Table 5. statistical analysis of orthogonal experiment for effect to ethanol concentration

operating variable	ethanol concentration, g/L at level i			R_j
	C_1	C_2	C_3	
substrate %w	14.30	14.19	12.68	1.62
coconut sugar %w	12.80	14.15	14.22	1.42
TY-DP %w	10.91	14.85	15.40	4.49
fermentation time, day	10.91	14.85	15.40	4.49

The optimal condition by using orthogonal experiment method according to effect of factors to TY-DP co-culture and ethanol concentration are 8%w pineapple peel, 3%w initial coconut sugar in LM-pH5, 6%w of TY-DP and 4 days fermentation. The ranking, from high to low, of influential boundary of Sm-SF affecting factors to ethanol concentration was TY-DP co-culture, incubation time, substrate and initial coconut sugar concentration, respectively.

3.2 Ethanol fermentation at the optimal condition

The ethanol concentration in Sm-SF by using starter co-culture TY-DP depends on enzymatic hydrolysis of fungi *Trichoderma reesei* RT-P1 which converted cellulose into reducing sugar and then sugar fermenting *Saccharomyces cerevisiae* RT-P2 was employed. Thus saccharification rate of cellulose increases with the increasing enzyme concentration and time [12]. Due to designed boundary time of fermentation was limited for 4 days in this work. The ethanol fermentation at the optimal condition was performed for 5 day.

The result of ethanol obtained under the optimal condition was presented in Fig. 1 which was found that starter co-culture TY-DP can convert reducing sugar to ethanol at the initial time of Sm-SF even though its lag phase is 1 day. The maximum ethanol concentration is 42 g/L or 52.5% dry weight of pineapple skin at 4 days fermentation.

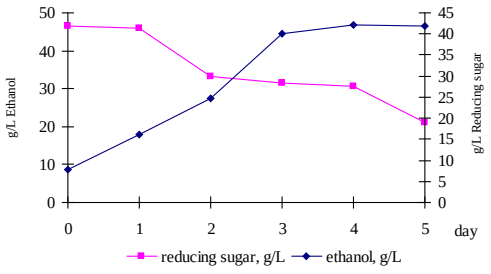

Fig. 1 ethanol and reducing sugar with time from pineapple peel in Sm-SF at the optimal condition

According to cellulase activity and growth of TY increased with time at 1 day incubation which was shown in Fig. 2. It might be due to the sufficient of micro and macronutrient in the fermentation medium at this optimal condition. Thus increasing ethanol concentration obtained from cellulose of pineapple peel hydrolysis by cellulase [9].

The different of ethanol fermentation from pineapple peel with co-culture of *Trichoderma reesei* RT-P1 and *Saccharomyces cerevisiae* RT-P2 in this study are cultivation method of starter co-culture, the composition of micro and macronutrients in liquid medium pH5 and initial coconut sugar concentration according to previous research; [5], [8], [10], [19-20]. Thus this co-culture TY-DP can be used as an alternative □ellulose for lignocellulosic materials hydrolysis to its ethanol production. The comparison of ethanol concentration in the present and previous studied was shown in Table 6.

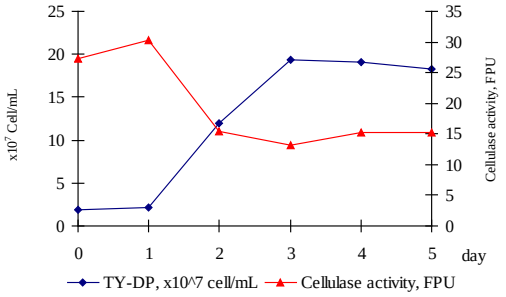

Fig. 2 cellulase activity and TY-DP concentration with time from pineapple peel in Sm-SF at the optimal condition

4. CONCLUSIONS

The optimal condition of ethanol fermentation is 8%w pineapple peel as a substrate, 6%w co-culture TY-DP, 3%w initial coconut sugar in LM-pH5 and 4 days incubation time, 42 g/L ethanol obtained or 52.5% dry weight of pineapple skin.

The direct use of co-culture TY-DP fermentation broths for the saccharification of pineapple peel resulted in about the same ethanol concentration as the use of cellulase from co-culture of *Aspergillus niger* and *Saccharomyces cerevisiae* [17] Recombinant *Klebsiella oxytoca* P2 and *Saccharomyces pastorianus* [19]. Therefore the new enzyme, TY-DP has high potential to ethanol production on cheap lignocellulosic substrate and easily used, could lead to more cost-effective production of bioethanol.