Energy balance for biodiesel production processes using microbial oil and scum

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Abstract

Biodiesel production using microbial oil is a promising technology. The main aim of this study is to check practical feasibility (in terms of energy balance) of different biodiesel production processes. Mass and energy balance of biodiesel production have been performed for 3 separate processes: (1) microbial lipid production from *T. oleaginosus* using waste substrates followed by INRS downstream process (2) microbial lipid production from pure substrate using *R. toruloides* followed by traditional and INRS downstream process and 3) oil extraction from scum and conversion to biodiesel. It was found that employing waste substrates like crude glycerol and municipal sludge in fermentation reduced the energy input by 50%. While employing biodegradable surfactants and petroleum-diesel as solvent (PD) for lipid extraction and recovery significantly reduced the energy input at cell wall disruption step. Biodiesel production from scum is a two-step process which is fast and energetically favorable.

Keywords: Energy balance; Biodiesel production; Waste sources; Scum

1 Introduction

Continuous use of fossil fuels is the major contributor of greenhouse gas emissions, which requires developing the alternate green sources of energy that should be sustainable and environmentally friendly. Biodiesel, fatty acid methyl esters (FAMEs), has grabbed great attention due to the advantages such as: it is renewable, sustainable, environmentally friendly (burns much cleaner than petroleum diesel), compatible with current commercial diesel engines, as well as has excellent lubricity and could provide energy density similar to diesel. As a feasible energy source, biodiesel production should be a sustainable and energetically favourable process (the difference between the energy output and the energy input is positive) (Thamsiriroj and Murphy, 2011; Zhang et al., 2013).

Current biodiesel feedstock like agriculture, fossils and wood cause environmental concerns like global warming, greenhouse gas emission and also leads to fast depletion of natural resources like deforestation. The soaring price of edible oil leads to biodiesel production unaffordable and present feedstock sources for fuel production. Moreover, some researchers have reported that biodiesel produced from two major raw materials, soybean and sunflower oils, was energetically unfavorable due to the low oil yield of the crops (energy loss of 32% for soybean and 118% for sunflower) (Pimentel and Patzek, 2005). Therefore, it has forced the researchers and engineers to search for replacement of the traditional oils and lipids as raw materials, which should be abundant, sustainable, and energetically favorable (positive energy balance). Oleaginous microorganisms have shown a great advantage as lipid source due to their faster growth rate and high lipid contents (up to 80% microorganism dry weight) compared to oilseed crops and animals (Gouda et al., 2008). However, techno-economic evaluation of microbial oil production using glucose as media revealed that unit production cost of biodiesel using microbial oil production with glucose as substrate was estimated to be $5.9/kg while commercial biodiesel price was $1.2/L (Apostolakou et al., 2009). Since biodiesel with microbial oil technology with glucose as substrate is an expensive process, alternatives for inexpensive sources should be looked upon.

Recently biodiesel production has been reported using renewable waste sources such as crude glycerol and municipal sludge (Chen et al., 2018b; Chen et al., 2017; Zhang et al., 2018; Zhang et al., 2017; Zhang et al., 2014). The wastewater sludge contains biodegradable carbon and nutrients (nitrogen, phosphorus and trace elements, etc.), which makes it a perfect raw material for growth of oleaginous microorganisms (Zhang et al., 2018). On the other

hand, crude glycerol generated as a by-product of biodiesel industry also pose a problem because crude glycerol is contaminated with various elements. The crude glycerol must be purified before any industrial use and the cost of purification is very high, which makes the process of glycerol utilization uneconomical (Cabanelas et al., 2013; Liang et al., 2010). Therefore, there is need to develop a process where there is no need to purify the crude glycerol solution or require minimum purification. Crude glycerol serves as a very good carbon source for bioconversion (Liang and Jiang, 2013). Co-fermentation of crude glycerol (carbon source) and wastewater sludge (carbon and nutrient sources) for lipid production using oleaginous microorganisms provides the solution to highly reduce the biodiesel production cost using heterotrophic microorganisms, and addresses the problem of food vs fuel crisis.

Moreover, studies have been reported on the energy balance for biodiesel production from microbial oil using co-fermentation of crude glycerol and wastewater sludge (Chen et al., 2018a; Zhang et al., 2013; Zhang et al., 2016). However, those studies were done considering traditional downstream operations for biomass settling and lipid extraction. Traditional biomass settling and cell wall disruption is achieved by centrifugation and utilization of toxic and expensive solvents, respectively. However, solvent application needs evaporation and solvent recovery which requires high energy input (Yellapu et al., 2018a). Along with these steps, biomass needs to be dried before cell wall disruption. These steps are energy intensive and therefore necessitates to look for alternatives (Chen et al., 2018a; Zhang et al., 2017). Recently in INRS lab, biomass settling has been performed using chemical coagulant, Calcium chloride and bio-flocculant (extra-polymeric substances or EPS) that will eliminate the requirement of centrifugation. The cell wall disruption was successfully performed by utilizing free nitrous acid or bio-surfactant n-lauryl sarcosine (Yellapu et al., 2016; Yellapu et al., 2018b). The released lipid after cell wall disruption was separated by phase separation with the aid of petroleum-diesel as solvent, which eliminated the requirement of organic solvents like chloroform, methanol and hexane (Yellapu et al., 2018b). Moreover, there will be no requirement of blending of petroleum diesel with biodiesel. However, to check practical feasibility of the new process (or INRS process), an energy balance needs to be performed. The energy balance will reveal whether the process is energetically favorable or not. Moreover, it will also reveal the main energy imparting steps and components in each step that will direct the future research for microbial oil-based biodiesel.

In this study, mass and energy balance for biodiesel production have been performed for 5 separate processes: (1) microbial lipid production using pure substrate (glucose and yeast extract) followed by conventional downstream process 2) microbial lipid production using pure substrate (glucose and yeast extract) followed by INRS downstream process (3) microbial lipid production using waste (sludge fortified crude glycerol) substrates followed by INRS downstream process 4) microbial lipid production using crude glycerol followed by INRS downstream process and 5) direct oil extraction from scum and conversion to biodiesel.

This study will compare the energy feasibility of several recently INRS biodiesel production processes using microbial oil – 1) waste substrates vs pure substrates (glucose) for microbial oil production, 2) lipid extraction using organic solvents vs lipid extraction using bio-surfactants and petroleum-diesel as solvent. This study also attempts to provide an insight of biodiesel production from wastes (wastewater sludge, crude glycerol and scum), and reveal which process of biodiesel production could be energetically feasible.

2 Methodology

2.1 Calculation basics

In this study, the energy and mass balance were calculated based on per tonne of biodiesel produced from each of the process: (1) microbial lipid production using waste (sludge fortified crude glycerol) substrates followed by INRS downstream process (2) microbial lipid production using pure substrate followed by conventional and INRS downstream process and 3) oil extraction from scum and conversion to biodiesel. The calculation started with raw materials and ends until the blended biodiesel was obtained. The electricity, steam or heating used in the process were considered as direct energy, which means that energy contents of these items are used in the calculation, while other materials (chemicals, solvents etc.) used during the production were considered to be indirect energy in which energy consumed during production of these materials was used in the calculation (Zhang et al., 2013). Following are the important energy terms used during making energy balance calculations:

Energy input: Sum of all energy inputs at every process step

Energy credit: Internal energy present in co-products

Net energy input: The difference between energy input and energy credit

Energy balance: Energy contained in produced biodiesel after subtracting the net energy input

Energy ratio: The ration between energy output and net energy input

For an energetically favourable process, net energy balance across the complete process should be positive or energy ratio (output/input) should be greater than 1.

2.2 Process description

2.2.1 Microbial lipid production using pure substrate (glucose and yeast extract) followed by conventional downstream process

For microbial lipid production, according to the study of Koutinas et al. (2014) the assumptions were taken. The lipid fermentation occurred for 134 h while 106.5 g/L total biomass and 71.9 g/L lipid was produced using *R. Toruloides*. The carbon source used in the fermentation was glucose (304 g/L) while yeast extract (15.7 g/L) and peptone (15.7 g/L) were used as nitrogen source. Media was sterilized at 121 °C before fermentation and 10% (v/v) inoculum was considered for the energy balance calculations. Fermentation was followed by centrifugation (biomass harvesting) and biomass drying (Fig. 1). The cell wall disruption was achieved by conventional process employing solvent mixture of chloroform-methanol (5 mL mixture/g biomass) at 60 °C (2:1 v/v) (Yellapu et al., 2016). Thereafter, lipids (dissolved in solvent) were separated from cell debris by centrifugation and the solvent mixture was simply evaporated and recovered for the next cycle. The extracted lipids (after solvent evaporation) were mixed in a reactor with methanol (6:1 M ratio of methanol to oil) and 1% (w/w of lipid) NaOH as catalyst for trans-esterification (Yellapu et al., 2016). The lipid extraction and trans-esterification efficiency was considered to be 100% and 97%, respectively (Yellapu et al., 2016). The biodiesel was mixed with petroleum-diesel (14.33 L PD/L biodiesel or BD) to get 7% v/v blended biodiesel (B-7). The blending of biodiesel was considered as for the INRS process using P-diesel (petroleum-Diesel), B-7 is the final product. In the conventional process for microbial oil biodiesel, 6 steps were involved to get blended biodiesel.

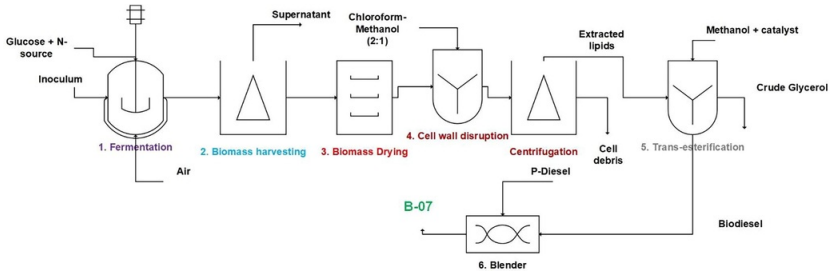

Fig. 1 Blended biodiesel production (B-7) using microbial oil with pure substrate (glucose) followed by conventional downstream process.

2.2.2 Microbial lipid production using pure substrate (glucose and yeast extract) followed by INRS downstream process

For microbial lipid production using *R. Toruloides*, according to the study of Koutinas et al. (2014) the assumptions were taken. The fermentation section is similar to that described above. Fermentation was followed by biomass settling which was performed using 70 mM calcium chloride followed by doses of EPS (119 mg/g biomass) (Yellapu et al., 2018b) (Fig. 2). The concentration of EPS and calcium chloride is dependent on the biomass concentration (more than 60 g/L biomass) obtained in fermented broth (Yellapu et al., 2018b). The settled biomass (166 g/L) was treated by N-lauryl sarcosine (40 mg N-LS/g biomass) (Yellapu et al., 2018b). The lipid was recovered using petroleum-diesel (PD) as solvent (14.33 mL PD/g lipid) where the N-LS treated biomass was treated with PD at 70 °C for 20 min (Yellapu et al., 2018b). The PD with lipid was separated from the cell debris through centrifugation. Although phase separation can be achieved without using centrifugation, to achieve faster phase separation and avoid loses in PD, centrifugation was employed. The recovered lipid with PD was reacted with methanol (6:1 M ratio of methanol: lipid) and 1% (w/w of lipid) NaOH as catalyst for trans-esterification. The lipid extraction and trans-esterification efficiency were considered to be 95% and 97%, respectively (Yellapu et al., 2018b). In the INRS process, only 4 steps would be required for blended biodiesel production eliminating the biomass drying and blending step.

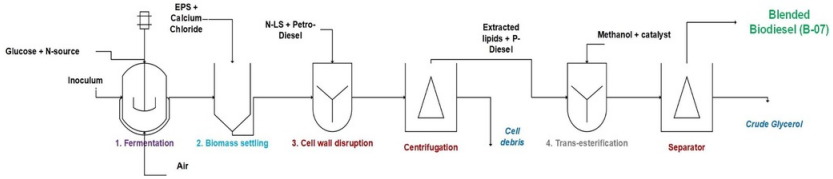

Fig. 2 Blended biodiesel production (B-7) using microbial oil with pure substrate (glucose) followed by INRS downstream process.

2.2.3 Microbial lipid production using waste substrates (sludge fortified with glycerol) followed by INRS downstream process

The process diagram remains same as that of Fig. 2. The difference is just that instead of glucose, sludge fortified with crude glycerol is used for fermentation and during lipid extraction, sequential treatment of N-LS and free nitrous acid is employed. Crude glycerol used during fermentation had (w/w) composition of 78.22% glycerol, 2.63% soap, 2.52% ash, 12.15% methanol and 1.56% water. For microbial lipid production, the fermentation operation was considered from Zhang et al. (2018) where lipid fermentation occurred for 48 h while 44.48 g/L total biomass and 17.37 g/L lipid was produced using *T. oleaginosus* (Zhang et al., 2018). The carbon source and nutrient source used in the fermentation was washed secondary municipal sludge (35 g/L sludge solids) while glycerol (40 g/L) was used as the additional carbon source whereas no additional nitrogen source and trace elements were added as sludge has sufficient nitrogen and trace metals for the microbial growth (Zhang et al., 2018). The pre-treatment of sludge was conducted using NaOH by bringing pH of the sludge medium to 12 and sterilization was performed at 121 °C for 30 min to increase the availability of the carbon substrate for bio-conversion. After sterilization, sulphuric

acid was used to bring pH of the medium to 6.5 from 12 (Zhang et al., 2018). Fermentation was followed by biomass settling which was performed using 52 mM calcium chloride followed by doses of EPS (39.9 mg/g biomass) (Yellapu et al., 2018b). The concentration of EPS and calcium chloride is dependent on the biomass concentration (more than 40 g/L biomass) obtained in fermented broth (Yellapu et al., 2018b). The settled sludge biomass (166 g/L) was treated by sequential treatment of free-nitrous acid (10 mg FNA/g biomass) and N-lauryl sarcosine (20 mg N-LS/g biomass) (unpublished data). The lipid was recovered using petroleum-diesel as solvent (14.33 mL PD/g lipid) where the treatment occurred at 70 °C for 20 min (Yellapu et al., 2018b). The PD with lipid was separated from the cell debris through centrifugation. Although phase separation can be achieved without using centrifugation, to achieve faster phase separation and avoid loses in PD, centrifugation was employed. The recovered lipid with PD was reacted with methanol (6:1 M ratio of methanol: lipid) and 1% (w/w of lipid) H_2SO_4 as catalyst for trans-esterification. The lipid extraction and trans-esterification efficiency were considered to be 92% and 97%, respectively (Yellapu et al., 2018b).

2.2.4 Microbial lipid production using crude glycerol media followed by INRS downstream process

The process diagram remains same as that of Fig. 2. The difference is just that instead of glucose, purified crude glycerol is used for fermentation and micro-organism used for lipid production is *T. oleaginosus*. The fermentation has been considered from Chen et al. (2018a) where 85% phosphoric acid was used for purifying crude glycerol through precipitation. The purified crude glycerol used during fermentation had (w/w) composition of 55% glycerol, 1.5% biodiesel, 4.2% ash, 18.5% methanol and 20.8% water. The fermentation was conducted using 50 g/L glycerol, 2.7 g/L KH_2PO_4, 0.95 g/L Na_2HPO_4 and 0.4 g/L NH_4Cl. 10.75 g/L biomass with 5.24 g/L lipid (47% lipid content) was obtained at 72 h. Fermentation was followed by biomass settling which was performed using 36 mM calcium chloride followed by doses of EPS (5.85 mg/g biomass) (Yellapu et al., 2018b). The concentration of EPS and calcium chloride is dependent on the biomass concentration (less than 20 g/L biomass) obtained in fermented broth (Yellapu et al., 2018b). The lipid extraction and trans-esterification were performed similar as described in Section 2.2.2.

2.2.5 Direct oil extraction from scum and conversion to biodiesel

Scum is a skimmed material, which floats on the surface of primary and secondary settling tanks in wastewater treatment plants. It is mainly composed of animal fat, vegetable oil, food wastes, plastic material, soaps, waxes and many other impurities discharged from restaurants, households and other facilities (Bi et al., 2015). Since it has high oil content, it can be used as substrate for biodiesel production. Fig. 3 shows production of blended biodiesel using scum (unpublished data). Seventy-five grams of scum solids were treated with 1 L petroleum diesel (as solvent) at 60 °C for 60 min to extract oil from the scum with 96% efficiency. Extracted lipids with PD were separated from the debris using centrifugation to achieve faster phase separation and avoid losses in PD. The recovered lipid with PD was reacted with methanol (6:1 M ratio of methanol: lipid) and 1% (w/w of lipid) H_2SO_4 as catalyst for trans-esterification. The trans-esterification efficiency was considered to be 95%.

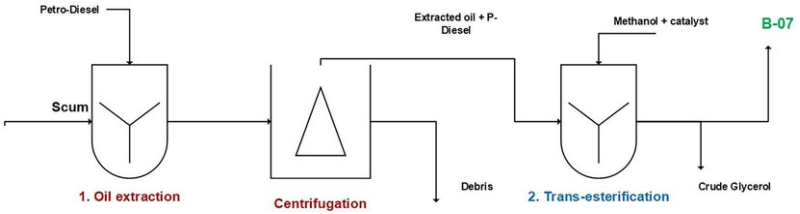

Fig. 3 Blended biodiesel production (B-7) using scum.

2.2.6 Trans-esterification of recovered lipids

The microbial lipids recovered by using petroleum diesel was treated with sulphuric aid as a catalyst dissolved in methanol (6:1 methanol to lipid molar ratio or 0.4 mL methanol per gram lipid) solution, and the final concentration of catalyst to lipid was 1% v/w (1 mL H_2SO_4/100 g lipid). The mixture was then heated to 70 °C for 2 h. After the reaction, the mixture was cooled to room temperature and 10 mL water (mL/g lipid) was added. After mixing, the mixture was kept for phase separation for 30 min. The top phase contained biodiesel in petroleum diesel (PD) and the bottom phase contained a residual catalyst, residual methanol, and glycerol. The biodiesel present in petroleum diesel was directly quantified by using Fourier transform infrared spectrometer (FT-IR) to determine the percentage of biodiesel present in the petroleum diesel. The characterization of biodiesel produced from scum and microbial oil is provided in Table 1.

Table 1 Characterization of biodiesel produced from microbial oil and scum.

Fatty acid	Biodiesel obtained from microbial oil	Biodiesel obtained from scum
C14:0 (%)	1.51	7
C15:0 (%)	3.23	–
C16:0 (%)	27.89	35

C16:1 (%)	20.11	–
C17:0 (%)	1.71	–
C18:0 (%)	11.33	10
C18:1 (%)	21.82	40
C18:2 (%)	2.42	8
C18:3 (%)	1.03	–
C20:1 (%)	1.31	–

3 Results and discussion

3.1 Microbial lipid production using pure substrate (glucose and yeast extract) followed by conventional downstream process

Mass balance and energy balance have been performed for producing 1 tonne of biodiesel or 14.2 tonne blended biodiesel B7 (Table 2). The mass of chemicals required for the biodiesel production were in column 'Amount supplied'. The energy input from chemical addition to fermentation was the energy consumed to produce the amount of chemicals. The energy content of glucose, yeast extract and peptone were considered from the literature. In the fermentation, all the media were sterilized (0.11 kg steam/m^3 with 80% energy recovery) before fermentation, and the energy content of steam is 26 MJ/kg steam (Chen et al., 2018a; Harding et al., 2007; Junker et al., 2006; Zhang et al., 2017). Energy input for agitation (7.3 W/m^3), and 0.5 vvm ~~(1 kW/m^3)~~ aeration (1 kW/m^3) has been considered from the literature (Harding et al., 2007; Junker et al., 2006). As from Table 2, it can be seen that total energy input for fermentation (production fermenter) was 30.68 GJ/tonne biodiesel. Out of which, glucose used as the carbon source was major energy contributing factor (60% of fermentation step) followed by aeration (0.5 vvm) which was dependent on the time of fermentation (132 h in this case). The seed fermentation energy input was considered 10% energy input of production fermenter as 10% (v/v) inoculum size was considered. After fermentation, broth will be concentrated using centrifugation (Jorquera et al., 2010). For biomass drying, compared to solar and natural gas drying, steam drying is more suitable method. It was which consumed 134 kWh to produce per tonne of dry biomass, hence, steam drying of the centrifuged biomass was adopted in these calculations (Zhang et al., 2013). Normally, the biomass is in bulk form after drying and in order to reduce the effect on the extraction efficiency, grinding (16 kWh/tonne product) should be performed to powder the biomass (Zhang et al., 2013). The energy input in centrifugation and drying step were 51.62 MJ and 287.4 MJ per tonne biodiesel, respectively. For the cell-wall disruption, chloroform and methanol (2:1 mixture) were used for the cell wall disruption at 60 °C. The energy content of methanol, chloroform and energy required for heating were considered from the literature (Zhang et al., 2017). Thereafter, centrifugation (1 kWh/m^3) was performed to remove the cell debris from the lipid-solvent mixture. The solvent mixture was also evaporated and recovered for the next cycle (19.5 kWh/tonne of solvent recovered) (Batan, 2010). The total energy input at lipid extraction step was 99.11 GJ/tonne biodiesel. Out of which, chloroform and methanol used for lipid extraction were major energy contributing steps (99% of lipid extraction step). Once the lipids were obtained after solvent evaporation, trans-esterification was performed to get biodiesel and crude glycerol. Energy content of methanol, sulphuric acid, energy imparted during heating and mixing were considered according to Batan et al. (2010). During trans-esterification, methanol used was major contributing factor and total energy input at trans-esterification step was 7.28 GJ/tonne biodiesel. The crude biodiesel was blended to get B-7 biodiesel. The energy input during blending (0.03 kWh/kg biodiesel) was considered from Zhang et al. (2017). The energy input at blending step was 108 MJ/tonne biodiesel. During the blending, energy imparted by mixing was only considered. Petroleum-diesel was directly used during blending without any energy input. Hence, the energy input from the utilization of PD was assumed to be zero and the energy imparted from PD in blended biodiesel has also been considered to be zero.

Table 2 Mass and energy balance of traditional biodiesel production process using microbial oil (glucose as substrate).

Step	Items	Unit energy	Amount supplied	Energy input (MJ)	Energy input (%)
Production Fermentation (PF)	Working volume		14.34 m^3		
	Sterilization (MJ/kg)	26.00	1.58 kg	41.01	0.03
	Yeast extract (MJ/kg)	6.46	225 kg	1453.50	1.03
	Peptone (MJ/kg)	17.30	225 kg	3892.50	2.77
	Glucose (MJ/kg)	4.20	4363 kg	18324.60	13.03
	Agitation (W/m^3)	7.30	14.34 m^3	50.50	0.04
	Aeration (kW/m^3)	1.00	14.34 m^3	6917.62	4.92

	Step energy input (MJ)			**30679.73**	**21.82**
Seed fermentation	**10% of Production fermenter (PFMJ)**			**3067.97**	**2.18**
Biomass Harvesting	Centrifugation (kWh/m^3)	1.00	14.34 m^3	51.62	0.04
	Step energy input (PMJF)			**51.62**	**0.04**
Biomass Drying	Drying (kWh/tonne)	134	1.5 tonne	201	0.14
	Grinding (kWh/tonne)	16	1.5 tonne	86.4	0.06
	Step energy input (MJ)			**287.4**	**0.2**
Lipid extraction	Reaction volume		7.63 m^3		
	Chloroform (MJ/kg)	7.63	7577.56 kg	57816.77	41.12
	Methanol (MJ/kg)	20.00	2014.85 kg	40296.96	28.66
	Solvent recovery (kWWh/t)	19.50	9.6 tonne	673.9	0.48
	Heating (kW/m^3)	2.72	7.63 m^3	597.70	0.42
	Centrifugation (kWh/m^3)	1.00	7.63 m^3	27.47	0.02
	Step energy input (MJ)			**99113.97**	**70.5**
Trans-esterification	Methanol (MJ/kg)	20.00	326.63 kg	6532.60	4.64
	NaOH (MJ/kg)	18.50	21.96 kg	406.26	0.29
	Mixing (kWh/kg biodiesel)	0.03	1000 kg	108.00	0.08
	Heating (kJ/kg biodiesel)	240.00	1000 kg	240.00	0.17
	Step energy input (MJ)			**7286.86**	**5.18**
Blending	Mixing (kWh/kg biodiesel)	0.03	1000 kg	108.00	0.08
	Step energy input (MJ)			**108.00**	**0.08**
Total energy input (MJ)				140595.5	
Total energy input (GJ)				**140.6**	
Credits – crude glycerol (GJ)				3.85	
Net energy input (GJ)				136.75	
Net energy output (GJ)				37.80	
Energy gain (GJ)				**−98.95**	
Energy conversion efficiency				**0.28**	

The total energy imparted for production of 1 tonne pure biodiesel or 14.2 tonne blended biodiesel was 140.6 GJ. The net energy input has been calculated by subtracting the energy imparted by crude glycerol (credits, 10.19 MJ/kg) produced during trans-esterification process (Zhang et al., 2016). The net energy gain of the process was −98.95 GJ and energy conversion efficiency of the process was 0.28, making it energetically unfavorable process. During the whole production process, lipid extraction step contributed to 70.5% of total energy input where solvents used, chloroform and methanol were major energy contributing factors. Chloroform and methanol used during cell wall disruption contributed 41% and 28.6% of total energy for biodiesel production process. Second most energy intensifying step was production fermentation (21.82% of total energy) where glucose used as carbon source was most energy intensifying. This was followed by aeration which is dependent on the time of fermentation. From this section, it can be concluded that fermentation and lipid extraction steps are major energy contributing factors in biodiesel

production from microbial lipid.

3.2 Microbial lipid production using pure substrate (glucose and yeast extract) followed by INRS downstream process

In this scenario, petroleum-diesel was directly used during cell wall disruption without any energy input. Hence, the energy input from the utilization of PD was assumed to be zero and the energy imparted from PD in blended biodiesel has also been considered to be zero. Mass balance and energy balance have been performed for production of 1 tonne biodiesel or 14.2 tonne blended biodiesel B7 using glucose as the carbon substrate and the INRS downstream process (Table 3). The total energy input in the production fermenter was 32.29 GJ/tonne biodiesel using ~~sludge and crude glycerol~~ glucose as carbon source. Out of which, glucose used as the carbon source was major energy contributing factor (60% of fermentation step) followed by aeration (0.5 vvm) which was dependent on the time of fermentation (132 h in this case). The seed fermentation energy input was considered 10% energy input of production fermenter as 10% (v/v) inoculum size was considered.

Table 3 Mass and energy balance of ~~developed~~INRS biodiesel production process using microbial oil (glucose as substrate).

Step	Items	Unit energy input	Amount supplied	Total energy input (MJ)	Energy input (%)
Production Fermentation (PF)	Reaction volume		15.09 m^3		
	Sterilization (MJ/kg)	26.00	1.66 kg	43.16	0.11
	yeast extract (MJ/kg)	6.46	236.90 kg	1530.37	3.21
	peptone (MJ/kg)	17.30	236.90 kg	4098.37	8.59
	Glucose (MJ/kg)	4.20	4591.70 kg	19285.14	40.41
	Agitation (W/m^3)	7.30	15.09 m^3	53.14	0.11
	Aeration (KW/m^3)	1.00	15.09 m^3	7279.42	15.25
	Step energy input (MJ)			**32289.6**	**67.66**
Seed fermentation	**10% of Production fermenter (~~PF~~MJ)**			**3228.96**	**6.77**
Biomass settling	EPS (MJ/kg)	18.84	191.24 kg	3602.96	7.55
	CaCl2 (MJ/kg)	7.20	117.25 kg	844.20	1.77
	Step energy input (~~PF~~MJ)			**4447.16**	**9.32**
Lipid extraction	~~n~~N-LS (MJ/kg)	5.76	64.28 kg	370.25	0.78
	Petro-diesel (MJ/kg)	0.00	13239.54 kg	0.00	0.00
	Agitation (W/m^3)	7.30	16.63 m^3	0.31	0.00
	Heating (kW/m^3)	2.72	16.63 m^3	40.71	0.09
	Centrifugation (kWh/m^3)	1.00	16.63 m^3	59.87	0.13
	Step energy input (MJ)			**471.14**	**0.99**
Trans-esterification	Methanol (kg)	20.00	326.58 kg	6531.60	13.69
	NaOH (kg)	18.50	21.96 kg	406.26	0.85
	Mixing (kWh/kg biodiesel)	0.03	1000 kg	108.00	0.23
	Heating (kJ/kg biodiesel)	240.00	1000 kg	240.00	0.5
	Step energy input (MJ)			**7285.86**	**15.27**
Total energy input (MJ)				47722.72	

Total energy input (GJ) – Case A	**47.72**
Total energy input (GJ) – Case B	**43.33**
Credits -crude glycerol (GJ)	3.86
Net energy input (GJ) – Case A	43.86
Net energy input (GJ) – Case B	39.47
Total energy output (GJ)	37.80
~~Net~~ Energy gain (GJ) – Case A	**−6.06**
~~Net e~~Energy gain (GJ) – Case B	**−1.67**
Energy conversion efficiency – Case A	**0.86**
Energy conversion efficiency – Case B	**0.96**

Note: **Case A is for bio-flocculant based biomass settling while Case B is for centrifuge aided biomass harvesting.**

For biomass settling, energy content of extra-polymeric substances (EPS) has been calculated from SuperPro Designer v10 where total energy imparted during production of EPS using sludge fortified crude glycerol was calculated to be 18.84 MJ/kg. The total energy during biomass settling was calculated to be 4.45 GJ where energy content of EPS and calcium chloride were only the contributing factors. Energy input at biomass harvesting using EPS and $CaCl_2$ was higher than centrifugation (Table 2). Hence two cases have been considered in biomass harvesting: a) centrifuge aided biomass harvesting and b) EPS aided biomass settling.

During the lipid extraction process, the total energy input was 471.14 MJ/tonne biodiesel where energy content of N-LS were major contributing factors. The energy input during lipid extraction using conventional method was 99.1 GJ/tonne biodiesel due to energy content of solvents used (Table 2). Another item needs to be noted here is that in the conventional process the lipid extraction process was for 4 h and the solvents need to be evaporated and recycled. While N-LS aided lipid extraction process completes only in 40 min (20 min treatment with N-LS and 20 min treatment with PD for lipid recovery) and no solvent evaporation is required. Also, the energy imparted from use of PD is zero as same energy will be imparted from it in energy output. During the trans-esterification process for the INRS process, the energy input was 7.29 GJ/tonne biodiesel where methanol was major contributing factor.

For the INRS~~-~~ process (bio-flocculant based biomass settling with N-LS aided lipid extraction – Case A), net energy input was 43.86 GJ/tonne biodiesel (after accounting for credits stream of crude glycerol) while energy output was 37.8 GJ/tonne biodiesel with net energy gain of – 6.06 GJ/tonne biodiesel and energy conversion efficiency of 0.86 making the process energetically unfavorable. However, if centrifugation (Case B) was used for biomass harvesting, net energy input was 39.47 GJ/tonne (after accounting for credits stream of crude glycerol) with net energy gain of −1.67 GJ/tonne biodiesel and energy conversion efficiency of 0.96. The main contributing step in the INRS process with pure substrate was production fermentation (67.77%) followed by trans-esterification (15.27%) and biomass settling using EPS (9.32%). The main energy reduction in the INRS process with pure substrate was in lipid extraction step, which was only 0.99% of total energy input while it was 70.34% in the conventional process (Table 2). From the analysis of Tables 2 and 3, lipid extraction step was the major energy contributing factor in conventional process, organic solvents used accounted for major energy contributing factors. Biosurfactants like N-LS are less energy intensive and can result in faster lipid extraction. Employing petroleum-diesel for the lipid recovery in extraction process makes the process less energy intensive since it eliminates the requirement of blending and is source of energy in the energy output. Another conclusion can be drawn on time of cell wall disruption process. Larger cell disruption time will increase the energy input due to agitation and mixing. The conventional cell wall disruption process was conducted for 4 h while the INRS downstream process was conducted for 40 min only. It is evident from comparison of two tables (Tables 2 and 3) that INRS downstream process has eliminated the energy requirement of biomass drying and blending. Although energy input for biomass drying and blending were 0.29 GJ and 0.1 GJ/tonne biodiesel, respectively, but on a higher scale of production, they can be quite energy intensive.

3.3 Microbial lipid production using sludge fortified with crude glycerol media followed by INRS downstream process

To address the problems of high energy input during fermentation, microbial oil was produced using waste substrates and cell wall disruption was performed by using the bio-surfactants and recovered using petroleum-diesel. The following sub-section discusses the mass and energy balance of the INRS process.

It was assumed that the fermentation would take place near a waste treatment plant; thus, there was no energy input in sludge transportation. The energy input from chemical addition to fermentation was the energy consumed to produce the amount of chemicals (Chen et al., 2018a; Ito et al., 2005; Ledgard et al., 2011; Selembo et al., 2009; Zhang et al., 2017). Crude glycerol is a by-product of the biodiesel production process and requires substantial efforts and energy input to purify it. At present, it is considered as a waste. In these computations, the crude glycerol was directly used as substrate in fermentation without any energy input for purification. Hence, the energy input

from the utilization of crude glycerol was assumed to be zero and the crude glycerol generated in the process (transesterification) was also given an energy value of zero. Similarly, petroleum-diesel was directly used during cell wall disruption without any energy input. Hence, the energy input from the utilization of PD was assumed to be zero and the energy imparted from PD in blended biodiesel has also been considered to be zero. Mass balance and energy balance have been performed for production of 1 tonne biodiesel or 14.2 tonne blended biodiesel B7 using the INRS process with microbial oil produced from sludge fortified crude glycerol (Table 4). The total energy input in the production fermenter was 16.39 GJ/tonne biodiesel using sludge and crude glycerol as carbon source. Out of which aeration (0.5 vvm) and sodium hydroxide used for sludge pre-treatment were major contributing factors. The seed fermentation energy input was calculated to be 1.64 GJ (10% of production fermented) The energy input during production fermentation using pure substrate was 32.29 GJ/tonne biodiesel (Table 4)(This should be Table 3) while using waste substrate it was 16.39 GJ/tonne biodiesel only indicating that on employing waste substrates, fermentation energy input has been reduced to 50%.

Table 4 Mass and energy balance of ~~developed~~INRS biodiesel production process using microbial oil (sludge fortified crude glycerol as substrate).

Step	Items	Unit energy	Amount supplied	Energy input (MJ)	Energy input (%)
Production Fermentation (PF)	Working volume		64.5 m^3		
	Sterilization (MJ/kg)	26.00	7.10 kg	184.47	0.61
	NaOH (MJ/kg)	18.50	186 kg	3441.00	11.34
	H_2SO_4 (MJ/kg)	7.10	216 kg	1533.60	5.04
	Agitation (W/m^3)	7.30	64.50 m^3	81.36	0.27
	Aeration (kW/m^3)	1.00	64.50 m^3	11145.60	36.74
	Step energy input (MJ)			**16386.03**	**54**
Seed fermentation	**10% of Production fermenter (~~PF~~MJ)**			**1638.60**	**5.4**
Biomass settling	EPS (MJ/kg)	18.84	114.76 kg	2162.08	7.12
	$CaCl_2$ (MJ/kg)	7.20	367 kg	2642.40	8.71
	Step energy input (~~PF~~MJ)			**4804.48**	**15.83**
Lipid extraction	Working volume		14.93 m^3		
	N-LS (MJ/kg)	5.76	57.38 kg	330.51	1.09
	FNA (MJ/kg)	3.20	26.69 kg	85.41	0.28
	Petro-diesel (MJ/kg)	0	13239.54 kg	0.00	0.00
	Agitation (W/m^3)	7.3	14.93 m^3	0.27	0.00
	Heating (kW/m^3)	2.72	14.93 m^3	36.55	0.16
	Centrifugation (kWh/m^3)	1.00	14.93 m^3	53.75	0.18
	Step energy input (MJ)			**506.49**	**1.67**
Trans-esterification	Methanol (kg)	20.00	326.5 kg	6530.00	21.52
	Sulphuric acid (kg)	7.10	19 kg	134.90	0.44
	Mixing (kWh/kg biodiesel)	0.03	1000 kg	108.00	0.36
	Heating (kJ/kg biodiesel)	240.00	1000 kg	240.00	0.79
	Step energy input (MJ)			**7012.90**	**23.11**
Total energy input (MJ)				30348.5	

Net energy input – Case A (GJ)	**30.35**
Net energy input – Case B (GJ)	**25.6**
Net energy output – (GJ)	37.80
Energy gain – Case A (GJ)	**7.45**
Energy gain – Case B (GJ)	**12.2**
Energy conversion efficiency – Case A	**1.25**
Energy conversion efficiency – Case B	**1.48**

Note: **Case A is for bio-flocculant based biomass settling while Case B is for centrifuge aided biomass harvesting.**

For biomass settling, energy content of extra-polymeric substances (EPS) has been calculated from SuperPro Designer v10 where total energy imparted during production of EPS using sludge fortified crude glycerol was calculated to be 18.84 MJ/kg. The total energy during biomass settling was calculated to be 4.8 GJ where energy content of EPS and calcium chloride were only the contributing factors. Energy input at biomass harvesting using EPS and $CaCl_2$ was higher than centrifugation (Table 3). Hence two cases have been considered in biomass harvesting: a) centrifuge aided biomass harvesting and b) EPS aided biomass settling.

During the lipid extraction process, the total energy input was 506.5 MJ/tonne biodiesel where energy content of N-LS and FNA were major contributing factors. The energy input during lipid extraction using conventional method was 99.1 GJ/tonne biodiesel due to energy content of solvents used (Table 2). Another item needs to be noted here is that in the conventional process the lipid extraction process was for 4 h and the solvents need to be evaporated and recycled. While N-LS and FNA aided lipid extraction process completes only in 1 h (20 min treatment with N-LS, 20 min treatment with FNA and 20 min treatment with PD for lipid recovery) and no solvent evaporation is required. Also, the energy imparted from use of PD is zero as same energy will be imparted from it in energy output. During the trans-esterification process, the energy input was 7 GJ/tonne biodiesel where methanol was major contributing factor. While in the traditional process, energy input during trans-esterification was 7.29 GJ/tonne biodiesel which was due to higher energy content of NaOH as catalyst as compared to sulphuric acid. For the INRS process with waste substrates, (bio-flocculant based biomass settling with N-LS and FNA aided lipid extraction – Case A), net energy input was 30.35 GJ/tonne biodiesel while energy output was 37.8 GJ/tonne biodiesel with net energy gain of 7.45 GJ/tonne biodiesel and energy conversion efficiency of 1.25 making the process energetically favorable. However, if centrifugation (Case B) was used for biomass harvesting, net energy input was 25.6 GJ/tonne with net energy gain of 12.2 GJ/tonne biodiesel and energy conversion efficiency of 1.48. The main contributing step in the INRS process with waste substrate was production fermentation (54%) followed by trans-esterification (23.11%) and biomass settling using EPS (15.83%). The main energy reduction in the INRS process was in lipid extraction step, which was only 1.67% of total energy input while it was 70.34% in the conventional process.

From the analysis of Tables 3 and 4, it could be concluded that fermentation was one of the main energy contributing step in biodiesel production using microbial oil. Energy imparted to fermentation step is dependent on: a) lipid concentration obtained by oleaginous micro-organisms in the fermentation step as it determines the amount of final biodiesel produced, and hence the net energy output, b) choice of the carbon source for fermentation can reduce the energy input in fermentation step as crude glycerol produced during trans-esterification reaction can be utilized as the substrate with no net energy input. In addition, if the biodiesel plant is built near wastewater treatment plant, sludge can be utilized as the substrate with no net energy input while commercial substrates like glucose have high energy value which contributes in the fermentation step as discussed in Section 3.2 c) lipid to substrate yield is an important factor as high lipid to substrate yield demands lesser substrate to produce similar lipid concentration as compared to the one with low lipid to substrate yield and d) fermentation time: long period fermentation demands high electricity consumption, which increases energy input during agitation and aeration.

Sludge fortified crude glycerol was energetically favorable for biodiesel production with energy conversion efficiency of 1.48. Energy balance has been performed in the literature for co-digestion of sludge and crude glycerol for biogas production (Zhang et al., 2016). The energy conversion efficiency (output/input ratio) sludge fortified crude glycerol to biogas was 0.27 only making the process energetically unfavourable. Hence, it can be concluded that utilizing sludge and crude glycerol for biodiesel production would be 5.4 times more energetically favorable than co-digesting them for biogas production.

3.4 Microbial lipid production using crude glycerol media followed by INRS downstream process

Mass balance and energy balance using crude glycerol as substrate for lipid production has been performed for production of 1 tonne biodiesel or 14.2 tonne blended biodiesel B7 using the INRS process (Table 5). The total energy input in the production fermenter was 72.27 GJ/tonne biodiesel using crude glycerol as carbon source. Out of which aeration (0.5 vvm) and KH_2PO_4 used as trace element were major contributing factors. The seed fermentation energy input was calculated to be 7.23 GJ (10% of production fermented). The energy input during production fermentation using sludge fortified crude glycerol was 16.39 GJ/tonne biodiesel only (Table 4). Higher energy input using only crude glycerol (72.27 GJ/tonne biodiesel) compared to that with sludge fortified crude glycerol (16.39 GJ/tonne biodiesel) would be due to the high energy contributed from the utilization of trace elements and nitrogen source (ammonium chloride) used in the fermentation whereas no trace elements and additional N-source were used in case of sludge fortified crude glycerol as sludge has trace elements and sufficient nitrogen in it (Zhang et al., 2018). Also,

lower biomass and lipid concentration were obtained in crude glycerol medium due to that the high fermentation volume was required which increases energy requirement for agitation and aeration.

Table 5 Mass and energy balance of ~~developed~~INRS biodiesel production process using microbial oil (crude glycerol as substrate).

Step	Items	Unit energy input	Amount supplied	Total energy input (MJ)	Energy input (%)
Production Fermentation (PF)	Reaction volume		192.24 m^3		
	Sterilization (MJ/kg)	26.00	21.15 kg	549.81	0.59
	H_3PO_4 (MJ/kg)	5.30	1414.50 kg	7496.85	8.05
	KH_2PO_4 (MJ/kg)	10.30	969.84 kg	9989.35	10.72
	Na_2HPO_4 (MJ/kg)	8.21	341.24 kg	2801.58	3.01
	NH_4Cl (MJ/kg)	8.64	143.68 kg	1241.40	1.33
	Agitation (W/m^3)	7.30	192.24 m^3	363.75	0.39
	Aeration (kW/m^3)	1.00	192.24 m^3	49828.61	53.47
	Step energy input (MJ)			**72271.34**	**77.56**
Seed fermentation	**10% of Production fermenter (~~PF~~MJ)**			**7227.13**	**7.76**
Biomass settling	EPS (MJ/kg)	18.84	13.25 kg	249.63	0.27
	$CaCl_2$ (MJ/kg)	7.20	768.20 kg	5531.04	5.94
	Step energy input (~~PF~~MJ)			**5780.67**	**6.21**
Lipid extraction	N-LS (MJ/kg)	5.76	91.38 kg	526.35	0.56
	Petro-diesel (MJ/kg)	0	13239.54 kg	0.00	0.00
	Agitation (W/m^3)	7.30	15.55 m^3	0.29	0.00
	Heating (kW/m^3)	2.72	15.55 m^3	38.07	0.04
	Centrifugation (kWh/m^3)	1.00	15.55 m^3	55.98	0.06
	Step energy input (MJ)			**620.68**	**0.67**
Trans-esterification	Methanol (MJ/kg)	20.00	326.58 kg	6531.60	7.01
	NaOH (MJ/kg)	18.50	21.96 kg	406.26	0.44
	Mixing (kWh/kg biodiesel)	0.03	1000 kg	108.00	0.12
	Heating (kJ/kg biodiesel)	240.00	1000 kg	240.00	0.26
	Step energy input (MJ)			**7285.86**	**7.82**
~~Net~~Total energy input (MJ)				93185.69	
Net energy input (GJ) – Case A				**93.19**	
Net energy input (GJ) – Case B				**88.1**	
Net energy output (GJ)				37.80	
Energy gain (GJ) – Case A				**−55.39**	
Energy gain (GJ) – Case A				**−50.30**	

Energy conversion efficiency – Case A	**0.41**
Energy conversion efficiency – Case B	**0.43**

Note: **Case A is for bio-flocculant based biomass settling while Case B is for centrifuge aided biomass harvesting.**

For biomass settling, energy content of extra-polymeric substances (EPS) has been calculated from SuperPro Designer v10 where total energy imparted during production of EPS using sludge fortified crude glycerol was calculated to be 18.84 MJ/kg. The total energy during biomass settling was calculated to be 5.78 GJ/tonne biodiesel where energy content of calcium chloride was major contributing factor. Energy input at biomass harvesting using EPS and $CaCl_2$ was higher than centrifugation. Hence two cases have been considered in biomass harvesting: a) centrifuge aided biomass harvesting and b) EPS aided biomass settling.

During the lipid extraction process, the total energy input was 620.7 MJ/tonne biodiesel where energy content of N-LS was major contributing factor. During the trans-esterification process, the energy input was 7.29 GJ/tonne biodiesel where methanol was major contributing factor. For the biodiesel production process using crude glycerol (biomass settling using bio-flocculant – Case A), net energy input was 93.19 GJ/tonne biodiesel while energy output was 37.8 GJ/tonne biodiesel with net energy gain of −55.39 GJ/tonne biodiesel and energy conversion efficiency of 0.41 making the process energetically unfavorable. However, if centrifugation (Case B) is used for biomass harvesting, net energy input is 88.1 GJ/tonne with net energy gain of – 50.3 GJ/tonne biodiesel and energy conversion efficiency of 0.43. The main contributing step in the INRS process was production fermentation (77.56%) followed by trans-esterification (7.82%) and seed fermentation (7.76%). On analyzing the energy input from Tables 4 and 5, it is found that energy input of 62.84 GJ/tonne of biodiesel is saved when using sludge is used with crude glycerol for lipid production which is the additional energy required for the nutrients and trace elements during fermentation.

3.5 Direct oil extraction from scum and conversion to biodiesel

Similarly, as described above, it was assumed that the biodiesel production site was near the wastewater treatment plant; hence, no energy input would occur for scum transportation. Mass and energy balance for 1 tonne biodiesel production using scum was highlighted in Table 6.a. The mass of chemicals required for the biodiesel production are in column 'Amount supplied'. The unit energies of all chemical components, mixing, heating and agitation has been considered from Zhang et al. (2017). The total energy input in oil extraction step using petroleum-diesel was 383 MJ/tonne biodiesel where heating required for the reaction was major energy contributing component (72.9% of oil extraction step). Trans-esterification imparted 7.15 GJ energy/tonne biodiesel where methanol used was major contributing component followed by heating required for the reaction. In this process, only 2 steps were required for biodiesel production – oil extraction contributing 5% of total energy input and trans-esterification contributing to 94.9% of energy input. Methanol used during trans-esterification contributes to 88.47% of total energy input followed by heating required during trans-esterification and oil extraction, which contributes to 4.55%. The total energy input for scum to biodiesel was 7.5 GJ/tonne biodiesel while energy output of the process was 37.8 GJ/tonne biodiesel with net energy gain of 30.3 GJ and energy conversion efficiency of 5. The process is highly energetically favourable. Biogas is also produced from scum using anaerobic digestion. It will be interesting to compute energy balance for biogas production from scum and compare with biodiesel production from scum.

Table 6.a Mass and energy balance of biodiesel production process using scum.

Step	Items	Unit energy	Amount supplied	Energy input (MJ)	Energy input (%)
Oil extraction	Scum used		11 m^3		
	Reaction volume (including PD)		28.56 m^3		
	Petro-diesel (MJ/kg)	0	13971 kg	0.00	0.00
	Agitation (W/m^3)	7.30	28.56 m^3	0.75	0.01
	Heating (kW/m^3)	2.72	28.56 m^3	279.7	3.71
	Centrifugation (kWh/m^3)	1.00	28.56 m^3	102.8	1.36
	Step energy input (MJ)			**383.2**	**5.08**
Trans-esterification	Methanol (MJ/kg)	20	333.40 kg	6668	88.97
	Sulphuric acid (MJ/kg)	7.10	19.37 kg	137.53	1.82
	Mixing (kWh/kg biodiesel)	0.03	1000 kg	108.00	1.43

	Heating (kJ/kg biodiesel)	240	1000 kg	240.00	3.18
	Step energy input (MJ)			**7153.53**	**94.92**
~~Net~~Total energy input (MJ)				7536.8	
Net energy input (GJ)				**7.5**	
Net energy output (GJ)				37.8	
Energy gain (GJ)				**30.3**	
Energy conversion efficiency				**5**	

Biogas production from digesting scum in pilot-scale fed-batch mesophilic anaerobic digesters has been studied and reported (Alanya et al., 2013). Average 69% COD removal has been achieved at scum loading of 7 g COD eq/(L·day) with approximate methane yield of 250 L CH_4/kg COD fed. The digestion was assumed to be performed in a 30 m^3 digester fitted with agitation system. The scum used for methane conversion was 11.77 m^3 per day. The digestion was carried out under mesophilic conditions (35 °C), with a heating energy input of 1.16 kWh/m^3/°C. The scum entering the digester was assumed to be at temperature of 20 °C (Møller et al., 2008). The energy required for agitation was 0.4 kWh/m^3 while the energy input from pumping was assumed to be 0.2 kWh/m^3 (Møller et al., 2008; Zhang et al., 2016). The energy for biomass harvesting through centrifugation was considered to be 1 kWh/m^3 (Chen et al., 2018a) as the scum solids obtained after 69% COD removal and harvesting are rich in phosphorus and can be used as a source of fertilizer. The same has been accounted in calculations as energy credits. Biogas produced from scum contains the toxic gas H_2S, and upgrading the biogas quality by removing H_2S would require an energy input of normally 11% of the energy content of the total biogas produced (Berglund and Börjesson, 2006; Zhang et al., 2016).

Mass and energy balance for methane production has been tabulated in Table 6.b. The scum used for methane conversion was 11.77 m^3 which is the same amount of scum used for 1 tonne biodiesel production (Table 6.a). From 11.77 m^3 scum, 20.6 m^3 methane was produced. Total energy input for digestion step was 762.7 MJ per 20.6 m^3 methane production. Out of which heating required to maintain desired temperature was major energy contributing followed by agitation and pumping. Biomass harvesting and H_2S scrubbing (purification) contributed 42.37 MJ and 47.59 MJ respectively. Total energy input for 20.6 m^3 methane production was 853.63 MJ. Out of which, digestion of scum (89.35%) contributed the most of the energy input and followed by purification (5.69%) and biomass harvesting (4.96%). Based on energy density of methane 21.43 MJ/m^3, total energy output was 441.46 MJ. Since the scum after digestion was rich in P, it was considered as a credit in terms of phosphorus fertilizer. There was 25.54 kg COD of scum produced in the calculation, which has an energy density of 0.13 MJ/kg scum. The credit would be 3.32 MJ, and thus the net energy invested would be 850.31 GJ. Subtracting the energy invested from the energy output, the net energy obtained was – 408.85 MJ. The conversion efficiency was calculated to be 0.52 which was energetically unfavourable. To compare the Tables 6.a and 6.b, it suggests that scum to biodiesel was 9.6 times more energetically favorable than scum to methane using same amount of scum for both the process.

Table 6.b Mass and energy balance for methane production using scum.

Step	Items	Unit energy input	Amount supplied	Total energy input (MJ)	Energy input (%)
Digestion	Scum used		11.77 m^3		
	Scum (MJ/kg)	0.00	82.39 kg COD	0.00	0.00
	Agitation (kWh/m^3)	0.40	11.77 m^3	16.95	1.99
	Pumping (kWh/m^3)	0.20	11.77 m^3	8.47	0.99
	Heating (kWh/m^3)	1.16	11.77 m^3	737.27	86.37
	Step energy input (MJ)			**762.7**	**89.35**
Biomass harvesting	Centrifugation (kWh/m^3)	1.00	11.77 m^3	**42.372**	**4.96**
Purification	Purification (11% of energy output/methane yield)			**47.56**	**5.69**
Total energy Input (MJ)				**852.65**	
Total energy Input (GJ)				**0.85**	
Credits (MJ)				3.32	
Net energy input (GJ)				0.85	

Net Energy output (GJ)	0.44
~~Net e~~Energy gain (GJ)	**−0.41**
Energy conversion efficiency	**0.52**

4 Conclusion

In this study, energy balance was conducted for different biodiesel processes. It was found out that employing waste substrates like wastewater sludge and crude glycerol in fermentation (microbial oil production) instead of commercial substrates (glucose) reduced the energy input at fermentation by 50%. Employing biodegradable surfactants like N-LS and petroleum-diesel as solvent during lipid extraction and recovery process proved to be energetically favorable when compared to traditional lipid extraction process using organic solvents like chloroform and methanol. Energy balance of another process was conducted for scum to biodiesel production. The process was fast and highly energetically favorable.

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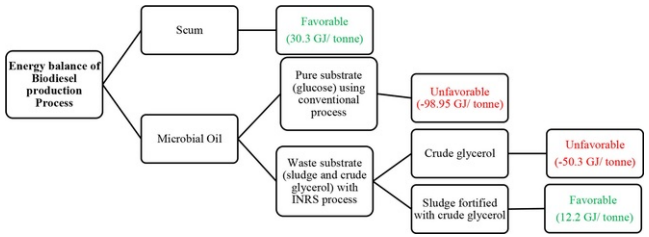
Graphical abstract



Highlights

- Biodiesel production from scum is more favorable than methane production.

- Lipid extraction using organic solvents makes process energy intensive.
- Sludge fortified crude glycerol reduces energy input at fermentation for microbial lipid production.
- Lipid extraction using N-LS and Petroleum diesel is quick and less energy intensive.

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