

Gate-to-gate life cycle assessment of biosurfactants and bioplasticizers production via biotechnological exploitation of fats and waste oils

Article

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Short title: LCA of biosurfactants and bioplasticizers production from fats and waste oils

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Abstract

BACKGROUND: This study investigated the biotransformation of fats and waste oils towards glycolipid biosurfactants and bioplasticizers. The ecological performance and environmental impacts of the bioprocesses were evaluated aiming to assess their present environmental status and thus suggest future improvements using LCA methodology.

RESULTS: Biosurfactants, namely rhamnolipids and sophorolipids were obtained via fermentation. Bioplasticizers, Fatty Acid Ethyl Esters (FAEE) and Monoglycerides

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(MAG), were developed via enzymatic catalysis with selected enzymes in mesophilic temperatures via ethanolysis and glycerolysis, respectively. The study revealed that air emissions, electricity and thermal energy requirements are the key contributors to the potential environmental impacts in the LCIA. More specifically, rhamnolipids production has less energetic needs compared to sophorolipids manufacturing, resulting thus to lower environmental impacts. The increased thermal requirements of MAG production phase is the main contributor to their negative environmental performance, with the overall energy consumption for MAG production being 3-fold higher than the FAEE formation phase.

CONCLUSIONS: The assessment identified that among the biosurfactant production processes, the sophorolipids production resulted to 22.7% higher environmental impact compared to rhamnolipids. Similarly, FAEE production can be classified as a more environmental friendly process compared to MAG, resulting to 67% lower environmental impact based on the environmental indicators assessed.

Keywords: biosurfactants; bioplasticizers; rhamnolipids; sophorolipids; enzymatic catalysis; life cycle assesment

INTRODUCTION

Our major source of energy derives from fossil fuels such as oil, coal and natural gas. However, as the world population continues to grow energy demands increase and the amount of fossil fuels is expected to fall short in the near future. In order to satisfy the ever-increasing demand of our modern society for energy, fuels and chemicals research has been focusing on the development of sustainable and innovative conversion processes based on biocatalysis of biodegradable feedstocks, such as waste streams and biomass¹. Current policies and legislation promote the exploitation of such side-streams in order to make a successful transition from petrochemical complexes to biorefineries that would have huge environmental and societal benefits^{2,3}.

Bio-based chemicals and materials, produced by biocatalysts such as yeast, bacteria and fungi, present the advantages of being biodegradable, biocompatible and environmentally friendlier than their fossil fuel-derived counterparts. However, their production cost is still higher compared to the respective petrochemical ones, since process technologies are still researched, developed and optimized. Biotechnological production of biosurfactants is such an example. Several studies have demonstrated the potential of mostly hydrophobic organic residues, rich in fats, as raw materials towards the production of such high value-added compounds obtained by fermentation and enzymatic processes. Their triglycerides, consisting of fatty acids, can be used as precursors by several microorganisms or as initial reactants in lipase-catalyzed processes for biosurfactants' production⁴⁻⁶.

Microbial biosurfactants are amphiphilic molecules, comprised of both hydrophilic and hydrophobic moieties, that have the ability to reduce surface and interfacial

tension. Several bacteria produce them as a key mechanism to facilitate hydrocarbon uptake through micelle solubilization or pseudosolubilization^{7,8}. They are characterized by a wide variety of chemical structures including glycolipids, lipopeptides, polymeric polysaccharide protein complexes, fatty acids and phospholipids. Their main advantages against their synthetic counterparts are: their biodegradable nature, low toxicity, ecological acceptability, biological activity as antifungal and antimicrobial compounds and sustainable production using renewable resources^{9,10}. Based on those features, their applications are quite diverse including their use in food, pharmaceutical and cosmetic industries, bioremediation, toiletries and household cleanup^{5,10-14}. Their global market by revenue is projected to reach \$2,477.4 Million by 2020, compared to \$1,870.1 Million in 2013, witnessing a compound annual growth rate (CAGR) of 4.1%¹⁵.

According to IUPAC (International Union of Pure and Applied Chemistry) a substance is defined as a plasticizer if when incorporated in a plastic material it enhances its flexibility, and processability due to its effect in reducing the second order transition temperature and the glass transition temperature (T_g)¹⁶. Plasticizers are an important class of low molecular weight non-volatile compounds and are widely used in polymer industries as additives¹⁷. Plasticizers demand continues to grow along with the plastic industry. However, critical issues regarding the use of phthalates (the most dominant plasticizers used globally) and their migration have arisen including their human- and eco-toxicity. This is one of the main reasons why a lot of attention has been focused on research in order to gradually replace them, with natural-based plasticizers that are characterized by low toxicity, low migration and high biodegradability, offering

competitive mechanical properties and produced in a cost-effective and sustainable manner¹⁸. It is worth noting that the bioplasticizers global market size is growing and is expected to reach up to \$1,140.3 Million by 2020, characterized by a CAGR of 9.8% between 2015-2020¹⁹.

Monoacylglycerols and their derivatives are non-ionic emulsifiers widely used for the pharmaceutical and especially the food industry. They are also of great importance in synthetic organic chemistry where they are utilized as synthetic intermediates and plasticizers for various materials^{20,21}. The synthesis of monoacylglycerols involves transesterification of triacylglycerols (TAGs) with glycerol at high temperatures and under nitrogen atmosphere employing inorganic alkaline catalysts²². However, several side-products are also obtained while low yields, low product quality and high-energy consumption are the major disadvantages of this conventional method. As an environmentally friendly alternative, enzymatic processes for monoacylglycerols synthesis based on lipase catalysis have been proposed. Those include, for example, selective glycerolysis/hydrolysis using regiospecific lipase enzymes, fatty acids esterification with glycerol and glycerolysis of fats/oils^{23,24}.

Fatty acid ethyl esters (FAEE) are produced via esterification of fats/oils using ethanol as the catalyst, a process also known as ethanolysis. Usually an excess of alcohol is necessary to push the reaction to completion. In the literature several studies point out the potential value of FAEE as plasticizers in polyvinyl chloride (PVC) based applications^{25,26}. FAEEs may derive from renewable, abundant and inexpensive raw materials and serve as alternatives to plasticizers such as phthalates.

So far, waste fats and oils have been mostly used for the production of animal feed,

for soapmaking and the production of free fatty acids (FFAs)²⁷. However, those by-products may be used for the production of high value-added biotechnological products, in an effort to reduce their high production cost. To this end, waste fats and oils of different origin, were screened and utilized in lab- and pilot-scale for the fermentative production of biosurfactants and enzymatic production of bioplasticizers in the framework of the EU-funded project Bio-SURFEST (FP7-SME-2011, Grant no: 286834).

A critical tool in order to evaluate and understand the benefits and hotspots during production is life cycle assessment (LCA). This technique allows the assessment of several environmental impact categories associated with all or specific stages of the product's life. However, since most bioprocesses are still under development and optimization, with only a few products being produced at pilot or industrial case, LCA analyses are currently being performed²⁸. In the case of biosurfactants only one study has been published recently, regarding the production of acetylated acidic sophorolipid biosurfactants and their application as a hand-washing detergent²⁹. Regarding bioplasticizers, which could eventually substitute phthalates, several have reached commercial scale production. However, LCA assessments on their production are still limited³⁰ or under investigation³¹.

The Bio-SURFEST project focused on the processes of biological production of glycolipid biosurfactants, in the form of rhamnolipids and sophorolipids, and also the enzymatic processes for production of FAEEs and MAGs. The ecological performance and environmental impacts of the production processes were estimated aiming to assess their present environmental status and thus enable future improvements by

using the existing midpoint approaches of LCA methodology with impact categories such as global warming, ozone depletion, photochemical oxidation, acidification, eutrophication and non-renewable fossil usage. Midpoints are considered to be links in the cause-effect chain (environmental mechanism) of an impact category, prior to the endpoints, at which characterization factors or indicators can be derived to reflect the relative importance of emissions or extractions³². To compile the LCI of each bio-product's manufacturing process, a series of process assumptions was made, the system boundaries for its production were determined and calculations were carried out based on the raw materials use and the electrical energy requirements. Biogenic emissions were also calculated for each process. In any case, the approach followed, and the results obtained from this study could be a useful tool for future studies on environmental performance of biosurfactants' and bioplasticizers' production.

EXPERIMENTAL

Life Cycle Assessment (LCA) methodology evaluates holistically the environmental consequences of a product system or activity, by quantifying inputs, in terms of materials and energy resources, and outputs such as emissions and wastes released to the environment assessing their environmental impacts. The environmental analysis developed in this work was carried out according to ISO 14040 guidelines and recommendations³³.

For this LCA study, the complete life cycle inventory (LCI) of large-scale biosurfactant and bioplasticizers production is unavailable at the early design stage, which makes an LCA from gate-to-gate the most appropriate and practical approach

for evaluating possible environmental impacts.

Gate-to-gate is a partial LCA looking at only one value-added process in the entire production chain. Since this calculation is a microscopic analysis, it is easy to identify a key unit³³. Therefore, LCI information from this methodology can be used more directly in exploring engineering and chemistry changes to improve manufacturing processes which is the fundamental part in the assessment boundaries.

The detailed operating strategy under which the systems was assessed, has been optimized and validated under the frame of Bio-SURFEST project, and is currently confidential due to patent application. Further information on the process description can be found in the projects web site (<http://www.biosurfest.com>).

Goal and scope

The goal of this study was to establish an up-to-date Life Cycle Inventory dataset to assess the potential environmental impacts of biosurfactants and bioplasticizers production. Moreover, the results of this gate-to-gate analysis consolidated with the environmental effect of the other production stages, could generate the full Life Cycle Assessment of the products after the development of the whole Life Cycle scenario from Cradle-to-Grave. It could also form the basis of future environmental product declarations.

Functional unit

The functional unit must represent the function of the options compared³⁴. In the case of biosurfactants, it was decided to use 1 kg of product as a functional unit for

environmental performance purposes and for expressing inputs, outputs and the environmental impact assessment. Similarly, for FAEE and MAG production it was decided to use as a functional unit 1 kg of produced FAEE and MAG, respectively. Due to lack of data on the industrial scale manufacture of biosurfactants and bioplasticizers, the operating parameters used for the evaluation of the processes were based on pilot plant results and prerequisites.

System description

The biotechnological products developed via the exploitation of waste oil and fats were developed in pilot scale, under the framework of the Bio-SURFEST project. The Life Cycle Analysis of the manufacturing stages for each of the products is described below.

Sophorolipids production

Based on the results from the pilot-scale production of sophorolipids, and the optimum operating conditions in which the highest yields of product recovery were achieved, the process is comprised of three stages. The first stage included the preparation of the pre-inoculum and its inoculation with the sophorolipids-producing yeast *Candida bombicola*. The pre-inoculum contained glucose, yeast extract and urea in order to accelerate yeast growth. During the second stage, an increase in the inoculum volume by extra addition of glucose and yeast extract, took place. The third stage was the main fermentation stage during which waste oil, glucose, yeast extract, potassium dihydrogen phosphate and magnesium sulfate heptahydrate were added.

Fermentation was conducted under aerobic conditions, continuous agitation and controlled pH and temperature at 4.5 and 30°C respectively for 168 hours.

Rhamnolipids production

Rhamnolipids production took place in three distinct phases using the rhamnolipid-producing bacterium *Pseudomonas aeruginosa*. The first phase was the preparation of the pre-inoculum with the addition of peptone, sodium chloride, di-sodium hydrogen phosphate dodecahydrate and potassium dihydrogen phosphate. At the second phase, the pre-inoculum was transferred in a larger volume of buffered peptone water, in order to form the inoculum. The third phase was the main fermentation processing, where the inoculation to the fermentation vessel, containing the growth medium, was performed. The substrate comprised of waste oil, yeast extract, potassium dihydrogen phosphate, ammonium nitrate and magnesium sulfate heptahydrate. The operating conditions for rhamnolipids production by *P. aeruginosa* were aerobic, with continuous agitation and temperature controlled at 30°C, while the whole cultivation period lasted 96 hours.

Fatty Acid Ethyl Esters (FAEE) production

The process of enzymatic ethanolysis, for the production of FAEE, consisted of three main phases and the formation of MAG, Triacylglycerol (TAG) and Diacylglycerols (DAG). The first phase was the preparation of raw materials with the specified physicochemical characteristics. The type of oil used in this case was waste oil mixture of animal and vegetable origin. In order to facilitate separation of the biocatalyst after

reaction completion, and its subsequent reuse, the immobilized enzyme lipase (from *Candida antarctica*) on polyacrylate beads was utilized. The second phase was the main reaction in which the influent mixture of EtOH:oil, at the optimum ratio, was mixed under mesophilic temperature conditions, followed by the addition of the enzyme. The duration of the reaction phase maximizing FAEE yields was defined to be 8 h. The third phase aimed at the product recovery in which FAEE recovery, biocatalyst regeneration and ethanol recovery took place. The product recovery phase was not part of this LCA survey.

Monoacylglycerol (MAG) production

With enzymatic glycerolysis MAG was produced either via reaction of fatty acids with glycerin, or through reaction of glycerin with triglycerides. The raw materials that were used for the glycerolysis process was industrial glycerol, sesame oil and the enzyme lipase, deriving from *Candida Antarctica*, immobilized on polyacrylate beads. During processing, the influent mixture reacted after the addition of catalyst. The reaction temperature was maintained at 40°C for 24 h. In the last phase, the separation of the produced MAG took place via sedimentation and removal of excess glycerol. The biocatalyst could be regenerated and reused.

System boundaries

In this study, the gate-to-gate system boundaries were set to include only the manufacturing process of biosurfactants and bioplasticizers.

In the case of biosurfactants production, the system boundaries included the pre-

inoculum and inoculum preparation as well as the biosurfactant (sophorolipids and rhamnolipids) production. Only the inputs (e.g. raw materials, energy) and outputs (e.g. emissions) associated with these processes were included within the boundaries. “Cradle” activities (e.g. oil production and purification, transport and storage) and “grave” activities (e.g. use and disposal phase) were not part of this study.

The system boundaries for bioplasticizers production were defined to include the raw materials preparation and mixing up to the reactions completion (FAEE and MAG production). The inputs used for the LCI database were the amounts of raw materials and the energy utilized, while the outputs included the emissions resulting from the processes. As in the case of biosurfactants production, the cradle and grave processes were not part of this study. Schematic diagrams of biosurfactants, FAEE and MAG production system boundaries are presented in Figure 1, 2 and 3.

Key assumptions

Some key assumptions were considered in order to conduct the environmental assessment.

Primary data on biosurfactants and bioplasticizers production were collected from the partners of Bio-SURFEST project, based on the optimum conditions of fermentation and enzymatic processes in pilot scale, since full-scale manufacturing is still under development. Certain elements such as solid wastes, wastewaters etc. were excluded from the study because it was not possible to define an appropriate pathway describing their further treatment or disposal with the current data.

Moreover, resource extraction and use and disposal activities were not included in

the assessment. For all processes, the calculation of the energetic needs and electricity consumption was carried out with the hypothesis that all processes are taking place in Greece in 2013, where electricity is generated mainly from lignite, leading thus to high CO₂ values.

Inventory data sources

Life Cycle Inventory data were determined for each of the products of this study and were obtained either directly from the partners of the Bio-SURFEST project, based on the optimum operating conditions of fermentation and enzymatic processes, or obtained from bibliographical sources.

The Ecoinvent v2.0 database, developed by the Swiss Centre of Life Cycle Inventories and available in SimaPro 7.3.3 software, was used to obtain data on the biogenic emissions of the processes and the electricity production by technology in Greece, based on annual averages. As it regards electricity production and distribution, the system did not take into account the energy transport and transformation, or distribution of energy losses. The energetic mixture considered for electricity production in Greece was lignite (54%), oil (11%) and natural gas (17%), while only 18% was provided by renewables³⁵. The life cycle inventory for each process is shown in detail in Table 1 and 2.

Impact assessment

Life Cycle Impact Assessment (LCIA) is the phase where the results of the inventory analysis are interpreted in terms of the impacts they have on the environment. The

impact assessment of the biosurfactants and bioplasticizers production was based on the internationally accepted Environmental Product Declarations (EPD 2008 V1.03) developed in-line with the International Standard ISO 14025 (Type III Environmental Declarations), and thus the following impact categories were identified: Global Warming Potential (kg CO₂ eq), Ozone Layer Depletion (kg CFC -11 eq), Photochemical Oxidation (kg C₂H₄ eq), Acidification Potential (kg SO₂ eq), Eutrophication (kg PO₄ eq) and non-renewable fossil (MJ eq).

RESULTS AND DISCUSSION

According to the goals of this study, the most pollutant stages during processing of waste oils and fats via the proposed biotechnological processes were identified. Moreover, the overall environmental performance of each process per kg of product produced was quantified per impact category.

Overview of results of the fermentation processes for sophorolipids and rhamnolipids production

In Table 3 and 4 the LCIA results of the fermentation processes for the production of sophorolipid and rhamnolipid biosurfactants, expressed per kg of product produced, are presented. The results indicate that the main environmental impact is due to the fermentation process, mainly because it is more energy intensive, compared to the phases of pre-inoculum and inoculum preparation for both processes. The main inputs for the calculation of the LCIA are the electricity consumption and the biogenic emissions (CO₂) generated by the metabolic activity of the strains. This makes the

fermentation process present an unacceptable environmental compatibility in the assessed boundaries.

Between the two processes, the rhamnolipid production process seems to have better environmental performance than sophorolipids production. Specifically regarding the global warming potential of 100 years, which is a key indicator in LCA studies and quantifies the amount of energy the emissions of 1 ton of a gas will absorb over 100 years, relative to the emissions of 1 ton of CO₂, the relative emissions in the rhamnolipid production case reach the value of 567.49 kg CO₂ eq. compared to sophorolipid production value of 734.68 kg CO₂ eq. This is attributed mainly due to the difference in the time required for their production during fermentation. In particular, the sophorolipids production is a longer process than rhamnolipids and, as a result, is more energy intensive because it requires higher amount of thermal energy in order to maintain the culture medium in steady temperature conditions.

This result is in agreement with a recent study, where a cradle-to-gate approach was developed to assess environmental impacts associated with the fermentation and purification process of acetylated acidic sophorolipid biosurfactants²⁹. Authors concluded that the production phase, including sophorolipids and dispenser production, is mostly responsible for the impact of every category. In addition, around 90% of the impact during the production phase was attributed to fermentation, especially due to the use of rapeseed oil (47%) and glucose (41%), with only 7% deriving from electricity. Large consumption of water for the production of rapeseed oil and glucose leads to most of the damage to ecosystems while most of the impact regarding resource use is caused by glucose and energy production.

Until today the number of LCA studies performed for the production of surfactants is limited, while LCA assessments are mainly focused on their application as detergents and cleaning products^{36–40}. Although there is still not a widely accepted definition for ‘green surfactants’, or else ‘biosurfactants’, for practical reasons, most of the times, biosurfactants are described as substances of biological origin⁴¹. For example, in their study Guilbot et al.³⁷ explored the case of an alkyl polyglucoside (APG), composed of cetearyl glucoside and cetearyl alcohol deriving from palm kernel oil. This surfactant, used as a self-emulsifier in cosmetics, is considered ‘green’ because of its vegetable origin and also due to its manufacturing process complying with the 12 rules of Green Chemistry^{37,42}. Although this study does not assess the production of microbial biosurfactants, valuable information on process hotspots may be obtained out of it that should be taken into account when assessing an LCA study on microbial biosurfactants. As it regards microbial biosurfactants, only one study, as mentioned above, has been published recently describing a cradle-to-grave approach for acetylated acidic sophorolipids where it was concluded that their environmental impact was similar to the one of chemical surfactants deriving from fossil resources²⁹. Therefore, the importance of a life cycle based method for sustainability assessment and development is highlighted⁴³.

Overview of results of the enzymatic processes

The LCIA results of the enzymatic processes for the production of MAG and FAEE, expressed per kg of product produced in each process, are presented in Table 5.

Products’ handling beyond the factory gate, is out of the system boundaries and on the

contrary with the biosurfactants production, in which the biogenic emissions are taken into account in the LCI formation, the physicochemical production of MAG and FAEE has no biogenic emissions which could be taken into account. As a result, the main environmental impact in glycerolysis and ethanolysis in this gate-to-gate analysis, is due to the energy requirements of the processes. As presented in Table 5, the GWP100 of MAG production has been estimated as 180.75 kg CO₂ eq., which is almost three-fold higher compared to the emissions arising by the FAEE manufacturing (59.59 kg CO₂ eq.).

The FAEE production stage takes place at 35°C for 8 h and thus, temperature has to be maintained stable for the whole reaction cycle. As a result, energy intensive equipment is needed for the temperature control of the process. Similarly, during MAG production the temperature has to be maintained at 40°C for 24 h, and as result the process has higher environmental impact than FAEE production. Consequently, the thermal needs of the process have the main effect on the overall process. Additionally, the use of sesame oil in MAG manufacturing has negative effect on the overall environmental process performance since it is a process competing with the food chain. As a result of the selected system boundaries, this parameter was not assessed. On the contrary, the reuse of waste oil mixtures for the production of FAEE will have possibly positive environmental effects, but also in this case the upstream processes, such as production of waste oil, was out of the present systems' boundaries.

Similar to results obtained from the fermentation processes, for rhamnolipids and sophorolipids production, the main hotspot identified in these enzymatic processes is the consumption of energy. The presented LCA was conducted considering the specific

electricity mix used in Greece. However, the electricity mix varies from one country to another. Improvements in energy efficiency, attributed to renewable energy sources such as solar photovoltaic panels, wind and nuclear power are expected to promote decarbonisation of the electricity mix due to the decrease in the share of lignite and hard coal ⁴⁴.

Currently bioplasticizers, alternative to conventional phthalate ones, have been studied regarding their impact on the environment using the LCA approach. In particular, SOFT-N-SAFE LIQ, an acetic acid ester of monoglycerides made from fully regenerated castor oil, was shown to have a lower environmental impact in terms of greenhouse gas emissions, non-renewable resource depletion and water resources ^{30,45}. Life cycle analysis is currently in progress for another biobased plasticizer, POLYSORB® ID37, which is composed of isosorbide diesters produced of vegetable origin fatty acids and isosorbide obtained by simple modification of sorbitol ³¹. Citroflex A-4, or else citric acid ester acetyltri-n-butyl citrate, is a widely used plasticizer and lubricant in food contact polymer applications ⁴⁶. It performs better over dibutyl phthalate in cellulose nitrate films and presents outstanding properties as a plasticizer in vinyl toys while it is characterized by excellent processing options, low toxicity, improved adherence to metals, low volatility and resistance to yellowing ⁴⁷. Till today, however, no LCA study has been reported for its production.

From the above it is clear that bioplasticizers produced from biobased raw materials are an upcoming field of study. Due to their biodegradable nature, low toxicity, low volatility, low migration and high heat stability are expected to outcompete phthalate plasticizers as an eco-sustainable solution. However, LCA studies regarding their

production are still very limited but of crucial importance in order to identify hotspots and potential bottlenecks that will help design and develop greener processes with societal and environmental benefits.

CONCLUSIONS

The study revealed that air emissions, electricity and thermal requirements, during their production, are the key contributors to the potential environmental impacts identified in this LCIA. Among the biosurfactant production processes, the sophorolipids production resulted to 22.7% higher environmental impact compared to the rhamnolipids production. Similarly, FAEE production can be classified as a more environmental friendly process compared to MAG production, resulting to 67% lower environmental impact based on the environmental indicators assessed. Environmental impacts due to energy consumption from all processes studied could be mitigated with the use of renewable energy sources to decrease the environmental footprint. Further analysis on their environmental performance and potential future work could extend the present study to include the entire cradle-to-grave analysis based on data derived from a full-scale production plant.

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FIGURE AND TABLE CAPTIONS

Figure 1. System boundaries for sophorolipids and rhamnolipids production

Figure 2. System boundaries for FAEE production

Figure 3. System boundaries for MAG production

Table 1. Life Cycle Inventory values per kg of sophorolipids and rhamnolipids produced at optimal operating conditions.

Table 2. Life Cycle Inventory, values per kg of FAEE and MAG produced at optimal operating conditions.

Table 3. LCIA results per kg of sophorolipids produced in Gate-to-Gate approach

Table 4. LCIA results per kg of rhamnolipids produced in Gate-to-Gate approach.

Table 5. LCIA results per kg of MAG and FAEE produced in Gate-to-Gate approach.

TABLE 1

<i>Raw Materials</i>	<i>Pre-Inoculum</i>	<i>Inoculum</i>	<i>Fermentation</i>	<i>Product</i>
Glucose (kg)	0.091	0.091	5.055	<i>Sophorolipids</i>
Urea (kg)	0.00091		0.364	
Yeast extract (kg)	0.0091	0.0182	0.455	
Water (L)	0.91	9.1	91	
Waste oil (kg)			0.6825	
Potassium dihydrogen phosphate (kg)			0.091	
Magnesium sulfate heptahydrate (kg)			0.0455	
Energy consumption (kWh)	39.76	122.86	433.25	
Peptone (kg)	12.5	125		<i>Rhamnolipids</i>
Di-potassium hydrogen phosphate (kg)			0.0625	
Pottasium dihydrogen phosphate (kg)	1.875		0.0625	
Yeast extract (kg)			0.625	
Water (L)	1.25	12.5	125	
Glucose (kg)			0.625	
Sodium chloride (kg)	6.25			
Waste oil (kg)			5	
Disodium phosphate dodecahydrate (kg)	11.25			
Ammonium nitrate (kg)			0.0625	
Copper(II) sulfate anhydride (kg)			0.00025	
Magnesium sulfate heptahydrate (kg)			0.001	
Manganese (II) sulphate 1-hydrate (kg)			0.00025	
Energy consumption (kWh)	39.76	98.38	248.57	

TABLE 2

<i>Raw Materials</i>	<i>Reaction phase</i>	<i>Product</i>
Waste oil (kg)	0.82	FAEE
Ethanol (kg)	0.18	
Enzymes (kg)	0.041	
Energy consumption (kWh)	60.6	
Sesame oil (kg)	2.1	MAG
Glycerol (kg)	0.4	
Water (L)	0.017	
Enzymes (kg)	0.105	
Energy consumption (kWh)	182	

TABLE 3

<i>Impact category</i>	<i>Unit</i>	<i>Total</i>	<i>Pre-inoculum</i>	<i>Inoculum</i>	<i>Fermentation</i>
Global warming (GWP100)	kg CO ₂ eq	734.6848584	39.48722555	129.1081318	566.0895011
Ozone layer depletion (ODP)	kg CFC-11 eq	2.61815E-05	1.40718E-06	4.60094E-06	2.01734E-05
Photochemical oxidation	kg C ₂ H ₄ eq	0.223587104	0.012017172	0.039291559	0.172278373
Acidification	kg SO ₂ eq	3.799283957	0.204200727	0.667658314	2.927424915
Eutrophication	kg PO ₄ ⁻³ - eq	0.149048208	0.008010918	0.02619264	0.114844651
Non renewable, fossil	MJ eq	11390.8092	612.2236585	2001.737314	8776.848223

TABLE 4

<i>Impact category</i>	<i>Unit</i>	<i>Total</i>	<i>Pre-inoculum</i>	<i>Inoculum</i>	<i>Fermentation</i>
Global warming (GWP100)	kg CO ₂ eq	567.4951172	39.50576349	97.71167591	430.2776778
Ozone layer depletion (ODP)	kg CFC-11 eq	2.02235E-05	1.40784E-06	3.48209E-06	1.53335E-05
Photochemical oxidation	kg C ₂ H ₄ eq	0.172706146	0.012022814	0.029736656	0.130946676
Acidification	kg SO ₂ eq	2.934693793	0.204296593	0.505297473	2.225099727
Eutrophication	kg PO ₄ ⁻³ - eq	0.115129813	0.008014679	0.019823126	0.087292009
Non renewable, fossil	MJ eq	8798.641384	612.5110772	1514.955758	6671.174549

TABLE 5

<i>Impact category</i>	<i>Unit</i>	<i>Total Impact for FAEE production</i>	<i>Total Impact for MAG production</i>
Global warming (GWP100)	kg CO ₂ eq	59.58836994	180.7514
Ozone layer depletion (ODP)	kg CFC-11 eq	2.12351E-06	6.44E-06
Photochemical oxidation	kg C ₂ H ₄ eq	0.018134567	0.055008
Acidification	kg SO ₂ eq	0.308149996	0.934722
Eutrophication	kg PO ₄ ⁻³ - eq	0.012088911	0.03667
Non renewable, fossil	MJ eq	923.8788293	2802.432



FIG. 1

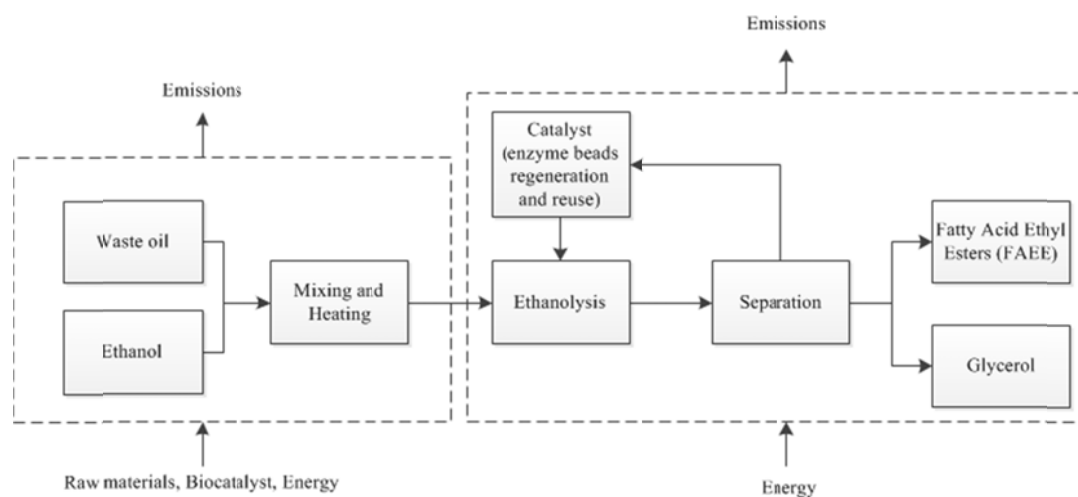


FIG. 2

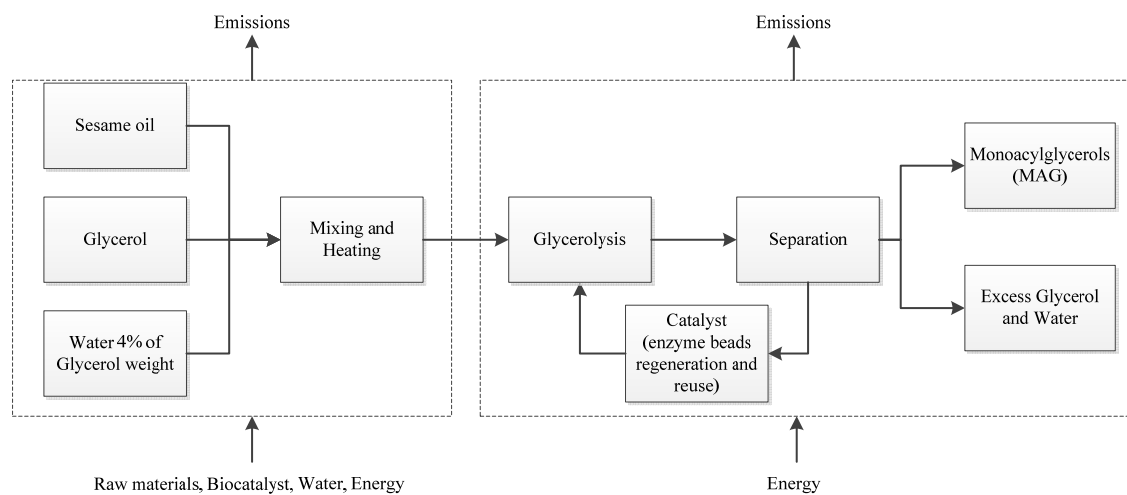


FIG. 3