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Bioplastic Sheet Production from 1,3-Propanediol Produced by Raw Glycerol Fermentation

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Abstract

1,3-Propanediol (PDO) was produced by *Enterobacter radicincitans* TISTR 1805 using crude glycerol fermentation for bioplastics sheet production. PDO production was scaled up to 150 L in a 300 L pilot scale fermentor by 5% crude glycerol at 37 ºC during 3 days. The PDO yield was 344.3 mmol mol^{-1} pure glycerol and the cell growth rate was 8.1-28.8 mg L^{-1} hr^{-1}. After that, the culture product was passed through purification process by diatomite filtration and fractional distillation at the range of 150-300 ºC, using the pressure of 10 mmHg. The purity of PDO was 98%. Preparation of polypropylene succinate (PPSu) using PDO was carried out by esterification and polycondensation processes. Then, the PPSu was blended with cellulose acetate and casted for bioplastic film. The preliminary result of biodegradation test in compost revealed that this film could be degraded 69-85% within 3 months at 58±2 ºC incubation. Furthermore, studies on primary testing of toxicity of residues after degradation on a tested animal, *Daphnia magna*, and a tested plant, *Brassica chinensis*, were also performed.

Keywords: 1,3 - Propanediol, PDO, Crude glycerol, Fermentation, *Enterobacter radicincitans*, biodegradable plastics

1. Introduction

Biodiesel, an alternative diesel fuel, is made from renewable biological sources. Glycerol is the principal by-product obtained during transesterification of vegetable oils and animal fats which represents 10% (v/v) of ester. The increasing need for renewable fuels throughout the world and the increasing demand and production of biodiesel (1). At present, there are 6 bio-diesel plants in Thailand producing 400,000 litres of bio-diesel daily. One of the major problems that many entrepreneurs are facing is a large amount of by product, especially crude glycerol from bio-diesel production process. Thus, if this by-product is not carefully undertaken in terms of utilization and good management, it will be an overall impact on the environment in the near future.

Several alternatives are being explored to utilize crude glycerol, and some methods have been established to produce 1,3-propanediol (PDO). PDO is a monomer with a variety of applications in several industries such as the synthesis of polyester, lubricant additive, solvent, and the main chemical for biodegradable plastic production. This monomer can be produced in either chemical synthesis, requires high temperature, high pressure and expensive catalysts, or biotechnology processes such as sugar fermentation. Since glycerol can be used as a carbon source in industrial microbiology, this by-product adds value to the productive chain of the biodiesel industry. This process is low cost raw materials and environmental benefits.

Biodegradable plastics are expected to be used as food packaging materials or agricultural materials which became waste along with organic waste such as food waste or the remaining parts of vegetables. These plastic wastes with organic waste can be added to or used as feedstock for biological recycling. Biodegradation is a process in which naturally-occurring microorganisms such as bacteria, fungi or algae acting on the material. Biodegradable plastics break down completely into non-plastic and nontoxic constituents like water, CO_2, CH_4 and biological materials (2).

Aliphatic polyester due to their favourable features of biodegradability and biocompatibility are becoming commercially available and increasing. Some of them are polycaprolactone (PCL), poly (hydroxybutyrate) (PHB), poly(lactide) and poly (butylene succinate) (PBSu). These polymers show a wide variety in their physical and mechanical properties directly comparable with that of many traditional and nonbiodegradable polymers (3). In this study, poly(propylene succinate) (PPSu) was prepared from 1,3-propandiol derived from by-product of biodiesel. The biodegradation was also measured.

2. Materials and methods

Microorganism, Fermentation and Purification

Enterobacter radicincitans TISTR 1805, which was isolated from waste water of biodiesel process, was used in this study. This bacterium was precultivated in 15 L of ENT, which was used as a mineral medium at 37°C. After 3 days incubation, the cells were inoculated into 150 L of ENT containing 5% by weight crude glycerol in a 300 L fermentor (approximately 10^8 cfu mL^{-1}) at 37°C, agitation speed 150 rpm for 3 days. Then, the supernatant was collected by diatomite filtration and passed through purification process by fractional distillation at the range of 150-300 °C, using the pressure of 10 mmHg.

Synthesis and film preparation

Synthesis of aliphatic polyester poly(propylene succinate) (PPSu) was performed following the two stages: esterification and polycondensation. The PDO from commercial and fermentation of crude glycerol was used for PPSu synthesis. The PPSu and cellulose acetate (CA) blends were prepared by solution cast method (3). The solution ratio of PPSu : CA: acetone as 1:1:25, 1:2:25 and 1:3:25 and then casted on the glass plate with doctor blade. Immersion the casting film in water for eliminates residual solvent. The film was dried under atmospheric condition. Chemical structures through Fourier transform infrared spectroscopy (FTIR, Shimadzu: Prestige-21).

Preliminary disintegration test in compost

The quality compost (referred to ISO 14855-1) was used for disintegration test of PPSu-PDO sheets. The mixture was combined with 500 g of dry weight compost and 12 plastic sheets, (2.5 x 15 cm.) in an anaerobic jar at 58 °C. The plastic samples were collected from the compost at 30, 60, 90 days of incubation. All samples were washed with water and dried in a desiccator until the weight was constant before analyzed for changes in chemical structure through Fourier transform infrared spectroscopy (FTIR), for mechanical strength through a tension test by ASTM D882. The degree of disintegration was estimated from the weight loss analysis of sample as Eq (1).

$$\text{Weight loss (\%)} = [(W_O - W_T)/W_O] \times 100 \qquad (1)$$

Where W_O is the weight of original film and W_T is the weight of the film after degradation.

Preliminary biodegradation test

The preliminary biodegradation test of PPSu-PDO sheets compared with positive reference (Cellulose microcrystalline, R+) and negative reference (Polyethylene sheet, R-). This method was estimated by modified procedure of static incubation-titrimetric determination (4). A mixture of 20 g wet weight compost and 0.2 g test sample per replication was put into a closed system with 1 N NaOH at 58°C. The CO_2 was trapped by 1 N NaOH solutions and titrated with 0.2 N HCl against phenolphthalein indicator after precipitation with 3N $BaCl_2$. It was measured every day until CO_2 was constant. The biodegradation was calculated using Eq (2) according to ISO 14855-1.

$$\text{\% biodegradation} = \frac{(CO_2)_T - (CO_2)_B \times 100}{Th\,CO_2} \qquad (2)$$

$$ThCO_2 = M_{TOT} \times C_{TOT} \times \frac{44}{12}$$

Where $(CO_2)_T$ is the amount of cumulative carbon dioxide evolved in each composting vessel containing test material, in grams per vessel.

$(CO_2)_B$ is the mean amount of cumulative carbon dioxide evolved in the blank vessel, in grams per vessel.

$ThCO_2$ is the theoretical amount of carbon dioxide

M_{TOT} is the total dry solid in the test material introduce into the composting vessels at the start of the test, in grams.

C_{TOT} is the proportion of total organic carbon in the total dry solid in the test material, in grams per vessel.

44 is the molecular weight of carbon dioxide and 12 is the molecular weight of carbon.

High performance liquid chromatography (HPLC)

To measure the concentration of glycerol and PDO, 10 μL of the culture supernatant was analyzed using HPLC (LC-10AD, Shimadzu) with a RI detector (RID-10A, Shimadzu) at 40°C. An Aminex HPX-87H (7.8 x 300 mm, BIO-RAD) column was used, and the liquid phase consisting of 0.005 N H_2SO_4 in water was supplied at a flow rate of 0.7 mL min^{-1}.

3. Results and discussion

Conversion glycerol to PDO

PDO production by *E. radicincitans* TISTR 1805 from crude glycerol fermentation in 150 L for 3 days was shown in table 1. The maximum growth for this bacteria strain was observed since the first day incubation, the cell growth rate was 8.1-28.8 mg L^{-1} hr^{-1}. The PDO yield was 344.3 mmol mol^{-1} pure glycerol. The purity of PDO was 98% after pass purification process.

Spectroscopic characterization

The FT-IR spectra of PPSu was shown in Fig 1. The peaks within 1,820-1660 cm^{-1} were assigned to carbonyl (C=O) indicate aliphatic ester group. The peaks at 1,300-1,000 cm^{-1} were assigned to C-O bonding. The peaks at 3,000-2,800 cm^{-1} were assigned to C-H aliphatic bonding. The FT-IR spectra were found in PPSu chemical structure.

Table 1. Growth of *E. radicincitans* TISTR 1805 and PDO productivity

Time (Days)	No. of cell (x 10^7cfu mL^{-1})	Yield of cell (mg g^{-1} glycerol)	Yield of PDO (mmol mol^{-1} glycerol)	Cell growth rate (mg L^{-1} h^{-1})	productivity rate (mg L^{-1} h^{-1})
T0	27.0	-	-	-	-
T1	50.0	16.5	344.3	28.8	529.7
T2	0.6	13.6	347.0	12.1	271.6
T3	2.3	13.7	335.1	8.1	174.9

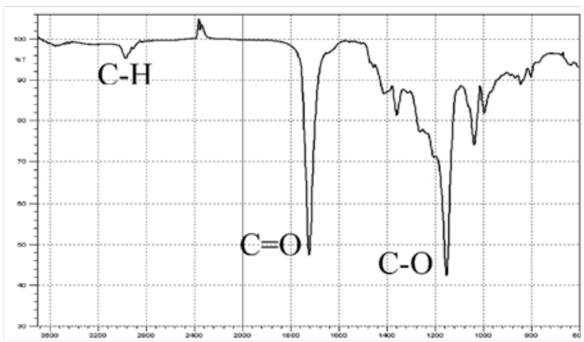

Figure 1. FT-IR spectra of PPSu

Preliminary disintegration test in compost

The PPSu and CA were blend to plastic sheet. The sample, PPSu-PDO-1 used PDO from commercial, while PPSu-PDO-2, PPSu-PDO-3 and PPSu-PDO-4 used PDO from fermentation of crude glycerol for PPSu synthesis. The R- was a polyethylene film as a negative control.

For preliminary disintegration test, the effect of composting on synthetic plastic sheet change in weight loss, mechanical strength and chemical structure were investigated. In table 2, the results shown the weight of plastic sheets from polymer blend could be decreased 69-85% within 3 months. Moreover, PDO from fermentation was degraded higher than PDO from commercial.

The mechanical strength of all plastic sheets from polymer blend was shown in table 3. It was found that the elongation decreased significantly within the incubation time, referred to the plastic film became brittle by composting.

FT-IR spectra of plastic blends from the disintegration test were shown in Fig 2. During composting conditions, there was a decrease in the absorption intensity of carbonyl (C=O) of ester groups compared with the original plastic blend. These results showed that the chemical structures of polymer blend were changed after disintegration due to hydrolysis and microorganisms in the compost effective to the ester bond.

Table 2. Weight loss (%) during incubation of plastic blends at 58 ± 2 °C

Sample	Weight loss (%)		
	30 days	60 days	90 days
R-	-	-	-
PPSu-PDO-1	19.74	32.08	49.15
PPSu-PDO-2	34.35	71.51	83.67
PPSu-PDO-3	33.13	57.21	69.66
PPSu-PDO-4	38.04	75.31	85.49

Table 3. Mechanical strength during incubation of plastic blends at 58 ± 2°C

Time (days)	R-		PPSu-PDO-1		PPSu-PDO-2		PPSu-PDO-3		PPSu-PDO-4	
	Stress	Strain	Stress	Strain	Stress	Strain	Stress	Strain	Stress	Strain
0	483.85 c	234.27 b	192.88	47.66	169.60	60.08	162.64	55.34	168.98	65.07
30	587.68 b	358.58 a	206.39	7.16	165.54	4.69	200.67	6.08	294.36	7.94
90	739.23 a	356.00 a	160.60	8.42	-	-	-	-	-	-
F-Test	*	*	ns	*	ns	*	ns	*	*	*
C.V.	0.53	0.58	0.11	1.84	0.31	2.10	0.70	2.00	0.68	1.92

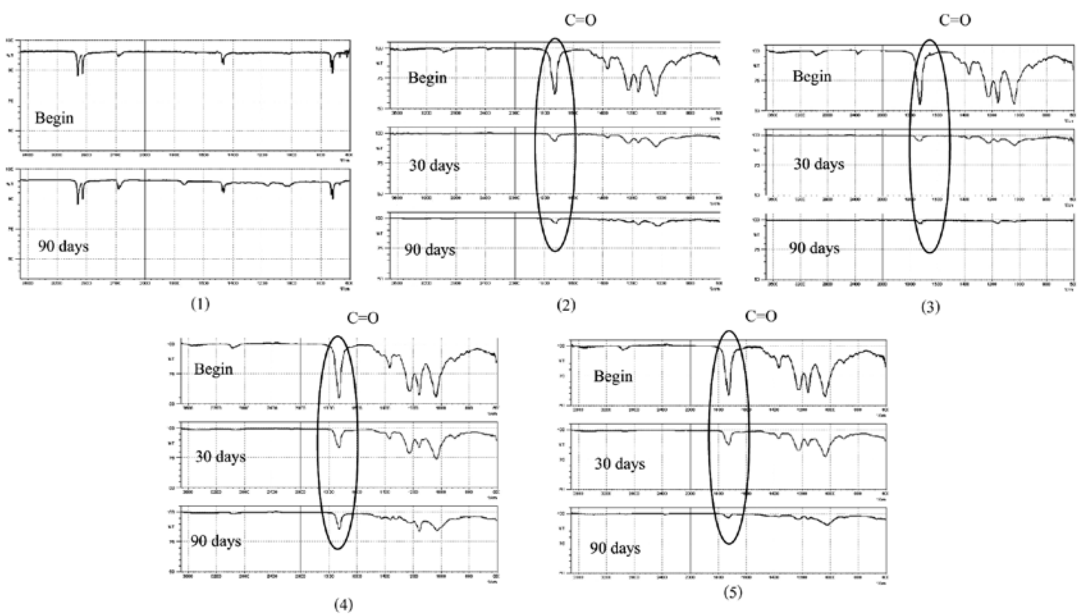

Figure 2. FT-IR spectra of plastic blends of facultative biodegradation test

(1) R-, (2) PPSu-PDO-1, (3) PPSu-PDO-2, (4) PPSu-PDO-3, (5) PPSu-PDO-4

Preliminary biodegradation test

Table 4 shows the biodegradation percentage of PPSu-PDO sheet. The results showed that all samples of PPSu-PDO sheets were biodegradable plastic when compared with polyethylene as the negative reference. The biodegradation percentage of polymer blends was greater than 60% of biodegradability near the positive reference.

The biodegradation process of this polymer blends was described by two stages. First, the ester bonds of polymer are broken down by hydrolysis degradation process with out enzyme, so the polymers molecular are decreased. And the second stage, the polymer chains are broken down into small particles, so they are used as food sources for microorganisms and carbon dioxide gas is released (5).

Table 4. Biodegradation during incubation of plastic blends at 58 ± 2°C

Samples	Biodegradation (%)
R+	74.60
R-	0.00
PPSu-PDO-1	62.11
PPSu-PDO-2	69.83
PPSu-PDO-3	73.45
PPSu-PDO-4	65.30

4. Conclusion

The PDO was produced by *E. radicincitans* TISTR 1805 using 5% crude glycerol fermentation was scaled up to 150 L. There was the PDO yield 344.3 mmol mol^{-1} pure glycerol and the cell growth rate was 8.1-28.8 mg L^{-1} hr^{-1}. The preliminary of disintegration and biodegradation test in compost revealed that the polymer blend could be degraded 69-85% within 3 months at 58±2 ºC incubation. Furthermore, studies on primary testing of toxicity of residues after biodegradation on a tested animal, *Daphnia magna*, and a tested plant, *Brassica chinensis*, were also performed.

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