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Life cycle assessment of biodiesel from estuarine microalgae

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ABSTRACT

Life cycle assessment (LCA) entails the analysis of potential environmental loads and natural resources utilised while manufacturing a product, which helps in sustainable production of biofuel and prudent management of natural resources. A variety of bio resources including microalgae are being explored for its potential as viable alternatives to conventional fossil fuels. This communication presents the lifecycle assessment of biodiesel production from microalgae and valorisation of other value-added products. Comparative assessment of feedstock cultivation was done by considering varied nutrient inputs – (i) no nutrient input (scenario 1), (ii) wastewater as nutrient input (scenario 2) and (iii) fertiliser input (scenario 3). Two different transesterification techniques followed for converting microalgal oil into biodiesel were i) acid catalyst and ii) biocatalyst. Environmental impacts of different scenarios considered were assessed using OpenLCA v1.10.3, which highlights higher eutrophication and photochemical oxidation related emissions for fertiliser input scenario with acid catalyst based transesterification. However, significant reductions in environmental impacts with minimal GHG footprint was observed with wastewater use for cultivating algae and transesterification through biocatalyst. Life cycle assessment of three different scenarios revealed a fossil energy requirement variation between 3.6 and 5.7 MJ/kg and the greenhouse gas emission (as kg equivalent CO_2 emissions) of 0.85–1.46 kg $CO_{2eq}.kg^{-1}$ of biodiesel. This highlights a reduction in fossil energy requirement of about 87.3% in the pilot substrate-based microalgal bioreactor. Wastewater – biocatalyst scenario exhibited a highest net energy ratio (NER) of 18.8 with an additional benefit of low cost remediation of wastewater.

1. Introduction

Rapid industrialisation, demographic transitions, improvements in human living standards, and burgeoning populations are acting as major drivers of escalating energy demand [1]. Dwindling stock of fossil fuels and the growing concerns of increasing GHG footprint has necessitated the exploration of sustainable fuel alternatives to meet the growing demand in the transportation sector and also to mitigate global warming and changes in climate [2]. This has given impetus to the use of renewable energies [3] which is projected to increase by 3.1% every year till 2050 to meet the global liquid fuel demand of >6052 Mtoe [4]. As per the sixth assessment report (AR6) by the Intergovernmental Panel on Climate Change (IPCC 2019), 23% of total CO_2 emissions are from anthropogenic activities, which resulted in 1.0 °C increase in global warming above pre-industrial levels. This rise in temperature is projected to reach 1.5 °C between 2030 and 2052 [5]. Thus, alternate energy options that are capable of simultaneous climate change mitigation and reduction in the dependence on fossil resources are quintessential. Biomass based feedstocks are being investigated as potential alternative to conventional energy sources. Biomass based bio energies are increasingly seen as low-carbon, distributed and renewable component of national energy resources [6] and are being investigated as potential alternatives to conventional energy sources. Bioenergy especially in the form of liquid biofuels is gaining attention at both national as well as global levels, which is evident from the recent biofuel policies promoting renewable energy sources towards clean energy. In this context, the National Energy Policy (NEP) drafted by NITI Aayog ((translated as "National Institution for Transforming India") – a policy think tank

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established by the Government of India in 2015 with an aim to achieve sustainable development goals by establishing cooperative federalism between the centre and the states) promotes the share of biofuel among other renewables and has set an indicative target of 20% ethanol blending in gasoline and 5% biodiesel blending in diesel [7]. The subsequent National Policy on Biofuels (2018, Government of India) aims at taking forward the indicative target of achieving 20% blending of biofuels with fossil-based fuels by 2030.

Biofuels are being extracted from sources that are of biological origin such as new or used vegetable oils and animal fats [8]. Evolution of biofuels is witnessed with the first generation biofuels from crops such as sugar beet (*Beta vulgaris*), cassava (*Manihot esculenta*), soybeans (*Glycine* max), wheat (*Triticum aestivum*), corn (*Zea mays*) and rapeseeds (*Brassica napus*). However, the conflict of food versus fuel, gave way to the exploration of biofuels from lignocellulosic residues (left-overs of agriculture) that are termed as second generation biofuels [9]. Then, the focus shifted to third generation feedstocks i.e. aquatic biomasses (microalgae and seaweeds) to minimise the possible shortage of land and fodder. Over the last ten years, there has been increased research efforts globally towards biofuel production. In India, large scale bioethanol (about 3 billion litres) is being produced using sugarcane molasses. India has emerged as the world's largest sugar producer (2019), overriding Brazil with 33 million metric tons (19% of global production of 180 million metric tons).

India's biodiesel production for the year 2019 was 190 million litres with installed production capacity varying between 11 million litres to 280 million litres. Indian biodiesel producers are using non-edible industrial oil, used cooking oil (UCO), animal fats and other tree-borne oils [10] as renewable feedstocks. Among other biomass energy feedstocks, the microalgae has emerged as a promising third generation feedstock with higher lipid yields and scope for higher CO_2 sequestration and ability to grow on non-arable lands [11]. Efforts of optimising process technologies that are useful in the conversion of algal biomass into biofuel and other bio-products are in progress to achieve commercial viability of microalgal biofuels. The success of alternate energy resources depends on the technical feasibility, economic viability, environmental soundness with the societal acceptance [12], which is assessed by assessing the energetics of the process and potential environmental impacts posed by each of the biofuel processes to the environment in a long run [11]. Table 1 lists the advantages and disadvantages of biodiesel and conventional diesel.

Life cycle assessment (LCA) has emerged as a fundamental tool to understand the relative environmental performance of various processing technologies at the systems level. LCA is being widely accepted, for computing GHG footprint of biomass based energies. However, given the variety of processes that are involved in bioenergy production processes, the GHG footprint can vary significantly for apparently very similar systems [13].

Table 1
Advantages of biodiesel over conventional fossil diesel.

Diesel type	Advantages	Disadvantages
Petroleum diesel	Less expensive than biodiesel owing to its reduced production costs	Takes millions of years to fossilise as particulate matter emissions ($PM_{2.5}$) of crude oil are high in conventional diesel, thus exhibiting higher carcinogenic toxicity during combustion.
Biodiesel	CO_2 sequestration is much higher than emission Quicker cycling time (5–7 days) (easy renewability) No arable land requirement for feedstock growth No human carcinogenic emissions such as organic hydrocarbons recorded during biodiesel combustion	Higher production costs compared to fossil diesel

1.1. Lifecycle assessment

LCA entails examining the environmental footprint of a product right from raw material/resource extraction stage to the final product and use of the product until its final disposal, thus encompassing the entire product system life cycle [12]. LCA quantifies and assesses all energy inputs and related outputs as environmental burdens of a product during its entire life cycle, thus enabling comprehensive comparisons of available technological options [14] to make informed decisions. LCA framework has been accepted internationally with the well-established best practices through environmental system standard - ISO 14040 and ISO 14044 for evaluating requirements and impact technologies, processes and products [15]. LCA is used to analyse the environmental impacts of a process, system and product as a whole evaluated for the entire product's lifetime. It involves four main phases: i) defining goal and scope; ii) compilation of life cycle inventory (LCI); iii) life cycle impact assessment (LCIA) and iv) interpretation [16]. The main reason in performing LCA is to compare and comprehensively evaluate the environmental footprint and societal aspects that are likely to be created by a product or service which enables possible comparisons with scope for choosing environmentally sound alternatives [13]. The various stages involved in LCA assessment of a product is given in Fig. 1. The scope of LCA can vary in terms of the adopted methods. A cradle-to-grave is regarded as a complete life cycle assessment starting from resource extraction (cradle) to the disposal phase (grave), whereas cradle-to-gate is a partial product life cycle that covers resource extraction (cradle) to the factory gate (excluding its use and disposal related emissions) [17]. From an energy (fuel) production perspective, these terms are modified as well-to-wheels and well-to-pump LCA. Well to wheel accounts for all the energy and emissions necessary to produce the fuel (well to pump) in addition to the operational energy and emissions associated with vehicle technologies considering emissions related to engine efficiency and tail-pipe [18].

1.2. LCA of various bio-energies

Biomass is a renewable energy resource that accounts for approximately 33% of the total energy needs of a developing nation [19]. Sustainability of biofuel produced from biomass based bioresources depends on i) precise assessment of feedstock (yield, etc.), ii) choosing technically feasible and cost-effective production processes and iii) environmentally sound conversion processes to minimise possible environmental implications. LCA involves assessing environmental loading from raw materials to the final product (cradle to gate). An integrated energy strategy implementation entails energy efficiency

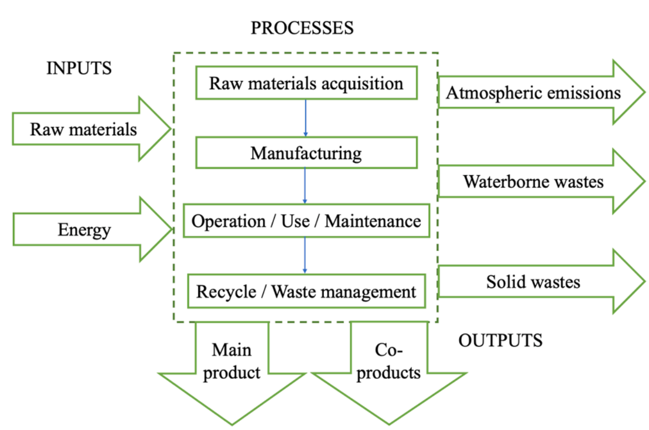


Fig. 1. Stages involved in LCA assessment process of a product [12].

improvement of end-use devices/equipment, optimisation of energy resources, maximisation of renewable resource usage, a well-balanced exploitation of biomass energy resources and reduction in the use of depletable resources [20]. As the biochemical composition of different biomasses are unique with variations in inherent properties, which are essentially influenced by variability in agroclimatic conditions, where the feedstock is grown, it is imperative to understand the pros and cons and the level of environmental implications of utilising the chosen biomass for bioenergy generation. For example, energetics analysis of paddy crop have shown an energy requirement of 32.14 GJ/ha for performing various agricultural activities including tilling to threshing [21]. Energy planning of any region should include climate and geographic factors apart from considering existing levels of energy consumption [22]. In terrestrial plant biomass based bioenergy production system, the cultivation stage is known to contribute to significant levels of GHG emission due to the use of energy intensive processes involving land preparation, sowing, harvest and transportation of feedstocks. Application of fertilisers and pesticides during cultivation stage also contribute significant levels of GHG emissions. Reports have shown higher GHG emissions during cultivation stage of sugarcane, released as a result of altered land-use, fuel and agro-chemical consumption. Bioelectricity and bioethanol are the two forms of bioenergy possible from sugarcane. A comparative environmental impact assessment through cradle to gate considering four different product routes from sugarcane bagasse demonstrated sugar production coupled with ethanol and biogas production to be beneficial from an environmental perspective [23]. LCA of bioethanol production from sugarcane bagasse using three alternative processes of separate hydrolysis and fermentation (process 1), simultaneous saccharification and fermentation (process 2) and Fischer Tropsch gasification (process 3) resulted in a higher energy efficiency (42.3%) in process 2, while depicting least potential for environmental impact in process 3 [24]. Stochastic environmental and economic assessment of an integrated sugarcane bioethanol and soyabean biodiesel production system showed economic feasibility in addition to reduced GHG emissions (18.6 gCO_{2e}/MJ_F) when compared to conventional sugarcane-to-ethanol process [25]. A comprehensive life cycle assessment carried out on Jatropha biodiesel production from the feedstocks grown under Indian agro-climatic conditions, considering irrigated and rain-fed scenarios, reveal GHG emission reduction of 40% to 107%, depending on the methods used for energy and emission distribution and irrigation [26].

1.3. LCA of microalgal biofuels

The third generation biofuel production system using microalgae has witnessed a variety of conversion technologies in an attempt towards large scale commercialisation. The conventional microalgae production system includes i) cultivation; ii) harvesting; iii) pre-treatment; iv) transesterification; v) purification. Microalgae cultivation utilises open ponds [27,28] or enclosed photobioreactors [29], while recent interests are towards attached biofilm cultivation [30,31]. Once the microalgae are grown, the cells are harvested using different cell concentration techniques such as centrifugation, gravity sedimentation, filtration, floatation and flocculation [32], which are energy intensive techniques requiring higher energy. Pre-treatment includes various cell disruption techniques such as sonication, bead beating, autoclaving, microwave treatment and pulsed electric field [33]. After pre-treatment, the cells are subjected to transesterification using acid, alkali, biocatalyst or supercritical CO_2 as catalysts to enhance the rate of reaction [34]. The final step of the biodiesel production process is the purification. Crude biodiesel containing traces of catalyst and crude glycerol are usually purified using water washing or dry washing technologies [35]. Life cycle and energy balance assessments of microalgae based biodiesel production is essential to assess the impacts of the processes which includes upstream (microalgal cultivation) and downstream (biofuel production) processes. Earlier studies on LCA of microalgal biofuels focussed on both qualitative analysis of different methodological aspects including system boundaries and co-product allocation [36] and comparison of the impacts with fossil fuel and other biofuels. In this regard, an earlier study [37] reviewed the environmental performance of microalgal biofuel over conventional fossil fuel as well as bioethanol, biodiesel derived from other terrestrial feedstocks and concluded that microalgae derived biodiesel was far more efficient than other terrestrial feedstocks in terms of reducing the land-use impacts. However when energy efficiencies of different biofuels are compared, other biofuels/fossil fuel out-perform algal biofuels, suggesting optimisation and careful choice of microalgae production pathways to improve energetic and environmental performance. The microalgal technologies that have been explored so far for downstream processing include: a) thermochemical and b) biochemical pathways. The thermochemical technologies include pyrolysis and hydrothermal liquefaction (HTL). The thermochemical conversion technologies result in energy rich biocrude, which is further transformed to a range of energy products. A more targeted approach of energy generation from microalgae is through lipid extraction and subsequent biodiesel production. Various physico-chemical methods of lipid extraction including grinding, sonication, bead-beating, organic solvent extraction, lyophilisation were experimented so far with combinations of different catalysts (acid, alkali, enzyme, supercritical CO_2). Earlier studies on LCA assessment of microalgae have mainly focussed on enhancing the biomass productivity by defining system boundaries until cultivation, while other studies have laid system boundary towards entire biofuel production process (including upstream and downstream). Life cycle analysis of microalgal cultivation system (photobioreactor) under artificial and natural light conditions demonstrated better energy balances and lesser environmental impacts for microalgae grown under natural light conditions [38]. Environmental assessment of four different microalgae to biofuel production processes leveraging GREET model resulted in an NER that ranged between 0.6 and 1.03 MJ/MJ of biofuel produced and associated GHG emissions varied between -46.5 and 496.7 g CO_{2e} MJ^{-1} with highest GHG emission was observed for transesterification using super critical CO_2 [39]. Net energy and GHG emission evaluation of biodiesel derived from marine microalgae *Nannochloropsis* sp. using a photobioreactor using GREET model exhibited a net energy of 0.93 MJ of energy consumed MJ^{-1} of energy produced with a CO_{2e} emission of 75 g per MJ of biodiesel produced [40]. A comparative life cycle assessment of microalgal biomass production while using different types of photobioreactors for algal cultivation resulted in a net energy ratio (NER) < 1 for tubular photobioreactor, and raceway ponds, while NER was found to be greater than one for flat-plate photobioreactors, [41]. NER is the ratio between the energy output of the biofuel and the overall energy input. If NER is less than one, then it represents an energetically unfavourable system as the energy required to produce the biofuel is greater than the energy contained in the biofuel [37]. The energy return on investment of a microalgal biocrude production facility through investigations of different cases (experimental, reduced input and highly productive), varied between 0.074 and 0.35 respectively [42]. Life cycle energy and GHG analysis performed for the microalgae *Chlorella vulgaris* in open raceway ponds resulted in 2.5 times as energy intensive as conventional diesel which highlights the necessity for decarbonisation in every step of the full production chain in order to realise the inherent environmental advantages of GHG emissions reduction through biomass [43]. Life cycle assessment of the effects of three different cell pre-treatment techniques on the marine microalga *Isochrysis galbana* revealed an increase in GHG emissions as a result of pre-treatment and suggests co-use of different pre-treatment processes to reduce the environmental impacts and to enhance energy efficiency [44]. Similarly, life cycle assessment of microalgae-based hydrotreated pyrolysis aviation fuel demonstrated the mass ratio of the pyrolysis biocrude to be the decisive factor in determining the environmental burden posed by different environmental processes [45]. The stochastic life cycle assessment considering the energy use and greenhouse gas emissions involved in upstream cultivation of microalgae demonstrated the advantage of using wastewaters from different origins for algal cultivation with the significant reduction in environmental burdens [46] and this did not include biofuel production (downstream processing) during the evaluation of environmental life cycle impact. Thus, performing a coherent LCA process of microalgae to biodiesel process requires a detailed modelling of each feedstock processing stages (growth, harvest, lipid extraction and biodiesel production) combined with a standard and consistent set of LCA boundary conditions. Despite having multiple process pathways, algae-based bioenergy products are facing multiple economic and environmental concerns. An LCA and environmental impact assessment study that compared the oil production from microalgae with other terrestrial oilseed crops/fossil fuels concluded that microalgae are competitive neither with oilseeds derived diesel nor with fossil fuels and suggest optimisation of the production chain and valorisation of value-added co-products for improving the overall energetics of microalgal biofuel [47]. Thus, to improve the economic and energetic performances of microalgal biofuels, recent research focus is towards algae based biorefineries. Biorefineries are integrated network of biomass processing facilities that converts biomass into various marketable products/energies through optimised use of resources and waste minimisation thereby maximising benefits and profitability [48]. An earlier study that reviewed numerous studies on technological and economic assessments of algal biofinery suggested a cascading principle to prioritise the production of high-value products before the extraction of energy [49]. Biorefineries are a nascent field of research that requires harmonisation of economic and environmental assessments in order to compare the tensions and trade-offs between different dimensions of sustainability [50].

The key to sustainable microalgal biofuel production lies in minimising the energy inputs as much as possible while enhancing the biomass productivity and energy output. Thus the focus of the present study was to evaluate the life cycle and environmental impacts of a substrate-based microalgal bioreactor when different nutrient sources were considered for cultivation of microalgae. This study unlike other life cycle assessment studies on microalgae intend to cover the complete energetic and environmental performance aspects of microalgal biofuel starting with feedstock cultivation till biodiesel production. Fig. 2 gives the detailed schematic illustration of different technological processes considered for life cycle and environmental impact assessment.

2. Materials and methods

2.1. Goal scope and definition

The environmental assessment presented in this study was performed using a cradle to gate LCA approach considering every process in the lifecycle starting from land preparation, microalgal cultivation to end-product production accounting of energy and emissions footprints.

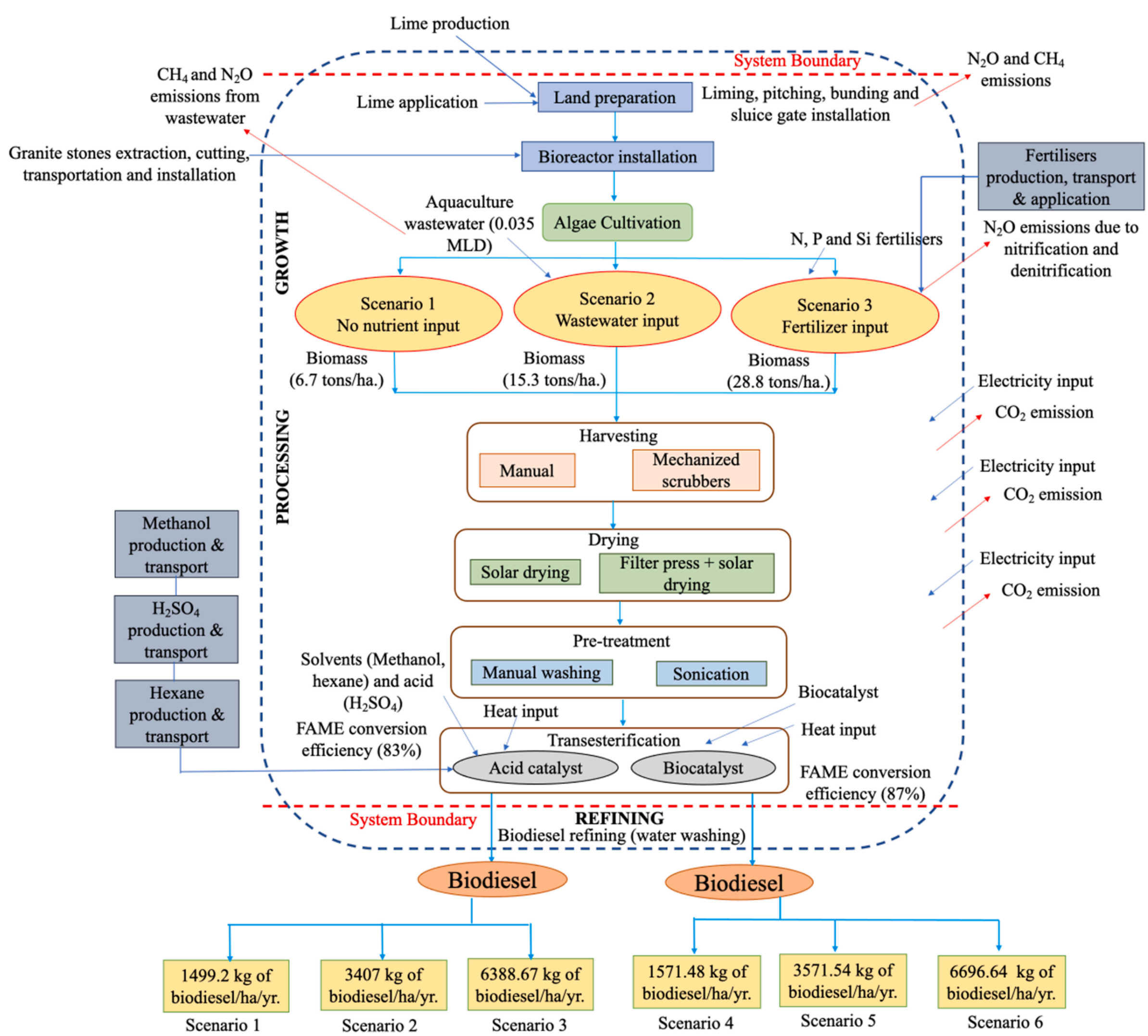


Fig. 2. Process-wise material input and associated emissions breakup for three different cultivation scenarios.

The functional unit for LCA was defined as the biomass achievable in one-hectare area in the flood plains along the coastlines of Karnataka for three different nutrient input scenarios i.e. i) without any external nutrient inputs, (ii) in wastewater (gives an additional scope to assess bioremediation potential with biofuel production) and (iii) with external inputs – synthetic fertiliser. The energy expended and kg CO_2 equivalent emissions were then computed for 1 kg of biodiesel basis (for comparison with published literatures). The microalgal biomass productivity considered for each of the scenario is given in Table 2.

2.2. Life cycle inventory (LCI)

The LCI plays a significant role in life cycle assessment by acting as a basis for life cycle impact assessment as well as economic analysis. The LCI associated with the GHG emission rates for producing microalgal biofuels was estimated following the IPCC guidelines (2006) for National Greenhouse Gas Inventories. The CO_2 equivalent emissions related to bioreactor (from land preparation activities such as pitching, bund formation, lime applications, etc.) were calculated considering the CH_4 and N_2O emissions.

The CH_4 and N_2O emissions were converted to its CO_2 equivalent values (considering respective gases' global warming potential (GWP) equivalent factors: GWP of 1, 32 and 298 for CO_2, CH_4, and N_2O respectively for 100-year time horizon [53]. The LCI data required for impact assessment involves the inputs and outputs of the processes involved directly in biofuel production as well as the accessory materials and process energies spent on important pre-requisites such as diesel, electricity, production of fertilisers (ammonium nitrate (AN), mono-ammonium phosphate (MAP) and Sodium silicate), chemicals (methanol, sulphuric acid and hexane), distilled water, granite stones, crushed lime were sourced from Indian and international databases (only when data is unavailable for Indian conditions). Table 3 lists the details of various processes and subprocesses considered for the generation of life cycle inventory of the substrate based microalgal biofuel production system.

2.3. Lifecycle energy analysis (LCEA)

The methods followed to quantify the energy expenditure incurred in different unit processes in the microalgal bioreactor are detailed in the following sub-sections.

2.3.1. Bioreactor design and implementation

A pilot scale bioreactor was designed for microalgal feedstock growth utilising granite stones having flat surface area as substrates and the process involved in feedstock cultivation to biodiesel production is considered for LCA analysis. The estimated energy consumption and associated GHG emissions (in terms of CO_{2e}) during land preparation and bioreactor installation for a functional unit of 1 ha is given in Table 4. The field bioreactor was assumed to be functional for 224 days excluding monsoon as the field conditions are not suitable for algal growth during monsoon due to low salinity. The stones were put onto a platform made of PVC mesh (4 ft. × 3.5 ft.) supported with a frame made up of PVC pipes immersed in water of the flood plains. The microalgae were allowed to naturally self-seed with no introduction of any external inoculum thus having a diverse polyculture in its biomass composition.

Table 2
Scenario-wise biomass productivity assumptions.

Scenario	Productivity ($g/m^2/d$)	Reference
Scenario 1	1.24	Experimental findings from field investigation
Scenario 2	8	[51]
Scenario 3	15	[52]

Microscopic examinations of cells that were adhered to the introduced substrata (granite stones) revealed the dominance of diatoms *Mastogloia* sp., *Epithema* sp. and *Navicula* sp., evident by its higher cell density. The growth cycle was estimated to be 5–7 days before it reached cell saturation on the substrata omitting the initial colonisation period of 2 weeks.

2.3.2. Growth, harvesting and processing of feedstock

Diatoms dominated other microalgae during cultivation and similar situation prevails with respect to species composition along the coastlines of Karnataka, India. Harvesting and downstream processing was carried out through manual harvesting and mechanised scrubbing in order to remove the attached microalgal cells at the end of each growth cycle (5–7 days). Drying of the algal biomass was done through i) direct solar drying; ii) filter press and subsequent solar drying followed by pre-treatment techniques of manual washing and sonication. Direct transesterification of dried algal biomass using acid (2% H_2SO_4) - and biocatalyst (fungi extracted extracellular lipase [66]) with FAME conversion efficiencies of 83–87% (based on conversion efficiencies of lab-scale experiments) was assumed for this study. GHG emissions during the feedstock cultivation varies based on nutrients inputs for microalgal growth. As solar energy was considered as the source of illumination, there was no conventional energy requirement during cultivation stage. The energy required at the time of harvesting and different downstream processing considered for each scenario was estimated by taking into account of the electricity (kWh) for usage of electrical appliances (as per manufacturer's specification) such as mechanised scrubbers, filter press, ultra-sonicator, fermenter and heat block (for transesterification) and the hours of operations of electricity used. This electricity consumption (kWh) value was converted into its corresponding energy (MJ). The energy required as heat for acid catalyst transesterification during lab-scale experiments, was used for energy estimation of the transesterification process during the life cycle. Tables 5 and 6 lists the scenario-wise energy usage with the environmental loadings (GHG emissions) for each stage and transesterification through acid and biocatalyst respectively.

2.3.3. Net energy ratio

Net energy ratio (NER) of microalgal biodiesel production is the ratio of energy output to direct energy input during different processes such as cultivation, harvesting, pre-treatment and transesterification (through acid or biocatalyst) as given in Eq. (1). The energy outputs include biodiesel and other co-products (such as biogas, glycerol etc.).

$$\text{Net energy ratio} = \frac{\sum \text{Energy output}}{\sum \text{Energy input}} \tag{1}$$

NER value greater than one indicates of an energetically favourable system.

2.3.4. GHG emission savings

Percentage reduction in environmental burden (or reduction in CO_{2e} emissions) (expressed as direct savings) while using biodiesel in the place of conventional fossil based diesel was calculated using the Eq. (2).

$$\text{Savings} = \left(\frac{FD - BD}{FD}\right) \times 100 \tag{2}$$

where, FD is the environmental burden posed by the fossil diesel and BD is the environmental burden caused by the proposed biodiesel production process.

2.4. Estimation of GHG emissions

Environmental loadings through GHG footprint (kg CO_{2e}) in the three input (no nutrient, wastewater and fertiliser input) scenarios was assessed to understand the variations in environmental loadings with

Table 3
LCA assessment of substrate-based microalgae production considering different processes involved.

Main Processes	Linked processes/ scenarios	Linked subprocesses	Material inputs	Energy Involved (MJ/ha)	CO_2 equivalent emissions (kg/ha)	Notes	References
Land Preparation	Pitching	Manual labour	3 days of labour (5 persons)	130.8	15.6	3 days labour involving 5 persons	[54]
	Lime ($CaCO_3$) application	Lime production	900 kg/ha of calcium carbonate required per ha.	2718	1170.9	3.02 MJ energy required/kg of lime (1.301 kg CO_2 emitted per kg of lime produced)	[55,56]
		Emissions from field application	900 kg of lime (225 kg/year)	–	108	0.12 t C is emitted/ton of lime (IPCC 2006)	[57]
		Manual labour	Field application of lime	34.88	5.2	5 persons for 1 day of labour - lime application	Data collected through personal interviews with shrimp farmers and counter-verified with wage rates of MNREGA Karnataka 2018.
	Bund formation	Manual labour	2 days of labour (3 people)	52.32	6.24	2 days labour for 3 persons work by installing laterite stone bricks around the field	
Bioreactor Installation	Granite stones	extraction/ cutting	Granite stones (~47.7 tons)	820.08	32.2		Extrapolated as per field studies. [58]
		Transport and Installation	truck transport and manual labour	147.6	9.3	4 L diesel is required to transport granite stone to the destination (4*36.9 MJ/L) of diesel	
Feedstock growth	Nutrient source	No nutrient input	–	–	–	–	
		Wastewater	Wastewater	–	114.86	CH_4 emissions due to discharge of wastewater in estuaries is 0.048 kg CH_4/kg COD and 0.019 N_2O/kg of N	[59]
		Fertiliser application	N: 2676 kg/ha/ y., P: 468.16 kg/ ha/y., Si: 1739 kg/ha/y.	–	6692	N_2O emitted as a result of nitrification and denitrification are 129.59 kg N_2O/ha. (N_2O direct and indirect) (calculated as per IPCC 2019).	[60-62]
Harvesting	Manual harvesting	Manual labour	–	558.08	66.56	A person breathes out an average of 1.04 kg CO_2 per day.	[55]
	Mechanical scrubbers	Electricity input	One person operating the scrubber for 8 hrs	46.08	9.1	12.8 kWh or 46.08 MJ of electricity is required for algae scrubber (8 h of operation)	From manufacturer's specification
		Spare replacement	–	–	–	–	
Drying	Solar drying	Energy input	–	–	–	–	
	Filter press		Electricity	71.28	14	–	[63,64]
	Oven drying			9726	1910	–	
Pre-treatment	Manual washing	–	–	–	–	–	
	Sonication	Energy input	Electricity	180	35.4	–	
Transesterification	Acid catalyst	Methanol (kg)	155.43	5206.9	1022	The volume of reactants and catalyst were taken based on optimisation experiments	50 kg/ton of methanol [63]
		Sulphuric acid (kg)	7.42	12.61	2.5		
		Hexane (kg)	66.23	33.1	6.5		
	Biocatalyst	Methanol (kg)	155.43	5206.9	1022		
		Biocatalyst (kg)	202.8	138.24	38.4	55% (w/w) of crude biocatalyst was considered for the analysis	5–30% w/w of lipase [65]
Biodiesel recovery/ purification	Water washing	Distilled water (kg)	153	330.48	1529	Water is required in the ratio of 1:10 to that of biodiesel	[35]

respect to type and quantum of nutrient inputs.

2.4.1. *Emissions from wastewater*

The sustained inflow of untreated wastewater to aquatic environment emits GHGs such as CO_2, CH_4 and N_2O during degradation of organic fractions. Higher GHG emissions are reported when organic matter is discharged into the waterbodies with water stagnation in nutrient-rich hypoxic waters such as eutrophic lakes, estuaries and rivers [67]. Emissions (in CH_4 and N_2O) due to the aquaculture wastewater use in the bioreactor for microalgae cultivation were calculated by accounting COD and TN (in kg) of aquaculture wastewater used during a year (224 days of facility operation) with CH_4 and N_2O emission factors

Table 4
Estimated energy and GHG emissions incurred during bioreactor installation.

Processes	Process Variables	Scenario 1–3		
		Material/Energy Input	Energy Expenditure (MJ/ha)	kg equivalent CO_2 emissions/ha
Land preparation	Pitching, bund, sluice gate formation and lime application	Manual labour, diesel consumption	843.35	166
Bioreactor installation	Granite stone extraction, production, transport and installation	Bioreactor construction material/manual labour/diesel consumption for JCB	2403	472

Table 5
Estimated energy and associated environmental loadings in acid catalyst based transesterification.

Processes	Process Variables	Scenario 1		Scenario 2		Scenario 3	
		Energy Expenditure (MJ/ha)		Energy Expenditure (MJ/ha)		Energy Expenditure (MJ/ha)	
Feedstock Growth	Nutrient input (wastewater/fertiliser)	–	–	–	–	–	–
Harvesting	Manual harvesting/Mechanised scrubbers	327.04[a]	1753[b]	327.04[a]	3984.3[b]	558.08[a]	7470[b]
Drying	Solar drying/Filter press + solar drying	–	460.1[c]	–	1045.6[c]	–	1960.5[c]
Pre-treatment	manual washing and sonication	248.16	248.1	564.03	564.03	1057.5	1057.5
Acid Catalysed Transesterification	Reaction	1602	1602	2160	2160	3060	3060
Biodiesel purification	Water washing	330.4	330.4	738	738	1152	1152
Processes	Process Variables	Scenario 1		Scenario 2		Scenario 3	
		GHG emissions (kg CO_{2e}/ha)		GHG emissions (kg CO_{2e}/ha)		GHG emissions (kg CO_{2e}/ha)	
Feedstock Growth	Nutrient input (wastewater/fertiliser)	–	–	869.16	869.16	6465.7	6465.7
Harvesting	Manual harvesting/Mechanised scrubbers	66.56	344	66.56	783	66.56	1467
Drying	Solar drying/Filter press + solar drying	–	90.4	–	205	–	385
Pre-treatment	manual washing and sonication	48.7	48.7	109	109	208	208
Acid Catalysed Transesterification	Reaction	315	315	424	424	601	601
Biodiesel purification	Water washing	64.9	64.9	145	145	226	226

a – Manual harvesting; b – mechanised scrubbers; c – filter press + solar drying.

Table 6
Estimated energy and associated environmental loadings in biocatalyst based transesterification.

Processes	Process Variables	Scenario 1		Scenario 2		Scenario 3	
		Energy Expenditure (MJ/ha)		Energy Expenditure (MJ/ha)		Energy Expenditure (MJ/ha)	
Feedstock Growth	Nutrient input (wastewater/fertiliser)	–	–	–	–	–	
Harvesting	Manual harvesting/Mechanised scrubbers	558.08[a]	1753[b]	558.08[a]	3984.3[b]	558.08[a]	7470[b]
Drying	Solar drying/Filter press + Solar drying	–	460.11[c]	–	1045.62[c]	–	1960.52[c]
Pre-treatment	manual washing and sonication	248.16	248.16	564.03	564.03	1057.5	1057.5
Acid Catalysed Transesterification	Reaction	164.4	164.4	373.7	373.7	700.7	700.7
Biodiesel purification	Water washing	330.5	330.5	738	738	1152	1152
Processes	Process Variables	Scenario 1		Scenario 2		Scenario 3	
		GHG emissions (kg CO_{2e}/ha)		GHG emissions (kg CO_{2e}/ha)		GHG emissions (kg CO_{2e}/ha)	
Feedstock Growth	Nutrient input (wastewater/fertiliser)	–	–	869.16	869.16	6465.7	6465.7
Harvesting	Manual harvesting/Mechanised scrubbers	66.56	344	66.56	783	66.56	1467
Drying	Solar drying/Filter press + solar drying	–	90.4	–	205	–	385
Pre-treatment	manual washing and sonication	48.7	48.7	109	109	208	208
Acid Catalysed Transesterification	Reaction	32.3	32.3	73.4	73.4	137.9	137.9
Biodiesel purification	Water washing	64.9	64.9	145	145	226	226

a – Manual harvesting; b – mechanised scrubbers; c – filter press + solar drying.

(as per IPCC 2019 refinement to the 2006 IPCC guidelines (Chapter 6)). The operational parameters considered for GHG emissions (kg CO_2 equivalents) from wastewater is given in the Table 7.

Table 7
Operational parameters of wastewater considered for GHG emission calculation.

Wastewater	Values	Reference
Flow rate (MLD)	0.035	Effluent discharge rate of commercial shrimp cultivation facility
COD (kg d^{-1})	0.40	[68]
TN (kg d^{-1})	0.60	[69]
CH_4 emission factor	0.048 kg CH_4/kg COD	IPCC 2019
N_2O emission factor	0.019 kg N_2O/kg TN	IPCC 2019
GWP of CH_4	25	IPCC 2006
GWP of N_2O	298	IPCC 2006

2.4.2. *Emissions due to N fertiliser*

GHG emissions due to fertiliser application in feedstock cultivation was calculated for Indian conditions based on the IPCC 2019 refinements to 2006 methods [67,70] IPCC 2006 guidelines) as listed in Table 8.

2.4.3. *N_2O emissions from fertiliser application*

N_2O is a greenhouse gas with global warming potential (GWP) of 298 kg $CO_{2eq}.kg^{-1}$ for 100 years period [60]. The nitrogen present in

Table 8
Emission factors for synthetic N fertiliser production used in this study.

N fertiliser product	Emission factor (kg $CO_{2eq}.kg^{-1}$N)	Reference
Ammonium nitrate	7.03	[70]
Mono ammonium phosphate	6.39	[71]

fertilisers gets released into the atmosphere as nitrous oxide (N_2O) on application due to nitrification and denitrification that are mainly triggered with N applications. The emission of N_2O can be of direct and indirect forms. Earlier studies [60] have shown both direct and indirect N_2O emissions from microalgal culture ponds during anoxic night time conditions. Direct N_2O emission depends on the amount of synthetic fertilisers and/or organic manure applied and is calculated based on the assumption that 0.003% of added nitrogen gets emitted as N_2O [55,60] as per Eq. (3). Indirect N_2O emissions were calculated by taking into account of the amount of nitrogen lost through leaching and volatilization [72] (Eq. (4)).

$$N_2O\ (\text{direct}) = \ \text{FSN} \ \times \ \text{EFN} \ \times \left(\frac{44}{28}\right) \ \text{kg N2O/ha} \tag{3}$$

where, FSN = Amount of synthetic fertiliser applied (kg N/ha); EFN = IPCC emission factor for added nitrogen (0.0298 kg N_2O-N/kg N).

Indirect N_2O is considered as a long-term fate of the nitrogen fertiliser. The fertilisers indirectly generate volatile N_2O due to microbial nitrification and denitrification.

$$N_2O\ (\text{indirect}) = (\text{FL} \times \text{EFL}) \times 44/28\ \text{kg N2O/ha} \tag{4}$$

where, FL = amount of N lost through leaching and NH_3 volatilisation (assumed as per [72]); EFL = IPCC emission factor for leached N (0.0075 kg N_2O-N/ha).

2.4.4. Emissions during downstream operations

Emissions due to the electrical power consumption during downstream operations was converted into its carbon equivalencies as kg equivalent CO_2 emissions as per IPCC 2006 guidelines for National Greenhouse gas emissions inventories [73].

2.5. Life cycle impact assessment

Sustainable development depends mainly on health of the renewable abiotic resources such as air, water, soil [74] and hence it is imperative to understand the impacts that would be posed to these abiotic factors as a result of the implementation of bioenergy projects. Lifecycle impact assessment (LCIA) aids in evaluating the potential impacts of a product/product system on the environment [17,75]. The four critical elements of LCIA are (i) classification, (ii) characterisation, (iii) normalisation and (iv) weighting. OpenLCA 1.10.3 software with Ecoinvent® 3.6 (academic free license version for non-OECD countries) database has been used for LCIA of biofuel from microalgae. Although biogenic CO_2 emissions were accounted in energy expenditure estimations, it is normally excluded from the inventory for estimating the life cycle impacts. Resource consumptions and various emissions related environmental impacts were accounted to evaluate the lifecycle impact categories. The functional unit considered for performing LCIA was taken as 1 ton of biodiesel manufactured under different scenarios. The impact categories relevant for microalgae-based biofuel production are as per CML – IA (developed by the Institute of Environmental sciences, Leiden University, The Netherlands) baseline model which are:

- Abiotic depletion: It refers to the depletion of non-renewable natural resources and expressed in kg of Antimony equivalents (kg Sb-eq.).
- Abiotic resource depletion (fossil fuel): This refers to non-renewable resource consumption especially fossil fuels and is a measure of scarcity of a substance. In this study, this impact category is related to the depletion of fossil diesel, coal due to the usage of conventional electricity, mineral acid such as sulphuric acid, and petroleum derivatives such as methanol and n-hexane in the microalgal biofuel production system. The consumption of each of these abiotic resources is aggregated with the characterisation factors expressed in terms of antimony equivalent (as per ISO 14042 LCA considered in CML – IA baseline method).
- Acidification: Acidification potential (AP) refers to the loss of base nutrients such as magnesium, calcium and potassium through leaching due to the increase in concentrations of acidic elements (hydrogen and aluminium). It is an air pollution index expressed as kg SO_2-eq., arising due to elements like nitrogen (N), sulphur (S), NO_x and NH_3. It is determined by its emissions to air in the form of sulphur dioxide (SO_2), Nitrogen oxides (NO_x) and ammonia NH_3 during its operation [76]. SO_2 and NO_x primarily originates from sulphur containing fossil fuels and its combustion in motor vehicles, whereas NH_3 emissions are majorly from animal husbandry and aquaculture practices.
- Eutrophication: It refers to nutrient enrichments in water bodies due to fertiliser addition, erosion of soil containing nutrients, sediment upwelling, deposition of nitrogen and is usually expressed as kg of phosphate equivalents (kg PO_4^{3-} eq.). The main pathway for aquatic eutrophication are through nitrates (N) and phosphates (P) diffusion from terrestrial sources into aquatic environments via NO_3 and PO_4^{3-} leaching.
- Photochemical oxidation referred to as summer smog or ground-level ozone is dependent on the amounts of carbon monoxide (CO), sulphur dioxide (SO_2), ammonium (NH_4), and volatile organic compound (VOC) emissions to the atmosphere. Photochemical oxidation in biodiesel production is mainly due to hexane use during oil extraction phase of the acid catalysed transesterification and is expressed as kg ethylene (C_2H_4) equivalent.

3. Results and discussions

3.1. Energy expenditure comparison

Fig. 3 illustrates the process-wise energy consumption in the microalgal bioreactor of one-hectare for different nutrient input scenarios and transesterification (using acid or enzyme (lipase) as catalyst). A functional unit of one-hectare was considered for lifecycle energy estimations. The results revealed that the energy required for biodiesel production using acid-catalyst was maximum (11,738 MJ/ha) for scenario 3 (fertiliser use during algal cultivation).

However, the energy consumption obtained in this study is the least ever reported (4547–17,946 MJ/ha) for microalgal cultivation compared to the earlier studies [42,43,52,77-79]. The reason for this lesser energy consumption trend could be attributed to the optimal choice of various unit processes during each stage of the microalgal biofuel production. For instance, a static substrate-based bioreactor which does not require any external energy input for its operation was used in cultivation stage. Conventional microalgae cultivation using open raceway ponds (ORPs) or photobioreactors (PBRs) requires humongous energy for its effective operation. ORPs require paddle wheels for water circulation and to avoid cell shading, while PBRs use pumps, compressors and artificial lighting for its operation. The lifecycle assessment of microalgal biodiesel using a hybrid cultivation system of PBR and open raceway pond [77] estimated an energy requirement of 35 MJ kg^{-1} of biodiesel for cultivation process alone. The study also projected a ten times higher energy expenditure for photobioreactors than that of the open ponds. Similarly, the energy consumption and GHG emission of a mechanised rotating biofilm bioreactor used for microalgal cultivation was 46.8 MJ kg^{-1} and CO_{2e} emissions of 7.8 kg CO_{2e} kg^{-1} of biodiesel respectively. Next to cultivation is the harvesting stage, where attached cultivation of microalgae was known to offer a critical advantage of mechanical harvesting of biomass in its dewatered form, unlike suspended growth systems that has to undergo an energy intensive dewatering processes [80]. Electrical energy consumption of 81.6 GJ ha^{-1} was reported for algal biomass harvesting using centrifuge [81] for an one hectare green wall panel photobioreactor, which is ~18 times higher than the average energy consumption for harvesting estimated in the present study. Combinations of solar energy and filter press were used for drying of the microalgal biomass which resulted in

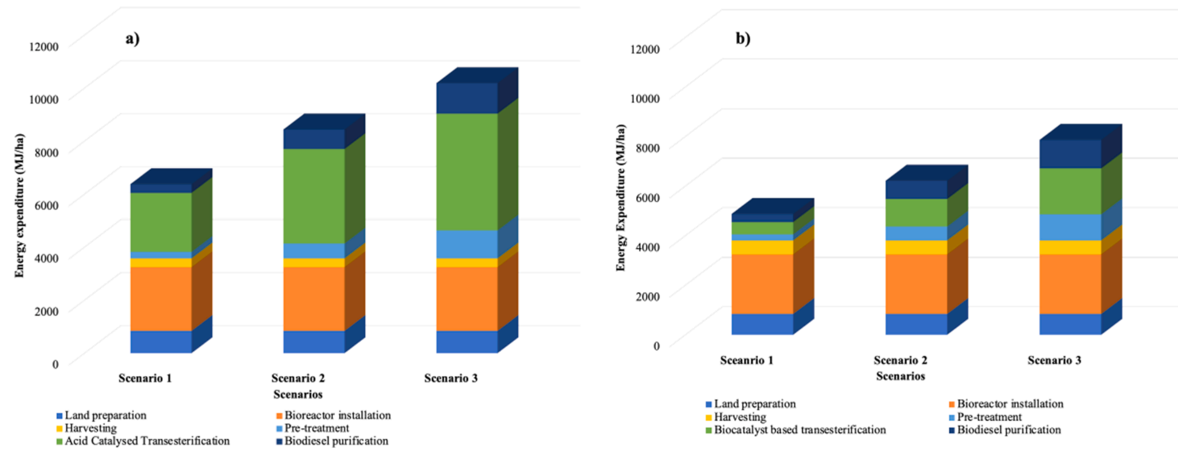


Fig. 3. Process-wise energy expenditure breakup for a) acid catalyst and b) biocatalyst scenarios.

minimal energy input of 248 MJ ha^{-1} to 1960 MJ ha^{-1} compared to the earlier reports of 84% energy and environmental burden during microalgal cultivation and drying stages [82]. Drying accounted for 16 MJ kg^{-1} of biodiesel in a tubular airlift PBR and an open raceway pond cultivation system [77]. The pre-treatment process considered in this study was manual washing followed by sonication. An energy input of 0.04–0.16 MJ kg^{-1} of biodiesel was required for pre-treatment which is lesser when compared to 1.7 MJ kg^{-1} of biodiesel reported for cell homogenisation of *Chlorella vulgaris* biomass [83]. The energy requirement for transesterification using acid and enzyme (biocatalyst) ranged between 1.81 and 2.13 MJ kg^{-1} of biodiesel. Generally an elevated temperature of 60°–85 °C is required for an effective functioning of the acid catalyst with energy of 2226–4406 MJ ha^{-1}, while biocatalyst functions well at room temperatures (25°–32 °C) with energy of 495–1852 MJ ha^{-1}. Table 9 compares the total energy requirement for biodiesel production from feedstocks of different generations (on per hectare basis), which highlights ~8–25 times higher energy inputs (1,61,094–4,64,300 MJ ha^{-1}) compared to the present endeavour based on sustainable and less energy intensive processes of (i) attached (biofilm) cultivation under natural sunlight conditions involving polycultures, (ii) mechanized scrubbers for biomass harvesting, (iii) solar drying and manual washing for biomass processing/pre-treatment and (iv) use of biocatalyst based transesterification.

The overall energy requirement for cultivation, harvesting and biocatalyst based transesterification was the least (4547 MJ/ha) for scenario 1 due to requirement of milder operating conditions (slightly above room temperature). The higher energy consumption in scenario 3 was due to the electricity consumption involved in acid catalysed transesterification and harvesting using mechanised scrubbers. Comparison of energy expenditures with other first and second biodiesel feedstocks showed comparable energy requirement for all the three scenarios and much lesser range when compared with microalgal biodiesel energy requirement (Table 10). The energy required for producing 1 kg of biodiesel is the least (3.6–5.7 MJ kg^{-1}) compared to other feedstock soybean (29.36 MJ/kg), Jatropha (12.93 MJ/kg), Pongamia (11.607 MJ/kg) and conventional diesel (48.97 MJ/kg) (Table 10).

Table 9
Comparison of energy expenditure in different biodiesel feedstock.

Feedstock	Energy Expenditure (MJ/ha/year)	Reference
Soybean	4031	[84]
Soybean	5569	[85]
Soybean	7651	[86]
Soybean	15,673	[87]
Canola	7651	[14]
Canola	6815	
Canola	6148	
Rapeseed	9199	[88]
Jatropha	76,500	[89]
Microalgae	1,61,094	[52]
Microalgae	4,64,300	[81]
Microalgae	4547–17,946	Present study

3.1.1. Comparison of energy expenditures under different process combinations

A combination of different process parameters in upstream and downstream processes such as manual harvesting/mechanised scrubbers, solar drying/filter press while using acid catalyst and biocatalyst was compared to assess the energy expenditures involved in maintaining the microalgal bioreactor (Fig. 4(a-b)).

When the amount of energy required per hectare area was considered under three different nutrient input scenarios, the energy requirement was the highest (17,946 MJ ha^{-1}) for scenario 3 (fertiliser inputs) irrespective of the process combinations and the conventional electricity requirement in downstream processing of the biomass including harvesting, drying and transesterification. The combination of manual harvesting, solar drying and biocatalyst was found be the best case scenario irrespective of the type of nutrient input. This is comparable to an earlier study that compared inorganic (acid) and biological (enzyme) catalysis for the production of biodiesel from rapeseed oil showed enzymatic production of biodiesel to be environmentally more favourable with significant improvements recorded over conventional acid catalyst in all impact categories [92].

3.1.2. Comparison of GHG emissions for different process combinations

Comparisons of GHG emissions for different process combinations are illustrated in Fig. 5(a-b), which illustrates of higher GHG emissions 7742–9990 kg CO_{2e} ha^{-1}) for scenario 3 irrespective of the process combinations. The process combinations of manual harvesting, solar drying and the use of biocatalyst exhibited the least CO_{2e} emissions (0.5 CO_{2e}/kg of biodiesel) due to use of solar energy for microalgal biomass drying which would cut down on electricity consumption. In addition, the requirement of milder operating conditions for enzymatic

Table 10
Energy requirement comparison with fossil diesel and different generation biodiesel.

Feedstock	Energy Requirement (MJ/kg of biodiesel)	Reference
Conventional diesel	48.97	[85]
Soybean biodiesel	29.36	[85]
Jatropha	12.93	[90]
Pongamia	11.607	[91]
Beef Tallow	30.65	[85]
Microalgae	203.25	[79]
Microalgae	3.6–5.7	Present study

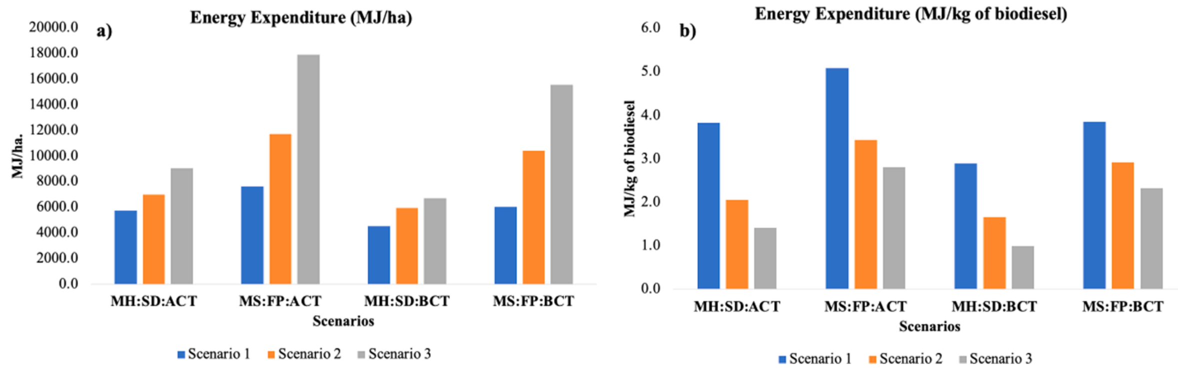


Fig.4. (a-b). Energy expenditure under different process combinations. MH – manual harvesting; SD – solar drying; ACT – acid catalysed transesterification; MS – mechanised scrubbers; FP – filter press; BCT – biocatalyst based transesterification.

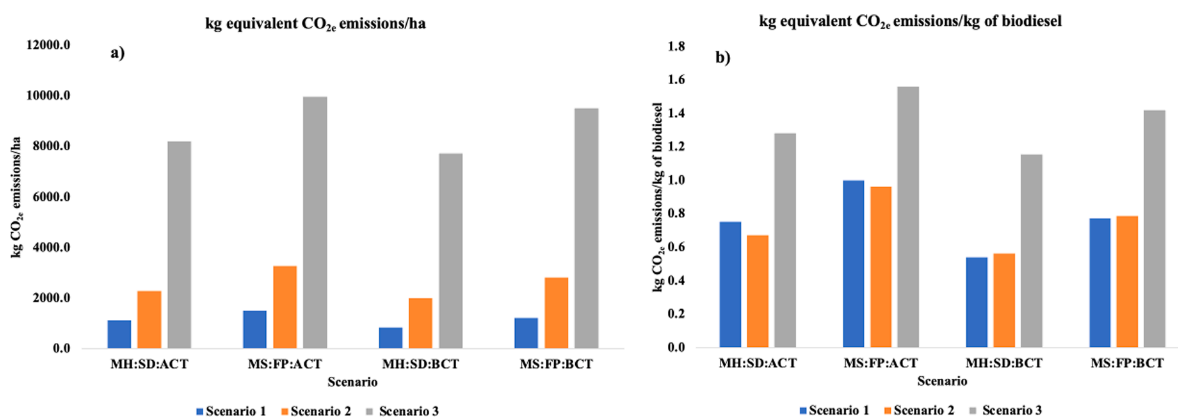


Fig. 5. (a-b). GHG emissions under different process combinations.

transesterification also aids in the reduction of energy and its associated GHG emissions. Comparisons of GHG emissions during transesterification using alkali and enzyme catalyst showed a CO_{2e} emission of 4.150 and 4.050 kg CO_{2e} kg^{-1} of biodiesel [92] which is ~2.5 times higher compared to the present study. Relatively lesser GHG emission reported for biocatalyst based transesterification than alkali catalyst corroborates with the present study.

3.1.3. Net energy ratio (NER)

Biofuel production system with NER greater than unity tends to be economically viable. The net energy ratios of different biomass productivity scenarios considered in this study using acid catalyst and biocatalyst is given in Fig. 6. The NER was calculated considering the energy output from three energy products (biodiesel, biogas and crude glycerol) possible from the microalgal biomass harvestable from one hectare plot bioreactor. The energy content of biodiesel, biogas and crude glycerol considered for calculation were 39 MJ kg^{-1}, 30 MJ m^{-3} and 25.3 MJ kg^{-1} respectively. The NER for all the scenarios was greater than 1, depicting a positive energy system unlike other conventional microalgae cultivation systems which suffer poor net energy ratio (usually <1). The result demonstrated a highest NER for wastewater input - biocatalyst scenario (NER = 18.8), which is higher than NER of conventional fossil diesel (5.26), owing to the milder operating conditions required during transesterification process with optimal biomass yield achievable by using aquaculture wastewater as a source of nutrient. NER of 12 and 14 for acid and biocatalysts respectively for scenario 3 despite a higher biomass and FAME conversion efficiency was due to the higher energy inputs in downstream processing such as harvesting and acid/biocatalyst transesterification. An earlier study using biofilm photobioreactor for microalgal biomass production have shown a NER of 6.0 [93] and 1.65 in a rotating algal biofilm reactor with cotton ducts as substrate for algal attachment and hydrothermal liquefaction for biocrude production [80].

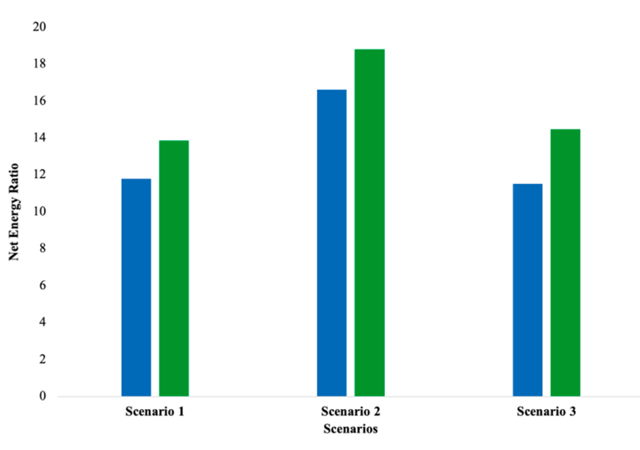
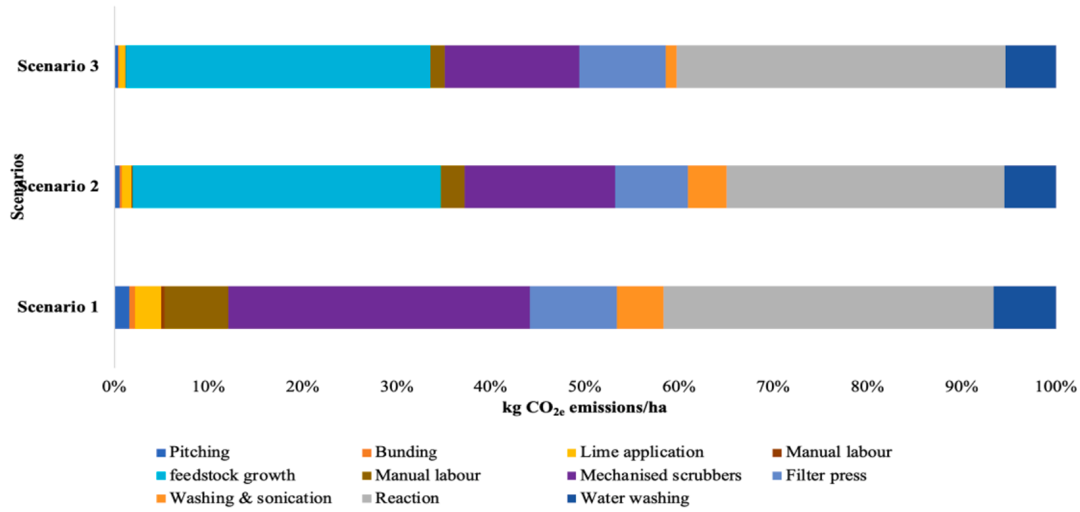


Fig. 6. NER of different scenarios using acid and biocatalyst.

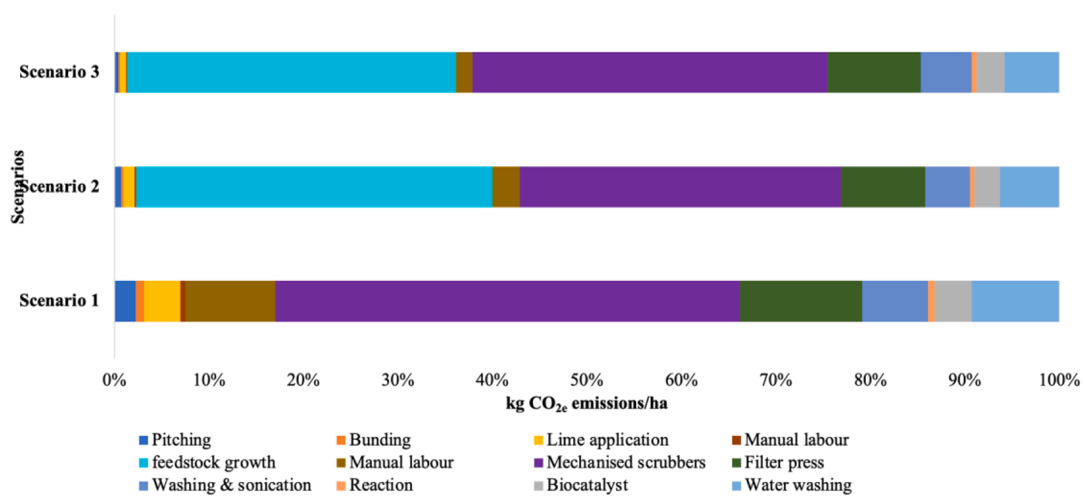
3.1.4. GHG emissions

Figs. 7a and 7b illustrates the process-wise GHG emission share for different scenarios with acid/biocatalyst based transesterification which show a similar trend. The GHG emissions (kg CO_{2e}) was found to be the least for baseline (850 kg CO_{2e}/ha) and highest for scenario 3 (fertiliser) input (9990 kg CO_{2e}/ha). In all these scenarios, energy required for mechanised scrubbing operation and the energy for acid catalysed transesterification was found to contribute to a maximum in the GHG emissions. In scenario 2, the relative contribution of GHG emissions was



(a). Process-wise kg equivalent CO_2 emissions for acid catalyst scenario

Fig. 7a. Process-wise kg equivalent CO_2 emissions for acid catalyst scenario.



(b). Process-wise kg equivalent CO_2 emissions for biocatalyst scenario

Fig. 7b. Process-wise kg equivalent CO_2 emissions for biocatalyst scenario.

from wastewater (32.3%). The major share of GHG emission (67.9%) for scenario 3 was from fertiliser application and transesterification reaction. As the application of fertiliser is known to induce acidification in the soil leading to eutrophication and reduced fertility of the soil, while using aquaculture wastewater with an optimal production of microalgal biomass, was found to be an ideal scenario for achieving microalgae biomass as a source of energy as well as mitigates environmental burden through bioremediation. Significant reduced environmental burden through integration of algal cultivation with wastewater treatment were reported in the substrate based microalgal biorefinery [80] and open pond reactors [94].

Table 11 lists a detailed breakup of energy expenditure incurred at different processing stages of microalgal biodiesel production with plant-based (soybean) biodiesel and conventional fossil diesel. The results exhibit a total energy requirement that varies between 3.6 and 5.7 MJ/kg of biodiesel for different scenarios in the substrate-based bioreactor, which is lesser compared to soybean biodiesel (27.3 MJ/kg) with the maximum share during soybean agriculture (24.25 MJ/kg). A reduction of about 87.3% of fossil energy is possible when microalgal biodiesel is used in place of petroleum derived diesel, which requires 48.09 MJ of energy [86].

Table 12 lists the process-wise CO_{2e} emission comparisons of microalgal biodiesel (present study) with soybean biodiesel and fossil diesel. Substrate based microalgal bioreactor eliminates the requirement of energy intensive harvesting operations, and the GHG emission is the lowest (0.85–1.46 kg CO_{2e} per kg of biodiesel) and also lesser compared to all values reported in microalgal biodiesel production so far. Microalgae also plays a vital role in environmental carbon mitigation [95] through efficient carbon sequestration as estimates indicate of 1 kg microalgal biomass sequestering about 1.83 kg CO_2 [96] and accounts for 40% of global carbon sequestration. Bio-fixation of 8.3 kg of CO_2 as organic carbon in the form of biomass for producing 1 kg of biodiesel, with 4.54 kg of dried microalgal biomass. Evidences prove that the microalgae's biological fixation capability by utilising solar energy is

Table 11
Stage-wise energy expenditure comparison with soybean biodiesel and fossil diesel.

Processes	MJ/kg of biodiesel				MJ/kg fossil diesel
	S1	S 2	S3	Soybean Biodiesel	Fossil diesel
Land preparation & bioreactor installation/Soybean agriculture/crude oil production	2.07	0.91	0.48	24.25	45.38
Microalgal feedstock growth/ soybean transport and crushing/crude oil transport	–	–	1.01	0.15	2.54
Harvesting	1.12	0.49	0.26		
Drying (Filter press + solar drying)	0.29	0.13	0.06		
Pre-treatment	0.16	0.07	0.04		
Transesterification/soybean oil conversion	1.81	2.13	2.06		
Biodiesel refining/soybean biodiesel refining/crude oil refining	0.21	0.20	0.17	2.9	0.17
Total Energy expenditure (MJ/ kg)	5.66	3.61	4.07	27.3	48.09

S1 – S3: Scenario 1 – Scenario 3.

Table 12
Stage-wise GHG (CO_{2e}) emissions comparison with soybean biodiesel and fossil diesel.

Processes	kg CO_2e emissions/kg of biodiesel				kg CO_2e emissions/kg fossil diesel
	S1	S2	S3	Soybean Biodiesel	Fossil diesel
Land preparation & bioreactor installation/ Soybean agriculture/ crude oil production	0.49	0.22	0.12	4.8	0.5
Microalgal feedstock growth/soybean transport and crushing/ crude oil transport	–	–	0.20	0.029	8.94
Harvesting	0.22	0.10	0.05		
Drying (Filter press + solar drying)	0.06	0.03	0.01		
Pre-treatment	0.17	0.07	0.04		
Transesterification/ soybean oil conversion	0.49	0.40	0.42		
Biodiesel refining/soybean biodiesel refining/crude oil refining	0.04	0.04	0.04	0.57	0.033
Total (CO_2e emissions/kg)	1.46	0.85	0.87	5.399	9.47

S1 – S3: Scenario 1 – Scenario 3.

10–50 times greater than that of the terrestrial plants [97]. Biodiesel reduces the net CO_2 emissions by 78.45% compared to petroleum diesel [86]. The use of biodiesel (B100) have shown to considerably decrease lifecycle emissions, especially total particulate matters such as carbon monoxide and sulphur oxides.

3.1.5. Lifecycle impact assessment

3.1.5.1. Abiotic resource depletion. Fig. 8 illustrates the different impact categories. The ADP in terms of mineral resources ranged between 0.0032 and 0.033 kg Sb eq./ton of biodiesel, a very minimal quantity considering the scale of biodiesel production possible from one hectare plot bioreactor. The ADP of the analysed ethanol project using sugarcane bagasse varied between 0.003 kg Sb-eq./kg bioethanol [9] and the ADP of microalgal biodiesel was found to be 0.0039 kg Sb-eq./kg of biodiesel which is much higher than the present study (0.0032 g Sb eq./kg of biodiesel).

The ADP in terms of fossil fuel requirement varied between 1.06E + 02 and 2.77E + 02 MJ/ton of biodiesel produced and found to be the highest for scenario 3 (acid catalyst) especially due to the higher fossil energy requirement for harvesting and downstream processing. Studies have shown that biodiesel consumes 45% less fossil fuel than that of what is expended in conventional diesel [98]. The ADP for biocatalyst scenarios (1–3) were the lowest pertaining to milder operating conditions during enzyme catalysed transesterification. LCA of microalgal biogas production system had shown an abiotic resource depletion due to the use of conventional electricity spent during microalgal harvesting [99].

3.1.5.2. Acidification potential. In this study, the estimation of acidification potential in the form of SO_2 equivalent emissions were calculated by taking into consideration of i) the use of fossil fuel and electricity usage, ii) fertiliser, limestone application, iii) the use of sulphuric acid during acid catalyst transesterification. The results revealed that, a greater proportion of the acidification potential (SO_{2e}) was through fossil electricity related emissions with 3.61–7.85 kg SO_2e/ton (or 3.6–7.85 g SO_{2e}/kg of biodiesel) was estimated for different scenarios. In scenario 2, the NH_3 volatilisation and N_2O emissions from aquaculture wastewater was found to induce an acidification potential of 5.86 kg SO_2e/ha. The acidification potential of scenario 3 (fertiliser input - acid catalyst) scenario was found to be the highest (7.85 kg SO_2e/ton). Acidification is mainly due to the use of synthetic fertiliser in upstream operation and electricity consumption during transesterification (downstream operations). Acidification potential of bioethanol derived from sugarcane bagasse as reported in earlier studies range from 2.34 to 11.66 g SO_{2e}/kg of bioethanol [9]. Acidification potential of jatropha biodiesel was found to be 58.5 g SO_{2e}/kg of biodiesel [100]. Another study [101] where environmental impact assessment of soybean biodiesel was carried out, the results revealed an acidification potential of 13.8 g SO_{2e}/kg of soybean biodiesel. Acidification potential of microalgal biodiesel was estimated to be 23.4 g SO_{2e}/kg of biodiesel produced which is much higher than the impact levels estimated in this present study [102].

3.1.5.3. Eutrophication. In this study, eutrophication potential (EP) of the proposed substrate-based bioreactor system was calculated as per ISO 14042 following CML – IA baseline method (Figs. 7a and 7b). The eutrophication potential of scenario 3 while using acid as well as biocatalyst was found to be higher (3.57–4.24 kg PO_4^{3-} eq./ton of biodiesel) or 3.57–4.24 g PO_4^{3-} eq per kg of biodiesel as there are likely chances of leaching of unutilised nutrients entering the receiving waterbodies which can lead to undesirable aquatic biomass growth. The analysis of the potential impacts due to eutrophication by microalgal biodiesel production [102] has shown 4.85 g PO_4^{3-} eq./kg of biodiesel which is comparable to the current study. The eutrophication potential of soybean biodiesel ranges between 0.32 and 3.08 g PO_4^{3-} eq/kg of biodiesel [103,104].

3.1.5.4. Photochemical oxidation. The photochemical oxidation expressed as kg C_2H_4 eq. was found to be the highest (7.30E−01 kg C_2H_4 eq./ton of biodiesel) or 0.730 g C_2H_4 eq./kg of biodiesel for fertiliser input during cultivation and acid catalyst in transesterification scenario. The photochemical oxidation was found to be minimal for biocatalyst scenario as there is no involvement of hexane in enzymatic transesterification of biodiesel. Photochemical oxidation potential of soybean, jatropha and microalgae are 3.31, 2.80 and 2.69 kg C_2H_4 eq. per ton of biodiesel due to the use of hexane during the transesterification process [102].

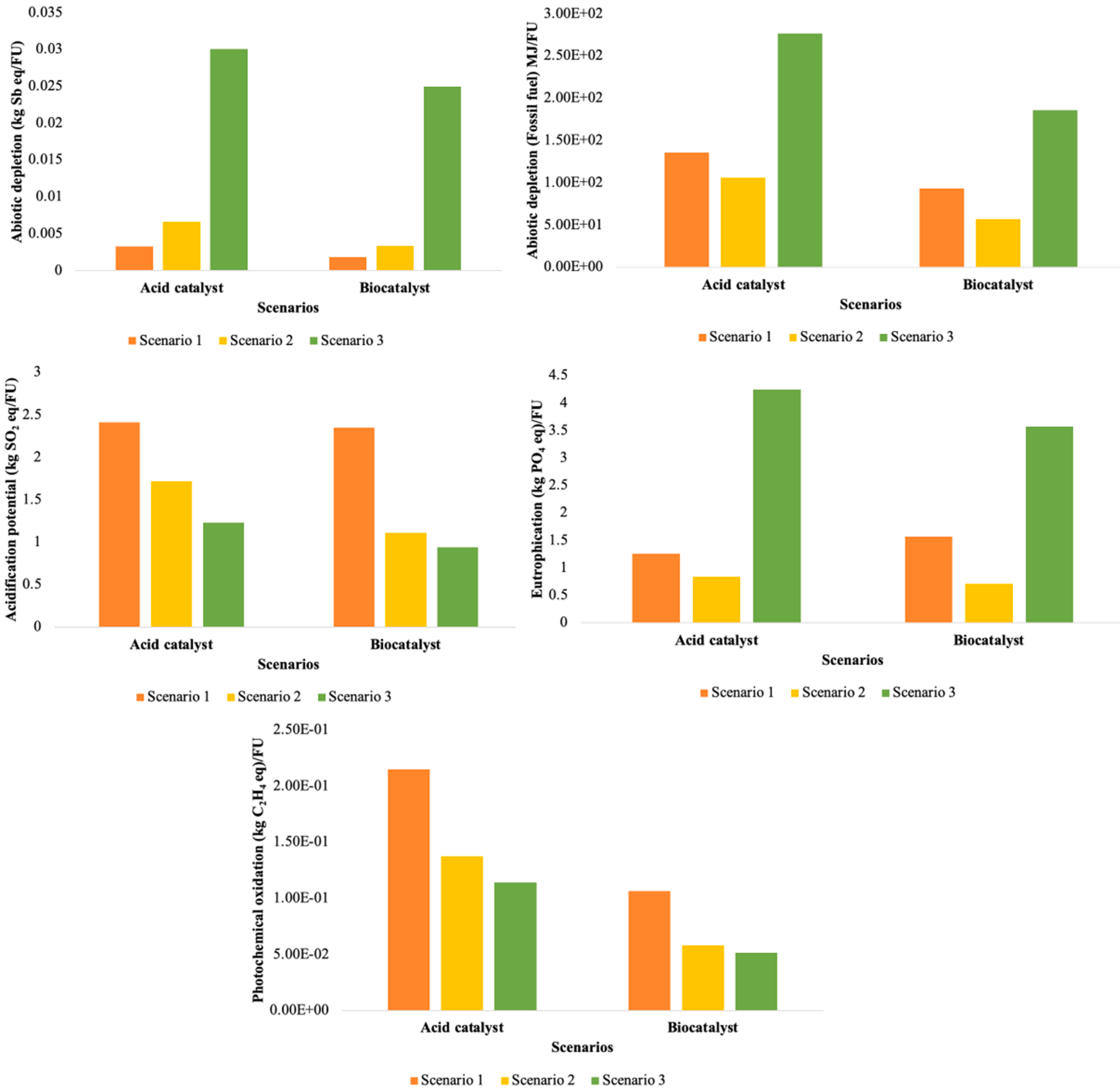


Fig. 8. Comparative results of the lifecycle environmental impacts of different scenarios considered for substrate based microalgal biodiesel production. *FU = 1 ton of biodiesel produced under different scenarios.

3.2. GHG emission mitigation with biodiesel

Replacement of environmentally burdening conventional fossil fuels with alternate green renewable energies would result in GHG emission mitigation (otherwise known as direct GHG emission savings, expressed as %). Direct GHG emission savings that is possible by replacing fossil diesel with microalgal biodiesel (calculated as per Eq. (2)) is given in Fig. 9. A direct emission savings of 67.9% to 85.4% for different scenarios is possible with the biofuel production process. LCA carried out on a hybrid microalgal cultivation system have shown a direct GHG emission savings of 42% [77] and 78% in biodiesel using the microalgae *Chlorella vulgaris* cultivated in a raceway pond compared to conventional biodiesel. Net GHG emission savings estimated on biodiesel production using rapeseed, soybean, palm and sunflower ranged between 20% and 38% when compared to fossil diesel [105].

The LCA of biodiesel from microalgae depicted energy minimisation and reduced GHG emissions with assured environmental benefits in the long run. The study substantiates the scope for microalgal biodiesel production with very minimal energy expenditures, which has been a hurdle for large scale conventional suspended mode microalgal

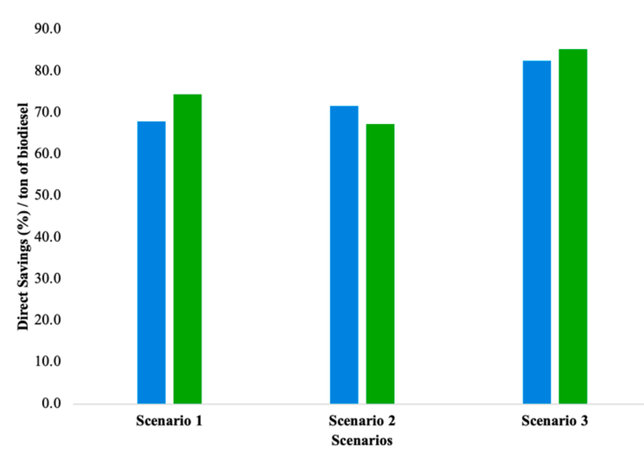


Fig. 9. Direct savings in environmental burden (as percentage) possible from biodiesel.

cultivation systems. Similarly an environmentally favourable condition was reported [92] for biocatalyst than acid catalyst based production of biodiesel from rapeseed oil.

The current study corroborates with the earlier study [78] that analysed the environmental impacts of biodiesel production using microalgae and highlights the need for decreasing the energy and fertiliser consumption to make microalgal biofuel an attractive energy option. Estimates on annual diesel consumption projections for the year 2040 revealed an increase in diesel consumption from 7.04 to 8.45 Exajoules (10^{18} J) [4,106]. Biodiesel reduces lifecycle CO_2 emissions as its combustion is offset by the carbon dioxide absorbed from growing the microalgal biomass. An earlier study showed a reduction in CO_2 emission by 74% compared with petroleum diesel.

Estimates show a requirement of 162.6 MT of conventional diesel for the year 2020, and for proportional B20 blending [107] as per the biofuel policy, GoI, 2018 requires about 32.53 MT of biodiesel. The use of B100 results in substantial reduction in lifecycle emissions of total particulate matter in terms of carbon monoxide (CO) and sulphur oxides (SO_x). The current study highlights that the potential of microalgal biofuel is quite promising both from an economic and life cycle/environmental perspective, evident from the results of the present study. Wise choice of process technologies for cultivation of microalgae as well as its subsequent conversion into biofuel and other value-added products would ensure economic viability of microalgal biodiesel.

4. Conclusions

Microalgae is a promising biodiesel feedstock especially from the perspective of decarbonising through reductions in fossil fuels use in the transportation sector and added benefits of sequestration of carbon during cultivation. LCA aided in assessing the environmental soundness of the bioenergy project. Critical challenges of microalgae based biofuel systems are optimisation of energy inputs for biomass harvesting and subsequent processing. The current study through substrate-based bioreactor demonstrates the economic viability through appropriate technologies for feedstock cultivation, harvesting and also transesterification. This study is based on the integrated approach of field data based on the pilot scale bioreactor and the review of literatures for the assessment of energetics and associated environmental impacts considering different cultivation approaches and transesterification (acid versus biocatalyst) process. The results revealed the lowest possible GHG emissions (0.85 kg CO_{2e} kg^{-1} of biodiesel) of all considered scenarios for scenario 2 (wastewater input). Wastewater use in cultivation of microalgae has additional benefit of remediation, which gives scope for decentralised wastewater treatment through microalgal bioreactors. The use of biocatalyst for transesterification was found to substantially reduce the environmental loadings compared to acid catalyst due to its milder operating conditions. The GHG emission mitigation of 67.9–85.4% is possible when microalgal biodiesel is substituted in the place of conventional fossil diesel. A substantially higher NER (18.8) for wastewater/biocatalyst scenario proves its economic feasibility which is mainly influenced by minimal energy requirement for harvesting, drying and transesterification. Thus, LCA of microalgal biodiesel generated using feedstocks grown in the proposed substrate-based microalgal cultivation system demonstrates sustainability in terms of technical feasibility, economic viability, environmentally sound and social acceptance, with the scope for sustainable bioresource utilisation while empowering rural economy, especially ensuring the livelihood through decentralised job opportunities to coastal women.

5. Research ethics

The publication is based on original research and has not been submitted elsewhere for publication or web hosting

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Declaration of Competing Interest

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

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