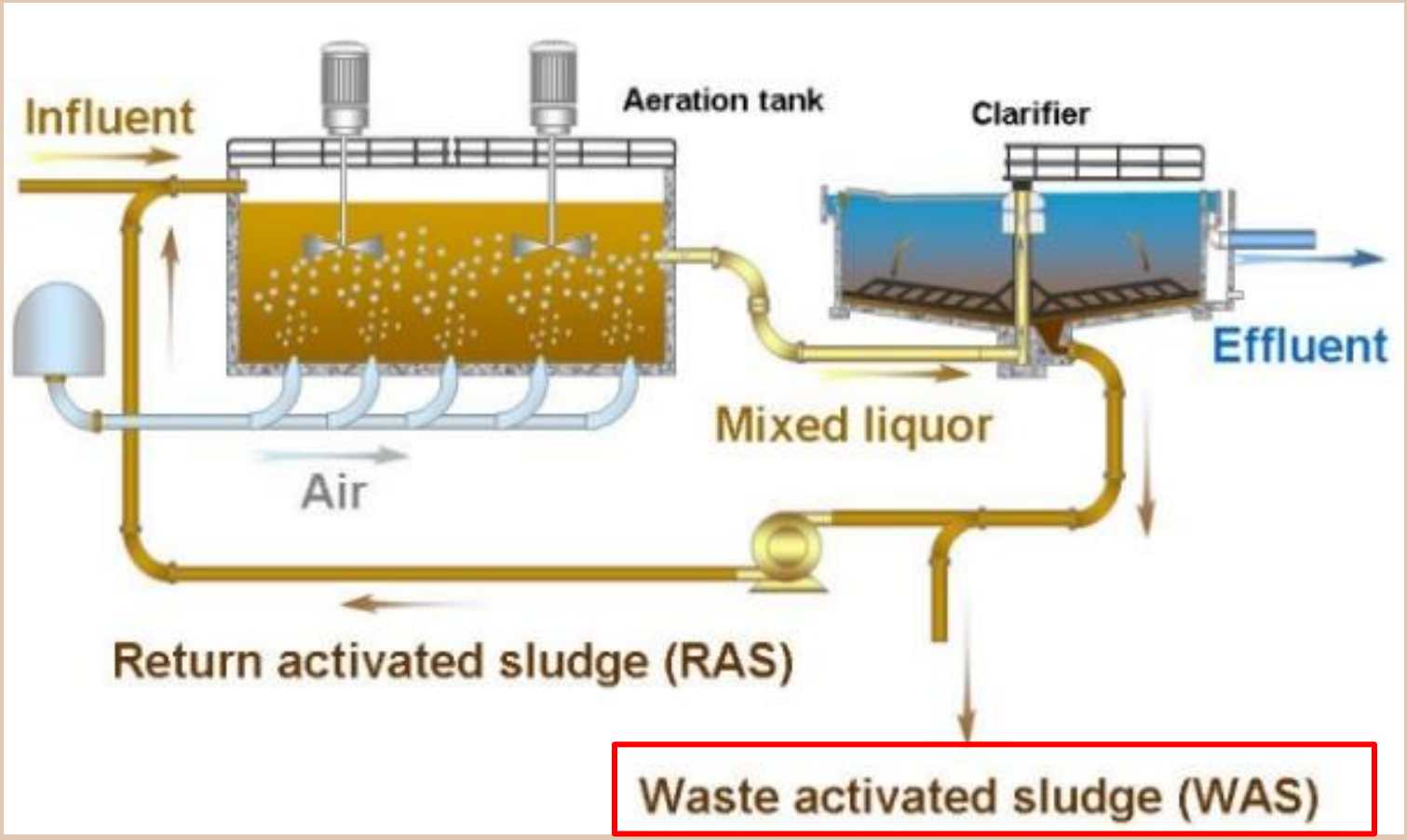
Paper industry

Industry (factory)	small	medium	large
Paper and paper product	51	43	3

Ministry of industry, 2558

Activated sludge process



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

Belt press

https://spanish.alibaba.com

Ingredient

Paper sludge

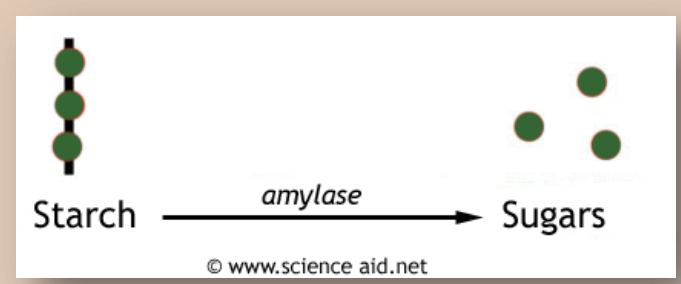
In paper sludge, group OH on cellulose chain cause bond with H in water molecule (Jirawatcharch, 2014).

Cassava waste

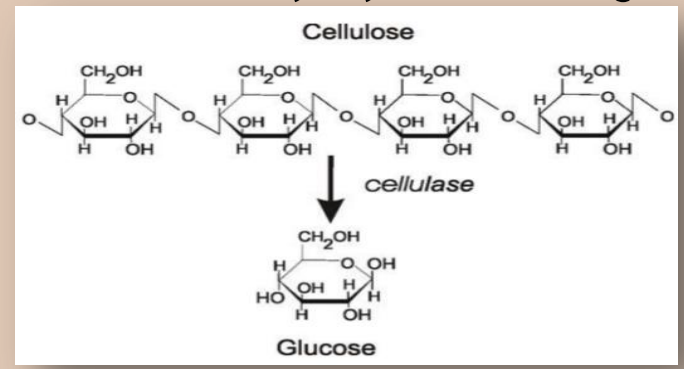
In cassava waste, the average of protein, lipid, fiber and starch is 2.37, 0.39, 13.99 and 50.19% respectively (Kosoom, 2009)

Enzyme

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.



Pornchalermpong, 2012

- 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.

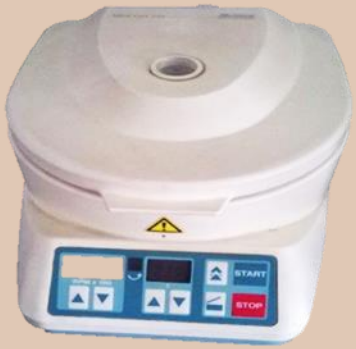
- Choubane *et al*. (2016) screening and phenotypic diversity of amylase producing rhizospheric bacteria from some north african plants. The result showed that *Caratonia ciliqua* and *Ficus carica* gave the best amylase production.

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

Laminar Flow

Spectrophotometer

Electrophoresis and power supply

MATERIALS

shaker incubator

Autoclave

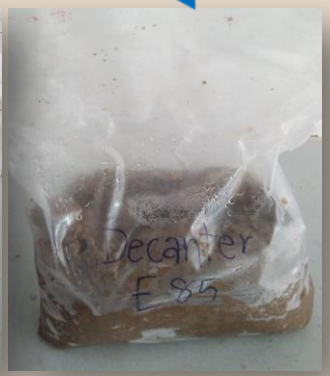
water bath

Transilluminator

METHODS

Cassava waste

AS E85



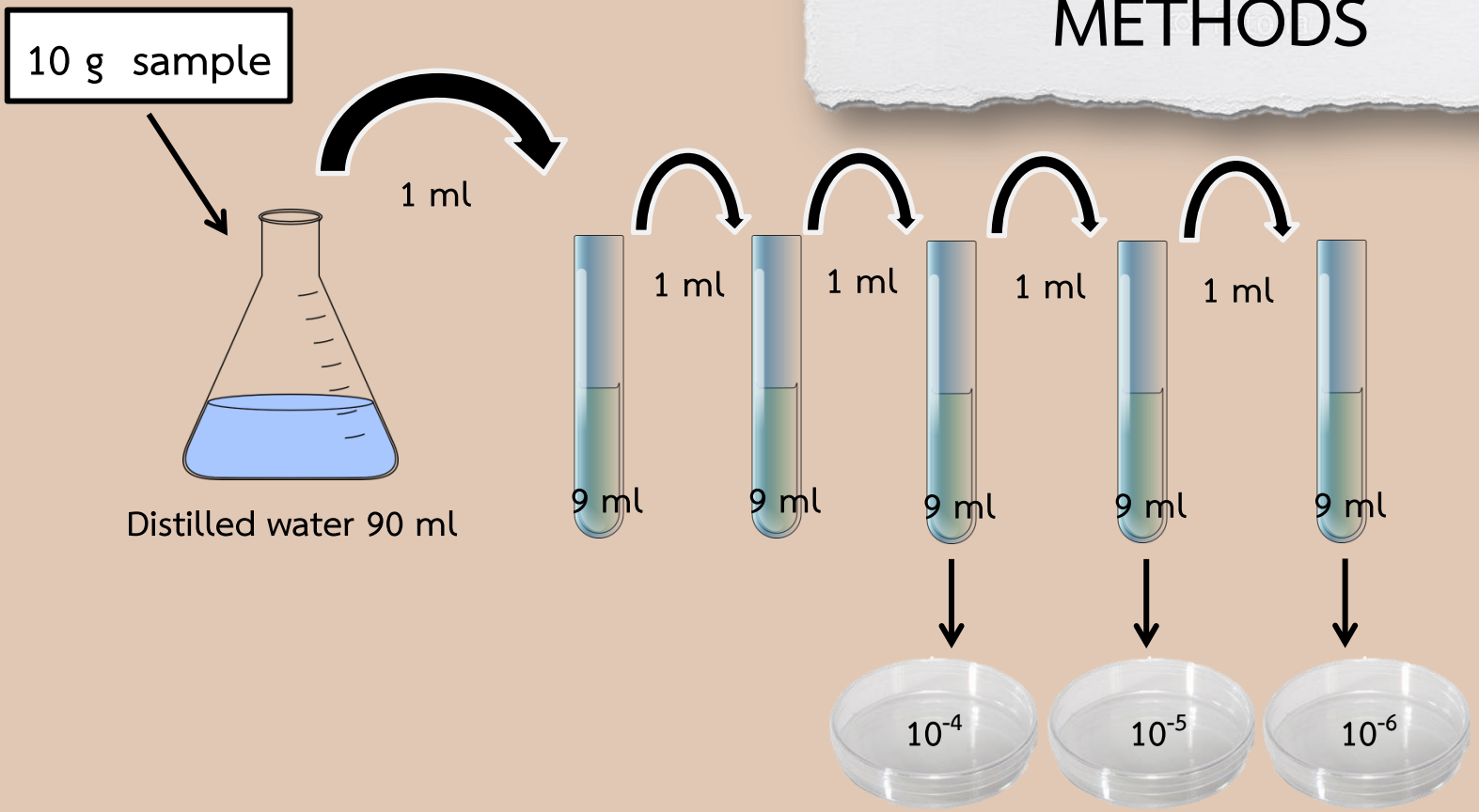
Decanter E85

Paper sludge

ETP2

ETP2A

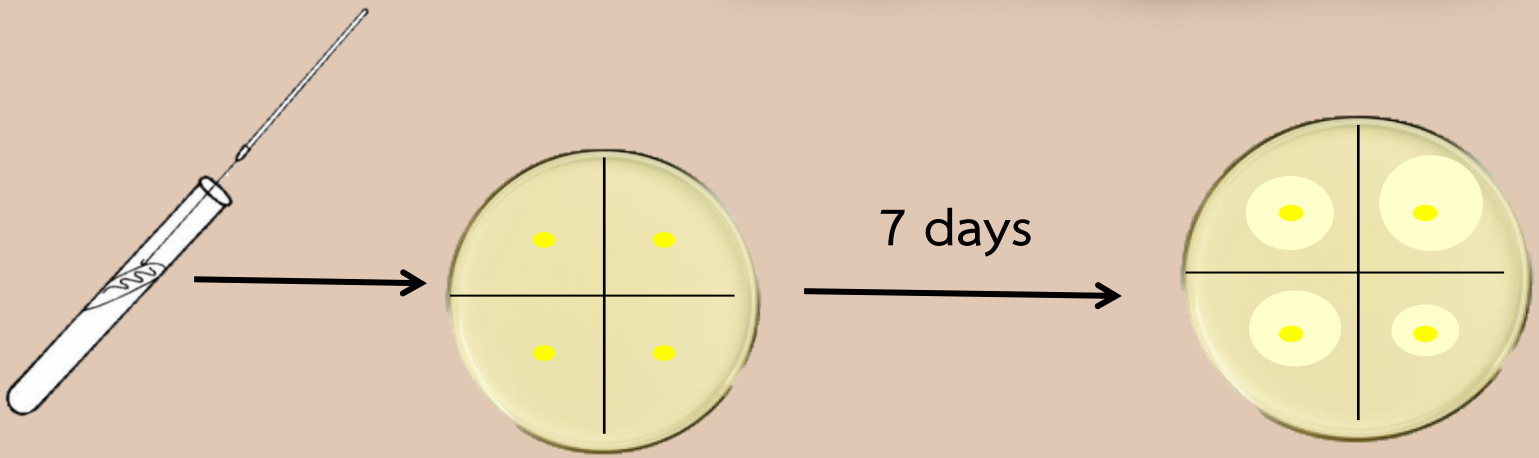
METHODS



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

Paper Sludge (ETP2 , ETP2A) → Carboxymethyl Cellulose

METHODS



Cassava waste → Starch agar →

Iodine solution

Paper Sludge → CMC →

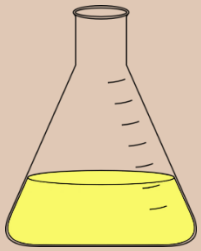
0.1% congo red

(15 minute)

+

To wash by NaCl 2-3 times

METHODS

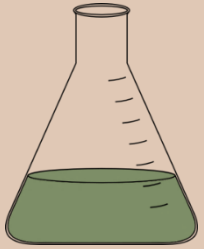


Nutrient broth 100 ml

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

Bacteria that grow in cassava waste

Bacteria that grow in paper sludge

METHODS

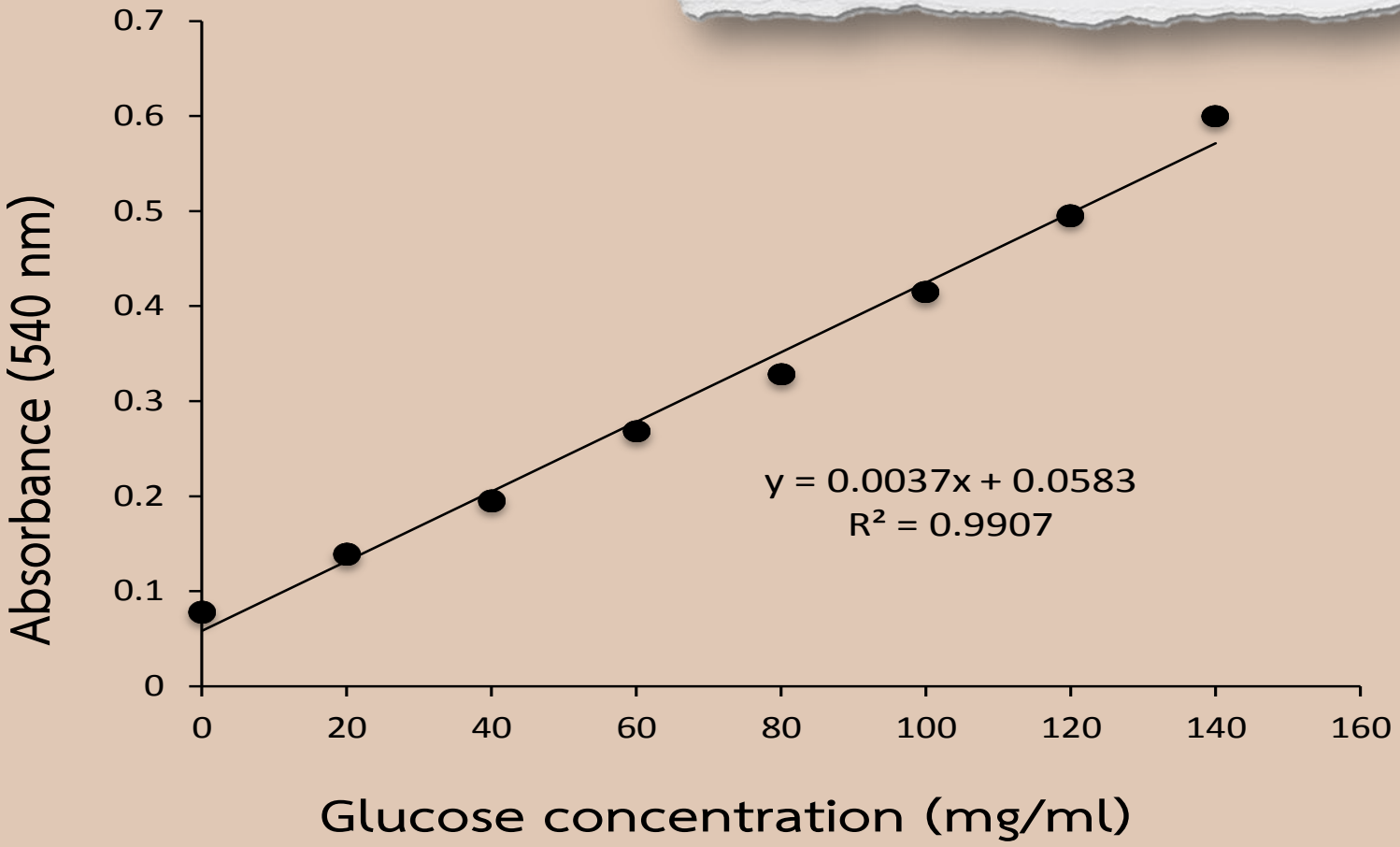
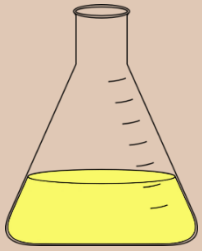


Figure 1 Standard curve of glucose concentration

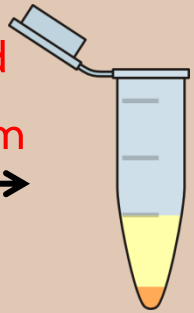
METHODS

Genomic DNA extraction from bacteria for identification



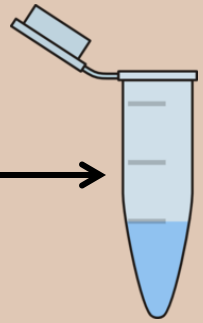
Nutrient broth 24 hours

Centrifuged at 8,000 rpm

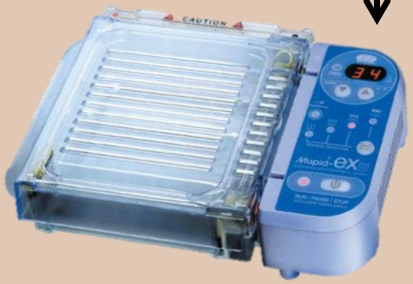


Precipitate

NucleoSpin tissue

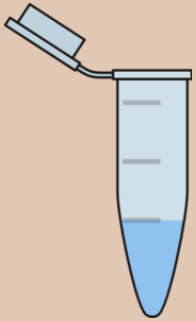


DNA



Electrophoresis

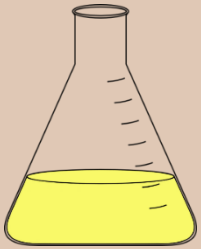
Transilluminator



-20°C

METHODS

Inoculum production



Culture of each isolate
nutrient broth
for 24 hours

Spread plate →

Count colony

→ Plot graph

↓

+

Fly ash 10 g + Peat 10 g

RESULT

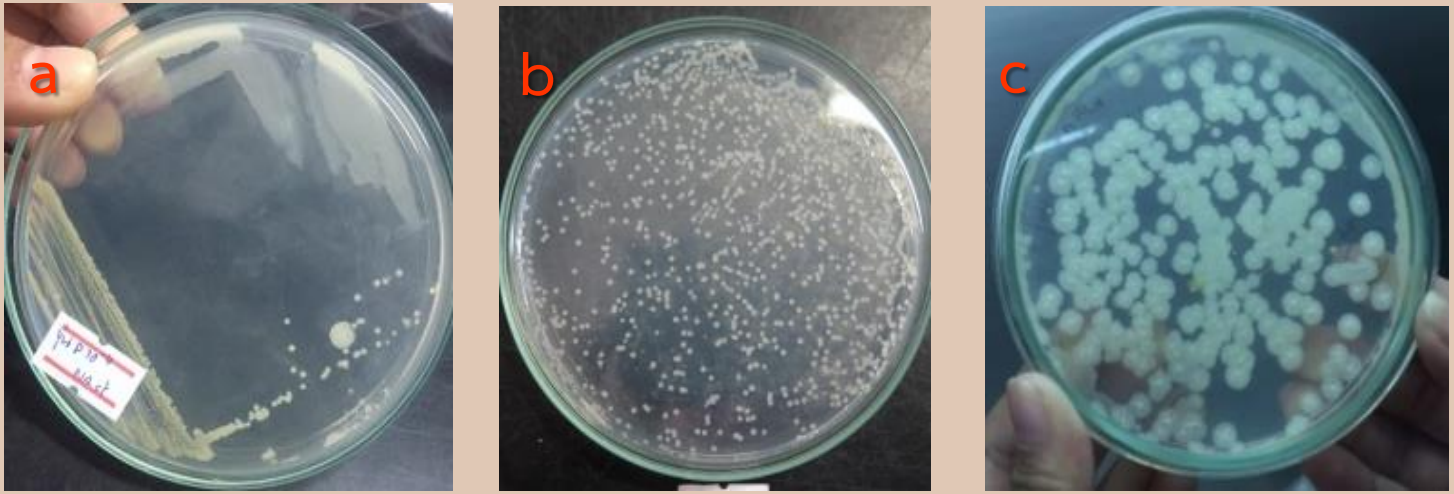


Figure 2 The colony of starch degrading bacteria on starch agar; a= SD1, b= SD2, c=SA1

RESULT

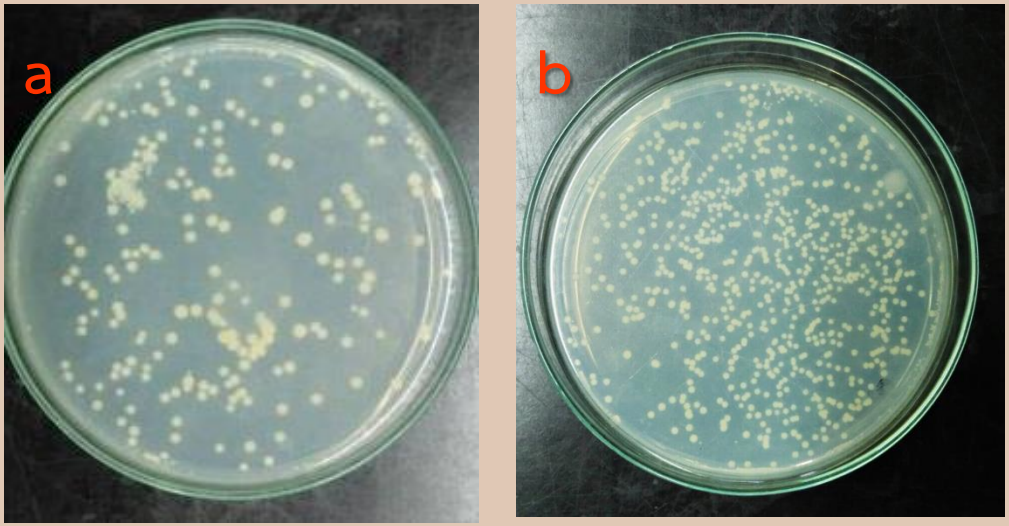


Figure 3 The colony of cellulose degrading bacteria on CMC medium ; a= CE1 , b= CE2

RESULT

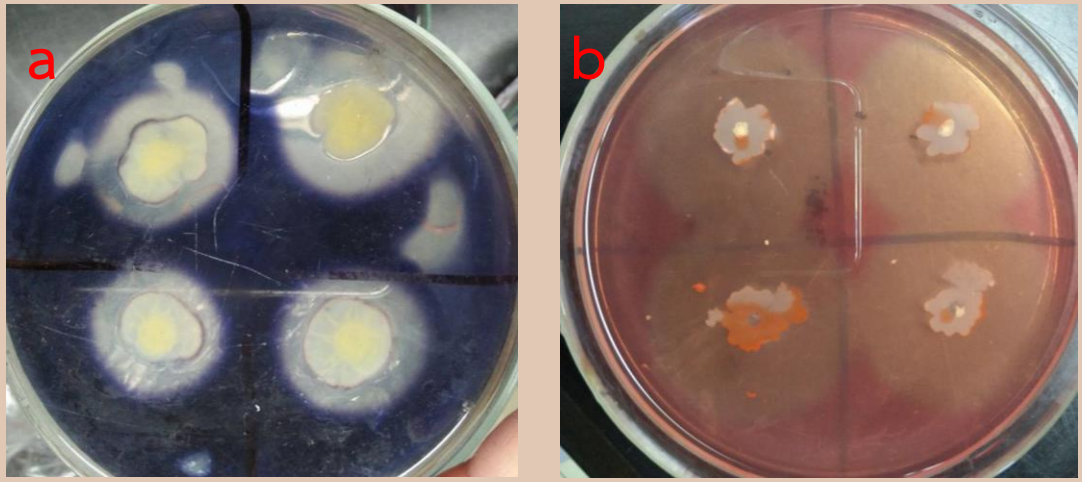


Figure 4 The clear zone of bacteria on starch agar and CMC medium a= bacteria from cassava waste , b= bacteria from paper sludge

RESULT

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

Treatment	Colony (cm.)	Clear zone (cm.)	Ratio
SD1	0.4^{b}	1.1^{b}	3.0^{b}
SD2	0.2^{b}	1.3^{b}	5.7^{a}
SA1	1.6^{a}	2.7^{a}	1.7^{b}
F-test	**	**	*
CV (%)	18.741	15.809	33.124

** : Statistical difference at P<0.01

* : Statistical difference at P<0.05

RESULT

Table 2. The average of colony width, clear zone and ratio of clear zone width per colony width on CMC medium

Treatment	Colony (cm.)	Clear zone (cm.)	Ratio
CE1	0.1[b]	0.6[b]	4.8
CE2	1.2[a]	3.7[a]	3.2
F-test	**	**	ns
CV (%)	17.765	5.371	35.872

** : Statistical difference at P<0.01

ns : No statistical difference

RESULT

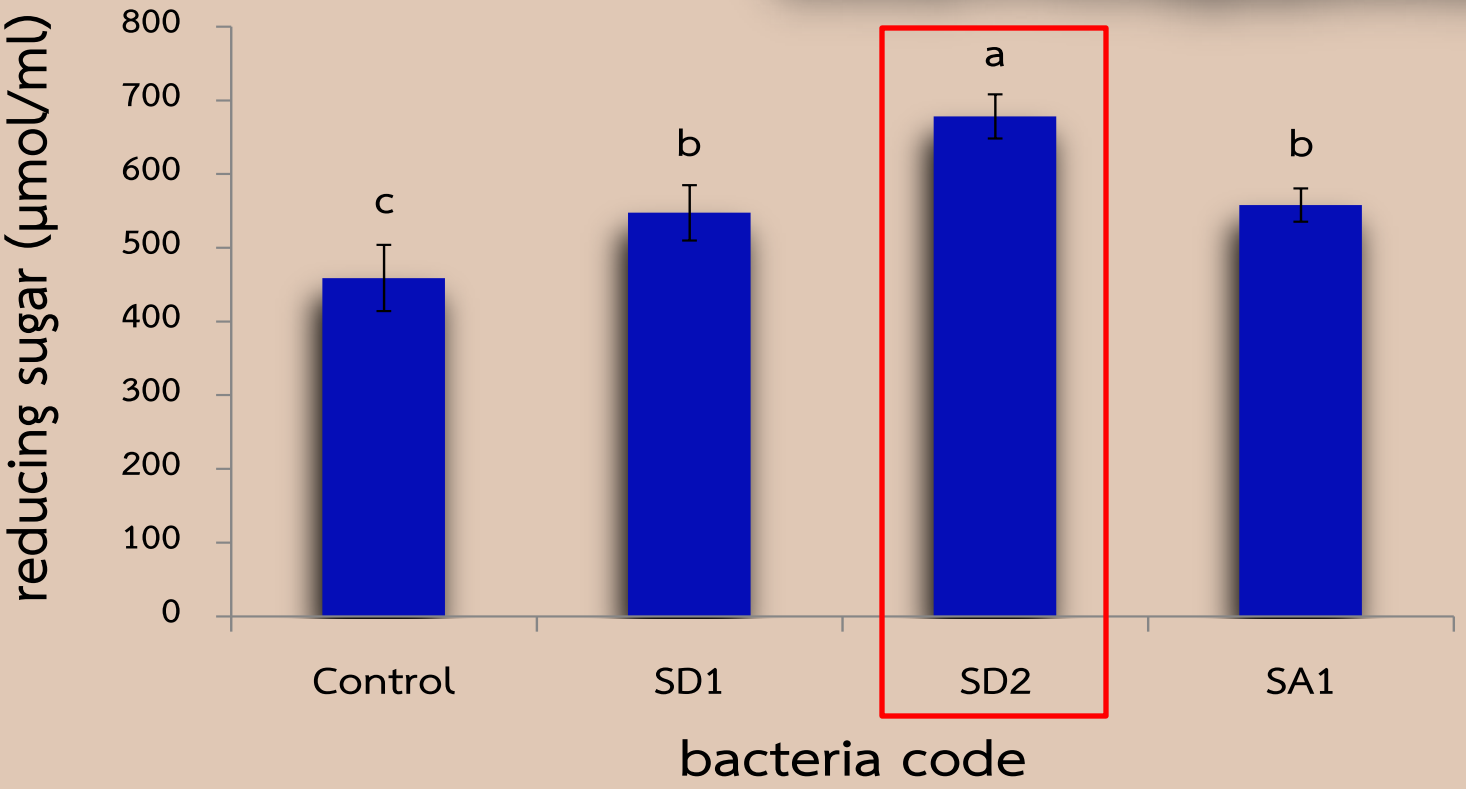


Figure 5 Reducing sugar releasing in CMC medium by using Decanter E85 as substrate

RESULT

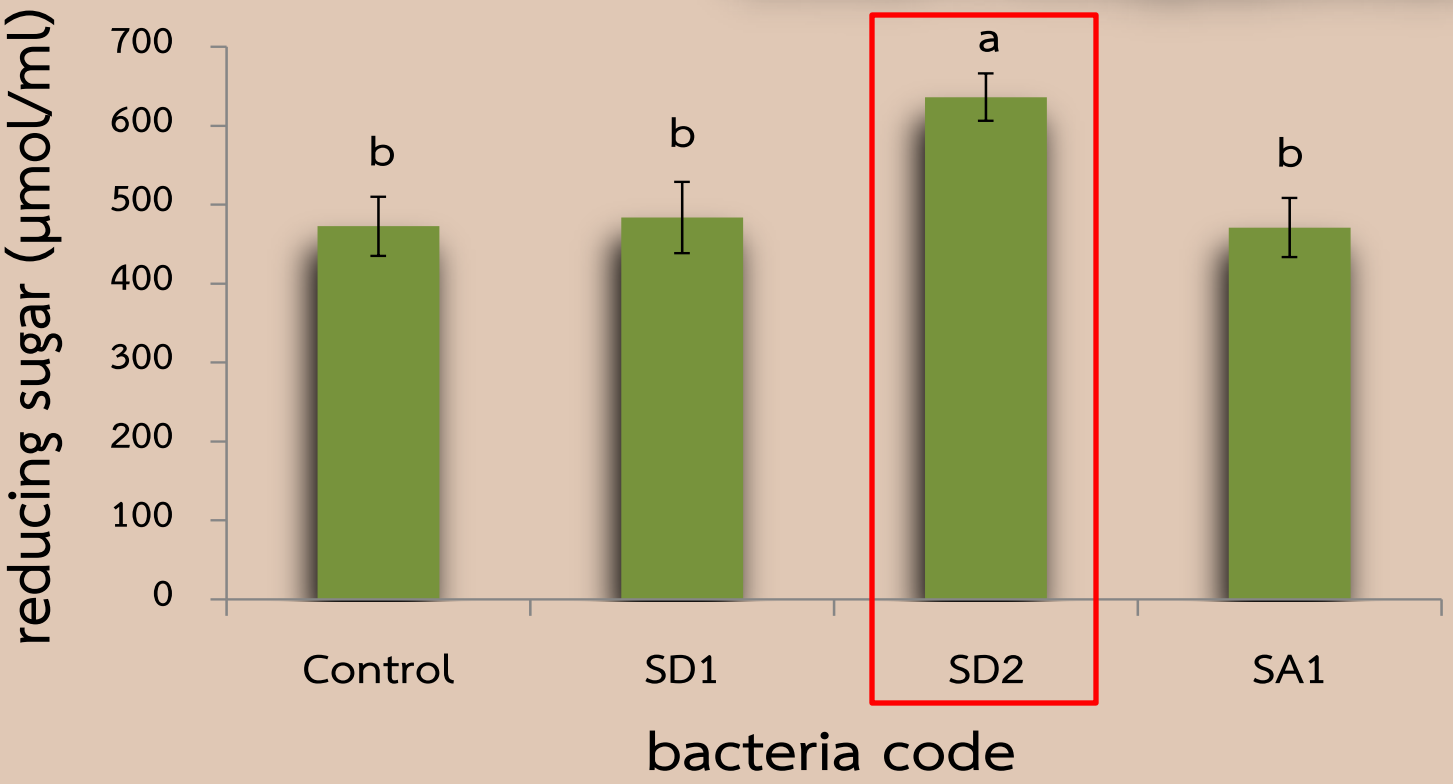


Figure 6 Reducing sugar releasing in CMC medium by using AS E85 as substrate

RESULT

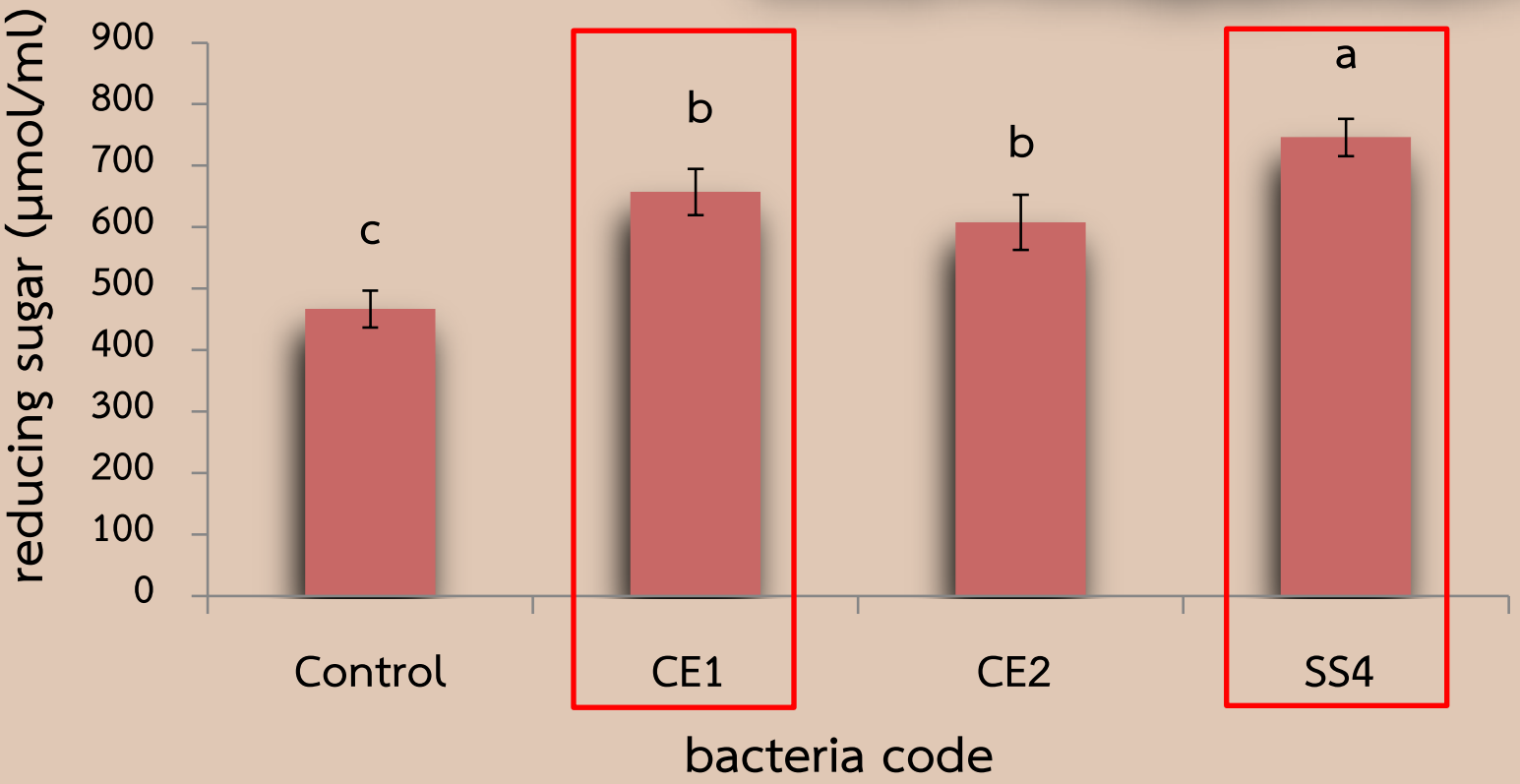


Figure 7 Reducing sugar releasing in CMC medium by using ETP2 as substrate

RESULT

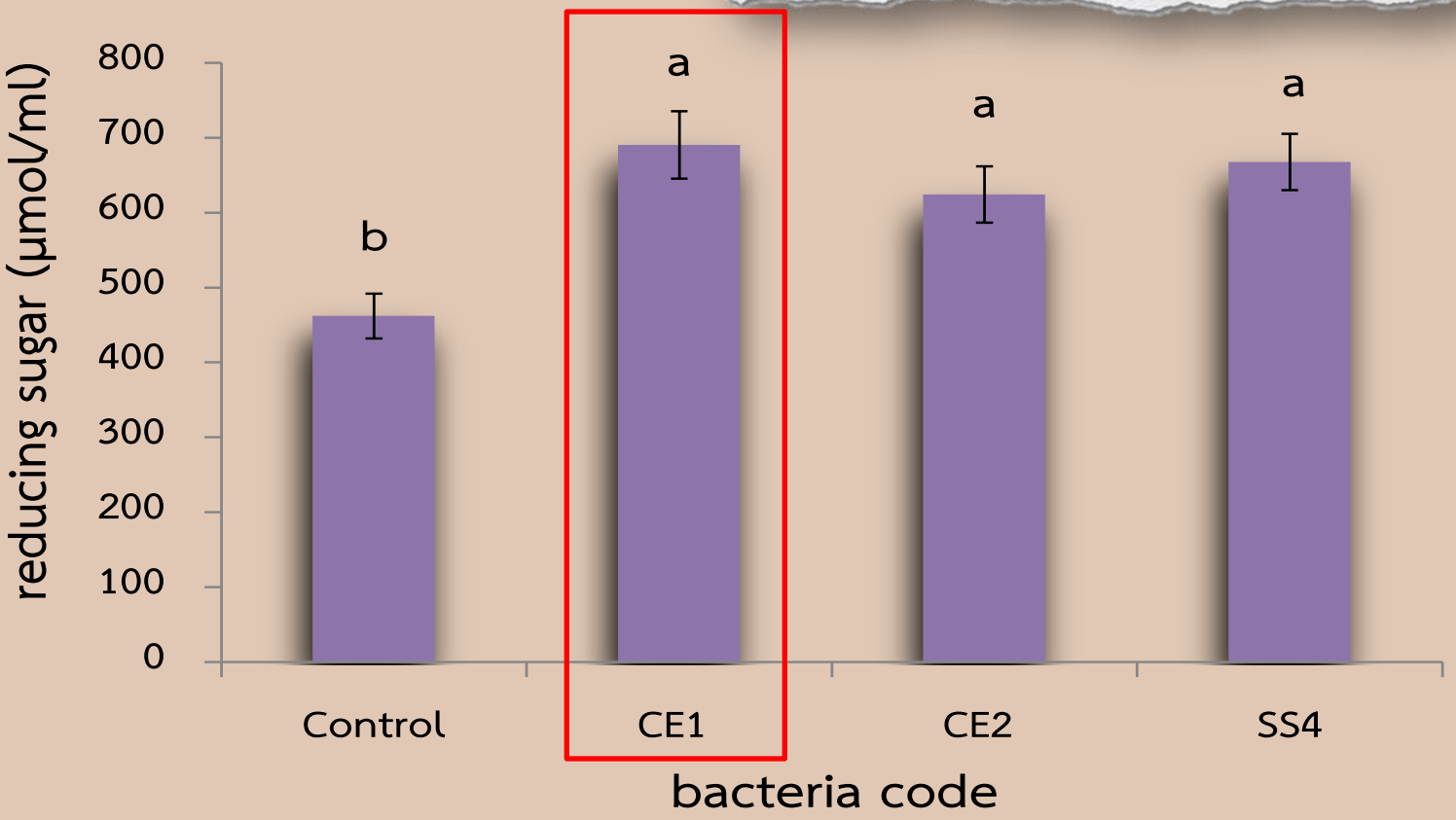


Figure 8 Reducing sugar releasing of reducing sugar in CMC medium by using ETP2A as substrate

RESULT

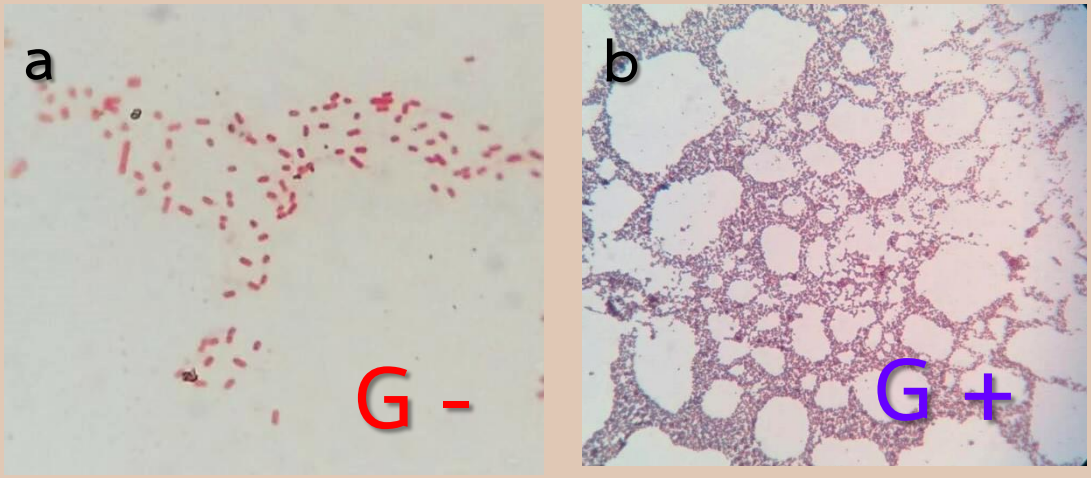


Figure 9 Gram stain of bacteria degraded cassava waste and paper sludge. a = SD2 and b = CE1

RESULT

Table 3 Identification of bacteria degraded cassava waste and paper sludge

Isolate	Species	Identification (%)
SD2	*Klebsiella pneumoniae*	99
CE1	*Staphylococcus Kloosii*	99
SS4	*Bacillus megaterium*	99

RESULT

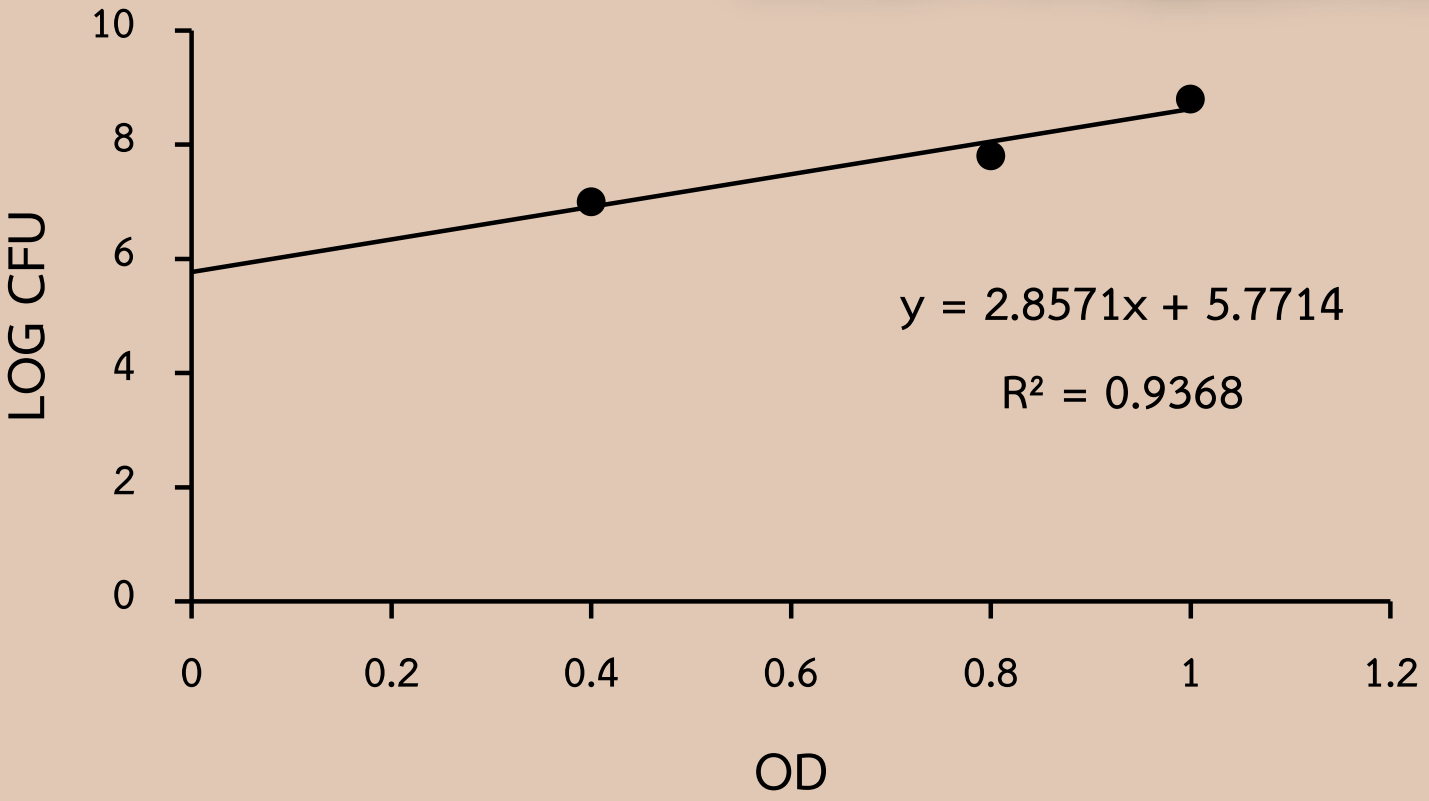


Figure 9 Standard curve of microorganism at different OD

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