

NH₄SO₄), 15 g/L mono potassium phosphate (NPK: 0-52-34), 30 g/L coconut-palm sugar and 1 L pure water.

2.4 Solid-state fermentation (S-SF)

Two plates of TY-P starter culture were inoculated with 1000 mL sterilized liquid medium pH 5 and stirred for 30 minutes. Then 200 mL of this starter culture (10⁹ cell/ml inoculation size of TY-P) was used by mixing with 200 g sterilized cassava waste in a glass bowl then covered with perforated rape film, and incubated at 26°C for 5-7 day. At this time the TY-FS, fresh starter co-culture was extremely green, and then drying in hot oven at 50°C for 2 hours to produce cellulase product, TY-DP.

2.5 Submerge-state fermentation (Sm-SF)

The fermentation affecting factors to ethanol concentration was substrate concentration, enzyme loading, initial fermentation liquid pH value and reaction temperature [12]. The L₉(3⁴) orthogonal experiment design was applied in submerge-state fermentation by using pineapple peel as a substrate. The experiment was carried out in 250 mL shaking flasks of fixed 100 mL liquid medium pH 5 at 32°C, with 3 levels weight of pineapple peel (8, 10 and 12 g), coconut sugar (2, 3 and 4 g) in LM-pH 5, TY-DP inoculation sizes (4, 5 and 6 g) and incubation time (2, 3 and 4 days) as presented in Table 1.

2.6 Analytical methods

The spore concentration which prepared in pure water suspension was determined using a haemocytometer (Boeco, Germany) with a microscope (40X). The reducing sugars were estimated with 3, 5-dinitrosalicylic acid (DNS) reagent [13]. The cellulase activity was measured as filter paper hydrolyzing activity, using a 1×6 cm strip of Whatman no. 1 filter paper, expressed in filter paper unit (FPU) according to the method of Ghose (1987) [14]. Ethanol concentrations were estimated by the dichromate colorimetric method, which based on the complete oxidation of ethanol by dichromate in the present of sulfuric acid to from acetic acid [15].

Table 1. Factors and levels of orthogonal experiment method

Factors	Level		
	C ₁	C ₂	C ₃
Substrate, %w	8	10	12
Enzyme loading TY-DP, %w	4	5	6
coconut sugar, %w	1	2	3
fermentation time, day	2	3	4

3. RESULT AND DISCUSSION

3.1 Effects of condition factors on Sm-SF

The cellulose, hemi-cellulose and lignin content of pineapple peel are 71.7, 17.6 and 10.7 %w, respectively.

The quantity of TY-DP (×10⁷cell/mL) and ethanol concentration (g/L) were used as response values to find the optimum condition on Sm-SF which was shown in Tables 2 and Table 3, respectively. The statistical analysis of the orthogonal experiments for Sm-SF of pineapple peel with TY-DP co-culture in 100 mL LM-pH5 were presented in Table 4 and Table 5, respectively.

Table 2. conditions and TY co-culture of orthogonal experiment

No.	substrate %w	coconut sugar %w	TY-DP %w	time day	TY co-culture cell/mL
1	8	1	6	4	12.5×10 ⁷
2	10	1	4	2	3.25×10 ⁷
3	12	1	5	3	1.00×10 ⁷
4	8	2	5	3	6.25×10 ⁷
5	10	2	6	4	4.38×10 ⁷
6	12	2	4	2	1.50×10 ⁷
7	8	3	4	2	9.25×10 ⁷
8	10	3	5	3	5.00×10 ⁷
9	12	3	6	4	2.75×10 ⁷

Table 3. conditions and ethanol concentration of orthogonal experiment

No.	substrate %w	coconut sugar in LM-pH 5 %w	TY-DP %w	time day	Ethanol conc. g/L
1	8	1	6	4	14.24
2	10	1	4	2	8.18
3	12	1	5	3	15.98
4	8	2	5	3	13.57
5	10	2	6	4	19.39
6	12	2	4	2	9.47
7	8	3	4	2	15.08
8	10	3	5	3	15.00
9	12	3	6	4	12.58

Table 4. statistical analysis of orthogonal experiment for effect to TY co-culture concentration

operating variable	TY co-culture ×10 ⁷ cell/mL at level i			R _j
	C ₁	C ₂	C ₃	
substrate	9.33	4.21	1.75	7.58
coconut sugar in LM-pH5	5.88	4.04	5.67	1.63
TY-DP co-culture	4.67	4.08	6.54	2.64
inoculation time	4.67	4.08	6.54	2.64