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Techno-economic analysis for extracellular-polymeric substances (EPS) production using activated sludge fortified with crude glycerol as substrate and its application in leachate treatment

Lalit R Kumar[a], Xiaolei Zhang[b], Rajwinder Kaur[a], Sravan K Yellapu[a], R.D. Tyagi[a]* and Patrick Drogui[a]

[a]*INRS Eau, Terre et Environnement, 490, rue de la Couronne, Québec, Canada, G1K 9A9*

[b]*School of Civil and Environmental Engineering, Harbin Institute of Technology (Shenzhen), Shenzhen, Guangdong 518055, China*

**Corresponding author: Tel: 1-(418) 654-2617; Fax: 1-(418) 654-2600. Email:Rd.tyagi@ete.inrs.ca*

ABSTRACT

Economic assessment of bio-flocculant production process has been carried out by SuperPro Designer where extracellular-polymeric substances (EPS) were produced using activated sludge fortified with crude glycerol in fermenter followed by centrifugation. Considering EPS concentration of 60 g/L in production fermenter at 96 h, the unit production cost for slime EPS was estimated to be $ 0.95/L. The unit price of S-EPS was sensitive to inoculum size and EPS productivity (EPS concentration and fermentation time) in the fermented broth. Economic analysis was also conducted for EPS aided leachate treatment. The unit leachate treatment cost was 7.78 $/m^3 and was sensitive to S-EPS unit production cost. To get same leachate treatment cost as current industrial practice (4 $/m^3), S-EPS unit production cost should lower down to $ 0.5/L. The process has several advantages: 1) sludge and crude glycerol valorization for bio-flocculant production 2) Leachate treatment using environment friendly bio-flocculant.

Keywords: Extracellular-polymeric substances, Waste carbon sources, Leachate treatment, Economic analysis

1. Introduction

Flocculating agents are widely used in industrial processes including wastewater treatment, downstream processing and food and fermentation processes. They are generally classified in three categories: a) inorganic flocculants such as aluminium sulfate and polyaluminium chloride, b) organic synthetic high-polymer flocculants such as polyacrylamide derivatives and polyethyleneimine and c) naturally occurring flocculants such as chitosan, sodium alginate and microbial flocculants like EPS (Nouha et al., 2018). 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 (Yu et al., 2009) (Salehizadeh & Shojaosadati, 2001). 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-flocculation ability with excess sludge due to presence of high molecular weight macromolecules (330-1200 kDa) and trivalent cations in it (Yu et al., 2009). To minimize the use of synthetic flocculants in sludge settling applications, a novel alternative approach is to use eco-friendly bio-coagulants/ bio-flocculants like EPS. The role of extracellular polymeric substances (EPS) produced by sludge microorganisms during the wastewater treatment process have been extensively studied (Nouha et al., 2018). Recently, a demand of biopolymers for various industrial, biotechnological and environmental applications like flocculation, settling, dewatering of sludge, dyes and metal removal from wastewater has rekindled the interest in EPS production (Nouha et al., 2017). The main characteristic of EPS is to enhance aggregation of bacterial cells and suspended solids (SS). EPS contains relatively high quantity of hydroxyl (-OH) and carboxyl (-COO) groups (Nouha et al., 2016a; Ram et al., 2018; Salim et al., 2012; Yu et al., 2009). The presence of these groups is favorable for flocculation process to provide the required surface charges, which helps in further binding with suspended particles to foster floc formation. Adhesion and cohesion occur between EPS and the biomass along with suspended solids by complex interactions such as London forces, electro-statics interactions and hydrogen bonding, which leads to the formation of flocs (Yellapu et al., 2019). These

EPS properties make them suitable for many applications such as sludge flocculation, biomass settling, dewatering, metal binding and removal of toxic organic compounds (Nouha et al., 2018; Yellapu et al., 2019). EPS can be applied in different forms – dry EPS (powder form), broth EPS (fermented broth used directly for application) and slime EPS (liquid EPS obtained after centrifugation of fermented broth).

Since EPS production from commercial carbon sources is expensive (Nouha et al., 2018), therefore researchers are exploring waste carbon sources like wastewater and industrial sludge (Nouha et al., 2018; Nouha et al., 2017). In INRS laboratory, slime EPS (or S-EPS) has been produced using industrial activated sludge fortified with crude glycerol as source of carbon and other nutrients. The S-EPS produced using sludge was used to treat leachate where effective removal of COD, metals, nitrates, colour and odour has been obtained (Kaur et al., 2019). Although studies have already been performed for EPS producing strain isolation and EPS production using wastewater sludge (Nouha et al., 2018; Nouha et al., 2017), a reliable techno-economic evaluation for application of EPS (produced from sludge) in the leachate treatment process has not been performed to check its industrial feasibility. This study focuses to investigate the; a) economic analysis of S-EPS production using activated sludge fortified with crude glycerol and comparison with chemical coagulants and b) economic analysis of application of the produced EPS for leachate treatment. Techno-economic feasibility study of a newly developed process (EPS aided leachate treatment) is essential for its eventual application. The techno-economic evaluation study reveals important process parameters, which should be optimized by the researchers for making the process (technology) feasible at a commercial scale.

2. Materials and Methods

2.1 Simulation description and assumptions

In this study, using SuperPro designer v10, a process was simulated to produce slime EPS using activated sludge fortified with crude glycerol as a raw material. The simulations were performed for the production of 1.1 million L of slime EPS with 65 g/L EPS concentration. The EPS production capacity is based on the requirement to treat 800 m^3 of leachate generated per day in a compost facility (Quebec, Canada). The EPS production plant operate continuously for 6 months as the leachate treatment in CANADA occurs for 6 months (no treatment occurs during winter and autumn). A plant continuously operating for 6 months per year would result in 80 batches while time between inoculation of two batches was 53.64 h. Two production fermenters of 20 000 L capacity (producing 60 g/L EPS in fermented broth) would be used for processing back-to-back batches while each production fermenter will be conducted for 96 h (Figure 1). Since the final product is slime EPS (crude) which is in liquid form, thus, the unit production cost has been calculated in $/L. For leachate treatment, 800 m^3 raw leachate needs to be treated per day (Quebec, Canada) (Kaur et al., 2019). Raw leachate is untreated leached coming from the compost facility. The simulations were made to treat 144 000 m^3 leachate in 6 months.

2.2 Process Description

The EPS production process can be divided in three-unit operations: (1) Inoculum development (2) Production fermenter, and (3) Centrifugation or the product recovery.

2.2.1 Inoculum development

Inoculum development was done in a series of reactors operated at 30°C for 24 hours. Inoculum size of 10% v/v was used for the next seed fermenter. The activated sludge with 5-10 g/L suspended solid (SS) concentration was obtained from an industry in Quebec, Canada. It was settled overnight to obtain 15 g/L SS concentration and further washed with water to remove the toxic elements present in the sludge. Washed sludge (SS-15 g/L) was pre-treated with 0.11g $Ca(OH)_{2}$/g solids for sludge hydrolysis and thus sterilization was performed at 121^{o}C for 30 min to increase the carbon and nutrients availability for bio-transformation. Washed sludge (SS-15 g/L) was fortified with crude glycerol (20 g/L) to supplement additional carbon required for EPS production. The crude glycerol used had (w/v) composition: 50% glycerol, 18% (w/v) water and 3.25% w/v potassium. The EPS production bacterial strain, BS4 was isolated in INRS laboratory from wastewater sludge (Subramanian et al., 2010). The additional minerals such as ammonium chloride (1 g/L), mono potassium phosphate (2.4 g/L), magnesium sulphate (0.5 g/L) and sodium phosphate (6 g/L) were sterilized and supplemented to enhance the growth of microorganism.

The process starts with 500 mL shake flask with 160 mL culture, which is used as an inoculum for 2 L fermenter with working volume of 1.6 L. A 2 L inoculum volume is used to inoculate 20 L fermenter with working volume of 160 L. A 20 L inoculum volume is used to inoculate 200 L fermenter (inoculum volume), which is further used for 2000 L fermenter inoculation.

2.2.2 Production fermenter

Washed sludge (15 g/L SS) fortified with crude glycerol (20 g/L) along with ammonium chloride (4.8 g/L), mono potassium phosphate (10.4 g/L), magnesium sulphate (2.16 g/L) and sodium phosphate (26 g/L) were used for the production fermenter. The activated sludge was washed and pre-treated (sterilized) with 0.11 g $Ca(OH)_{2/}$ g sludge solids. The sludge and trace elements were sterilized at 121^{o}C for 30 min before transfer of non-sterilized crude glycerol and inoculum to the fermenter. The reactor pH was adjusted and maintained at 6.8. During 6-12 h, DO (dissolved oxygen) decreases from 90% to 35% and later, it was maintained in the range of 30%-40%.

A 20 000 L reactor with working volume of 15 750 L was used as the main production fermenter. The fermentation was conducted for 96 hours at 30°C at pH of 6.8. The extracellular polymeric substance (EPS) are extra-cellular products and EPS concentration achieved after 96h fermentation was considered to be 60 g/L. Crude glycerol was imparted based on consumption and total glycerol consumed during 96 h fermentation was 188.5 g/L. However, to recover the slime EPS (S-EPS), there is a need to separate the EPS from the cell biomass.

2.2.3 EPS recovery by centrifugation

Centrifugation was performed on 15 750 L (after every 53.64 h) of fermented broth using continuous bowl centrifuge, which was assumed to be operated at the efficiency of 95%. The centrifugation was performed at 15 000 g for 4 hours with the processing rate of 4 m^3/h. The purchase cost of centrifuge is dependent on the processing capacity of centrifuge. In the process, the factor deciding the time between inoculation of two batches is the fermentation time, hence the centrifuge with lower processing capacity was used. Per batch, 13 846 L of slime EPS was obtained in supernatant with 65 g/L EPS concentration along with 3387 kg of remaining centrifuged solids. Time between inoculation of two batches was 53.64 hours, which means centrifugation was performed after every 2 days. Table 1 gives process timeline for processing of 1 batch.

2.2.4 Leachate treatment by EPS and coagulant

The process developed at INRS treated the leachate obtained from the composting plant located in Quebec Canada. The raw leachate was high on colour, turbidity, organic carbon, ammoniacal nitrogen and metals. The treatment process used 2 g/L $FeSO_4$ (as chemical coagulant) of the leachate followed by agitation for 1.5 min (Kaur et al., 2019). The EPS with optimum concentration of 0.5 g/L was added to the leachate followed by agitation at 120 rpm for 5 min and then slow agitation for 8 h followed by gravitational settling for 30 min. Slow agitation helps in binding of opposite charges in coagulant, EPS and particles in leachate. Thereafter, settling of flocs occurred leading to clear water. The characteristics of raw leachate and treated leachate obtained by EPS treatment are presented in Table 2. The leachate treatment using EPS resulted in high removal rates for metals, colour, turbidity and nitrate.

The characterization of S-EPS produced from BS4 has been conducted in INRS laboratory. The kaolin clay flocculation activity of S-EPS with cation and without cation was 63.9% and 50.4%, respectively while zeta potential was -40 mV at pH 7. The mechanism behind the removal of organic matter and other contaminants is that the introduction of positively charged coagulants ($FeSO_4$) destabilizes the stable negative charge of the target particles by compressing the double layer. This upset decreases the distance or repulsion between particles, in turn decreasing the zeta potential. The particles are then able to get close enough together due to van der Waals forces, and the particles begin to flocculate (Kaur et al., 2019). The mechanism responsible for the removal of nitrates is biosorption, because leachate consists of various positively charged cations, such as K^+, Na^+, Al^{3+}, Mg^{2+} and Ca^{2+} (Kaur et al., 2019). Functional anionic groups such as carboxyl and hydroxyl present in EPS offer cation exchange potential that forms complexes with these metal ions. These positively charged cations also help in attracting anions (nitrates–nitrites) by columbic forces and produce adsorption sites capable of chemical interaction with anions (Kaur et al., 2019). The high protein and polysaccharide content of EPS plays an important role in the removal of metals. The FTIR conducted by Nouha et al. (2016b) revealed that C═O (carbonyl groups),─OH (hydroxyl groups), and amide groups located on the protein fraction of EPS can remove heavy metals by means of electrostatic interaction. In addition to proteins with various groups, polysaccharides containing C-O-C (ether) groups, alcohols with ─OH (hydroxyl) groups, phenolic alcohols with C═O (carboxyl) groups, and sulfur- and phosphorus-containing groups are also involved in the complexation reaction. Nucleic acids, especially DNA, present in EPS also provide binding sites for metals due to the presence of phosphorus groups in the sugar–phosphorus backbone, which makes DNA anionic in nature.

Moreover, uronic acids and nucleotides consisting of phosphorus groups present in EPS are negatively charged or anionic components and bind with multivalent cations to remove metals by electrostatic interaction (Nouha et al., 2018).

2.3 Economic Evaluation

Simulations were performed to get streamwise mass flow details for every unit operation. The annual operational cost was calculated using all the significant components of production like raw material cost, labour cost, laboratory quality control (QC), waste disposal cost, utilities cost and facility dependent cost into account (Gubicza et al., 2016; Koutinas et al., 2014; Kumar et al., 2019b; Wu et al., 2019). The unit production cost of EPS was calculated from annual production cost and the amount of EPS produced.

3. Results and discussion

3.1 S-EPS production using co-fermentation of sludge and crude glycerol

3.1.2 Equipment purchase cost

Stainless steel grade 304 (SS304) is chosen as material of construction for the equipment. The SS304, with its chromium-nickel content and low carbon, is the most versatile and widely used type of stainless steel. It contains 18% chromium and 8% nickel. SS304 is resistant to oxidation, corrosion, and durable (Phadnis et al., 2003) for this type of application. All the fermenters and vessels had height to diameter ratio (H/D) of 3 and were built at design pressure of 1.5 bar. The equipment purchase cost was estimated in US$. The assumed prices for the equipment were derived from quotations provided by different manufacturers.

The total equipment purchase cost has been divided into various sub-sections – process equipment, cleaning-in-place (CIP) generation system, water purification system and distributed control system (DCS). Total equipment purchase costs were estimated to be 3.43 million $ (Table 3a).

Process equipment contributes to 88% of total equipment purchase costs and CIP generation system with a tank, skid, transfer pump, heating element and a PLC (programmable logic controller) contributes to 5.17%. The plant would be operated through complete automation with a DCS (distributed control system with software, analog input/output and personal computer), which costs around 0.13 million dollars contributing 3.93 % of total equipment purchase costs. For the plant operations, purified water is used for CIP and to produce the purified water, water purification unit has been considered. Water purification unit (using reverse osmosis) contributes to 2.91% of total equipment purchase costs. In the process equipment, two fermentation reactors accounted for the largest contributor to the equipment costs (46.61%). Two continuous bowl centrifuges required for EPS recovery costs 8.74 % of the total equipment costs. Out of the two continuous centrifuges, one is operational and the other considered as standby. Two tanks of 20 000 L (each capacity similar to the production fermenter) were also accounted into main process equipment – one tank for feeding sludge into the production fermenter and another tank used as a harvest vessel. Two tanks account for 13.98% of total equipment purchase costs. Four lobe pumps (300 LPM) were used for inoculum transfer, media transfer to production fermenter, transfer of fermented broth to centrifuge.

3.1.2 Direct Fixed Cost (DFC)

Direct fixed cost of the plant comprises different plant cost elements: a) Plant direct cost, which includes equipment purchase cost, cost of installation, piping, instrumentation, building, facilities etc. b) Plant indirect cost, which includes plant construction and engineering cost and c) Contractor fee and contingency fees.

The direct fixed cost (DFC) of the plant comprises with total plant direct cost (TPDC), total plant indirect cost (TPIC) and contractor fee and contingency (DFC). The TPDC comprises of equipment purchase cost, equipment installation cost, their instrumentation, insulation, electrical connection, cost for building development, improvement of the yard and other auxiliary charges. In this process plant, typical scheme of calculations was used, which often are used as rule of thumb for such bioprocesses

(Table 3b). The scheme of calculation of the plant direct cost is designed using (Chen et al., 2018); Kumar et al. (2019a). The TPIC includes engineering and construction cost. The engineering cost required to properly engineer the plant for required production is separately accounted *(8% of total plant direct cost).* Contingency fee is also incorporated to account for variation in the cost-estimate, which was considered to be 15% of additional sum of indirect and direct plant cost. Direct Fixed Cost (DFC) = TPDC + Construction cost + Engineering cost + Contractor fee + contingency. The DFC for the process was calculated to be 12.54 million dollars (Table 3b).

3.1.3 Annual operating cost

The annual operational cost was calculated using all the significant components of production, which include: raw material cost, labour cost, quality control, waste treatment, facility dependent and utilities. The cost of different raw materials (crude glycerol, $Ca(OH)_2$, water, sludge transportation cost, $Na_2HPO_4.12H_2O$, NH_4Cl, KH_2PO_4 and $MgSO_4.7H_2O$) was taken for bulk price from the internet and fed into the software for calculating annual requirement of raw material. Industrial water consumption cost was considered from '**Ministère des affaires municipales et des régions**'. The cost of treating aqueous waste was taken from Kumar et al. (2019a) and the average salary of plant operators was considered from database of the software. Since the plant was operated for 6 months, salary of plant operators has been adjusted accordingly.

Annual expenditure for raw material purchase was 206 594 \$. Considering the raw material acquisition cost, the most cost intensifying factor was water (0.07 \$/L), which was used for cleaning of seed vessels and production fermenters (52.82%) after every batch followed by sodium phosphate (1.2 \$/kg) used during fermentation accounted for 16.38 % and crude glycerol (0.15 \$/kg), which accounted for 16.18%. The activated sludge, which was used as principal carbon source as well as source of other nutrients, has no or zero cost. However, the cost of sludge transportation within a range of 50 km has been assumed, which is 200 $/ trip (cost/rent of the truck that will transport the sludge). The sludge transportation cost accounted for 7.74% of raw material acquisition cost.

For all the heating and cooling requirements of the process, utilities like steam and NaCl brine are used in the process plant. Standard electrical power and steam are most frequently used for heat generation, and mechanical transport of materials. A total sum of 70330 $ is needed annually to run the plant. NaCl brine (0.25$/ MT) was used for cooling the fermenters (after sterilization of fermentation medium). Standard electricity (0.1 $/ kWh) to operate the centrifuge and main fermenters accounted for 64.71% of annual utility cost. The annual requirement of standard power (3 kW/m^3 fermentation broth during agitation and 30.76 kW during centrifugation), steam (24.16 kg/ h) and chilled water (9807.8 kg/h) was calculated from the software based on the process requirement.

Annual labour operating cost calculated from the software was 200 000$. Eight operators at average pay-scale of 50 000 $/yr (25 000 $ for 6 months) are required to operate the facility: 2 dedicated for seed fermenters, 3 dedicated for production fermenters, 1 dedicated for centrifuge, 1 for warehouse and 1 for purchase and accounts department. Supervisory and quality control labour has been considered to be 15% each of annual operating labour. Since the plant has been considered fully automated, the operators are on lower side.

Facility-dependent cost comprises of plant annual maintenance cost, insurances, local taxes and factory expenses. The maintenance cost is for proper running of the facility. This cost is 2% of the direct fixed cost (DFC). Taxes are also imposed on the facility dependent cost. Insurance charges, local taxes and other factory expenses are estimated as 0.5%, 0.5% and 1% of the DFC (direct fixed cost), respectively. These % has been considered assuming the plant will be operated for 6 months only. The total facility dependent cost for this facility was calculated to be 0.5 million dollars. Annually 520 m^3 of aqueous waste (generated from cleaning of fermenters) is generated by the process plant, which is disposed at the rate of $0.11/m^3.

A total sum of 1.04 million dollars was required to annually run the facility (Table 4a). Annual operating cost analysis of the process reveals that 48.3% of the total annual operating cost is because of facility dependent cost for maintenance and repair of the facility. Annual operating labour cost (including operating, supervisory labour and QC or quality control), account for 25 % of annual operating cost.

3.1.4 Unit production cost

In this scenario, S-EPS produced will be used to treat the industrial leachate. It is assumed that the slime EPS (or S-EPS) produced will not be sold to market. The total capital investment for the project is calculated on the basis of direct fixed capital cost to set-up the plant, working capital required to conduct trial and validation batches before actual commercialization of the plant (20% of DFC). The total investment to start the project is 15.05 million $. Through annual operating cost and quantity of EPS produced, the unit production cost calculated was 0.95 $/ L slime EPS (S-EPS) (Table 4b). It can be observed from Table 5a that bio-flocculant cost in this study was 3 times higher than cost of chemical flocculant and 2 times lower than plant-based bio-flocculant (Lee et al., 2018).

In the present study, fermentation section (including seed fermentation) contributed 86.6% of EPS production cost while centrifugation contributed 13.4% of total unit production cost. It indicates that to further reduce the unit production cost, fermentation parameters like EPS productivity and inoculum size can be further optimized to improve the process economics.

3.1.5 Sensitivity analysis

EPS concentration in fermented broth

Simulations were performed for different EPS concentration obtained in the production fermenter at 96 h. In current scenario, EPS concentration at 96 h was 60 g/L. An increase in the EPS concentration in the fermenter from 60 g/L to 80 g/L reduces the unit production cost from 0.95 $/L to 0.71 $/L (25% reduction). EPS concentration in fermentation governs size of fermenter (Lower

EPS concentration requires higher capacity fermenter) and amount of substrate used for fermentation (as size of fermenter increases, substrate requirement and cost also increase). If the EPS concentration in the fermenter decreases to 40 g/L or 25 g/L, the unit production cost is increased by 1.3 and 2.4 times, respectively.

EPS productivity in fermented broth

The effect of EPS concentration and fermentation time can be combined into EPS productivity (EPS produced per unit time per unit volume of the fermenter). EPS productivity governs the size of the fermenter, time of fermentation, number of batches produced annually, amount of EPS produced per batch, amount of substrate used for fermentation (as size of fermenter increases, substrate requirement and cost also increase). In the presence scenario, EPS productivity in the fermenter was 0.63 g/L/h, for which the unit production cost is 0.95 $/L. If EPS productivity increases to 0.83 g/L/h, the unit production cost decreases to 0.71 $/L. For EPS-productivity of 0.26 g/L/h and 0.42 g/L/h, the unit production cost becomes 2.27 $/L and 1.42 $/L, respectively. An increase in productivity decreases unit production cost.

Inoculum size

Inoculum size for the production fermenter is an important parameter to analyze because fermentation is the major contributing factor for unit production cost. The fixed capital cost and unit production cost for different inoculum sizes are compared in Table 5b. Decreasing the inoculum size %v/v, decreases the equipment purchase cost and capital investments. Moreover, reduction in inoculum volume decreases the facility dependent cost and utilities cost along with labour cost as it reduces the number of seed fermenters. Assuming the EPS concentration in production fermenter remains same, reducing inoculum volume from 10 (%v/v) to 2 (%v/v) reduces the unit production cost from 0.95 $/L to 0.86 $/L (a 9.47% cost reduction) and capital investments from 15.05 million $ to 13.57 million $. The difference in unit production cost may appear small but annual operating cost reduces by 100 000 $, which is significant at industrial scale. However, not much reduction was observed between inoculum size of 2% (v/v) and 1% (v/v). Further, experimental studies are required to investigate the impact of inoculum size on EPS yield and productivity.

3.2 EPS aided Leachate Treatment

3.2.1 Economic Evaluation

As per the requirement of raw leachate treatment, 800 m^3 leachate needs to be treated per day. Hence, the simulations were designed to treat 144 000 m^3 leachate in 6 months. Depending on amount of leachate treatment and time to process one batch (8.7 h), equipment sizing has been calculated. Leachate amount of 341 000 L needs to be treated in one batch. The total equipment purchase cost for one vessel with agitation and one clarifier (for settling of flocs) accounts to 87 000 $ (Table 6a). Direct fixed capital for leachate treatment has been calculated from the scheme used in Table 3b and was estimated to be 280 000 $. Direct fixed cost includes direct cost (installation, piping, instrumentation, electrical, auxiliary facilities), indirect cost (engineering and construction) and contingency.

Facility-dependent cost comprises of plant annual maintenance cost, insurances, local taxes and factory expenses. The maintenance cost is for proper running of the facility. This cost is 2% of the direct fixed cost (DFC). Taxes are also imposed on the facility dependent cost. Insurance charges, local taxes and other factory expenses are estimated as 0.5%, 0.5% and 1% of the DFC (direct fixed cost), respectively. Facility dependent cost has been considered assuming the plant will be operated for 6 months only as no treatment occurs in autumn and winter in CANADA. The total facility dependent cost for leachate treatment was calculated to be 11 200 dollars.

3.2.2 Operating cost for leachate treatment

Considering S-EPS purchase cost for leachate treatment to be 0.95 \$/L as obtained from Table 4b, the process was simulated to treat the leachate. The raw material acquisition cost for leachate treatment (EPS as bio-flocculant and $FeSO_4$ as coagulant) was 1.09 million \$, where EPS was major cost contributing factor in the raw material acquisition cost (95.28%). Total electricity consumed during agitation was 172 454 kW-h and considering the cost of 0.1\$/ kW-h, annual electricity consumption cost was found to be 17 245 \$. The facility dependent cost was calculated to be 11 200 \$. The annual operating cost for leachate treatment was found to be 1.12 million \$, where raw material acquisition cost contribution was found to be 97.46% while contribution of facility dependent cost and electricity were 1% and 1.54 %, respectively (Table 6b). Based on annual operating cost of 1.12 million \$ and annual leachate to be treated 144 000 m^3, the unit leachate treatment cost was found to be 7.78 \$/m^3. Since unit leachate treatment cost is heavily dependent on EPS production cost, sensitivity analysis needs to be performed.

3.2.3 Sensitivity analysis

S-EPS production cost

Since S-EPS production cost is major contributor in unit leachate treatment cost, simulations were performed for different S-EPS production cost. When S-EPS unit production cost is \$0.4/ L, leachate unit treatment cost is \$3.25/m^3. Unit treatment cost becomes \$6.49/m^3 when S-EPS price is \$0.79/ L and the unit treatment cost further increases to 10.82\$/m^3 when S-EPS cost is \$1.32/ L. The unit treatment cost increases with the increase in S-EPS unit production cost and is directly proportional to S-EPS production cost. In the above scenario, S-EPS production cost is \$ 0.95/L for which the unit treatment is \$ 7.78/m^3, which is around 1.9 times that of current industrial leachate treatment cost (\$ 4/m^3) in Quebec, CANADA.

Incubation time of treatment

The process developed for raw leachate at INRS optimized the incubation time for EPS and leachate interaction from 0.5 to 12 h (Kaur et al., 2019). Hence, the simulations were performed for different incubation time. The incubation time (current scenario) of 8 h resulted in leachate treatment cost of 7.78 $\$/m^3$. However, changing the incubation time affects the electricity consumption cost required for agitation. Incubation time of 12 h resulted in leachate treatment cost of 7.87 $\$/m^3$ (1.2% increase) while incubation time of 0.5 h resulted in leachate treatment cost of 7.61 $\$/m^3$ (2.19% decrease). The incubation time did not impact leachate treatment cost significantly as major cost contributing factor was EPS cost.

The settled sludge (obtained after leachate treatment) and residual sludge (obtained after broth centrifugation) are waste produced during process and can be used for S- EPS (slime EPS) production. Settled sludge is rich in metals, phosphorus, and nitrogen as well as carbon compounds, therefore it could be used as nutrient media for EPS production. In one of the studies, the sludge solids were recycled for EPS production in shake flask studies (Kaur et al., 2019). The EPS production of 4 g/L was observed at 72 h with centrifuged sludge as a media, which was enhanced to 13g/L with fortification of crude glycerol and additional minerals. However, studies need to be conducted for use of settled sludge (obtained after leachate treatment) and centrifuged solids (obtained from fermented broth) as EPS production medium in the fermenter. Recycling of centrifuged solids and settled sludge solids for EPS production can reduce the cost of trace elements added during fermentation.

4. Conclusion

Techno-economic evaluation revealed that S-EPS production cost was major contributing factor in the leachate treatment. To get same leachate treatment cost as current industrial practice (4 $\$/m^3$), S-EPS unit production cost should be 0.5 $/L. EPS productivity (EPS concentration and fermentation time) in the fermented broth and inoculum size should be further optimized to reduce S-

EPS unit production cost. Recycling of sludge solids, obtained after leachate treatment and centrifugation of fermented broth, as production medium may reduce S-EPS production cost.

5. Supplementary Files

Supplementary Figures S1 to S3 can be found online.

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Figures

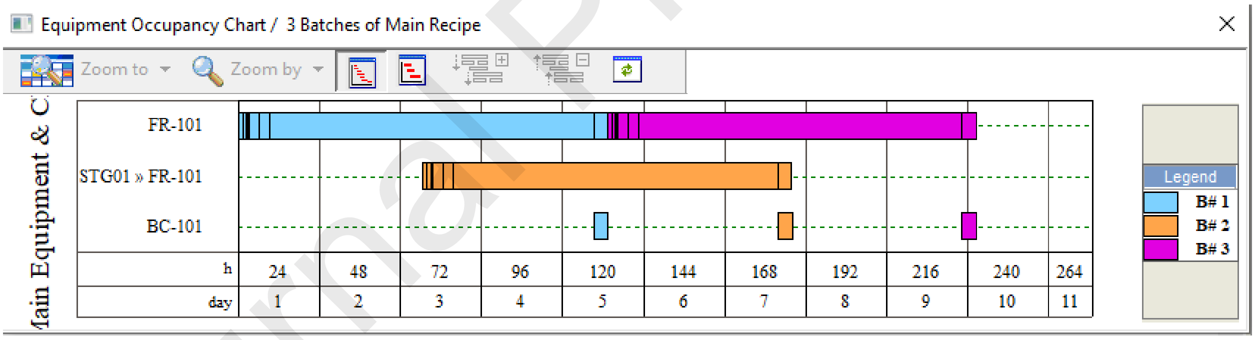


Figure 1: Gantt chart for processing of three consecutive batches

Tables

Table 1: Process Timeline for S-EPS production for 1 batch

Operation	Description
Seed Fermentations	
Cleaning in Place (CIP)	60 min - Cleaning of equipment with water (30% of equipment capacity) supplied @ 60°C
CHARGE-Sludge	30 min, Charge sludge and trace elements to the seed fermenter
Sterilization @ 121°C	120 min (holding time of 15 min)
COOL	Cooling to 30.0 °C (cooling time around 120 min)
TRANSFER-Inoculum	30 min - Crude glycerol and Inoculum transfer through lobe pump
Inoculum development	Inoculum development for 8 h at 30 °C
FR-101	**Main Fermenter**
CIP	60 min, Cleaning of equipment with water (with 30% of equipment capacity) supplied @ 60°C
CHARGE- Sludge	60 min, Charge sludge to main fermenter
Sterilization @ 121°C	180 min (including holding time of 15 min)
COOL	Cool to 30.0 °C (cooling time around 180 min)
TRANSFER-glycerol	30 min - Transfer crude glycerol to main fermenter
TRANSFER-Inoculum	30 min - Transfer inoculum from seed fermenter to the main fermenter through lobe pump
Fermentation	Fermentation for 96 h at 30.00 °C.
BC-101	**Centrifugation**
CENTRIFUGATION	Centrifuge fermented broth for 240 min to obtain slime EPS

Table 2: Characterization of raw leachate and EPS based leachate treatment

Parameter	**Units**	**Raw leachate**	**Leachate treatment with EPS**
COD	mg/L	2015	400 (84% removal)
Colour	UCV	2500	105 (95% removal)
Turbidity	NTU	96	4 (95% removal)
Total Kjeldhal nitrogen	mg N/L	425	90 (79% removal)
Ammoniacal nitrogen	Mg NH_3-N/L	327	82 (75% removal)
Total organic carbon	mg C/L	589	68 (88% removal)
Nitrate/Nitrite	mg N/L	3	0.15 (97% removal)
PO_4	mg P-PO_4/L	21	1.62 (96% removal)
S	mg/L	40	4 (90% removal)
Mg	mg/L	44	0.4 (90% removal)
Al	mg/L	0.16	0.12 (29% removal)
Na	mg/L	100	5 (95% removal)
K	mg/L	344	3.54 (99% removal)

Table 3a: Distribution of equipment purchase cost

Equipment	Capacity of Equipment	Unit cost of Equipment ($)	Number of units	Final cost ($)	Cost %
Process Equipment					
Seed flask (SFR-105)	500 mL	50	1	50	0
Seed Fermenter (SFR-104)	2 L	20 000	1	20 000	0.58
Seed fermenter (SFR-103)	20 L	50 000	1	50 000	1.46
Seed Fermenter (SFR-102)	200 L	90 000	1	90 000	2.62
Seed Fermenter (SFR-101)	2 000 L	300 000	1	150 000	8.74
Main fermenter (FR-101)	20 000 L	800 000	2	1 600 000	46.61
Harvest & feed vessel (HR-101 & FV-101)	20 000 L	300 000	2	600 000	13.98
Centrifuge (BC-101)	5 m^3/h	150 000	2	300 000	8.74
Lobe Pumps for transfer	300 LPM	50 000	4	200 000	5.83
Software & DCS		135 000	1	135 000	3.93
CIP system					
CIP tank including pump & PLC		160 000	1	160 000	4.66
CIP skid for transfer		1400	1	14 000	0.41
Heating element		1800	2	3 600	0.1
Water purification unit		100 000	1	100000	2.91
Total equipment cost (Million $)				**3.43**	100.00

Table 3b: Direct fixed cost of the plant

Direct fixed cost components		Million $
a. TOTAL PLANT DIRECT COST (TPDC)		
Equipment Purchase Cost, PC		3.43
Installation	30% of PC	1.03
Process Piping	30% of PC	1.03
Instrumentation	25% of PC	0.86
Insulation	8% of PC	0.27
Electrical	10% of PC	0.34
Building	20% of PC	0.69
Yard Improvement	10% of PC	0.34
Auxiliary Facilities	25% of PC	0.86
TPDC ($)		**8.86**
b. TOTAL PLANT INDIRECT COST (TPIC)		
Engineering	8% of TPDC	0.71
Construction	10% of TPDC	0.89
TPIC ($)		**1.59**
Total Plant COST (TPC=TPDC+ TPIC)		10.54
c. CONTRACTOR FEE & CONTINGENCY (CFC)		
Contractor's Fee	5% of TPC	0.52
Contingency	15% of TPC	1.57
CFC ($)		**2.09**
DIRECT FIXED COST (DFC= CFC+ TPC)		**12.54**

Table 4a: Distribution of Annual operating cost

Item	Description	Annual cost ($)	% of AOC
Operating Labour		200 000	19.26
Supervisory labour	15% of operating cost	30 000	2.89
Quality Lab Control	15% of operating cost	30 000	2.89
Raw material cost		206 594	19.89
Utilities		70330	6.77
Facility dependent		501 610	48.3
Waste treatment		57	0.01
Annual Operating cost, AOC (Million $)		**1.04**	**100**

Table 4b: Unit production cost for S-EPS production using activated sludge fortified with crude glycerol

Investment details		
Direct Fixed Capital	12.54	million $
Working Capital (20% of DFC)	2.51	million $
Total Investment	**15.05**	**million $**
Annual Production rates		
EPS slime	1.1`	Million L per year
Annual Operating cost		
Annual Operating Cost	1.04	million $/ year
Unit Production Cost	0.95	$/ L

Table 5a: Comparison of price of different types of flocculants

Types of flocculant		Price ($/kg)
Plant-based bio-flocculant (okra)	Aqueous bio-flocculant	31.4
	Dried bio-flocculant	37.3
Microbial bio-flocculant (EPS)	s-EPS (This study)	14.42 (equivalent cost from $/L)
Chemical	Cationic polyacrylamide	4.25
	Anionic polyacrylamide	4.08
	Non-ionic polyacrylamide	4.08
Food-grade bio-flocculants	Coconut shell activated carbon	52
	Chitosan	50
	Sodium Alginate	62.5
Starch-based bio-flocculant	Amylopectin	191

Table 5b: S-EPS price sensitivity to different inoculum size

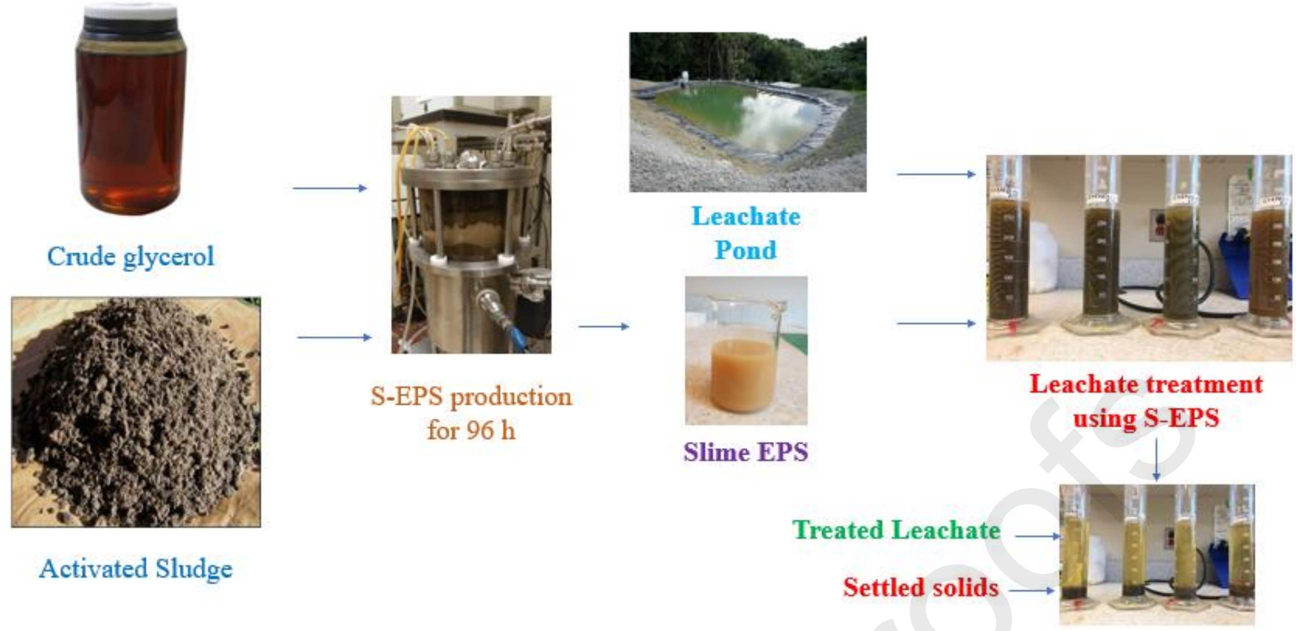
Inoculum %	**1 %**	**2 %**	**5 %**	**10 %**	**v/v**
Equipment purchase cost	3.08	3.1	3.23	3.4	million $
Direct Fixed Capital	11.25	11.31	11.88	12.54	million $
Working Capital (20% of DFC)	2.25	2.26	2.38	2.51	million $
Total Investment	**13.51**	**13.57**	**14.26**	**15.05**	**million $**
No of seed fermenters	3	3	4	5	
Annual Operating Cost	0.94	0.94	1	1.04	million $/ year
Unit production cost	0.86	0.86	0.9	0.95	$ per L

Table 6a: Equipment sizing for leachate treatment

Equipment	No of units	Material of construction	Design capacity	Purchase cost ($)
Vessel with agitation (R-101)	1	Carbon steel	400 000 L	50 000
Clarifier (CL-101)	1	Carbon steel	400 000 L	37 000
Total equipment purchase cost ($)				**87 000**

Table 6b: Annual operating cost for EPS aided leachate treatment

Cost Item	Cost ($)	%
Raw material acquisition	1091 613	97.46
Facility dependent	11 200	1.0
Utilities	17 245	1.54
Total (million $)	**1.12**	**100**



Highlights

- S-EPS was produced using activated sludge fortified with crude glycerol
- EPS productivity and inoculum size are important cost impacting parameters
- S-EPS cost was major cost impacting factor in the leachate treatment

Lalit R Kumar: Conceptualization, Methodology, Software, Formal Analysis, Writing – Original Draft

Xiaolei Zhang: Investigation, Supervision

Rajwinder Kaur: Writing - Review & Editing, Resources

Sravan K Yellapu: Data curation

RD Tyagi: Validation, Project Administrator, Funding Acquisition

Patrick Drogui: Visualization