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A review on homogeneous and heterogeneous catalytic microalgal lipid extraction and transesterification for biofuel production

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Content

Abstract

Extraction of lipids from the microalgal biomass for the alternative affordable clean energy industries hold a great potential, as there are possible cost–effective chemical conversion technical approaches have been utilized to produce the FAMEs *via* transesterification of the lipids. The extraction mainly involves the lipids *viz*. FFAs, phospholipids and TAGs that can reduce the required energy for the extraction process, notably to meet the growing demand of fossil–derived energies. Many approaches significanyly *via* catalytic, non–catalytic and enzymatic transesterification paths offer a sustainable bioenergy production from microalgal species. In this regard, the key considerations of this review mainly include the recent insights on the microalgal lipid extraction *viz*. solvent, Soxhlet, Bligh and Dyer's, *SC*–CO_2 (Supercritical CO_2), ILs (Ionic liquids solvent) methods and the conversion by tranesterification along with suitable mechanism *via* homo / heterogeneous acid / base catalysed, enzymatic, non–catalytic, mechanically / chemically catalysed *in–situ* techniques towards algal bioenergy production. Moreover, the technical advances in both extraction and conversion is essential for the renewable energy sector to commercialization.

Kerywords: Microalgae; Lipid extaction; Transesterification; Catalytic; Enzymatic; *In–situ* techniques.

Abbreviations

AEP	–	After extraction process
ASE	–	Accelerated solvent extraction
BEP	–	Before extraction process
CO_2	–	Carbon dioxide
ER mechanism	–	Eley–Rideal mechanism
FAMEs	–	Fatty acid methyl esters
Fas	–	Fatty acids
FFAs	–	Free fatty acids
GHGs	–	Greenhouse gases
ILs	–	Ionic liquids
LHHW mechanism	–	Langmuir–Hinshel–Wood–Hougen–Watson mechanism
RSO_3H	–	Organic sulphonic acid
SC–CO_2	–	Supercritical Carbon dioxide
SCM	–	Supercritical methanol
TAGs	–	Triacylglycerides

1. Introduction

Inorder to overcome the energy crisis, which has become a serious global issue for the 20th century, algae biofuels have been received a great attention with some challenges [1–11]. Using algae biomass for "only biofuel" seems not a viable option and therefore, researchers are forecasting the integration of green biorefinery (production of commercially viable green chemicals) along with the biofuel production [5–7, 11–17]. In this regard, microalage hold a very high potenttial to serve as renewable enregy source [11,12,18–20], however the production, cultivation and conversion technolgies are at infancy stage towards reality of algal biofuel utopia. Integrative approaches with simultaneous wastewater treatement and CO_2 remediation (biofixation) could make the algal biomass a valuable resource with commercial benefits [11,19,21,22]. Energy density of the algal biomass, by accumualting the major component lipid and triacylglycerides (TAGs), is mainly concerned for the increment of its heat and fuel value. This can be done by mixotrophic growth or certain physilogical triggers, such as light intensity, fatty acid composition of the microalage species which varies according to the species nature [23] and it's accumualtion that could also be altered by modifying the environmental factors mainly on certain elemmental concentrations like nitrogen (N) and phosphorous (P) in the medium and so on [21].

At present, commercially obtainable biodegradale microalgal bio–oil has been extracted using catalyzed transesterification of lipid TAGs of carbons $C_{14–20}$, which mainly consist of three fatty acids *viz.* $R_1–CO_2H$, $R_2–CO_2H$ and $R_3–CO_2H$ (where R_1, R_2 and R_3 = alkyl chains) into fatty acid methyl esters (FAMEs) with polyhydric glycerols as valuable by–products [24]. The FAMEs thus obtained suitably replace the petro–derived fuels and reduce the viscosity, volatility, high unsaturated characteristics, unburned hydrocarbons, emission of greenhouse gases and particulate matters [25]. The efficacy of homogenous / heterogeneous solid acid / base, enzyme catalyzed and *in–situ* transesterification reactions [26–40] has been reported in the literature for the potential extraction of microalgal bio–oil. In the case of production of biodiesel from microalgal biomass, even though the use of base catalysed technology possesses higher activity when compared to the acid catalysts through transeterification process, it is suitable only for the algal lipids of low free fatty acid (FFA) componenets due to the soap formation by means of partial saponification [35]. Furthermore, the acid catalyzed process in the biodiesel

production is facilitated *via* both the transesterification and esterification routes only [36, 41, 42].

Enzymatic transesterification is analogous to conventional process, except the use of bio–catalysts like *lipases* that effectively transesterify the TAGs with high FFAs [34, 43]. Main disadvantage of this process is its cost–intensivity, recycling process of the employed enzymes through enzyme immobilization that is mainly attributed to their routine consumption used suitably in the transesterification pathway [12]. In the case of cost–effective *in–situ* transesterification, there is an eco–friendly direct conversion *via* alcoholysis of algal lipids to FAMEs without solvent extraction; which is more effective towards microalgal lipids [44]. Demirbas reported that the transesterification of microalgal lipids in the presence of catalysts yields FAMEs, which are very similar to the petro–derived fuels and the process is comparatively effortless [25,45,46]. Considering the above facts, this review predominantly emphasizes an overview on chemistry involved in various lipid extraction methods and conversion technologies of the microalgal lipids into microalgal oil production *via* different transesterification techniques with suitable mechanistic pathways.

2. Lipid extraction methods from microalgae

The harvesting and pretreatment processes are followed for the extraction of algal lipids by means of suitable chemical and physical techniques. A number of significant methods are listed in **Table 1**. The technologies used should be of specificity far above the ground to diminish protein and carbohydrate infectivity. Moreover, the techniques should be cost–effective, secure, should require a little time and should not interact with algal lipids. A discussion about lipid extraction methods has presented in the following sections.

Table 1. Extraction methods for microalgal lipids (Adopted from modified Refs.[1,47]).

Extraction techniques	Solvent	Microalgal species	Efficiency / yield (wt. %)	Time (min.)	Temp. (°C)	Pressure (MPa)
Bead beater + solvent	Chloroform /Methanol	*Botryococcus braunii*	28.60	50.00	–	–
		Botryococcus sp.	28.10	–		
	CO_2 (Carbon di oxide)	*Chlorella vulgaris*	13.30 [a]			
Bligh and Dyer's method	–	*Chlorella vulgaris*	10.60 [a]			
Cold pressing	Ethanol	*Scenedesmus obliquus*	62.04±72.42		73–75	
Ionic liquids	[Bmim] $[CF_3SO_3]$[d]	*Chlorella vulgaris*	12.50 [a]		–	
	[Emim] $[MeSO_4]$[e]		11.90 [a]			
Organic solvent	1–butanol	*Chaetoceros muelleri*	94.00	60.00	70	
	Isopropanol/Hexane	*Chlorococcum* sp.	06.80	450.0	25	
	Hexane		01.50	–	–	
	Ethanol, 5 mL/g Dried microalgae	*Phaeodactylum tricornutum*	29.00	1440		
Soxhlet	DBU [b]/Octanol	*Botryococcus braunii*	81.00	240.0	60	
	Hexane	*Chlorococcum* sp.	03.20	330.0	–	
		Chlorella vulgaris	01.77	140.0	70	
	CO_2, 2.0 mL/min	*Isochrysis galbana*	04.00–10.00	–	40	69.0
	CO_2 / Ethanol		05.00–11.00 [a]		50	6.89

	Hexane	*Scenedesmus obliquus*	40.71±74.46	–	63–65	–
Supercritical fluid	CO_2, 10 g/min	*Crypthecodinium cohnii*	09.00	180.0	50	30.0
	CO_2	*Chlorococcum* sp.	05.80	80.00	60	10.0–50.0
		Nannochloropsis sp.	25.00	–	40	55.0
	Ethanol		90.21	–	–	–
	CO_2	*Spirulina maxima*	03.10	–	35	60.0
		Spirulina platensis	08.60	60.00	40	40.0
			90.00 [a]	15.00	55	70.0
	DCM [c]/ Methanol (9:1)	*Tetraselmischui*	15.00		99	10.3

[a] Production of oil; [b]1,8–diazabicyclo–[5.4.0]–undec–7–ene; [c] Dichloromethane; 1–butyl–3–methylimidazolium trifluoromethanesulfonate; [e] 1–Ethyl–3–methylimidazoliummethyl sulfate.

2.1. Solvent extraction method

Nature of the preferred solvent depends on the chosen species of microalgae for effective extraction since the extracted algal lipids have many types of interactions that is disruption of hydrophobic activity between non–polar solvents and neutral lipids, by the way there exists hydrogen bonding between polar organic solvents and polar lipids. Soxhlet and Bligh and Dyer's methods are the two characteristically employed traditional extraction methods for microalgal lipids. Both of the Soxhlet and Bligh and Dyer's methods employ a mixture of hexane and chloroform, methanol, benzene and ether for the extraction of lipids [48]. Among them, hexane has shown better results as it is less toxic, has low affinity towards non–lipid contamination and has higher selectivity for neutral lipid moieties [48]. Aminul Islam *et al*. extracted microalgal lipids of 55–75 % using Accelerated solvent extraction (ASE) process [49]. The extraction of algal oil using the solvents should be cheap, non–toxic, volatile, non–polar and deprived the extraction towards other non–lipid constituents of the algal cells. Further, the Soxhlet and Bligh and Dyer's traditional lipid extraction techniques are not suitable in the case of wet algal biomass since the surface charge prevents them contacting into the organic solvent phase, which leads to the low extraction and yield [48]. Thereby, the extraction process using both Supercritical CO_2 (*SC*–CO_2) and Ionic liquids (ILs) are green technical substitutes for the traditional lipid extraction techniques since these methods have high solvating tendency, low toxicity, inflammability and reactivity [50].

2.1.1. Soxhlet extraction method

Soxhlet extraction method employs the extraction of microalgal lipids, using hexane solvent lonesome/in combination with the oil press/expeller technique, follwed by the extraction of residual pulp using cyclo–hexane which does not limit the equilibrium of lipid mass transfer and reqiures a large volume of solvents. The used solvent and the lipids can be separated by distillation process and the yield is about 95 %. Holbrook *et al*.,[51] extracted the microalgal lipids from *Monoraphidium* sp. on a larger scale by refluxing it in its powder form with 1:10 (w/v) hexane and a mixture of methanol–NaOH (1 M) in the ratio of 10:56 w/w at 60 ºC for about 90 min. Then, they cooled the reaction mixture followed by vacuum centrifugation and mixed with a little water to separate the by–products and the un–reacted components from the oil content. This Soxhlet extraction

method is effecient as compared to the batch extraction, for instance a dried *Chlorococcum* sp. microalgal biomass yields about 0.015 g lipid/g, by means of Soxhlet method but which yields nearly about 0.057 g lipid/g using a batch technique. Though, its disadvantage falls with the continuous distillation process [1,47,48]. *Botryococcus braunii, Nannochloropsis* sp., *Arthrospira platensis* and some mixed cultures microalgae from South Coast of Yogyakarta, Indonesia, among all the species of microalgae studied, *Nannochloropsis* sp. was found to have the highest algal oil yield (0.0346 g dry algal oil/g dry microalgae) and theoretical calorific value (187.69 kcal/kg dry microalgae) [52].

2.1.2. Bligh and Dyer's method

A traditional lipid extraction technique is followed, which was developed by Folch and Bligh and Dyer [53,54]. They employed the use of co–solvents that is a mixture of polar and non–polar solvents (2:1), usually methanol–chloroform system for the dried microalgal biomass and the ratio should be 2:2:1.8. For the dry route [55], the solvent to tissue ratio must be around [(3+1):1] since water is not significant in comparision to the biomass tissue. Lam and Lee [44,56] followed Bligh and Dyer method with both dry and wet route (**Fig. 1**) and found that the lipid extraction yield is ~ 95% of the total lipids.

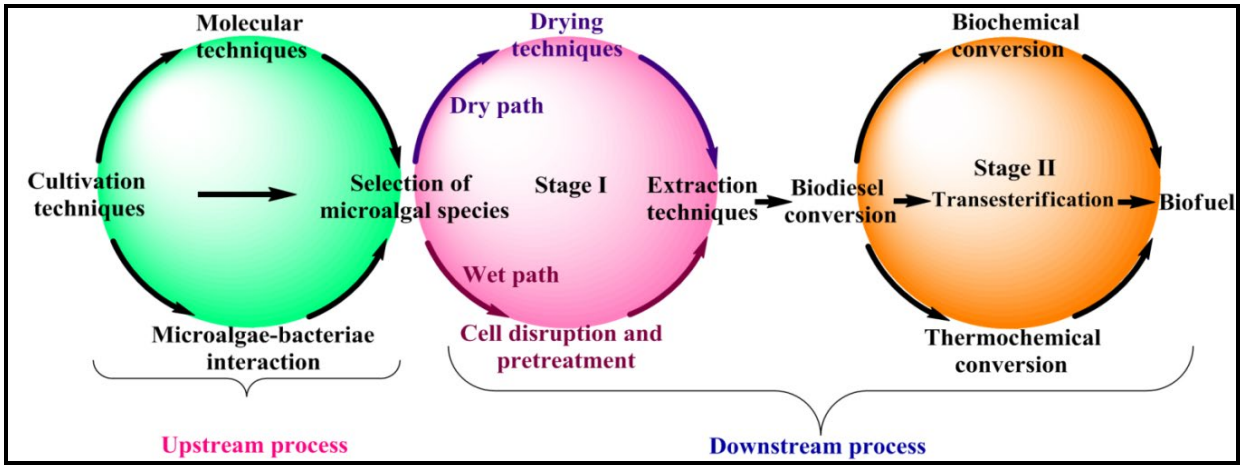


Fig. 1. Dry and wet route involved in microalgal lipid extraction and energy production process.

The separation of lipids, by–products and water can be done by homogenized centrifugation, which follows fractional distillation. Its major limitation is its duration, which falls in the range of 6–12 h and sometimes it extends up to12–24 h due to some eccentric loading of biomass. Consequently, there is a possibility for the dissolution of chlorophyll magnesium along with some other pigments, which simultaneously spoils the quality of the extracted lipids. Moreover, the solvents are expensive and perilous [1].

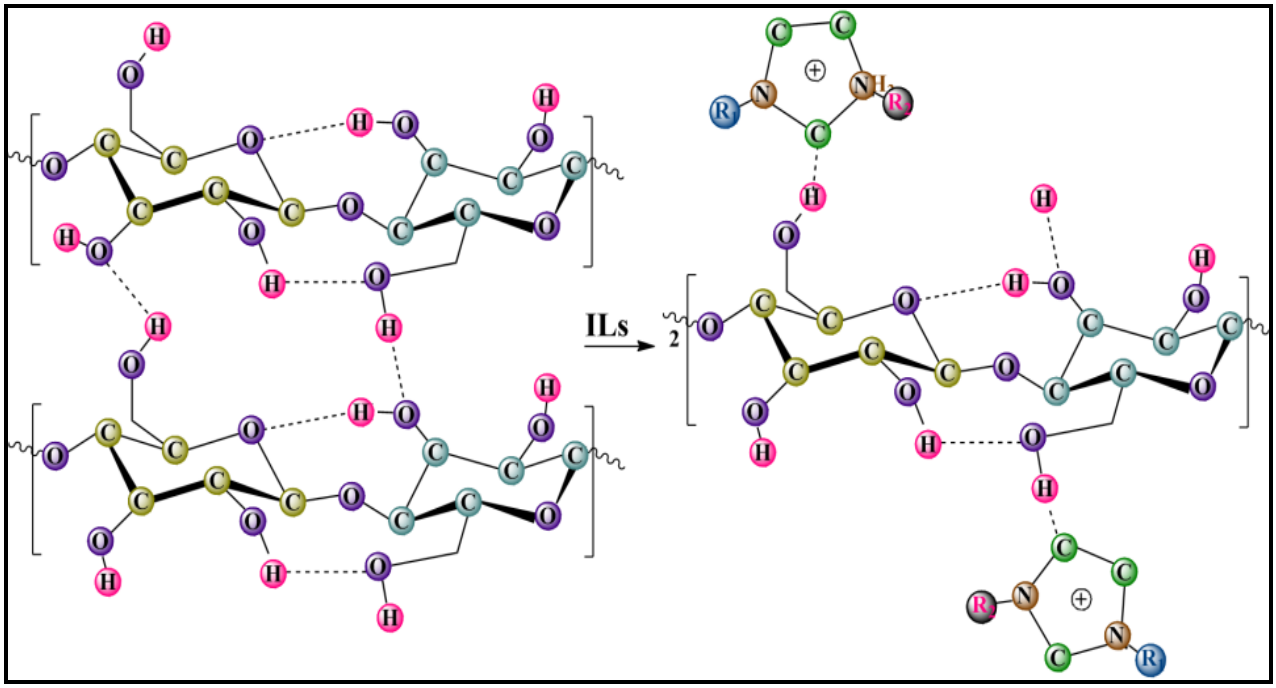
2.1.3. Supercritical CO_2 extraction (*SC*–CO_2) method

It facilitates the direct renovation of wet algal biomass into alkyl esters and neutral lipids (acyl glycerides) [57]. It involves the decompressed *SC*–CO_2 with a flow rate of 400 mL/min within the time duration of about 4.9–14.1 min at 60–80 ºC and the pressure ranges from 10–50 MPa in 80–120 min. Extraction of lipids from the microalgae biomass depends on the fluid density, consequently about 50 wt.% of bio-oil can be extracted. Moreover, the yield obtained is solvent free and the supercritical fluids are non–corrosive, non–toxic, non–inflammable and static. The yield increases with the decrease of temperature and decrease of pressure. The literature reveals that 90 % of oil can be recovered from *Spirulina platensis* in < 15 min at 55 ºC and 10–70 Mpa pressure, using SC–CO_2 but it took 6 h in Soxhlet method. In addition, the *Chlorococcum* sp. yields about 0.058 g lipids/g within 80 min, using this technique whereas 0.032 g lipids/g yield was obtained with Soxhlet extraction path [48]. Anyhow, the viscosity and cost–intensively are some of the main disadvantages of this method [57].

2.1.4. Ionic liquids (ILs) solvent extraction

Ionic liquids are non–aqueous liquid state salts, mainly consist of ions/short lived ion pairs which involve comparatively bulky asymmetric organic cations of nitrogen containing ring structure mutually with smaller inorganic/organic anions [14]. These are also called as future/designer solvents since such materials innovatively replace some of the toxic organic solvents. **Scheme 1** shows the dissolition of cellular components in ionic liquids through hydrogen bonding. The liquid state of ILs could be maintained at 0–140 ºC and these have melting point of < 100 ºC. The cations and anions are responsible for the polarity of the ILs and effective lipid extraction, respectively and methanol is used to reduce the high viscosity of Ils [48]. They have comparatively no vapor pressure, low

toxicity, exact solubility, electrical conductivity and hydrophobicity [58–62]. Kim *et al*., [59] extracted microalgal lipids of about 12.5 % and 11.9 %, using the ionic liquid [Bmim] [CF_3SO_3]: 1–butyl–3–methylimidazolium trifluoromethanesulfonate–methanol/ [Emim][$MeSO_4$]: 1–Ethyl–3–methylimidazolium methyl sulfate–methanol in the ratio 1:1, respectively. After that the lipid was estranged by centrifuged. The yield was high when compared to the Bligh and Dyer's method [59]. The hydrophobic ILs such as [Bmim][PF_6]: 1–butyl–3–methylimidazolium hexafluorophosphate and [Bmim][Tf_2N]: 1,2–dimethylimidazolium bis(trifluoromethylsulfonyl)imide possess low extraction yield, when compared to hydrophilic ILs namely [Bmim][Cl]: 1–butyl–3–methylimidazolium chloride and [Emim][AC]: 1–Ethyl–3–methylimidazolium acetate [59]. Though, only a few studies were found in the literature [63–65]. Kim and Choi [59] extracted microalgal lipids from *Chlorella vulgaris,* eco–friendly using this method. It is predicted that this method is one of the best among the various extraction techniques. **Table 2** shows comparative studies on the cost and energy efficiency the different extraction methods.



Scheme 1. Dissolution of cellular components in ionic liquids (ILs).

Table 2. Comparison of the cost and energy efficiency involved in different methods of lipid extraction (Adopted from modified Ref.[61]).

Methods	Efficiency rating	Cost concerned	Energy necessity	Remarks
Bead beating	Moderate	Cost–effective	Energy intensive	Difficult to scale up
Electroporation	Very high	Cost–intensive; Comparatively cost–effective operation	Less energy	Appears promising but comprehensive pilot–scale studies have to be carried out
Expeller press	Low–moderate	High	Energy intensive	Heat generation and possible damage of the compounds
Isotonic extraction	Moderate–high			Less hazardous
Microwave	Very high			Easy to scale up
Organic solvent extraction				Intensive Fire, health and environmental hazards; Regulatory issues
Osmotic shock method	Moderate–high	Very high	Less energy	Appears promising but comprehensive pilot–scale studies have to be carried out
Pressurized solvent extraction	High	High because of cumulative costs incurred by use of solvent as well as use of pressurized nitrogen	Energy intensive	Environmental hazards; regulatory issues
Sonication method		High		Poor product quality due to the damage during the process
Supercritical CO_2	Moderate			Environmental and safety issues

3. Conversion technologies for microalgal lipids into biofuels

Biofuels have been received much attention as renewable, biodegradable and non–toxic basis of fuels. Moreover it is not required to do any engine modificatios to use biofuels. Microalgal species are believed to be a lipid based sustainable feedstock for biofuel production in which a number of biochemical, physical, metabolic and genetic engineering approaches have frequently been employed to stimulate biosynthesis of superior algal triacylglycerol lipids (TAGs) under stress conditions, forced either by physical stimuli: pH, temperature, light intensity and or by chemical stimuli: nutrients stress (nitrogen and/or phosphorous starvation/deprivation) and heavy metals (**Table 3**) [18,66–69]. **Table 3** depicts that the microalgal growth rate increases with intense irradiation and increasing temperature. High pH stress activates successfully the accumulation of lipids, but inhibits the algal cell cycle and high salinity slows the algal growth [69].

Table 3. The impact of physico–chemical stress on microalgal lipid accumulation (Adopted from modified Refs. [68,70,71]).

Microalgal species	Stress
Physical stress: Irradiation	
Chaetoceros muelleri	Increase in monounsaturated FAs with UV–A radiation
Chaetoceros simplex	Increase of saturated fatty acid with high UV–B irradiation
Nannochloropsis sp.	Increase in the content of total lipids, about > 31.3% with 100 μM m^{-2} s^{-1}/18h light intensity: 6h, dark cycle
	Increase in the saturated FAs : PUFAs ratio by UV–A irradiation
Neochloris oleoabundans	19–25% increase in the TAG content with 050–200 μM m^{-2} s^{-1} of light intensity
	Increase in the biomass concentration from 1.2–1.7 g L^{-1} with increase of light intensity from 050–200 μM $m^{-2}s^{-1}$
Pavlova lutheri	Increase in total lipid content with high light intensities stress
	23–78% increase in the TAG content with 09–19 W m^{-2} increase in light intensity
Scenedesmus sp.	Lipid and TAG content increased from 26–41% and 16–32%, respectively with increase in light intensity from 050–250 μM m^{-2} s^{-1}

Selenastrum capricornutum	Increase in linoleate FAs (18:02) with dark treatment stress Increase in biomass concentration 2.5–3.6 g L^{-1} with 050–250 μM m^{-2} s^{-1} increase of light intensity
Tetraselmis sp.	Increase of saturated as well as monounsaturated FAs and decrease of PUFAs with UV–B irradiation
Thalassiosira pseudonana	Increase of polar lipids (79–89% of total lipid) with 100 μM m^{-2} s^{-1}/ 12:12h, 100 μMm^{-2} s^{-1}/24:00h and 50 Mm^{-2} s^{-1} /24:00h light: dark, harvested at the logarithmic phase Increase of TAGs (22–45% of total lipid) with 100 μM m^{-2} s^{-1}/ 12:12h, 100 μM m^{-2} s^{-1}/24:0h and 50 μM m^{-2} s^{-1}/24:0h light: dark, harvested at the stationary phase
Temperature	
Chlamydomonas reinhardtii	56–76% of TAG content with 17–32 °C increased temperature
Chlorella ellipsoidea	Increase of unsaturated FAs with decreased temperature (chilling sensitivity)
Cryptomonas sp.	Increase of lipid productivity by 12.70% at 27–30 °C temp. range
Isochrysis sp.	Increase of lipid production by 21.70% within the temp. range of 27–30 °C
Monoraphidium sp.	Lipid content decreased from 33–9% with increase of temperature from 25–35 °C. Increased biomass concentration with increase in temperature from 25–30 °C but then decreased with further raise of temperature up to 35 °C
Nannochloropsis oculata	Increase in lipid production by 14.92% with temp. range of 20–25 °C Decreased lipid content from 15–8% with increase of temperature from 15–20 °C but then raised up to 14% with further increase of temperature to 25°C Increased specific growth rate with raise in temperature from 15–20 °C but then decreased with further raise in temperature to 25 °C
Rhodomonas sp.	Increase in lipid production by 15.50 % with temp. range of 27–30 °C
Scenedesmus sp.	Decreased lipid content from 35–22% with increase of temperature

	from 20–30 °C
Selenastrum capricornutum	Increase in oleate FAs (18:1) with temp. range of from 10–25 °C
Salinity	
Botryococcus braunii	Increased TAG content from 05–31% with an increased concentration of NaCl from 0–0.7 M Decreased growth rate, significantly with an increase of NaCl concentration from 0–0.7 M
Chlorococcum sp.	Increased lipid content from 10–30% with an increased concentration in NaCl from 0–2 % Concentration of biomass significantly decreased, around 4–folds with an increased concentration of NaCl from 0–2 %
Dunaliella salina	Increased concentration of C_{18} FAs with culture, transferred from 029.2 g L^{-1}–204.5 g L^{-1} NaCl (from 0.5–3.5 M NaCl)
Dunaliella tertiolecta	Increased TAG contents from 40–57%, with an increased concentration in NaCl from 0.5–1.0 M Similar growth rate over 0.5–1.0 M range of salinity
Hindakia sp.	3–folds higher lipid production, compared to N starvation by 8.8 g L^{-1} NaCl (0.15 M NaCl)
Nannochloropsis salina	Increased lipid contents, highest at 34 g L^{-1}
Nitzschia laevis	Increased neutral and polar unsaturated FAs with 10g L^{-1}– 20g L^{-1} increase of NaCl (from 0.17–0.34 M NaCl)
Schizochytrium limacinum	Increased greatly in saturated FAs (C15:0 and C17:0) with 09–36 g L^{-1}salinity at 16–30 °C range of temp.
pH	
Coelastrella sp.	TAG content increased with increase in pH
Neochloris oleoabundans	Increased TAG content, from 13–35% with increased pH from 8.10–10.0
Scenedesmus obliquus	TAG content increased with increase in pH
Scenedesmus sp.	Increase in TAG accumulation
Chemical stress: Nitrogen stress	
Chlorococcum infusionum	Lipid productivity : 15–40%
Chlorococcum oleofaciens	Lipid productivity : 127 (mg L^{-1} d)
Chlorella sorokiniana	Lipid production : 85 %

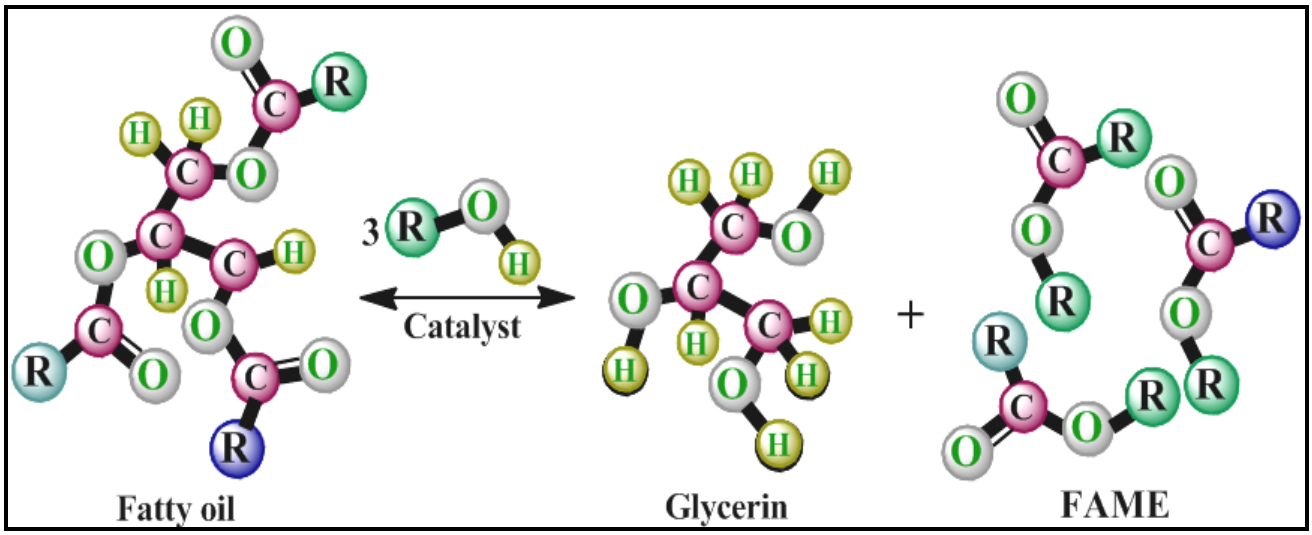
Chlorella sp.	Lipid productivity : 54 %
Chlorella vulgaris	Lipid productivity : 146 –78 %
Dunaliella tertiolecta	Fivefold increase in lipid fluorescence
	Increased lipid content from 10–48%, after 4–days nitrogen depletion
Neochloris oleoabundans	Productivity of lipids: 131 (mg L^{-1} d)
	Accumulation of TAGs, increased from 1.50–12.4% w/w
	Increased TAG contents from 08–26%, after 3–days nitrogen depletion
	Productiion of biomass decreased from 220–197 mg L^{-1} d^{-1}, after 3–days nitrogen depletion
Nannochloropsis sp.	Increased lipid contents from 39–69 %, after nitrogen depletion
	Decreased producton of biomass, after nitrogen depletion
Parachlorella kessleri	Lipid productivity : 0–29 %
Scenedesmus dimorphus	Lipid production : 111 (mg L^{-1} d)
Scenedesmus naegleii	Lipid productivity: 83 %
	Nitrogen and phosphorus stress
Scenedesmus sp.	Lipids content increased 30 % and 53 %, respectively
Chaetoceros sp.	Phosphorus limitation
Isochrysis galbana	Increase in total lipids
Phaeodactylum tricornutum	Increase in total lipids content
Monodus subterraneus	Increase in TAGs accumulation
Chlorella kessleri	Increase in unsaturated fatty acids
Sulphur stress	
Chlamydomonas reinhardtii	2–Folds increase in the phosphatidylglycerol
	Increase of TAGs
Silicon stress	
Cyclotella cryptica	Increase in total lipids from 27.6–54.1 %

Temperature tolerance is incredibly significant while choosing the algal strains, which response to nitrogen starvation/deprivation and is crucial for better–quality of the biofuel feedstock [67]. Among the macronutrients in the medium, nitrogen acts an imperative role, towards the microalgae lipids and carbohydrates accumulation. It can

modify chlorophyll and proteins/peptides as nitrogen source. Consequently, there is a conversion of algal carbon skeleton into lipid and carbohydrate. In addition, nitrogen starvation triggers the lipid accumulation [69]. There are nearly about 300,000 algal species, which contain 60 % lipids to yield biofuel [48]. The aviation industry has already been testeing the agal biofuels as a jet fuel without even the engine modifications [18] and they possess the benefits of suffeciently lower flash point as well as freezing point (**Table 4**), and higher energy densities with reduced emissions of CO_2, up to about 78 %, when compared to the recently utilized petro-derived fuels [68,72]. **Table 5** shows the pros- and cons- of algal derived biofuels. Due to high viscosity of such biofuels, they usually have been blended with conventional diesel and different methods, namely chemical conversion / transesterification, biochemical conversion and thermochemical conversion can be employed to minimize the viscosity.

3.1. Chemical conversion / Transesterification

Chemical conversion / Transesterification converts the raw and viscous microalgal lipids effectively to lower molecular weight fatty oil alkyl esters (FAMEs). It involves alcoholysis and interesterification of TAGs using a solvent in the presence of a catalyst (**Scheme 2**) which can be acidic/basic/enzymatic [1,47,48,73,74]. It can stimulate the rate of reaction by simultaneous esterification and transesterification of TAGs.



Scheme 2. Transesterification of viscous microalgal lipids.

Table 4. Comparison of properties of microalgal biodiesel and petro diesel to ASTM Standard (D6751–02) (Adopted from modified Refs. [70–72]).

Properties	Unit	Microalgal biodiesel	Petrodiesel	ASTM Standard method	Limits
Acid number	mg KOH/g	0.022–0.003	0.5	D 664	0.80 max
Boiling point	ºC	182–338	188–343	–	–
Calorific (heating) value	MJ/kg	41	40–45	–	–
Carbon residue	wt.%	–	0.05 max %mass	D 4530	0.050 max
Cetane number	–	48–65	40–55	D 613	47 min
Cloud point	ºC	–5.2 to 3.9	–35 to 5	D 2500	Report to customer
Cold filter plugging point	ºC	–	–7 to –2	–3 (max.–6)	0 to –15
Copper(Cu)	wt.%	0.042	–	–	–
Copper strip corrosion	(3h at 50 ºC)	1ppm	No. 3 max	D 130	No. 3 max
Density	kg/L	0.864	0.838	–	0.86–0.9
Flash point, closed cup	ºC	>160	75	D 93	130 min
Free glycerine	wt.%	0.009–0.014% (m/m)	–	D 6584	0.020
Fuel composition	–	C_{12}–C_{22} FAME	C_{10}–C_{21} HC	–	–
H:C ratio	–	1.81	1.81	–	–
Nickel (Ni)	wt.%	0.074	–	–	–
Phosphorus (P)	wt.%	<0.1 ppm	–	D 4951	0.0010
Pour point	ºC	–16	–17	–	–

Solidifying point	–	–12	–50 to 10	–	–
Specific gravity	kg/L	0.88	0.85	–	0.88
Stoichiometric Air/Fuel Ratio (AFR)	–	13.8	15	–	–
Sulfated ash	wt.%	<0.005	0.0015 max	D 874	0.020 max
Total glycerine	wt.%	0.091–0.102% (m/m)	–	D 6584	0.240
Total sulfur	wt.%	0.6–5.1 ppm	–	D 5453	0.05 max
Vacuum distillation end point	% distilled	–	–	D 1160	360 ºC max, at T–90
Viscosity (mm^2/s) at 40ºC	mm^2/s	4.519–4.624	1.9–4.1	D 445	1.9–6.0

Table 5. Advantages and disadvantages of microalgal based biofuels (Adopted from modified Ref. [1]).

Advantages	Disadvantages
More cost effective	Difficult to harvest due to microscopic size of most planktonic microalgae
Less water demand than land crops;	Salt precipitation on the bioreactor walls; Precipitates on pump sand valves;
Algae can grow on brackish water from saline aquifers or in seawater;	
This may solve some of the water availability problems	Presence of salts in the final biomass, which will likely have to be purged with steam
High growth rate; No sulfur content	Low biomass concentration
High–efficiency CO_2 mitigation	There is a need to develop techniques for growing a single species;
	Reducing evaporation losses and increasing the utilization of CO_2
Growing algae do not require the use of herbicides / pesticides	Drying and extraction is difficult;
	In dry extraction (drying the algae by using the sun or artificially), they receive a much lower yield;
	When using artificial dryers (using electricity) it takes more energy to extract than the energy you can get from the yield
Capability of performing the photobiological production of biohydrogen	Not cost effective
Non–toxic and highly biodegradable biofuels	Natural algal strands are not favoured probably due to their low productivity for target organisms;
	Most of microalgae species are unadapted to local climate sand outdoor cultivation

Easy to provide optimal nutrient levels due to the well–mixed aqueous environment as compared to soil	Limited genomic data for algal species
Ability to adjust harvest rates to keep culture densities at optimal levels at all times; Especially with the continuous culture systems, such as raceway ponds and bioreactors, harvesting efforts can be controlled to match productivity	Microalgae grown in open pond systems are prone to contamination
High levels of poly unsaturates in algae biodiesel is suitable for cold weather	Biodiesel performs poorly compared to its mainstream alternative
Continuous production avoids establishment periods of conventional plants	Large scale extraction procedures for microalgal lipids are complex and still in development stage.
A high per–acre yield (7–31timesgreaterthanthenextbestcrop–palm oil)	Produce sun stable biodiesel with many polyunsaturates
Algae oil extracts can be used as livestock feed and even processed into ethanol	A lack of data on large–scale cultivation
Algae–based fuel properties allow use in jet fuels.	Large–scale production could present many other drawbacks

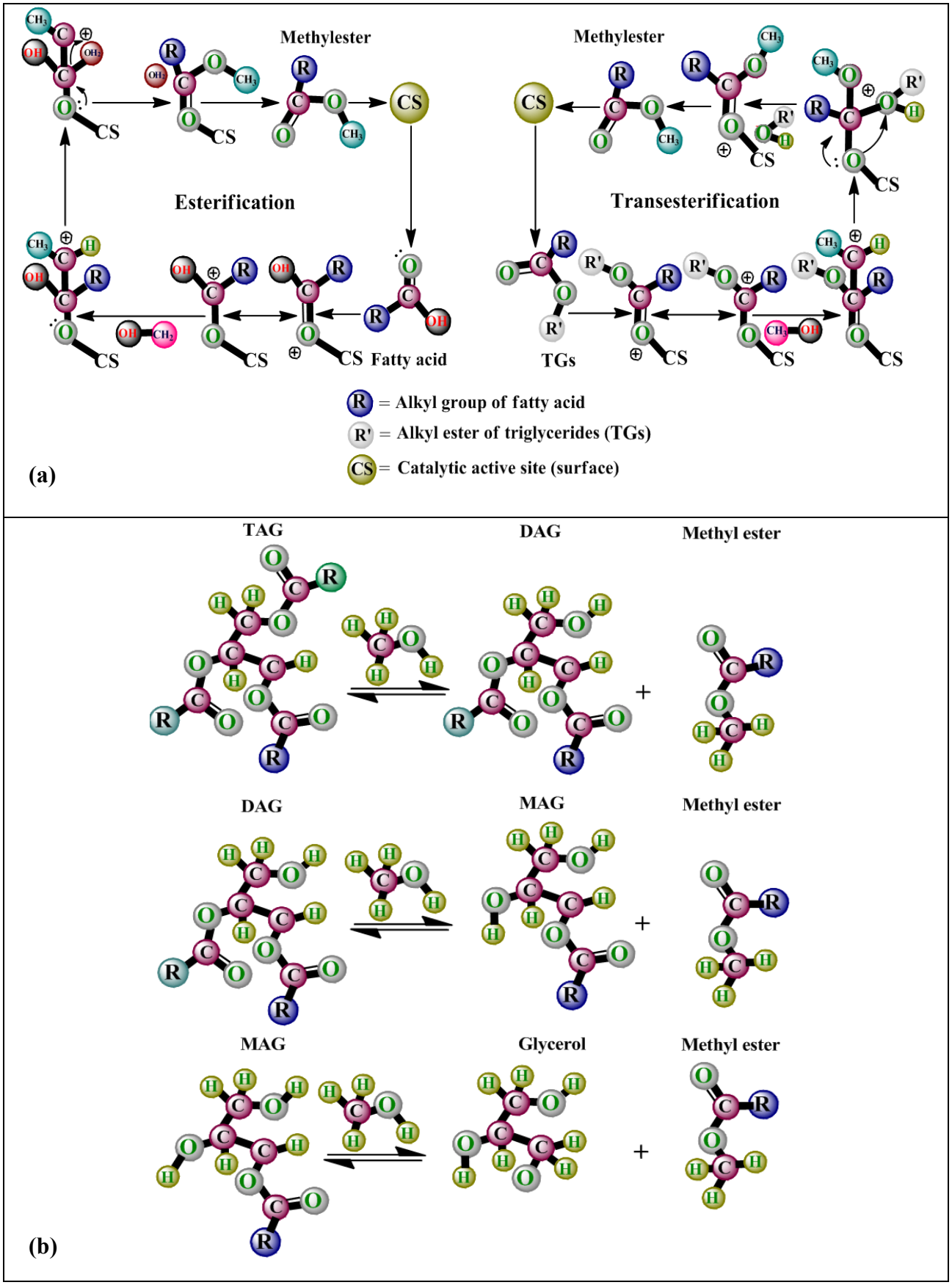


Fig.2. (a) Proposed mechanism of esterification and transesterification of algal feedstocks and (b) pathways of transesterification process respectively.

The proposed mechanism of this method is shown in **Fig. 2**. The disadvantages of this reaction are the recovery of the catalyst and moisture along with FFAs content, which affects the high quality of biodiesel production (4.3 MJ/L) [47, 48]. The use of acid/base/enzymes in the transesterification process with improved efficiency and cost effectiveness has been highlighted in the following sections. Transesterification is a sequence of three pathways: TAG is first converted to DAG and FAME; then DAG is converted to MAG and an additional FAME; finally, MAG is converted to glycerol which is the by–product and results in the last FAME (**Fig**. **2b**). The acyl acceptors (C=O) have been employed in this conversion are CH_3OH (methanol), C_2H_5OH (ethanol), $CH_3CHOHCH_3$ (isopropanol), $CH_3(CH_2)_3OH$ (n–butanol), $(CH_3)_2CHCH_2OH$ (*iso*–butanol), $(CH_3)_2CH(CH_2)_2OH$ (*iso*–amyl alcohol) *etc*. The solvents enhance the solubility of hydrophobic TAGs with hydrophilic alcohols and the novel solvents used in this process are $CH_3OCOOCH_3$ (dimethyl carbonate), $CH_3COOC_2H_5$ (methyl acetate) and $C_2H_5COOC_2H_5$ (ethyl acetate), which can effectively eliminate glycerol and micro–emulsion form at the time of biodiesel production.

3.1.1. Homogeneous catalysed tranesterification

The conventional microalgal oil production is still dominated by the use of homogeneous catalysed tranesterification by means of acid and base catalysed tranesterification.

3.1.1.1. Acid catalysed tranesterification

In the case of oils that contain excess free FFAs, can not be converted easily into biodiesel since the production of soap inhibits separation of the formed ester, glycerol and water wash [73]. In such cases, some typical Bronsted acid catalysts like H_2SO_4 (sulphuric acid), HCl (hydrochloric acid), $Fe_2(SO_4)_3$ (ferric sulphate), H_3PO_4 (phosphoric acid), BF_3 (boron trifluoride) and RSO_3H (organic sulphonic acid) are commonly employed. The steps involved are the protonation of carbonyl group of the ester and it results in the carbocation formation, followed by the generation of tetrahedral inetrmediate with alcohol by the nucleophilic attack, which loses a proton to form FAMEs (**Fig. 3a**). After the equilibrium attainment, the catalyst and the formed water contents can be removed by centrifugation process. It is relatively having slow reaction

rate (4000 times slower than the base catalysis) by means of the fact that alcohol to oil molar ratio but it can be prevented by addition of excess alcohol further, the acid catalysts are more corrosive [29,75]. The literature reveals that a molar alcohol:oil ratio of 30:1 in a temperature range of 55–80 ºC with 0.5–1 M% concentration of catalyst is obligatory to attain 99% conversion in 50 h [29,73] and 250 mg lipid molecules of *Chaeotoceros mulleri* produces 10 mg of FAME in the presence 0.6 N HCl–methanol acid catalysts [47]. It has the advantage that the biodiesel can be produced from low cost feedstock as such catalysts prevent the conversion of FFAs to excess soap [48]. The major disadvantages of using such homogeneous acid catalysts are the requirement of high temperature for long time reaction which corrodes the reaction vessel and removal of chemical waste, formed by neutralization of the acid catalysts. It is often desirable to substitute this process with heterogeneous acid catalysts.

3.1.1.2. Base catalysed tranesterification

Many base catalysts are effectively preferable and have been frequently employed in the process of homogeneous base catalyzed transesterification for the biodiesel production. The most common catalysts are sodium aluminate carbonate/hydroxide/methoxide/ethoxide ($NaAlO_2$/Na_2CO_3/NaOH/$NaOCH_3$/$NaOC_2H_5$), potassium carbonate/hydroxide/methoxide (K_2CO_3/KOH/$KOCH_3$) [29,76,77]. $Iron^{III}$ oxide and Cu/Zn/Sn/Pb oxides. However, these catalysts always produce water by the reaction with alcohols, which leads to the hydrolysis of ester to yield soap formation. The general proposed mechanism involves four main paths (**Fig. 3b**). The pre step involves the formation of alkoxide and then the formation of protonated catalyst, followed by the tetrahedral inetrmediate formation by the nucleophilic attack from the alkoxide on the carbonyl group of TAGs. In the third step, the formation of alkyl ester and the concerned anion of DAGs is terminated by deprotonation and regeneration of the catalyst. Sodium and potassium alkoxides are very effective catalysts but their hydroxides are cheaper than their alkoxides. However, these catalysts always produce water by the reaction with alcohols, which leads to the hydrolysis of ester to yield soap as shown in the following reaction (**Scheme 3**).

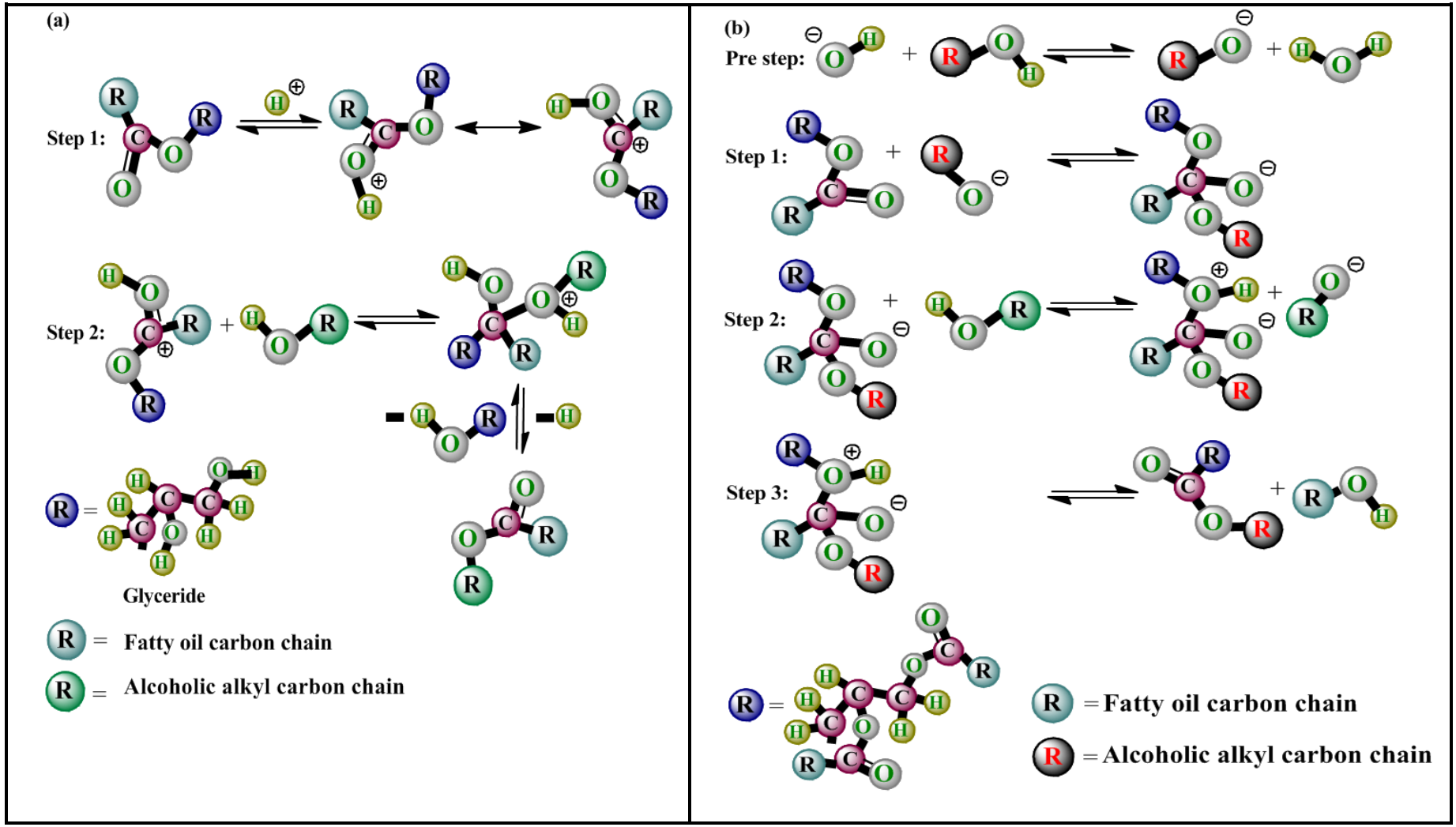
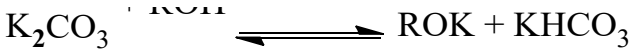


Fig.3. **(a)** Proposed acid and (b) base catalyzed pathways of transesterification process respectively.

Scheme 3. Hydrolysis of ester and formation of soap.

K_2CO_3 yields high amount of fatty acid alkyl esters since the formed bicarbonate, instead of water, reduces the ingredients that incite soap formation during transesterification. It is given in the following reaction as [77]:



Chaeotoceros mulleri produces 3.3 mg of FAME in the presence of NaOH base catalysts. Its disadvantages fall with the removal of chemical wastes, shaped by the neutralization of the base catalysts and high energy requirement. It has some advantages also like its low operating temperature, which is around 60 ºC and 90–98% conversion rate to FAMEs. Moreover, it has been recommended that such a reaction will be performed merely with purified microalgal oil by means of low FFAs *i.e.*, < 0.5 wt. %. It is hard to make up a combined route for simultaneous elimination and decontamination of FFAs as of glycerol by–product, which softens the FAMEs back into the solvent stage, since it prevents the large–scale production [48].

3.1.2. Heterogeneous catalysed tranesterification

Heterogeneous catalysis is an eco–friendly technique since the catalysts are non–corrosive, easily separable from the products, recyclable, cost–effective and very last longer than homogeneous catalysts. In general, the homogeneous catalyzed transesterification process has some drawbacks like (i) excess FFAs as well as high water contents of microalgae oil, (ii) high purity of the microalgae and (iii) side reactions like saponification and hydrolysis of microalga oil leads to difficult separation of biodiesel and glycerol from reactant mixture. Microalgal oils with higher FFA content will lead to form soap quikly, the formed soap leads to increase the viscosity and form a gel in the reactant mixture, which reduce the production of FAME yields. Thereby, the formed soap inhibits the separation of biodiesel from the reactant mixture as well as the separation of glycerin and wash water as it causes more wastewater from purification, which

consequently affects the activity of homogeneous catalysts [78]. Furthermore, the homogeneous catalysts are moderately miscible in both biodiesel as well as glycerol, which makes difficut to recovery of the biodiesel as well as glycerol from the reactant mixture, that leads to increase the viscosity of the mixture as well as increase the separation of product cost [79]. Hence, the heterogeneous catalysis is widely employed for transesterifciation of microalgal oil into biodiesel. It is an eco–friendly technique since the catalysts provides (i) high activity, (ii) specific selectivity, (iii) noncorrosive nature, (iv) easily separable from the products, (v) high recyclable, (vi) low cost–effective, (vii) ecofriendly with less environmental effects, (viii) water adaptability due to the presence of a large number of active acid or basic sites as compared to the homogeneous catalysts, *etc*. Recently, the numerous heterogeneous catalysts have been utilized commerically for the production of biodiesel like alkali–doped oxide (say Li doped CaO), alkali earth metal oxides (say CaO, MgO, SrO, BaO, *etc*), acid solids, mixed metal oxides and hydrotalcites [80,81]. In modern heterogeneous based catalytic transesterification process, both adsorption of the raectants (microalgal oil) followed by desorption of products (biodiesel and by–products) on the solid catalystic surface involves two types of mechanisms namely (i) Eley–Rideal (ER) and (ii) Langmuir–Hinshel–Wood–Hougen–Watson (LHHW) [82]. In the ER mechanism, the transesterification reaction is carried out by a direct pickup of reactant species from the surface by a liquid phase molecule, whereas in the LHHW mechanism, initially both the reactants as well as solid catalysts are undergoing adsorption (either physical or chemical adsorption), then reacted and followed by desorption of the products from the surface of the catalysts.

3.1.2.1. Heterogeneous acid catalysis

It is the potential substitute of homogeneous acid catalysis. Not many researchers reported such type of catalysis. Moreover, the solid acid catalysts are preferred over liquid acid catalysts as they possess multiple sites with different strengths of Bronsted / Lewis acidity (**Fig. 4a**). Bronsted acid catalysts are promising in promoting simultaneous esterification and transesterification with cheap feedstocks of higher FFAs concentration [1,48]. Both the reactants fatty acids and alcohol are very lipophilic in nature. In the process, one inaccessible Bronsted acid site is surrounded by a hydrophobic atmosphere. The adsorption of hydrophobic tail of the FFAs is parallel to the hydrophobic surface

(**Fig. 4b**) [83]. Then, there are a few acid sites in the locality and the adsorption of FFAs is perpendicular to the surface with the tails, forming a restricted hydrophobic background. Finally, very acidic and/or hydrophilic structures, adjacent to acid sites and/or hydroxyl groups, the formed water by–product from the esterification may be adsorbed on the surface, while the catalyst might lose its activity as the layer of water prevents the contact of FFAs and alcohol to the catalytic surface. A Lewis acid catalyst is more vigorous when compared to the Bronsted acid catalyst, while it is hazardous to poisoning from water and/or FFAs.

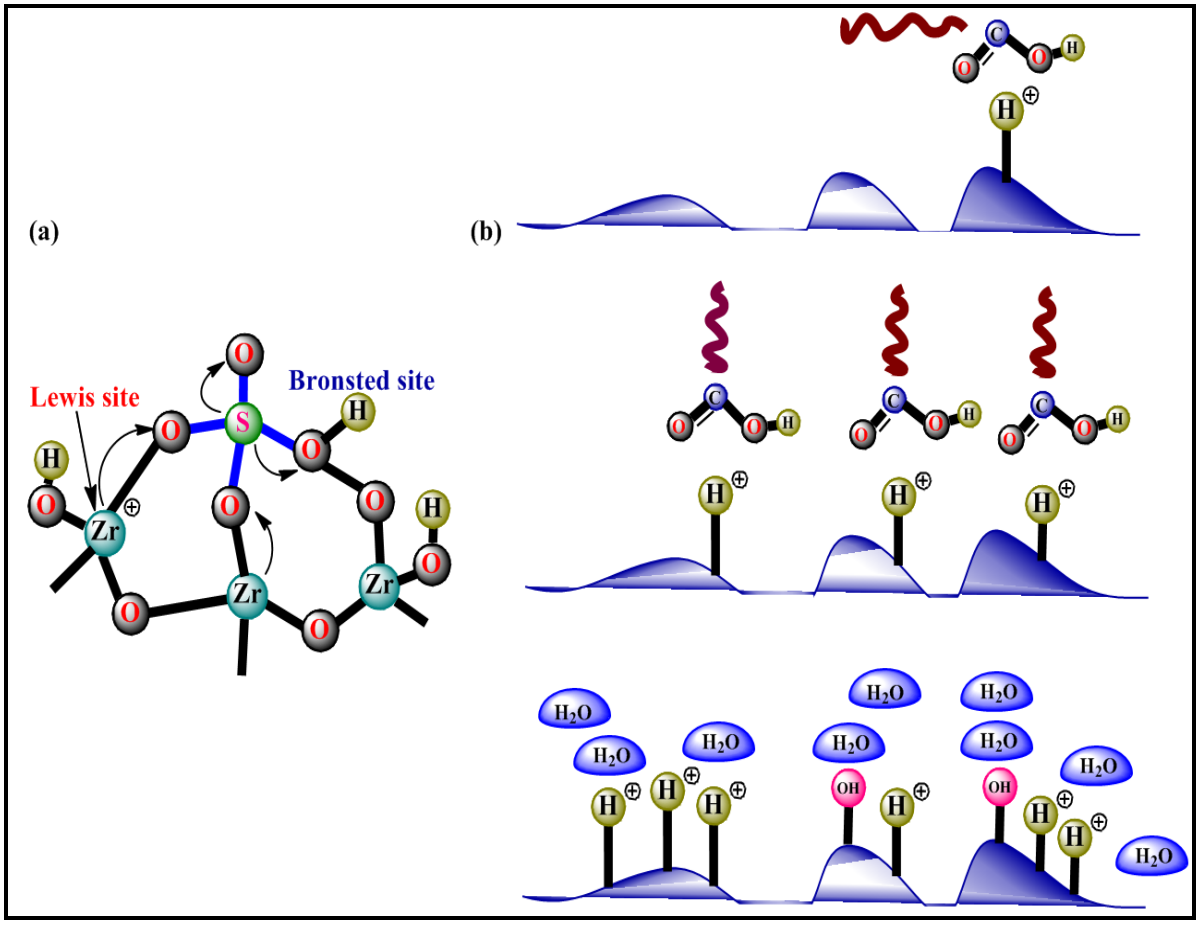


Fig. 4. (a) Predicted Bronsted and Lewis active sites in sulphonated zirconia solid acid catalyst and (b) Influence of the surface hydrophobicity in the solid acid catalytic activity respectively (Adopted from modified Ref. [47]).

The solid acid catalysts can be easily separable and adoptable to recycling. In addition, they catalyze the TGAs transesterification as well as FFAs esterification. The literature has proved that solid acid catalysts namely sulfated/tungstate zirconia, sulfated tin oxide and sulphonated polystyrene/saccharides play an effective role in the microalgal

biodiesel production of 90.2 % at 350–400 ºC and 2500 psi (17.23 Mpa) [1,48]. Its disadvantages are low reaction rate and possible unpleasant side reaction. Furthermore, its mechanisms have not fully understood [73].

3.1.2.2. Heterogeneous base catalysis

Heterogeneous base catalysts are more active than homogeneous acid catalysts. Alkaline earth metal oxides have been used as catalysts for glycerol transesterification with TAGs. A variety of heterogeneous base catalysts, employed in the industries, are bimetallic Sn–Ni, exchange resins, organometallic compounds, $P(RNCH_2CH_2)_3N$, multi–functionalized, organosulphonix–acid functionalized mesoporous silica and mixed oxides [48]. *Nannochloropsis* sp. yields biodiesel using Mg–Zr catalyst [48]. Iron supported on mesoporous silica nanoparticles (Fe–MSN) catalysis involves Mars–Van Krevelen mechanism, in which the surface of iron is partially oxidized by the carboxylic group of the substrate during transesterification. Kandel *et al.,* employed mesoporous silica Fe–MSN nanoparticles in the conversion of microalgal feedstock to biodiesel [84]. Their proposed mechanism involves the conversion of oleic acid into *n*–nonadecane and *n*–heptadecane from the intermediates of 1–nonadecanol and octadecanal, respectively (**Fig. 5a**). The mechanism of oleic acid hydro–treatment with Fe–MSN fits into the model proposed by Langmuir– Hinshelwood, in which two reactants bind at two different sites (**Fig. 5b**). Arvindnarayan *et al*. reported the bio–oil production from *Botryococcus braunii* by transesterification in the presence of Ni/H_2 catalystsupported with N(II)–Schiff base promotor [70,71]. The algal feed stock consists of major unsaturated constituents of C_{16}, C_{18} andC_{20} and minor saturated fatty acids constituents of C_{14}, C_{16} and C_{18} as di/triglycerides. The catalyst on hydro–treatment (30 bar H_2) catalyzes the hydrogenation of unsaturated fatty acids to *trans* unsaturated and saturated acids, which is further hydrogenated to aldehyde and turn to alcohol intermediates as a result of algal bio–oil production (**Fig. 5c**). For example, *cis*–oleic acid on hydrogenation gives saturated stearic and unsaturated *trans*–vaccenic acid. The di/triglycerides also undergo hydrotreatment to produce higher alkanes and were cracked into lower alkanes(**Fig. 5d**). Furthermore, this base catalyzed tranesterification has proved higher reaction rate, during tranesterification. Both the proposed pathways are compaeable (**Fig. 5e**) [70,71,84]. Moreover, **Table 6** depicts the reported list of heterogeneous solid acid/base catalysts [84].

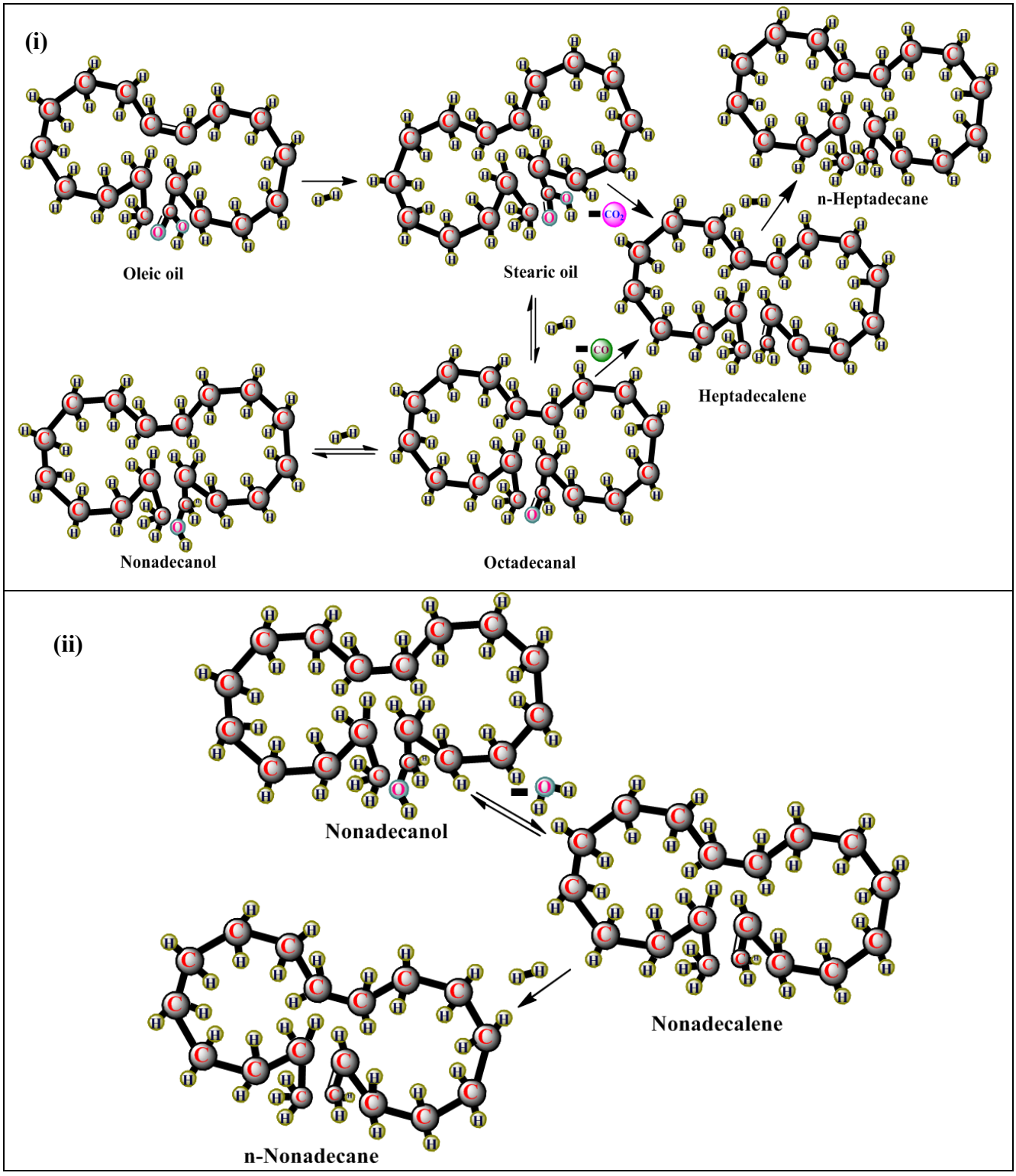


Fig. 5a. Proposed heterogeneous base catalysis using mesoporous silica Fe–MSN nanoparticles Fe–MSN in the production of biodiesel from algal feedstock. (i) Conversion of oleic oil to nonadecanol and (ii) Conversion of nonadecanol *n*–nonadecane respectively (Adopted from modified Ref. [84]).

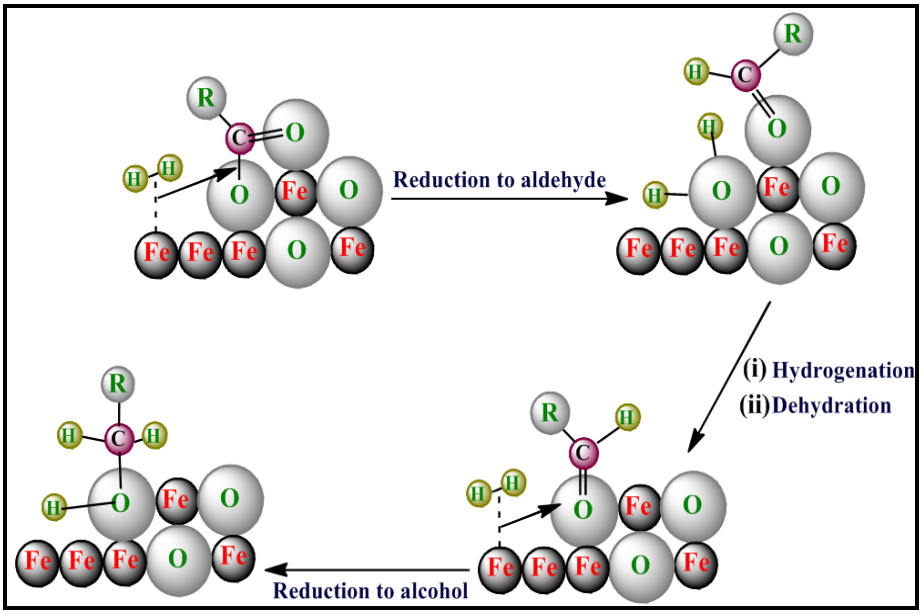


Fig. 5b. Langmuir–Hinshelwood model of oleic acid hydrotreatment with Fe–MSN (Adopted from modified Ref.[84]).

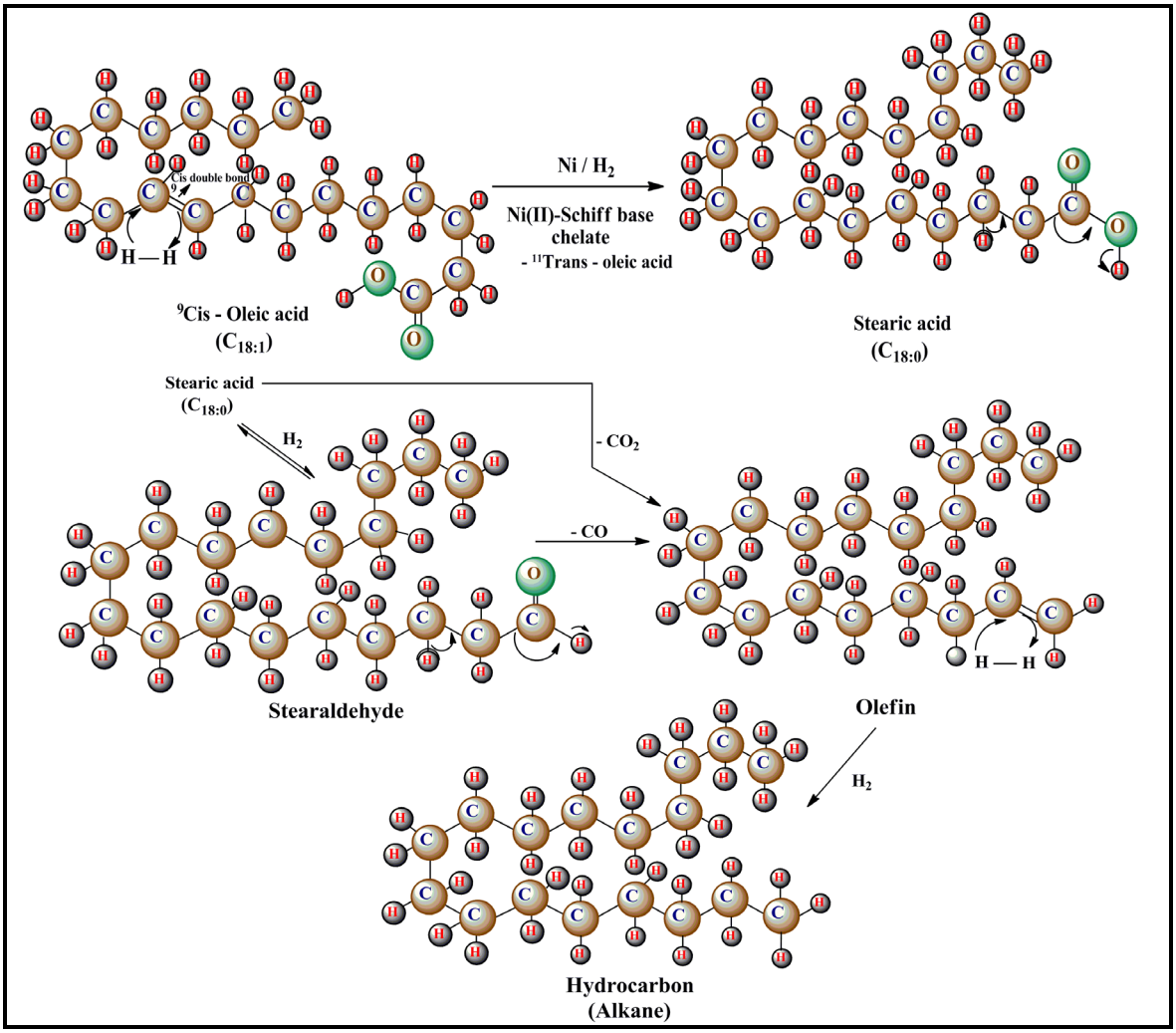


Fig. 5c. Proposed mechanism for formation of algal bio–oil from algal feed stock. (Adopted from modified Ref. [70]).

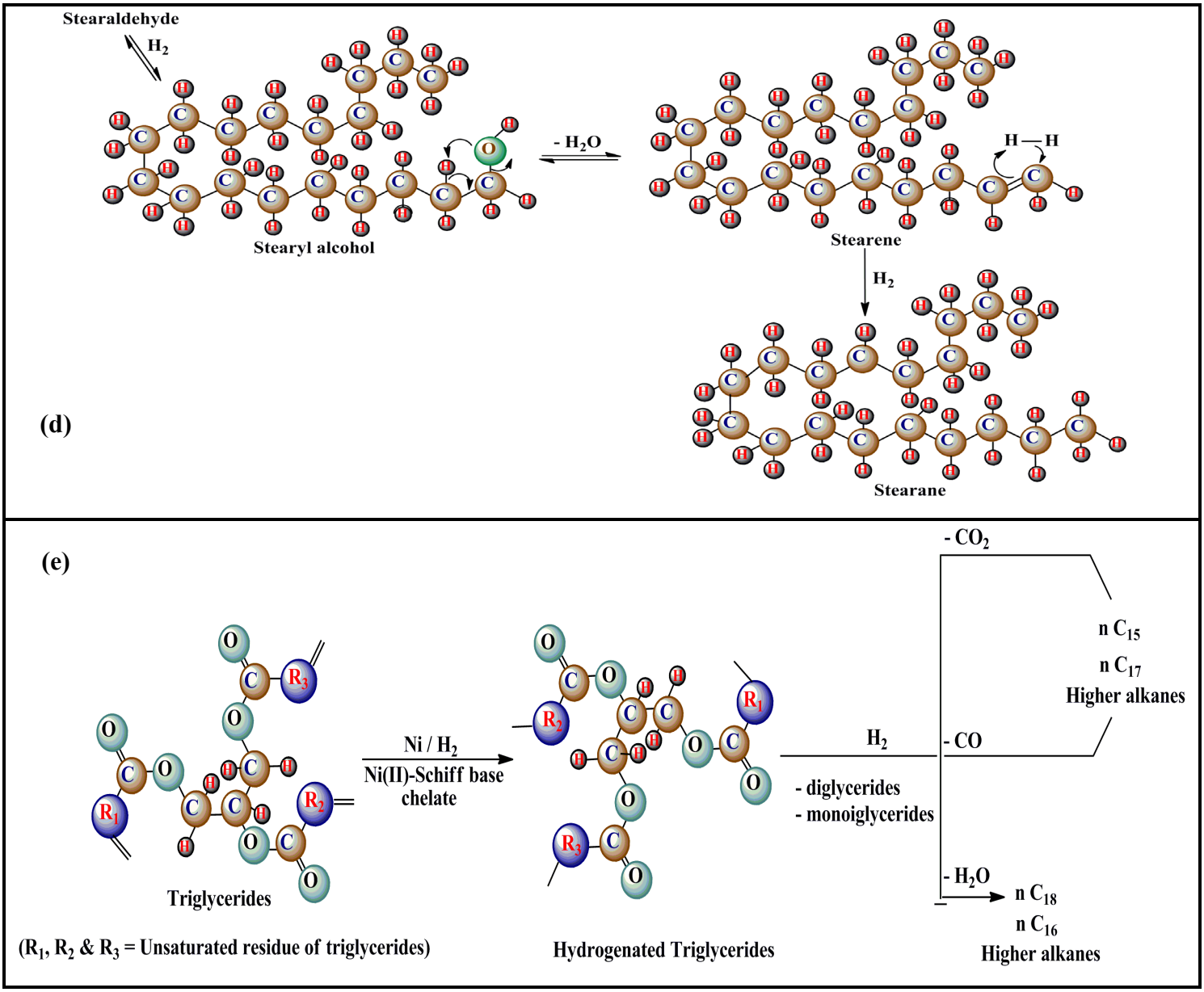


Fig. 5. **(d)** Proposed mechanism for formation of algal bio–oil from algal feed stock and **(e)** Mechanism involved in algal oil production due to tri / diglycerides respectively (Adopted from modified Ref. [70]).

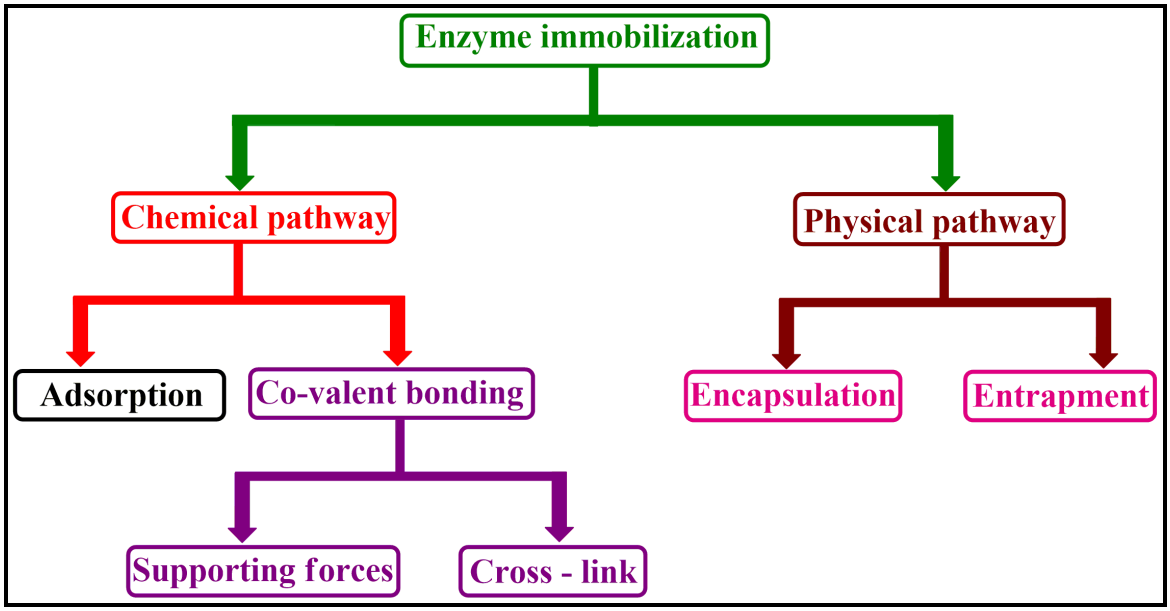
Table 6 The list of some significant heterogeneous solid acid/base catalysts.

Solid acids	Solid bases
Zinc acetate supported over silica: $Zn(Ac\ O)_2$–SiO_2 & Copper supported over silica: Cu–SiO_2	Oxides of group IIA elements: CaO, MgO, SrO & BaO; Carbonates of group IA elements: K_2CO_3
Free sulphated tin oxide supported over alumina: SO_4^{2-}–SnO_2/ Al_2O_3 & Free sulphated tin oxide supported over silica: SO_4^{2-}– SnO_2/SiO_2	Carbonates of group IIA elements:$CaCO_3$, $MgCO_3$, $SrCO_3$, $BaCO_3$ & Li–promoted oxides of group IIA elements.
Heteropoly acids and their derivatives: $H_3PW_{12}O_{40}$ –Phosphotungstic acid & $H_4SiW_{12}O_{40}$–Silicotungstic acid	Metal complexes: Schiff base meal complexes
Organosulphonic acids supported over mesoporous silica /alumina: R–SO_3H–SiO_2/Al_2O_3	Free and mixed transition metal oxides: ZnO, CuO, $CaLaO_3$,$CaCeO_3$, $CaZrO_3$, $CaMnO_3$ &$CaTiO_3$
Nafion (sulfonated tetrafluoroethylene based fluoro polymer–copolymer) : $C_7HF_{13}O_5S\cdot C_2F_4$	Basic zeolites, Mg–Zr &Aluminates of Zinc (Spinel): $ZnAl_2O_4$
Sulfated zirconia mixed with other transition metal (M) Oxides : SO_4^{2-}–ZrO_2/WO_3 & SO_4^{2-}–ZrO_2/MO_3	Cs–exchanged sepiolite: $Mg_4Si_6O_{15}(OH)$& Iron supported on mesoporous silica nanoparticles (Fe–MSN)
Sulfated zirconia supported over silica: SO_4^{2-}–ZrO_2 / SiO_2 / Al_2O_3	Hydrotalcites: (Mg–Al)& bimetallic Sn–Ni
Microporous aluminosilicates (Zeolitic materials): HeY, HBeta, ZSM–5, H–MOR, ETS–10 and ETS–4.	Quanidine anchored cellulose or other Polymers,Metal generated salts of primary amino acids:Organometallic compounds: $P(RNCH_2CH_2)_3N$

3.2. Enzymatic tranesterification

Homogeneous as well as heterogeneous acid/base catalysis can be effectively replaced by a suitable biocatalyst *i.e.*, enzyme–based tranesterification [85]. These

reactions possess tolerancy towards cocentration of FFAs and water. Its mild reaction condition along with moderate temperature (35–45 ºC) and pressure requirement make it an attractive substitute for homogeneous acid/base catalysis. Further, no saponification occurs, so there is no need for additional purification/separation processes for the formed products / wastes [48,77]. Extra/Intra cellular non–stereospecific lipases extracted/remained inside from/in *Mucormiehei*, *Rhizopusoryzae*, *Candida antarctica*and *Pseudomonas cepacia* belong to a group of hydrolytic enzymes and are the most commonly employed enzymes. Based on region–selectivity, the lipase activity falls under three categories: hydrolysis on R_1/R_3 ester bond of TAGs *i.e.*, S_N–1,3–specific; hydrolysis on R_2 ester bond of TAGs *i.e.*, S_N–2–specific; hydrolysis on non–specified bond positions of TAGs *i.e.,* non–specified [48]. The enzymes can be denatured and destabilized by the products of transesterification and are expensive. Additionally, the reusability of the enzyme is based on an immobilized structure and indispensable cost–effective analysis. Further, the literature reveals that usage of the solvents may be toxic, inflammable and have to be eliminated from the ester. Therefore, supercritical fluids, eco–friendly solvents have been recommended as alternative sources [48,76]. Moreover, the cells of *Rhizopus* species are used as an effective catalyst in the production of algal biofuels [1]. Enzyme immobilization can be carried out by chemical and physical pathways **(Scheme 4)**.



Scheme 4. Enzyme immobilization pathways.

Recent research has interestingly been demonstrated about a suitable biocatalysis in the transesterification of *Chlorella vulgaris*, which is a modified nano form of super magnetic biocatalysis to yield biodiesel from its microalgal bio–oil. The biocatalysis involves a kind of magnetic core namely MNP of composition formula Fe_3O_4, consequently has functionalized with MNP–AP named, 3–amino propyl triethoxysilane, as well as MNP–AP–GA; glutaraldehyde and an enzyme *Rhizopus oryzae lipase* immobilization. The advantages of biocatalysis mainly depend on the functional groups as these functional have been provided a wider space for enzyme activity like grafting of AP and GA on MNP surface to produce the accessibility with more active sites. Further, the chance of enzyme leaching can be prevented by the function of biocatalyst active groups, for instance the existence of the dipole–dipole interactiosn in between the enzyme and MNP systems by means of $–NH_2$ (amino) groups of AP and –HC=O (aldehyde) of GA, apart from the formation of covalent linkage between –HC=O groups of GA and $–NH_2$ groups of ROL to increase the air stability of biocatalyst throughout the reaction. The literature says that such a biocatalyst can be stable up to five cycles along with the combination of free as well as immobilized lipase thereby about 57.2% yield of biodiesel from the algal lipid molecules can be attained [86]. Chen *et al.,* [87] investigated the biocatalysis of microalgal lipids for two steps transesterification and esterification by the utilization of *Aspergillus niger* derived free lipase (first step) and *Candida antarctica* derived immobilized lipase (second step). It was found that the solvent–free second step was performed for the esterification of FFAs. Moreover, the optimization of certain reaction conditions *viz*. strategy on addition of methanol as a reactant, addition of a water–absorbent as molecular sieve, methanol to lipid stoichiometric ratio, dosage of enzyme as biocatalyst, and temperature to increase the activity of the biocatalyst as well as the yield of biodiesel and it was nearly about 78% for the first step, while about 97% for the second step. A comparable study was performed with *Scenedesmus obliquus* lipids by employing *Pseudomonas fluorescens* derived free and *Candida* sp. derived immobilized lipase. A 90% yield of biodiesel was procured by means of *Pseudomonas fluorescens* derived immobilized lipase over a batch reaction of four cycles [86]. Bharathiraja *et al.,* [88] investigated the yield of biodiesel from three marine macroalgae namely *Enteromorpha compressa*, *Gracilaria edulis* and *Ulva lactuca*. Then, they compared both the activity and stability of two dissimilar biocatalysts *viz*. a recombinant *Pichia pastori*s derived intracellular *Cal* A and *Cal* B lipase and *Candida antarctica* derived immobilized lipase through the entire reaction of the production of biodiesel as

the biocatalyst was secured by means of a polar cellelular membrane, which restrains the free accessibility of the superfluous reactants on the active sites to increase the biodiesel yield as well as the stability of biocatalyst, while a higher activity of the immobilized lipase was achieved by means of denaturation with solvent ethanol. Another research compared the activities of both *Aspergillus* sp. derived free and *Candida* sp. derived immobilized whole–cell lipases in the case of for *Scenedesmus obliquus* lipids conversion to biodiesel and these biocatalysts were reusable for two cycles of the reaction. The prolonged accessibility with the immobilized lipase can be achieved by purity and polarity of the algal lipids, the methanol reactant and solvent hexane [86]. Moreover, such features only determine the reaction rate of biodiesel conversion and it was confirmed by an investigation on *Rhizopus oryzae* derived lipase biocatalyst in the conversion of *Nannochloropsis gaditana* lipids to yield the biodiesel *via* two approaches. First approach involves the extraction of lipids, using v/v ethanol–hexane mixture of and consequently was purified by means of crystallization with acetone, whereas the second approach involves the same steps but hexane was the only solvent for lipid extraction. Afterwards, the polar lipids were precipitated in the form of phospholipids at the same time as neutral saponifiable lipids solubilized using acetone in their purest form with less polarity in the presence of biocatalyst *viz*. *Rhizopus oryzae* derived immobilized lipase. Whereas, less conversion efficiency and also less stability were observed with non–immobilized *Rhizopus oryzae* derived whole–cell biocatalyst, here v/v ethanol–hexane mixture was used for the lipid extraction as the polar lipids eventually deactivates the immobilized *Rhizopus oryzae* derived lipase, sooner. But it was confirmed that the lipid extraction using hexane as the only solvent provides the stability up to three cycles of batch reaction and increased activity of *Rhizopus oryzae* derived non–immobilized whole–cell biocatalyst due to the presence of polar lipids [86]. Lee *et al*., [89] enhanced the activity of immobilized lipase (Novozyme 435) biocatalyst for the transesterification *Chlorella* sp. derived triglycerides, using DMC (dimethyl carbonate) as the reaction solvent medium and it acts as (CH_3–C=O–) acyl acceptor. The biodiesel yield can be improved by a simultaneous formation of CO_2 (carbon dioxide) since it shifts the reaction equilibrium towards the product formation side. Consequently, it was observed that the very high stability of immobilized biocatalyst even after over ten cycles of batch reactions [86].

3.3. *In–situ* tranesterification (Direct tranesterification)

This direct transesterification/reactive extraction is a process which involves simultaneous extraction and transesterification processes as in the case of SCM (supercritical methanol) with the advantages of minimum use of solvents, simpler products separation and gained reaction time [90]. Biodiesel yield is affected with the wet algal feedstock used, while better yield is observed for dry biomass due to effective percolation of chemicals. Wahlen et al. employed this technique to the wet microalgal biomass with 90 % water content *using Chaetoceros gracilis, Phaeodactylum tricornutum, Tetraselmis suecica, Neochloris oleoabundans, Chlorella sorokiniana, Synechocystis* sp*., Synechococcus elongatu*s and a mixed culture from municipal wastewater lagoon and found that the yield was ranged from 40 (*Synechococcus elongatu*s)–80 % (*Chaetoceros gracilis*) [90]. It has the advantage that it is the best method for producing biodiesel from both pure and mixed cultures of microalgae species, which consumes less volume of solvents when compared to the traditional transesterification [91]. The disadvatages are the requirement of dewatering and drying before extraction, the necessity of high amount of methanol– sulphuric acid and high energy demand. The steps involved in this process are shown in **Fig. 6** [92,93]. Here, two types of approaches have been discussed; they are mechanically and chemically catalyzed *in–situ* transesterification.

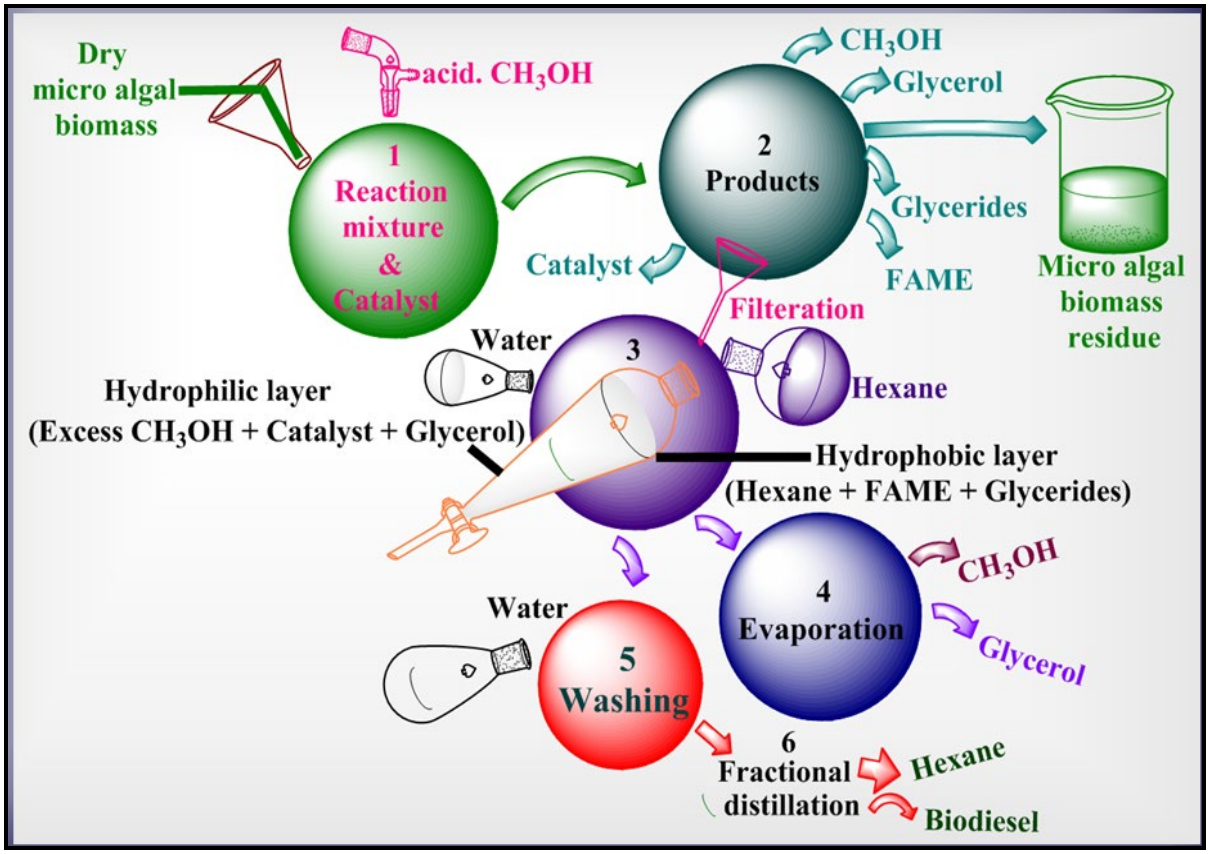


Fig. 6 Steps involved in in–situ transesterification for biodiesel production (Adopted from modified Ref. [93]).

3.3.1. Mechanically catalysed *in–situ* tranesterification

Chemical interactions play an imperative role in mechanically catalyzed transesterification, which also involves modest mechanical processes also it depends on the reaction parameters such as reaction time and temperature. It yields low, when compared to the solvent extraction, but the involvement of mechanical forces increase the lipid yield to some extent. Further these forces improve the solvent penetration towards the cell wall. Effective lipid extraction can be achieved by the addition of certain strong acids/bases such as sulphuric acid/sodium hydroxide. Patil et al. extracted 80.1 % of algal lipids dried from *Nannochloropsis* species *via* microwave–assisted *in–situ* transesterification by the adaptation of algae–to–methanol ratio of 1:12 (w/v), KOH concentration of 2 % by weight, and a reaction time of 4–5 min at 60–64 ºC [94]. In addition, Ehimen *et al*., improved the *in–situ* transesterification of *Chlorella* sp. by sonication (24 kHz) and the yield was 91–96 % with the time duration of 20 min–2 h [95]. Higher yield was reported in 0–2 h at 60 ºC when the reaction was ultrasound–

assisted transesterification in which algae to methanol molar ratio is much higher (1:105–1:315), but lower than that used in microwave–assisted transesterification on the conversion with w/v is (1:1.3–1:4) [48,59].

3.3.2. Chemically catalysed *in–situ* tranesterification

Drying of the microalgal biomass is required before employing the chemically catalyzed *in–situ* transesterification, since there are possibilities of inhibition due to hydrolysis by means of the reaction of water with TAG to yield DAG and FFA consequently esterification instead at the time of transesterification process [59,96]. In recent years, chemically catalyzed *in–situ* transesterification through co–solvents and ionic liquids have been carried out for effective extraction to improve FAME yield. In the co–solvent systems, which serve as the eco–friendly lipid extractor, the mixture of two organic solvents must be miscible with methanol, immiscible with water, performed by a strong acid/base in the absence of water. Xu *et al.,* and Lee *et al.,* employed toluene/toluene system and DCM/methanol for *Spirulina*, *Cladofora* and *B. braunii* respectively [97–99]. In the case of ILs, effective bio–oil extraction occurs. Moreover, ILs have many advantages, which have already been mentioned in the above discussion, especially their recyclability prevents the fabrication of wastes [58,59]. The conversion hypothesis involves biodegradation of lignin from lignocellulosic microalgal biomass at ambient pressure towards cost–effectiveness. The ILs also possesses the capability to mobilize both the acidic and basic catalysts and to recycle the catalysts without further addition of chemicals. Young et al. investigated this reaction with [Emim] derivatives, methanol and acetyl chloride to get the yield of 85–100 %, using the molar ratio 1000:1–3000:1 towards alcohol: TAG [100]. The only disadvantage falls with the cost–intensivity. **Table 7** summarizes different extraction methods with their lipid productivity.

Microalgal cell structure was investigated before and after the transesterification process to get better drying of microalgal biomass for enhanced yield. It was observed with SEM analysis that up to 150 ºC/50 min pretreatment, the algal cell morphology was very comparable to untreated algal cells and some intercellular constituents were identifiable at 200–225 ºC. After 225 ºC/10 min, a gradual modification in morphology was observed and results areshown in **Fig.7** for *Chlorella vulgaris, Chlorella* sp*.,* FD and SD green algae [70,71,99]. It is evident from the results that it could clarify the

increasing/decreasing yield of treated algae as a function of lipid retention. In addition, is is suggested that the extracted cells may also be employed again to tranesterification process before typical burning and discarding the algal residue [24,77].

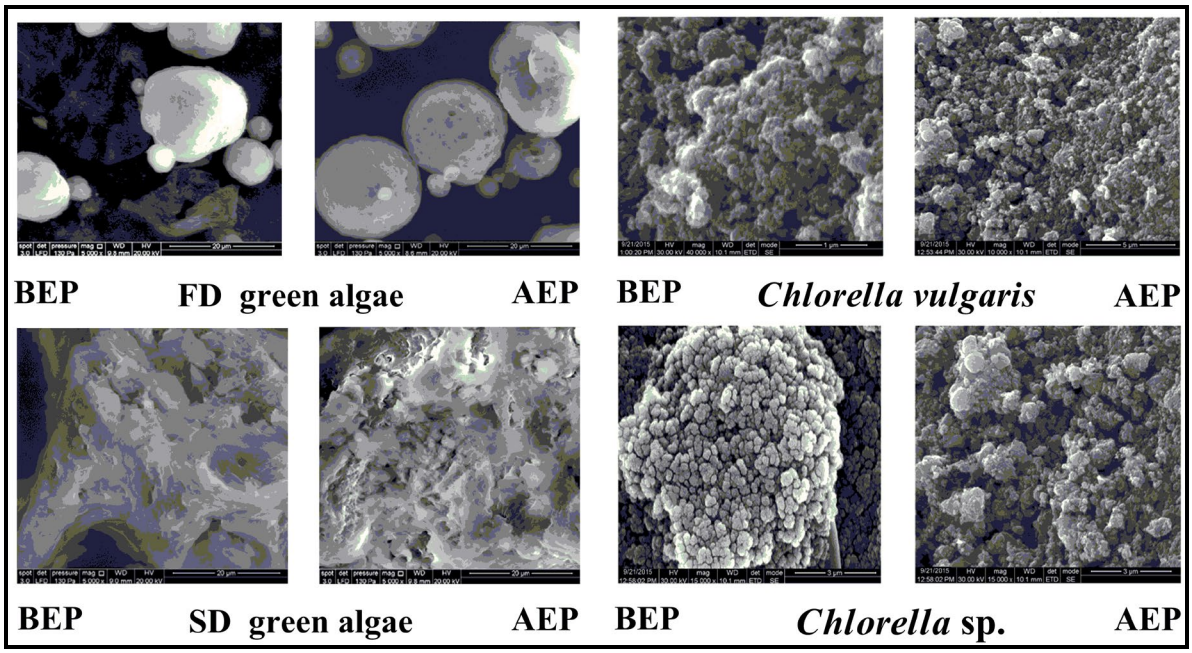


Fig. 7. SEM photographs of morphology of microalgal cells BEP (before extraction process) and AEP (after extraction process) respectively (Adopted from modified Refs. [70,71]).

Table 7 Different oil extraction techniques with their lipid productivity (Adopted from modified Ref. [47]).

Extraction process	Technique	Circumstances	Lipid productivity (%)
Chemical method	n–Hexane–Soxhlet extractor	–	95–99
	Chloroform, ethanol; deionized water	8h	49± 72.4
	Aqueous oil	2h	38
	Ultrasound assisted aq. oil	050 ºC; pH=9; 6h	67
	Acetone; n–hexane	–	–
	Subcritical ethanol	20:1(v/w) Ethanol:alga;105 ºC; 100 min	73
Enzymatic method	Aqueous enzymatic oil–cellulase/hemicellulose	060 ºC; pH=4.5; 2h	
	Aqueous enzymatic oil–alk. protease	060 ºC; pH=7.0; 2h	86
		050 ºC; pH=9.0; 6h	64
	Ultrasound–alk. protease	–	74
Mechanical methods	Engine driven	–	68
	Screw press	–	79–80
	Ram press	–	63
Microwave method	B20 co–solvent	080 ºC;1.2kW; 2.45GHz;15 min hold; 30 min cool–down	13±0.8
		100 ºC; 1.2kW; 2.45GHz;15 min hold; 30 min cool–down	17 ± 1.6
		120 ºC; 1.2kW; 2.45GHz;15 min hold; 30 min cool–down	12 ± 2.0
	B40 co–solvent	080 ºC;1.2kW; 2.45GHz;15 min hold; 30 min cool–down	32 ± 6.0
		100 ºC; 1.2kW; 2.45GHz;15 min hold; 30 min cool–down	38 ± 8.0

		120 ºC; 1.2kW; 2.45GHz;15 min hold; 30 min cool–down	57 ± 8.0
	Chloroform + ethanol	080 ºC;1.2kW; 2.45GHz;15 min hold; 30 min cool–down	16 ± 0.7
		100 ºC; 1.2kW; 2.45GHz;15 min hold; 30 min cool–down	46 ± 2.2
		120 ºC; 1.2kW; 2.45GHz;15 min hold; 30 min cool–down	53 ± 3.0
Super critical method	*SC*–CO_2	080 ºC; 250 bar	14

4. Non–catalytic tranesterification

The catalysts diminish the time requisite of transesterification, while they uphold some barrier at the time of purification of the products. To evade such a drawback, observed with catalysts, non–catalytic process, that involves supercritical alcohol, has been employed for the efficient single step conversion of algal lipids to ester within a short duration [48]. Methanol is used at a critical temperature for the simultaneous extraction and transesterification for algal lipids of wet algal biomass in a short reaction time which is cost–effective and in the way of ease of separation product [94,101]. It involves the principle that at high temperature, water–methanol mixture shows both hydrophobic and hydrophilic distinctiveness. Patil et al. found from *Nannochloropsis oculata* that under optimum conditions, SCM yields 84.2 % in 25 min. at 250 ºC by an algae to methanol ratio of 1:8 (w/v). Transesterification with SCM has not yet been extensively studied [94]. The main drawbacks of this method are the high energy necessity, hard recovery of by–products and the requirement of wastewater treatment. Yew *et al.,* [102] performed a thermal–assisted (at 36.5 °C) Fenton reaction for the optimum recovery of microalgae *Chlorell sorokiniana* derived lipids (lipid concentration at 977.41 mg/g ; lipid recovery at 53.31%) using non–catalytic transesterification through ultra–sonication for large scale production. Though, this kind of non–catalytic transesterification with ultrasound is rapid and cost–effective, it is applicable only for the microorganism with cell wall membrane.

5. Pros and cons in algae transesterification

Generally for algae lipid transesterification, hereby two methods have mentioned. The first method involves a two–step conventional process. The extracted algae lipids can be converted to biodiesel via catalytic transesterification. The second method involves single step / direct i.e., in situ conversion of the selected algae biomass to biodiesel with the presence of an acid catalyst. The two methods mainly depend on price as well as time duration by the processes, yield of biodiesel and selectivity [86]. In the case of two–step conventional process, recovery of bio–oil from the selected algae biomass can be performed in the first step via extraction, followed by solid–liquid separation. Recovery of algal bio–oil significantlly conists of drying of algae biomass, cell wall disruption, and extraction using a suitable solvent mwdium. Drying of algae before extraction *via*

centrifuges, freeze drying, heated–drum drying, spiral–plate, spray drying, pressure, sun drying, vacuum and membrane filtration involves dewatering process that obviously improves the efficiency of extraction process, since the non–existence of water content in the selected algae biomass enhances the mass transfer between algae cellular lipids and thereby increases the bio–oil yield. Amongt the above mentioned drying techniques, the first five processes are cost–intensive, whereas others are not but for sun–drying process more time and wide drying surface area are required. However, these five technologies bring high algae lipid extraction efficiency [103]. On comparision to both the traditional and chemical methods, the solvent extraction, microwave and ultrasonic are commercially very effecient for disrupting the algae cell wall [86]. Furthermore, the solvent extraction includes kind of toxic solvents like chloroform, hexane, methanol, *etc*., which all are not eco–friendly. Similar way, certain facts exist in the chemical method are high temperature, and long time duration, and are not desirable [104].

The second step in the conventional method, the extracted algae lipids can be undergone catalytic transesterification. All the above mentioned catalysis possess their own pros and cons for algae lipid transesterification to biodiesel conversion for sustainablity. The homogeneous transesterification catalysis– commercially lead to the feasible mass transfer between lipids and biocatalysts under mild reaction conditions that mainly includes high reactivity of the catalyst, low temperature and pressure with short reaction time duration [86]. The saponification is one of the crucial fact in homogeneous catalysis, especially with basic medium and the challenges are purification of the product as well as separation recovery and reusability of the catalyst from the reaction mixture. Further, the acidic homogeneous catalysis is corrosive [86]. The heterogeneous transesterification catalysis– prominently involves ease of catalyst separation, reusability of the catalyst and negligible waste generation. Additionally, some solid catalysts there can handle both transesterifications of algae lipids as well as esterification of FFAs in the algaefeedstock to biodiesel and thereby observed increase in the efficiency of this process. Though, occasionally weak porous nature of heterogeneous catalyst makes a limitation in mass transfer between algel lipids and catalytic active sites. Thereby the process become cost–intensive as the reaction conditions should be harsher to improve the catalytic efficiency [105]. Enzymatic catalysis *i.e.,* biotransesterification is highly efficient for the production of pure products and recyclability, without soap formation, though, thr catalysts are very expensive at an industrial scale [86]. In the case of *in–situ*

algae transesterification *i.e.*, a combination of extraction of algal lipids and transesterification process to yield biodiesel with the existence of an acid catalyst and methanol / supercritical conditions. Further, this single step process needs a shorter time duration, lower solvent and reagents, when compared to the two–step conventional process. But the co–existence of both lipids and FAs (fatty acids) of the reaction mixture requires more energy for the final extraction process of oil and is more difficult and hazardous [86,106].

6. Perspectives and Concluding remarks

Academic as well as commercial research towards algae–derived sustainable fuel \ meets the demands and the most implemented / cost–effective alternative source for transportation fuels due to the consideration of fossil fuel reserves as anthropogenic origin that rapidly warning global pediatric health *via* climate changes by means of CO_2 emissions [107]. The microalgal oil has been occupied a crucial role to achieve renewable fuel target. The catalyst separation, bio–oil purification–coupled continuous extraction facilitate commercial acheivement. Moreover, the solid acid and base catalysis enhance the improvements in commercial homogeneous as well as heterogenneous catalysis. But, the improvemrnts to design catalysed bio–oil production, mainly with heterogeneous catalysis is critical one [108]. However, the surface hydrophobicity on heterogeneous active catalytic centres strongly effect transesterification process through the expulsion of water away from those sites thereby, porevents unwanted reverse hydrolysis. Solid catalysts are capable for carrying out a simultaneous FFA and TAG esterification and transesterification, respectively in mild reaction conditions is a major challenge. The study reveals that the solid acids can be employed to hydrolyse algae feedstocks, consequently followed by esterification to yield FAME from the FFAs. It is well known fact that the unavailability of a perfect reactor technology for the extraction process of natural oils / fats beacause each technique possesses its own pros and cons. Technical advancement for both material chemistry and reactor engineering can be pursued if the optimization and developement in renewable bio–energy sector facilitate distributed biodiesel production and demand [109]. It requires a sound knowledge in catalysis, molecular simulation, chemical and genetic engineering to conquer innovative reactor technologies and to produce suitable catalysts. Increase in the utilization of waste / low grade oil sources mostly invole a challenge towards heterogeneous catalysis in terms of

improved upstream purification of bio–oil, because the existing impurities like acid, heavy metals, moisture, *etc*., enhance rapid on–stream deactivation. The long term utilization of bio–oil for a high performance engine should prove less problematic because of the FAMEs are made up of long chain esters ($4C_{18}$) [110]. So far, further improvement requires both government policies and incentive schemes. Thereby, the acheivement to develop not only for homogeneous catalysed, enzymatic, heterogeneous catalysed, non catalytic, mechanically catalysed in–situ as well as chemically catalysed in–situ transesterification / reactors, but also to carry out various processes involved in the extraction techniques for solvent, *SC*–CO_2 and ILs solvent to improve the economic viability in favour of bio–oil producrion [111,112].

Generally, all the techniques have extensively been applied for industrial scale owing to its simplicity and cost–effective fabrication, amongst the low efficiency and instability. Futher, the improved reactor design for the industrial scale to update different extraction techniques of natural oils and conversion technologies into biodiesel. The reactors should be more efficient for blending for quick mixing, space for construction and low maintenance are mostly desirable. The issues associated with the reactors are low efficiency and difficulty to control the ongoing process. By employing reaction–separation reactors, higher conversion can be achieved since there is a possibility of excellent mixing [113]. Eventhough, controlling power as well as temperature, low reproducibility are the main associated issues with such reactors. The developement in advanced transesterification reactor should meet the reduction of capital cost, consumption of energy as well as water, space for construction, reaction time and environmental hurdles, simultaneously should be improved for biodiesel quality and conversion efficiency. Additinally, advanced engineering approaches and emerging technologies eventually replaces the traditional ones. It can be proved that such approaches are commercially profitable and environmentally sustainable. Moreover, choosing appropriate sustainable microalgal feedstock is considered to be a crucial issue in bio–oil production since it only defines the cost of productivity [114]. Current focus is on microalgae as the third generation feedstock for biofuel production, using various technologies. So, these microalgae have been considered as a future alternative renewable energy sources and are concerned noteworthy research due to minimum green gas emission, maximum bio–absorption of CO_2 and the increasing energy demand of fossil fuels. The crucial ingredients of micro algae lipids are the FFAs and TGs and they can be

converted effectively in to FAMEs by means of the above discussed techniques. This review deals some significant lipid extraction methods as well as certain conversion technologies for the microalgal lipids into microalgal bio–oil production. Biodiesel production from a sustainable feedstock requires low water footprint for its cultivation and low cost techniques in oil extraction for large–scale biorefineries, consequently provide environmental a well as economical benefits. Such a feedstock should minimize greenhouse gases (GHGs) emissions atleast by 35%, when compared to petro–derived fuels. Feedstock collection is a critical management for biodiesel production using different kinds of transesterification reactors *viz.* cavitational, microwave, rotating, simultaneous reaction–separation, tubular/plug–flow reactors by means of homogeneous catalysed, enzymatic, heterogeneous catalysed, non–catalytic, mechanically catalysed *in–situ* as well as chemically catalysed *in–situ* transesterification to carry out various processes involved in the extraction techniques for solvent, supercritical CO_2 and Ionic liquids solvent. Though, more concentration is essential for a detailed characterization of microalgal biomass, lipids and their biodiesel since very little information in literature is available on the same.

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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 article.

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