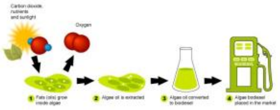
LOSCHMIDT LABORATORIES

6. Lecture – Biofuels



Bi7430 Molecular Biotechnology

Biofuels

- Alternatives to fossil fuels (crude oil, coal,…)
- Plant and animal biomass
- Primary biofuels like wood or crop waste used since ancient ages
- Most of the currently used biofuels are plant-based
- Algae and bacteria are promising sources of biofuels for the future

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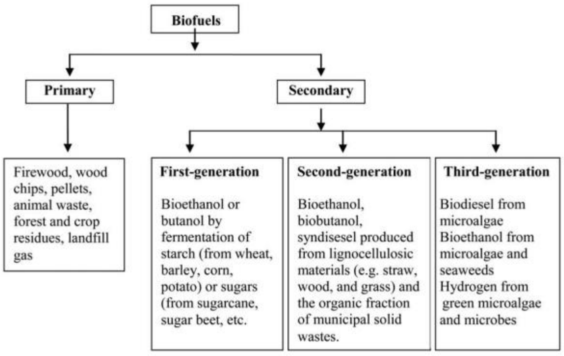
Generations of biofuels



Biofuels

Primary

Firewood, wood chips, pellets, animal waste, forest and crop residues, landfill gas

Secondary

First-generation

Bioethanol or butanol by fermentation of starch (from wheat, barley, corn, potato) or sugars (from sugarcane, sugar beet, etc.

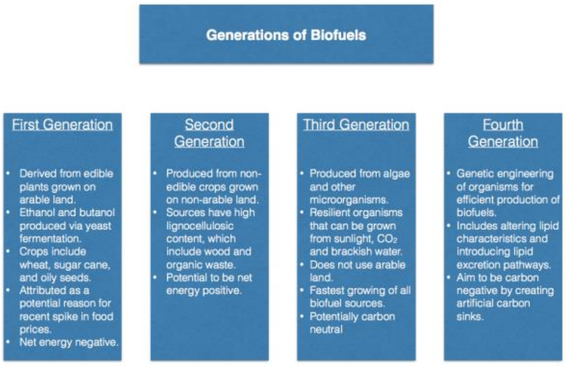
Second-generation

Bioethanol, biobutanol, syndiesel produced from lignocellulosic materials (e.g. straw, wood, and grass) and the organic fraction of municipal solid wastes.

Third-generation

Biodiesel from microalgae
Bioethanol from microalgae and seaweeds
Hydrogen from green microalgae and microbes

Generations of biofuels



Generations of Biofuels

First Generation

- Derived from edible plants grown on arable land.
- Ethanol and butanol produced via yeast fermentation.
- Crops include wheat, sugar cane, and oily seeds.
- Attributed as a potential reason for recent spike in food prices.
- Net energy negative.

Second Generation

- Produced from non-edible crops grown on non-arable land.
- Sources have high lignocellulosic content, which include wood and organic waste.
- Potential to be net energy positive.

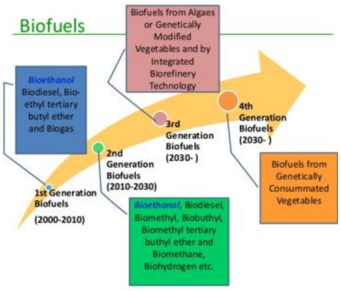
Third Generation

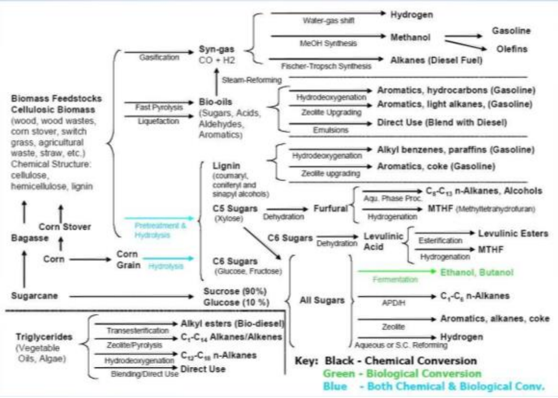
- Produced from algae and other microorganisms.
- Resilient organisms that can be grown from sunlight, CO_2 and brackish water.
- Does not use arable land.
- Fastest growing of all biofuel sources.
- Potentially carbon neutral

Fourth Generation

- Genetic engineering of organisms for efficient production of biofuels.
- Includes altering lipid characteristics and introducing lipid excretion pathways.
- Aim to be carbon negative by creating artificial carbon sinks.

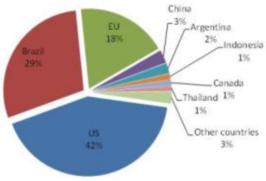
Generations of biofuels





Biofuels in the world

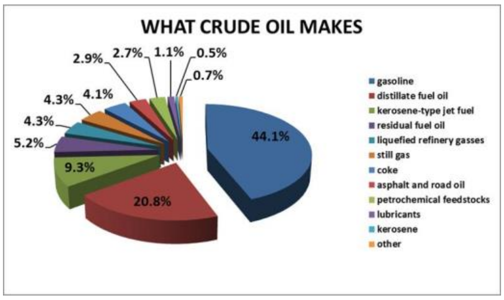
- Vast majority of the biofuels production is based in the US, Brazil and Europe



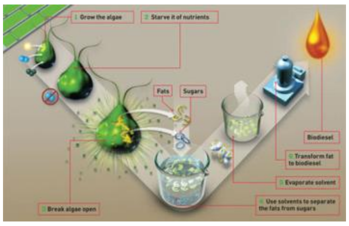
Why are biofuels important?

- Renewable sources of energy
- Lowering of carbon emissions
- Lower energy demands than ´traditional´ processes
- Biomass can be used for extraction of biologically active compounds and as biofuel
- Waste is biodegradable or can be used further

Crude oil consumption

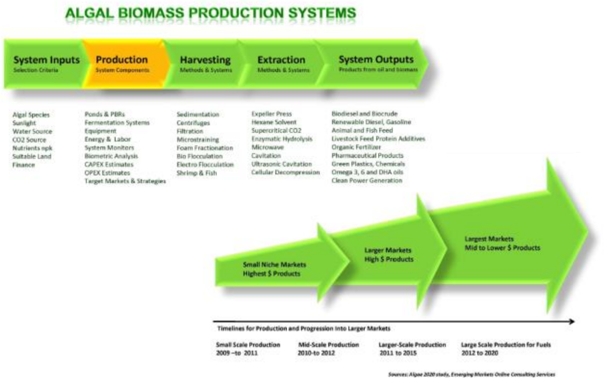


Algae as biofuels sources



Advantages and disadvantages of biofuel production using microalgae.

Advantages	Disadvantages
High growth rate	Low biomass concentration
Less water demand than land crops	Higher capital costs
High-efficiency CO_2 mitigation	
More cost effective farming	



ALGAL BIOMASS PRODUCTION SYSTEMS

System Inputs Selection Criteria	Production System Components	Harvesting Methods & Systems	Extraction Methods & Systems	System Outputs Products from oil and biomass
Algal Species	Ponds & PBRs	Sedimentation	Expeller Press	Biodiesel and Biocrude
Sunlight	Fermentation Systems	Centrifuges	Hexane Solvent	Renewable Diesel, Gasoline
Water Source	Equipment	Filtration	Supercritical CO2	Animal and Fish Feed
CO2 Source	Energy & Labor	Microstraining	Enzymatic Hydrolysis	Livestock Feed Protein Additives
Nutrients npk	System Monitors	Foam Fractionation	Microwave	Organic Fertilizer
Suitable Land	Biometric Analysis	Bio Flocculation	Cavitation	Pharmaceutical Products
Finance	CAPEX Estimates	Electro Flocculation	Ultrasonic Cavitation	Green Plastics, Chemicals
	OPEX Estimates	Shrimp & Fish	Cellular Decompression	Omega 3, 6 and DHA oils
	Target Markets & Strategies			Clean Power Generation

Timelines for Production and Progression Into Larger Markets

Small Scale Production	Mid-Scale Production	Larger-Scale Production	Large Scale Production for Fuels
2009 –to 2011	2010-to 2012	2011 to 2015	2012 to 2020

Sources: Algae 2020 study, Emerging Markets Online Consulting Services

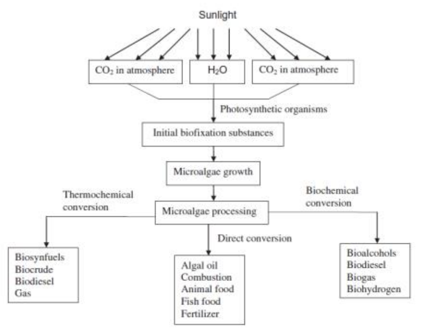
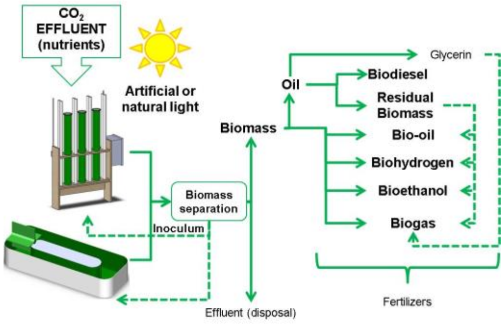


Fig. 1. Carbon dioxide fixation and main steps of algal biomass technologies.



CO_2
EFFLUENT
(nutrients)

Artificial or natural light

Biomass separation

Inoculum

Effluent (disposal)

Biomass

Oil

Glycerin

Biodiesel

Residual Biomass

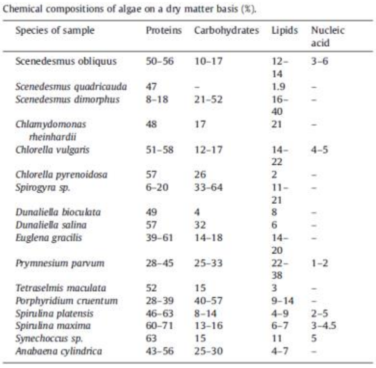
Bio-oil

Biohydrogen

Bioethanol

Biogas

Fertilizers



Chemical compositions of algae on a dry matter basis (%).

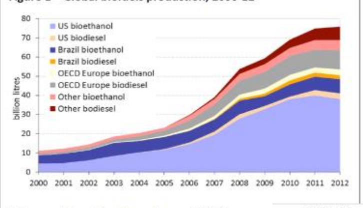
Species of sample	Proteins	Carbohydrates	Lipids	Nucleic acid
Scenedesmus obliquus	50–56	10–17	12–14	3–6
Scenedesmus quadricauda	47	–	1.9	–
Scenedesmus dimorphus	8–18	21–52	16–40	–
Chlamydomonas rheinhardii	48	17	21	–
Chlorella vulgaris	51–58	12–17	14–22	4–5
Chlorella pyrenoidosa	57	26	2	–
Spirogyra sp.	6–20	33–64	11–21	–
Dunaliella bioculata	49	4	8	–
Dunaliella salina	57	32	6	–
Euglena gracilis	39–61	14–18	14–20	–
Prymnesium parvum	28–45	25–33	22–38	1–2
Tetraselmis maculata	52	15	3	–
Porphyridium cruentum	28–39	40–57	9–14	–
Spirulina platensis	46–63	8–14	4–9	2–5
Spirulina maxima	60–71	13–16	6–7	3–4.5
Synechoccus sp.	63	15	11	5
Anabaena cylindrica	43–56	25–30	4–7	–

Biodiesel

- Methylesters of unsaturated fatty acids
- Better biodegradability than fossil-based diesel
- High energy capacity
- Can corrode the engine parts
- Higher health hazard than fossil fuels

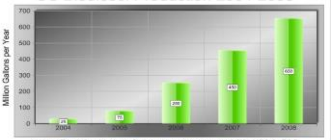
- In the EU 5 % of biodiesel has to be mixed with liquid fossil fuels

Figure 1 – Global biofuels production, 2000-12



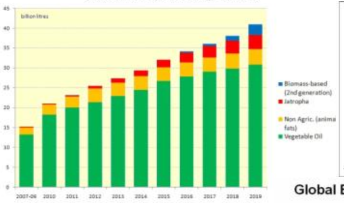
Data source: International Energy Agency, 2000-12.

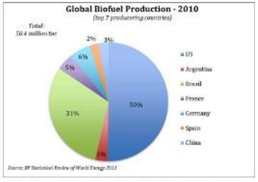
US Biodiesel Production 2004-2008



source Emerging Markets Online, Algae 2020 study, NBB, USDA, FAO

Global biodiesel production by feedstock





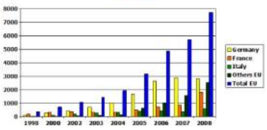
Global Biofuel Production - 2010

(top 7 producering countries)

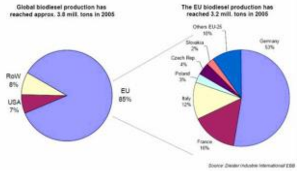
Source: BP Statistical Review of World Energy 2011

EU and Member States' Biodiesel Production ('000 t)

Source: EBB

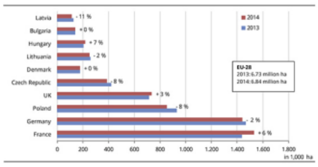


Global Biodiesel Production by Country



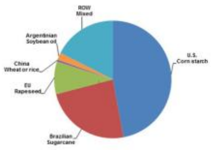
Source: Diesel Industrie International/ EBB

Increase in EU rapeseed area in 2014



Source: European Commission, AMI

Global Biofuels Market Share by Feedstock, World Markets: 2011



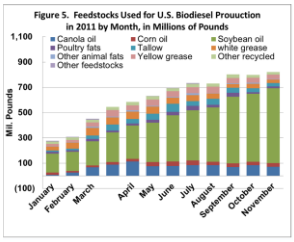
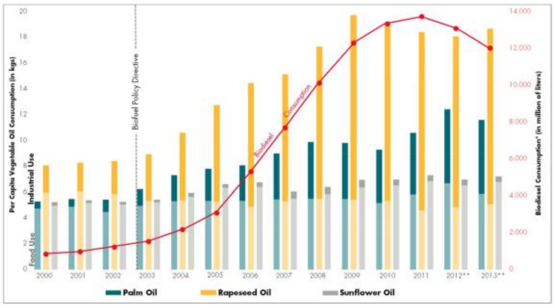


Figure 5. Feedstocks Used for U.S. Biodiesel Prouuction in 2011 by Month, in Millions of Pounds

Figure 3. EU Per Capita Consumption of Vegetable Oil and Biodiesel

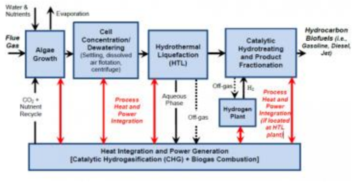


*Biodiesel consumption is total industrial consumption, converted from '000Barrels a day using EIA's unit conversion of 158.99 liters per barrel.

**2012-13 biodiesel consumption based on percent change from USDA estimates.

Sources: Vegetable Oil Consumption, USDA Foreign Agricultural Service, Production, Supply and Distribution database; per capita calculated using World Bank, World Development Indicators data on population. Biodiesel Consumption is from US Energy Information Administration, International Energy Statistics.

Algae processing



- Water removal is important
- 20% humidity after dewatering

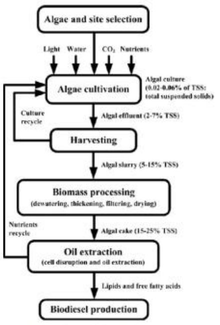


Fig. 1. Microalgae biodiesel value chain stages.

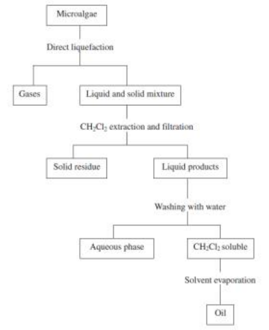


Fig. 3. Direct liquefaction of microalgae and oil from liquefaction products by CH_2Cl_2 extraction.

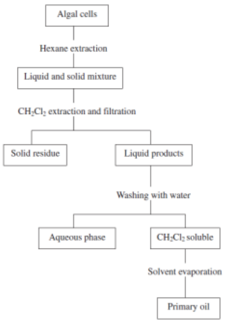


Fig. 4. Primary oil from algal cells by liquefaction of hexane extraction.

Comparison of microalgae with other biodiesel feedstocks.

Plant source	Seed oil content (% oil by wt in biomass)	Oil yield (L oil/ha year)	Land use (m^2 year/kg biodiesel)	Biodiesel productivity (kg biodiesel/ha year)
Corn/Maize (*Zea mays* L.)	44	172	66	152
Hemp (*Cannabis sativa* L.)	33	363	31	321
Soybean (*Glycine max* L.)	18	636	18	562
Jatropha (*Jatropha curcas* L.)	28	741	15	656
Camelina (*Camelina sativa* L.)	42	915	12	809
Canola/Rapeseed (*Brassica napus* L.)	41	974	12	862
Sunflower (*Helianthus annuus* L.)	40	1070	11	946
Castor (*Ricinus communis*)	48	1307	9	1156
Palm oil (*Elaeis guineensis*)	36	5366	2	4747
Microalgae (low oil content)	30	58,700	0.2	51,927
Microalgae (medium oil content)	50	97,800	0.1	86,515
Microalgae (high oil content)	70	136,900	0.1	121,104

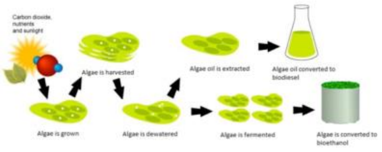
Yields of bio-oil by pyrolysis from alga samples at different temperatures (K).

Sample	575	625	675	725	775	825	875
Cladophora fracta	10.5	23.5	33.2	43.4	48.2	46.8	44.6
Chlorella protothecoides	12.8	27.4	38.4	50.2	55.3	53.7	51.6

Table 1 | Comparative study between algal biomass and terrestrial plants for biodiesel production.

Feedstock	Conditions	Biodiesel	Reference
ALGAE			
Spirulina platensis	Reaction temperature 55°C, 60% catalyst concentration, 1:4 algae biomass to methanol ratio, 450 rpm stirring intensity	60 g/kg lipid	Nautiyal et al. (2014)
Nannochloropsis sp.	Oil extraction with n-hexane, acidic transesterification	99 g/kg lipid	Susilaningsih et al. (2009)
Scenedesmus sp.	Alkaline (NaOH), temperature of 70°C	321.06 g/kg lipid	Kim et al. (2014)
	Acidic (H_2SO_4) catalyst, temperature of 70°C	282.23 g/kg lipid	
Nannochloropsis salina	Freeze drying of biomass, extraction with chloroform–methanol (1:1 ratio), alkali transesterification	180.78 g/kg lipid	Muthukumar et al. (2012)
Chlorella marina		100 g/kg lipid	
TERRESTRIAL PLANTS			
Madhuca indica	0.30–0.35 (v/v) methanol-to-oil ratio, 1% (v/v) H_2SO_4 as acid catalyst, 0.25 (v/v) methanol, 0.7% (w/v) KOH as alkaline catalyst	186.2 g/kg lipid	Ghadge and Raheman (2005)
Pongamia pinnata	Transesterification with methanol, NaOH as catalyst, temp. 60°C	253 g/kg lipid	Mamilla et al. (2011)
	Acid-catalyzed esterification by using 0.5% H_2SO_4, alkali-catalyzed transesterification	193.2 g/kg lipid	Naik et al. (2008)
Azadirachta indica	Reaction time of 60 min, 0.7% H_2SO_4 as acid catalyst, reaction temperature of 50°C, and methanol: oil ratio of 3:1	170 g/kg lipid	Awolu and Layokun (2013)
Soybean	Hydrotalcite as basic catalyst, methanol/oil molar ratio of 20:1, reaction time of 10 h	189.6 g/kg lipid	Martin et al. (2013)

Bioethanol



- Production depends on content of fermentable sugars
- Production higher than 4 % (40 g/L) is necessary to make the proces economically feasible

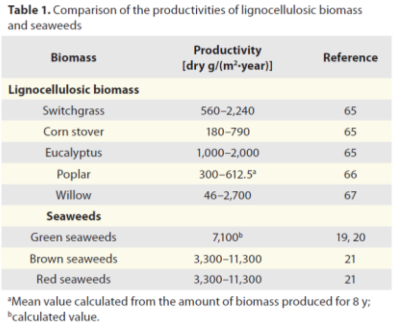


Table 1. Comparison of the productivities of lignocellulosic biomass and seaweeds

Biomass	Productivity [dry g/(m^2·year)]	Reference
Lignocellulosic biomass		
Switchgrass	560–2,240	65
Corn stover	180–790	65
Eucalyptus	1,000–2,000	65
Poplar	300–612.5[a]	66
Willow	46–2,700	67
Seaweeds		
Green seaweeds	7,100[b]	19, 20
Brown seaweeds	3,300–11,300	21
Red seaweeds	3,300–11,300	21

[a] Mean value calculated from the amount of biomass produced for 8 y; [b] calculated value.

Bioethanol production

- Cells are pretreated using acid or enzymatic hydrolysis
- Hydrothermal pretreatment may be applied

- Ethanol fermentation by bacteria or yeast
  - *Saccharomyces cerevisiae*
  - or technical cultures
- Mannitol cannot be converted by *S. cerevisiae*

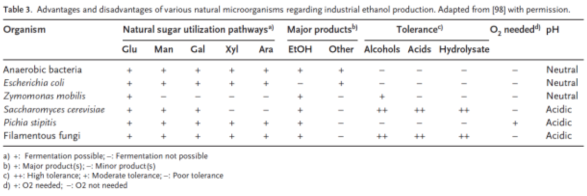


Table 3. Advantages and disadvantages of various natural microorganisms regarding industrial ethanol production. Adapted from [98] with permission.

Organism	Natural sugar utilization pathways[a)]					Major products[b)]		Tolerance[c)]			O_2 needed[d)]	pH
	Glu	Man	Gal	Xyl	Ara	EtOH	Other	Alcohols	Acids	Hydrolysate		
Anaerobic bacteria	+	+	+	+	+	+	+	–	–	–	–	Neutral
Escherichia coli	+	+	+	+	+	–	+	–	–	–	–	Neutral
Zymomonas mobilis	+	–	–	–	–	+	–	+	–	–	–	Neutral
Saccharomyces cerevisiae	+	+	+	–	–	+	–	++	++	++	–	Acidic
Pichia stipitis	+	+	+	+	+	+	–	–	–	–	+	Acidic
Filamentous fungi	+	+	+	+	+	+	–	++	++	++	–	Acidic

a) +: Fermentation possible; –: Fermentation not possible
b) +: Major product(s); –: Minor product(s)
c) ++: High tolerance; +: Moderate tolerance; –: Poor tolerance
d) +: O2 needed; –: O2 not needed

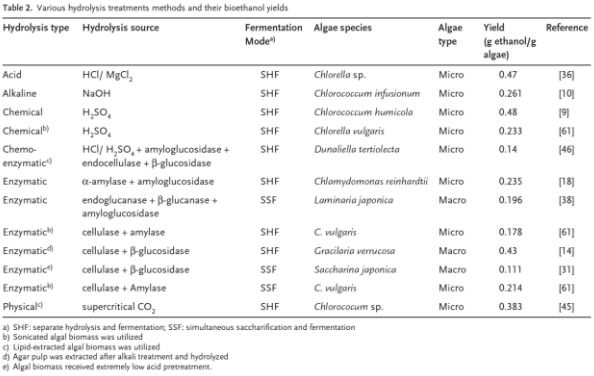


Table 2. Various hydrolysis treatments methods and their bioethanol yields

Hydrolysis type	Hydrolysis source	Fermentation Mode[a)]	Algae species	Algae type	Yield (g ethanol/g algae)	Reference
Acid	HCl/ $MgCl_2$	SHF	*Chlorella* sp.	Micro	0.47	[36]
Alkaline	NaOH	SHF	*Chlorococcum infusionum*	Micro	0.261	[10]
Chemical	H_2SO_4	SHF	*Chlorococcum humicola*	Micro	0.48	[9]
Chemical[b)]	H_2SO_4	SHF	*Chlorella vulgaris*	Micro	0.233	[61]
Chemo-enzymatic[c)]	HCl/ H_2SO_4 + amyloglucosidase + endocellulase + β-glucosidase	SHF	*Dunaliella tertiolecta*	Micro	0.14	[46]
Enzymatic	α-amylase + amyloglucosidase	SHF	*Chlamydomonas reinhardtii*	Micro	0.235	[18]
Enzymatic	endoglucanase + β-glucanase + amyloglucosidase	SSF	*Laminaria japonica*	Macro	0.196	[38]
Enzymatic[b)]	cellulase + amylase	SHF	*C. vulgaris*	Micro	0.178	[61]
Enzymatic[d)]	cellulase + β-glucosidase	SHF	*Gracilaria verrucosa*	Macro	0.43	[14]
Enzymatic[e)]	cellulase + β-glucosidase	SSF	*Saccharina japonica*	Macro	0.111	[31]
Enzymatic[b)]	cellulase + Amylase	SSF	*C. vulgaris*	Micro	0.214	[61]
Physical[c)]	supercritical CO_2	SHF	*Chlorococum* sp.	Micro	0.383	[45]

a) SHF: separate hydrolysis and fermentation; SSF: simultaneous saccharification and fermentation
b) Sonicated algal biomass was utilized
c) Lipid-extracted algal biomass was utilized
d) Agar pulp was extracted after alkali treatment and hydrolyzed
e) Algal biomass received extremely low acid pretreatment.

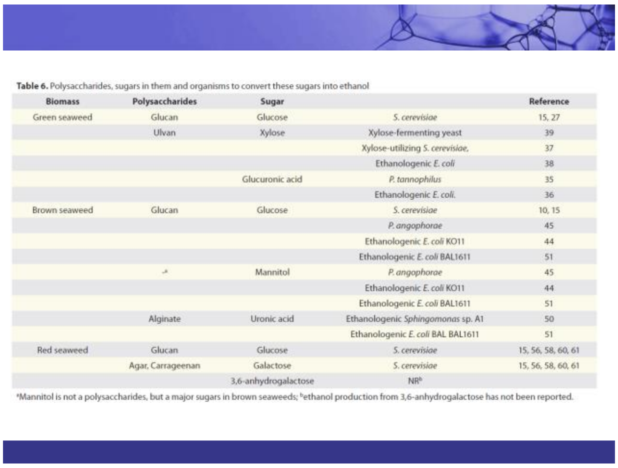
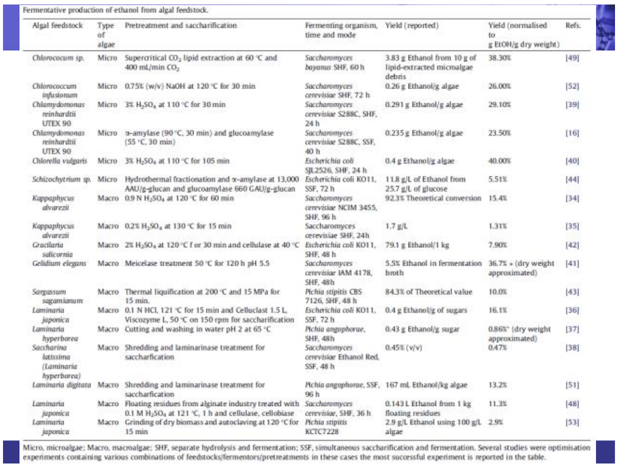


Table 6. Polysaccharides, sugars in them and organisms to convert these sugars into ethanol

Biomass	Polysaccharides	Sugar		Reference
Green seaweed	Glucan	Glucose	*S. cerevisiae*	15, 27
	Ulvan	Xylose	Xylose-fermenting yeast	39
			Xylose-utilizing *S. cerevisiae,*	37
			Ethanologenic *E. coli*	38
		Glucuronic acid	*P. tannophilus*	35
			Ethanologenic *E. coli.*	36
Brown seaweed	Glucan	Glucose	*S. cerevisiae*	10, 15
			P. angophorae	45
			Ethanologenic *E. coli* KO11	44
			Ethanologenic *E. coli* BAL1611	51
	-[a]	Mannitol	*P. angophorae*	45
			Ethanologenic *E. coli* KO11	44
			Ethanologenic *E. coli* BAL1611	51
	Alginate	Uronic acid	Ethanologenic *Sphingomonas* sp. A1	50
			Ethanologenic *E. coli* BAL BAL1611	51
Red seaweed	Glucan	Glucose	*S. cerevisiae*	15, 56, 58, 60, 61
	Agar, Carrageenan	Galactose	*S. cerevisiae*	15, 56, 58, 60, 61
		3,6-anhydrogalactose	NR[b]	

[a] Mannitol is not a polysaccharides, but a major sugars in brown seaweeds; [b] ethanol production from 3,6-anhydrogalactose has not been reported.



Fermentative production of ethanol from algal feedstock.

Algal feedstock	Type of algae	Pretreatment and saccharification	Fermenting organism, time and mode	Yield (reported)	Yield (normalised to g EtOH/g dry weight)	Refs.
Chlorococum sp.	Micro	Supercritical CO_2 lipid extraction at 60 °C and 400 mL/min CO_2	*Saccharomyces bayanus* SHF, 60 h	3.83 g Ethanol from 10 g of lipid-extracted microalgae debris	38.30%	[49]
Chlorococcum infusionum	Micro	0.75% (w/v) NaOH at 120 °C for 30 min	*Saccharomyces cerevisiae* SHF, 72 h	0.26 g Ethanol/g algae	26.00%	[52]
Chlamydomonas reinhardtii UTEX 90	Micro	3% H_2SO_4 at 110 °C for 30 min	*Saccharomyces cerevisiae* S288C, SHF, 24 h	0.291 g Ethanol/g algae	29.10%	[39]
Chlamydomonas reinhardtii UTEX 90	Micro	α-amylase (90 °C, 30 min) and glucoamylase (55 °C, 30 min)	*Saccharomyces cerevisiae* S288C, SSF, 40 h	0.235 g Ethanol/g algae	23.50%	[16]
Chlorella vulgaris	Micro	3% H_2SO_4 at 110 °C for 105 min	*Escherichia coli* SJL2526, SHF, 24 h	0.4 g Ethanol/g algae	40.00%	[40]
Schizochytrium sp.	Micro	Hydrothermal fractionation and α-amylase at 13,000 AAU/g-glucan and glucoamylase 660 GAU/g-glucan	*Escherichia* coli KO11, SSF, 72 h	11.8 g/L of Ethanol from 25.7 g/L of glucose	5.51%	[44]
Kappaphycus alvarezii	Macro	0.9 N H_2SO_4 at 120 °C for 60 min	*Saccharomyces cerevisiae* NCIM 3455, SHF, 96 h	92.3% Theoretical conversion	15.4%	[34]
Kappaphycus alvarezii	Macro	0.2% H_2SO_4 at 130 °C for 15 min	Saccharomyces cerevisiae SHF, 24h	1.7 g/L	1.31%	[35]
Gracilaria salicornia	Macro	2% H_2SO_4 at 120 °C f or 30 min and cellulase at 40 °C	*Escherichia coli* KO11, SHF, 48 h	79.1 g Ethanol/1 kg	7.90%	[42]
Gelidium elegans	Macro	Meicelase treatment 50 °C for 120 h pH 5.5	*Saccharomyces cerevisiae* IAM 4178, SHF, 48h	5.5% Ethanol in fermentation broth	36.7% * (dry weight approximated)	[41]
Sargassum sagamianum	Macro	Thermal liquification at 200 °C and 15 MPa for 15 min.	*Pichia stipitis* CBS 7126, SHF, 48 h	84.3% of Theoretical value	10.0%	[43]
Laminaria japonica	Macro	0.1 N HCl, 121 °C for 15 min and Celluclast 1.5 L, Viscozyme L, 50 °C on 150 rpm for saccharification	*Escherichia coli* KO11, SSF, 72 h	0.4 g Ethanol/g of sugars	16.1%	[36]
Laminaria hyperborea	Macro	Cutting and washing in water pH 2 at 65 °C	*Pichia angophorae*, SHF, 48h	0.43 g Ethanol/g sugar	0.86%* (dry weight approximated)	[37]
Saccharina latissima (Laminaria hyperborea)	Macro	Shredding and laminarinase treatment for saccharfication	*Saccharomyces cerevisiae* Ethanol Red, SSF, 48 h	0.45% (v/v)	0.47%	[38]
Laminaria digitata	Macro	Shredding and laminarinase treatment for saccharfication	*Pichia angophorae*, SSF, 96 h	167 mL Ethanol/kg algae	13.2%	[51]
Laminaria japonica	Macro	Floating residues from alginate industry treated with 0.1 M H_2SO_4 at 121 °C, 1 h and cellulase, cellobiase	*Saccharomyces cerevisiae*, SHF, 36 h	0.143 L Ethanol from 1 kg floating residues	11.3%	[48]
Laminaria japonica	Macro	Grinding of dry biomass and autoclaving at 120 °C for 15 min	*Pichia stipitis* KCTC7228	2.9 g/L Ethanol using 100 g/L algae	2.9%	[53]

Micro, microalgae; Macro, macroalgae; SHF, separate hydrolysis and fermentation; SSF, simultaneous saccharification and fermentation. Several studies were optimisation experiments containing various combinations of feedstocks/fermentors/pretreatments in these cases the most successful experiment is reported in the table.

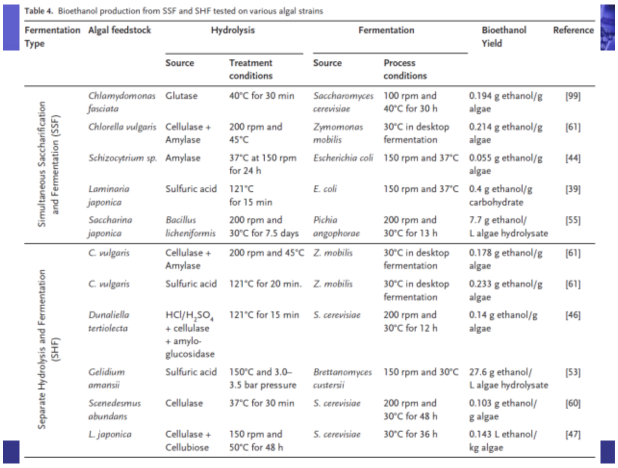
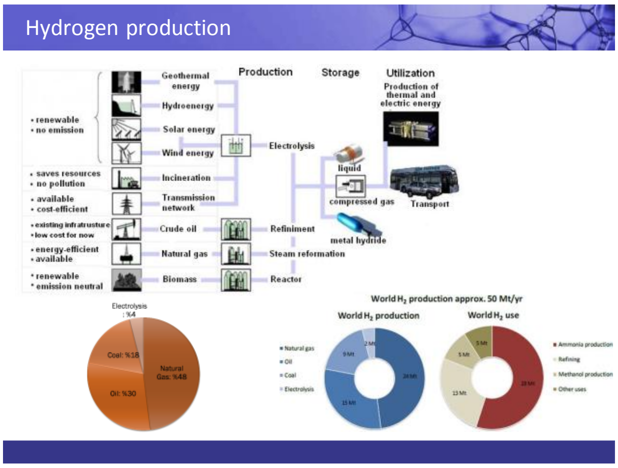


Table 4. Bioethanol production from SSF and SHF tested on various algal strains

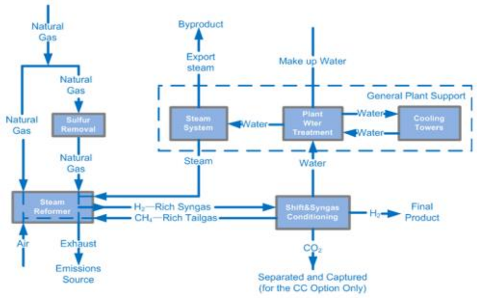
Fermentation Type	Algal feedstock	Hydrolysis		Fermentation		Bioethanol Yield	Reference
		Source	Treatment conditions	Source	Process conditions		
Simultaneous Saccharification and Fermentation (SSF)	*Chlamydomonas fasciata*	Glutase	40°C for 30 min	*Saccharomyces cerevisiae*	100 rpm and 40°C for 30 h	0.194 g ethanol/g algae	[99]
	Chlorella vulgaris	Cellulase + Amylase	200 rpm and 45°C	*Zymomonas mobilis*	30°C in desktop fermentation	0.214 g ethanol/g algae	[61]
	Schizocytrium sp.	Amylase	37°C at 150 rpm for 24 h	*Escherichia coli*	150 rpm and 37°C	0.055 g ethanol/g algae	[44]
	Laminaria japonica	Sulfuric acid	121°C for 15 min	*E. coli*	150 rpm and 37°C	0.4 g ethanol/g carbohydrate	[39]
	Saccharina japonica	*Bacillus licheniformis*	200 rpm and 30°C for 7.5 days	*Pichia angophorae*	200 rpm and 30°C for 13 h	7.7 g ethanol/ L algae hydrolysate	[55]
Separate Hydrolysis and Fermentation (SHF)	*C. vulgaris*	Cellulase + Amylase	200 rpm and 45°C	*Z. mobilis*	30°C in desktop fermentation	0.178 g ethanol/g algae	[61]
	C. vulgaris	Sulfuric acid	121°C for 20 min.	*Z. mobilis*	30°C in desktop fermentation	0.233 g ethanol/g algae	[61]
	Dunaliella tertiolecta	HCl/H_2SO_4 + cellulase + amylo-glucosidase	121°C for 15 min	*S. cerevisiae*	200 rpm and 30°C for 12 h	0.14 g ethanol/g algae	[46]
	Gelidium amansii	Sulfuric acid	150°C and 3.0–3.5 bar pressure	*Brettanomyces custersii*	150 rpm and 30°C	27.6 g ethanol/ L algae hydrolysate	[53]
	Scenedesmus abundans	Cellulase	37°C for 30 min	*S. cerevisiae*	200 rpm and 30°C for 48 h	0.103 g ethanol/ g algae	[60]
	L. japonica	Cellulase + Cellubiose	150 rpm and 50°C for 48 h	*S. cerevisiae*	30°C for 36 h	0.143 L ethanol/ kg algae	[47]

Hydrogen production

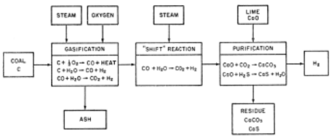


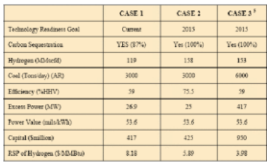
Hydrogen production from natural gas

- $CH_4 + H_2O \rightleftharpoons CO + 3\,H_2$ (at 700 – 1100 °C) – steam reforming

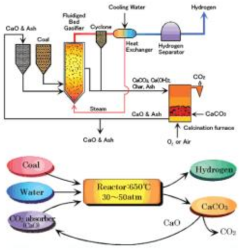


Hydrogen from coal

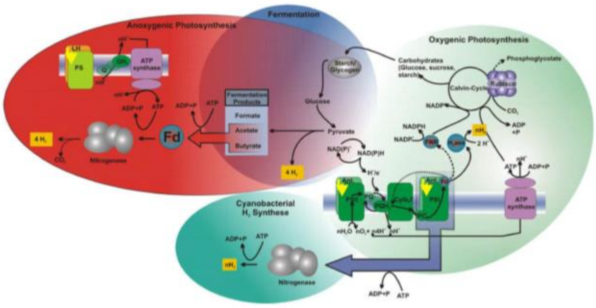




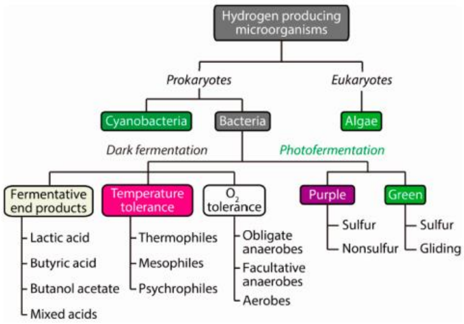
	CASE 1	CASE 2	CASE 3 [5]
Technology Readiness Goal	Current	2015	2015
Carbon Sequestration	YES (87%)	Yes (100%)	Yes (100%)
Hydrogen (MMscfd)	119	158	153
Coal (Tons/day) (AR)	3000	3000	6000
Efficiency (%HHV)	59	75.5	59
Excess Power (MW)	26.9	25	417
Power Value (mils/kWh)	53.6	53.6	53.6
Capital ($million)	417	425	950
RSP of Hydrogen ($/MMBtu)	8.18	5.89	3.98

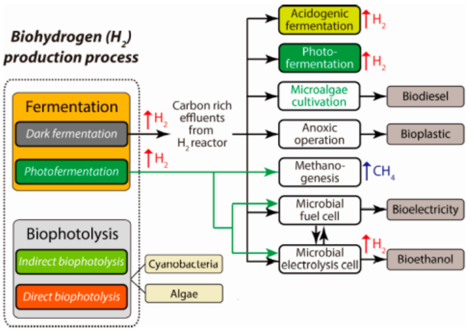


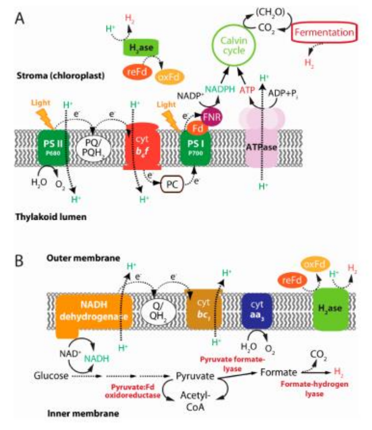
Biohydrogen production



Biohydrogen production







Nitrogenase in cyanobacteria

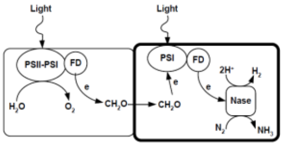
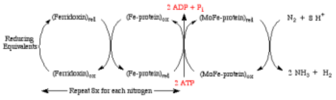


Fig. 2. Nitrogenase(Nase)-mediated hydrogen evolution in a heterocyst of nitrogen-fixing heterocystous cyanobacteria [10, 30, 32]. The oxygen and hydrogen evolution are carried out separately and the energy-rich carbohydrate (CH_2O) is used as the electron source in the oxygen-free heterocyst.

$$N_2 + 8H^+ + 8e + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi \qquad \ldots 2$$

$$8H^+ + 8e + 16ATP \rightarrow 4H_2 + 16ADP + 16Pi \qquad \ldots 3$$



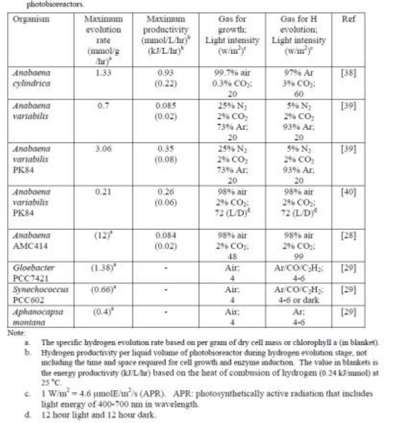


Table 1. Hydrogen evolution via direct biophotolysis by cyanobacteria in laboratory photobioreactors.

Organism	Maximum evolution rate (mmol/g /hr)[a]	Maximum productivity (mmol/L/hr)[b] (kJ/L/hr)[b]	Gas for growth; Light intensity (w/m²)[c]	Gas for H evolution; Light intensity (w/m²)[c]	Ref
Anabaena cylindrica	1.33	0.93 (0.22)	99.7% air 0.3% CO_2; 20	97% Ar 3% CO_2; 60	[38]
Anabaena variabilis	0.7	0.085 (0.02)	25% N_2 2% CO_2 73% Ar; 20	5% N_2 2% CO_2 93% Ar; 20	[39]
Anabaena variabilis PK84	3.06	0.35 (0.08)	25% N_2 2% CO_2 73% Ar; 20	5% N_2 2% CO_2 93% Ar; 20	[39]
Anabaena variabilis PK84	0.21	0.26 (0.06)	98% air 2% CO_2; 72 (L/D)[d]	98% air 2% CO_2; 72 (L/D)[d]	[40]
Anabaena AMC414	(12)[a]	0.084 (0.02)	98% air 2% CO_2; 48	98% air 2% CO_2; 99	[28]
Gloebacter PCC7421	(1.38)[a]	-	Air; 4	Ar/CO/C_2H_2; 4-6	[29]
Synechococcus PCC602	(0.66)[a]	-	Air; 4	Ar/CO/C_2H_2; 4-6 or dark	[29]
Aphanocapsa montana	(0.4)[a]	-	Air; 4	Ar; 4-6	[29]

Note:

a. The specific hydrogen evolution rate based on per gram of dry cell mass or chlorophyll a (in blanket).

b. Hydrogen productivity per liquid volume of photobioreactor during hydrogen evolution stage, not including the time and space required for cell growth and enzyme induction. The value in blankets is the energy productivity (kJ/L/hr) based on the heat of combusion of hydrogen (0.24 kJ/mmol) at 25 °C.

c. 1 W/m² = 4.6 µmolE/m²/s (APR). APR: photosynthetically active radiation that includes light energy of 400-700 nm in wavelength.

d. 12 hour light and 12 hour dark.

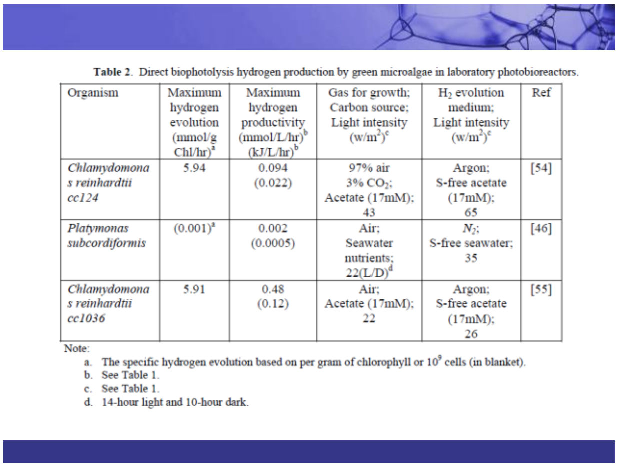


Table 2. Direct biophotolysis hydrogen production by green microalgae in laboratory photobioreactors.

Organism	Maximum hydrogen evolution (mmol/g Chl/hr)[a]	Maximum hydrogen productivity (mmol/L/hr)[b] (kJ/L/hr)[b]	Gas for growth; Carbon source; Light intensity (w/m²)[c]	H_2 evolution medium; Light intensity (w/m²)[c]	Ref
Chlamydomonas reinhardtii cc124	5.94	0.094 (0.022)	97% air 3% CO_2; Acetate (17mM); 43	Argon; S-free acetate (17mM); 65	[54]
Platymonas subcordiformis	(0.001)[a]	0.002 (0.0005)	Air; Seawater nutrients; 22(L/D)[d]	*N_2*; S-free seawater; 35	[46]
Chlamydomonas reinhardtii cc1036	5.91	0.48 (0.12)	Air; Acetate (17mM); 22	Argon; S-free acetate (17mM); 26	[55]

Note:

a. The specific hydrogen evolution based on per gram of chlorophyll or 10^9 cells (in blanket).
b. See Table 1.
c. See Table 1.
d. 14-hour light and 10-hour dark.

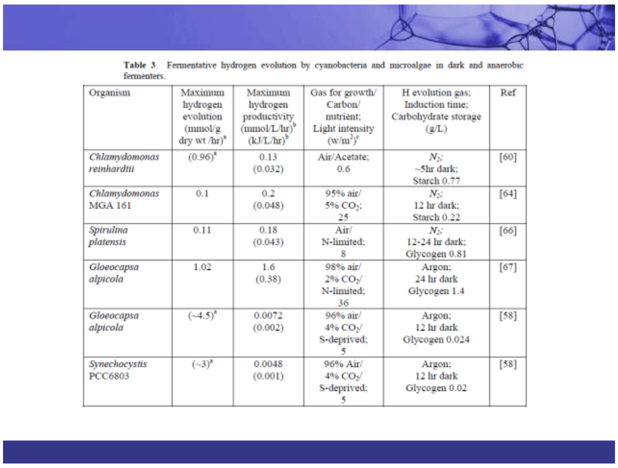


Table 3. Fermentative hydrogen evolution by cyanobacteria and microalgae in dark and anaerobic fermenters.

Organism	Maximum hydrogen evolution (mmol/g dry wt /hr)[a]	Maximum hydrogen productivity (mmol/L/hr)[b] (kJ/L/hr)[b]	Gas for growth/ Carbon/ nutrient; Light intensity (w/m²)[c]	H evolution gas; Induction time; Carbohydrate storage (g/L)	Ref
Chlamydomonas reinhardtii	(0.96)[a]	0.13 (0.032)	Air/Acetate; 0.6	*N_2*; ~5hr dark; Starch 0.77	[60]
Chlamydomonas MGA 161	0.1	0.2 (0.048)	95% air/ 5% CO_2; 25	*N_2*; 12 hr dark; Starch 0.22	[64]
Spirulina platensis	0.11	0.18 (0.043)	Air/ N-limited; 8	*N_2*; 12-24 hr dark; Glycogen 0.81	[66]
Gloeocapsa alpicola	1.02	1.6 (0.38)	98% air/ 2% CO_2/ N-limited; 36	Argon; 24 hr dark Glycogen 1.4	[67]
Gloeocapsa alpicola	(~4.5)[a]	0.0072 (0.002)	96% air/ 4% CO_2/ S-deprived; 5	Argon; 12 hr dark Glycogen 0.024	[58]
Synechocystis PCC6803	(~3)[a]	0.0048 (0.001)	96% Air/ 4% CO_2/ S-deprived; 5	Argon; 12 hr dark Glycogen 0.02	[58]

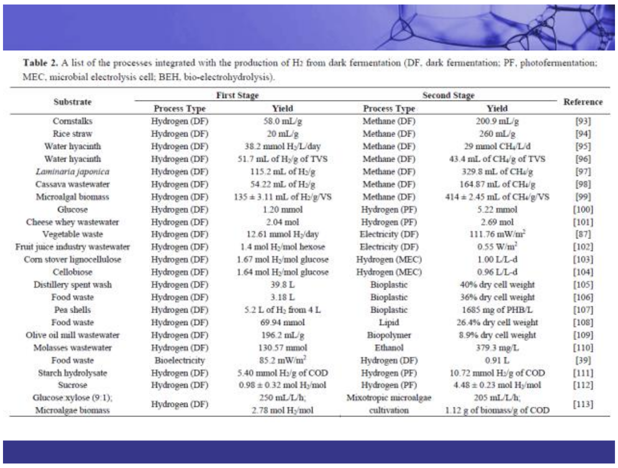
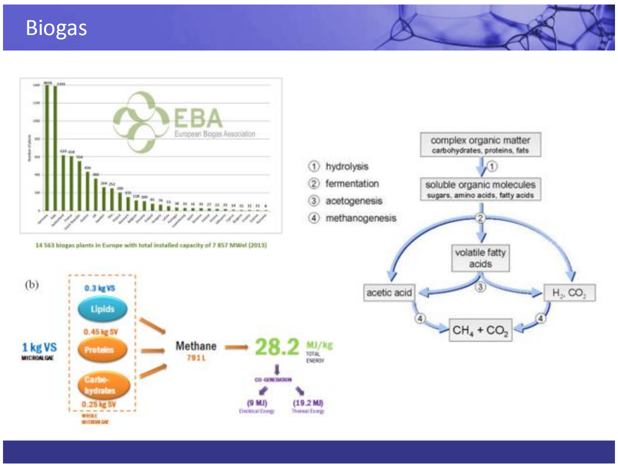


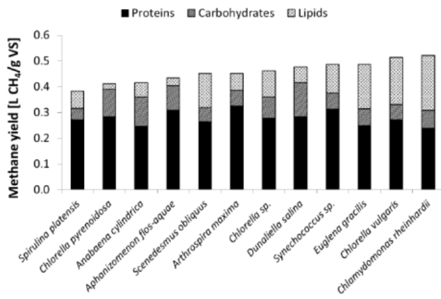
Table 2. A list of the processes integrated with the production of H_2 from dark fermentation (DF, dark fermentation; PF, photofermentation; MEC, microbial electrolysis cell; BEH, bio-electrohydrolysis).

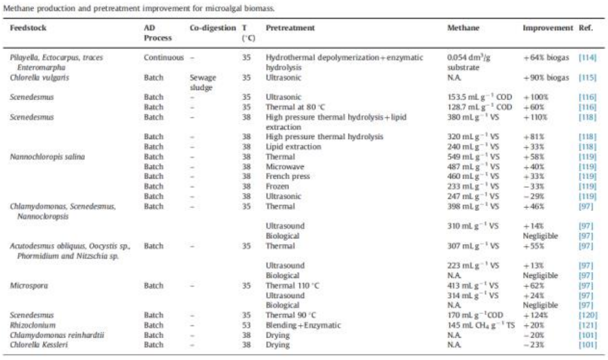
Substrate	First Stage		Second Stage		Reference
	Process Type	Yield	Process Type	Yield	
Cornstalks	Hydrogen (DF)	58.0 mL/g	Methane (DF)	200.9 mL/g	[93]
Rice straw	Hydrogen (DF)	20 mL/g	Methane (DF)	260 mL/g	[94]
Water hyacinth	Hydrogen (DF)	38.2 mmol H_2/L/day	Methane (DF)	29 mmol CH_4/L/d	[95]
Water hyacinth	Hydrogen (DF)	51.7 mL of H_2/g of TVS	Methane (DF)	43.4 mL of CH_4/g of TVS	[96]
Laminaria japonica	Hydrogen (DF)	115.2 mL of H_2/g	Methane (DF)	329.8 mL of CH_4/g	[97]
Cassava wastewater	Hydrogen (DF)	54.22 mL of H_2/g	Methane (DF)	164.87 mL of CH_4/g	[98]
Microalgal biomass	Hydrogen (DF)	135 ± 3.11 mL of H_2/g/VS	Methane (DF)	414 ± 2.45 mL of CH_4/g/VS	[99]
Glucose	Hydrogen (DF)	1.20 mmol	Hydrogen (PF)	5.22 mmol	[100]
Cheese whey wastewater	Hydrogen (DF)	2.04 mol	Hydrogen (PF)	2.69 mol	[101]
Vegetable waste	Hydrogen (DF)	12.61 mmol H_2/day	Electricity (DF)	111.76 mW/m²	[87]
Fruit juice industry wastewater	Hydrogen (DF)	1.4 mol H_2/mol hexose	Electricity (DF)	0.55 W/m²	[102]
Corn stover lignocellulose	Hydrogen (DF)	1.67 mol H_2/mol glucose	Hydrogen (MEC)	1.00 L/L-d	[103]
Cellobiose	Hydrogen (DF)	1.64 mol H_2/mol glucose	Hydrogen (MEC)	0.96 L/L-d	[104]
Distillery spent wash	Hydrogen (DF)	39.8 L	Bioplastic	40% dry cell weight	[105]
Food waste	Hydrogen (DF)	3.18 L	Bioplastic	36% dry cell weight	[106]
Pea shells	Hydrogen (DF)	5.2 L of H_2 from 4 L	Bioplastic	1685 mg of PHB/L	[107]
Food waste	Hydrogen (DF)	69.94 mmol	Lipid	26.4% dry cell weight	[108]
Olive oil mill wastewater	Hydrogen (DF)	196.2 mL/g	Biopolymer	8.9% dry cell weight	[109]
Molasses wastewater	Hydrogen (DF)	130.57 mmol	Ethanol	379.3 mg/L	[110]
Food waste	Bioelectricity	85.2 mW/m²	Hydrogen (DF)	0.91 L	[39]
Starch hydrolysate	Hydrogen (DF)	5.40 mmol H_2/g of COD	Hydrogen (PF)	10.72 mmol H_2/g of COD	[111]
Sucrose	Hydrogen (DF)	0.98 ± 0.32 mol H_2/mol	Hydrogen (PF)	4.48 ± 0.23 mol H_2/mol	[112]
Glucose:xylose (9:1); Microalgae biomass	Hydrogen (DF)	250 mL/L/h; 2.78 mol H_2/mol	Mixotropic microalgae cultivation	205 mL/L/h; 1.12 g of biomass/g of COD	[113]

Biogas

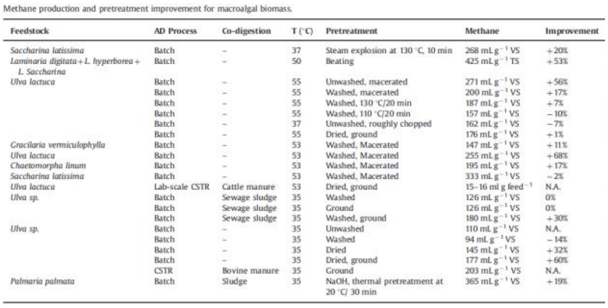


14 563 biogas plants in Europe with total installed capacity of 7 857 MWel (2013)




Methane production and pretreatment improvement for microalgal biomass.

Feedstock	AD Process	Co-digestion	T (°C)	Pretreatment	Methane	Improvement	Ref.
Pilayella, Ectocarpus, traces Enteromarpha	Continuous	–	35	Hydrothermal depolymerization + enzymatic hydrolysis	0.054 dm^3/g substrate	+64% biogas	[114]
Chlorella vulgaris	Batch	Sewage sludge	35	Ultrasonic	N.A.	+90% biogas	[115]
Scenedesmus	Batch	–	35	Ultrasonic	153.5 mL g^{-1} COD	+100%	[116]
	Batch	–	35	Thermal at 80 °C	128.7 mL g^{-1} COD	+60%	[116]
Scenedesmus	Batch	–	38	High pressure thermal hydrolysis + lipid extraction	380 mL g^{-1} VS	+110%	[118]
	Batch	–	38	High pressure thermal hydrolysis	320 mL g^{-1} VS	+81%	[118]
	Batch	–	38	Lipid extraction	240 mL g^{-1} VS	+33%	[118]
Nannochloropis salina	Batch	–	38	Thermal	549 mL g^{-1} VS	+58%	[119]
	Batch	–	38	Microwave	487 mL g^{-1} VS	+40%	[119]
	Batch	–	38	French press	460 mL g^{-1} VS	+33%	[119]
	Batch	–	38	Frozen	233 mL g^{-1} VS	−33%	[119]
	Batch	–	38	Ultrasonic	247 mL g^{-1} VS	−29%	[119]
Chlamydomonas, Scenedesmus, Nannocloropsis	Batch	–	35	Thermal	398 mL g^{-1} VS	+46%	[97]
				Ultrasound	310 mL g^{-1} VS	+14%	[97]
				Biological		Negligible	[97]
Acutodesmus obliquus, Oocystis sp., Phormidium and Nitzschia sp.	Batch	–	35	Thermal	307 mL g^{-1} VS	+55%	[97]
				Ultrasound	223 mL g^{-1} VS	+13%	[97]
				Biological	N.A.	Negligible	[97]
Microspora	Batch	–	35	Thermal 110 °C	413 mL g^{-1} VS	+62%	[97]
				Ultrasound	314 mL g^{-1} VS	+24%	[97]
				Biological	N.A.	Negligible	[97]
Scenedesmus	Batch	–	35	Thermal 90 °C	170 mL g^{-1}COD	+124%	[120]
Rhizoclonium	Batch	–	53	Blending + Enzymatic	145 mL CH$_4$ g^{-1} TS	+20%	[121]
Chlamydomonas reinhardtii	Batch	–	38	Drying	N.A.	−20%	[101]
Chlorella Kessleri	Batch	–	38	Drying	N.A.	−23%	[101]


Methane production and pretreatment improvement for macroalgal biomass.

Feedstock	AD Process	Co-digestion	T (°C)	Pretreatment	Methane	Improvement
Saccharina latissima	Batch	–	37	Steam explosion at 130 °C, 10 min	268 mL g^{-1} VS	+20%
Laminaria digitata + L. hyperborea + L. Saccharina	Batch	–	50	Beating	425 mL g^{-1} TS	+53%
Ulva lactuca	Batch	–	55	Unwashed, macerated	271 mL g^{-1} VS	+56%
	Batch	–	55	Washed, macerated	200 mL g^{-1} VS	+17%
	Batch	–	55	Washed, 130 °C/20 min	187 mL g^{-1} VS	+7%
	Batch	–	55	Washed, 110 °C/20 min	157 mL g^{-1} VS	−10%
	Batch	–	37	Unwashed, roughly chopped	162 mL g^{-1} VS	−7%
	Batch	–	55	Dried, ground	176 mL g^{-1} VS	+1%
Gracilaria vermiculophylla	Batch	–	53	Washed, Macerated	147 mL g^{-1} VS	+11%
Ulva lactuca	Batch	–	53	Washed, Macerated	255 mL g^{-1} VS	+68%
Chaetomorpha linum	Batch	–	53	Washed, Macerated	195 mL g^{-1} VS	+17%
Saccharina latissima	Batch	–	53	Washed, Macerated	333 mL g^{-1} VS	−2%
Ulva lactuca	Lab-scale CSTR	Cattle manure	53	Dried, ground	15–16 ml g feed^{-1}	N.A.
Ulva sp.	Batch	Sewage sludge	35	Washed	126 mL g^{-1} VS	0%
	Batch	Sewage sludge	35	Ground	126 mL g^{-1} VS	0%
	Batch	Sewage sludge	35	Washed, ground	180 mL g^{-1} VS	+30%
Ulva sp.	Batch	–	35	Unwashed	110 mL g^{-1} VS	N.A.
	Batch	–	35	Washed	94 mL g^{-1} VS	−14%
	Batch	–	35	Dried	145 mL g^{-1} VS	+32%
	Batch	–	35	Dried, ground	177 mL g^{-1} VS	+60%
	CSTR	Bovine manure	35	Ground	203 mL g^{-1} VS	N.A.
Palmaria palmata	Batch	Sludge	35	NaOH, thermal pretreatment at 20 °C/ 30 min	365 mL g^{-1} VS	+19%

Current approaches in biofuels production

- Single gene targeted approaches
  - Insertion of specific enzyme
  - Engineering of RUBISCO and/or PS II
  - Enzyme engineering
- Systemic approaches, metabolic engineering
  - Multiple insertions/deletions
  - Novel metabolic pathways
  - Tampering the central carbon metabolism

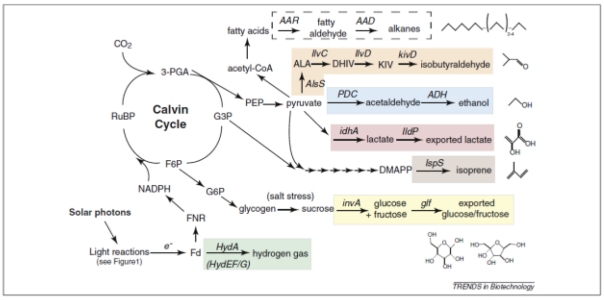
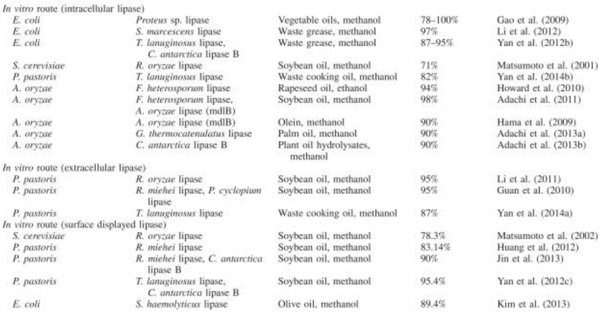


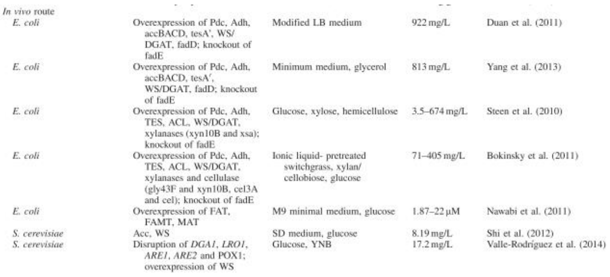
Figure 2. Schematic representation of engineered biochemical pathways in cyanobacteria. Core metabolism of photosynthetic processes is shown in black text. Branch points utilized for the production of various compounds discussed in this review are indicated (highlighted pathways) with relevant enzymes catalyzing specific reactions indicated in italics. Abbreviations: 3-PGA, 3-phosphoglycerate; AAD, aldehyde decarbonylase; ADH, alcohol dehydrogenase II; ALA, 2-acetolactate; AlsS, acetolactate synthase; DHIV, 2,3-dihydroxy-isovalerate; F6P, fructose 6-phosphate; FNR, ferredoxin NADP+ reductase; G6P, glucose 6-phosphate; HydA, [FeFe] hydrogenase; HydEF/G, hydrogenase maturation factors; ldhA, lactate dehydrogenase; IlvD, dihydroxy-acid dehydratase; IlvC, acetohydroxy acid isomeroreductase; PDC, pyruvate decarboxylase; PEP, phosphoenolpyruvate.

- Single gene targeted approaches



In vitro route (intracellular lipase)				
E. coli	*Proteus* sp. lipase	Vegetable oils, methanol	78–100%	Gao et al. (2009)
E. coli	*S. marcescens* lipase	Waste grease, methanol	97%	Li et al. (2012)
E. coli	*T. lanuginosus* lipase, *C. antarctica* lipase B	Waste grease, methanol	87–95%	Yan et al. (2012b)
S. cerevisiae	*R. oryzae* lipase	Soybean oil, methanol	71%	Matsumoto et al. (2001)
P. pastoris	*T. lanuginosus* lipase	Waste cooking oil, methanol	82%	Yan et al. (2014b)
A. oryzae	*F. heterosporum* lipase	Rapeseed oil, ethanol	94%	Howard et al. (2010)
A. oryzae	*F. heterosporum* lipase, *A. oryzae* lipase (mdlB)	Soybean oil, methanol	98%	Adachi et al. (2011)
A. oryzae	*A. oryzae* lipase (mdlB)	Olein, methanol	90%	Hama et al. (2009)
A. oryzae	*G. thermocatenulatus* lipase	Palm oil, methanol	90%	Adachi et al. (2013a)
A. oryzae	*C. antarctica* lipase B	Plant oil hydrolysates, methanol	90%	Adachi et al. (2013b)
In vitro route (extracellular lipase)				
P. pastoris	*R. oryzae* lipase	Soybean oil, methanol	95%	Li et al. (2011)
P. pastoris	*R. miehei* lipase, *P. cyclopium* lipase	Soybean oil, methanol	95%	Guan et al. (2010)
P. pastoris	*T. lanuginosus* lipase	Waste cooking oil, methanol	87%	Yan et al. (2014a)
In vitro route (surface displayed lipase)				
S. cerevisiae	*R. oryzae* lipase	Soybean oil, methanol	78.3%	Matsumoto et al. (2002)
P. pastoris	*R. miehei* lipase	Soybean oil, methanol	83.14%	Huang et al. (2012)
P. pastoris	*R. miehei* lipase, *C. antarctica* lipase B	Soybean oil, methanol	90%	Jin et al. (2013)
P. pastoris	*T. lanuginosus* lipase, *C. antarctica* lipase B	Soybean oil, methanol	95.4%	Yan et al. (2012c)
E. coli	*S. haemolyticus* lipase	Olive oil, methanol	89.4%	Kim et al. (2013)

- Systemic approaches, metabolic engineering



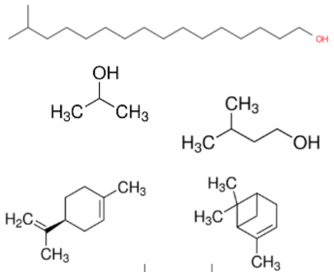
In vivo route				
E. coli	Overexpression of Pdc, Adh, accBACD, tesA', WS/DGAT, fadD; knockout of fadE	Modified LB medium	922 mg/L	Duan et al. (2011)
E. coli	Overexpression of Pdc, Adh, accBACD, tesA′, WS/DGAT, fadD; knockout of fadE	Minimum medium, glycerol	813 mg/L	Yang et al. (2013)
E. coli	Overexpression of Pdc, Adh, TES, ACL, WS/DGAT, xylanases (xyn10B and xsa); knockout of fadE	Glucose, xylose, hemicellulose	3.5–674 mg/L	Steen et al. (2010)
E. coli	Overexpression of Pdc, Adh, TES, ACL, WS/DGAT, xylanases and cellulase (gly43F and xyn10B, cel3A and cel); knockout of fadE	Ionic liquid- pretreated switchgrass, xylan/cellobiose, glucose	71–405 mg/L	Bokinsky et al. (2011)
E. coli	Overexpression of FAT, FAMT, MAT	M9 minimal medium, glucose	1.87–22 µM	Nawabi et al. (2011)
S. cerevisiae	Acc, WS	SD medium, glucose	8.19 mg/L	Shi et al. (2012)
S. cerevisiae	Disruption of *DGA1*, *LRO1*, *ARE1*, *ARE2* and POX1; overexpression of WS	Glucose, YNB	17.2 mg/L	Valle-Rodríguez et al. (2014)

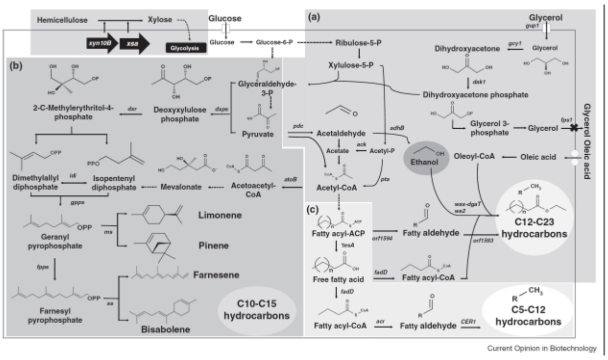
Current approaches in biofuels production

- Designing photosynthetic microorganisms for production of photobiological solar fuels
- Microbial fuel cells (electrobiofuels)
- Technical cultures of engineered (and natural) strains of microorganisms
- Systems metabolic engineering of bacteria and yeast, creation of cell factories for high-value desired chemicals

Biofuels produced by engineered microbes

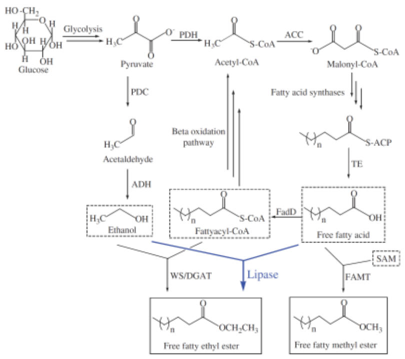
- Lipids and fatty acids
- Fatty alcohols
- Ethanol, isopropanol
- Butanol, methylbutanol
- Hexanol, octanol
- Alkanes, alkenes
- Isoprenoids



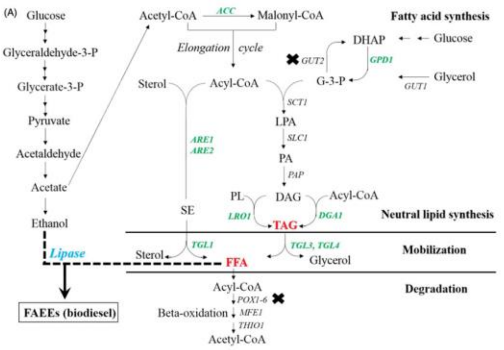


Current Opinion in Biotechnology

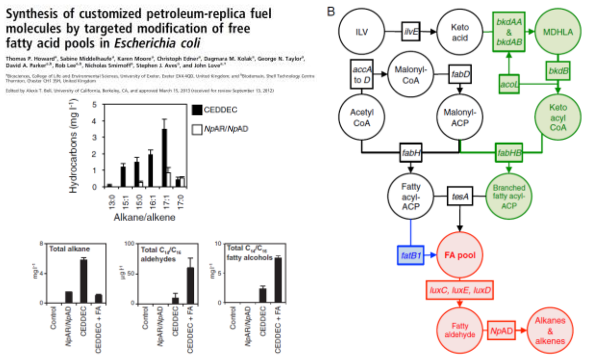
Biodiesel in engineered *E. coli*



Biodiesel from *Y. lipolytica*



Biodiesel in *E. coli*



Synthesis of customized petroleum-replica fuel molecules by targeted modification of free fatty acid pools in *Escherichia coli*

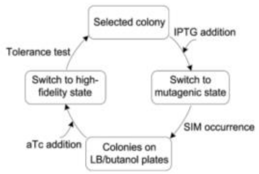
Thomas P. Howard[a], Sabine Middelhaufe[a], Karen Moore[a], Christoph Edner[a], Dagmara M. Kolak[a], George N. Taylor[a], David A. Parker[a,b], Rob Lee[a,b], Nicholas Smirnoff[a], Stephen J. Aves[a], and John Love[a,1]

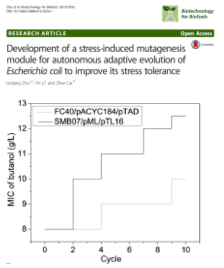
[a]Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter EX4 4QD, United Kingdom; and [b]Biodomain, Shell Technology Centre Thornton, Chester CH1 3SH, United Kingdom

Edited by Alexis T. Bell, University of California, Berkeley, CA, and approved March 15, 2013 (received for review September 13, 2012)

Stress engineering

- Biofuels producing bacteria may suffer from presence of the target compound
- Stress tolerance engineering is important
  - Targeted metabolic engineering
  - Stress-induced mutagenesis





Biotechnology for Biofuels

RESEARCH ARTICLE Open Access

Development of a stress-induced mutagenesis module for autonomous adaptive evolution of *Escherichia coli* to improve its stress tolerance

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Research review paper

A state of the art review on microbial fuel cells: A promising technology for wastewater treatment and bioenergy

Zhuwei Du[a], Haoran Li[a], Tingyue Gu[b,*]

[a] National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100080, People's Republic of China

[b] Department of Chemical and Biomolecular Engineering, Ohio University, Athens, Ohio 45701, USA

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Questions