Techno-economic evaluation of simultaneous production of extra-cellular polymeric substance (EPS) and lipids by *Cloacibacterium normanense* NK6 using crude glycerol and sludge as substrate

S. K. Ram, L. R. Kumar, R. D. Tyagi and P. Drogui

S. K. Ram
L. R. Kumar
R. D. Tyagi (corresponding author)
P. Drogui
Centre Eau Terre Environnement,
INRS,
Canada
E-mail: rd.tyagi@ete.inrs.ca

ABSTRACT

This study used technical, economic analysis tool, SuperPro designer in evaluating a novel technology for simultaneous production of extracellular polymeric substance (EPS) and bio-diesel using crude glycerol and secondary sludge. As renewable energy sources are depleting, the process utilizes municipal sewage sludge for production of EPS and bio-diesel along with crude glycerol, which is a waste by-product of biodiesel industry providing an alternate way for disposal of municipal sludge and crude glycerol. Newly isolated *Cloacibacterium normanense* NK6 is used as microorganism in the study as it is capable of producing high EPS concentration, using activated sludge and crude glycerol as the sole carbon source. The technology has many environmental and economic advantages like the simultaneous production of two major products: EPS and lipids. Sensitivity analysis of the process revealed that biomass lipid content is a most significant factor where unit cost production of biodiesel was highly sensitive to lipid content during bioreaction. B7 biodiesel unit production cost can be lowered from 1$ to 0.6$ if the lipid content of the biomass is improved by various process parameter modifications.

Key words | bio-diesel, crude glycerol valorization, EPS (extracellular polymeric substance), lipids, sludge valorization

INTRODUCTION

Recent age of industrialization has surged the global energy demands, and our current resources are incapable of meeting them. The rising population, advancements in technology, and consumer based economy has given rise to serious environmental issues like *solid waste management*, *scarcity of non-renewable fuel resources*, and *global warming*. It is desirable to have eco-friendly biotechnical processes that can address these issues simultaneously without any economic strain.

In the arena of municipal solid waste management, sludge dewatering and disposal is a serious problem. Activated sludge is a flocculated suspension composed of microorganisms that can produce extracellular polymeric substances (EPS). EPS is a complex mixture of high molecular weight compounds including polysaccharide, protein, nucleic acids, and humic substances. They can protect the cells from the external environment and used as energy and carbon source when the substrate is limited (Wingender *et al.* 2012). It has been reported that EPS produced by bacteria plays an important role in controlling the flocculation and floc forming properties, including settling and dewatering (More *et al.* 2012; Nouha *et al.* 2016a, 2016b). Flocculating agents are widely used in industrial processes including wastewater treatment, downstream processing and food and bioreaction processes. They are generally classified in three categories: a) inorganic flocculants such as aluminum sulfate and poly aluminium chloride, b) organic synthetic high-polymer flocculants such as polyacrylamide derivatives and polyethylene imine and c) naturally occurring flocculants such as chitosan, sodium alginate and microbial flocculants like EPS. Among them, the synthetic organic flocculants are widely applied due to their higher efficiency and low cost. However, they inherit the drawback of being less biodegradable and producing carcinogenic monomers

during degradation (Salehizadeh & Shojaosadati 2001; Yu *et al.* 2009). Hence, the use of microbial flocculants is bound to increase as they are biodegradable and their monomer units are harmless to the ecosystem (Salehizadeh & Shojaosadati 2001). EPS has displayed high bio-flocculability with excess sludge due to the presence of high molecular weight macromolecules (330–1,200 kDa) and trivalent cations in it (Yu *et al.* 2009). Moreover, EPS can be produced at high rates and has easy recovery from the bioreaction broth.

For many microorganisms, EPS production is stimulated by carbon availability concomitant with the limitation of other nutrients, such as nitrogen, oxygen or phosphorus (Kumar *et al.* 2007). Meanwhile, fourth generation microbial oil is emerging to be the alternative for vegetable oil or animal fat oil as a feedstock for biodiesel production. Therefore, in the recent era, lipid accumulation by microbes is receiving considerable attention because of their potential as a source of biofuel production. Numerous studies have successfully transferred microbial oil to biodiesel (Meng *et al.* 2009; Gao *et al.* 2014). Lipids serve as storage materials in some lipid accumulating yeasts and bacteria. Many studies observed that under nitrogen limiting and carbon-excess conditions, organisms started to store lipids (Zhang *et al.* 2014). Therefore, the limitation of nitrogen source could be the key for simultaneous production of EPS and lipid. The bacterium *Cloacibacterium normanense* NK6 has been reported to produce high EPS concentration using activated sludge and crude glycerol as the carbon source (Klai *et al.* 2015). The crude glycerol feeding was used for EPS synthesis. High and reproducible EPS productivity (0.28 g/L.h) was achieved with initial glycerol concentrations of 20 g/L. During the course of bioreaction, *C. normanense* is also reported to produce up to 8 g/L lipids. Production of an additional metabolite like lipid can increase the revenue of a process developed to produce the two products simultaneously.

In order to realize laboratory research into practical reality, it is essential to assess the bioprocess thereby developed, in terms of its process cost economy, energy economy and the environmental impact. An encouraging evaluation of laboratory research will aid to the rapid technology transfer which can benefit the society at large. In the current study, an intensive evaluation of the bioprocess has been conducted in terms of its economic aspects. A sensitivity analysis of the process is presented to identify the cost intensifiers in the process, where R&D (Research and development) can focus on further minimizing the cost.

METHODS

Simulation description

In this study, using SuperPro designer, a process was simulated to produce EPS and lipids simultaneously. The simulations were performed for the annual production of 1 million tons of biodiesel B7 (biodiesel blend of 7.5% biodiesel and 92.5% petroleum diesel with some trace impurities of salt, methanol and unreacted oil). The plant was assumed to operate continuously for 350 days per year. The final product composition produced by the plant were Biodiesel B7 and EPS (99% pure dried formulation). Annually 0.2 million tons of EPS and 1 million tons of B7 biodiesel were produced. The biomass obtained after lipid extraction is assumed to have nutritious value and can be sold as animal feed at the rate of $0.7/kg due to its high protein content (Poultry feed normally available at $2.4/kg, www.walmart.ca, ID 095668105525). After trans-esterification, crude glycerol is produced as waste stream. This stream is considered to be valorized as a substrate for production of several metabolites during bioreaction. Thus, it was assumed to fetch additional revenue based on selling price of $0.1/kg (Yang *et al.* 2012).

Process description

The complete process can be divided into three sections, (1) Bioreaction, (2) EPS extraction and (3) Lipids Extraction. Crude glycerol obtained from (Biocardel) was used as the carbon source along with municipal secondary sludge. The composition of crude glycerol is given in Table 1.

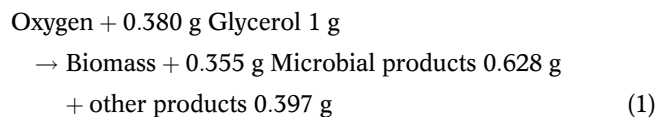### Bioreaction, media, substrates and reaction stoichiometry

The bioreaction (Figure 2(a)) was conducted in aerobic conditions (Klai *et al.* 2015). DO (Dissolved oxygen) of the reactor was maintained >40% for adequate availability of

Table 1 | Composition of crude glycerol used in the bioreaction

Composition of crude glycerol

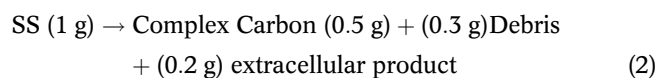| Component	% (w/w)	% (w/v) (density 1.2 g/mL)
Glycerol	78	93.6
Methanol	1.28	1.536
Salts	0.12	0.144
Soap	2.4	2.88
Water	18.2	21.84

oxygen to the microbes. The initial concentration of glycerol was 45 g/L. Municipal sludge at a suspended solids concentration of 25 g/L was charged into the reactor. Peptone (1.5 g/L) was used as a complex nitrogen source to enhance the biomass development during the bioreaction. The reactor pH was maintained at 7.0 using 4 M H_2SO_4 and 4 M NaOH. The reactor contents were sterilized before inoculation. During sterilization, the sludge underwent thermal hydrolysis and the suspended solids concentration reduced to 12 g/L. The sterile media containing sterilized sludge solids and supplemented crude glycerol was inoculated with 10% v/v seed culture (until pre-log phase) of *C. normanense* NK6. The bioreaction was conducted until 96 hours at 30 °C (Klai *et al.* 2015).

After bioreaction, the biomass (or suspended solids) concentration increased to 27.8 g/L (Figure 1). During the course of bioreaction, the 22.4 g/L extracellular EPS (loosely bound) is produced. The biomass simultaneously accumulated 28% (w/w) lipids yielding a final concentration of 8 g/L of microbial lipid produced in the process. The biomass generation followed reaction stoichiometry as shown in Equation (1); this equation is based on the yield factors obtained from the experimental results in the laboratory.

$$\text{Oxygen} + 0.380 \text{ g Glycerol 1 g}$$
$$\rightarrow \text{Biomass} + 0.355 \text{ g Microbial products } 0.628 \text{ g}$$
$$+ \text{ other products } 0.397 \text{ g} \tag{1}$$

The other products besides biomass and valuable metabolites are gases produced during bioreaction and other extra-cellular metabolites (proteins, toxins etc.). The stoichiometry is based on the assumption that per unit generation of the biomass requires slight excess than one unit of oxygen. During growth of *E. coli* on glycerol, 14.9 g of biomass is generated per unit oxygen atom (16 g) metabolized (Hempfling & Mainzer 1975). However, such stoichiometry was difficult to determine for complex carbon substrate due to nature of the complexity of the reaction. For the process simulation, it was assumed that carbon source was completely exhausted at the end of the reaction. Biomass yield (including intracellular lipid) was 0.355 g-biomass/g-glycerol. The lipid content was 28.1% (w/w) of biomass on a dry basis. Therefore, during the sterilization, conversion of suspended solids to complex carbon source was accounted as per following stoichiometric equation (Equation (2)).

$$\text{SS (1 g)} \rightarrow \text{Complex Carbon (0.5 g)} + (0.3 \text{ g})\text{Debris}$$
$$+ (0.2 \text{ g}) \text{ extracellular product} \tag{2}$$

where, Complex carbon (soluble products) can be assimilated by bacteria while debris and extracellular products are other metabolites and structural components of biomass (inert microbial products), which can't help in biomass growth. The stoichiometric equation assumed 100% hydrolysis of a unit gram of biomass having an elemental composition comprising 50% in the form of carbon (Andersen & Hessen 1991). During the bioreaction, a large quantity of crude glycerol (440 kg per ton of B7 biodiesel) was required to produce one ton of final product (B7 biodiesel). Using mass balance across the bioreaction, unit process quantities of each raw material required to produce one kg of final product (biodiesel B7) was calculated. Due to the undefined complex nature of sludge hydrolysate individual stoichiometry for biomass conversion using complex
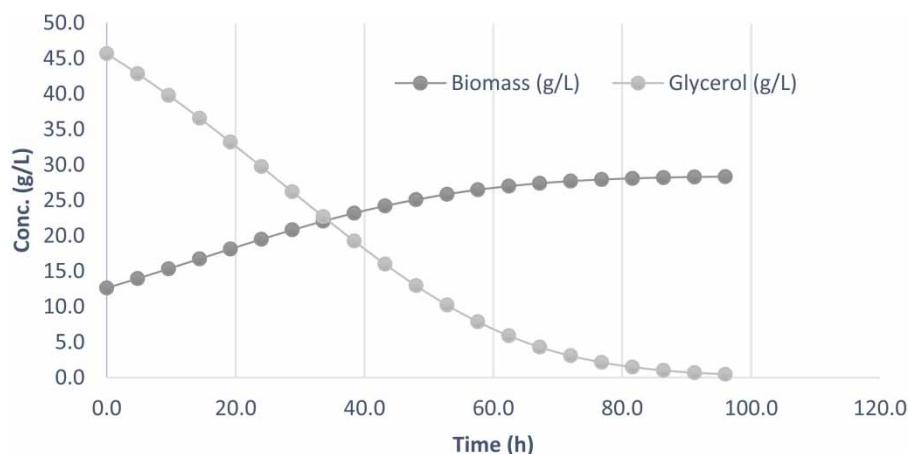

Figure 1 | Simulated bioreaction profile for the growth of *C. normanense* using SuperPro Designer as per the growth profile suggested by Nouha *et al.* (2016a, 2016b).
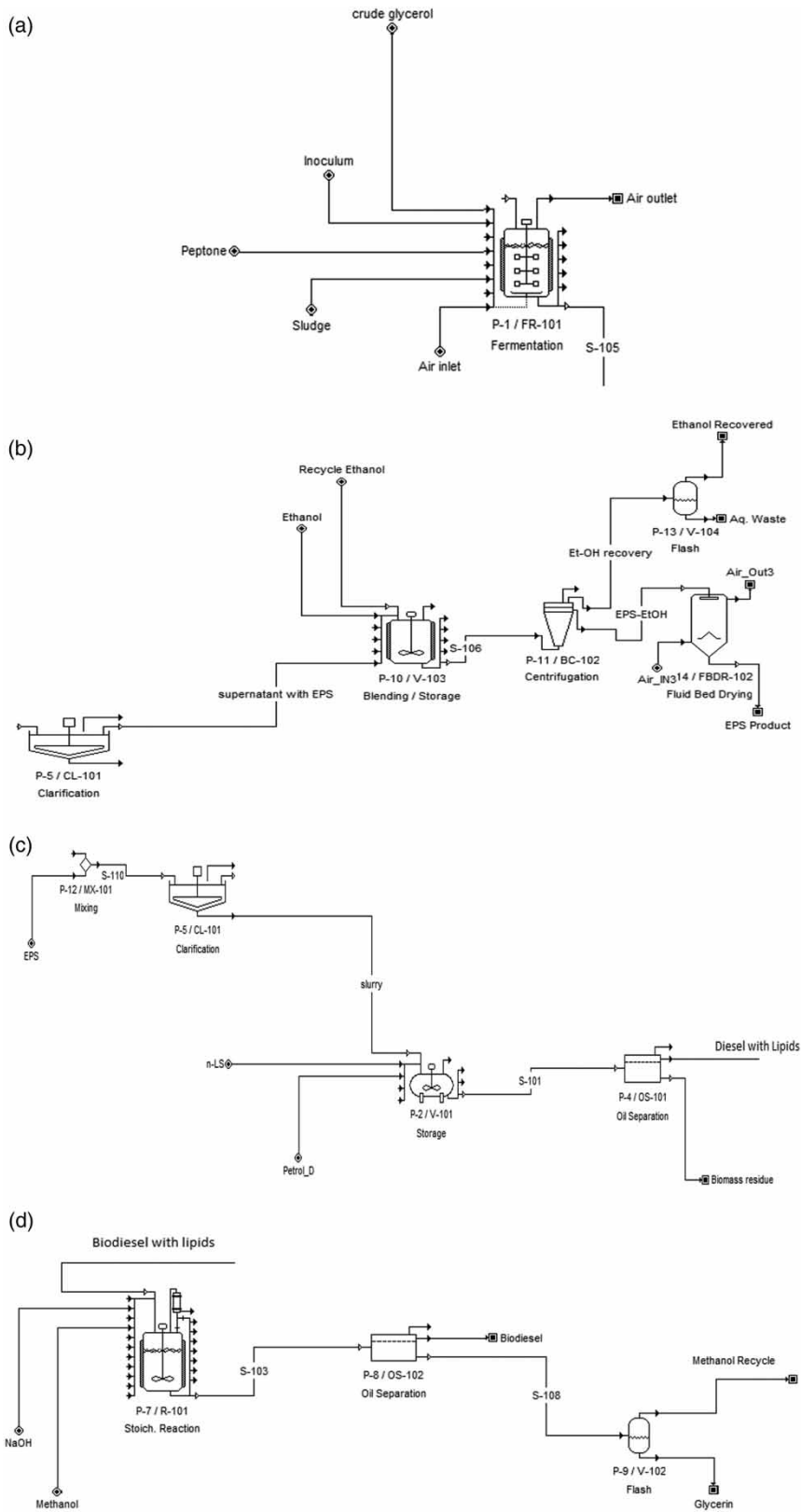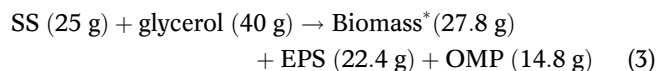
Figure 2 | (a) bioreaction process for simultaneous production of lipid and EPS (b) EPS extraction using ethanol precipitation method (c) Lipid extraction using petro-D solvent and (d) Base-catalyzed trans-esterification method.

carbon released after hydrolysis of sludge solids wasn't possible but experimental data was used to indicate the overall stoichiometry of the process in one liter reaction volume basis (Equation (3)).

$$\text{SS (25 g)} + \text{glycerol (40 g)} \rightarrow \text{Biomass}^{*}\text{(27.8 g)}$$
$$+ \text{EPS (22.4 g)} + \text{OMP (14.8 g)} \quad (3)$$

where SS is dry weight of sludge solids; glycerol is the major carbon source furnished by crude glycerol, Biomass* is the biomass containing lipid. This biomass includes the residual biomass (not hydrolyzed from sludge) and newly synthesized microbial biomass. EPS is extracellular polysaccharides which is one of the major product, and OMP is other microbial products which can encompass extracellular metabolites (proteins, toxins, etc.), and respiratory gaseous products released during the course of bio reaction. At a process scale, depending upon the suspended solids concentration in sludge and glycerol concentration in crude glycerol we can comment how many tons of sludge and crude glycerol is required for production of one ton of each product.

EPS extraction

EPS was extracted from the supernatant of the bioreaction broth by ethanol precipitation (Figure 2(b)) method as described by (Klai *et al.* 2015). For the extraction of EPS, the supernatant of the bioreaction broth after centrifugation was mixed with two volumes of denatured ethyl alcohol. After the solid-liquid separation from the broth (containing loosely bound soluble EPS), only 87% of the bioreaction volume was available for EPS extraction while rest of the volume was in the form of cell mass volume. Only soluble EPS was extracted from the broth because major fraction (90–95%) of EPS was present in loosely bound form while an insignificant (5–10%) concentration of tightly bound EPS was produced by the microbe. Extraction of tightly bound EPS would have rendered the process uneconomical. The complete volume was blended and then incubated at sub-zero temperatures for precipitation of EPS. The EPS precipitation was assumed to go to 100% completion. After cold incubation, the EPS was separated from the ethanol by simple centrifugation step, which was assumed to be operating at 90% efficiency. The supernatant was sent to ethanol recovery units by flash distillation column which was assumed to evaporate 99% of the ethanol from the ethanol-water mixture. The EPS pellets obtained from the

centrifugation unit were further dried to concentrate in a fluid bed dryer. The drying efficiency of the unit process was 90% (with respect to water) whereas it removed ethanol present in the pellets with 100% efficiency. The fluid bed dryer was operated at an evaporation rate of 100 $(\text{kg/h})/\text{m}^3$. The obtained EPS pellets were then stored as the final product. Depending upon the purpose of EPS further modification and formulation can be done. This EPS product can be directly used in municipal wastewater treatment facilities for sludge flocculation.

Lipid extraction

After the bioreaction, the broth was subjected to solid-liquid separation using EPS assisted flocculation and sedimentation. If required, 5.85 mg-EPS/g-suspended solids were added to the broth (Figure 2(c)). After 30 minutes of settling, the biomass concentration of the concentrated fraction was increased from 28 g/L (broth concentration) to 161 g/L. The concentrated biomass slurry was further treated with n-LS (N Lauroyl Sarcosine), a detergent which has been reported to facilitate cell lysis (Yellapu *et al.* 2016). N-LS is effective only after heat treatment of cells. To break the cells, 920 µg-nLS/g-suspended solids was added to the slurry and agitated for 10 minutes at 30 °C. The degree of cell lysis was assumed to be 100%. The stoichiometry followed by the cell lysis process can be depicted by the following equation (Equation (4)).

$$\text{(1 g) Biomass} \rightarrow \text{(0.72 g) Debris} + \text{(0.28 g) Lipid} \quad (4)$$

After the cell lysis, 12.75 mL/ gm-lipid of petroleum diesel was added to the cell lysate to dissolve the microbial lipids into oil phase. The mixture was further reacted for 30 minutes. After mixing the oil-water, the mixture was sent to a phase separation tank which was assumed to operate at 98% efficiency. Petroleum diesel separates out from the cell debris in the oil separation tank as the top layer. The Debris includes other structural components of cell mass including proteins, carbohydrates, humic substances, etc. Petroleum diesel containing microbial oil was directed to the trans-esterification module where base catalyzed trans-esterification took place for biodiesel production.

Trans-esterification for biodiesel production

Microbial lipids dissolved in petroleum diesel were trans-esterified (Figure 2(d)) to biodiesel in the presence of 1%

(w/v) base catalyst (NaOH). Six moles of methanol (mono-alkyl alcohol) were added for each mole of microbial lipid. The Trans-esterification reaction was carried out at 50 degree Celsius for one hour. The reaction scheme is presented in Equation (5). After transesterification, the FAMEs (fatty acid methyl esters) were transferred to petroleum diesel while polar molecules like residual glycerol, catalyst (NaOH), and water form a separate hydrophilic phase. The oil-water phase separation tank operated with an efficiency of 99%. The oil phase was separated in another oil separation unit. The oil phase was composed of 7% (w/w) FAMEs in petroleum diesel making it a B7 standard (EN 590, Europe Standard for biodiesel) biodiesel which can be marketed directly for use in fuel engines. Methanol was used further for secondary valorization and use.

Lipid (891.50 g) + Methanol (96.12g)
$$\rightarrow \text{Glycerol (92.12g)} + \text{FAMEs (895.5g)} \qquad (5)$$
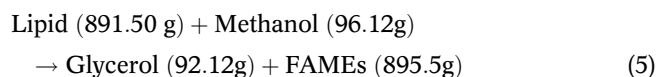
Batch scheduling and logistics

The plant operates for 350 days a year. The batch recipe time or the time required for one batch to finish is 102.79 hours including 96 hours of bioreaction and 7.75 hours of cycle time, i.e., the minimum time difference required between inoculations of two reactors. Consequently, a total of 1,072 batches were completed in one year. To obtain 1 million tons of B7 Biodiesel production, a batch volume of 9,169 m^3 is required, and each reactor was assumed to have bioreaction volume of 350 m^3 thereby requiring 26 parallel stacked reactor series. The complete scheduling of each process operation is presented by the Gantt chart of the process (Figure 3). The batches are starting continuously in series one after the other. In reality, several parallel stacks of reactors will be inoculated simultaneously to achieve the desired production rate. Inoculum for the final production bioreactor was prepared by series of six bioreactors each with 10% v/v inoculum of *C. normanense* For each inoculum, the culture was grown until 24–36 hours (until the pre-log phase of microbial growth) and then immediately transferred to next reactor. For example, *C. normanense* was inoculated first in a bio-reactor with working volume of 1 L and was transferred as 10% v/v inoculum into next reactor with final working volume 10 L. It was serially done in six subsequent steps till the final bioreaction working volume was 9.169 m^3 (26 reactors

of 350 m^3 volume) which will be operated for 96 h for lipid and EPS production.

Economic evaluation

Economic evaluation of the process was performed by estimating capital cost of the plant, operating cost of plant and revenue generation. Economic evaluation of the process was conducted using MS-Excel, and Intelligen Inc. Super Pro Designer. All the data were obtained from updated 2016 databases. All equipment costing was carried out by power law model using the Equation (6).

$$C = C_o \left(\frac{S}{S_0} \right)^n \qquad (6)$$

where, C is the cost of equipment of dimension values S whereas C_o and S_o are known as the cost and scale information, respectively, available presently via literature (Sweeting 1997).

The cost of the substrate was taken from literature and inbuilt cost databases (2016–17) used by the software. Prices of raw materials were taken from different bulk supplier's websites (like Alibaba.com). Simulations were performed to get streamwise mass flow details after every unit steps. The annual operational cost was calculated using all the significant components of production like raw material cost, labour cost, laboratory quality control (QC) analysis charges, waste disposal cost, utilities, transport and miscellaneous cost into account. Revenues production rates were obtained by the rigorous mass balance across the process and were multiplied with a unit price of the commodities available in the market to get annual revenues and credits. The rate of revenue return (selling prices) for biodiesel B7 and EPS were set to 1.0 $/kg and $4.5/kg, respectively. The process also earns some credit from its by-product streams, which can be used in other processes. After trans-esterification crude glycerol was generated, which was used again as substrate. The crude glycerol stream was assumed to fetch ¢ 15/kg. The biomass residue obtained after lipid extraction was assumed to have a credit worth of ¢ 70/kg.

Revenue & profitability analysis

To evaluate the profitability of the gross process margin, return on investment (ROI), gross profit and payback time was calculated according to following scheme.
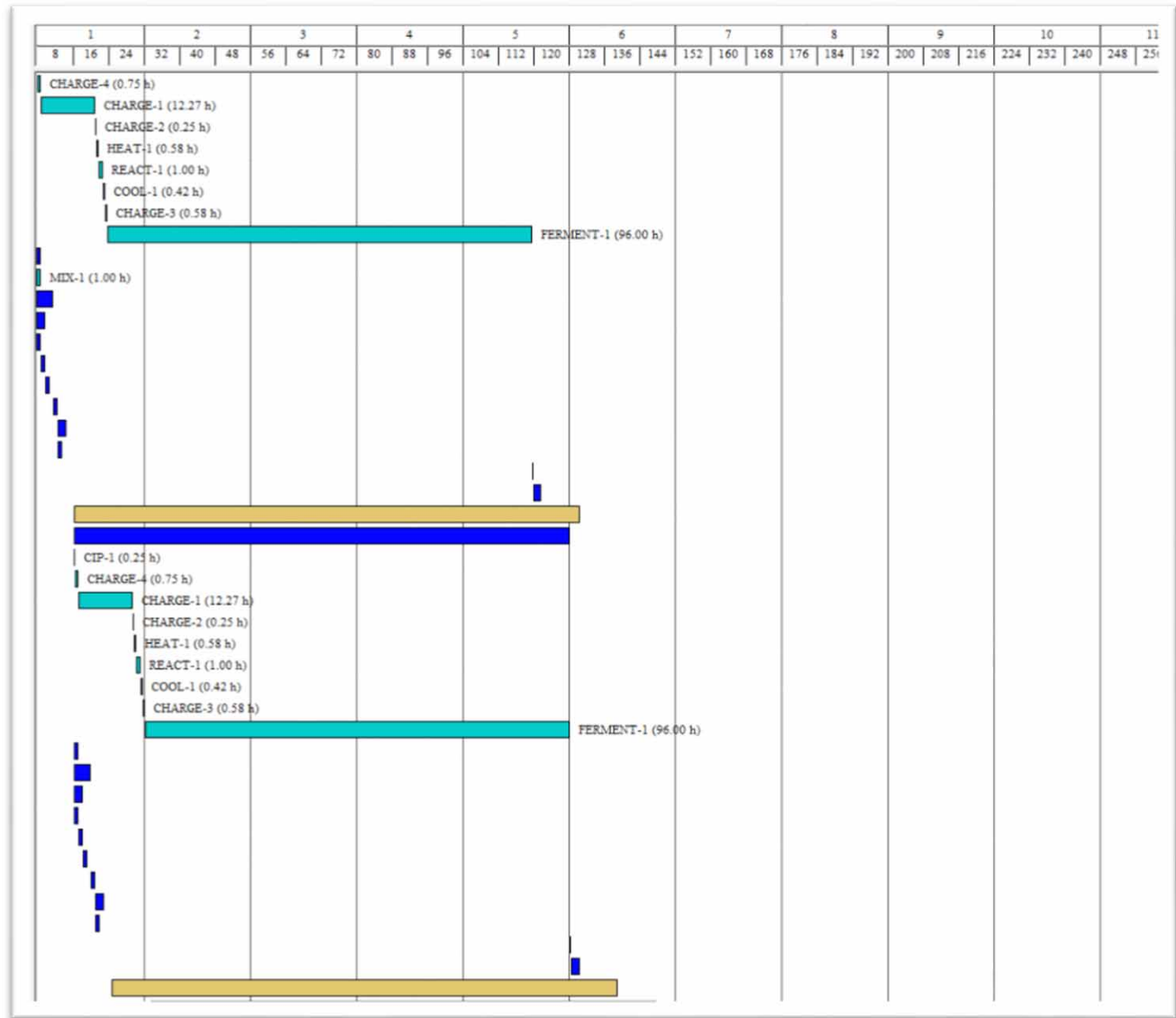
Figure 3 │ The Gantt chart for the process showing two batches in detail, Green bars: unit steps, blue bars unit Process, and Yellow bars are Batches.

(Equations (7)–(13)).

$$Gross\ Profit = Revenue(s) - Annual\ operating\ cost \qquad (7)$$

$$Gross\ profit\ \% = \frac{Gross\ profit}{revenue} * 100 \qquad (8)$$

$$ROI = \frac{Gross\ profit}{Total\ Investment} * 100 \qquad (9)$$

$$Payback\ time\ (years) = \frac{Total\ Investmet}{Gross\ profit} \qquad (10)$$

$$Actual\ AOC\ = working\ capital \qquad (11)$$

$$net\ AOC = Actual\ AOC \\ - Revenue\ (residual\ Solids + Glycerol + EPS) \qquad (12)$$

$$net\ Unit\ cost\ = net \frac{AOC}{total\ product} \qquad (13)$$

In the above scheme, revenue is the total value of the sales of the products and credits annually. Annual operating

cost of the process comprises all the cost incurred for raw materials, utilities, labor employment, waste treatment, facility use and maintenance, QC and analysis, failed product disposal and other miscellaneous expenses.

RESULTS AND DISCUSSION

Capital cost of the process plant

The total cost required acquiring the facility and its equipment for the plant was 39.7 million dollars. The major cost of the equipment was contributed by centrifuge units which accounted for 34.6% of the total equipment cost. After ethanol addition for EPS extraction (2:1 ratio, ethanol: broth), 3 batch volumes needed to be centrifuged to obtain the EPS pellets; therefore, it required 196 centrifuges to accommodate the large volume. Other major equipment cost factor was 89 blending tanks making up 17.9% of the cost which were required to mix ethanol and supernatant of the bioreaction broth for EPS extraction. Third major cost factors were 26 units of large 350 m^3 bioreactors to

achieve the required production. All the other cost factors are listed in Table 2. The unlisted equipment was estimated to be 20% cost of the total purchased equipment. The total plant direct cost (TPDC) includes cost elements which were incurred for the establishment of the facility. The TPDC comprises of equipment purchase cost, equipment installation cost, their instrumentation, insulation, electrical connection, the cost of building development, improvement of the yard and other auxiliary charges. In this processing plant, the typical scheme of calculations was used, which often are used as a rule of thumb for such bioprocesses. The scheme of calculation of plant direct cost is shown in Table 3 (SuperPro Module 2016). Accordingly, 118.3 million dollars was required as TPDC. There are various other indirect cost factors involved, which should be accounted. The engineering cost required to properly engineer the plant for required production was separately accounted. Further construction of the whole facility requires other additional charges, which can be accounted under the head of the construction cost, contractor's fee, and extra contingency. The scheme of calculation for indirect fixed cost estimation is also presented in Table 3.

Table 2 | List of equipment and FOB cost of purchase used in the process

Qty.	Name	Description	Unit Cost ($)	Total (1000X $)	% of Total Purchase cost
26	FR-101	Fermenter Vessel Volume = 342.25 m^3	145,000	3,770	9.50
33	PFF-101	Mixing Tank Vessel Volume = 99.71 m^3	90,000	2,970	7.48
1	OS-101	Oil separator Volume 1,157.76 m^3	52,000	52	0.13
22	R-101	Stirred Reactor Vessel Volume = 38.25 m^3	100,000	200	0.50
1	OS-102	Oil separator Volume 1,157.35 m^3	60,000	60	1.75
8	V-102	Flash Drum Vessel Volume = 1.69 m^3	30,000	240	0.60
89	V-103	Blending Tank Vessel Volume = 299.65 m^3	80,000	7,120	17.93
196	BC-103	Bowl Centrifuge Throughput = 119.68 m^3/h	70,000	13, 720	34.56
108	V-102	Flash Drum Vessel Volume = 1.69 m^3	38,000	4, 104	10.34
1	FBDR-101	Fluid Bed Dryer Dryer Volume = 40.27 m^3	40,000	40	0.10
18	R-104	Clarifier Surface Area = 2,446 m^2	45,000	810	2.04
		Unlisted Equipment	148,400	6, 617	16.67
Total Purchase Cost, PC (S)				39,703	

Table 3 | Total fixed cost calculation for the whole process

TOTAL PLANT DIRECT COST (TPDC)	Million $
Equipment Purchase Cost, PC	39.70
Installation (10% of Equipment purchase cost, PC)	3.31
Process Piping (50% of Equipment purchase cost, PC)	19.85
Instrumentation (35% of Equipment purchase cost, PC)	13.89
Insulation (40% of Equipment purchase cost, PC)	15.88
Electrical (3% of Equipment purchase cost, PC)	1.19
Building (10% of Equipment purchase cost, PC)	3.97
Yard Improvement (45% of Equipment purchase cost, PC)	17.87
Auxiliary Facilities (15% of Equipment purchase cost, PC)	5.95
TPDC ($)	**118.32**
TOTAL PLANT INDIRECT COST (TPIC)	
Engineering (25% of Total Plant Direct Cost, TPDC)	29.58
Construction (35% of Total Plant Direct Cost, TPDC)	41.41
TPIC ($)	**70.99**
Total Plant COST (TPC = TPDC + TPIC)	**189.30**
CONTRACTOR FEE & CONTINGENCY (CFC)	
Contractor's Fee (5% of Total Plant Cost)	94.65
Contingency (10% of Total Plant Cost)	18.93
CFC ($)	89.92
DIRECT FIXED COST (DFC = CFC + TPC)	**279.22**

The final, direct fixed cost (DFC) of the plant is the sum of direct and indirect cost. DFC = TPDC + Construction cost + Engineering cost + Contractor fee + contingency. The DFC for the process was calculated to be 279 million dollars (Table 3).

Operating cost of the process plant

Facility dependent cost tab is the cost factor, which is incorporated to account for the maintenance and proper running of the facility. Every facility depreciates from its initial establishment value (scrap value) to account for this, depreciation and taxes imposed on the facility dependent cost, is introduced. This comprises of maintenance cost which is 6% of the DFC, further depreciation cost is 1% of the DFC. Insurance charges, local taxes, and other factory expenses were estimated as 1%, 2% and 5% of the DFC respectively. The total facility dependent cost for this facility was calculated to be 39 million dollars.

The facility was assumed to be majorly automated, but still, 18 dedicated workers were employed to maintain the facility. The cost of labour was assumed to be standard $60/hour for highly qualified process engineers as per North American standards that include base pay, bonuses, insurances and miscellaneous contingencies. A total sum of $9.3 million per annum was required as labour cost expenses. Further additional labour was required to maintain the quality standards of the final products, which is done by QC and quality analysis department which account for 15% of the labour cost making a total sum of labour related cost to be 10.7 million dollars.

The process majorly capitalizes over its waste streams like residual biomass and waste glycerol streams. One aqueous waste stream was generated eventually when all the valuable components like EPS product and ethanol have been recovered from the supernatant fraction of the bioreaction broth. Annually 71 million tons of aqueous waste was generated by the process plant which was assumed to be discarded after treatment. This aqueous waste wasn't further valorized. The treatment of this waste required 25 million dollars for their safe disposal at the rate of $3/m^3 (http://www.costwater.com/runningcostwastewater.htm).

For all the heating and cooling requirements of the process various utilities like steam, chilled water, etc. were used in the process plant. Standard electrical power and steam are most frequently used for heat generation and mechanical transport of materials. For the cooling purpose, chilled water or glycol were used to maintain lower temperatures. 58.6% of the total utility cost was required for standard electricity as in the process EPS extraction required a large number of centrifuges for the EPS separation. A total sum of 537 million dollars was needed annually to run the plant. The distribution and rates of utility charges are given in Table 4.

The bioreaction process utilized crude glycerol, a waste by-product of biodiesel industry to produce EPS and lipid. The bioreaction process was further supplemented with municipal secondary sludge as a secondary carbon source. This strategy not only reduces the amount of crude glycerol

Table 4 | Utilities cost distribution for the overall process

Utility	Unit cost ($)	Annual Amount	Ref Unit	Annual Cost (Million $)	% of Total Utility Cost
Std. Power	0.1	3,152,464,815	k-Wh	315.25	58.60
Steam	10	12,154,105	MT	121.54	22.59
Chilled Water	0.06	1,686,143,438	MT	101.17	18.81
Glycol	0.35	70,210,011	MT	24.57	4.57
				537.96	100.00

required for product formation but also provide an alternative to municipal sludge disposal. The process valorizes the two waste streams (crude glycerol and municipal sludge). According to the process simulation for producing 1 kg of biodiesel B7 (7% FAMEs), 6.4 kg of sludge (with 2.5% w/w dry SS), 1.3 kg of ethanol and nearly 1 kg of petro-diesel was required. The distribution of other raw material requirement is provided in Table 5.

High quantity of sludge and ethanol was required for the process, 90% of the ethanol used was recovered via flash distillation drums. Considering the cost factors, ethanol accounted for the significant fraction of the raw material cost with its share of 68% (of raw material cost). The second most cost intensifying factor was petroleum diesel, which was used as the solvent for microbial extraction in

this process ending up as the part of the product, which accounted for 25.08% of the raw material cost. Further, 4.84% of the cost was contributed by crude glycerol which acted as the major carbon source. The details of the price assumptions taken from the wholesale market websites like www.alibaba.com are listed in Table 6. Annual expenditure for raw material purchase was 1,874.4 million dollars.

It is essential to ameliorate the lipid content of the biomass by improving the process conditions. The biomass yield from crude glycerol was at the lower end which can be improved further. Application of a cheaper nitrogen source will further reduce the cost of production. Petroleum diesel was integrated into the final product, which acted as solvent for extraction thus it can be ignored as cost intensifying factor. The extraction method of EPS was contributing significantly to the cost of the process which should be addressed to achieve a lower unit cost of production.

Annual operating cost

Annual operating cost analysis (Table 7) of the process reveals that 75.28% of the total annual operating cost was because of raw material required in the process. A total sum of 2,489 million dollars was required for annual operation of the plant.

The power cost requirements were the second highest cost contributors because the process dealt with 1 volume of supernatant and 2 volumes of ethanol to extract EPS at very low temperatures. A large volume of ethanol (double) was added to the supernatant fraction of the bioreaction

Table 5 | Raw material requirement distribution for one kg of biodiesel (BD7) production

Material	Value	Unit
Sludge	162.5	g
Ethanol	1.3	Kg
Petro-Diesel	914	g
Crude Glycerol	440	g
Water	267	g
Peptone	11	g
Methanol	40.8	g
Sodium Hydroxide	12	g
EPS	1.3	g
n-LS	0.20	g

Table 6 | Material cost assumption and total material cost data for the whole process

MATERIAL REQUIRED (ENTIRE PROCESS)

Bulk Material	Unit Cost ($/kg)	Annual Amount	Unit (1,000 metric Tons MT)	Annual Cost (Million $)	%
Water	0.003	344.26	MT	1.03	0.06
Crude glycerol	0.16	567.09	MT	90.73	4.84
Peptone	0.85	14.74	MT	12.53	0.67
Sludge	0	8,231.71	MT	0.00	0.00
n-LS	0.79	0.27	MT	0.21	0.01
Petro-Diesel	0.4	1,175.43	MT	470.17	25.08
Methanol	0.24	52.59	MT	12.62	0.67
Sod. Hydro	0.2	15.46	MT	3.09	0.16
Ethanol	0.75	1,712.04	MT	1, 284.03	68.50
EPS	In house-free	1.72	MT	0.00	0.00
				1,874.43	100.00

Table 7 | Annual operating cost analysis for the overall process

Process cost component	Total amount (Million $)	% of total operating cost
Raw Material	1,874.43	75.28
Utilities	537.96	21.61
Labour Dependent	10.73	0.43
Failed Product Disposal	1.00	0.04
Waste Treatment	25.08	1.01
Facility Dependent	39.09	1.57
QC/QA Lab	1.61	0.26
Total (working capital)	**2,489.89**	

broth which required a proportionally increasing amount of, handling equipment, power for operation, heating and cooling utilities to maintain the low temperatures. It is essential to find methods of EPS extraction other than using ethanol to obtain maximum EPS possible at relatively less extreme conditions. Other methods like EDTA, heating, etc. should be optimized first, to get desired quality and quantity of EPS.

Revenue and profitability analysis

The profitability of the process (Table 8) is highly dependent on the relative prices set to sell the final product, which decides the annual revenue of the process and eventually decides the gross profits and payback periods. During annual operation of the process, 1 million tons of B7 biodiesel, 0.2 million tons of EPS as main products were produced. Apart from EPS and B7 biodiesel, the process yielded 1.59 million tons of residual solid and 0.18 million tons of crude glycerol as waste which were assumed to give a credit value (as revenue) of $0.7/kg and $0.1/kg respectively.

By setting the current price to $0.7/kg, $1/kg and $0.15/kg for residual biomass, EPS and crude glycerol respectively, the annual operating cost reduced to 1,106 million dollars from earlier 2,489 million dollars. The cost of unit production without any credits was $1.9/kg whereas when revenues from glycerol, EPS, and residual mass was considered; it was only $0.86/kg-B7 Biodiesel.

The payback time and return of interest depend upon what price you are ready to sell the B7 biodiesel which is governed by the market forces. Price of petroleum Diesel is often in range of $1 - $1.1/kg at pumping stations. If the price of Biodiesel B7 was set to $1/kg, then process plant had 1,563 million dollars of gross profit which converts to a gross margin of 59% and ROI turns out to be 56.4%. In this fashion, the payback of the entire investment could be achieved in 1.77 years.

Table 8 | Profitability analysis of the overall process

Profitability analysis		
Direct Fixed Capital	279.22	Million $
Working Capital	2,489.89	Million $
Total Investment	2,769.12	Million $
Revenue & Credits Production rates		
Revenue Biodiesel	1,286, 109.85	MT per year
Credits Resi. Solids	1,592, 235.09	MT per year
Crude Glycerol waste credit	186, 065.66	MT per year
EPS Product	241, 127.15	MT per year
Revenue/ Credit Price		
Biodiesel B7	1	$/kg
Residual solids	0.7	$/kg
Crude glycerol	0.15	$/kg
EPS	1	$/kg
Revenue Obtained		
Biodiesel B7	1,286.11	Million $/year
Residual solids	1,114.56	Million $/year
Crude glycerol	27.91	Million $/year
EPS	241.13	Million $/year
Total revenues	2,669.71	Million $/year
Annual Operating cost		
Actual AOC	2,489.89	Million $/year
Net AOC	1,106.29	Million $/year
Unit Production Cost	1.94	$ per kg
Net Unit production cost	0.86	$ per kg
Unit production revenue	2.08	$ per kg
Unit profit	1.22	$ per kg
Gross Profit	1,563.42	Million $/year
Gross Margin	58.56	%
Return on Investment	56.46	%
Payback time	1.77	Years

Sensitivity analysis

Three parameters were varied to see the impact on unit production cost of B7 biodiesel: the scale of operation, bioreaction time and lipid content.

Scale of operation

The scale of operation also affects the unit cost of production (Figure 4). As we increased the scale of production (annual production level of the plant), the unit

Figure 4 | Sensitivity analysis for scale of operation.

cost of production decreased. As per the sensitivity analysis of the presented process, the unit cost of production was <$1/kg when the plant produced at least 100, 000 tons of B7 biodiesel per year which was also termed as the minimum scale of operation for the profitable market as the marketed price of petroleum diesel is $1.0–$1.1/kg.

Bioreaction time

The unit production cost of B7 Biodiesel varies with bioreaction time (Figure 5(a)). Assuming the same biomass concentration profile was achieved in 72 hours and 48 hours, the price of B7 Biodiesel production reduced from $0.86/kg to $0.85/kg (for 72 hours) and $0.75/kg (for 48 hours). Since as per the annual operating cost analysis, the significant fraction of the cost was due to the substrate it is obvious that increasing number of batches of operation

will not have a significant effect on cost and the cost of production will remain proportionally high. Only 12% reduction in the cost of production was observed by bringing the bioreaction time from 96 hours to 48 hours.

Lipid content

When lipid content of the cell biomass was varied from 28% to 60% the unit cost of production improved significantly. When lipid content was increased from 28% to 60%, a significant (20%) reduction in the cost of production was observed (Figure 5(b)). This is quite evident by the fact that increasing the lipid content of biomass will require less bioreaction volume for the same production level, which will consequently affect the operating cost of equipment required to handle that volume and also this implies a significant reduction in the amount of raw material required for lipid extraction and trans-esterification.

EPS and residual solids cost

The sensitivity of price of B7 biodiesel is shown in Figure 6. It is evident from the figure that the cost of production of B7 biodiesel is highly sensitive to cost of residual solids as compared to the EPS produced as by product in this process. This can be accounted by the fact that amount of residual solids (obtained after lipid extraction) produced at the end of the process is 6–7 times higher than EPS. As we vary the selling price of residual solids from $0.1/kg to $1.5/kg the cost of B7 biodiesel production reduces from $1.6/kg
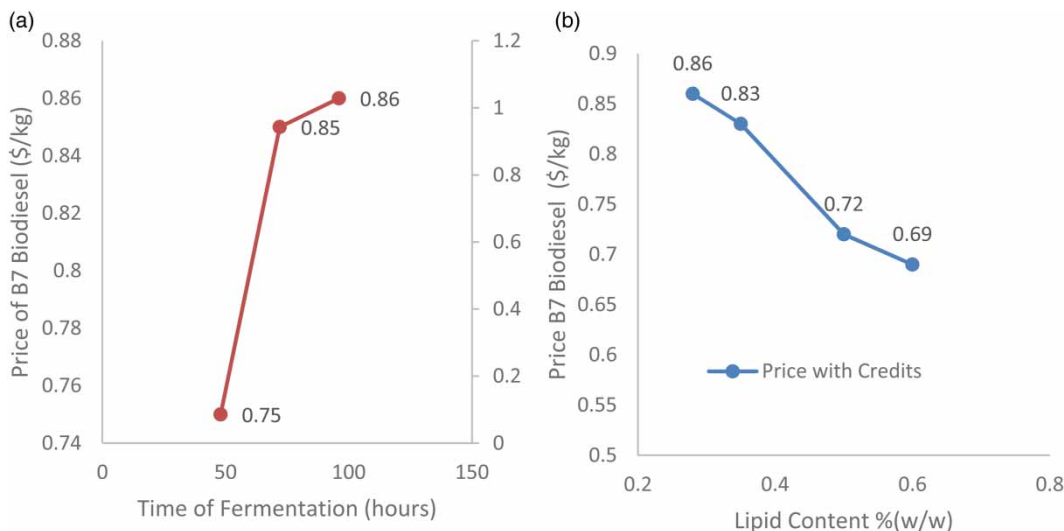

Figure 5 | (a) Sensitivity analysis for bioreaction time (b) lipid content of the biomass.
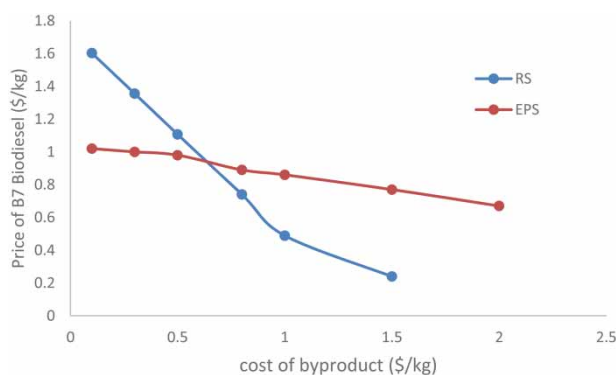
Figure 6 | sensitivity of B7 biodiesel cost of production w.r.t. changes in residual solids (RS) cost and EPS cost.

to $0.24/kg. When EPS cost is increased from $0.1 to $2.0/kg the cost of production of B7 biodiesel changed from $1.02 to $0.67/kg only. Thus it is important for the viability of the process plant that a good market for the residual solids should be produced to sell out the residual solids at highest possible prices.

CONCLUSIONS

Following conclusions can be drawn on techno-economic analysis of the process with the simultaneous production of EPS and biodiesel from municipal sludge and crude glycerol:

The process serves many advantageous purpose (1) production of bio flocculants, EPS (2) production of comparatively greener biodiesel B7 (3) alternate disposal or bio valorization of municipal secondary sludge (4) bio valorization of crude glycerol, waste stream of biodiesel industry (5) byproduct stream of residual bio-solids rich in proteins and other nutrients which can be used as manure or animal feed.

1. Crude glycerol and municipal secondary sludge provide an economical alternative to an expensive carbon source, such as glucose during bioreaction.
2. Ethanol accounted for 68% of the total raw material cost which is very high. This process required the addition of twice the volume of the bioreaction broth to precipitate the EPS. This increased the volume of liquid to handle which simultaneously increased the cost of centrifugation, heating, cooling, and mixing of the broth.
3. In total purchase cost, major cost (31%) was due to cost of centrifuges required for EPS pellet separation, and another 16% of the cost was required to handle

(mixing) the massive volume of the broth. The process can be made further very economical if alternate methods are employed to reduce volume handling in EPS extraction. Other chemical methods like EDTA extraction or Ultrafiltration methods should be tested to achieve maximum EPS from the broth.

4. The sensitivity analysis of the process revealed that unit cost of biodiesel was most sensitive to lipid content obtained in bioreaction where cost reduction of 20% was observed when lipid content was increased from 28% to 60%. B7 biodiesel cost can be lowered to as low as $0.6/kg given the lipid content of the biomass is improved by various process modifications.

Many dedicated researchers are working on this domain, and a lot of research is in a progressive state. Given the process is optimized considering the economics of the process, certain improvements in the process can make it economically competitive with its petrochemical counterparts.

REFERENCES

Andersen, T. & Hessen, D. O. 1991 Carbon, nitrogen, and phosphorus content of freshwater zooplankton. *Limnology and Oceanography* **36** (4), 807–814.

Gao, Q., Cui, Z., Zhang, J. & Bao, J. 2014 Lipid fermentation of corncob residues hydrolysate by oleaginous yeast *Trichosporon cutaneum*. *Bioresource Technol.* **152**, 552–556.

Hempfling, W. P. & Mainzer, S. E. 1975 Effects of varying the carbon source limiting growth on yield and maintenance characteristics of *Escherichia coli* in continuous culture. *Journal of Bacteriology* **123** (3), 1076–1087.

Klai, N., Hoang, N. V., Yan, S., Tyagi, R. D. & Surampalli, R. Y. 2015 Characterization of extracellular polymeric substances (eps) produced by *Cloacibacterium normanense* isolated from wastewater sludge for sludge settling and dewatering. *Civil Environ. Eng.* **5**, 6.

Kumar, A. S., Mody, K. & Jha, B. 2007 Bacterial exopolysaccharides-a perception. *J. Basic Microbiol.* **47**, 103–117.

Meng, X., Yang, J., Xu, X., Zhang, L., Nie, Q. & Xian, M. 2009 Review biodiesel production from oleaginous microorganisms. *Renewable Energy.* **34**, 1–5.

More, T. T., Yan, S., Hoang, N. V., Tyagi, R. D. & Surampalli, R. Y. 2012 Bacterial polymer production using pre-treated sludge as raw material and its flocculation and dewatering potential. *Bioresource Technol.* **121**, 425–431.

Nouha, K., Hoang, N. V. & Tyagi, R. D. 2016a Fourier transform infrared spectroscopy and liquid chromatography–mass spectrometry study of extracellular polymer substances produced on secondary sludge fortified with crude glycerol. *J Material Sci Eng* **5**, 240.

Nouha, K., Kumar, R. S. & Tyagi, R. 2016b Heavy metals removal from wastewater using extracellular polymeric substances produced by *Cloacibacterium normanense* in wastewater sludge supplemented with crude glycerol and study of extracellular polymeric substances extraction by different methods. *Bioresource Technology* **212**, 120–129.

Salehizadeh, H. & Shojaosadati, S. 2001 Extracellular biopolymeric flocculants: recent trends and biotechnological importance. *Biotechnology Advances* **19** (5), 371–385.

Sweeting, J. 1997 *Project Cost Estimating: Principles and Practice.* IChemE.

Wingender, J., Neu, T. R. & Flemming, H.-C. 2012 *Microbial Extracellular Polymeric Substances: Characterization, Structure and Function.* Springer Science & Business Media.

Yang, F., Hanna, M. A. & Sun, R. 2012 Value-added uses for crude glycerol–a byproduct of biodiesel production. *Biotechnology for Biofuels* **5** (1), 13.

Yellapu, S. K., Bezawada, J., Kaur, R., Kuttiraja, M. & Tyagi, R. D. 2016 Detergent assisted lipid extraction from wet yeast biomass for biodiesel: a response surface methodology approach. *Bioresource Technology* **218**, 667–673.

Yu, G.-H. *et al.*. 2009 Characteristics of extracellular polymeric substances (EPS) fractions from excess sludges and their effects on bioflocculability. *Bioresource Technology* **100** (13), 3193–3198.

Zhang, X., Yan, S., Tyagi, R. D., Surampalli, R. Y. & Valéro, J. R. 2014 Lipid production from *Trichosporon oleaginosus* cultivated with pre-treated secondary wastewater sludge. *Fuel* **134** (Octobre), 274–282.