Identifying economical route for crude glycerol valorization: Biodiesel versus polyhydroxy-butyrate (PHB)

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Abstract

Crude glycerol, a by-product of biodiesel industry, has been used for production of biodiesel and polyhydroxy-alkanoates. But question is: which product is economically favorable using crude glycerol as substrate? In this study, energy balance and economic assessment has been carried out for crude glycerol valorization for B10 biodiesel and polyhydroxy-butyrate (PHB) production. For same quantity of crude glycerol utilized, energy ratio for B10 production was higher than PHB production while unit production cost for B10 was lower than that of PHB. For 50 million L plant capacity of biodiesel, unit production cost was 0.77 \$/L B10 while for 2 million kg plant capacity of PHB, unit production cost was 4.88 \$/kg PHB. Thus, in present scenario production of biodiesel seems economically better than production of PHA with crude glycerol as raw material. This study is useful for researchers, environmental scientists and industries in identifying effective route for crude glycerol valorization.

Keywords: Crude glycerol; Economic evaluation; Biodiesel; Polyhydroxy-butyrate (PHB)

1 Introduction

Crude glycerol is a by-product of biodiesel industry, produced through *trans*-esterification of lipids. Around 1 tonne of crude glycerol is generated, for every 10 tonnes of biodiesel produced during the *trans*-esterification reaction. Annually, 5.87 billion pounds of crude glycerol is produced according to the demand for 8 billion gallons of biodiesel production. Due to continuous production of crude glycerol as by-product during *trans*-esterification, a substantial decrease in price of crude glycerol (from 0.25 \$/lb to 0.05 \$/lb) has been observed (Anitha et al., 2016; Mota et al., 2017; Vivek et al., 2017). The purification cost of crude glycerol to attain a commercial level can be as high as 50.85 \$/kg (Chol et al., 2018) while the market price of pure glycerol is 1–15 \$/kg. But crude glycerol can be used as low-cost carbon source for generation of biomass and microbial products through fermentation (Gao et al., 2016; Gong et al., 2015, 2016; Leite et al., 2015). Besides, crude glycerol conversion to value-added products provides an alternative route for crude glycerol disposal (Chen et al., 2020b; Jiang et al., 2020; Rodrigues et al., 2020). Over the past decade, the crude glycerol has been used by researchers for production of lipids (Chen et al., 2020a; Kumar et al., 2021) and polyhydroxy-alkanoates (PHA) through fermentation (Koller et al., 2017; Yadav et al., 2020). The microbial lipids can be *trans*-esterified to produce biodiesel which is bio-degradable, renewable and low-carbon emitting fuel. On the other, PHAs are renewable and biodegradable plastics and have applications in packaging materials, disposable items, and in medical science (Alcântara et al., 2020; Sabapathy et al., 2020; Yadav et al., 2020).

Both biodiesel and PHA are renewable, biodegradable and have environmental advantages. Although crude glycerol has been used by many researchers for production of either biodiesel or PHA, the question is: from economic view point which product is favorable if crude glycerol is used as substrate? In this study, energy balance and cost analysis have been performed for biodiesel and PHA production using crude glycerol as carbon substrate. Best upstream and downstream processes have been selected from the literature to perform these analyses. Through energy balance and cost estimation, the best product (out of biodiesel and PHA) has been identified through crude glycerol valorization. The study will be useful for industries and environmental scientists for identifying economical route for crude glycerol valorization. Besides, it will also be helpful to lab researchers to identify best product for utilizing crude glycerol as substrate for future research.

2 Materials and methods

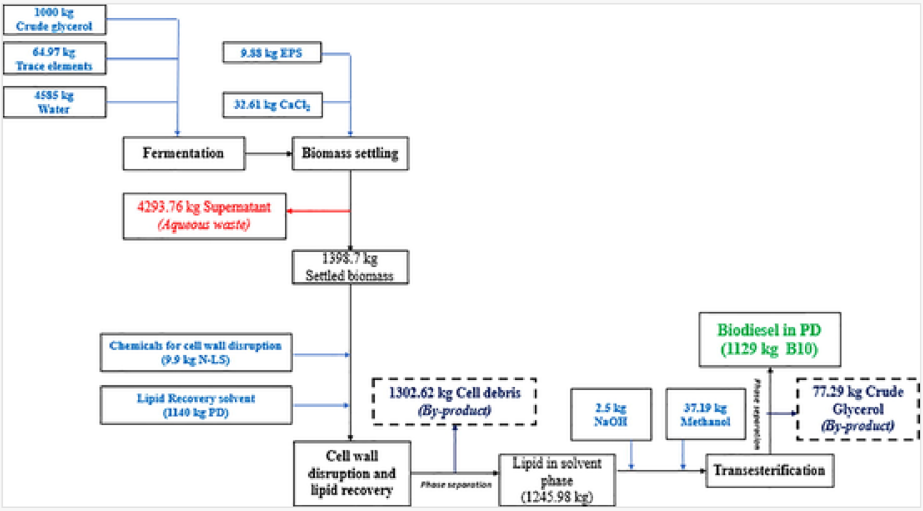
2.1 Process justification and description

2.1.1 Crude glycerol to biodiesel

The fermentation process for microbial lipid production was selected from study of Chen et al. (2017). This study was selected due to the fact that high lipid productivity (0.42 g/L/h) was obtained in short fermentation time (52 h). The downstream processing for biodiesel production was selected from Yellapu et al. (2019). The biomass settling was performed using bio-flocculant, extracellular polymeric substances (EPS) instead of expensive centrifugation. While lipid was extracted from wet biomass using surfactant n-lauryl sarcosine (N-LS) and lipid was recovered using petro-diesel (PD). This downstream process has already been proven energetically and economically favorable over conventional solvent based lipid extraction from dried biomass (Kumar et al., 2020a, 2019b). Other lipid extraction processes like enzymatic method, bead-milling and sonication are either expensive or highly energy consuming (Kim et al., 2013; Yellapu et al., 2018).

The fed-batch fermentation for microbial lipid production was adopted using *T. oleaginosus* and reported by (Chen et al., 2017). The crude glycerol had (w/v) composition: 13.24% glycerol, 10.37% water, 23.58% soap, 3% ash, 31.14% methanol and 3.1% NaOH. The trace elements provided for cell growth at 0 h were: 5.4 g/L KH_2PO_4, 1.9 g/L Na_2HPO_4, 3.23 g/L NH_4Cl, 0.2 g/L $MgSO_4 \cdot H_2O$ and 0.8 g/L peptone. At 52 h, 177 g/L crude glycerol (including FFA and glycerol) was utilized while 43.82 g/L biomass and 21.87 g/L lipid was produced (Chen et al., 2017). Calcium chloride (52 mM) in combination with extracellular polymeric substances (39.9 mg EPS/g biomass) were used for biomass settling (Yellapu et al., 2019). The concentration of $CaCl_2$ and EPS was dependent on the biomass concentration (more than 40 g/L biomass). The settled biomass (177 g/L) was treated with N-lauryl sarcosine (40 mg N-LS/-g biomass) under agitation for lipid extraction. Petroleum-diesel (PD) was used as solvent for lipid recovery (10 mL PD/g lipid) at 70 °C under agitation for 15 min (Yellapu et al., 2019). After phase separation, the lipid-PD mixture was reacted with methanol (6:1 M ratio of methanol: lipid) for *trans*-esterification in presence of catalyst (1% w/w NaOH). The lipid extraction efficiency of 95% and *trans*-esterification efficiency of 97% was obtained, respectively (Yellapu et al., 2019). Final product produced was B10: 10% Biodiesel (fatty acid methyl ester) and 90% PD. The composition of final stream was 10.06% fatty acid methyl ester (FAMEs/ biodiesel) and 89.94% PD. The process flow diagram for B10 biodiesel production is shown in Fig. 1.

Fig. 1



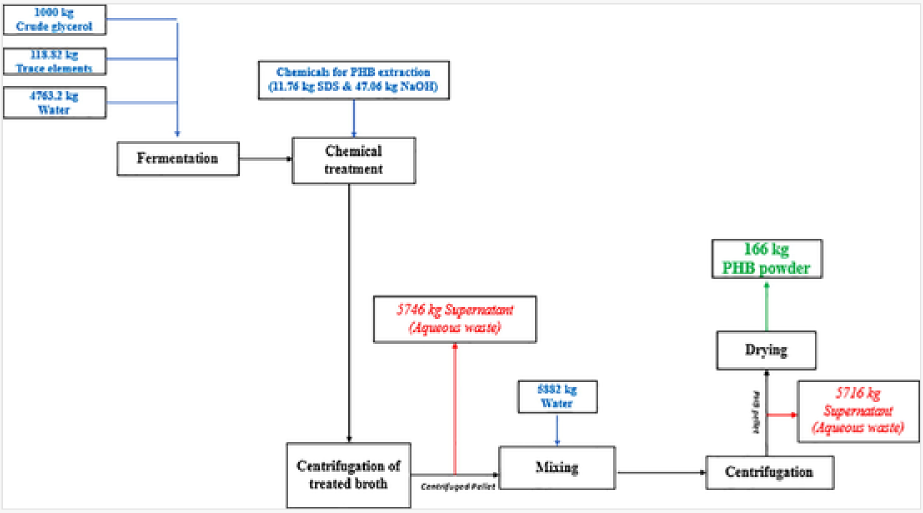
Biodiesel production through valorization of crude glycerol.

2.1.2 Crude glycerol to PHA

The fermentation process for polyhydroxybutyrate (PHB) production was chosen from study of (Cavalheiro et al., 2009). This study was chosen as high PHB productivity (1.1 g/L/h) was obtained in short fermentation time (35 h). Biomass concentration of 76.2 g/L and PHB concentration of 38.1 g/L (50% PHB content) was produced in 35 h. For PHA extraction, several methods have been reported: alkali based, acid based, surfactant based and solvent based (Dietrich et al., 2017; Righi et al., 2017). Solvent based extraction is environment unfriendly as toxic solvents are harmful for human health and environment (Dietrich et al., 2017). Sodium hydroxide and sodium dodecyl sulphate (NaOH-SDS) based extraction has been reported to be the most favorable due to low extraction costs, less greenhouse gas (GHG) emission and non-renewable energy use when compared to other extraction processes (Fernández-Dacosta et al., 2015). Another advantage of using the above extraction process is direct extraction from fermented broth eliminating the requirement of biomass drying and biomass settling.

The fed-batch fermentation for P3HB poly(3-hydroxybutyrate) production was conducted using *C. necator* DSM 545 (Cavalheiro et al., 2009). The crude glycerol solution obtained from one of the biodiesel industries contained 1174.5 g/L glycerol, 3% (w/w) Na, <0.2% (w/w) methanol and <1.5% (w/w) monoacylglycerides. The trace elements supplied at 0 h were: 4 g/L $(NH_4)_2SO4$, 13.3 g/L KH_2PO_4, 1.7 g/L citric acid and 1.2 g/L $MgSO_4 \cdot H_2O$ At 35 h of fermentation, 170 g/L glycerol was utilized while 76.2 g/L biomass and 38.1 g/L PHB was produced (Cavalheiro et al., 2009). The fermented broth was subjected to chemical treatment with sodium hydroxide (NaOH, 0.2 M) and sodium dodecyl sulphate (SDS, 0.2 w/v%) at 30 °C for 1 h (Fernández-Dacosta et al., 2015). This was followed by centrifugation for removal of supernatant from treated cell mass. Thereafter, solid impurities (including cell debris) were removed from centrifuged pellet by mixing it with water followed by centrifugation. This was followed by PHB drying for removal of residual humidity (Fernández-Dacosta et al., 2015). After drying, PHB powder with 99.9% purity was obtained with 73.5% overall extraction yield (Fernández-Dacosta et al., 2015). The loss in PHB occurred during centrifugation and washing step. The process flow diagram for PHB production is shown in Fig. 2.

Fig. 2



PHB production through valorization of crude glycerol.

2.2 Mass and energy balance

Mass and Energy balance was computed based on per tonne of crude glycerol utilized for production of B10 biodiesel and PHB. The energy balance have been performed according to Kumar et al., (2019b). The positive net energy balance (energy output-energy input) or energy ratio (energy output/energy input) higher than 1 are required to make the process energetically favorable.

2.3 Simulation description

In this study, using SuperPro designer (v10), two processes utilizing crude glycerol were simulated to produce blended biodiesel B10 and poly(3-hydroxybutyrate), respectively. The simulations were performed to utilize 2000 tonnes of crude glycerol (produced in Quebec province, Canada) per year. The plants were assumed to operate continuously for 350 days per year.

For biodiesel plant, 2.6 million L B10 was produced annually based on annual utilization of 2000 tonnes crude glycerol. For microbial lipid production, two fermenters with 50,000 L capacity were used for processing consecutive batches. The biodiesel plant continuously operating for 350 days/year would result in 274 batches, while there was time gap of 30.42 h between inoculation of two batches. The process timeline for processing of 1 batch of B10 has been indicated in Table 1a.

Table 1a

Process Timeline for processing of 1 batch of B10.

Operation	Description
FR-101	**Production Fermenter**
Cleaning in place	60 min, Cleaning of equipment with 1 M NaOH solution supplied at 60 °C
CHARGE crude glycerol	30 min, Charge crude glycerol to production fermenter
Sterilization at 121 °C	180 min (including holding time of 15 min)
COOL	180 min, Cooling to 30 °C
CHARGE trace elements	15 min, Charge trace elements to main fermenter
Inoculum TRANSFER	30 min, Transfer inoculum from seed fermenter to main fermenter
Fermentation	52 h, Fermentation at 30 °C

Transfer-out	60 min, Transfer the fermented broth to a clarifier
CLR-101	**Biomass settling in a clarifier**
Settling	30 min, settling using EPS and $CaCl_2$
R-101	**Lipid extraction**
Agitation	15 min, Treatment of settled biomass by N-LS under agitation
Lipid recovery	15 min, Lipid recovery using petroleum diesel under heating (70 °C)
OS-101	**Phase separation I**
Phase Separation	60 min, Separation of PD-lipid mixture from the cellular debris
R-102	**Trans-esterification**
Trans-esterification	120 min, Production of FAME and glycerol
OS-102	**Phase separation II**
Phase Separation	60 min, Separation of B10 blended biodiesel from crude glycerol

For PHB plant, 331700 kg P3HB was produced annually based on annual utilization of 2000 tonnes crude glycerol. For PHB production, two 40,000 L capacity fermenters were used for processing consecutive batches. The PHB plant continuously operating for 350 days per year would result in 381 batches, while there was time difference of 22 h between inoculation of two batches. The process timeline for processing of 1 batch of PHB has been indicated in Table 1b.

Table 1b

Process Timeline for processing of 1 batch of PHB.

Operation	Description
FR-101	**Production Fermenter**
Cleaning in place	60 min, Cleaning of equipment with 1 M NaOH solution supplied at 60 °C
CHARGE crude glycerol	30 min, Charge crude glycerol to production fermenter
Sterilization at 121 °C	180 min (including holding time of 15 min)
COOL	180 min, Cooling to 30 °C
CHARGE trace elements	15 min, Charge trace elements to main fermenter
Inoculum TRANSFER	30 min, Transfer inoculum from seed fermenter to main fermenter
Fermentation	35 h, Fermentation at 34 °C
Transfer-out	60 min, Transfer the fermented broth to digester
R-101	**PHB extraction**
Extraction	60 min, Broth treatment with NaOH and SDS under agitation
BC-101	**Centrifugation 1**
Centrifugation	240 min, Centrifugation of digested broth to remove liquid
MX-101	**Mixing**
Mixing	60 min, Mixing of centrifuged pellet with water

BC-102	**Centrifugation 2**
Centrifugation	240 min, Centrifugation of mixture to remove solid impurities
RDR-101	**Drying**
Drying	360 min, Drying of washed PHB pellet

2.4 Economic evaluation

All the substantial components such as raw material cost, utilities cost, facility dependent, labor cost, laboratory quality control (QC) and waste disposal cost were taken into account while estimating the annual operating cost. The unit production cost was calculated from annual operating cost and the quantity of product produced (Kumar et al., 2020b).

3 Results and discussion

3.1 Mass and energy balance

3.1.1 Crude glycerol to biodiesel

Mass and energy balance have been performed for biodiesel production using 1 tonne crude glycerol (Table 2a). The mass of chemicals required for the biodiesel production is represented in the 'amount supplied' column. Two scenarios were considered for the energy balance: Case A: Crude glycerol with no energy input and Case B: Crude glycerol with energy input (Table 2a). The energy input of other chemicals used was the energy consumed to produce the amount of chemicals (Zhang et al., 2017). The energy consumed during aeration, agitation and mixing were taken from the reported study of Zhang et al., (2019). The fermentation medium was sterilized (0.11 kg steam/m^3 with 80% energy recovery) before fermentation, and the energy content of steam was 26 MJ/kg steam (Chen et al., 2018b; Harding et al., 2007; Junker et al., 2006; Zhang et al., 2017). For the lipid production by fermentation process, the total energy input was 1.73GJ/tonne crude glycerol utilized (Case A, Table 2a). The aeration and trace elements added during fermentation were main energy contributing factors. Inoculum volume of 10%v/v was considered and the energy input in inoculum production was 173 MJ (which is 10% of the production fermenter). During biomass settling, the total energy input was calculated to be 377 MJ. The energy content of $CaCl_2$ and EPS were the main contributing factors. During lipid recovery, 1% loss ($0.01 \times 1140 = 11.4$ kg) of PD has been considered. This loss was taken as an energy input (Case A, Table 2a). The remaining diesel (99%) used during lipid recovery remained as fuel in the blended biodiesel (B10). Therefore, the energy input and energy output for 99% PD has not been taken into account. During the lipid extraction process, the total energy input was 573 MJ, which was mainly due to loss of PD during lipid recovery. In addition, during the *trans*-esterification process, the methanol was a major contributing factor responsible for the energy input of 830 MJ.

Table 2a

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Mass and energy balance for B10 production using 1 tonne crude glycerol.

Step	Items	Unit energy input	Amount supplied	Energy input (MJ) Case A	Energy input (MJ) Case B
Production Fermentation	Reaction volume (m^3)		5.65		
	Sterilization (MJ/kg)	26.00	0.62 kg	16	16
	Crude glycerol (MJ/kg)	0 (Case A) & ~~{~~8.29 (Case B)~~}~~	1000 kg	0	8290
	KH_2PO_4 (MJ/kg)	10.30	30.51 kg	314	314
	Na_2HPO_4 (MJ/kg)	8.21	10.73 kg	88	88

	NH_4Cl (MJ/kg)	8.64	18.08 kg	156	156
	Peptone (MJ/kg)	17.30	4.52 kg	78	78
	Magnesium Sulphate (MJ/kg)	10.65	1.13 kg	12	12
	Agitation (W/m^3)	7.30	5.65 m^3	8	8
	Aeration (kW/m^3)	1.00	5.65 m^3	1058	1058
	Energy input in Lipid Production (MJ)			**1730**	**10,020**
Seed Fermentation	**10% of Production fermenter (MJ)**			**173**	**1002**
Biomass settling	EPS (MJ/kg)	14.36	9.88 kg	142	142
	$CaCl_2$ (MJ/kg)	7.20	32.61 kg	235	235
	Energy input in Biomass Settling (MJ)			**377**	**377**
Lipid extraction	N-LS (MJ/ kg)	5.76	9.90 kg	57	57
	Loss in PD (MJ/ kg)	45.00	11.4 kg	513	513
	Agitation (W/m^3)	7.30	1.38 m^3	0.02	0.02
	Heating (kW/m^3)	2.72	1.38 m^3	3	3
	Energy input in Lipid Extraction (MJ)			**573**	**573**
Trans-esterification	Methanol (MJ/kg)	20.00	37.19 kg	744	744
	NaOH (MJ/kg)	18.50	2.50 kg	46	46
	Mixing (kWh/ kg FAME)	0.03	114 kg	12	12
	Heating (kJ/kg FAME)	240.00	114 kg	27	27
	Energy input in Trans-esterification (MJ)			**830**	**830**
Total energy input (MJ)				**3683**	**12,802**
FAME & B10 produced (kg)				**114 & 1129**	**114 & 1129**
Energy input (MJ) /kg FAME				**32.31**	**112.3**
Net energy input (GJ)				**3.68**	**12.8**
Net energy output (GJ)				**4.31**	**4.31**
Net Energy gain/ loss (GJ)				**0.63**	**−8.29**
Energy Ratio				**1.17**	**0.34**

The main contributing step in B10 biodiesel production was fermentation (46.98%) followed by *trans*-esterification (22.53%) and lipid extraction process (15.57%) (Case A, Table 2a). For biodiesel process, 114 kg FAMEs (or 1129 kg B10) were produced using 1 tonne crude glycerol where net energy input was 3.68 GJ/tonne crude glycerol utilized, while energy output (calculated from 37.8 MJ/kg FAME) was 4.31 GJ/tonne crude glycerol utilized. The net energy gain (energy output − energy input) was 0.63 GJ/tonne crude glycerol (Case A, Table 2a) and energy ratio (energy output/ energy input) was 1.17, making the process energetically favorable (Case A, Table 2a). It indicates that B10 production process using crude glycerol was energetically favorable, while not considering the energy input due to crude glycerol. If energy input of crude glycerol was accounted in energy balance for biodiesel production (Case B, Table 2a), energy input in production fermenter increases from 1.73 GJ to 10.02 GJ/tonne crude glycerol utilized. For the complete process, total energy input increases from 3.68 GJ to 12.8 GJ while net energy loss was − 8.29 GJ and energy ratio was 0.34 (Case B, Table 2a). If energy input of crude glycerol was accounted, it would make B10 production from crude glycerol energetically unfavorable.

3.1.2 Crude glycerol to PHA

Mass and energy balance have been performed for PHB production using 1 tonne crude glycerol (Table 2b). The mass of chemicals needed for the PHB production is represented in the ‘amount supplied’ column. Two scenarios were considered for the energy balance: Case A: Crude glycerol with no energy input and Case B: Crude glycerol with energy input (Table 2b). The total energy input in the production fermenter was 2.17 GJ/tonne crude glycerol utilized (Case A, Table 2b). Aeration and trace elements added during fermentation were major contributing factors. The seed fermentation energy input was calculated to be 217 MJ (10% of production fermenter) because 10% (v/v) inoculum size was considered. During PHB extraction process, the total energy input was 1.56 GJ, where energy content of NaOH and SDS were main contributing factors. During PHB washing, the total energy input was 22 MJ where, centrifugation (1 kWh/m^3) was major contributing factor. For drying of PHB pellet, rotary air dryer (60 °C) was considered where 134 kWh energy input was required to produce per tonne of product. The energy input for PHB drying was 80 MJ. The main contributing step in PHB production was fermentation process (53.68%) followed by PHB extraction (38.44%) and inoculum development (5.37%) (Case A, Table 2b). For PHB process, 166 kg PHB was produced using 1 tonne crude glycerol where net energy input was 4.05 GJ/tonne crude glycerol utilized while energy output (calculated from 22 MJ/kg PHB) was 3.65 GJ/tonne crude glycerol utilized. The net energy loss (energy output − energy input) was −0.4 GJ/tonne crude glycerol and energy ratio (energy output/ energy input) was 0.9 making the process energetically unfavorable (Case A, Table 2b). If energy input of crude glycerol was accounted in energy balance for PHB production (Case B, Table 2b), energy input in production fermenter would increase from 2.17 GJ to 10.46 GJ/tonne crude glycerol utilized. For the complete process, total energy input increases from 4.05 GJ to 13.17 GJ while net energy loss was − 9.52 GJ and energy ratio was 0.28 (Case B, Table 2b).

Table 2b

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Mass and energy balance for PHB production using 1 tonne crude glycerol.

Step	Items	Unit energy input	Amount supplied	Energy input (MJ) Case A	Energy input (MJ) Case B
Production Fermentation	Reaction volume (m^3)		5.88 m^3		
	Sterilization (MJ/kg)	26	0.65 kg	17	17
	Crude glycerol (MJ/kg)	0 (Case A) & ~~{~~8.29 (Case B)~~}~~	1000 kg	0	8290
	KH$_2$PO$_4$ (MJ/kg)	10.3	78.24 kg	806	806
	Ammonium sulphate (MJ/kg)	8.93	23.53 kg	210	210
	Citric acid (MJ/kg)	10.21	31.25 kg	319	319
	Magnesium sulphate (MJ/kg)	10.65	7.06 kg	75	75
	Agitation (W/m^3)	7.3	5.88 m^3	5	5
	Aeration (kW/m^3)	1	5.88 m^3	741	741
	Energy input in Production Fermenter (MJ)			**2173**	**10,463**
Seed Fermentation	**10% of Production fermenter (MJ)**			**217**	**1046**
PHB extraction	NaOH (MJ/kg)	18.5	47.06 kg	871	871
	SDS (MJ/kg)	56.5	11.76 kg	664	664
	Mixing (W/m^3)	7.3	5.88 m^3	0.15	0.15
	Centrifugation	1	5.88 m^3	21	21

	(kWh/m^3)				
	Energy input in PHB Extraction (MJ)			**1556**	**1556**
PHB washing	Water (MJ/m^3)	0.04	5.88 m^3	0.24	0.24
	Mixing (W/m^3)	7.3	5.88 m^3	0.15	0.15
	Centrifugation (kWh/m^3)	1	5.88 m^3	21	21
	Energy input in PHB washing (MJ)			**22**	**22**
PHB Drying	Drying (kWh/tonne)	134	0.166 tonne	80	80
	Energy input in PHB drying (MJ)			**80**	**80**
Total Energy input (MJ)				**4049**	**13,168**
PHB produced (kg)				**166**	**166**
Unit energy input (MJ/kg PHB)				**24.39**	**79.32**
Net energy input (GJ)				**4.05**	**13.17**
Net Energy output (GJ)				**3.65**	**3.65**
Net Energy loss (GJ)				**−0.4**	**−9.52**
Energy ratio				**0.9**	**0.28**

Per tonne crude glycerol utilized, PHB production process using crude glycerol was energetically unfavorable while B10 production was energetically favorable (Case A). The energy contribution of production fermenter was 46.98% and 53.68% for B10 and PHB, respectively (Case A). The high contribution of production process (fermenter) in PHB was due to high concentration of trace elements required for cell growth of *C. necator*.

3.2 Economic evaluation

3.2.1 Equipment sizing

3.2.1.1 B10 biodiesel process

All seed and production fermenters were built with design pressure of 1.5 bars and H/D ratio (Height to diameter) of 3. Production fermenter (FR-101) of capacity 50,000 L was considered for handling 39,097 L fermented broth. Two production fermenters were considered for processing consecutive batches. A clarifier (CLR-101) with design capacity of 45,000 L was used for biomass settling. Based on the settling velocity of 7.5 cm/min in lab-scale experiments, settling time for 2.25 m high clarifier has been calculated to be 30 min. A lipid extraction vessel (R-101) of 25,000 L capacity was considered for handling 16,700 L reaction mixture. A phase separation tank (OS-101) of 20,000 L design capacity was considered for separation of PD-lipid mixture from cell debris. It was assumed to operate for 60 min at 99% efficiency. A *trans*-esterification tank (R-102) of 15,000 L capacity was considered for handling 9830 L reaction mixture. Second phase separation (OS-102) of 12,000 L capacity was considered for phase separation of B10 and crude glycerol. Stainless steel grade 304 (SS304) was chosen as material of construction for the equipment, which is unaffected by oxidation, corrosion, and durable for this type of application (Phadnis et al., 2003). The clarifier and phase separation tanks were made of carbon steel (CS) as they were only used for settling and phase separation, respectively.

3.2.1.2 PHB process

All seed and production fermenters were built with design pressure of 1.5 bars and H/D ratio (Height to diameter) of 3. Production fermenter (FR-101) of 40,000 L design capacity was considered for handling 31,372 L fermented broth. Two production fermenters were considered for processing consecutive batches. A chemical reactor (R-101) of 40,000 L capacity was considered for PHA extraction. A continuous bowl centrifuge (BC-101) of 10 m^3/h capacity was considered for processing 31,528 L of reaction mixture in 4 h. A mixing tank (MX-101, design capacity 35,000 L) was considered for mixing centrifuged pellet with water. Another continuous bowl centrifuge (BC-102) of 10 m^3/h capacity was considered for processing 31,500 L wash water in 4 h. The centrifuges were considered to be operated at 87.5% efficiency. A rotary drying equipment (drum area 32.73 m^2) was used for drying of PHA pellet. All equipment were made of SS-304 material except mixing tank which was made of CS.

3.2.2 Equipment purchase cost

3.2.2.1 B10 biodiesel process

The assumed prices for the equipment were considered from literature and quotations provided by different manufacturers. The total equipment purchase cost (estimated in US$) involves process equipment, water purification system, cleaning-in-place (CIP) generation system, and distributed control system (DCS). Total equipment purchases costs (EPC) for biodiesel process were calculated to be 4.44 million $ (Table 3a). Process equipment contributes to 90.98% of total equipment purchase costs. Among the process equipment, two production fermenters contribute most (62.5% of EPC) followed by four seed fermenters for seed fermentation (11.5% of EPC). Lipid extraction equipment contributes to 6.71% of EPC followed by *trans*-esterification equipment (4.96% of EPC). Lobe pumps for transfer of inoculum and fermented broth contribute to 4.51% of EPC. CIP generation system with a skid, tank, transfer pump, heating element and a PLC (programmable logic controller) to 3.72% of EPC. The DCS (control system with software, analog input/output and personal computer) would be used for complete automation of plant, which costs around 0.13 million $ contributing 3.04% EPC. For the plant operations, water purification unit (using reverse osmosis) has been considered, which contributes to 2.25% of EPC.

Table 3a

Distribution of equipment purchase cost for B10 process (Freight on Board).

Unit operation	Equipment	Capacity of equipment	Price of equipment ($)	Number of units	Final cost ($)	Cost %
Seed Fermentation	Shake flask (SFR-105)	1 L	50	1	50	0
	Seed Fermenter (SFR-104)	5 L	25,000	1	25,000	0.56
	Seed Fermenter (SFR-103)	50 L	50,000	1	50,000	1.13
	Seed Fermenter (SFR-102)	500 L	87,469	1	87,469	1.97
	Seed Fermenter (SFR-101)	5000 L	348,220	1	348,220	7.85
Production Fermenter	Production Fermenter (FR-101)	50,000 L	1,386,290	2	2,772,579	62.5
	Lobe pump for transfer	500 LPM	50,000	4	200,000	4.51
Biomass settling	Clarifier (CLR-101)	45,000 L	35,000	1	35,000	0.79
Lipid Extraction	Lipid extraction vessel (R-101)	25,000 L	285,816	1	285,816	6.44
	Oil separator 1 (OS-101)	20,000 L	12,000	1	12,000	0.27
Trans-esterification	Trans-esterification vessel (R-102)	15,000 L	210,367	1	210,367	4.74
	Oil separator 2 (OS-102)	12,000 L	9500	1	9500	0.21
Software & DCS			135,000	1	135,000	3.04
CIP systems	CIP tank including pump & PLC		160,000	1	160,000	3.61
	CIP skid for transfer		1400	1	1400	0.03

	Heating element		1800	2	3600	0.08
Water purification unit			100,000	1	100,000	2.25
Total equipment purchase cost (Million $)					**4.44**	

3.2.2.2 PHB process

EPC for PHB process was estimated to be 4.71 million $ (Table 3b). Process equipment contributes to 91.5% of total equipment purchase costs. Among the process equipment, two production fermenters contribute most (51.51% of EPC) followed by PHA extraction equipment contributes to 14.42% of EPC. Four seed fermenters for seed fermentation contribute to 9.37% of EPC. PHA washing equipment contributes to 7.65% of EPC. PHA drying equipment contributes to 4.31% of EPC. Lobe pumps for transfer of inoculum and fermented broth contribute to 4.25% of EPC. CIP generation system, DCS and water purification unit contribute to 3.5%, 2.87% and 2.12% of EPC. The difference between EPC of PHA and biodiesel process was 0.27 million $. It was due to expensive equipment used in downstream processing of PHA like continuous centrifuge and rotary drying.

Table 3b

Distribution of equipment purchase cost for PHB process (Freight on Board).

Unit Operation	Equipment	Capacity of actual equipment	Price of actual equipment ($)	Number of units	Final cost ($)	Cost %
Seed Fermentation	Shake flask (SFR-105)	1 L	50	1	50	0
	Seed Fermenter (SFR-104)	4 L	20,000	1	20,000	0.42
	Seed Fermenter (SFR-103)	40 L	40,000	1	40,000	0.85
	Seed Fermenter (SFR-102)	400 L	76,508	1	76,508	1.62
	Seed Fermenter (SFR-101)	4000 L	304,585	1	304,585	6.47
Production Fermenter	Production Fermenter (FR-101)	40,000 L	1,212,573	2	2,425,147	51.51
	Lobe pump for transfer	500 LPM	50,000	4	200,000	4.25
PHA Extraction	PHA extraction vessel (R-101)	40,000 L	378,929	1	378,929	8.05
	Centrifugation (BC-101)	10 m^3/h	300,000	1	300,000	6.37
PHA washing	Mixing tank (MX-101)	40,000 L	60,000	1	60,000	1.27
	Centrifugation (BC-102)	10 m^3/h	300,000	1	300,000	6.37
Drying	Rotary dryer (RDR-101)	32.73 m^2	203,000	1	203,000	4.31
Software & DCS			135,000	1	135,000	2.87
CIP systems	CIP tank including pump & PLC		160,000	1	160,000	3.40
	CIP skid for transfer		1400	1	1400	0.03

	Heating element		1800	2	3600	0.08
Water purification unit					100,000	2.12
Total equipment purchase cost (Million $)						**4.71**

3.2.3 Direct fixed cost

The direct fixed cost of the plant comprises of plant direct cost, plant indirect cost and contractor and contingency fees. The scheme of calculation of plant direct cost was taken from Chen et al. (2018a) and is presented in Table 4. The total plant direct cost (TPDC) comprises of equipment purchase and installation cost, their instrumentation, electrical connection, insulation, building development cost, improvement of the yard and additional auxiliary charges. In addition, engineering and construction are other indirect cost factors, which should be also accounted. Moreover, the engineering cost (needed to properly engineer the plant for required production) was also separately accounted*, which was 8% of the total plant direct cost.* The contingency fee, which was 15% of sum of indirect and direct plant cost was also incorporated. Direct Fixed Cost (DFC) = TPDC + Construction cost + Engineering cost + Contractor's fee + contingency. The DFC for B10 production plant was calculated to be 16.21 million $ (Table 4). The DFC for PHB production plant was calculated to be 17.20 million $ (Table 4). Higher DFC for PHB producing plant was due to higher equipment purchase cost.

Table 4

Direct fixed cost of B10 and PHB production plant.

Direct fixed cost components		**B10**	**PHB**
a. TOTAL PLANT DIRECT COST (TPDC)			
Equipment Purchase Cost, EPC (Million $)	~~4.44~~	4.44~~71~~	4.71
Installation	30% of EPC	1.41	1.41
Process Piping	30% of EPC	1.41	1.41
Instrumentation	25% of EPC	1.18	1.18
Insulation	8% of EPC	0.38	0.38
Electrical	10% of EPC	0.47	0.47
Building	20% of EPC	0.84	0.84
Yard Improvement	10% of EPC	0.47	0.47
Auxiliary Facilities	25% of EPC	1.18	1.18
TPDC (Million $)		**11.44**	**12.15**
b. TOTAL PLANT INDIRECT COST (TPIC)			
Engineering	8% of TPDC	0.92	0.97
Construction	10% of TPDC	1.14	1.21
TPIC (Million $)		**2.06**	**2.19**
Total Plant COST (TPC = TPDC + TPIC)		13.5	14.33
c. CONTRACTOR FEE & CONTINGENCY (CFC)			
Contractor's Fee	5% of TPC	0.68	0.72
Contingency	15% of TPC	2.03	2.15

CFC (Million $)	**2.7**	**2.87**
DIRECT FIXED COST (DFC = CFC + TPC)	**16.21**	**17.20**

3.2.4 Annual operating cost

3.2.4.1 B10 biodiesel process

The cost of different raw materials was taken from bulk price from the internet and incorporated into the software for calculation of the annual raw material acquisition cost. For estimation of labor cost, the inbuilt database of software was used to consider the average salary of plant operators. The cost for treatment of liquid waste was taken from Kumar et al. (2019a) and incorporated into the software to calculate waste treatment cost per year. For B10 production, a total amount of 5.71 million $ was needed to run the facility annually (Table 5). The major part (50.97%) of the total annual operating cost for B10 production was due to utilities. While, 17.73% and 14.19% of total operating cost involves raw material acquisition and facility dependent cost, respectively.

Table 5

Annual operation cost for B10 and PHB production.

Cost item	B10	PHB
Operating Labor ($)	750,000	750,000
Supervisory Labor ($)	112,500	112,500
QC Labor ($)	112,500	112,500
Raw material cost ($)	1,012,524	1,002,406
Utilities ($)	2,910,613	4,073,463
Facility dependent ($)	810,298	860,022
Waste treatment ($)	1480	2759
Annual operating cost (Million $)	**5.71**	**6.91**

The total expenditure for raw material purchase in B10 production was 1.01 million $ per year. The crude glycerol (used during fermentation, 0.15 $/kg) and slime-EPS (used during biomass settling, 0.95 $/L) accounts for 29.63% and 27.24% of raw material cost, respectively. Sodium hydroxide solution (1 M) used for cleaning of fermenters accounted for 11.66% of raw material acquisition cost.

The utilities such as steam and brine solution were used for heating and cooling requirements of the process. The steam and standard electrical power are most commonly used for mechanical transport of materials and heat generation. A total amount of 2.91 million $ was needed annually for utilities to run the B10 plant. The 85.55% of annual utility cost was required for steam (12 $/MT) to sterilize the fermenters and for heating during lipid extraction and *trans*-esterification process), while 7.43% of annual utility cost was accounted for brine solution (0.25 $/MT, metric ton). Brine solution was required for cooling of fermentation medium after sterilization. In addition, 7.02% of annual utility cost was accounted for standard electricity (0.10 $/kW-h) used during agitation of fermentation, lipid extraction and *trans*-esterification process. Since all three utilities were used during fermentation step, fermentation operation was major contributing factor in annual utility cost.

For B10 facility, fifteen operators were considered to operate the facility (8 responsible for production and seed fermenters, 2 responsible for settling and lipid extraction, 1 required for *trans*-esterification, 2 for the accounts and 2 for warehouse). Operating labor cost was calculated to be 750,000 $, based on average salary of 50,000 $ per operator.

The 15% each of annual operating labor cost was considered for supervision and QC labor. Since the B10 plant was considered fully automated, the number of operators was on the lower side.

The facility-dependent cost consists of plant annual maintenance cost, local taxes, factory expenses and insurances. The maintenance cost (required for proper running of the facility) is 2% of DFC. The 1% of the DFC was calculated for local taxes, insurance charges and other factory expenses. The total facility dependent cost for B10 facility was estimated to be 0.81 million \$. Since fermentation was responsible for 62.5% of the total equipment purchase cost, facility dependent cost was arising mainly from the maintenance and repair of fermenters.

For one batch of B10, 49.1 m^3 of aqueous waste was generated from cleaning-in place (CIP) of fermenters and supernatant (obtained after biomass settling). Annually 13,453 m^3 of aqueous waste was generated by B10 process plant, which was treated at the rate of 0.11 $\$/m^3$. Annual waste treatment cost for B10 plant was 1480 \$.

3.2.4.2 PHB process

For PHB production, a total sum of 6.91 million \$/year was required to run the facility (Table 5). Annual operating cost analysis of PHB production process reveals that the utilities account for 58.92% of the total annual operating cost. While, 14.5% and 12.44% of total operating cost involves raw materials and facility dependent cost, respectively. The expenditure for raw material purchase in PHB production was 1 million \$ annually. The crude glycerol (0.15 \$/kg) and KH_2PO_4 (1.5 \$/kg) used during fermentation accounts for 29.93% and 23.79% of raw material cost, respectively. Water (0.01 \$/kg) used for washing of centrifuged pellet accounted for 21.21% of raw material acquisition cost.

For PHB facility, fifteen operators (8 responsible for seed and production fermenters, 2 responsible for PHB extraction and washing, 1 dedicated for drying, 2 for the accounts and 2 for warehouse) were considered to operate the facility. The operating labor cost was calculated to be 750,000 \$ based on average salary of 50,000 \$ per operator. The 15% each of annual operating labor cost was considered for supervision and QC labor.

The total facility dependent cost for PHB facility was estimated to be 0.86 million \$. Higher facility dependent cost in PHB plant was due to higher DFC in PHB plant. For one batch of PHB, 65.83 m^3 of aqueous waste was generated arising from cleaning-in place (CIP) of fermenters and supernatant (obtained after centrifugation and washing). Annually 25 082 m^3 of aqueous waste is generated by PHB process plant, which was treated at the rate of 0.11 $\$/m^3$. Annual waste treatment cost for PHB plant was 1480 \$. Due to additional amount of water used for washing in PHB process, higher amount of aqueous waste was generated leading to higher treatment costs in PHB plant.

A total amount of 4.07 million \$ was needed annually for utilities to run the PHB plant. Steam used during sterilization of fermenters and drying of PHB pellet account for 85.06% of annual utility cost. Brine solution was used during cooling of fermentation and centrifugation, which accounts for 6.25% of utility cost annually. Standard electricity, required during agitation and centrifugation account for 7.02% of annual utility cost.

In PHB process, additional brine solution was required during centrifugation for maintaining lower temperature (30 °C) as heat was generated during the process. Additional steam (12 \$/MT) was required for drying of PHB pellet. The increased steam requirement for operation of rotary drying, increased electricity and brine solution requirement for centrifuge operation were main reasons for increased utility cost in PHB process. While in B10 process, simple equipment has been used in downstream processing of B10 production process, which require less utilities for operation. Another observation can be drawn from time of downstream operation. For B10 process, time for downstream operation for one batch was 5 h while for PHB process, was 16 h. The increased time of downstream operation in PHB process increases the utility requirement, affecting annual cost for utilities. The difference in annual operating cost for B10 and PHB production (1.2 million \$) was mainly due to higher annual utility costs in PHB process.

3.2.5 Unit production cost

The unit production cost was calculated based upon operating cost and the amount of product produced per year (Table 6). It was revealed that unit production cost of biodiesel was 2.2 \$/L B10 while unit production cost of PHB was estimated to be 20.84 \$/kg. In B10 process, crude glycerol (during *trans*-esterification) and cell debris (during lipid extraction) were generated as by-products. The debris includes structural components of cell mass including proteins, carbohydrates, humic substances due to which it can be sold as animal feed supplement (Ram et al., 2018). After incorporating revenues from sale of crude glycerol (0.15 $/kg) and cell debris (0.7 $/kg), net unit production cost reduces to 2.12 $/L B10 (Table 6). Considering market price of PHB to be 11.42 $/kg (Nieder-Heitmann et al., 2019) & 1.2 $/L for B10 (Kumar et al., 2020a), greater annual losses are likely to be incurred in PHB production plant (Table 6). It indicates that for same quantity of crude glycerol utilized, B10 production was more economical than PHB production. However, in current scenario, both B10 and PHB are not profitable because for an enterprise to be profitable, unit production cost should be lower than the market price.

Table 6

Unit production cost for B10 and PHB.

Parameter	B10	PHB
Annual crude glycerol utilized	2000000 kg	2000000 kg
Annual Production rates		
Annual production	2.6 million L	331700 kg
Annual Operating cost		
Annual Operating Cost	5.71 million $	6.91 million $
Unit Production Cost	**2.2 $/L B10**	**20.84 $/kg PHB**
Additional revenues		
Crude glycerol (0.15 $/kg)	96448 kg	–
Cell debris (0.7 $/kg)	265780 kg	–
Net production cost (after incorporating revenue)	5.5 million $	6.91 million $
Net unit production cost	**2.12 $/L B10**	**20.84 $/kg PHB**
Annual loss	2.39 million $	3.1 million $

3.2.6 Sensitivity analysis

3.2.6.1 Plant capacity

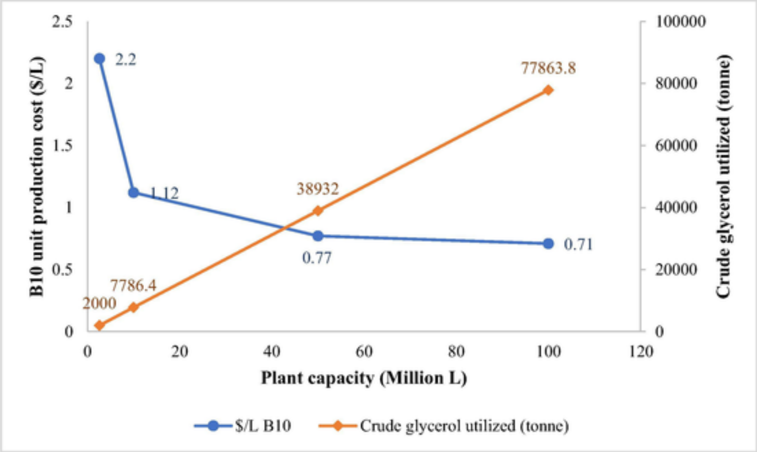
The equipment cost for higher capacities plant was carried out by power law model using Eq. (1).

$$C = C_o\left(\frac{S}{S_0}\right)^n \quad (1)$$

where c, s and n are cost of equipment, size of equipment and size exponent factor, respectively. The n value normally falls between 0.5 and 1.0 with an average value for vessels of around 0.6 (Chen et al., 2018a; Ram et al., 2018).

For B10 plant, increasing the plant capacity from 2.6 million L/ year to 100 million L/ year decreases the unit production cost from 2.2 $/L to 0.71 $/L (67.73% reduction) (Fig. 3). At 50 million L plant capacity, B10 unit production cost (0.77 $/L B10) was less than market price (1.2 $/L B10). The B10 manufacturer should target plant capacity of 50 million L or more for the enterprise to be profitable. Fig. 3 also displays annual crude glycerol utilized for different plant capacities of B10.

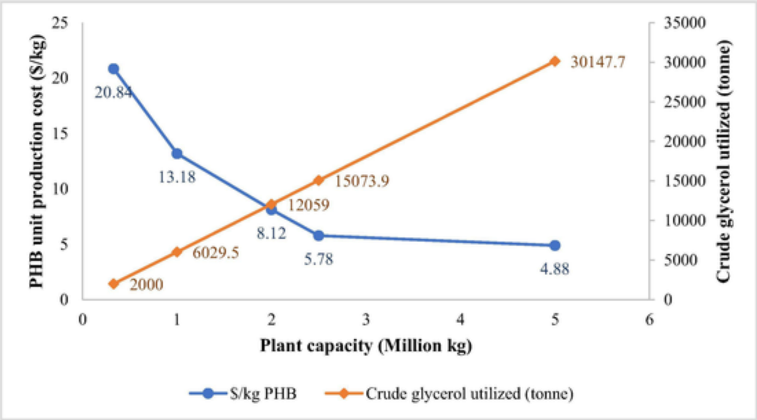
Fig. 3



Impact of B10 plant capacity on unit production cost and annual utilization of crude glycerol.

For PHB plant, increasing the plant capacity from 331700 kg/ year to 5 million kg/ year decreases the unit production cost from 20.84 $/kg to 4.88 $/kg (76.58% reduction) (Fig. 4). At 2 million kg plant capacity, PHB unit production cost (8.12 $/kg PHB) was less than market price (11.42 $/kg PHB). The PHB manufacturer should target plant capacity of 2 million kg or more for the enterprise to be profitable. Fig. 4 also displays annual crude glycerol utilized for different plant capacities of PHB.

Fig. 4



Impact of PHB plant capacity on unit production cost and annual utilization of crude glycerol.

3.2.6.2 PHB extraction yield

In the present scenario, PHB extraction (PHB recovery) yield was 73.5% due to losses during continuous centrifugation. To investigate the effect of extraction yield (%) on PHB unit production cost, simulations were performed for 5 million kg PHB plant capacity (utilizing 30147.7 tonnes crude glycerol annually). If extraction yield can be enhanced from 73.5% to 95%, PHB unit production cost lowers down from 4.88 $/kg to 4.47 $/kg (8.4% reduction).

4 Conclusion

For annual utilization of same quantity of crude glycerol, B10 production was more economical and energetically favorable than PHB production. The main reason for increased production costs in PHB process~~ease~~ was greater requirement of utilities during centrifugation and PHB drying. For 50 million L plant capacity of biodiesel, unit production cost was 0.77 $/L B10 while for 2 million kg plant capacity of PHB, unit production cost was 4.88 $/kg PHB. PHB production cost will decrease if PHB extraction yield can be enhanced.

CRediT authorship contribution statement

Lalit R. Kumar: Conceptualization, Data curation, Formal analysis, Methodology, Software, Writing - original draft, Writing - review & editing. **Rajwinder Kaur:** Formal analysis, Visualization. **R.D. Tyagi:** Funding acquisition, Project Administrator, Supervision, Validation. **Patrick Drogui:** Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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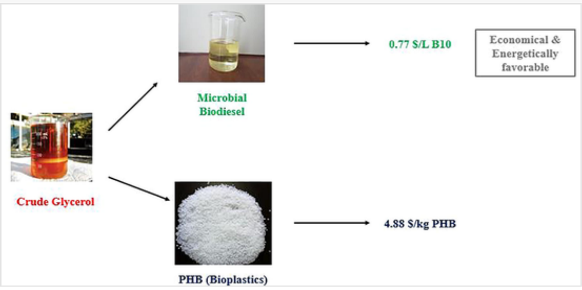
Graphical abstract



Highlights

- Biodiesel and PHB were produced from crude glycerol.
- Biodiesel production was more economical than PHB production.
- Biodiesel production was more energetically favorable.
- Unit production cost was sensitive to plant capacity.
- PHB production cost was sensitive to PHB extraction yield.

Queries and Answers

Q1

Query: Your article is registered as a regular item and is being processed for inclusion in a regular issue of the journal. If this is NOT correct and your article belongs to a Special Issue/Collection please contact l.daniel@elsevier.com immediately prior to returning your corrections.

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Q2

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Q3

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Answer: Yes