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Advances in algal biomass pretreatment and its valorisation into biochemical and bioenergy by the microbial processes

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Abstract

Urbanization and pollution are the major issues of the current time own to the exhaustive consumption of fossil fuels which have a detrimental effect on the nation's economies and air quality due to greenhouse gas (GHG) emissions and shortage of energy reserves. Algae, an autotrophic organism provides a green substitute for energy as well as commercial products. Algal extracts become an efficient source for bioactive compounds having anti-microbial, anti-oxidative, anti-inflammatory, and anti-cancerous potential. Besides the conventional approach, residual biomass from any algal-based process might act as a renewable substrate for fermentation. Likewise, lignocellulosic biomass, algal biomass can also be processed for sugar recovery by different pre-treatment strategies like acid and alkali hydrolysis, microwave, ionic liquid, and ammonia fiber explosion, etc. Residual algal biomass hydrolysate can be used as a feedstock to produce bioenergy (biohydrogen, biogas, methane) and biochemicals (organic acids, polyhydroxyalkanoates) via microbial fermentation.

Keywords: Algal biomass, fermentation, polyhydroxyalkanoates, pretreatment, valorisation

1. Introduction

Excessive exploitation of natural resources and burning of fossil fuels are the major causes of environmental pollution and greenhouse gas (GHG) emissions that can be minimized with the use of renewable and green energy sources (Bhatia et al., 2021b; Rasool et al., 2021). Global warming and the greenhouse effect are mainly attributed to the increase in the concentration of GHG and carbon dioxide (CO_2) identified as one of the largest contributors (M. Kumar et al., 2018). CO_2 assimilation via chemical reactions, and physical storage lack environmental feasibility due to the requirement of large space, investment, and CO_2 spillage (Bhatia et al., 2019). In comparison, biological sequestration is environmentally as well as economically suitable (Liu et al., 2021). Algae and cyanobacteria can assimilate environmental CO_2 at 10-50 times faster rate than terrestrial plants (Onyeaka et al., 2021). Algal based process seems more profitable and feasible due to its autotrophic nature along with the production of various commercially valuable compounds e.g. mono/poly-unsaturated, omega-3 fatty acids, carotenoids, sterols, phyco-proteins, etc., (Mehariya et al., 2021; Patel and Kannan, 2021; Zhou et al., 2022). Algal cultivation methods have been categorized into open and closed cultivation systems. In open pond system algae is cultivated in ponds, and tanks under natural illumination (Saengsawang et al., 2020). The system is economic and cost effective but prone to contamination along with variable yield due to uncontrolled growth conditions (Bhatia et al., 2021a). In contrast, closed cultivation systems /bioreactors-based cultivation provides a controlled growth environment to attain optimum yield (Patel et al., 2022).

Microalgae occupy ~49-132 times less space for higher productivity of oil per unit area as compared to the other crops (Mathimani and Pugazhendhi, 2019; Trivedi et al., 2015). Besides the production of valuable biochemicals, algae are also used in wastewater treatment and pollutant degradation through phycoremediation (Bhuyar et al., 2021; Chandel et al., 2022; Rout et al., 2022). Algal biomass is mainly comprised of three macromolecules

including carbohydrate (8-20%), protein (30-70%), lipids (6-25%), along with a minor fraction of minerals (Viswanathan, 2017). After extraction of required biomolecules, residual biomass can also be used as raw material and transformed into various secondary products like biogas, ethanol, butanol, etc. via fermentation (Guenka Scarcelli et al., 2021; Khammee et al., 2021; Naresh Kumar et al., 2020). The algal cell wall is a complex structure and requires various pretreatment methods such as physical, chemical, biological, and physicochemical to depolymerize the polymeric structure and release free sugars (de Carvalho et al., 2020; Rebello et al., 2020). During the pretreatment, the production of various inhibitors was also reported which can affect microbial fermentation and productivity (Ahuja et al., 2022a; Jung et al., 2019). Detoxification of algal hydrolysate is required before utilizing it as a feedstock for microbial fermentation. Various detoxification methods like the use of activated biochar, liming and rotatory evaporation methods can be used to remove inhibitors (Ahuja et al., 2022b; Kubisch and Ochsenreither, 2022). There are many reports on the valorization of algal biomass hydrolysate to bioenergy (bioalcohol, biogas, and biohydrogen) and biochemicals (organic acids, and biopolymers) using microbial fermentation (Anh et al., 2020; Khammee et al., 2021; Monlau et al., 2021; Nagarajan et al., 2021).

In the present review, the recent advances in algal biomass pretreatment methods and valorization of algal biomass hydrolysate into commercially valuable biochemicals and bioenergy resources using microbial fermentation are summarized.

2. Algal biorefinery and life cycle analysis

Algae are a rich source of omega 3 fatty acids, proteins, carbohydrates, and other bioactive molecules hence itself used as nutraceuticals, feed in aquaculture, cattle feed, fertilizer, and plant bio-stimulant in agriculture (Araújo et al., 2021). Several reports are available for the production of astaxanthin from *Haematococcus pluvialis,* β-carotene from *Dunaliella salina,*

linoleic acid, and α- linoleic acid from *Chlorella vulgaris,* γ-linoleic acid from *Arthrospira platensis,* etc., (Camacho et al., 2019; Kratzer and Murkovic, 2021; Uma et al., 2022). Due to diverse commercial products, algae represent a huge market qualitatively as well as quantitatively (Table 1). According to the market survey report, algal products hold a market share of US$ 4.7 billion which is expected to increase by 6.3% to attain US$ 6.4 billion by 2026 (FB 6208, 2021). In some cases, whole biomass is consumed as food while in other cases target compounds are extracted mostly by soxhlet extraction using solvents of different polarities. Due to energy investment and associated risk of heat in presence of solvents, other green technologies have been developed which emphasized the use of supercritical fluid, application of high pressure, microwave, ultrasound, and electric field for the extraction of bioactive compounds with high yield (Saengsawang et al., 2020; Soquetta et al., 2018). Algal biomasses left behind after extraction have carbon rich bio-polymers like carbohydrates, proteins, and lipids. Due to the availability of fermentable sugars and nutrients, microbial biomass can be used as a substrate for secondary fermentation. Algal biomass also required pretreatments like lignocellulosic biomass to release the sugars and make them available for fermentation.

Life cycle analysis (LCA) involves the evaluation of the environmental impact of a product or process from its origin to its final disposal (Di Maria et al., 2020). The various steps include material extraction, processing, manufacture, distribution, and use with the inclusion of various guidelines to validate their utility. Algal based biorefinery goes through stages of algal growth, harvesting, and extraction. LCA analysis shows that the cultivation of algae in wastewater reduces the expenses required to set up a photobioreactor. The selection of pretreatment strategy depends on biomass type and product intended e.g., chemo-enzymatic pretreatment methods are used for biomass pretreatment when carbohydrate rich residual biomass is used as a substrate for fermentative production of biochemicals and

biofuels, whereas for lipid extraction mechanical disruption method is preferred (Rebello et al., 2020). Similarly, for harvesting of algal biomass flocculation and pH adjustment methods are preferred as compared to energy consuming centrifugation method. In relation to pretreatment, biological methods have been found more effective for better yield and productivity with energy consumption. In a study, it was reported that conversion of microalgae to butanol poses less environmental effect on climate and human health as compared to biodiesel production (Wu et al., 2019).

3. Algal biomass pretreatment methods

The algae cell wall is comprised of a fibrillar skeleton and an amorphous matrix. Fribillar component is made up of cellulose, mannan, and xylans. In terms of biopolymers, algal biomass is comprised of 5-23% carbohydrates followed by 7-23% lipids and 6-52% proteins (Viswanathan, 2017). Some of the algal species like *Chlorella* and *Nannochloropsis* etc. also contained lignin like biopolymer called sporopollenin or algaenan (Vassilev and Vassileva, 2016; Viswanathan, 2017). Algal cell wall thickening affects algal growth, harvesting, and extraction process. Medium engineering and metabolic engineering techniques can be applied to manipulate the algal cell wall constituents. Photosynthesis provides energy and carbon-containing precursors required for the biosynthesis of starch, lipids, and cell wall components. Jeong et al., (2017) reported mild nitrogen deficiency induces cell wall thickening *Nannochloropsis* sp. due to increased transcription level of genes coding for UDP-Glc pyrophosphorylase and cellulose synthase. The complex nature of biomolecule and inert lignin-like material hinders the accessibility for microbes thus pretreatment becomes necessary to improve the digestion and release sugars which can be further used to produce various commercially valuable compounds (Fig. 1). There are various pretreatment methods have been reported and each has its own merits and demerits (Table. 2).

3.1 Physical methods

Biomass can be processed with physical treatment to break the bonds via thermal and radiation to release fermentable sugars from polymers. Physical treatment improves the surface topography and accessibility of biomass for chemical, biological, or other hydrolyzing agents (Aftab et al., 2019). Thermal treatment can be further categorized into low temperature (<100 ºC), high temperature (>100 ºC), and steam explosion. Treatment at low temperature solubilizes the extracellular components while high temperature results in cell disruption (Passos et al., 2015). Thermal treatment improved the digestibility and improved biogas production but suffered from high energy and associated cost (Nanda et al., 2014).

The microwave offers an effective way of localized heat transfer using electromagnetic waves that utilize the dielectric properties of the target material. Microwaves impose an electric field of 2450 MHz with oscillation of 4.9×10^9 times per second which has the potential to disrupt the structure of biomass (Puligundla et al., 2016). Yin and colleague reported that microwave pretreatment of macroalgae biomass *Laminaria japonica* resulted in improved hydrogen production during the fermentation (Yin et al., 2019). For higher sugar recovery, microwave treatment of biomass may be assisted with acid, alkali, or other hydrolyzing agents (Aftab et al., 2019). Yuan and Macquarrie hydrolyzed brown seaweed *Ascophyllum nodosum* biomass by microwave assisted acid hydrolysis. Saccharification of biomass offered maximum sugar recovery of 127 mg/g biomass when treated with 0.4 M H_2SO_4 (3.13% w/v) at 150 °C for 1 min along with 0.00, 0.01, and 1.8 g/L furfural, hydroxymethyl furfural, and phenolic respectively. The maximum ethanol conversion efficiency of 60.7% (5.57 g/L) was achieved from hydrolysate by *Saccharomyces cerevisiae* ATCC no. 200062 without detoxification (Yuan and Macquarrie, 2015).

165 **3.2 Chemical methods:** There are different chemical-based methods such as acid/base
166 hydrolysis, ozonation, ionic liquid treatment, etc. have been reported for pretreatment of algal
167 biomass (Rebello et al., 2020; Taherzadeh et al., 2020).
168 *Acid hydrolysis:* It acts directly on cellulose and hemicellulose fraction in biomass and
169 hydrolyses the bonds that aid in depolymerization and release of monomeric sugars. The
170 yield of C-5 and C-6 in hydrolysate depends upon acid concentration and treatment
171 conditions (Phwan et al., 2019). The major drawback of acid hydrolysis is the production of
172 sugar and lignin derivatives including furfurals, 5-hydroxymethylfurfural, and phenolic acids
173 which acts as microbial inhibitors. Hydrochloric acid and sulfuric acid are the common acids
174 used for biomass hydrolysis however oxalic acid, and maleic acid might offer efficient
175 hydrolysis and less toxicity for fermentation (Aftab et al., 2019). The reaction between acidic
176 proton and oxygen involved in bond formation between two glucose units, forms conjugated
177 acid. Cleavage of the C-O bond is followed by the release of proton and the formation of
178 sugar molecules in presence of water (Joksimović and Marković, 2007).
179 Laurens et al., have found that late harvest of *Scenedesmus* and *Chlorella* improved
180 the biofuel yield. Hydrolysis of biomass with 10% H_2SO_4 released >90% of available glucose
181 in hydrolysate from biomass (Laurens et al., 2015). Phwan and colleagues compared the
182 sugar yield from *Chlorella* biomass after treatment with dilute sulfuric acid and acetic acids
183 of different concentrations ranging from 1%, 3%, 5%, 7%, and 9% (v/v). Sugar recovery
184 from both the acids was almost equal at all the concentrations however H_2SO_4 was more
185 efficient (Phwan et al., 2019).
186 *Alkali hydrolysis:* Sodium hydroxide is the most common alkali used for biomass processing
187 but calcium hydroxide is the cheapest as recovery and reuse are possible (Aftab et al., 2019).
188 In alkaline treatment alkali targets lignin where excess OH^- makes hydrogen bond between
189 cellulose and hemicellulose and ester bond between saponified hemicellulose and lignin

190 sensitive and weak followed by solubilisation of majority of lignin (Zhang, 2021).
191 Comparative evaluation of acid and alkali methods revealed that alkaline treatment (1.2 %
192 alkali at 140 ^{o}C for 30 min) was more efficient and exhibited sugar recovery of 23.67 wt%
193 sugars/g algal biomass (Rehman and Anal, 2018). Enzymatic hydrolysis of alkali pretreated
194 *Chlorella* biomass with cellulase cocktail from *Trichoderma longibrachiatum* resulted in
195 >84% hydrolysis and offered maximum reducing sugar of 413.42 ± 7.62 mg/g biomass
196 (Kassim et al., 2019). Maceiras et al., reported maximum reducing sugars *i.e.* 1.02 ± 0.01 g/L
197 recovered from algal biomass after treatment with 1% NaOH in ration 1:10 (alkali: biomass)
198 in a microwave of 249 W for 12.5 (Maceiras et al., 2021).
199 *Ozonation:* Ozone pretreatment is one of the approaches employed to improve biomass
200 digestibility without generating inhibitors as in the case of acid hydrolysis. Ozone acts as an
201 oxidizing agent and acts on lignin and liberates lower molecular weight fragments. Ozone is
202 soluble in water hence biomass moisture content affects lignin oxidization and oxidation
203 lowered in high moisture content as water film restricts the ozone interaction with biomass
204 (Travaini et al., 2015). Ozonolysis in presence of an alkaline environment promotes
205 delignification (Aftab et al., 2019). Keris-Sen and Guro reported that ozonolysis of algal
206 biomass with different O_3 dosages of 0.25-2.0 g O_3/g DWB improved enzymatic
207 saccharification and carbohydrate recovery. Ozonolysis of algal biomass with 0.5 g O_3/DWB
208 for 4 h offered a maximum glucose yield of 80.6% (w/w) of total carbohydrates (Keris-Sen
209 and Gurol, 2017).
210 *Ionic liquid:* Ionic liquids are the solvents that offered the benefit of ions (cations or anions)
211 with high thermal stability and polarity at less melting point, and negligible vapor pressure.
212 Different ionic liquids which have the capability to solubilize cellulose consist of
213 morpholinium^{+}, phosphonium^{+}, imidazolium^{+}, pyridinium^{+}, or ammonium^{+} based cations and
214 anions that can form a hydrogen bond with the hydroxyl group of cellulose (Hou et al., 2017).

215 Anions that form strong hydrogen bonding are not always capable of dissolving cellulose as
216 cations can also indirectly influence the dissolving ability of ILs. Ionic liquids disrupt the
217 native structure of biomass by competing for hydrogen bonding and manipulate the regular
218 structure (Aftab et al., 2019). To et al., compared different ionic liquids for lipid and sugar
219 extraction from *C. vulgaris* and *Spirulina platensis.* Choline amino acid based ionic liquids
220 treatment of *Chlorella* and *Spirulina* recovered 30.6% and 51% total lipids and 71% and 26%
221 total sugars (Q. To et al., 2018). Zhou and colleagues employed repeated use of ionic liquids
222 for lipid extraction from microalgal biomass. Treatment of biomass with ionic liquid
223 [BMIM][$MeSO_4$] at 70 °C for 2 h showed the best lipid extraction efficiency (17% total
224 lipids of dry weight). The 1 : 7 and 1 : 3 were the optimized ration of ionic liquid for the
225 complete extraction of the lipids (Zhou et al., 2019). Weldemhret et al., also reported that
226 ionic liquid (IL) pretreatment deconstructs the *Gelidium amansii* cell wall. Among ILs
227 screened, [Bmim]Ac was the most effective IL with 99% dissolution along with methanol as
228 antisolvent offered 78% reconstitution. Hydrolysis of IL-treated biomass with α-
229 neoagarobiose hydrolase 'AhgI' offered maximum yields of 56.5% D-Gal and 33.7% of 3,6-
230 anhydro-L-galactose (Weldemhret et al., 2019).

231 **3.3 Biological methods**

232 In comparison to chemical and physical treatment methods, biological methods offered a
233 green solution for biomass processing along with operation under mild operating conditions.
234 The transformation value of a feedstock relies upon the availability of target compounds like
235 cellulose, and xylans. Cellulases, xylanases, hemicellulases, pectinases, glucosidases, and
236 amylases are the major enzymes that can be used to hydrolyze the cellulose and xylan in algal
237 biomass. Some of the organisms can accumulate comparatively higher structural components
238 in response to environmental or stimulus response. *Chlamydomonas reinhardtii* UTEX90 can
239 accumulate up to 44% of starch that can be used as fermentable feedstock. Treatment of *C.*

240 *reinhardtii* biomass with thermostable α-amylase from *Bacillus licheniformis* and
241 amyloglucosidase from *Aspergillus niger* released glucose directly as a result of starch
242 hydrolysis and offered a maximum glucose yield of 235 mg/g algal biomass (Choi et al.,
243 2010). Similarly, Mahdy et al. also reported the treatment of algal biomass with
244 carbohydrases and proteases for solubilization of organic matter. Protease treatment leads to
245 54% hydrolysis of organic matter and resulted in 6.3 folds higher methane yield (Mahdy et
246 al., 2016). Monjed et al., isolated crude enzyme extracts from *Penicillium chrysogenum*, *A.*
247 *tubingensis*, *A. fumigatus*, and *Thermomyces lanuginosus* for the treatment of *C. vulgaris*
248 biomass (Monjed et al., 2021). The crude enzyme from *A. fumigatus* was most efficient in
249 biomass saccharification at 37 °C and extracted 67% of sugars from intact biomass and 94%
250 from defatted biomass. It also exhibited complete saccharification from intact biomass at 50
251 °C (Monjed et al., 2021).

252 **3.4 Physicochemical methods**

253 *Ammonia Fiber Explosion (AFEX):* AFEX combined the use of NH_3 (chemical agent) and
254 pressure (physical factor) for biomass processing. In AFEX, liquid (anhydrous) ammonia is
255 mixed with moist biomass and kept at a moderate operating pressure of 6.5-45 bar and
256 temperatures of 60–200 °C for about 5–30 min (Bensah and Mensah, 2018). Followed by an
257 incubation period, pressure will be dropped suddenly to atmospheric pressure that led to
258 depolymerization of biomass. Pretreatment of biomass with ammonia creates an
259 interconnected nanoscale network by breaking the carbohydrate-lignin ester bonds and is
260 responsible for the removal of lignin along with decrystallization of cellulose and loss of
261 some hemicelluloses. Usually, less than 2 Kg ammonia is required per Kg of dry biomass
262 (Bensah and Mensah, 2018). The use of AFEX in the case of algal biomass is not well
263 established but some findings have shown the efficiency of free ammonia (Singh et al.,
264 2022). Wang and colleague have shown that poor digestion of algal biomass by methanogens

265 might be responsible for low methane production. It was found that treatment with free
266 ammonia (240530 mg NH_3-N/L) for 24 h increased the algae solubilisation from 0.01
267 SCOD/g TOD (SCOD: soluble chemical oxygen demand; TCOD: total chemical oxygen
268 demand) to 0.05-0.06 g SCOD/g TCOD. Further, the biomass hydrolysis rate (k) and
269 methane potential (P0) were increased from 0.21/d and 132 L CH_4/kg TCOD respectively in
270 control to 0.33-0.50/d and 140-154 L CH_4/kg TCOD respectively in the treated group (Wang
271 et al., 2019).

272 *CO_2 explosion /Supercritical carbon dioxide:* Supercritical fluids are the substances that
273 remained in homogeneous phase-condition above their critical temperature and pressure and
274 exhibit characteristics including diffusivity, viscosity, and density between gases and liquids.
275 In general, the efficiency of treatment increased with temperature but carbohydrates might
276 start degrading at a higher temperature range and form furan derivatives and organic acids.
277 Among different supercritical fluids, supercritical carbon dioxide (scCO2) is commonly used
278 due to moderate critical conditions (31.1 °C and 74 bar), low-toxicity, non-flammability, and
279 wide availability (Escobar et al., 2020). The $scCO_2$ act by rapid expansion within biomass
280 fiber to disrupt the native structure of biomass. Due to zero dipole moment, $scCO_2$ mainly
281 targets non-polar or weakly polar compounds however its solvation power may be modulated
282 in the presence of co-solvents. In presence of moisture in biomass, $scCO_2$ forms carbonic acid
283 and promotes hemicellulose hydrolysis (Escobar et al., 2020; Li et al., 2014). Michalak et al.,
284 treated marine macroalgae including *Polysiphonia*, *Ulva,* and *Cladophora* species, collected
285 from Baltic Sea for the extraction of plant growth stimulatory compounds. The dried algal
286 biomass was exposed to CO_2 in the ratio of 78.7 Kg CO_2/Kg biomass at a pressure of 500 bar.
287 Particle size has a direct effect on extraction as maximum extraction of 17.9 g/Kg biomass
288 was achieved from fine grained biomass. Composition analysis revealed the presence of
289 phytohormones along with macro and microelements (Michalak et al., 2016).

290 **4. Algal biomass hydrolysate valorization by microbial fermentation**

291 Algal biomass can be fermented into biofuels, biohydrogen, biomethane, biogas, biodiesel,
292 and organic acids – thus turning trash into cash (Tables 3 and 4). Algae-based biorefineries
293 thus offer a solution for meeting future needs and moving towards sustainability (Fig. 2)
294 (Rajesh Banu et al., 2020).

295 **4.1 Bioenergy**

296 High dependence on nonrenewable fossil fuels to fulfill the energy requirement is a matter of
297 concern economically as well as environmentally. The negative impacts can be minimized by
298 the use of renewable, cleaner, and greener alternative fuels. Algal biomass can be used as
299 feedstock for microbial fermentation to produce biogas, bioalcohol, biohydrogen, etc.
300 Monlau et al., used defatted and enzymatic hydrolyzed algal biomass of *Chlorella*
301 *protothecoides* for biogas production using a continuous stirred tank bioreactor under
302 anaerobic conditions and reported biogas production rich in methane (196 ± 4 Nm^3 CH_4/t VS)
303 (Monlau et al., 2021). Biomethane production from raw *Scenedesmus* sp. and residues
304 obtained after amino acid and lipid extraction have been compared in another study. The raw
305 residues showed a methane yield of 140±29.4 L_{CH4}/kg VS while amino acid extracted algal
306 residues showed methane yields of 272 ±7.3 L_{CH4}/kg VS and lipid extracted residues showed
307 a yield of 212 ±5.6 L_{CH4} kg/VS (Ramos-Suárez and Carreras, 2014).

308 Generation of hydrogen from dark fermentation involves hydrogenase enzyme.
309 Protons accept excess electrons generated by the oxidation of organic substrates and generate
310 hydrogen in this process (Ghimire et al., 2015). The dark fermentation process has gained
311 attention because of its simplicity and negative energy balance. Hydrogen is produced either
312 by acetate or butyrate pathways. Theoretically, 1 mol of glucose yields 4 mol of H_2 through
313 the acetate pathway and 2 mol of H_2 through the butyrate pathway. Fermentation is achieved
314 using hydrogen producing bacteria (Table. 3) (Sambusiti et al., 2015). Different algal biomass

315 *Chlorella, Chlamydomonas, Scendesmus* sp., have been explored for bioethanol production
316 mainly because of their high carbohydrate content using *S. cerevisiae* and *Zymomonas* sp.
317 (Harun et al., 2010). *L. japonica* biomass has been fermented after the addition of mannitol,
318 using *S, cerevisiae* KCCM50550, and an ethanol yield of 2.59 g/L was obtained (Lee and
319 Lee, 2012). Park et al, has used a genetically engineered strain of *Klebsiella oxytoca* capable
320 of utilizing mixed sugars present in *Golenkinia* sp. hydrolysates for 2-3 butanediol production
321 (Park et al., 2017).

322 **4.2 Organic acids:** Algal biomass can be fermented into high valuable organic acids like
323 pyruvic acid, succinic acid, and lactic acid. These acids are the building blocks for the
324 synthesis of commercially valuable products in the food and pharmaceutical industries. Citric
325 acid has been synthesized from red algae *Gelidiella acerosa* with a yield of 0.357g/g, using
326 *A. niger* (Ramesh and Kalaiselvam, 2011). Enzyme hydrolysed mixture of *L. digitate* has
327 been used for the production of succinic acid (0.865 g/g) by using *Actinobacillus*
328 *succinogenes* DSM2257 strain (Alvarado-Morales et al., 2015). Acid treated and enzyme
329 hydrolysed Green alga *Ulva reticulate* can be fermented using *Halomonas* sp. BL6 to yield
330 pyruvic acid (0.368 g/g) (Anh et al., 2020). *C. sorokiniana* SLA-04 and *S. obliquus* UTEX
331 393 have also been shown to undergo auto fermentation under alkaline conditions for lactic
332 acid, acetate, and formic acid synthesis. The yield of acetate and formic acid can be increased
333 under anoxic conditions (Pendyala et al., 2020). Apart from these several genetically
334 modified strains have been used to increase the production of organic acids. An example is
335 the deletion of *aceE* (pyuruvate dehydrogenase), *ldhA* (D- lactate dehydrogenase), *poxB*
336 (pyruvate oxidase), and *pps* (PEP synthase) genes, associated with byproducts accumulation
337 and knocking out genes *ptsG*, *manZ* and *glk* to enhance xylose utilization, which has been
338 shown to increase the yield of propionic acid (Maleki et al., 2018; Zhang et al., 2021).

339 **4.3 Biopolymers**: Polyhydroxyalkanoates (PHAs) are synthesized and stored by
340 microorganisms as carbon and energy reserves (Lee et al., 2022). PHA offers an ecofriendly
341 solution to petroleum-based plastic, owing to its high functionality, biocompatibility, and
342 biodegradability (Liu et al., 2021). Industrial production of biopolymers is expensive as
343 almost 40% of the cost is due to feedstock. The solution is offered by the use of agricultural
344 waste or nonfood feedstock like macro and microalgae (Bhatia et al., 2021a). The latter is a
345 more promising approach, due to its high sugar and low lignin content (Khomlaem et al.,
346 2020). The polysaccharides present in algae are finally converted to glucose, followed by its
347 conversion to acetyl-CoA. The next step in the synthesis of PHA is the conversion of acetyl
348 CoA to acetoacetyl-CoA by the enzyme β-ketothiolase. Acetoacetyl CoA reductase converts
349 it to 3-hydroxybutyryl-CoA and is finally polymerized to polyhydroxybutyrate by PHA
350 synthase (Noreen et al., 2016). Algal biomass can be pre-treated using acid and high-
351 temperature conditions and further fermented using PHA producing bacteria like
352 *Alphaproteobacteria*, *Gammaproteobacteria*, *Cupriavidus necator*, *Bacillus megaterium*,
353 *Haloferax mediterranei*, *Paracoccus* sp. LL 1, recombinant *E. coli,* etc. (Alkotaini et al.,
354 2016; Fradinho et al., 2013). Biorefineries can be strengthened using co-production
355 approaches, which generate more valuable chemicals. Khomlaem et al., (2020) reported
356 coproduction of PHA (3.62 g/L) and carotenoid (6.08 mg/L) from the fermentation of
357 defatted *Chlorella* sp. using *Paracoccus* sp. LL 1 at 5 L scale.

358 **5. Technological challenges and future perspective:** Algal biomass based bioproducts and
359 biorefinery offer a renewable solution to meet future energy and high-value chemical and
360 products demands but its commercialization is still challenging. This is mainly owed to
361 multiple factors like the high cost of cultivation of algae, their seasonal harvesting time which
362 influences their biochemical composition, taxonomic structure, more energy-consuming
363 processing techniques, cost of equipment, and high downstream processing costs (Zhou et al.,

2022). Open and closed installations are thus preferred to control the algal proliferation. These include concrete ponds, circular ponds with stirrers, photobioreactors, biooil reactors, and plate bioreactors. But this adds to the additional cost of the process. The high cost of cultivation can be overcome by utilizing the natural algae from water blooms. But most of the natural algae have high ash content compared to carbon and nitrogen content. Secondly, their harvesting is challenging, which increases the cost of operation. Traditional harvesting techniques include the use of centrifugation, sedimentation, floatation, and flocculation techniques and all of them have their own set of drawbacks (Zhou et al., 2022). Uses of chemicals and metals further contaminate the biomass, making the downstream processing expensive. Future studies can be focused on the development of such harvesting technologies. One such innovative harvesting technique is the use of iron-based magnetic nanoparticles, used to harvest *C. vulgaris* and *S. platensis* (Almomani, 2020). Alternatively, biological flocculation offers a promising solution that can be explored in the future. Flocculating microorganisms like *Aspergillus* sp. UMNF01 has been used to flocculate non-flocculating algae *C. vulgaris* UMN235 (Zhou et al., 2012).

For the economic feasibility of algal biorefineries, the challenge remains to scale up the production of bioproducts with high purity, maximized yield, and productivity (Zhou et al., 2022). The yield can be improved by the addition of co-substrates along with algal biomass (Dębowski et al., 2013). The focus should be on decreasing the energy consumption and cost of the overall process. Attention can be focused on the coupling of biodiesel and methane production, which has been shown to increase the energy produced by up to 40%, making it more economical (Bohutskyi et al., 2015). Pretreatment processes also affect the overall economics of the process and have disadvantages. The use of physical methods increases the cost of energy input and they are ineffective in removing lignin and inhibit cellulases. On the other side, acidic chemicals are corrosive and lead to the formation of

inhibitors while alkaline hydrolysis leads to low digestibility. The use of ionic liquids is expensive and has low biodegradability.

For sustainable development, it is imperial to integrate the conversion processes and work on the generation of multi-product algal biorefineries. The focus should be emphasized on the regulation of metabolic flux to increase yields of desirous products and optimization of these processes. Techno–economic feasibility of these processes should also be accessed to make the scale up process feasible at a commercial scale.

6. Conclusions

The feasibility of the commercialization of algal biotechnology depends on the utilization of biomass and the synthesis of multi-products of high-value products. The product recovery from algal biomass can be improved by using advanced extraction methods. Leftover algal biomass after extraction of valuable molecules should be further utilized as feedstock for microbial fermentation to produce other valuable bioenergy resources and biochemicals. Although there are many reports on the valorisation of algal biomass still there are few efforts were made to integrate the algal-based product production and valorization of residual algal biomass to improve the overall economics of the process.

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877 **Table. 1 Commercial market demand of various algal products.**

Algal products	Organisms	Product name/Brand	Manufacture/s	Application	Market demand	References
Diatomaceous earth	Diatoms	Charcoal white	Coalgate India	Personal Care	US$ 1048.46 million	(QYR17046401, 2022)
		Nature's Whitening toothpaste	Cali white USA	Personal Care		
		Detoxifying Sea Algae Mask	Prolixr Global Bees India	Cosmetic		
		Daily microfoliant face scrub	Dermalogica USA	Cosmetic		
		Filter Press	Dutch Filtration Netherlands	Filtration system		
		Celpure	ErtelAlsop USA	Filtration system		
Astaxanthin	*Haematococcus pluvialis*	Astaxanthin	Now Food Bloomingdale	Healthcare	US$ 647 million	(FB 5254, 2021)
		Himalayan Organics Naturally Sourced Astaxanthin	Vlado Sky Enterprises India	Healthcare		
		Astaxanthin All-In-One Collagen Gel	DHC Japan	Cosmetic		
		Astaxanthin & Bilberry	Oriflame	Healthcare		

		Extract				
		Acai Clarifying Wash – Clear	UltraLuxe USA	Cosmetic		
Fatty acids, DHA, EPA, PUFA	*Schizochytrium* sp.	Ovegha	Unived India	Healthcare	US$ 1.86 billion	(IMARC, 2022)
Whole algae biomass	*Spirulina platensis*	Spirulina capsule	Sunova Organic India	Healthcare	US$ 393.6 million	(A03875, 2021)
		Acigon	Sanofi Aventis Pharma India	GERD, acidity	US$ 343 billion	(Maia-20389119, 2022)
Alginates	Sargassum	Alginate salt	Qingdao Hyzlin Biology Development Co.,Ltd. China	Thickening agent		
Carrageenans	*Eucheuma cottonii*	Carrageenans	FoodChem China	Thickening agent	US$ 761.2 millions	(Global Carrageenan Market Research Report 2020)
Agarose	Red seaweed	SeaKem	Lonza Switzerland	Base gel preparation	US$ 101.9 million	(QYR-18331892, 2021)

878

879 **Table. 2 Advantages and disadvantages of biomass pretreatment methods.**

Treatment method	Merits	Demerits	References
Microwave assisted treatment	• Rapid and high rate of localized heating • Excellent control of the operation	• Overheating and uneven distribution of heat in the sample • High cost of operation and energy intensive	(Priecel and Lopez-Sanchez, 2019)
Ultrasonication	• Economical and clean technology with the least use of solvent • Can transfer high energy rapidly	• Heat transfer is not uniform • Low yield and scale-up is a big challenge • Not suitable for complex substrate	(Carreira-Casais et al., 2021)
Acid hydrolysis	• Effective in the hydrolysis of cellulose • Efficiency can be improved when operated with a steam blast and easy to scale up	• Generated inhibitors like HMF, furfurals, and phenolics • High concentrations can be corrosive	(Nazari et al., 2018)
Alkali treatment	• Alkali act on lignin from the biomass • Easy scale up • Hydrolysis of de-lignified biomass offered higher sugar recovery	• High concentrations can be corrosive • Recovery of alkali is not possible • Also extract the hemicellulose fraction • At higher pH, bio-molecules undergo decarboxylation rather than dehydration	(Bhatia et al., 2020)
Ozonation	• Remove lignin from biomass • No toxic byproducts generated	• For sugar recovery, biomass must be treated further	(Aftab et al., 2019)

Ionic liquids	• Operated under mild temperature and pressure • Higher dissolution rate • High thermal stability	• High cost of operation • Need a large amount of ozone • Low efficiency of treatment • High pollution rate • Scale-up feasibility is a challenge	(Hou et al., 2017;)
Biological method	• Operated at low temperature • Low energy operation • Target specific compounds • Microbes can consume sugars along with biomass hydrolysis	• Slow process rate hence high treatment time • Not feasible for commercial applications • Time consuming	(Sindhu et al., 2016)
AFEX	• Cost effective and simple as no liquid/water stream involved • Less energy intensive, offered high yield and easy to scale	• Not suitable for high lignin content • Requires high solid loading, pressures as well as high ammonia loadings • Ammonia is toxic and its recycling is challenging	(Peral, 2016)
CO_2 explosion	• Low cost of operation • Non-toxicity, and non-flammability	• Operated at very high pressure	(Gu et al., 2013)
Hydrothermal	• Wet biomass can be used • Effective and high rate of hydrolysis • Less time consuming and easy to scale up	• Energy inefficient operated at high T (>400 ºC) and P (>15 MPa) • High nitrogen and oxygen content make products unstable	(Chen et al., 2016)

880

881 **Table. 3 Valorization of algal biomass into bioenergy.**

Product	Algae	Pretreatment method	Microorganism used	Fermentation condition	Comments	Yield	References
Ethanol	Wastewater algal biomass (*C. dorsoventralis*, *G. emersonii, C. proboscideum*, *S. obliquus*, *Micractinium* sp., *Desmodesmus* sp., and *Chlorella* sp.).	Set A: glucose Set B: Dried biomass Set C: 1M H_2SO_4 at 121 ºC for 15 min followed by enzymatic treatment	*Clostridium phytofermentens* DSM1183	Batch culture	Acid treatment followed by enzymatic treatment improved the sugar recovery and ethanol yield	4.6 g/L	(Fathima et al., 2016)
	Nannochloris	2% H_2SO_4 at 155 °C for 15 min	*S. cerevisiae* D5A	Batch culture	Salt addition has no impact on sugar utilization rates or ethanol production	0.19 g/g biomass	(Knoshaug et al., 2018)
Butanol	*C. vulgaris* JSC-6	1% NaOH followed by 3% H_2SO_4	*Clostridium acetobutylicum* ATCC824	Batch culture	High nitrogen content in algal biomass suppressed the butanol production	13.1 g/L	(Wang et al., 2016)
	C. vulgaris	2% H_2SO_4 at 121	*C.*	Batch culture	No detoxification is	8.05 g/L	(Gao et al.,

		ºC for 20 min of defatted biomass (solvent/ ionic liquid extraction) and detoxified with L-493 resin	*saccharobutylicum* (DSM 13864)		required for ionic liquid and solvent extracted biomass	(Acid hydrolysis) 4.99 g/L (ionic liquid)	2016)
2,3 butanediol	*Golenkinia* sp.	1.5 N H_2SO_4 hydrolyzed algae	GalP expressing, ΔmgsA *Klebsiella oxytoca*	Batch culture	Multisugar utilization with a faster process rate were observed compared to wild type	2.76 g/L	(Park et al., 2017)
Biohydrogen	*Ulva reticulate*	Acidic - hydrogen peroxide (H_2O_2) induced microwave pretreatment	Digested sludge	Batch culture	Microwave improved liquefication of biomass by thermal effect	92.5 mL H_2/g COD	(Dinesh Kumar et al., 2019)
	Chaetomorpha antennina	surfactants (ammonium dodecyl sulfate—ADS) aided microwave	Methanogenic bacteria	Batch culture	The addition of surfactant improves the solubilization of organic matter during microwave treatment	74.5 mL/g COD	(Kumar et al., 2019)

		disintegration					
	U. reticulata	Chemo-mechanical pretreatment (CMP)	Methanogens	Batch culture	Extraction of volatile fatty acids was almost double than MP only and thus better for bio-H_2 production	63 mL/g COD	(M. D. Kumar et al., 2018)
	A. platensis and *L. digitata*	Acid hydrolysis	Anaerobic sludge	Batch culture	The optimum C/N ratio was 26.2 for optimum H_2 production	85 mL/g VS	(Xia et al., 2016)
Biomethane	*C. protothecoides*	Defatted and enzymatic hydrolyzed algal biomass	Anaerobic fermentative bacteria	Batch culture	Almost complete utilization of nutrients into multiple products including diesel, methane, and fertilizer	196 ± 4 Nm^3 CH_4/t VS	(Monlau et al., 2021)
	N. gaditana	Lipid extracted biomass under mesophilic and	Anaerobic inoculums	Batch culture	Deoiling affects the structure integrity without cell lysis and	360 mL CH4/g VS	(Capson-Tojo et al., 2017)

		thermophilic conditions.			improve hydrolysis		
Biogas	*C. protothecoides*	Defatted and enzymatic hydrolyzed algal biomass	Anaerobic fermentative bacteria	Batch culture	Almost complete utilization of nutrients and conversion into multiple products including diesel, methane, and fertilizer	1.7 ± 0.1 NL/days	(Monlau et al., 2021)
	Ulva sp.	Solid state fermentation (SSF)	*Aspergillus fumigatus*	Batch culture	SSF offered a higher biogas yield than acid/alkali treatment	153 ± 3 mL CH4 g/VS	(Ben Yahmed et al., 2017)

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884 **Table. 4 Valorization of algal biomass into biochemicals.**

Products	Algae	Pretreatment method	Microorganism used	Fermentation condition	Comments	Yield	Product
Lactic acid	De-oiled algal cake	0.4 % (w/v) pepsin, 1200 U α-amylase, 5 U cellulase	*Lactobacillus casei*	37 °C at 200 rpm, pH 6.5 for 24 h	Triple digestion with pepsin, cellulose, and amylase improved digestion	11.17 g/L	(Overbeck et al., 2016)
	Dried *Gracilaria* sp. *Sargassum siliquosum,* and *U. lactuca*	0.4 N HCl, 121 ºC for 60 min cellulase treatment	*Lactobacillus acidophilus* BCRC 10695 and *L. plantarum* BCRC 12327	30 °C for 48 h.	Agitation reduced the LA production	19.32 g/L	(Lin et al., 2020)
	Ulva sp.	Acid hydrolysis with H_2SO_4	*Lactobacillus* sp. and *Weissella* sp	Batch culture	Lactic acid production was higher in red algae over green and brown	0.85 g/g	(Nagarajan et al., 2022)
Succinic acid (SA)	*S. latissima*	Enzymatic hydrolysis	*Actinobacillus succinogenes* 130Z	Anaerobically 37 °C at 200 rpm, pH- 6.8 up to 48h.	Seasonal variation affected the SA production from biomass	0.92 g/g	(Marinho et al., 2016)
	Defatted	Acid hydrolysis	*A. succinogenes*	Batch culture	Direct transesterification	0.67g/g	(Sorokina et

	Micractinium sp. IC-44 biomass	with 2% H_2SO_4	130Z	150 rpm at 37 °C for 48 h	of biomass with [BMIM][HSO_4] offered a higher FAME yield	fermentable sugar	al., 2020)
	Desmodesmus sp.	2% H_2SO_4 hydrolysed algae, heated at 155 °C for 15 min	*Actinobacillus succinogenes* ATCC130Z	37 °C at 150 rpm,	Oxalic acid used for pretreatment reduces the salt load in the conversion process	0.3 g/g biomass	(Knoshaug et al., 2018)
Pyruvic acid	*U. reticulata*	0.5 M H_2SO_4, 120 ºC for 90 min + 70 IU/g Viscozyme L	*Halomonas* sp. BL6	32 °C, pH- 9.0 for 72 h	Ulve hydrolysate supported microbial growth as well as pyruvate production in comparison to pure glucose	0.368 g/g	(Anh et al., 2020)
Butyric acid	*G. amansii*	180 mM H_2SO_4 at 150 ºC for 5 min+ 100 g/L Viscozyme L at 45 ºC for 48 h at 150 rpm	*Clostridium acetobutylicum* KCTC1790	Anaerobically 37 °C at 150 rpm, pH- 6.3 for 216 h	3% AC treatment removes HMF with the least effect on sugars	0.25 g/g	(Ra et al., 2017)
	Chlorella sp.	Acidogenic conditions	Anaerobic mixed cultures	Anaerobically 35 °C at 150 rpm, pH- 7.0 for 150 h in	Hydrogen production was higher without endo-nutrients	0.05 g/g	(Usmanbaha et al., 2019)

				batch cultures			
Carotenoid	*L. japonica*	0.3 N H_2SO_4 120 ºC for 20 min	*Paracoccus* sp. LL1	Fed batch culture	Acid treated biomass exhibited high disorder with ridges and fractures	2.3 g/L	(Muhammad et al., 2020)
PHA	*L. japonica*	0.3N H_2SO_4 120 ºC for 20 min	*Paracoccus* sp. LL1	Fed batch culture	Acid treated biomass exhibited high disorder in the cell wall structure	4.98 g/L	(Muhammad et al., 2020)
	G. amansii	94 mM treated with H_2SO_4	*Bacillus megaterium* KCTC2194	pH stat Fed Batch culture	No need of enzymatic hydrolysis or inhibitor removal.	8.2 g/L	(Alkotaini et al., 2016)
	Algal biodiesel waste residue	-	*Halomonas daqingensis*	Batch culture	Thermal degradation temperature 290 ºC	0.236 g/L	(Dubey and Mishra, 2021)

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886 **Fig. 1 Schematic presentation of algal biomass pretreatment and product production.**

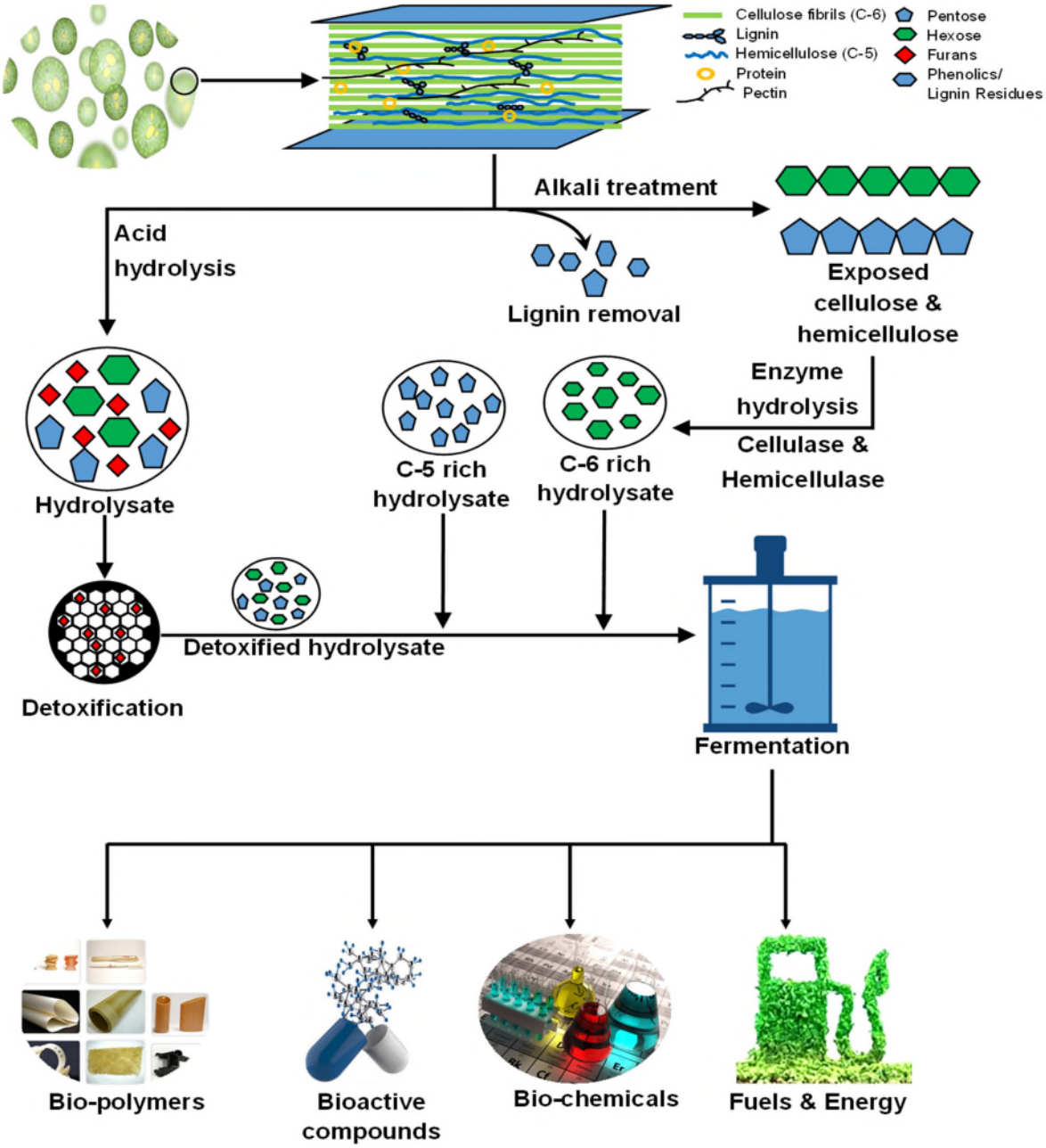

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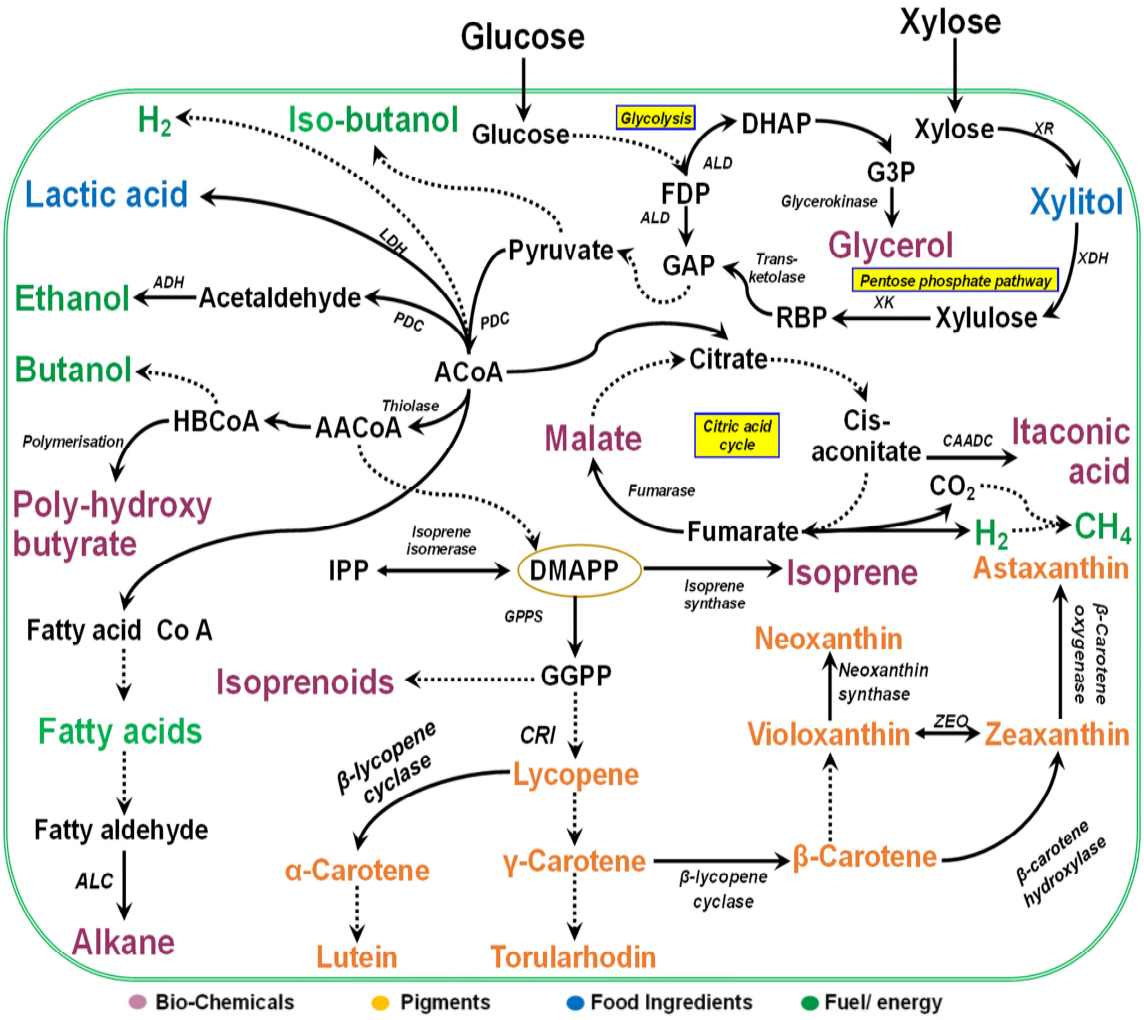
Fig. 2 Valorisation of algae biomass to valuable products by microbial fermentation.



FDP: Fructose diphosphate; ACoA: Acetyl-CoA; GGPP: 2-Geranylgeranyl pyrophosphate; GAP: Glyceraldehyde-3-phosphate; PDC: Pyruvate decarboxylase complex; LDH: Lactase dehydrogenase; ALC: Aldehyde decarbonylase; ALD: Aldolase; ADH: Alcohol dehydrogenase; AACoA: Aceto acetyl-CoA; HBCoA: Hydroxy butyryl-CoA; XR: Xylose reductase; RBP: Ribulose-5-phosphate; XDH: Xylitol dehydrogenase; XK: Xylulose-5-kinase; DHAP: Dihydroxy acetone phosphate; G3P: Glycerol-3-phosphate; OAA: Oxalo acetic acid; CAADC: Cis-aconitic acid decarboxylase; IPP: Iso pentyl pyrophosphate; DMAPP: Di methyl allyl pyrophosphate; ZEO: Zeaxanthin epoxidase.

Credit author statement

Shashi Kant Bhatia: Conceptualization, Writing - Review & Editing. **Vishal Ahuja:** Writing - Review & Editing. **Neha Chandel:** Writing - Review & Editing. **Ranjit Gurav:** Writing - Original Draft. **Ravi Kant Bhatia:** Writing - Review & Editing, **Muthuswamy Govarthanan:** Writing - Original Draft. **Vinay Kumar Tyagi:** Writing - Original Draft. **Vinod Kumar:** Writing - Original Draft. **Arivalagan Pugazendhi:** Conceptualization, Review & Editing, **J Rajesh Banu:** Conceptualization, Review & Editing. **Yung-Hun Yang:** Conceptualization, Writing - Review & Editing.

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