

1 A brief review on bioethanol production using marine
2 biomass, marine microorganism and seawater

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9

10 **Abstract**

11

12 This review introduces a new approach of completely marine based
13 bioethanol production by analyzing and evaluating the recent trends in
14 bioethanol fermentations using algae, marine microorganisms and the
15 replacement of freshwater with seawater. Both macroalgae and microalgae
16 have been successfully used for bioethanol production. Marine yeasts showed
17 excellent tolerance to salt and inhibitors, and fit for seawater fermentation.
18 The combination of marine biomass, marine microorganism and seawater has
19 a potential for a greener bioethanol production.

20

21 **Keywords**

22 Seaweed; pretreatment and enzymatic hydrolysis; bioenergy; yeast

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24

25 **1. Introduction**

26

27 Increasing concerns over energy shortages and environmental pollution has
28 led to a growing focus on the development of renewable energy sources, such
29 as solar, wind, bioenergy and geothermal energy. When compared with other
30 renewable energy sources, biofuels especially bioethanol, have several unique
31 advantages, such as its use as a liquid fuel, which can be directly used in
32 existing vehicle engines, it can be distributed via the existing fossil fuel
33 system and encourages rural economy. The increasing demand for bioethanol
34 has led to the excessive usage of food material and arable land for production.
35 This has resulted in food price rises and has restricted the growth of the
36 bioethanol industry.

37

38 A promising alternative choice of bioethanol production is the development
39 of a marine resource based bioethanol production process, as shown in Figure
40 1. Marine biomass, specifically microalgae and macroalgae, are fast growing
41 photosynthetic species which contain little or no lignin content, and require
42 no arable land and minimum nutrients for their cultivation. They are
43 considered as the 3rd generation of bioethanol feedstock [1]. In the past
44 decade, there has been an increase in research focus on bioethanol
45 production from marine biomass. Besides marine biomass, marine-derived
46 microorganisms have unique properties, such as high osmotic tolerance,
47 utilization of particular sugars and production of special enzymes [2]; these
48 properties provide extra benefits for bioethanol production, especially when
49 using marine biomass. Seawater is an abundant under estimated resource.

50 The use of seawater as a substitute for freshwater in bioethanol production
51 was suggested to reduce the water footprint of bioethanol production.

52

53 This paper reviews the latest progress in bioethanol production using marine
54 biomass, marine microorganisms and seawater. It also discusses future
55 trends in marine resources based bioethanol production.

56

57 **2 Bioethanol production using marine biomass**

58

59 **2.1 Macroalgae (seaweed)**

60 Macroalgae can be divided into three types, brown (*Phaeophyta*), red
61 (*Rhodophyta*) and green (*Chlorophyta*). In order to evaluate the bioethanol
62 production potential, the composition and carbohydrate profile of various
63 seaweed species have been determined (Table 1). Although the results did
64 not always concur, in general, seaweed contains 23.8-67% carbohydrate, 4.8-
65 23% protein, 0.53-4.8% lipid and 14-42% ash content (w/w dry weight basis,
66 (dw), based on 90% of the values listed in Table 1). When comparing sugar
67 composition, brown seaweed typically contains alginate, mannitol, laminarin,
68 fucoidin and cellulose; red seaweed typically contains carrageenan, agar,
69 cellulose and lignin and green seaweed typically contains mannan, ulvan,
70 starch and cellulose, though there is considerable variation [9]. Similar to
71 lignocellulosic bioethanol production, pretreatment and saccharification are
72 required to hydrolyze the seaweed into a fermentable sugar solution. Dilute
73 acid pretreatment using sulfuric acid and moderately high temperatures
74 (100-150°C) is a typical pretreatment method for converting seaweed into a

75 hydrolysate suitable for conversion into bioethanol [10, 11]. Other
76 pretreatment methods developed for lignocellulosic bioethanol production
77 process, such as alkali [12] and microwave [13] pretreatments have also been
78 successfully applied to seaweed hydrolysis processes. A subsequent
79 enzymatic saccharification step is normally required after pretreatment.
80 Using a cocktail of cellulosic enzyme solution, an overall hydrolysis yield over
81 90% has been achieved [11]. Utilization of seaweed specific enzymes, such as
82 alginate lysase [14] and laminarinase [15] have also been reported, which
83 effectively hydrolyzed brown seaweeds.

84

85 Subsequent to pre-treatment and saccharification, seaweed hydrolysates
86 have been evaluated in various fermentation models for bioethanol
87 production. Figure 2 plots bioethanol concentration and overall bioethanol
88 yield, the two crucial economic indicators in seaweed to bioethanol
89 fermentations. In general, relatively low bioethanol concentration of less than
90 30 g/L was observed (Figure 2). When the hydrolysate was concentrated, e.g.
91 by rotary evaporation, the initial sugar content in the hydrolysate was
92 enhanced and a bioethanol concentration of 65 g/L has been reported [16].
93 Bioethanol yields of 28% (w/w) have been reported, which is decent
94 comparing to the theoretical maximum overall bioethanol yield of 38% (w/w)
95 (Figure 2).

96 *Saccharomyces cerevisiae* is the most commonly used microorganism due to
97 its high glucose fermentation capacity. However, existing *S. cerevisiae* strains
98 are inefficient in fermenting algae specific sugar monomers, such as mannitol
99 and laminaran. Therefore, non-*S. cerevisiae* strains, such as *Pichia angophorae*

100 [17] and *Defluviitalea phaphyphila* [18] have been investigated to promote
101 conversion of mannitol, laminaran and alginate contained in seaweed
102 hydrolysates. Another promising strategy is the construction of macroalgae
103 sugar utilization pathways in high ethanol producing strains. Enquist-
104 Newman et al., (2013) constructed an alginate transportation and metabolism
105 system in *S. cerevisiae*, which efficiently converted 4-deoxy-L-erythro-5-
106 hexoseulose uronate (DEHU) and mannitol into bioethanol [19]. In a novel
107 process, a genetically modified *Escherichia coli* strain (*E coli* KO11) was
108 developed, which hydrolyzed, transported and converted alginate into
109 bioethanol simultaneously [20]. A bioethanol concentration of 4.7% (v/v) was
110 obtained with a yield of 0.281 g bioethanol per g dry weight macroalgae.

111

112 **2.2 Microalgae**

113

114 Microalgae have attracted great attention for biodiesel production due to
115 their fast growing character and their high lipid content in certain species,
116 such as *Chlorella sp.* [21]. Apart from lipid, some microalgae species, e.g.
117 *Synechococcus sp.* accumulated 60% carbohydrate content in favorable
118 culture conditions [22]. In a recent paper, a microalgae, designated SP2-3
119 containing 70% (w/w, dw) carbohydrate content was identified, indicating it
120 could be a promising marine feedstock for bioethanol production [23]. When
121 compared with macroalgae or terrestrial biomass, microalgal cell wall is
122 relatively easy to break down following a lysozyme, dilute acid or a
123 combination of both pre-treatment [23]. Early research on the hydrolysis of a
124 green microalgae *Chlamydomonas reinhardtii* with 3% (w/w) H₂SO₄ at 110°C

125 for 30 minutes led to a hydrolysate with a glucose concentration of 28.5 g/L
126 [24]. Subsequent fermentation of the hydrolysates by *S. cerevisiae* resulted in
127 a bioethanol production of 14.6 g/L, which corresponds to 0.292 g bioethanol
128 per g biomass (dw) [24]. Since then, various microalgae, such as
129 *Cyanobacterium synechococcus* sp. [22] *Chlorella* sp. [25], have been explored
130 for bioethanol production. These results have been summarized in Table 2
131 and recent articles [1, 23]. Normally, a microalgae hydrolysate contains
132 around 10-30 g/L sugars, and 3.6-14.6 g/L bioethanol was obtained with a
133 typical bioethanol to biomass yield of 0.2-0.3 (w/w, dw). When the
134 hydrolysate was concentrated, the sugar content can reach 137 g/L and
135 produce a bioethanol titre of up to 61.2 g/L [23].

136

137 **3 Marine microorganisms in bioethanol production**

138

139 The majority of microorganisms that are used for bioethanol synthesis have
140 been isolated from terrestrial environments. Hydrolysates derived from
141 marine biomass typically contain a different spectrum of sugar monomers
142 from hydrolysates from terrestrial plants [9] and as a result terrestrial
143 microorganisms struggle to utilize these sugars efficiently. An alternative
144 approach other than genetically modifying a microorganism is to screen for
145 new microorganisms which could utilize sugars present in the marine
146 biomass-derived hydrolysates. Isolation of marine-derived yeast was first
147 reported in 1894, since then, hundreds of marine yeasts had been isolated,
148 and some of these have been successfully used for bioethanol, pharmaceutical
149 and industrial enzyme production [2, 32]. Recently, Zaky et al., (2014)

150 compared various marine yeast isolation methods and developed an efficient
151 three-step protocol for marine yeast isolation [2]. Applying this method to 14
152 geographically different marine samples, over 100 marine yeasts were
153 isolated, of which 17 displayed efficient sugar utilization strains and were
154 subsequently identified [33]. Fermentations using *S. cerevisiae* AZ65, one of
155 the isolates in the above study produced 97.41 g/L bioethanol from a glucose
156 based medium in 15 L fermenters [34]. Obara et al. (2012) reported
157 fermentations of a concentrated paper shredder scrap hydrolysate using
158 marine-derived *S. cerevisiae* which achieved 122.5 g/L of bioethanol [35].
159 When this strain was used to ferment a mixture of seaweed hydrolysate
160 (*Undaria pinnatifida*) and paper shredder, 87.7 g/L bioethanol was produced
161 [36]. Besides *S. cerevisiae*, marine-derived microorganisms, such as *Pichia sp.*,
162 *Candida sp.* *Yarrowia sp.* and *Wickerhamomyces sp.* have also been
163 investigated for their suitability for bioethanol production [2].
164 The utilization of marine microorganisms in marine biomass hydrolysate was
165 recently explored. Khambhaty et al. (2013) reported fermentations of red
166 seaweed *Kappaphycus sp.* hydrolysate which contained 5.5% sugar and
167 11.25% salt by a marine-derived *Candida sp.* and 12.3 g/L bioethanol was
168 observed [37]. A thermophilic marine bacterium *Defluviitalea phaphyphila*
169 was isolated, which converted un-hydrolyzed brown seaweed powder (*S.*
170 *japonica*) to bioethanol with a yield of 0.25 g/g seaweed (dw) [18].

171

172 Marine microorganisms have also been used in enzymatic hydrolysis
173 processes and used as gene donors for the construction of novel bioethanol
174 producing strains. Trivedi et al., (2015) demonstrated the enzyme solution

175 obtained from a marine fungus *Cladosporium sphaerospermum* hydrolyzed
176 green seaweed *Ulva fasciata* [38]. The enzyme solution maintained 74-94% of
177 its activities in ionic liquid (IL), indicating it could be used together with IL for
178 biomass hydrolysis. Parab et al., (2017) successfully used an enzyme solution
179 produced from a marine bacterium *Bacillus sp.* BT21 for the hydrolysis of red,
180 green and brown seaweeds (*Ahnfeltia plicata*, *Ulva lactuca* and *Padina*
181 *tetraströmatica*) [39]. Sugar yields of 0.23, 0.10 and 0.073 g/g biomass (dw)
182 respectively were observed. Inulinase genes originated from marine-derived
183 yeasts *Pichia guilliermondii* [40] and *Candida membranifaciens* [41] were
184 successfully expressed in *Saccharomyces sp.* W0, respectively. The
185 transformants *Saccharomyces sp.* Inu-66 and W14-3-INU-112 both produced
186 over 12% (v/v) ethanol from Jerusalem artichoke derived inulin solution.

187

188 **4 Use seawater in bioethanol fermentation**

189

190 Seawater, which represents 97% of world's total water, is a potentially
191 important marine resource for bioethanol industry. With the successful
192 demonstration of using marine biomass and marine yeast for bioethanol
193 production, the further replacement of freshwater with seawater would lead
194 to a fully marine based process. The replacement of freshwater by seawater
195 in bioethanol fermentation using marine yeast *S. cerevisiae* AZ65 showed no
196 inhibitory effect. In 15 L batch fermentations using a sugarcane molasses
197 derived medium prepared in seawater, marine yeast *S. cerevisiae* AZ65
198 produced 52.2 g/L of bioethanol after 48 hours of culture (unpublished data).

199

200 **5 Challenges and opportunities**

201 Marine biomass is a promising feedstock for bioethanol production. It is
202 estimated that macroalgae has the potential of producing 23.4 m³/ha/y
203 bioethanol, which is 10.6 and 2.5 folds higher than those for corn and sugar
204 cane, respectively [15]. However, currently marine biomass has an annual
205 production of only 27 million tons (wet weight) [42], in comparison, sugar
206 cane production was 1.68 billion tons in 2012 [43]. Unlike major terrestrial
207 crops, which had been bred and screened for increasing productivity for
208 thousands of years, marine biomass are under-investigated, especially in
209 terms of breeding. This indicates that the potential for marine biomass
210 productivity could be improved dramatically and this development will have
211 a crucial impact on bioethanol production and growth of the industry. The
212 near 90% (w/w) water content in both microalgae [21] and macroalgae [44]
213 is a concern for industrial bioethanol production. A low cost, highly efficient
214 dewatering technology has yet to be developed. A combination of new strain
215 discovery, especially marine yeasts isolation, gene discovery and therefore
216 strain development of novel microorganisms which have the capacity to use
217 the full range of algae sugars would improve marine bioethanol production
218 and perspectives. The replacement of freshwater by seawater in bioethanol
219 industry could reduce the bioethanol production water footprint and possibly
220 provide freshwater for other sectors, possibly achieving bioethanol
221 production from sole marine resource. Integrating bioethanol production
222 with the existing algae industry, CO₂ fixation or wastewater treatment would
223 be an attractive approach [45, 46].

224

225 The utilization of macroalgae and microalgae for bioethanol production has
226 been reviewed in this paper. Significant improvement has been achievement
227 recently both in fermentation process optimisation and strain development.
228 Marine microorganisms and seawater have been demonstrated to be able to
229 used in algal biofuel fermentation. The development of an algae-based
230 biorefinery, extracting or producing value-added chemicals together with
231 completely marine based bioethanol fermentation would improve the overall
232 economic feasibility of algal biofuel production [47].

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239 **References**

240 1. S. A. Jambo, R. Abdulla, S. H. Mohd Azhar, H. Marbawi, J. A. Gansau, P. Ravindra,
241 A review on third generation bioethanol feedstock. Renewable Sustainable
242 Energy Rev., 65 (2016) 756–769
243 **

244 This paper reviews latest research progress in bioethanol production from
245 marine biomass. It focuses on algae hydrolysis and the subsequent bioethanol
246 fermentations. It summarises case studies of hydrolysis yield and bioethanol
247 production yield using both microalgae and macroalgae.

248
249 2. A. Zaky, G. Tucker, Z. Daw, C. Du, Marine yeast isolation and industrial
250 application. FEMS Yeast Res. 14(2014) 813–825
251 *

252 This review summarizes marine yeast isolation method and the application of
253 marine yeasts in biofuel production, synthesis of pharmaceutical compounds
254 and industrial enzymes.

255
256 3. H. Mæhre, M. Malde, K. Eilertsen, E. Elvevoll, Characterization of protein,
257 lipid and mineral contents in common Norwegian seaweeds and evaluation of
258 their potential as food and feed. J. Sci. Food Agric. 94 (2014) 3281–3290.
259 <http://dx.doi.org/10.1002/jsfa.6681>.

260
261 4. M. Ghadiryanfar, K.A. Rosentrater, A. Keyhani and M. Omid, A review of
262 macroalgae production, with potential applications in biofuels and
263 bioenergy. Renew. Sust. Energ. Rev. 54 (2016) 473–481.

264
265 5. E. Kostas, D. White, C. Du, D. Cook, Selection of yeast strains for bioethanol
266 production from UK seaweeds. J Appl Phycol 28 (2016) 1427–1441

267
268 6. E. Kostas, D. White, D. Cook, Development of a bio-refinery process for the
269 production of speciality chemical, biofuel and bioactive compounds from
270 *Laminaria digitata*. Algal Res 28 (2017) 211-219

271
272 7. M. Song, H. Duc Pham, J. Seon, H. Chul Woo, Marine brown algae: a
273 conundrum answer for sustainable biofuels production, Renew. Sust. Energ.
274 Rev. 50 (2015) 782–792, <http://dx.doi.org/10.1016/j.rser.2015.05.021>.

275
276 8. M.D.N. Meinita, B. Marhaeni, D.F. Oktaviani, G.-T. Jeong, Y.-K. Hong,
277 Comparison of bioethanol production from cultivated versus wild *Gracilaria*
278 *verrucosa* and *Gracilaria gigas*. J. Appl. Phycol. (2017).
279 <https://doi.org/10.1007/s10811-017-1297-x>

280
281 9. K. A. Jung, S. R. Lim, Y. Kim, J. M. Park, Potentials of macroalgae as
282 feedstocks for biorefinery. Bioresour. Technol., 135 (2013), 182–190.

283
284 10. F. Fernand, A. Israel, J. Skjermo, T. Wichard, K.R. Timmermans, A. Golberg

285 Offshore macroalgae biomass for bioenergy production: environmental
286 aspects, technological achievements and challenges. *Renew. Sustain. Energy*
287 *Rev.*, 75 (2017) 35-45
288

289 11. R. Jiang, K.N. Ingle, A. Golberg, Macroalgae (seaweed) for liquid
290 transportation biofuel production: what is next? *Algal Res.*, 14 (2016) 48-57
291 **
292 This recent review focuses on liquid biofuel production from macroalgae
293 (seaweed). It compares various pretreatment method, fermentation types and
294 bioethanol yield in recent papers related to seaweed based bioethanol
295 production.
296

297 12. S. Kumar, R. Gupta, G. Kumar, D. Sahoo, R. Kuhad, Bioethanol production
298 from *Gracilaria verrucosa*, a red algae, in a biorefinery approach, *Bioresour.*
299 *Technol.* 135 (2013) 150-156
300 <http://dx.doi.org/10.1016/j.biortech.2012.10.120>.
301

302 13. Y. Yuan, D. Macquarrie, Microwave assisted acid hydrolysis of brown
303 seaweed *Ascophyllum nodosum* for bioethanol production and
304 characterization of alga residue. *ACS Sustainable Chem. Eng.*, 3 (2015) 1359-
305 1365 DOI: 10.1021/acssuschemeng.5b00094
306

307 14. M.R. Ryu, E.Y. Lee, Saccharification of alginate by using exolytic
308 oligoalginate lyase from marine bacterium *Sphingomonas* sp. MJ-3, *J. Ind. Eng.*
309 *Chem.* 17 (2011) 853-858.
310

311 15. J. Adams, J. Gallagher, I. Donnison, Fermentation study on *Saccharina*
312 *latissima* for bioethanol production considering variable pre-treatments, *J.*
313 *Appl. Phycol.* 21 (2009) 569-574, [http://dx.doi.org/10.1007/s10811-008-](http://dx.doi.org/10.1007/s10811-008-9384-7)
314 [9384-7](http://dx.doi.org/10.1007/s10811-008-9384-7).
315

316 16. P.I. Hargreaves, C.A. Barcelos, A.C.A. da Costa, N. Pereira Jr., Production of
317 ethanol 3G from *Kappaphycus alvarezii*: evaluation of different process
318 strategies, *Bioresour. Technol.* 134 (2013) 257-263,
319 <http://dx.doi.org/10.1016/j.biortech.2013.02.002>
320

321 17. S.J. Horn, I.M. Aasen, K. Østgaard, Ethanol production from seaweed
322 extract, *J. Ind. Microbiol. Biotechnol.* 25 (2000) 249-254,
323 <http://dx.doi.org/10.1038/sj.jim.7000065>.
324

325 18. S.-Q. Ji, B. Wang, M. Lu, F.-L. Li, Direct bioconversion of brown algae into
326 ethanol by thermophilic bacterium *DeFluviitalea phaphyphila*. *Biotechnol*
327 *Biofuels* (2016) 9:81 <https://doi.org/10.1186/s13068-016-0494-1>
328

329 19. M. Enquist-Newman, A.M.E. Faust, D.D. Bravo, C.N.S. Santos, R.M. Raisner,
330 A. Hanel, P. Sarvabhowman, C. Le, D.D. Regitsky, S.R. Cooper, L. Peereboom, A.
331 Clark, Y. Martinez, J. Goldsmith, M.Y. Cho, P.D. Donohoue, L. Luo, B.
332 Lamberson, P. Tamrakar, E.J. Kim, J.L. Villari, A. Gill, S.A. Tripathi, P.
333 Karamchedu, C.J. Paredes, V. Rajgarhia, H.K. Kotlar, R.B. Bailey, D.J. Miller, N.L.

334 Ohler, C. Swimmer, Y. Yoshikuni, Efficient ethanol production from brown
335 macroalgae sugars by a synthetic yeast platform, Nature 505 (2014) 239–243,
336 doi:10.1038/nature12771

337 *

338 This is an interesting article provides a case study on the construction of a
339 genetically modified yeast strain for the conversion of brown seaweed
340 monomers to bioethanol.

341

342 20. A.J. Wargacki, E. Leonard, M.N. Win, D.D. Regitsky, C.N.S. Santos, P.B. Kim,
343 S.R. Cooper, R.M. Raisner, A. Herman, A.B. Sivitz, A. Lakshmanaswamy, Y.
344 Kashiyama, D. Baker, Y. Yoshikuni, An engineered microbial platform for
345 direct biofuel production from brown macroalgae, Science 335 (2012) 308–
346 313, doi:10.1126/science.1214547

347

348 **

349 This is an interesting article describes a case study on the construction of an *E*
350 *Escherichia coli* strain, that directly fermented brown algae (*Saccharina*
351 *japonica*) to bioethanol.

352

353 21. T., Mata, A. Martins, N. Caetano. Microalgae for biodiesel production and
354 other applications: a review. Renew Sustain Energy Rev 14 (2010) 217–232.

355

356 22. K.B., Möllers, D., Cannella, H., Jørgensen, N.U., Frigaard, Cyanobacterial
357 biomass as carbohydrate and nutrient feedstock for bioethanol production by
358 yeast fermentation, Biotechnol Biofuels (2014) 7:64.
359 <https://doi.org/10.1186/1754-6834-7-64>

360

361 23. L., Sanchez Rizza, M.E., Sanz Smachetti, M., Do Nascimento, G.L., Salerno, L.,
362 Curatti. Bioprospecting for native microalgae as an alternative source of
363 sugars for the production of bioethanol. Algal Res. 22 (2017) 140–147.

364 <http://dx.doi.org/10.1016/j.algal.2016.12.021>.

365 *

366 This is an interesting article discusses the isolation of a microalgae containing
367 70% carbohydrate on cell dry weight basis. It also summarises recent case
368 studies on bioethanol fermentation using microalgae.

369

370 24. M.T. Nguyen, S.P. Choi, J. Lee, J.H. Lee, S.J. Sim, Hydrothermal acid
371 pretreatment of *Chlamydomonas reinhardtii* biomass for ethanol production, J.
372 Microbiol. Biotechnol. 19 (2009) 161–166.

373

374 25. K.H. Kim, I.S. Choi, H.M. Kim, S.G. Wi, H.-J. Bae, Bioethanol production from
375 the nutrient stress-induced microalga *Chlorella vulgaris* by enzymatic
376 hydrolysis and immobilized yeast fermentation, Bioresour. Technol. 153
377 (2014) 47–54.

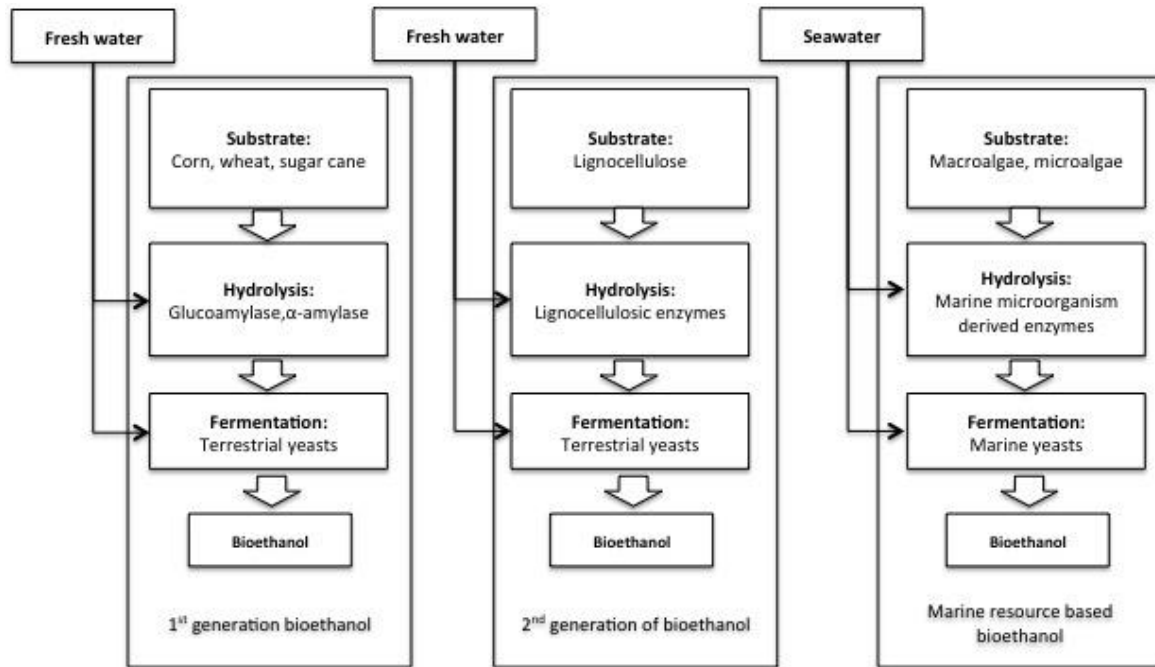
378

379

- 380 26. S.J. Sim, S.P. Choi, M.T. Nguyen, Enzymatic pretreatment of
381 *Chlamydomonas reinhardtii* biomass for ethanol production. *Bioresour.*
382 *Technol.* 101 (2010), 5330–5336
383
- 384 27. S.H. Ho, S.W. Huang, C.Y. Chen, T. Hasunuma, A. Kondo, J.S. Chang,
385 Bioethanol production using carbohydrate rich microalgae biomass as
386 feedstock. *Bioresour. Technol.* 135 (2013), 191–198.
387
- 388 28. R. Harun, M.K. Danquah. Influence of acid pre-treatment on microalgal
389 bio- mass for bioethanol production. *Process Biochem.* 46 (2011), 304–309.
390
- 391 29. R. Harun, M.K. Danquah, G.M. Forde, Microalgal biomass as a fermentation
392 feedstock for bioethanol production. *J. Chem. Technol. Biotechnol.* 85 (2010),
393 199–203
394
- 395 30. Z. Reyimu, D. Ozçimen, Batch cultivation of marine microalgae
396 *Nannochloropsis oculata* and *Tetraselmis suecica* in treated municipal
397 wastewater toward bioethanol production. *J. Clean. Prod.* 150 (2017) 40-46
398
- 399 31. S.H. Ho, P.J. Li, C.C. Liu, J.S. Chang, Bioprocess development on microalgae-
400 based CO₂ fixation and bioethanol production using *Scenedesmus obliquus*
401 CNW-N. *Bioresour. Technol.* 145 (2013), 142–149.
402
- 403 32. Z. Chi, G.L. Liu, Y. Lu, H. Jiang, Z.M. Chi, Bio-products produced by marine
404 yeasts and their potential applications, *Biores. Technol.* 202 (2016) 244-252.
405
- 406 33. A. S. Zaky, D. Greetham, E. J. Louis, G. A. Tucker, C. Du, A new isolation and
407 evaluation method for marine derived yeast spp with potential applications in
408 industrial biotechnology, *J. Microbial. Biotechnol.* 26(2016) 1891–1907. doi:
409 10.4014/jmb.1605.05074.
410
- 411 34. A. S. Zaky, G. A. Tucker, C. Du, Use of marine yeast for the efficient
412 production of bioethanol from seawater-based media. *New Biotechnol.* 33
413 (2016), S52-S53, doi:10.1016/j.nbt.2016.06.906
414
- 415 35. N. Obara, M. Ishida, N. Hamada-Sato, N. Urano. Efficient bioethanol
416 production from scrap paper shredder by a marine *Saccharomyces cerevisiae*
417 derived C-19. *Stud. Sci. Technol.* 1 (2012) 127–132.
418
- 419 36. N. Obara, M. Okai, M. Ishida, N. Urano. Bioethanol production from mixed
420 biomass (waste of *Undaria pinnatifida* processing and paper shredding) by
421 fermentation with marine-derived *Saccharomyces cerevisiae*. *Fish. Sci.*
422 81(2015) 771-776
423
- 424 37. Y., Khambhaty, D., Upadhyay, Y., Kriplani, N., Joshi, K., Mody, M., Gandhi,
425 Bioethanol from macroalgal biomass: utilization of marine yeast for
426 production of the same. *Bioenergy Res.* 6(2013) 188–195.
427

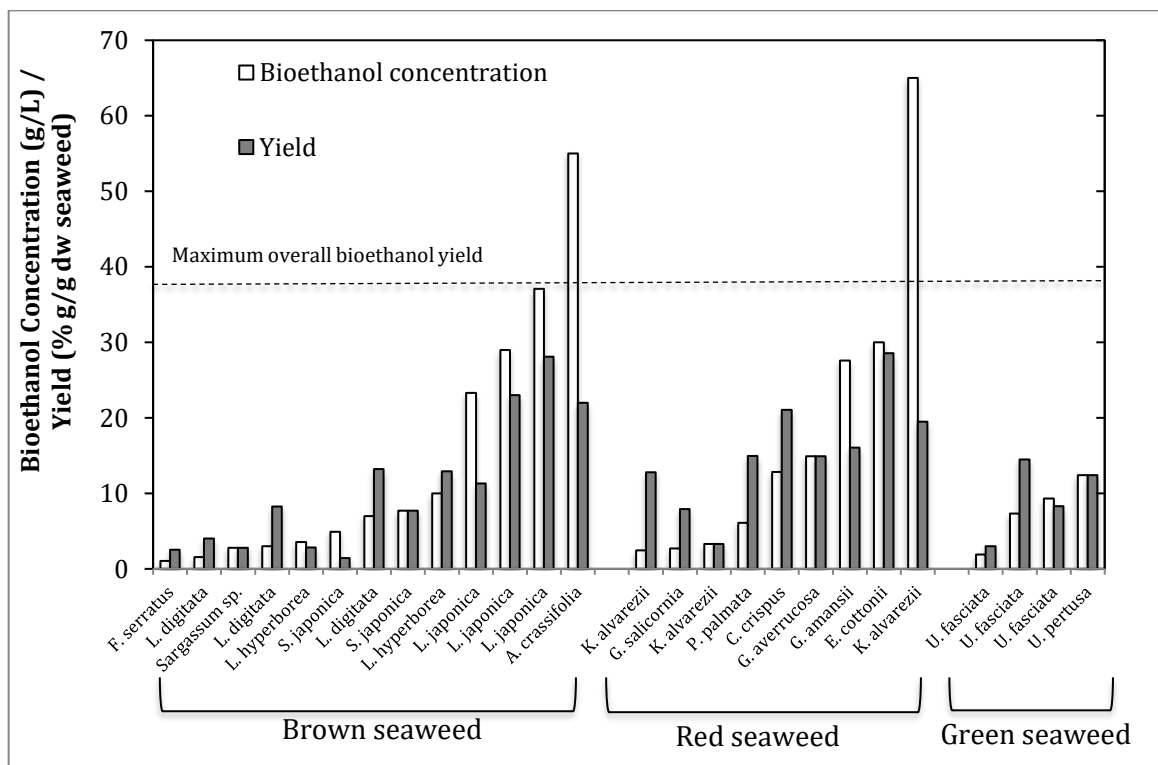
428 38. N. Trivedi, V. Gupta, C.R.K. Reddy, B. Jha, Enzymatic hydrolysis and
429 production of bioethanol from common macrophytic green alga *Ulva fasciata*
430 *Delile*, Bioresour. Technol. 150 (2013) 106–112,
431 <http://dx.doi.org/10.1016/j.biortech.2013.09.103>.
432
433 39. P. Parab, R. Khandeparker, U. Amberkar, V. Khodse, Enzymatic
434 saccharification of seaweeds into fermentable sugars by xylanase from
435 marine *Bacillus sp.* strain BT21. 3 Biotech 7 (2017) 296
436
437 40. T. Zhang, Z. Chi, C.H. Zhao, Z.M. Chi, F. Gong, 2010. Bioethanol production
438 from hydrolysates of inulin and the tuber meal of *Jerusalem artichoke* by
439 *Saccharomyces sp.* W0. Bioresour. Technol. 101 (2010) 8166–8170.
440
441 41. L.L. Zhang, G.L. Liu, Z. Chi, G.Y. Wang, Z.P. Wang, Z.M. Chi. Cloning and
442 characterization of an inulinase gene from the marine yeast *Candida*
443 *membranifaciens* sub sp. *flavinogenie* W14-3 and its expression in
444 *Saccharomyces sp.* W0 for ethanol production. Mol. Biotechnol. 57(2015) 337–
445 347.
446
447 42. FAO (2016). The state of world fisheries and aquaculture 2016
448 <http://www.fao.org/3/a-i5555e.pdf>, last access 26/01/2018
449
450 43. K.A. Jung, S.R. Lim, Y. Kim, J.M. Park, Potentials of macroalgae as
451 feedstocks for biorefinery, Bioresour. Technol. 135 (2013) 182–190.
452
453 44. J.A. Gallagher, L.B. Turner, J.M. Adams, P.W. Dyer, M.K. Theodorou.
454 Dewatering treatments to increase dry matter content of the brown seaweed,
455 kelp (*Laminaria digitata* ((Hudson) JV Lamouroux)) Bioresour. Technol. 224
456 (2017) 662–669.
457
458 45. R. Shukla, M. Kumar, S. Chakraborty, R. Gupta, S. Kumar, D. Sahoo, R.C.
459 Kuhad, Process development for the production of bioethanol from waste
460 algal biomass of *Gracilaria verrucosa*, Bioresour. Technol. 220 (2016) 584–
461 589.
462
463 46. S. Chia, H. Ong, K. Chew, P. Show, S. Phang, T. Ling, D. Nagarajan, D. Lee, J.
464 Chang, Sustainable approaches for algae utilisation in bioenergy production,
465 Renewable Energy (2017) <https://doi.org/10.1016/j.renene.2017.04.001>.
466
467 47. A. Raheem, P. Prinsen, A.K. Vuppaladadiyam, M. Zhao, R. Luque, A review
468 on sustainable microalgae based biofuel and bioenergy production: Recent
469 developments. J. Clean. Prod. 181 (2018). 42–59
470 <https://doi.org/10.1016/j.jclepro.2018.01.125>.
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475 Figure 1. Schematic diagram of marine resource based bioethanol production
476 processes in comparison with the 1st and 2nd generation bioethanol
477 production processes.
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481 Figure 2 Comparison of bioethanol concentration (g/L) and overall
 482 bioethanol yield (g bioethanol per g dry weigh seaweed) in fermentations
 483 using seaweed hydrolysates [5,6,11]. The theoretical maximum overall
 484 bioethanol yield of 38% (w/w) was calculated based on the carbohydrate
 485 content in seaweed (67% w/w) and bioethanol to sucrose yield of 0.568 g/g.
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490 Table 1 The carbohydrate, protein, lipid and ash composition of macroalgae,
 491 (dry weight basis, %).

Seaweed sp.	Carbohydrate (w/w)	Protein (w/w)	Lipid (w/w)	Ash (w/w)	REF
Brown seaweed					
<i>Alaria esculenta</i>		9.11	1.3	24.56	[3]
<i>Ascophyllum nodosum</i>	39.5-60.6	4.8-9.8	1.9-4.8	18-24	[4]
<i>Fucus serratus</i>	26.4	9.6	2.8	18.8	[5]
<i>Fucus vesiculosus</i>		6.11	3.51	20.92	[3]
<i>Laminaria digitata</i>		5.31	1.13	24.43	[3]
<i>Laminaria digitata</i>	21.7	26.8	1.9	24.3	[5]
<i>Laminaria digitata</i>	46.6	12.9	1	26	[6]
<i>Laminaria digitata</i>		4.63	0.53	26.5	[4]
<i>Laminaria hyperborea</i>		5.02	1.42	28.75	[3]
<i>Laminaria japonica</i>	51	8	1		[1]
<i>Laminaria sp.</i>	60	12	2	26	[4]
<i>Macrocystis sp</i>	41.7	17.3		41.1	[4]
<i>Pelvetia canaliculata</i>		5.72	5.81	21.24	[3]
<i>Saccharina</i>	40.8-67.0	8.4-14.8	1.3-2.4	14.3	[7]
<i>Sargassum ilicifolium</i>	32-33	8-9	2		[1]
<i>Undaria</i>	26.5-42.8	12.0-23.0	1.1-4.5	22.4	[7]
<i>Undaria pinnatifida</i>	43	24	3-4		[1]
Green seaweed					
<i>Ulva sp.</i>		13.6	2.7	30.2	[4]
<i>Ulva lactuca</i>	59	17	3-4		[1]
<i>Ulva lactuca</i>		8.65	2.62	29.31	[3]
<i>Ulva lactuca</i>	23.8	16.4	1	21.5	[5]
<i>Enteromorpha intestinalis</i>		11.33	1.03	55.29	[3]
<i>Cladophora rupestris</i>		3.42	0.63	77.8	[3]
Red seaweed					
<i>Chondrus crispus</i>	21.8	19.9	0.48	19	[5]
<i>Eucheuma cottonii</i>	26	09-10	1		[1]
<i>Gelidium amansii</i>	66	20	0.2		[1]
<i>Gracilaria gigas</i>	64.71	12.63	1.31	19.59	[8]
<i>Gracilaria sp.</i>		11.4		37.7	[4]
<i>Gracilaria verrucosa</i>	60.81	9.86	0.8	13.85	[8]
<i>Palmaria palmata</i>		12.26	1.33	42.23	[3]
<i>Palmaria palmata</i>	39.4	22.9	3.3	25.7	[5]
<i>Vertebrata lanosa</i>		11.56	1.3	28.78	[3]

Table 2 Comparison of bioethanol production using microalgae feedstock.

Microalgae species	Pretreatment		Fermentation		Bioethanol		REF
	Method	Sugar	Strain	Condition	Titre (g/L)	Yield (g/g)	
<i>Chlamydomonas reinhardtii</i> UTEX 90	3% H ₂ SO ₄ , 110°C, 30 min	0.58 g/g	<i>S. cerevisiae</i>	30°C, 24 h	14.6	0.292	[24]
<i>Chlamydomonas reinhardtii</i> UTEX 90,	0.005% a-amylase, 90°C, 30 min	N/A	<i>S. cerevisiae</i>	30°C, 40 h, 160 rpm	N/A	0.235	[26]
<i>Chlorella vulgaris</i>	240 IU/mg substrate pectinase, 50°C, 200 rpm, 72 h,	0.148 g/g	<i>S. cerevisiae</i>	30°C, 48 h	N/A	0.069	[25]
<i>Chlorella vulgaris</i> FSP-E	1% (w/v) H ₂ SO ₄ , 121°C, 20 min, pH 6.0	0.477 g/g	<i>Z. mobilis</i>	30°C, 24 h	11.7	0.233	[27]
<i>Chlorella vulgaris</i> FSP-E	2% (w/v) cellulase + amylase, 45°C, 200 rpm, pH 6.0	0.461 g/g	<i>Z. mobilis</i>	30°C, 24 h	4.3	0.214	[27]
<i>Chlorococcum humicola</i>	3% (w/v) H ₂ SO ₄ , 160°C, 15 min, pH 7.0	N/A	<i>S. cerevisiae</i>	30°C, 50 h, 200 rpm	7.2	0.520	[28]
<i>Chlorococcum sp.</i>	Supercritical CO ₂ extraction of lipid, 60°C	N/A	<i>S. cerevisiae</i>	30°C, 60 h, 200 rpm	3.8	0.380	[29]
<i>Cyanobacterium</i> <i>synechococcus sp</i>	Sonication, lysozyme and a-glucanase	N/A	<i>S. cerevisiae</i>	34°C, 72 h, 160 rpm	30.0	0.270	[22]
<i>Desmodesmus sp.</i>	10% dry w/v, 2% (v/v) H ₂ SO ₄ , 120°C, 30 min, followed by lyophilization	137.2 g/L*	<i>S. cerevisiae</i>	28°C, 30 h, 120 rpm	61.2	0.310	[23]
<i>Nannochloropsis oculata</i>	0.75% (w/v) NaOH, room temperature, 10 min	1-2.4% (dw)	<i>S. cerevisiae</i>	30°C, 48 h 150 rpm	N/A	0.037	[30]
<i>Scenedesmus obliquus</i> CNW-N	0.5-5% (w/v) H ₂ SO ₄ , 121°C, 20 min, pH 6.0		<i>Z. mobilis</i>	30°C, 24h	N/A	0.213	[31]
<i>Tetraselmis suecica</i>	0.75% (w/v) NaOH, room temperature, 10 min	3.4-27% (dw)	<i>S. cerevisiae</i>	30°C, 48 h, 150 rpm	N/A	0.073	[30]

* After concentration by lyophilization.