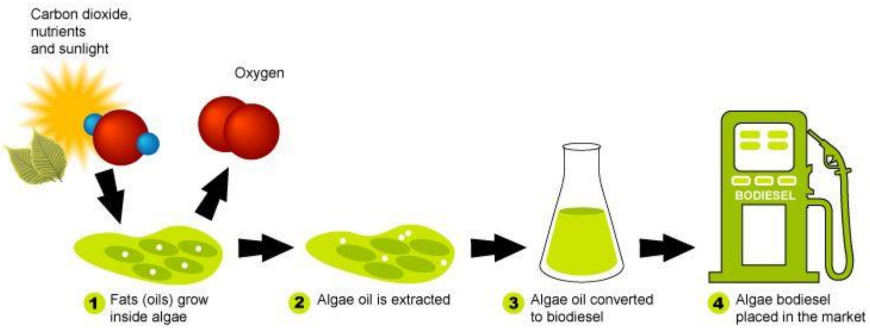
LOSCHMIDT
LABORATORIES

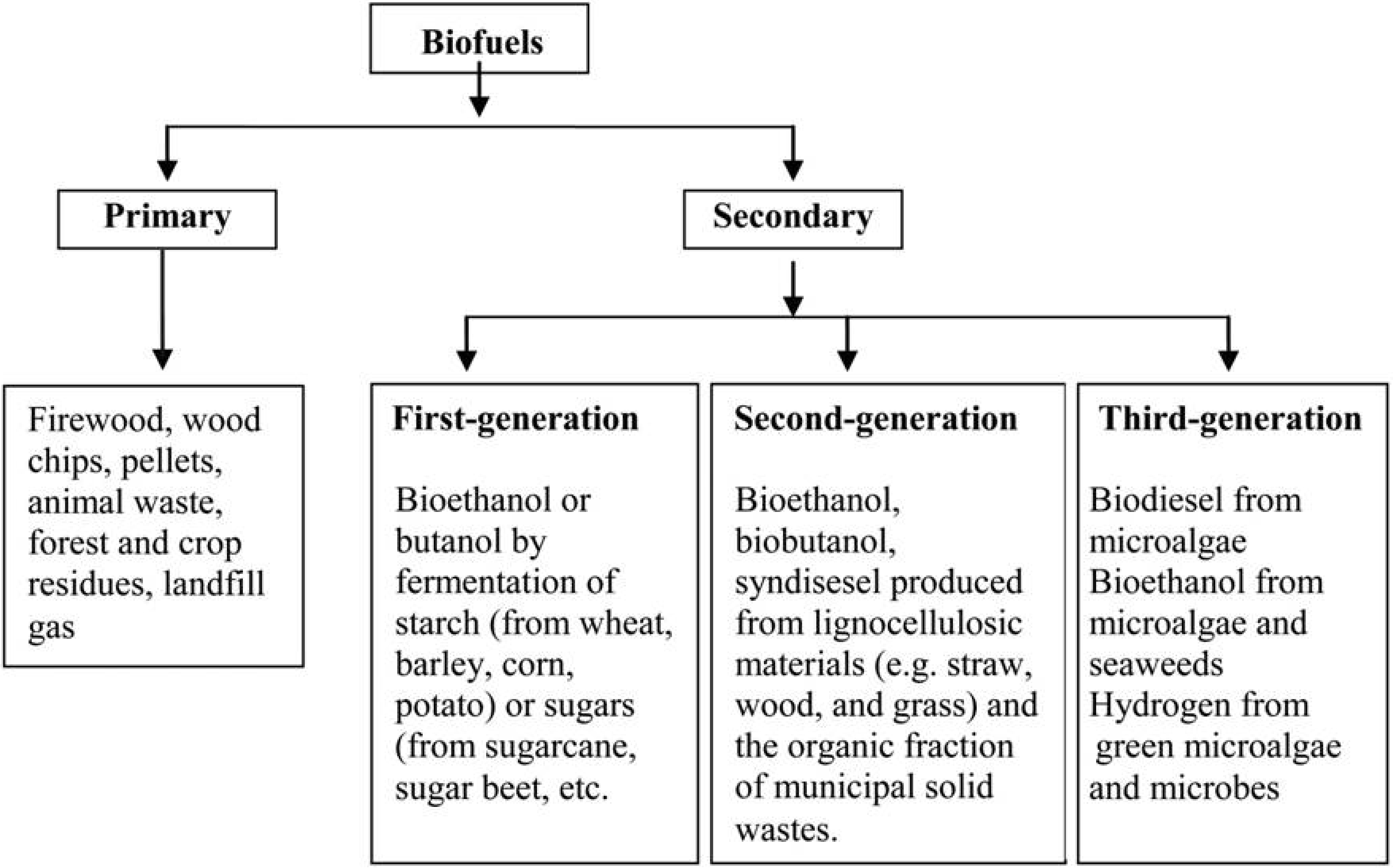
Bi9540 Biotechnology and practical use of algae and fungi

Lecture 7 – Biofuels



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 are promising sources of biofuels for the future



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, syndisesel 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

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.

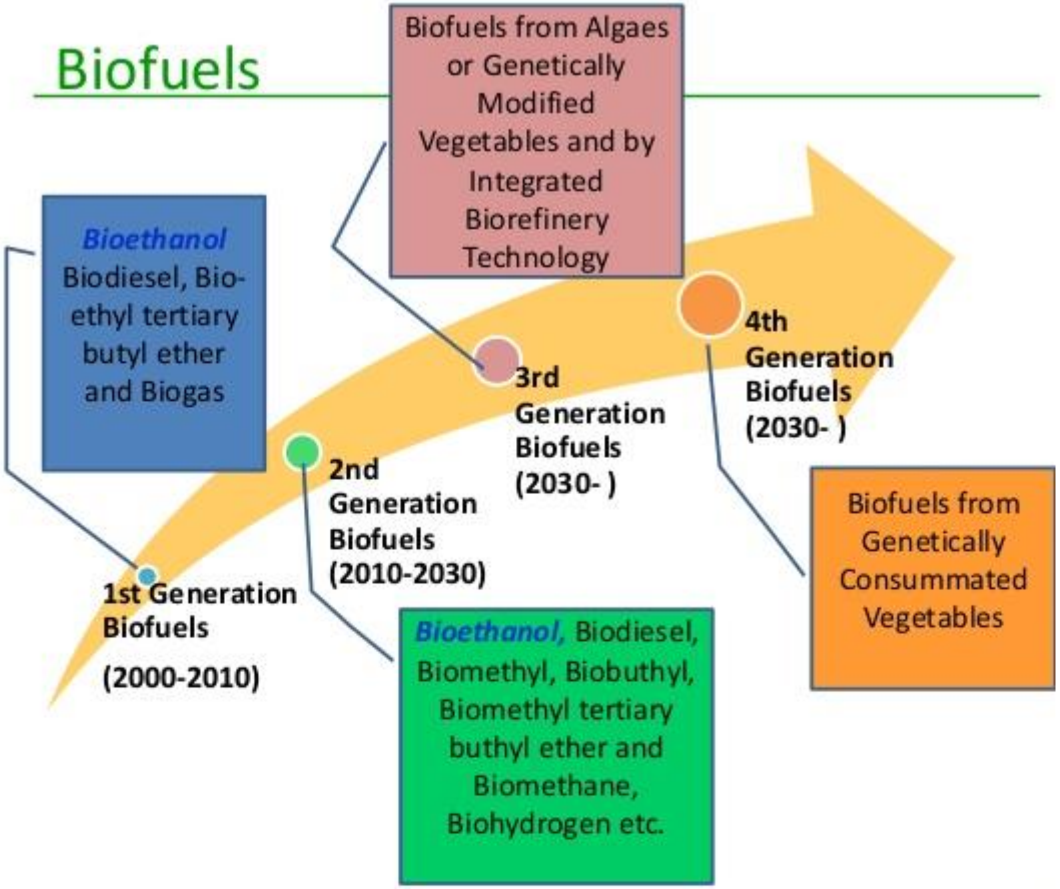
Third Generation

- 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.

Biofuels



1st Generation Biofuels

(2000-2010)

Bioethanol
Biodiesel, Bio-ethyl tertiary butyl ether and Biogas

2nd Generation Biofuels (2010-2030)

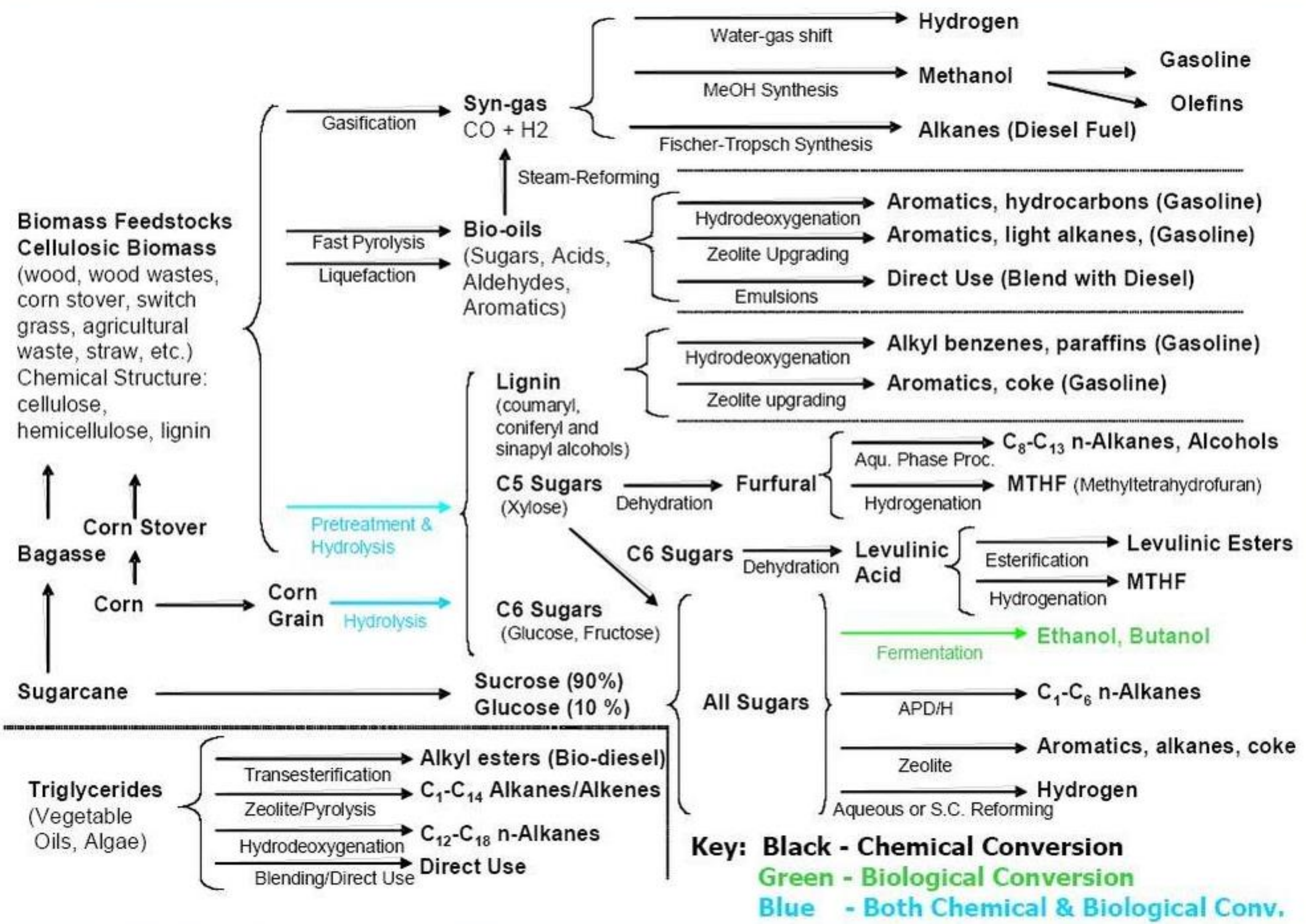
Bioethanol, Biodiesel, Biomethyl, Biobuthyl, Biomethyl tertiary buthyl ether and Biomethane, Biohydrogen etc.

3rd Generation Biofuels (2030-)

Biofuels from Algaes or Genetically Modified Vegetables and by Integrated Biorefinery Technology

4th Generation Biofuels (2030-)

Biofuels from Genetically Consummated Vegetables



Biomass Feedstocks Cellulosic Biomass
(wood, wood wastes, corn stover, switch grass, agricultural waste, straw, etc.)
Chemical Structure: cellulose, hemicellulose, lignin

Bagasse

Corn Stover

Corn

Sugarcane

Gasification → **Syn-gas** CO + H2

- Water-gas shift → **Hydrogen**
- MeOH Synthesis → **Methanol** → **Gasoline**, **Olefins**
- Fischer-Tropsch Synthesis → **Alkanes (Diesel Fuel)**

Steam-Reforming

Fast Pyrolysis, Liquefaction → **Bio-oils** (Sugars, Acids, Aldehydes, Aromatics)

- Hydrodeoxygenation → **Aromatics, hydrocarbons (Gasoline)**
- Zeolite Upgrading → **Aromatics, light alkanes, (Gasoline)**
- Emulsions → **Direct Use (Blend with Diesel)**

Pretreatment & Hydrolysis

Lignin (coumaryl, coniferyl and sinapyl alcohols)

- Hydrodeoxygenation → **Alkyl benzenes, paraffins (Gasoline)**
- Zeolite upgrading → **Aromatics, coke (Gasoline)**

C5 Sugars (Xylose) → Dehydration → **Furfural**

- Aqu. Phase Proc. → **C_8-C_{13} n-Alkanes, Alcohols**
- Hydrogenation → **MTHF** (Methyltetrahydrofuran)

C6 Sugars → Dehydration → **Levulinic Acid**

- Esterification → **Levulinic Esters**
- Hydrogenation → **MTHF**

Corn Grain → Hydrolysis → **C6 Sugars** (Glucose, Fructose)

Sucrose (90%)
Glucose (10 %)

All Sugars

- Fermentation → **Ethanol, Butanol**
- APD/H → **C_1-C_6 n-Alkanes**
- Zeolite → **Aromatics, alkanes, coke**
- Aqueous or S.C. Reforming → **Hydrogen**

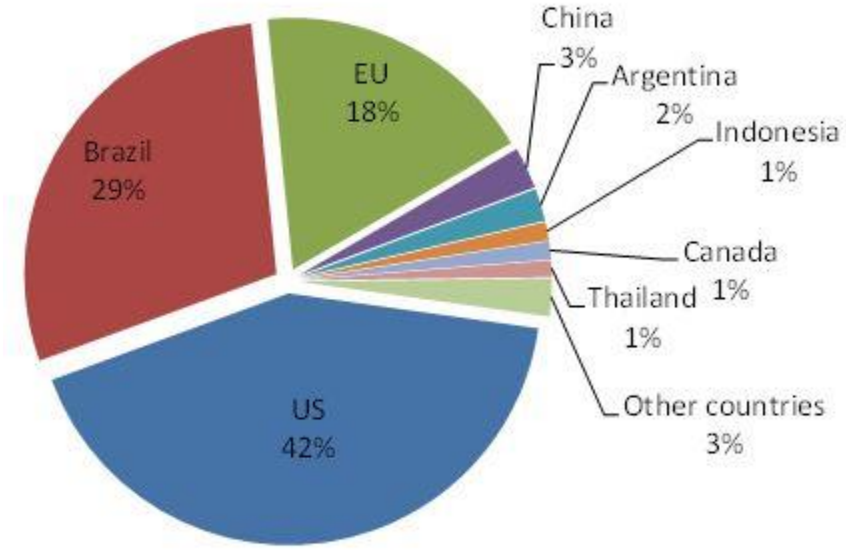
Triglycerides (Vegetable Oils, Algae)

- Transesterification → **Alkyl esters (Bio-diesel)**
- Zeolite/Pyrolysis → **C_1-C_{14} Alkanes/Alkenes**
- Hydrodeoxygenation → **C_{12}-C_{18} n-Alkanes**
- Blending/Direct Use → **Direct Use**

Key: Black - Chemical Conversion
Green - Biological Conversion
Blue - Both Chemical & Biological Conv.

Biofuels in the world

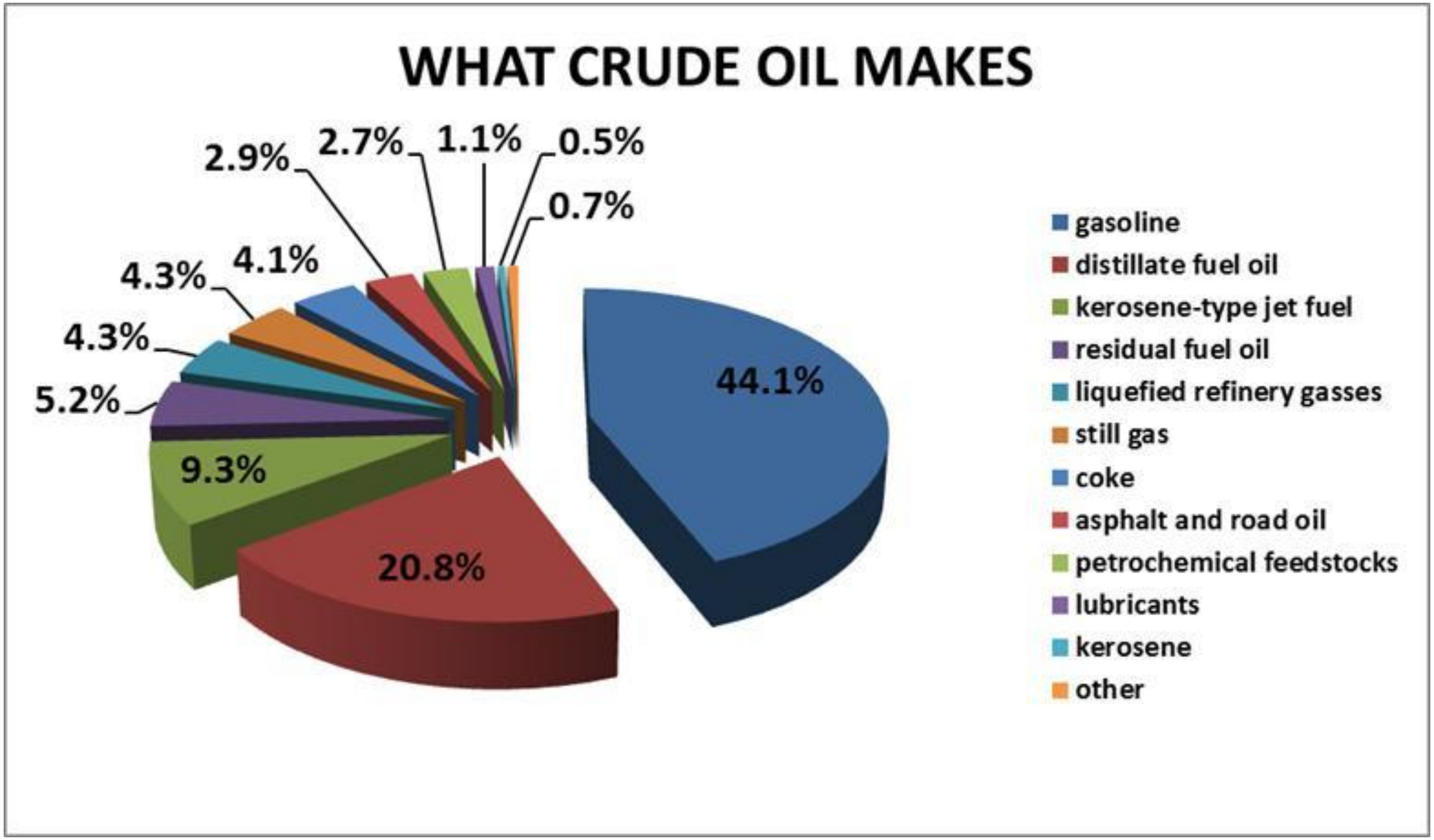
- Vast majority of the biofuels production is based in the US, Brazil and Europe



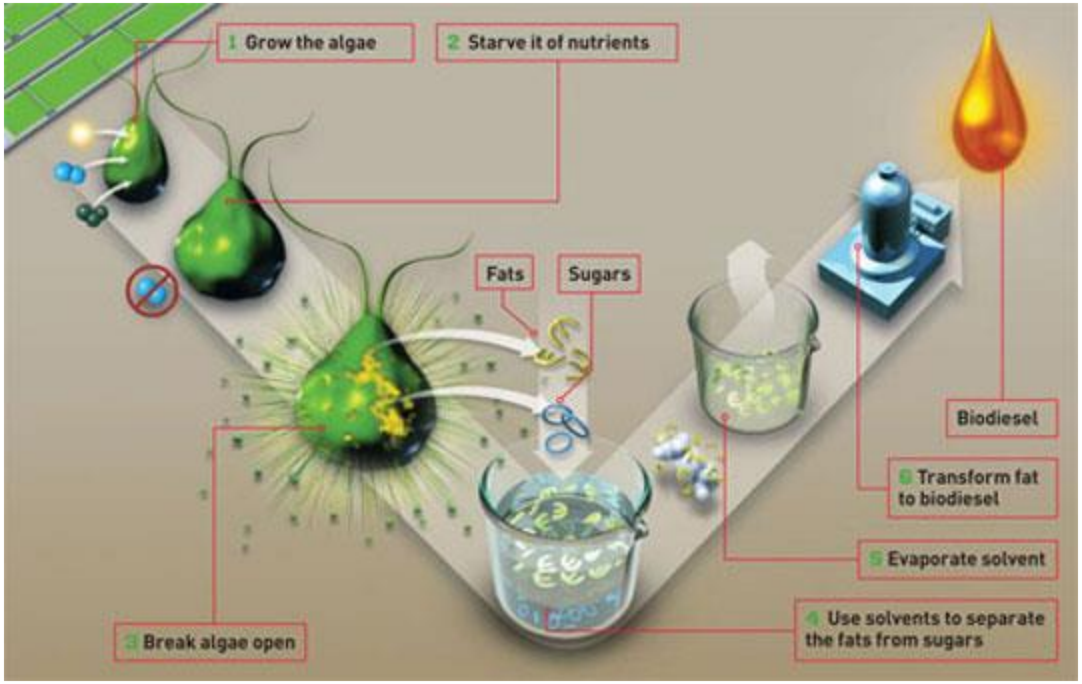
Why are biofuels important?

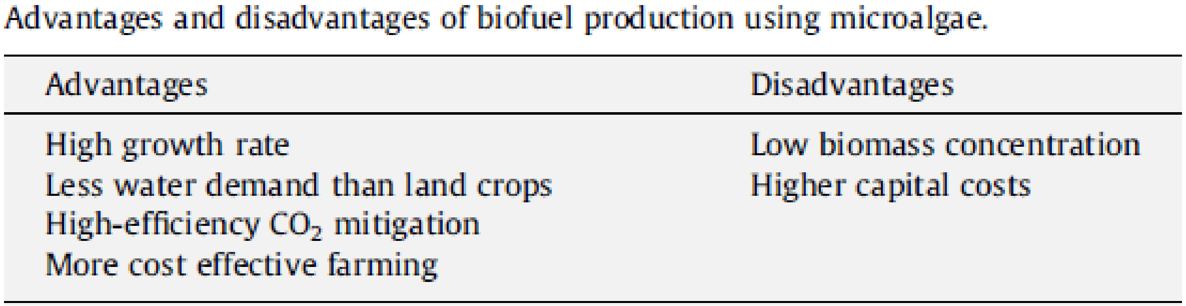
- Renewable sources of energy
- Lowering of carbon emissions
- Lower energy demands than ´traditional´ processes
- Biomass can be used for extraction of biologically active compounds and as biofuel
- Waste is biodegradable or can be used further

Crude oil consumption



Algae as biofuels sources





Advantages and disadvantages of biofuel production using microalgae.

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

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

Source: Algae 2020, Emerging Markets Online Consulting Services

Small Niche Markets
Highest $ Products

Larger Markets
High $ Products

Largest Markets
Mid to Lower $ Products

Timelines for Production and Progression Into Larger Markets

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

Sources: Algae 2020 study, Emerging Markets Online Consulting Services

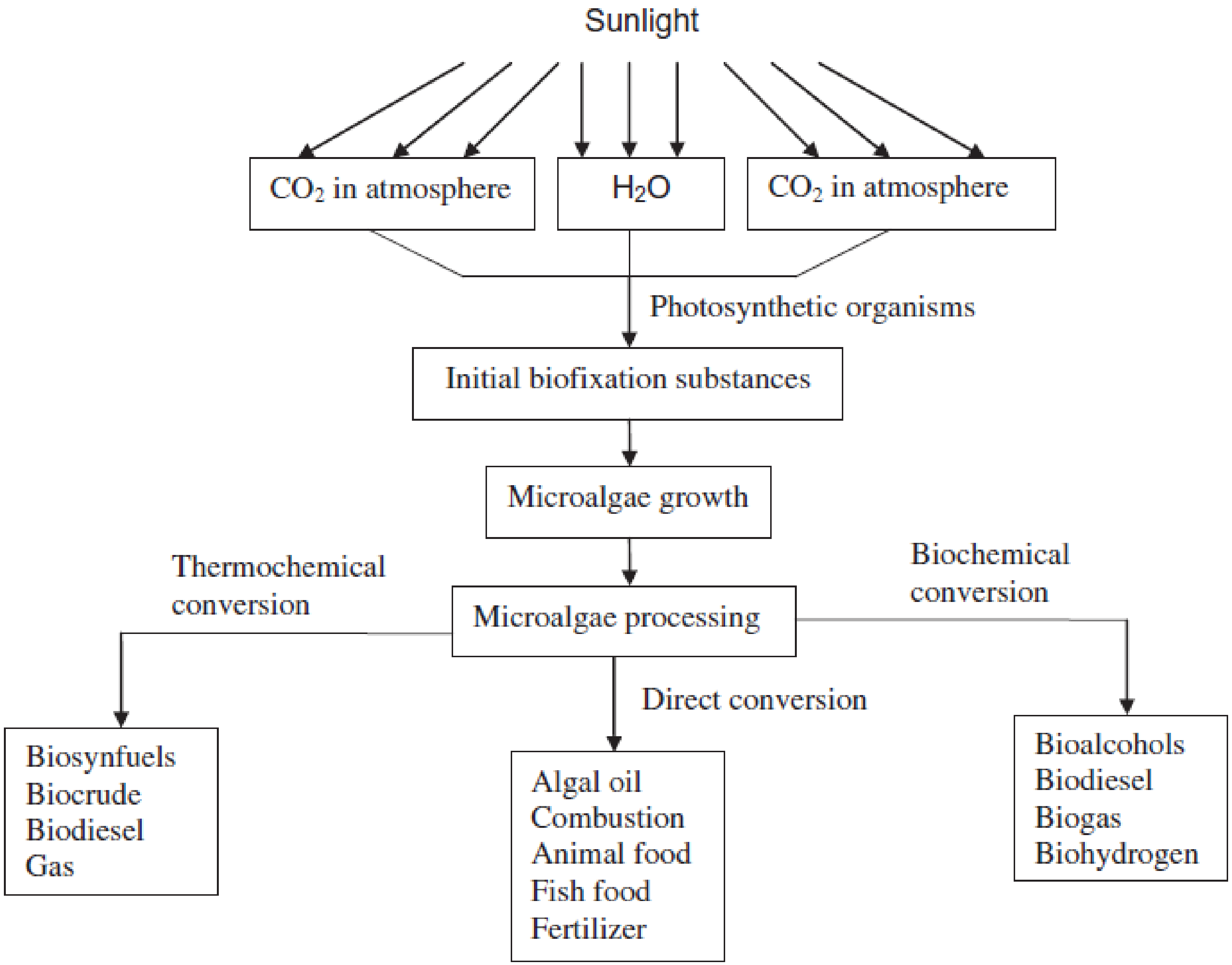
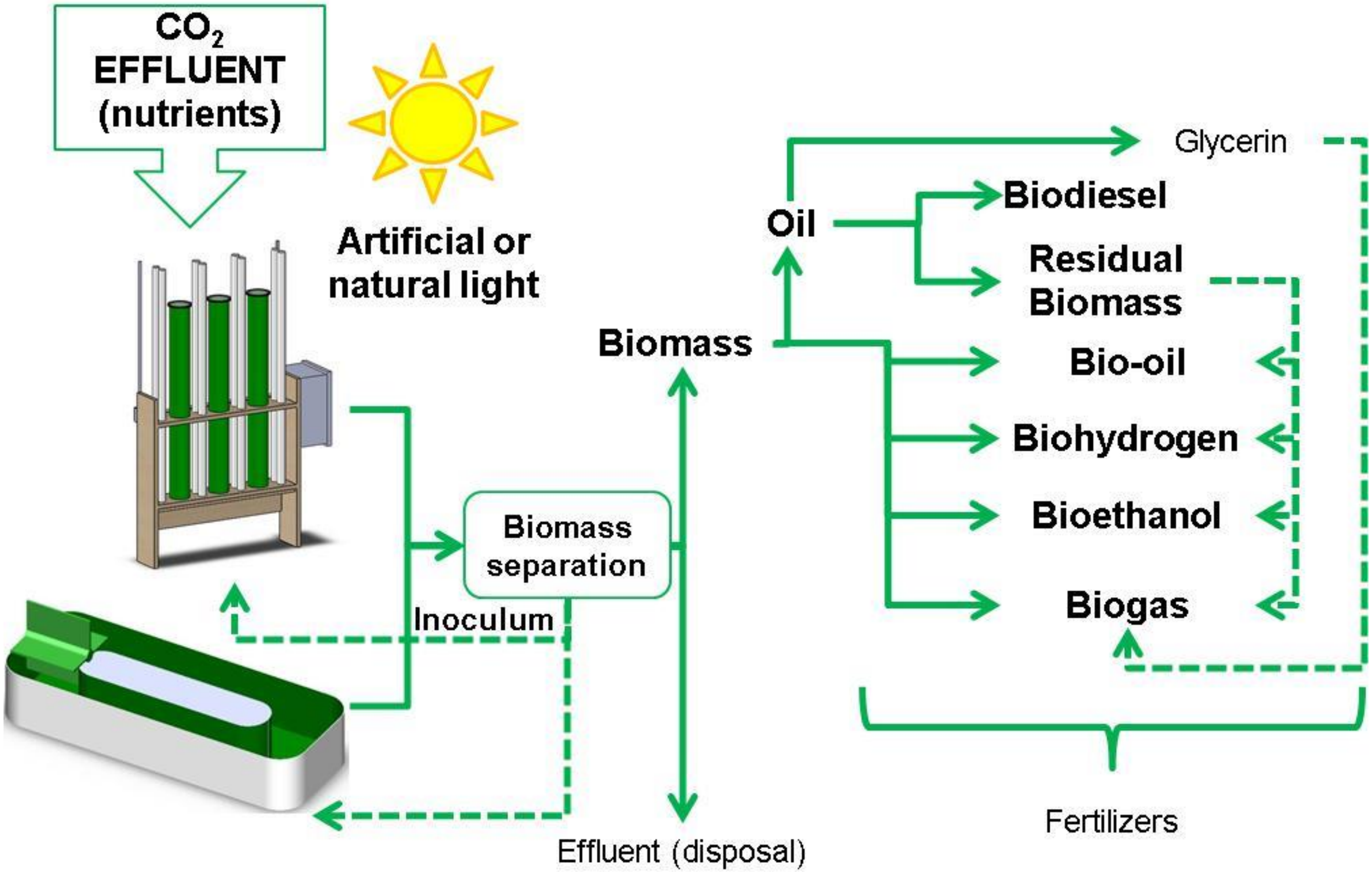


Fig. 1. Carbon dioxide fixation and main steps of algal biomass technologies.



CO_2
EFFLUENT
(nutrients)

Artificial or natural light

Biomass separation

Inoculum

Biomass

Effluent (disposal)

Oil

Glycerin

Biodiesel

Residual Biomass

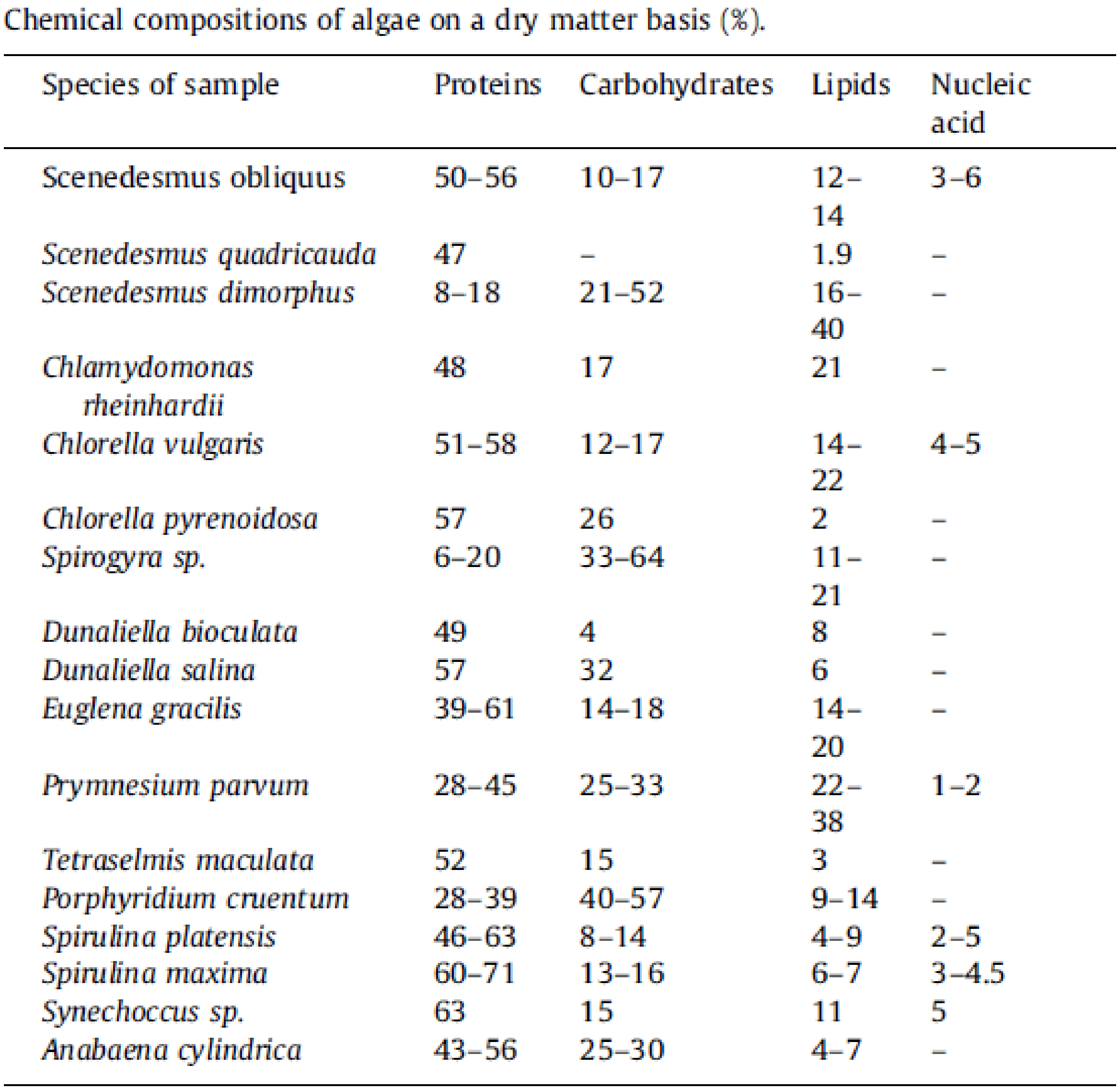
Bio-oil

Biohydrogen

Bioethanol

Biogas

Fertilizers



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

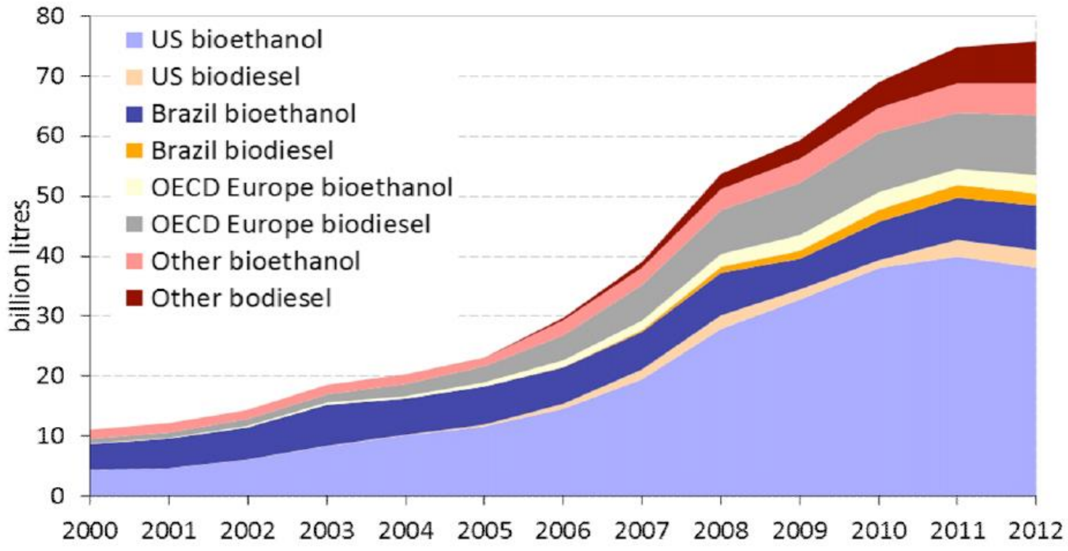
Species of sample	Proteins	Carbohydrates	Lipids	Nucleic acid
Scenedesmus obliquus	50–56	10–17	12–14	3–6
Scenedesmus quadricauda	47	–	1.9	–
Scenedesmus dimorphus	8–18	21–52	16–40	–
Chlamydomonas rheinhardii	48	17	21	–
Chlorella vulgaris	51–58	12–17	14–22	4–5
Chlorella pyrenoidosa	57	26	2	–
Spirogyra sp.	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

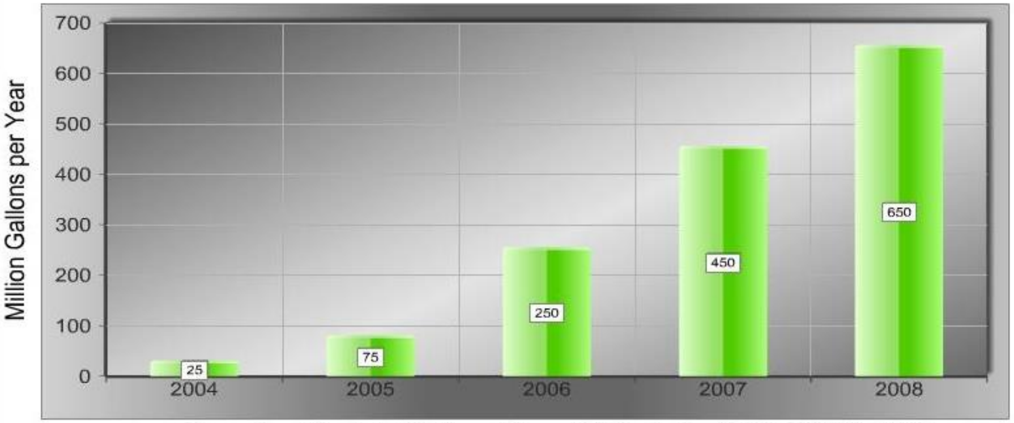
- In the EU 5 % of biodiesel has to be mixed with liquid fossil fuels

Figure 1 – Global biofuels production, 2000-12



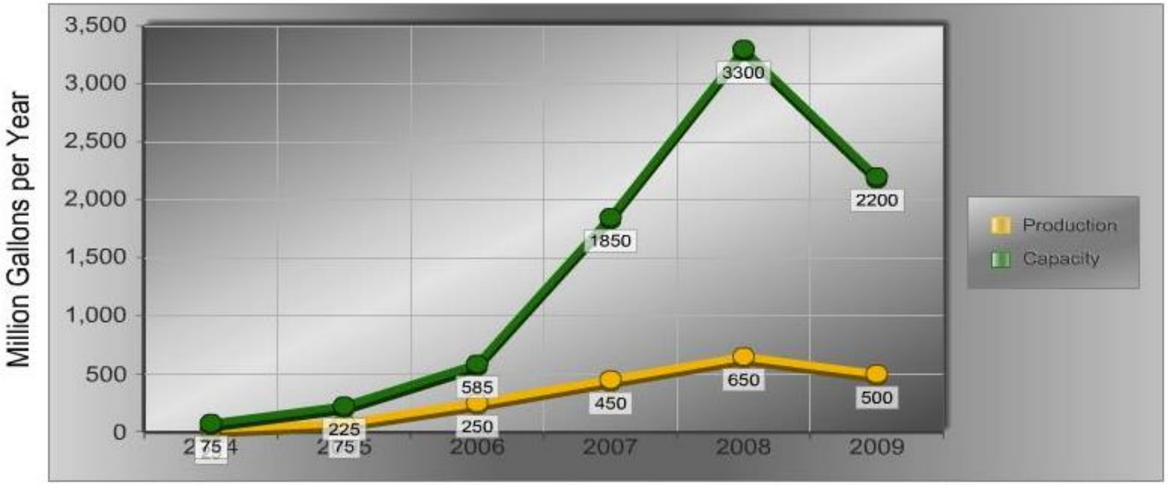
Data source: International Energy Agency, 2000-12.

US Biodiesel Production 2004-2008



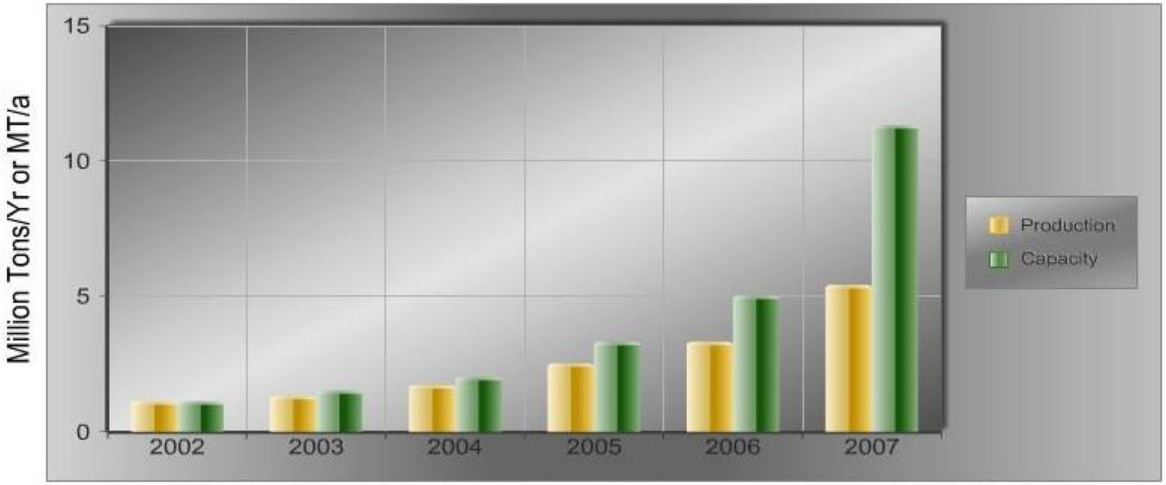
source Emerging Markets Online, Algae 2020 study, NBB, USDA, FAO

US Biodiesel Production and Capacity



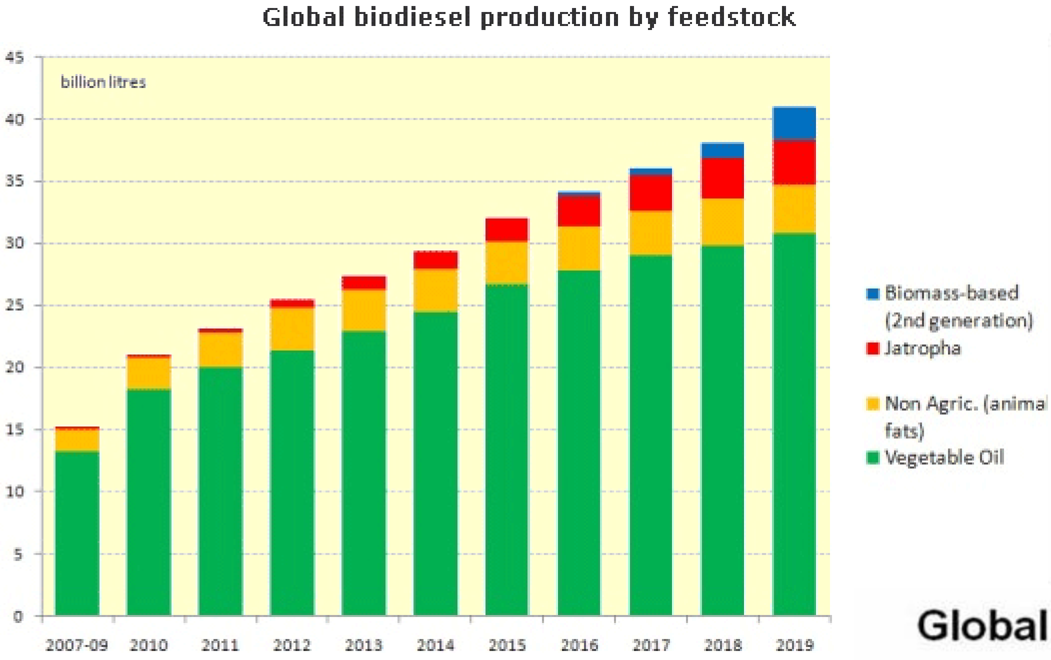
source Emerging Markets Online Consulting Services, Algae 2020 study

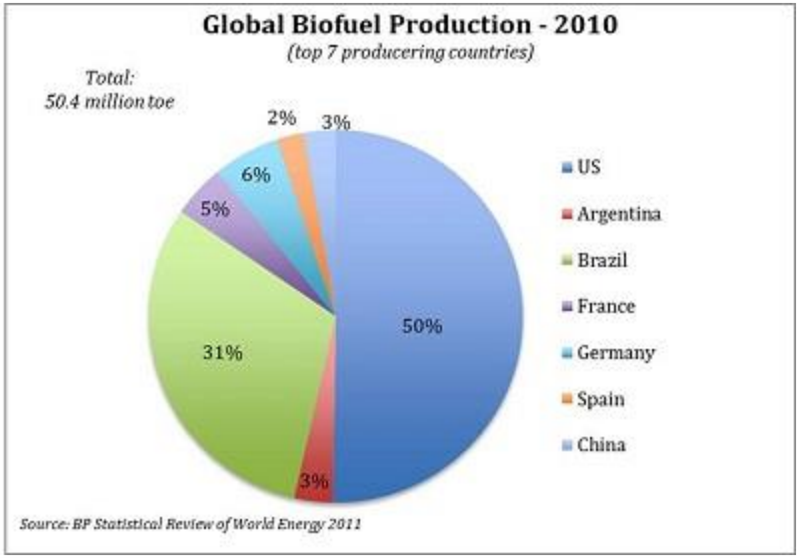
Europe Biodiesel Production and Capacity



sources Biodiesel 2020: A Global Market Survey, EBB, USDA, OilWorld, FAS

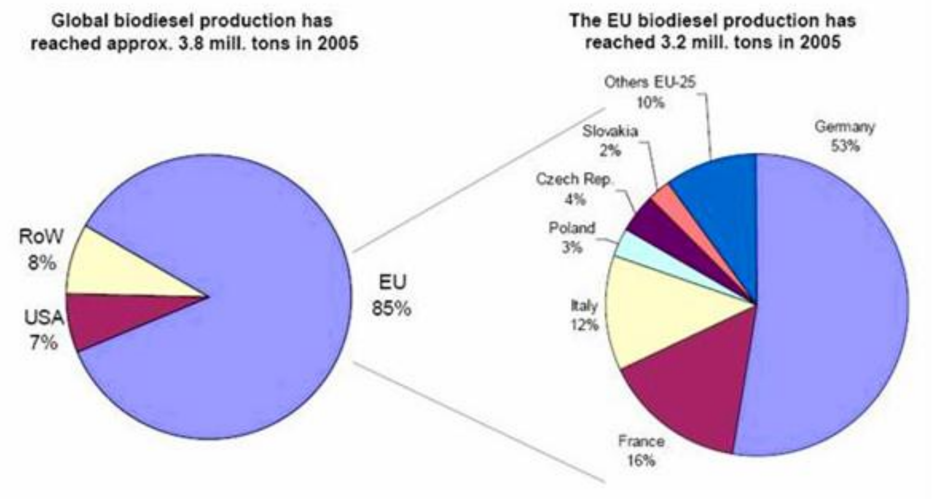
Global biodiesel production by feedstock





Source: BP Statistical Review of World Energy 2011

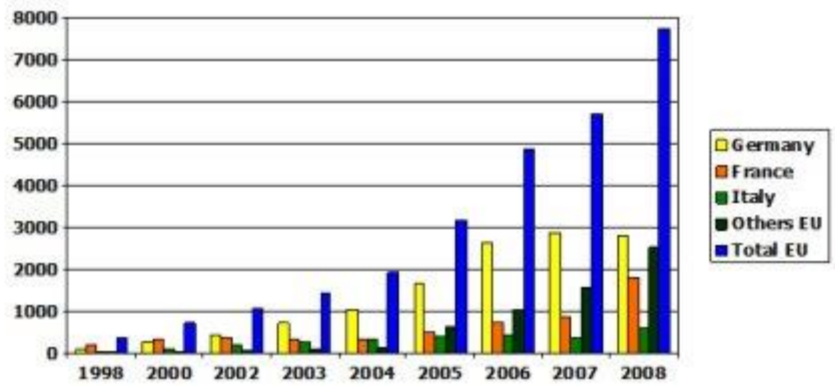
Global Biodiesel Production by Country



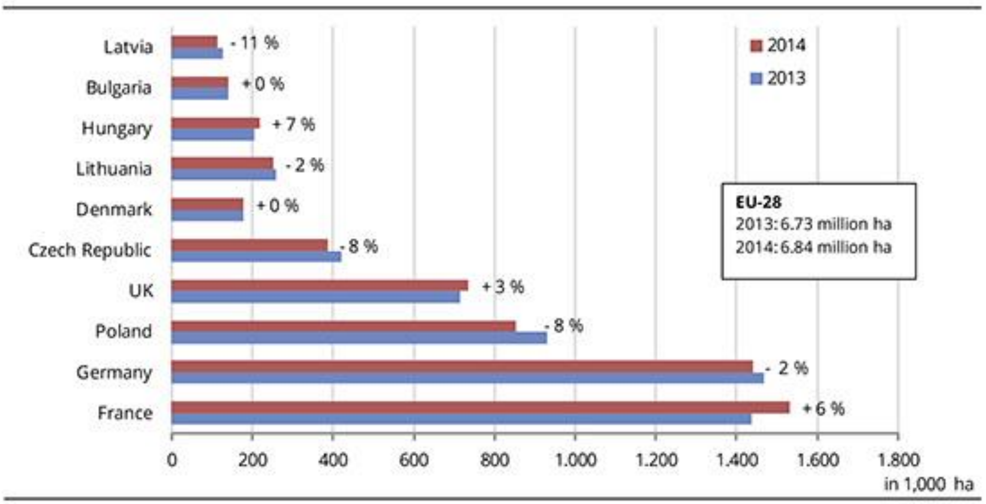
Source: Diester Industrie International/ EBB

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

Source: EBB



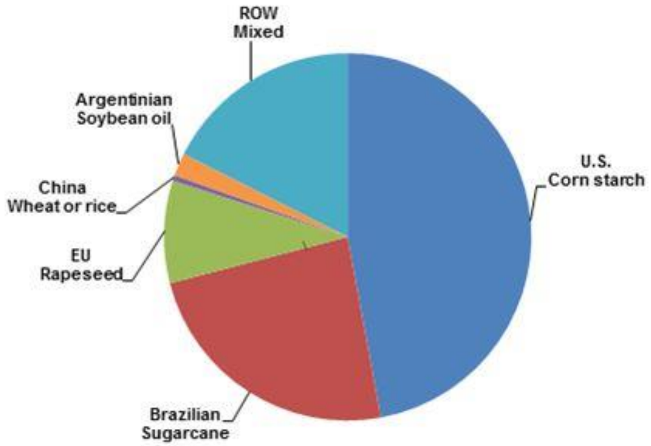
Increase in EU rapeseed area in 2014



EU-28
2013: 6.73 million ha
2014: 6.84 million ha

Source: European Commission, AMI

Global Biofuels Market Share by Feedstock, World Markets: 2011



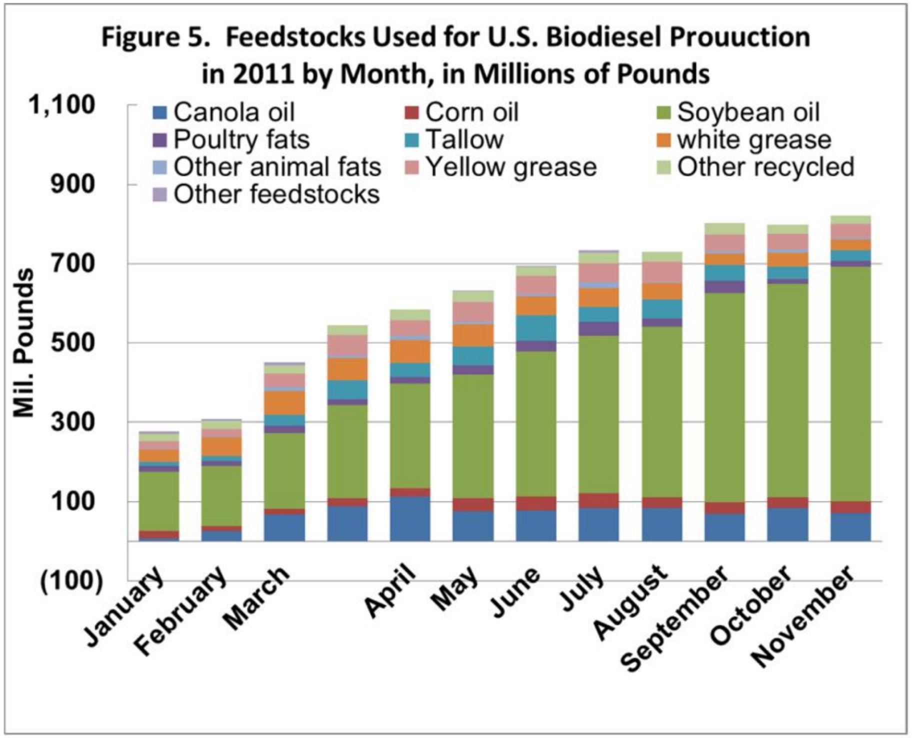
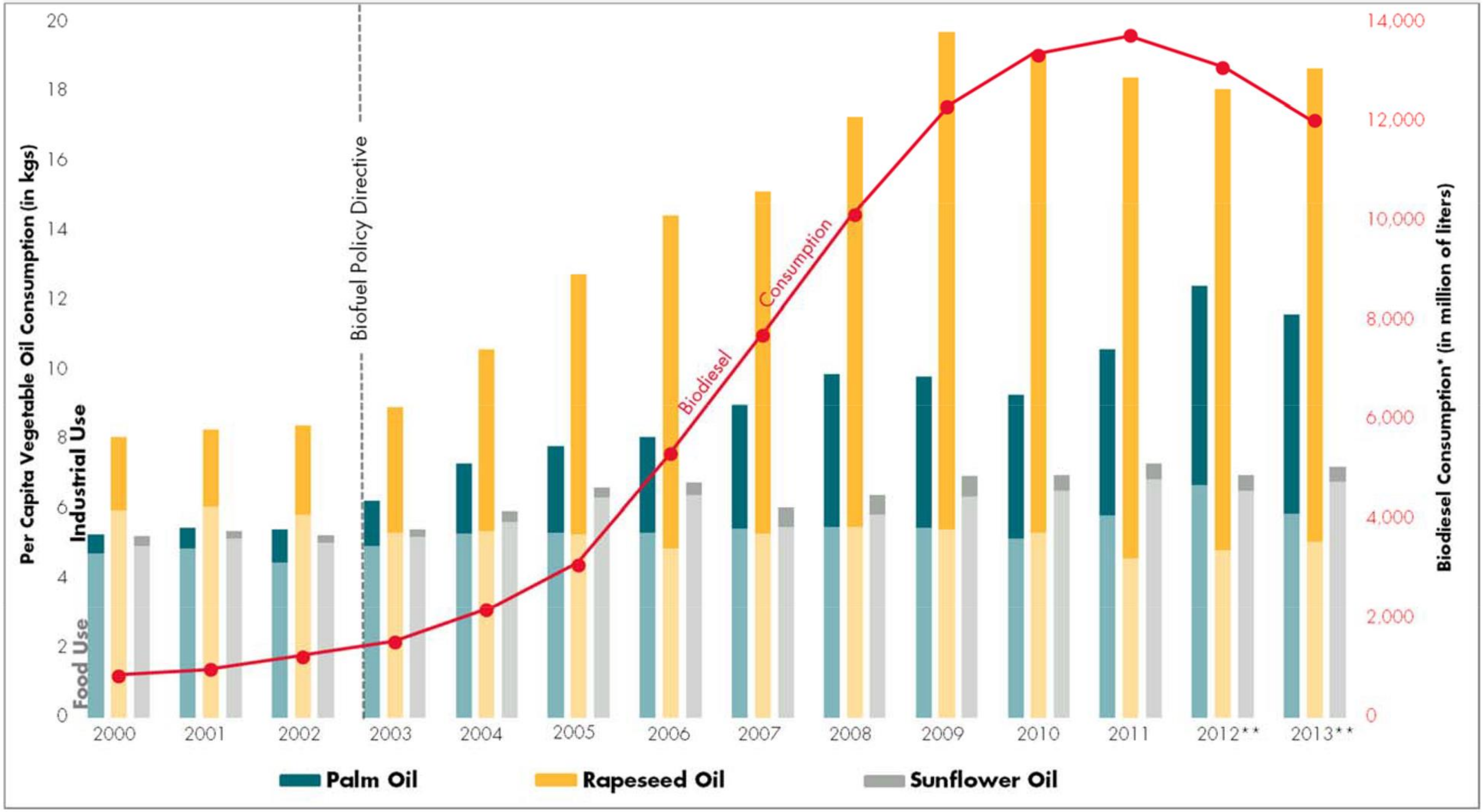


Figure 3. EU Per Capita Consumption of Vegetable Oil and Biodiesel

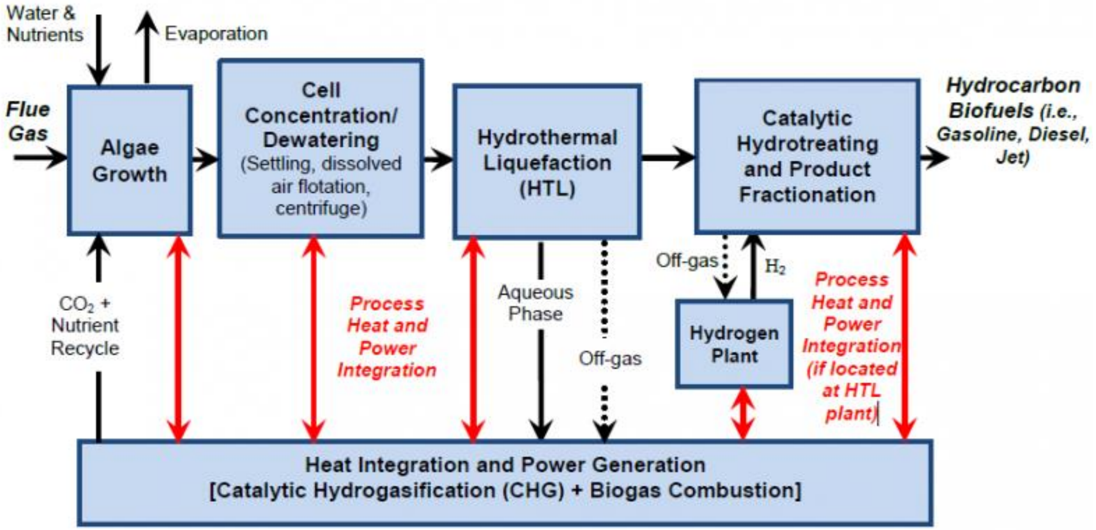


*Biodiesel consumption is total industrial consumption, converted from '000Barrels a day using EIA's unit conversion of 158.99 liters per barrel.

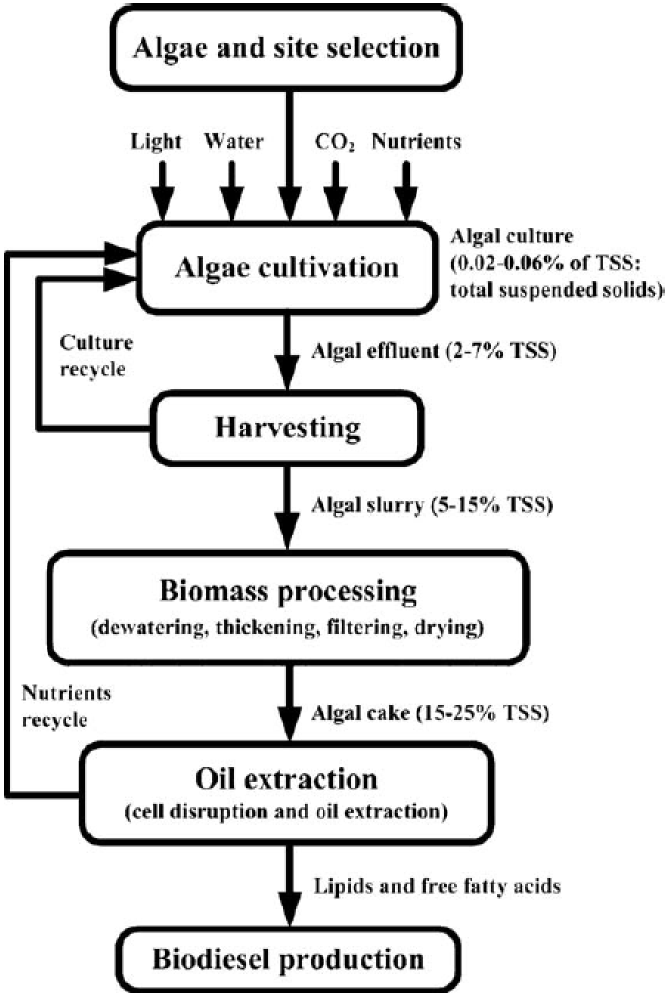
**2012-13 biodiesel consumption based on percent change from USDA estimates.

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



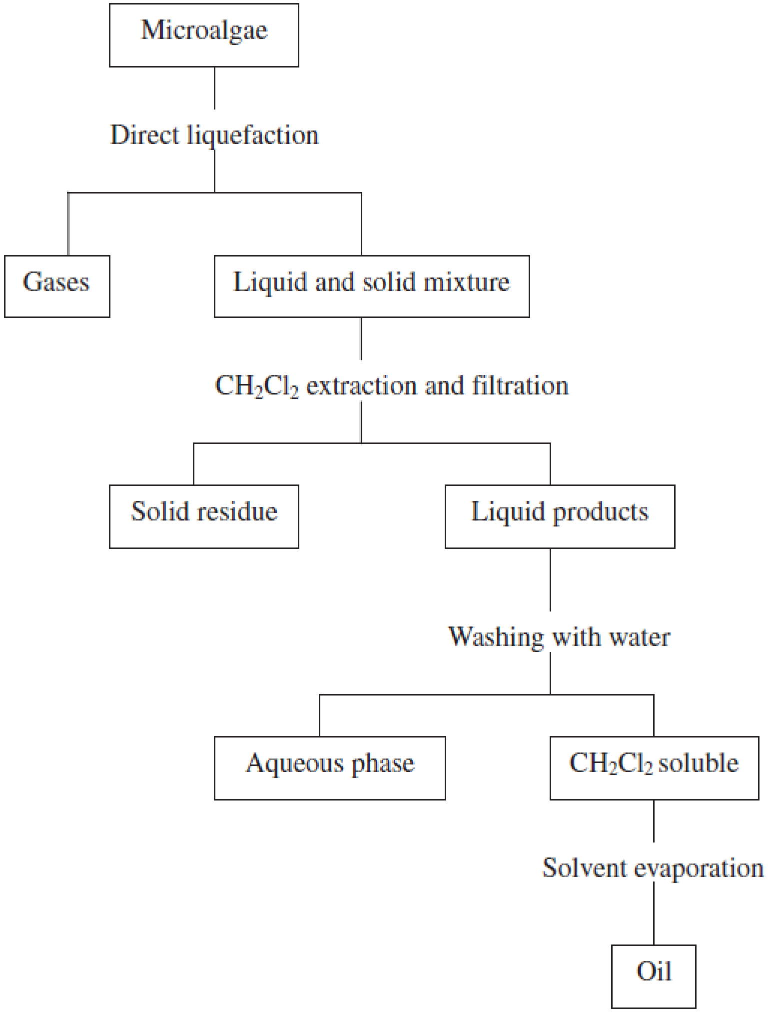
Water & Nutrients
Evaporation
Flue Gas
Algae Growth
CO2 + Nutrient Recycle
Cell Concentration/ Dewatering (Settling, dissolved air flotation, centrifuge)
Process Heat and Power Integration
Hydrothermal Liquefaction (HTL)
Aqueous Phase
Off-gas
Catalytic Hydrotreating and Product Fractionation
Off-gas
H_2
Hydrogen Plant
Process Heat and Power Integration (if located at HTL plant)
Hydrocarbon Biofuels (i.e., Gasoline, Diesel, Jet)
Heat Integration and Power Generation
[Catalytic Hydrogasification (CHG) + Biogas Combustion]



Algae and site selection
Light
Water
CO_2
Nutrients
Algae cultivation
Algal culture (0.02-0.06% of TSS: total suspended solids)
Culture recycle
Algal effluent (2-7% TSS)
Harvesting
Algal slurry (5-15% TSS)
Biomass processing (dewatering, thickening, filtering, drying)
Nutrients recycle
Algal cake (15-25% TSS)
Oil extraction (cell disruption and oil extraction)
Lipids and free fatty acids
Biodiesel production

Fig. 1. Microalgae biodiesel value chain stages.

- Water removal is important
- 20% humidity after dewatering



Microalgae

Direct liquefaction

Gases

Liquid and solid mixture

CH_2Cl_2 extraction and filtration

Solid residue

Liquid products

Washing with water

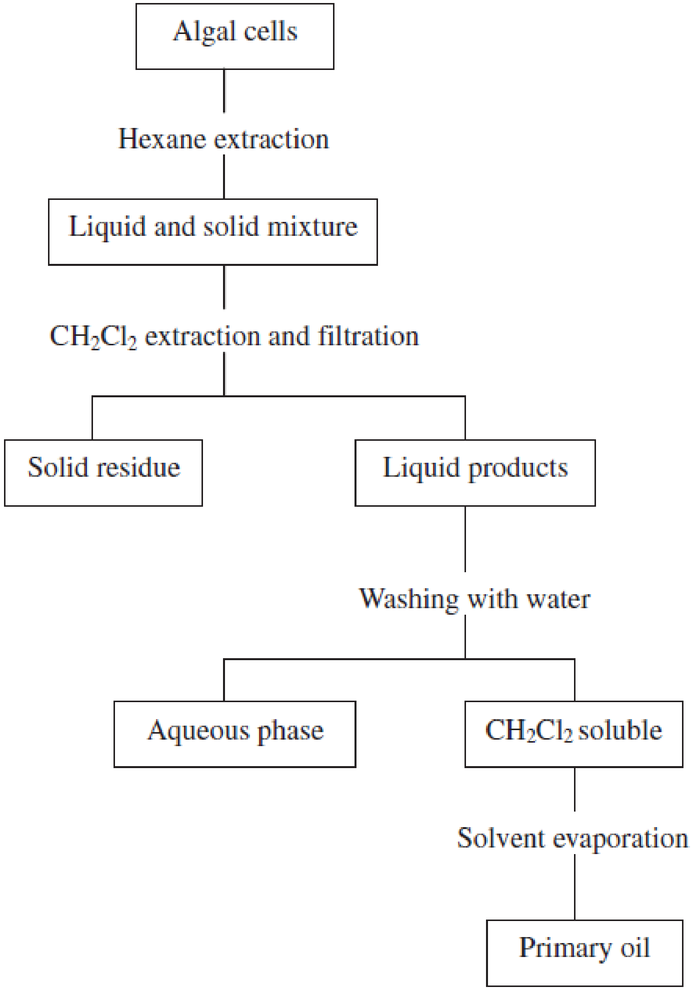
Aqueous phase

CH_2Cl_2 soluble

Solvent evaporation

Oil

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



Algal cells

Hexane extraction

Liquid and solid mixture

CH_2Cl_2 extraction and filtration

Solid residue

Liquid products

Washing with water

Aqueous phase

CH_2Cl_2 soluble

Solvent evaporation

Primary oil

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

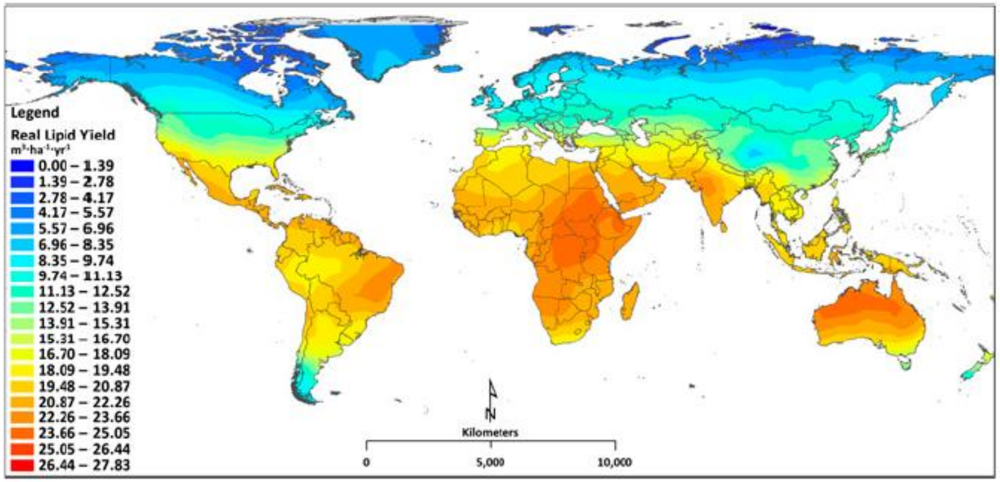
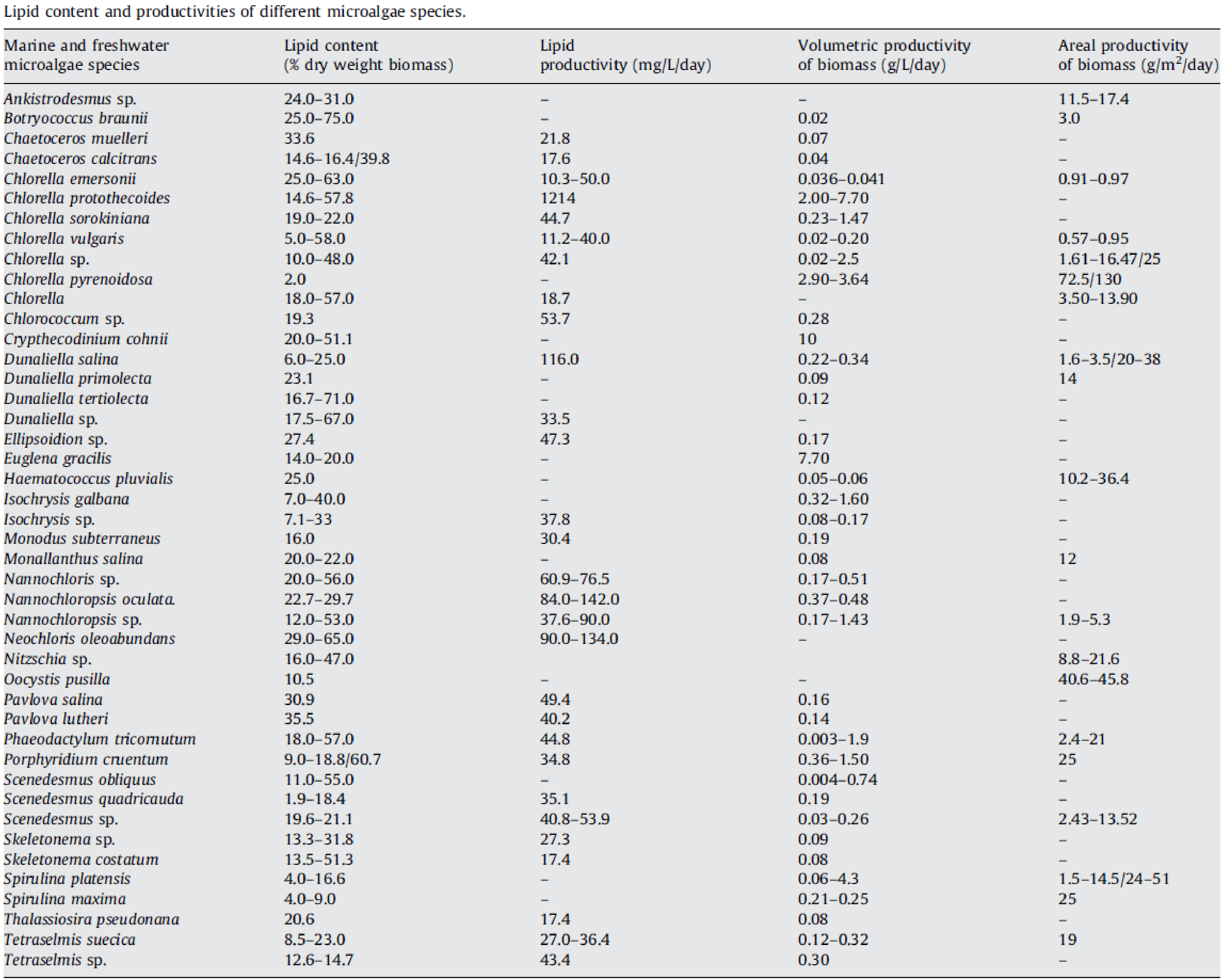


Fig. 1. World map of the current near-term lipid productivity potential from microalgae based on a validated biological growth model representative of *Nannochloropsis* cultivated in a photobioreactor. Results are based on the simulation of 4,388 geographical locations.

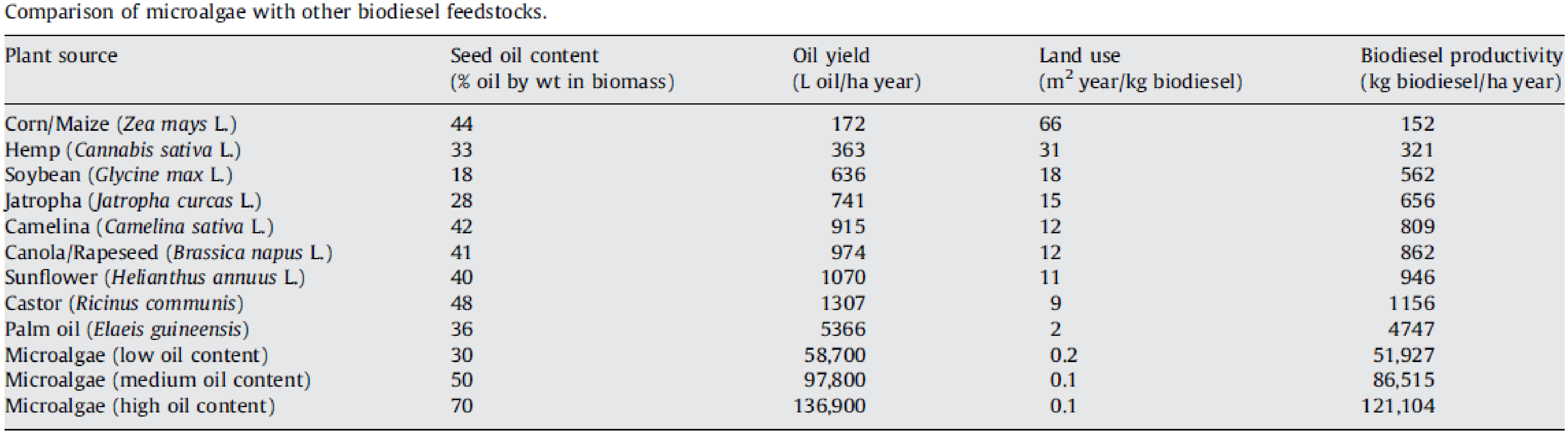
Table 1. Average microalgae lipid yields in cubic meters per hectare^{-1} per meter^{-1} (corresponding biomass yields in grams per meter^{-2} per day^{-1}) of various regions around the world with respective high and low monthly lipid yields

	Lipid and biomass yield		
Location	Maximum monthly	Average monthly	Lowest monthly
Kisumu, Kenya	2.47 (15.9)	2.28 (14.8)	2.07 (13.3)
Learmonth, Australia	2.61 (18.0)	2.16 (14.0)	1.49 (9.64)
Trivandrum, India	2.42 (15.6)	2.08 (13.4)	1.75 (11.3)
Cali, Columbia	2.27 (14.6)	2.04 (13.2)	1.91 (12.3)
Hawaii, United States	2.36 (15.3)	1.97 (12.8)	1.50 (9.95)
Yuma, AZ, United States	2.68 (17.3)	1.80 (11.7)	0.68 (5.16)
Poltavka, Russia	2.30 (14.8)	1.06 (6.84)	0.46 (3.23)
Bagaskar, Finland	2.19 (14.1)	0.77 (5.00)	0.55 (3.86)
Punta Arenas, Chile	1.77 (11.9)	0.77 (5.07)	0.51 (3.25)



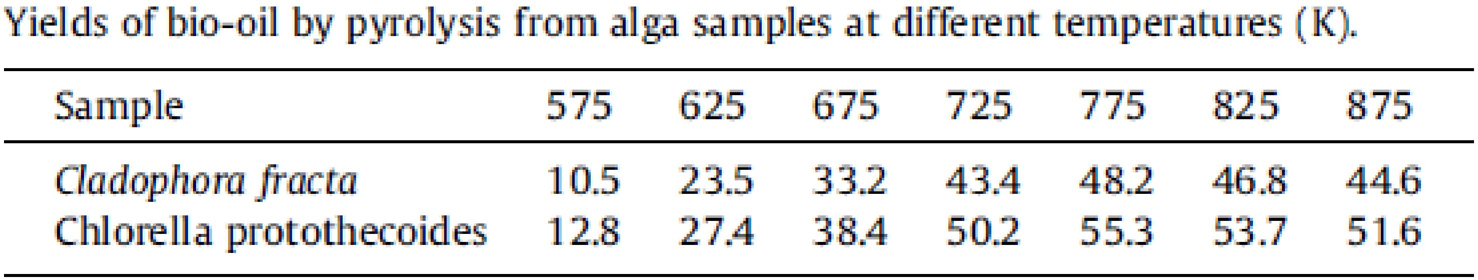
Lipid content and productivities of different microalgae species.

Marine and freshwater microalgae species	Lipid content (% dry weight biomass)	Lipid productivity (mg/L/day)	Volumetric productivity of biomass (g/L/day)	Areal productivity of biomass (g/m^2/day)
Ankistrodesmus sp.	24.0–31.0	–	–	11.5–17.4
Botryococcus braunii	25.0–75.0	–	0.02	3.0
Chaetoceros muelleri	33.6	21.8	0.07	–
Chaetoceros calcitrans	14.6–16.4/39.8	17.6	0.04	–
Chlorella emersonii	25.0–63.0	10.3–50.0	0.036–0.041	0.91–0.97
Chlorella protothecoides	14.6–57.8	1214	2.00–7.70	–
Chlorella sorokiniana	19.0–22.0	44.7	0.23–1.47	–
Chlorella vulgaris	5.0–58.0	11.2–40.0	0.02–0.20	0.57–0.95
Chlorella sp.	10.0–48.0	42.1	0.02–2.5	1.61–16.47/25
Chlorella pyrenoidosa	2.0	–	2.90–3.64	72.5/130
Chlorella	18.0–57.0	18.7	–	3.50–13.90
Chlorococcum sp.	19.3	53.7	0.28	–
Crypthecodinium cohnii	20.0–51.1	–	10	–
Dunaliella salina	6.0–25.0	116.0	0.22–0.34	1.6–3.5/20–38
Dunaliella primolecta	23.1	–	0.09	14
Dunaliella tertiolecta	16.7–71.0	–	0.12	–
Dunaliella sp.	17.5–67.0	33.5	–	–
Ellipsoidion sp.	27.4	47.3	0.17	–
Euglena gracilis	14.0–20.0	–	7.70	–
Haematococcus pluvialis	25.0	–	0.05–0.06	10.2–36.4
Isochrysis galbana	7.0–40.0	–	0.32–1.60	–
Isochrysis sp.	7.1–33	37.8	0.08–0.17	–
Monodus subterraneus	16.0	30.4	0.19	–
Monallanthus salina	20.0–22.0	–	0.08	12
Nannochloris sp.	20.0–56.0	60.9–76.5	0.17–0.51	–
Nannochloropsis oculata.	22.7–29.7	84.0–142.0	0.37–0.48	–
Nannochloropsis sp.	12.0–53.0	37.6–90.0	0.17–1.43	1.9–5.3
Neochloris oleoabundans	29.0–65.0	90.0–134.0	–	–
Nitzschia sp.	16.0–47.0			8.8–21.6
Oocystis pusilla	10.5	–	–	40.6–45.8
Pavlova salina	30.9	49.4	0.16	–
Pavlova lutheri	35.5	40.2	0.14	–
Phaeodactylum tricornutum	18.0–57.0	44.8	0.003–1.9	2.4–21
Porphyridium cruentum	9.0–18.8/60.7	34.8	0.36–1.50	25
Scenedesmus obliquus	11.0–55.0	–	0.004–0.74	–
Scenedesmus quadricauda	1.9–18.4	35.1	0.19	–
Scenedesmus sp.	19.6–21.1	40.8–53.9	0.03–0.26	2.43–13.52
Skeletonema sp.	13.3–31.8	27.3	0.09	–
Skeletonema costatum	13.5–51.3	17.4	0.08	–
Spirulina platensis	4.0–16.6	–	0.06–4.3	1.5–14.5/24–51
Spirulina maxima	4.0–9.0	–	0.21–0.25	25
Thalassiosira pseudonana	20.6	17.4	0.08	–
Tetraselmis suecica	8.5–23.0	27.0–36.4	0.12–0.32	19
Tetraselmis sp.	12.6–14.7	43.4	0.30	–



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

Alkali and acid catalysed transesterification for the production of biodiesel from algae.

Algal feedstock	Pretreatment and oil extraction	Catalyst; conditions	Acyl donor; co-solvent	Yield (reported)	Refs.
Dictyochloropsis splendida	Drying followed by chloroform–methanol (2:1 v/v)	NaOH	Methanol; none	Not reported	[61]
Wild mixed cultures	Air drying for 120 min hexane extraction	KOH, 6 h.	Ethanol; none	Not reported	[123]
Stichococcus bacillaris	Freeze-drying followed by chloroform–methanol (2:1 v/v)	NaOH, 65 °C, 3 min.	Methanol; none	Not reported	[124]
Spirulina sp.	*In situ* process	NaOH, 24 °C, 1 h.	Methanol toluene (1:2); none	86% Overall biodiesel yield (% conversion of TAGs) after 2 cycles	[73]
Dunaliella tertiolecta	Glass beads cell disruption followed by chloroform–methanol (2:1 v/v) oil extraction	CH_3ONa, 110 °C, 5 h	Methanol: THF	23.6 ± 0.5 FAME/dry cell weight	[125]
P. canaliculata, F. spiralis and mixed macroalgae	*In situ* process from dry biomass	NaOH, 60 °C, 11 h	Methanol (300:1 M ratio to oil); none	17.1% FAME yield	[62]
Chlorella protothecoides	Freeze-drying followed by hexane extraction	H_2SO_4 (1:1 M ratio to oil); 30 °C, 4 h.	Methanol (56:1 M ratio to oil); none	Not Reported	[81–88]
Chlorella pyrenoidosa	*In situ* process	H_2SO_4; 90 °C, 2 h	Methanol (165:1 M ratio to oil); hexane	95% Oil conversion	[76]
Chlorella pyrenoidosa	Drying followed by chloroform–methanol (2:1 v/v)	H_2SO_4; 100 °C, 4 h	Methanol (30:1 M ratio to oil); none	Not reported	[90]
Chlorella sp.	*In situ* process	H_2SO_4 (1:1 M ratio to oil); 60 °C, 4 h	Methanol (315:1 M ratio to oil); none	92.22% Gravimetric	[91]
Schizochytrium limacinum	In situ process from dry biomass	H_2SO_4; 90 °C, 40 min	Methanol; chloroform	>100% Oil conversion 67% Biomass to FAME	[74]
Unknown algae	In situ process from dry biomass	H_2SO_4; 65 °C, 2 h	Methanol (220:1 M ratio to oil); none	98% Theoretical yield	[80]
Pure and Mixed cultures	In situ process from dry biomass	H_2SO_4 1.8% (v/v); 80 °C, 20 min.	Methanol; none	36% (w FAME/w algae) for diatoms 10.7% (w FAME/w algae) for wastewater lagoon	[68]
Nannochloropsis oculata	In situ process	HCl 80 °C for 2 h	Methanol: chloroform (10:1); none	23.07 ± 2.76% w/w	[75]

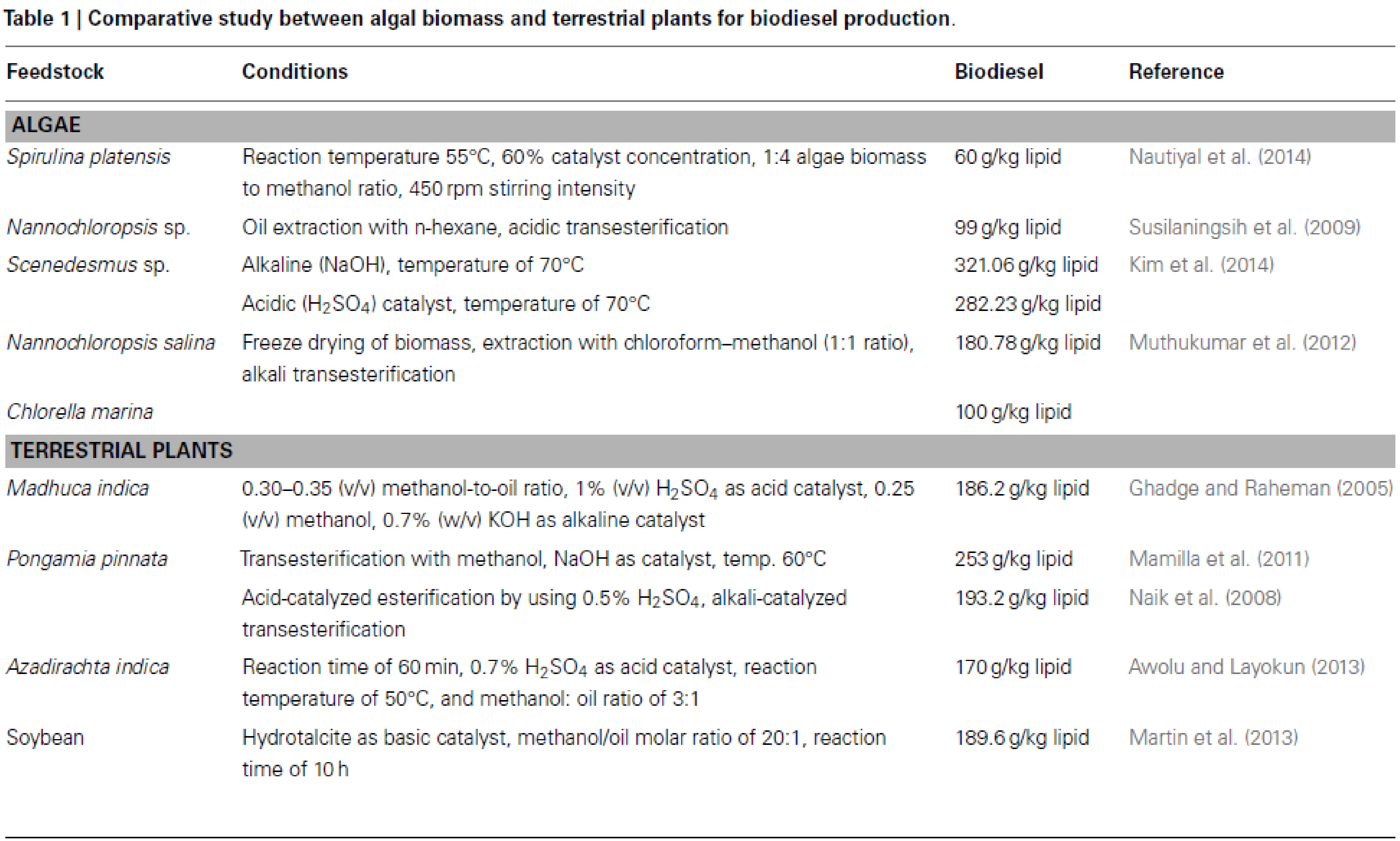
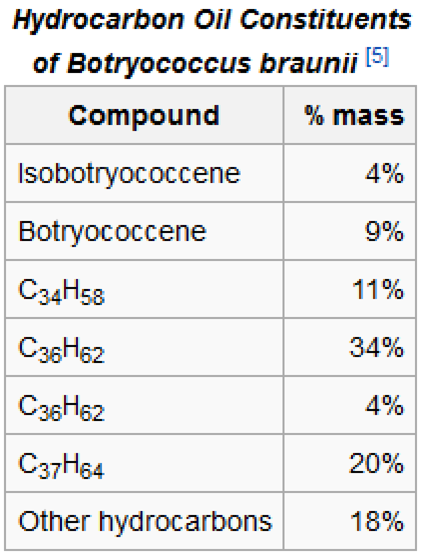


Table 1 | Comparative study between algal biomass and terrestrial plants for biodiesel production.

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)

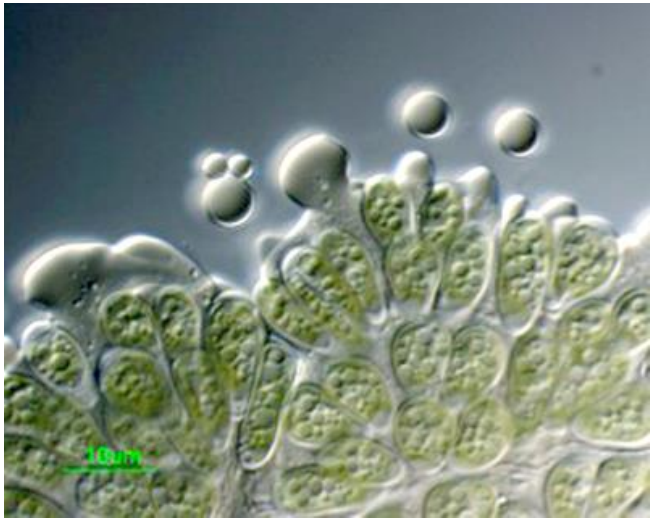
Botryococcus braunii

- Green oil producing alga
- Oils are not suitable for transesterification
- Triterpenes can be used for refinement
    - Octane
    - Kerosene
    - Diesel

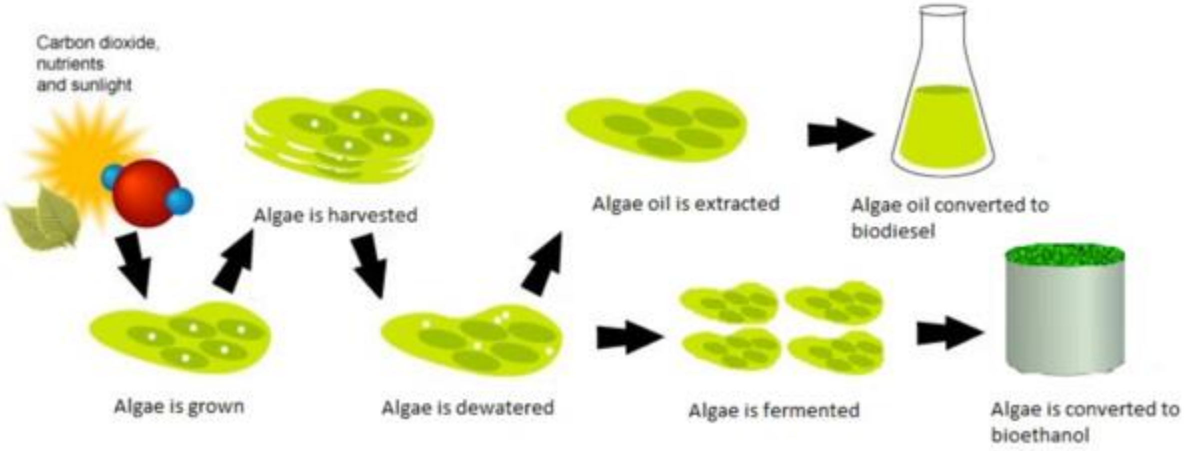


Hydrocarbon Oil Constituents of Botryococcus braunii [5]

Compound	% mass
Isobotryococcene	4%
Botryococcene	9%
$C_{34}H_{58}$	11%
$C_{36}H_{62}$	34%
$C_{36}H_{62}$	4%
$C_{37}H_{64}$	20%
Other hydrocarbons	18%



Bioethanol



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

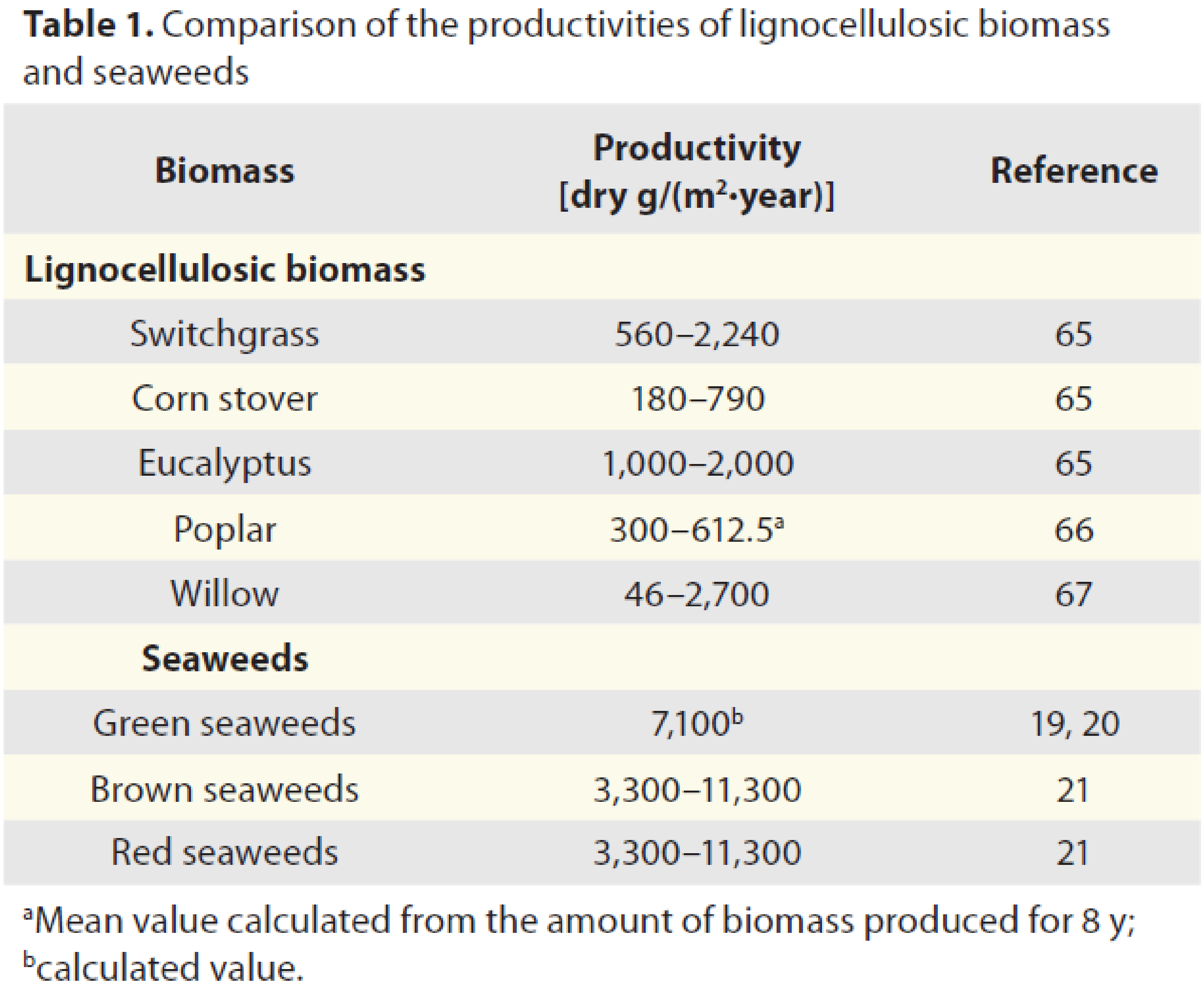


Table 1. Comparison of the productivities of lignocellulosic biomass and seaweeds

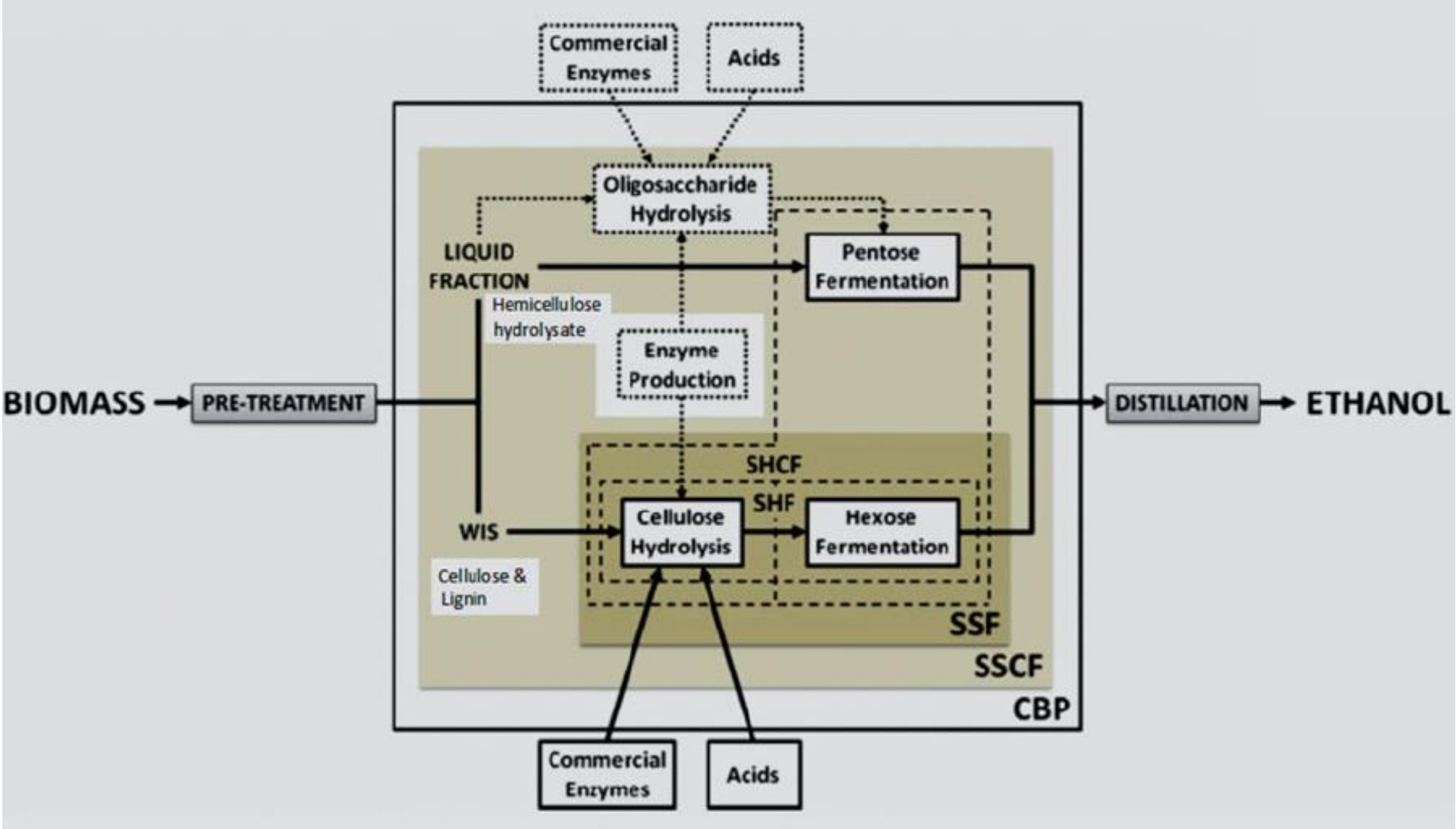
Biomass	Productivity [dry g/(m²·year)]	Reference
Lignocellulosic biomass		
Switchgrass	560–2,240	65
Corn stover	180–790	65
Eucalyptus	1,000–2,000	65
Poplar	300–612.5[a]	66
Willow	46–2,700	67
Seaweeds		
Green seaweeds	7,100[b]	19, 20
Brown seaweeds	3,300–11,300	21
Red seaweeds	3,300–11,300	21

[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*


Commercial Enzymes
Acids
Oligosaccharide Hydrolysis
LIQUID FRACTION
Hemicellulose hydrolysate
Pentose Fermentation
Enzyme Production
BIOMASS
PRE-TREATMENT
DISTILLATION
ETHANOL
SHCF
SHF
WIS
Cellulose Hydrolysis
Hexose Fermentation
Cellulose & Lignin
SSF
SSCF
CBP
Commercial Enzymes
Acids

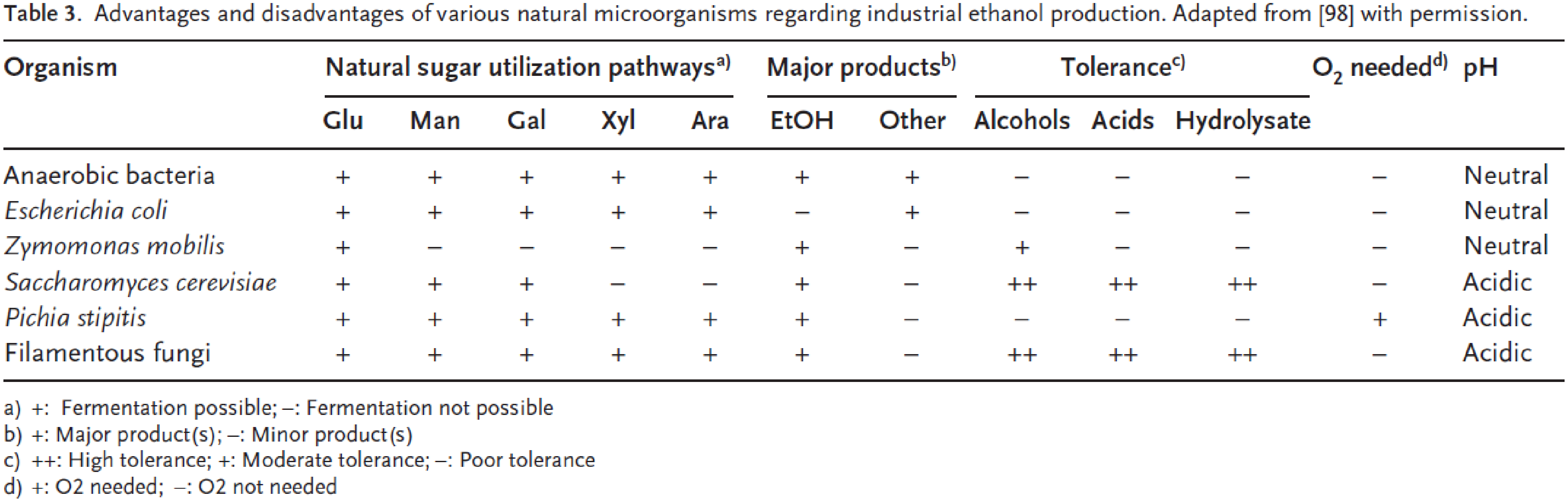


Table 3. Advantages and disadvantages of various natural microorganisms regarding industrial ethanol production. Adapted from [98] with permission.

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

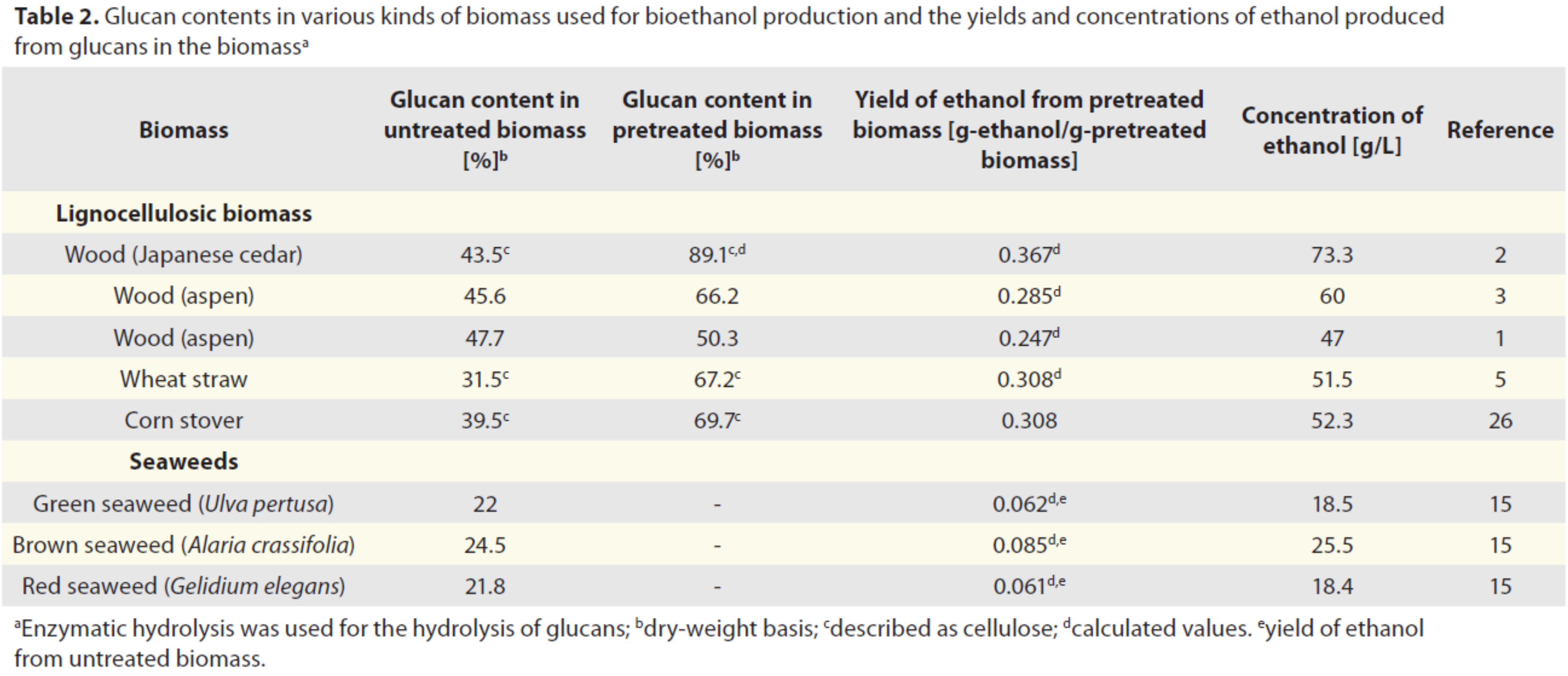


Table 2. Glucan contents in various kinds of biomass used for bioethanol production and the yields and concentrations of ethanol produced from glucans in the biomass[a]

Biomass	Glucan content in untreated biomass [%][b]	Glucan content in pretreated biomass [%][b]	Yield of ethanol from pretreated biomass [g-ethanol/g-pretreated biomass]	Concentration of ethanol [g/L]	Reference
Lignocellulosic biomass					
Wood (Japanese cedar)	43.5[c]	89.1[c,d]	0.367[d]	73.3	2
Wood (aspen)	45.6	66.2	0.285[d]	60	3
Wood (aspen)	47.7	50.3	0.247[d]	47	1
Wheat straw	31.5[c]	67.2[c]	0.308[d]	51.5	5
Corn stover	39.5[c]	69.7[c]	0.308	52.3	26
Seaweeds					
Green seaweed (*Ulva pertusa*)	22	-	0.062[d,e]	18.5	15
Brown seaweed (*Alaria crassifolia*)	24.5	-	0.085[d,e]	25.5	15
Red seaweed (*Gelidium elegans*)	21.8	-	0.061[d,e]	18.4	15

[a] Enzymatic hydrolysis was used for the hydrolysis of glucans; [b] dry-weight basis; [c] described as cellulose; [d] calculated values. [e] yield of ethanol from untreated biomass.

Table 3. Yields and concentrations of sugars produced by hydrolysis of brown seaweeds

Seaweed	Hydrolysis	Conditions	Sugars produced	Yield of sugar [g-sugar/g-seaweed]	Concentration of sugar [g/L]	Reference
Undaria pinnatifida	Acid[a]	120°C for 24 h	Glucose	0.065	3.3[m]	43
			Xylose	0.002	0.1[m]	
			Fructose	0.004	0.2[m]	
Undaria pinnatifida	Enzymatic[b]	45°C for 60 min	Glucose	0.014	0.7[m]	43
Laminaria japonica	Enzymatic[c] after acid pretreatment[d,e]	Enzymatic hydrolysis at 50°C for 48 h after acid pretreatment at 121°C for 1 h	Glucose	0.2775[l]	5.55[m]	10
Laminaria japonica	Acid[f] and enzymatic[g]	Acid hydrolysis at 121°C for 15 min and enzymatic hydrolysis at 50°C for 24 h	Glucose	0.0698	6.98[m]	44
	0.0698		Mannitol	0.3054	30.54[m]	
Sargassum fulvellum	Acid[f] and enzymatic[g]	Acid hydrolysis at 121°C for 15 min and enzymatic hydrolysis at 50°C for 24 h	Glucose	0.0596	5.96[m]	44
			Mannitol	0.0215	2.15[m]	
Undaria pinnatifida	Enzymatic[h] after removal of alginate[i]	Enzymatic hydrolysis at 40°C for 15 h after removal of alginate at 80°C[j]	Glucose	0.13	0.130[m]	14
Alaria crassifolia	Enzymatic[k]	50°C for 120 h	Glucose	0.224[m]	67.2	15

[a] Sulfuric acid was used at 5% (v/v); [b] Celluclast 1.5 L and Novozyme 188 were used as enzymes; [c] commercial cellulase and cellobiase were used as enzymes; [d] sulfuric acid was used for the acid pretreatment at a concentration of 0.1% (w/v); [e] acid-insoluble residue was washed and used for the enzymatic hydrolysis; [f] hydrochloric acid was used at a concentration of 0.1 N; [g] Viscozyme L and Celluclast 1.5 L were used as enzymes; [h] commercial cellulase was used as the enzyme; [i] sodium carbonate was used for the removal of alginate at a concentration of 1% (w/v); [j] treatment time for the removal of alginate is not described;[14] [k] Meicelase was used as the enzyme; [l] yield of ethanol from pretreated seaweed; [m] calculated values.

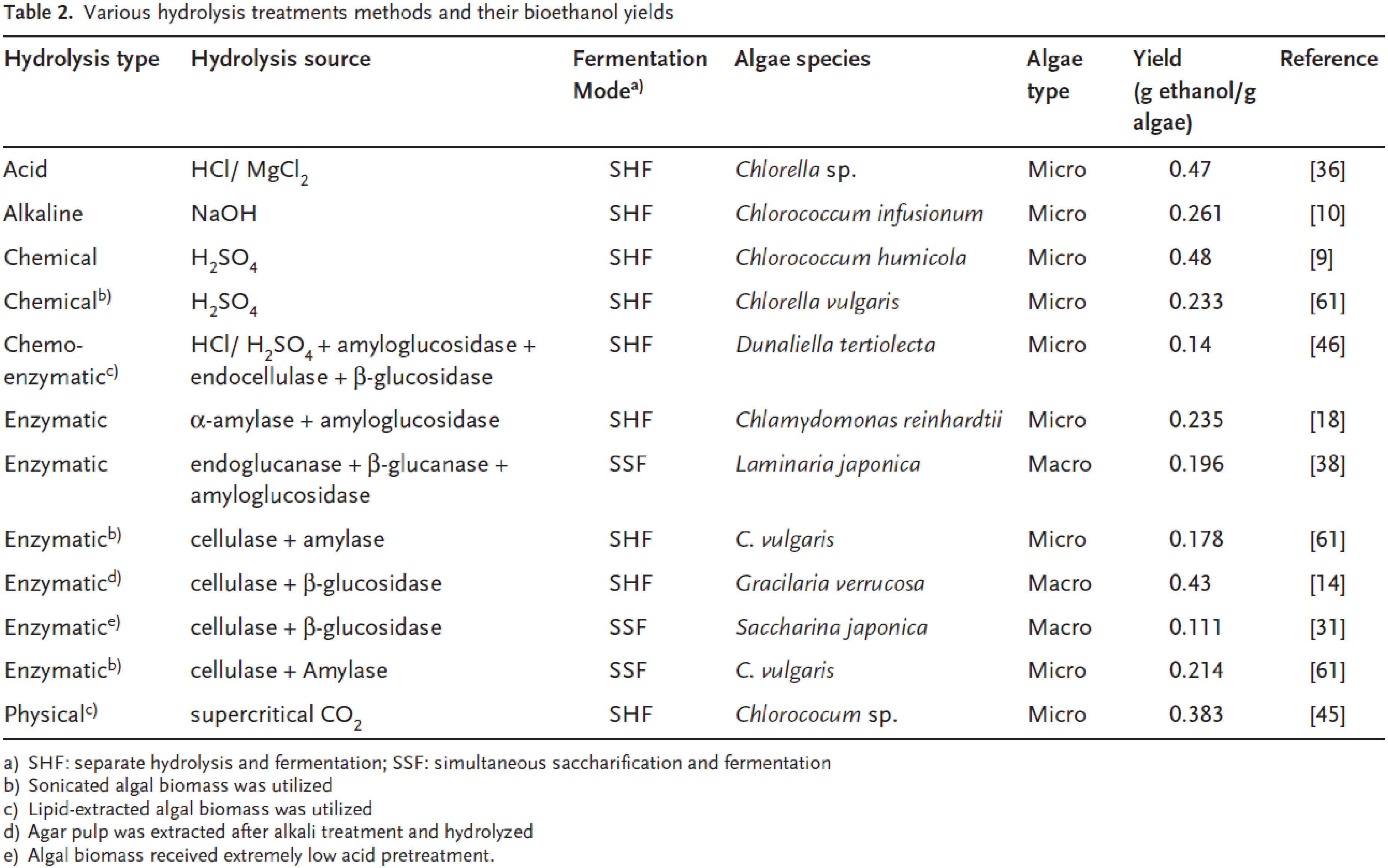


Table 2. Various hydrolysis treatments methods and their bioethanol yields

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 5. The highest concentrations of ethanol produced from green, brown and red seaweeds

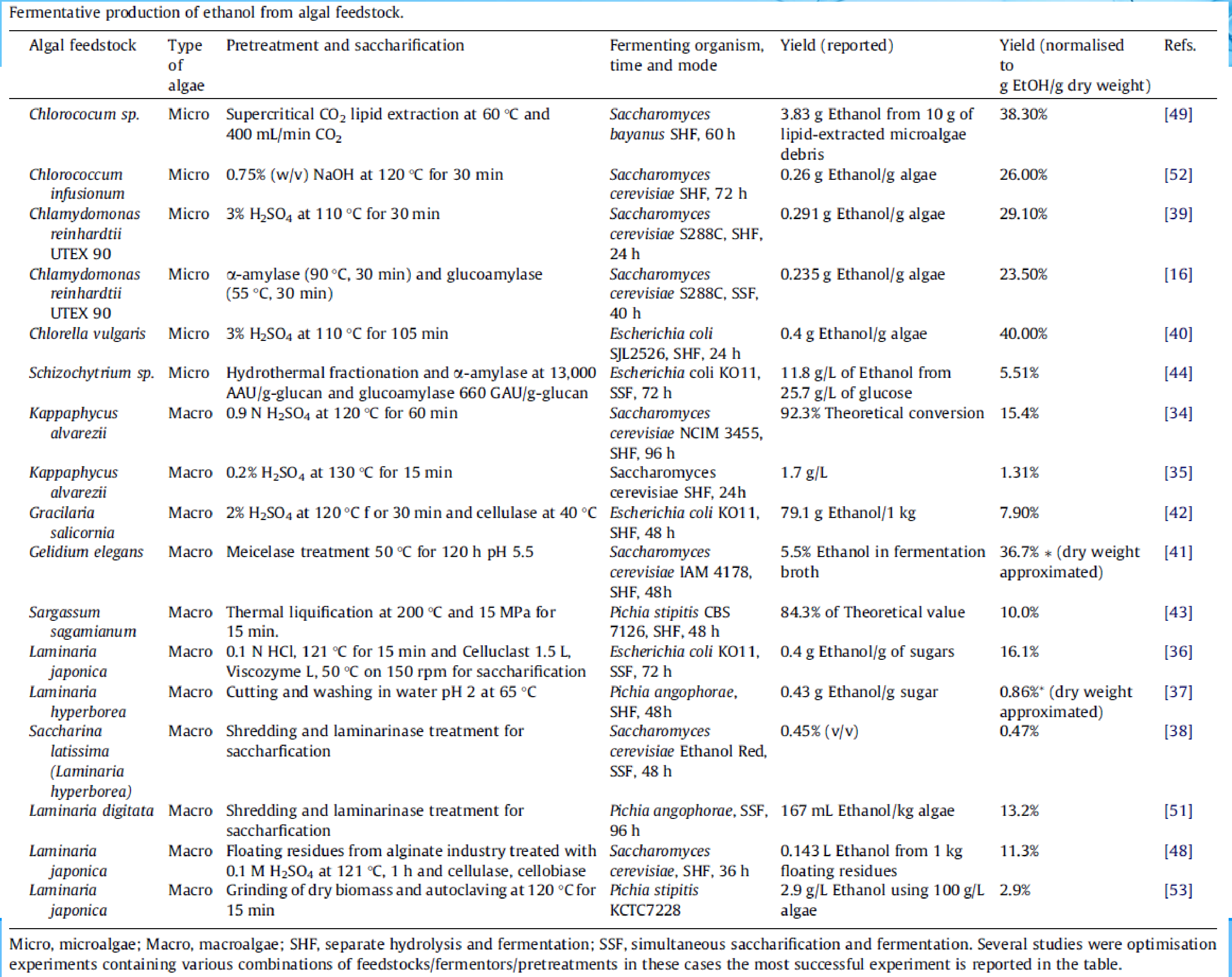
Seaweed	Carbohydrates converted to ethanol	Ethanol concentration [g/L]	Ethanol yield [g-ethanol/g-seaweed]	Reference
Ulva pertusa	Glucans	27.5	0.092[b]	15
(Green seaweed)				
Laminaria japonica[a]	Glucose	37.8	0.291[b]	51
(Brown seaweed)	Mannitol			
	Alginate			
Gelidium elegans	Glucans	55	0.183[b]	15
(Red seaweed)	Agar (galactose)			

[a]Described in that study as *Saccharina japonica*; [b]calculated values.

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 + amyloglucosidase	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]

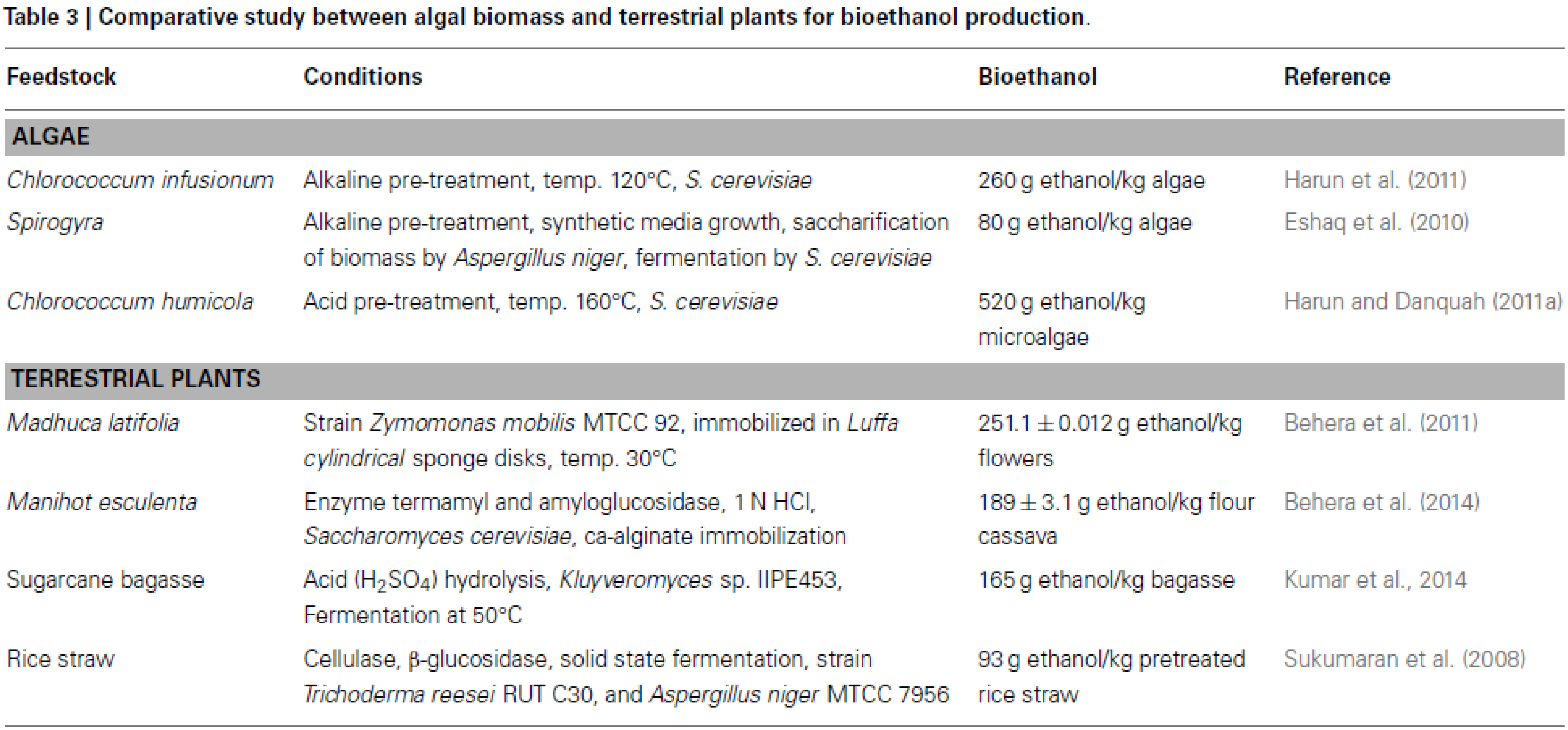
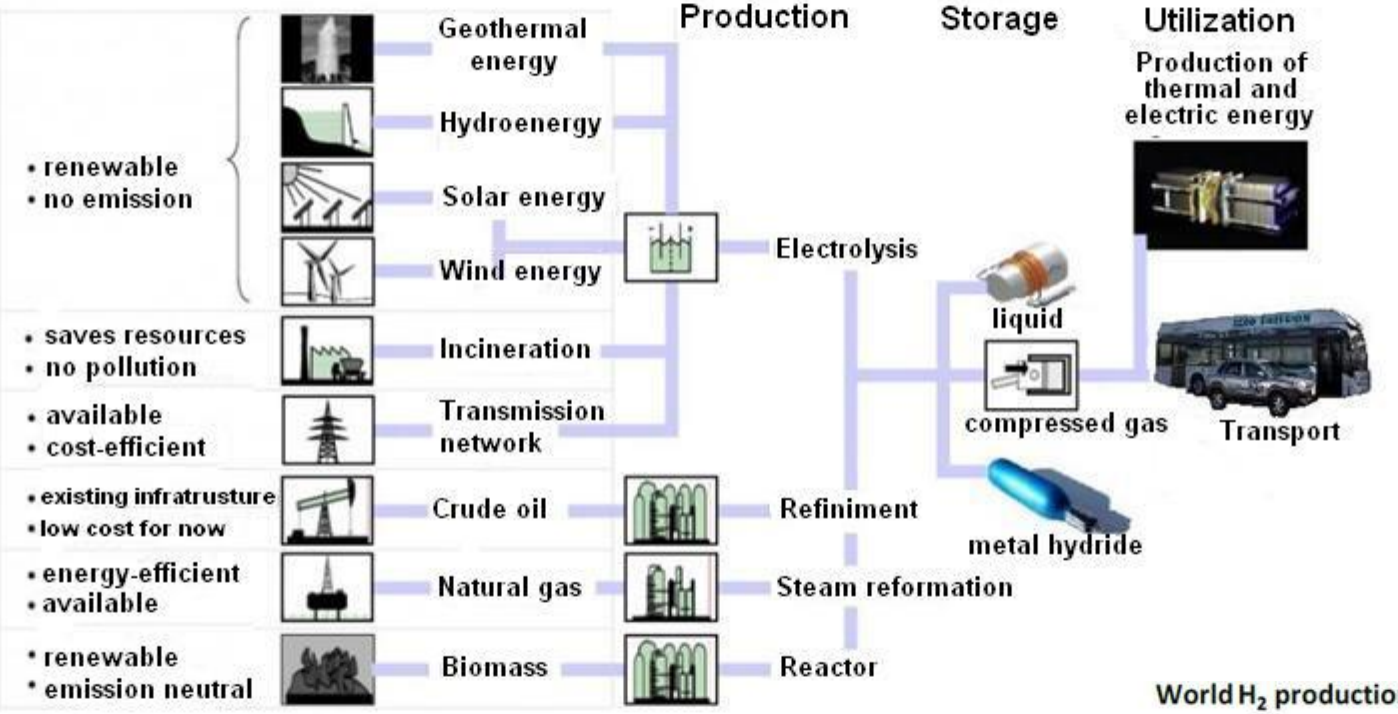


Table 3 | Comparative study between algal biomass and terrestrial plants for bioethanol production.

Feedstock	Conditions	Bioethanol	Reference
ALGAE			
Chlorococcum infusionum	Alkaline pre-treatment, temp. 120°C, *S. cerevisiae*	260 g ethanol/kg algae	Harun et al. (2011)
Spirogyra	Alkaline pre-treatment, synthetic media growth, saccharification of biomass by *Aspergillus niger*, fermentation by *S. cerevisiae*	80 g ethanol/kg algae	Eshaq et al. (2010)
Chlorococcum humicola	Acid pre-treatment, temp. 160°C, *S. cerevisiae*	520 g ethanol/kg microalgae	Harun and Danquah (2011a)
TERRESTRIAL PLANTS			
Madhuca latifolia	Strain *Zymomonas mobilis* MTCC 92, immobilized in *Luffa cylindrical* sponge disks, temp. 30°C	251.1 ± 0.012 g ethanol/kg flowers	Behera et al. (2011)
Manihot esculenta	Enzyme termamyl and amyloglucosidase, 1 N HCl, *Saccharomyces cerevisiae*, ca-alginate immobilization	189 ± 3.1 g ethanol/kg flour cassava	Behera et al. (2014)
Sugarcane bagasse	Acid (H_2SO_4) hydrolysis, *Kluyveromyces* sp. IIPE453, Fermentation at 50°C	165 g ethanol/kg bagasse	Kumar et al., 2014
Rice straw	Cellulase, β-glucosidase, solid state fermentation, strain *Trichoderma reesei* RUT C30, and *Aspergillus niger* MTCC 7956	93 g ethanol/kg pretreated rice straw	Sukumaran et al. (2008)

Hydrogen production

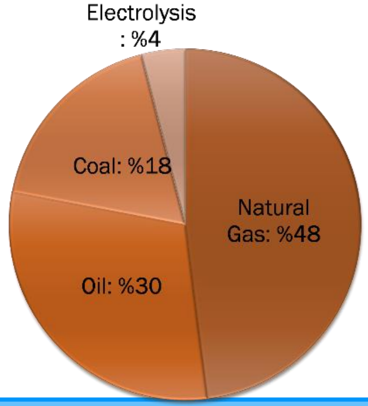


Production **Storage** **Utilization**

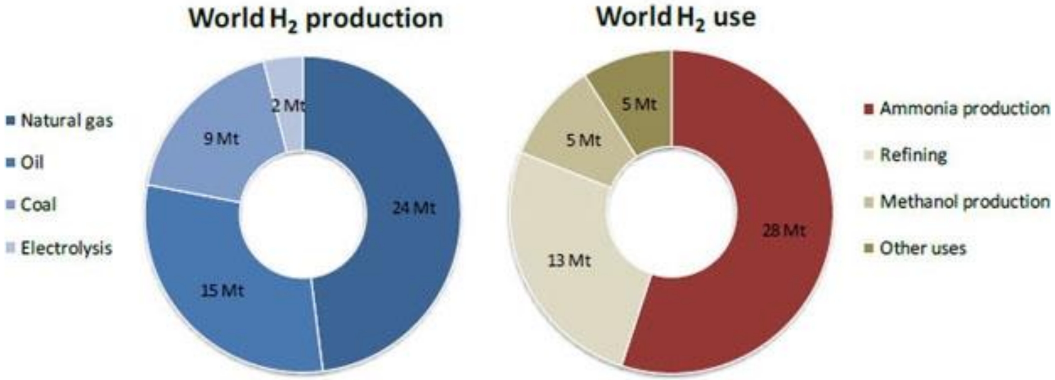
• renewable • no emission	Geothermal energy Hydroenergy Solar energy Wind energy	Electrolysis
• saves resources • no pollution	Incineration	Electrolysis
• available • cost-efficient	Transmission network	Electrolysis
• existing infratrusture • low cost for now	Crude oil	Refiniment
• energy-efficient • available	Natural gas	Steam reformation
• renewable • emission neutral	Biomass	Reactor

Storage: liquid, compressed gas, metal hydride

Utilization: Production of thermal and electric energy; Transport

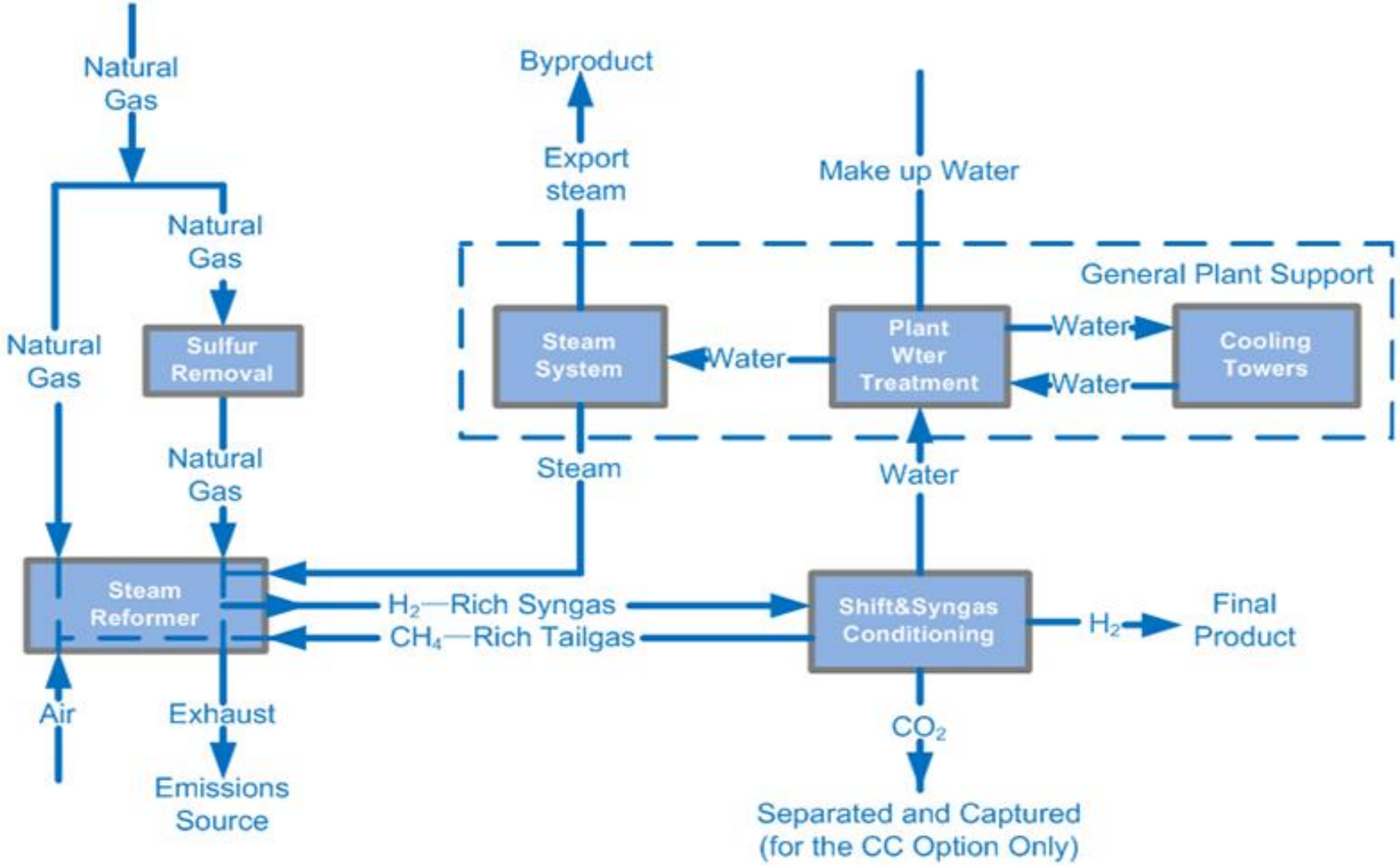


World H_2 production approx. 50 Mt/yr

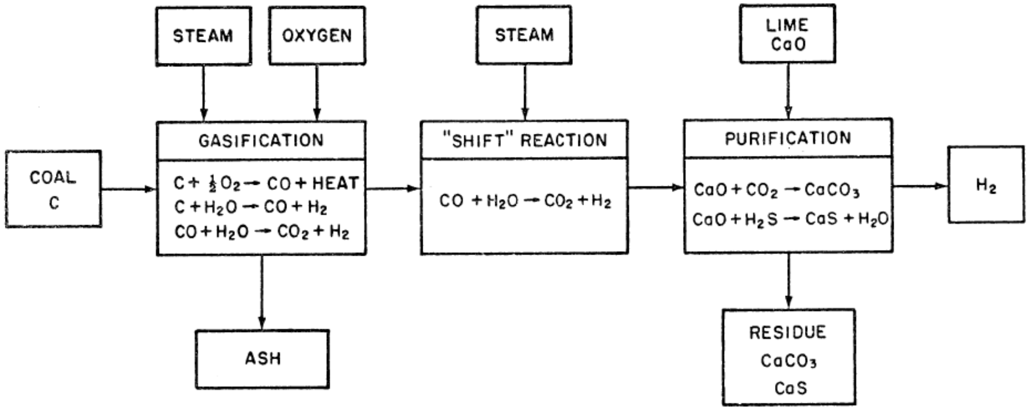


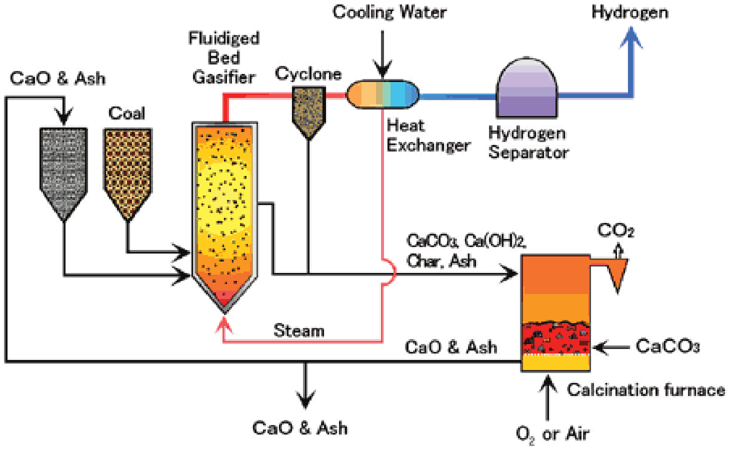
Hydrogen production from natural gas

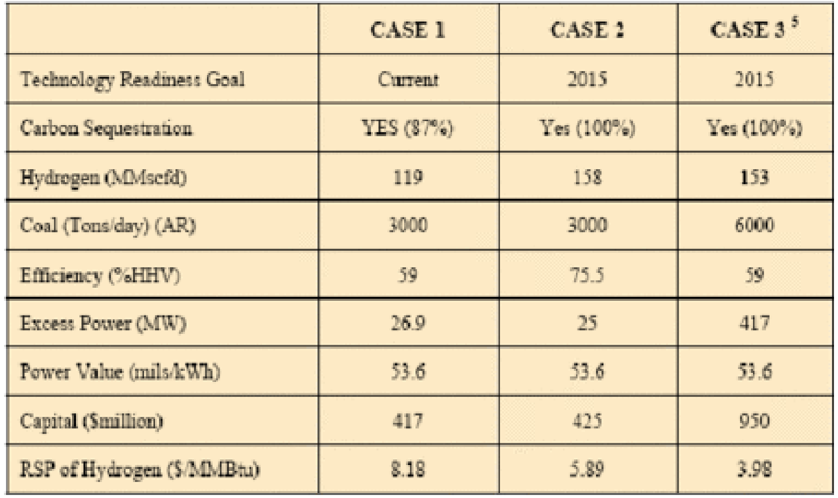
- $CH_4 + H_2O \rightleftharpoons CO + 3\ H_2$ (at 700 – 1100 °C) – steam reforming



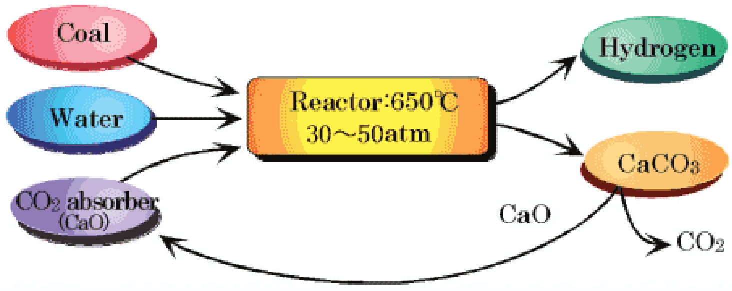
Hydrogen from coal



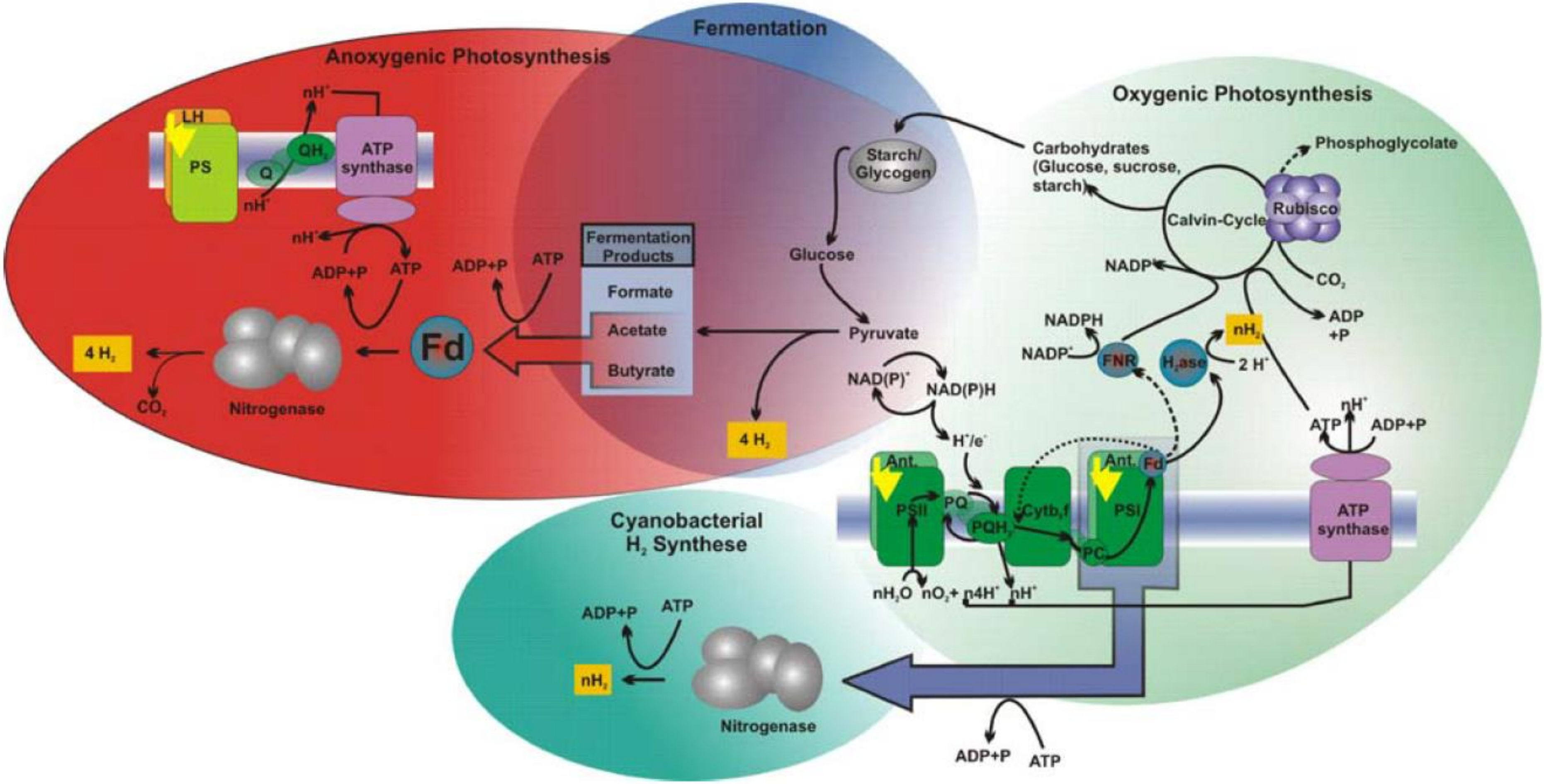




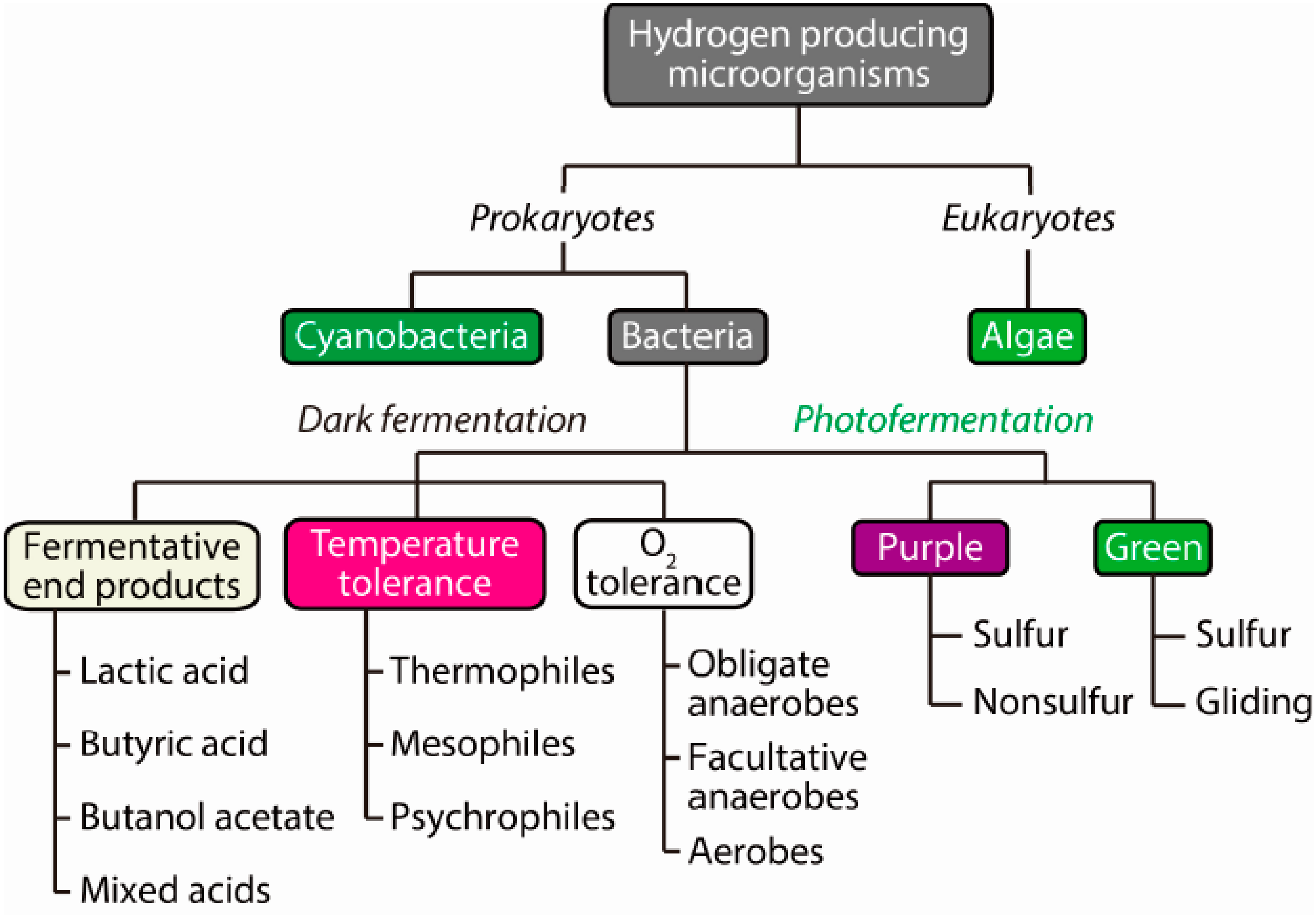
	CASE 1	CASE 2	CASE 3 [5]
Technology Readiness Goal	Current	2015	2015
Carbon Sequestration	YES (87%)	Yes (100%)	Yes (100%)
Hydrogen (MMscfd)	119	158	153
Coal (Tons/day) (AR)	3000	3000	6000
Efficiency (%HHV)	59	75.5	59
Excess Power (MW)	26.9	25	417
Power Value (mils/kWh)	53.6	53.6	53.6
Capital ($million)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



Biohydrogen production



Biohydrogen production



Hydrogen producing microorganisms

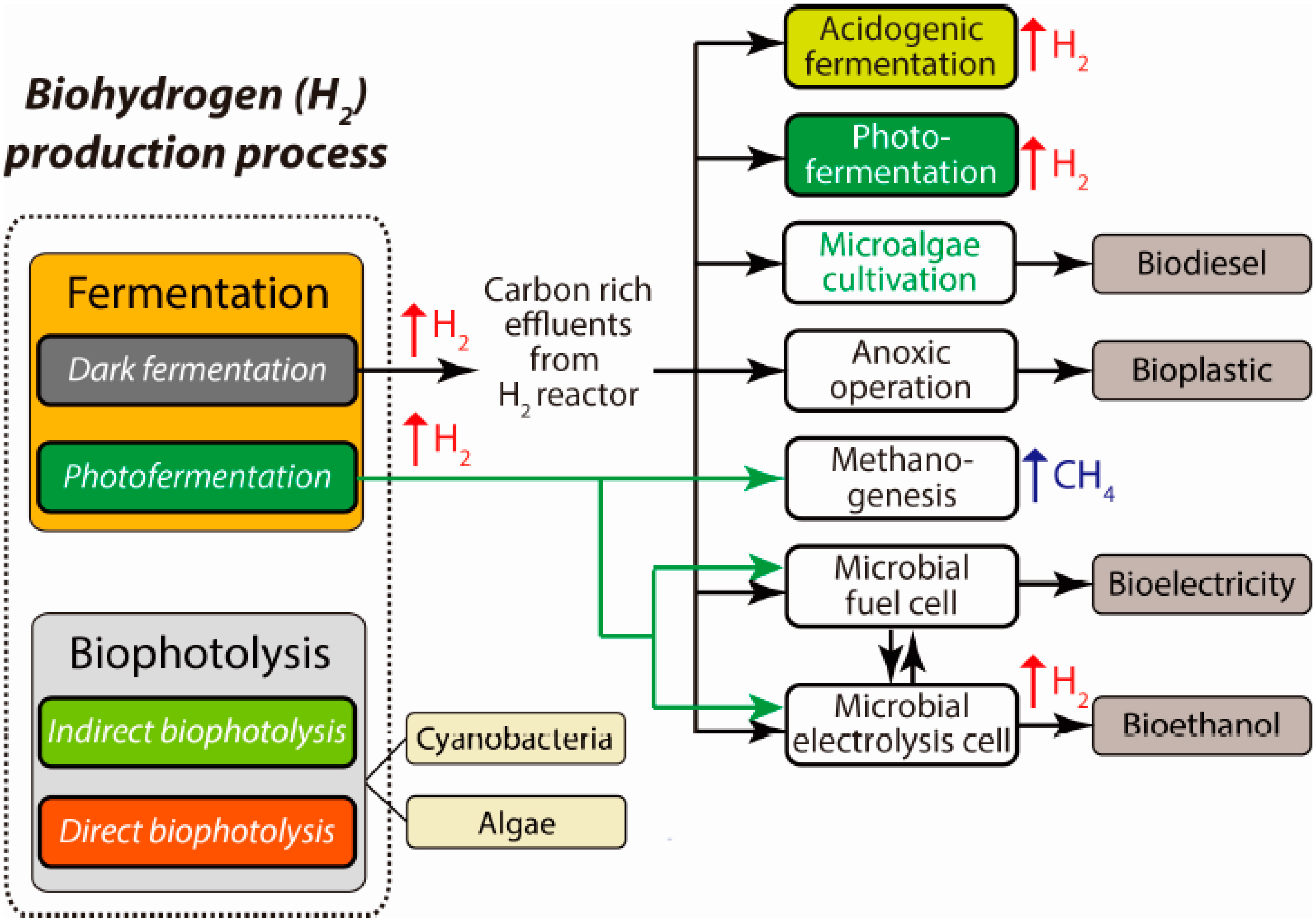
Prokaryotes

- Cyanobacteria
- Bacteria
  - *Dark fermentation*
    - Fermentative end products
      - Lactic acid
      - Butyric acid
      - Butanol acetate
      - Mixed acids
    - Temperature tolerance
      - Thermophiles
      - Mesophiles
      - Psychrophiles
    - O_2 tolerance
      - Obligate anaerobes
      - Facultative anaerobes
      - Aerobes
  - *Photofermentation*
    - Purple
      - Sulfur
      - Nonsulfur
    - Green
      - Sulfur
      - Gliding

Eukaryotes

- Algae

Biohydrogen (H_2) production process



Fermentation

Dark fermentation

Photofermentation

↑ H_2

↑ H_2

Carbon rich effluents from H_2 reactor

Biophotolysis

Indirect biophotolysis

Direct biophotolysis

Cyanobacteria

Algae

Acidogenic fermentation ↑ H_2

Photo-fermentation ↑ H_2

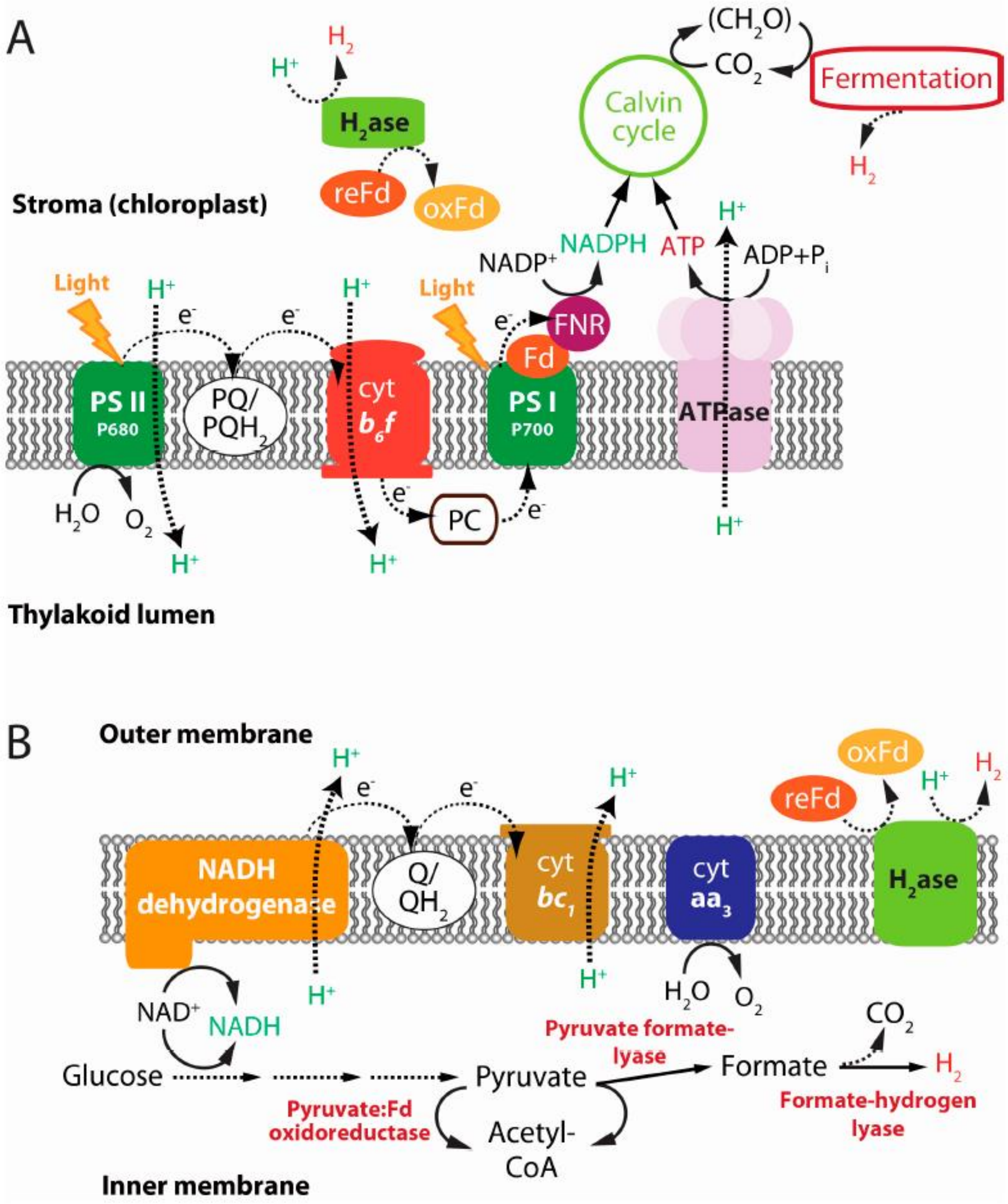
Microalgae cultivation → Biodiesel

Anoxic operation → Bioplastic

Methanogenesis ↑ CH_4

Microbial fuel cell → Bioelectricity

Microbial electrolysis cell ↑ H_2 → Bioethanol


A
H+
H2
H2ase
reFd
oxFd
(CH2O)
CO2
Calvin cycle
Fermentation
H2
Stroma (chloroplast)
NADP+
NADPH
ATP
H+
ADP+Pi
Light
H+
e-
e-
H+
Light
e-
FNR
Fd
PS II
P680
PQ/
PQH2
cyt
b6f
PS I
P700
ATPase
H2O
O2
H+
e-
PC
e-
H+
H+
Thylakoid lumen
B
Outer membrane
H+
e-
e-
H+
oxFd
reFd
H+
H2
NADH
dehydrogenase
Q/
QH2
cyt
bc1
cyt
aa3
H2ase
H+
H+
H2O
O2
NAD+
NADH
Glucose
Pyruvate
Pyruvate formate-lyase
Formate
CO2
H2
Pyruvate:Fd oxidoreductase
Acetyl-CoA
Formate-hydrogen lyase
Inner membrane

Nitrogenase in cyanobacteria

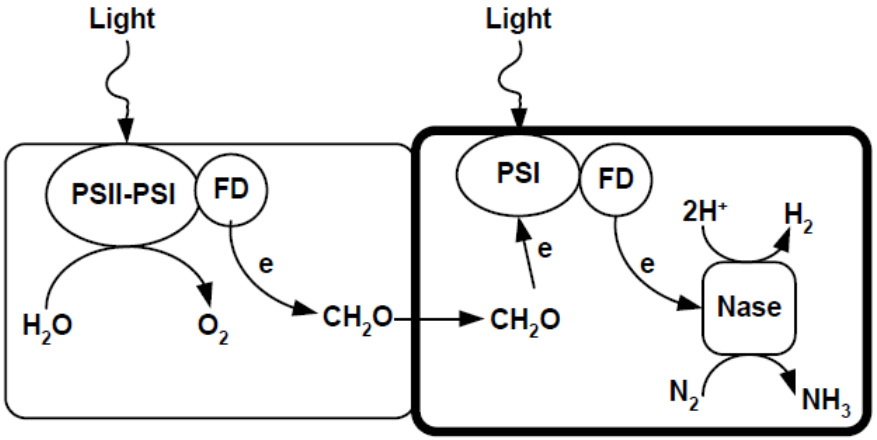
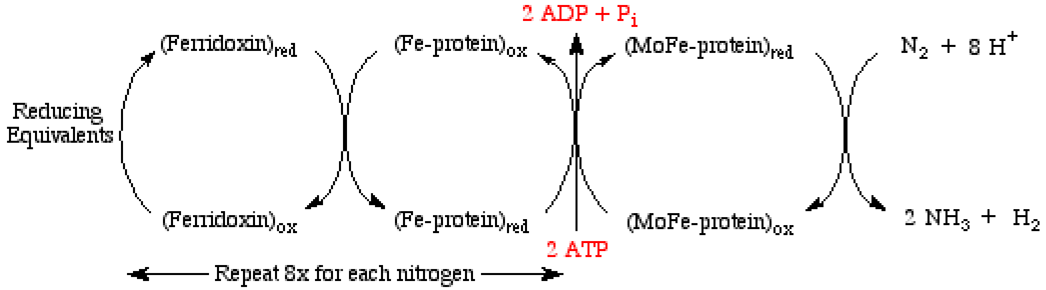


Fig. 2. Nitrogenase(Nase)-mediated hydrogen evolution in a heterocyst of nitrogen-fixing heterocystous cyanobacteria [10, 30, 32]. The oxygen and hydrogen evolution are carried out separately and the energy-rich carbohydrate (CH_2O) is used as the electron source in the oxygen-free heterocyst.

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

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



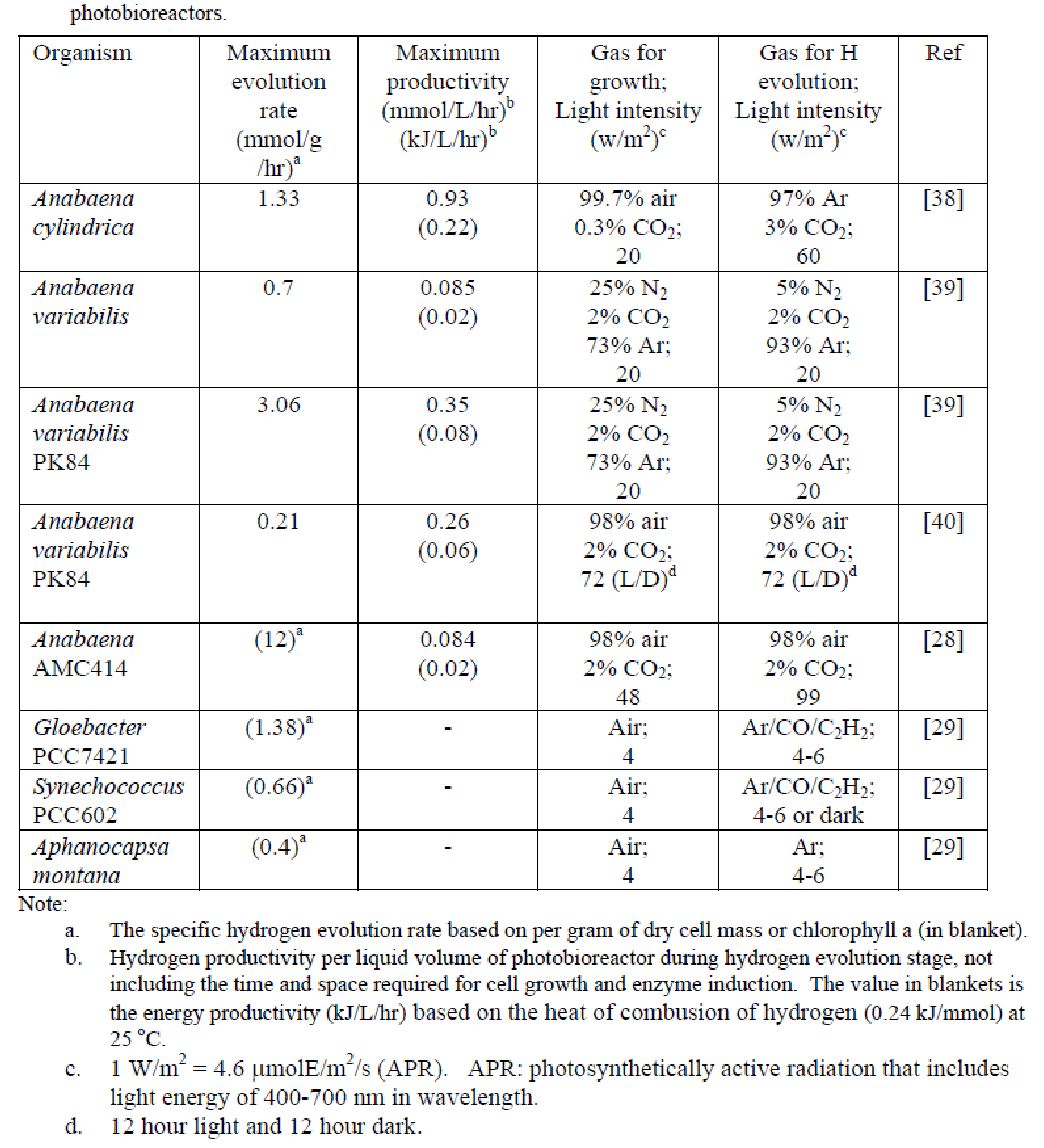


Table 1. Hydrogen evolution via direct biophotolysis by cyanobacteria in laboratory photobioreactors.

Organism	Maximum evolution rate (mmol/g /hr)[a]	Maximum productivity (mmol/L/hr)[b] (kJ/L/hr)[b]	Gas for growth; Light intensity (w/m^2)[c]	Gas for H evolution; Light intensity (w/m^2)[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/m^2 = 4.6 µmolE/m^2/s (APR). APR: photosynthetically active radiation that includes light energy of 400-700 nm in wavelength.

d. 12 hour light and 12 hour dark.

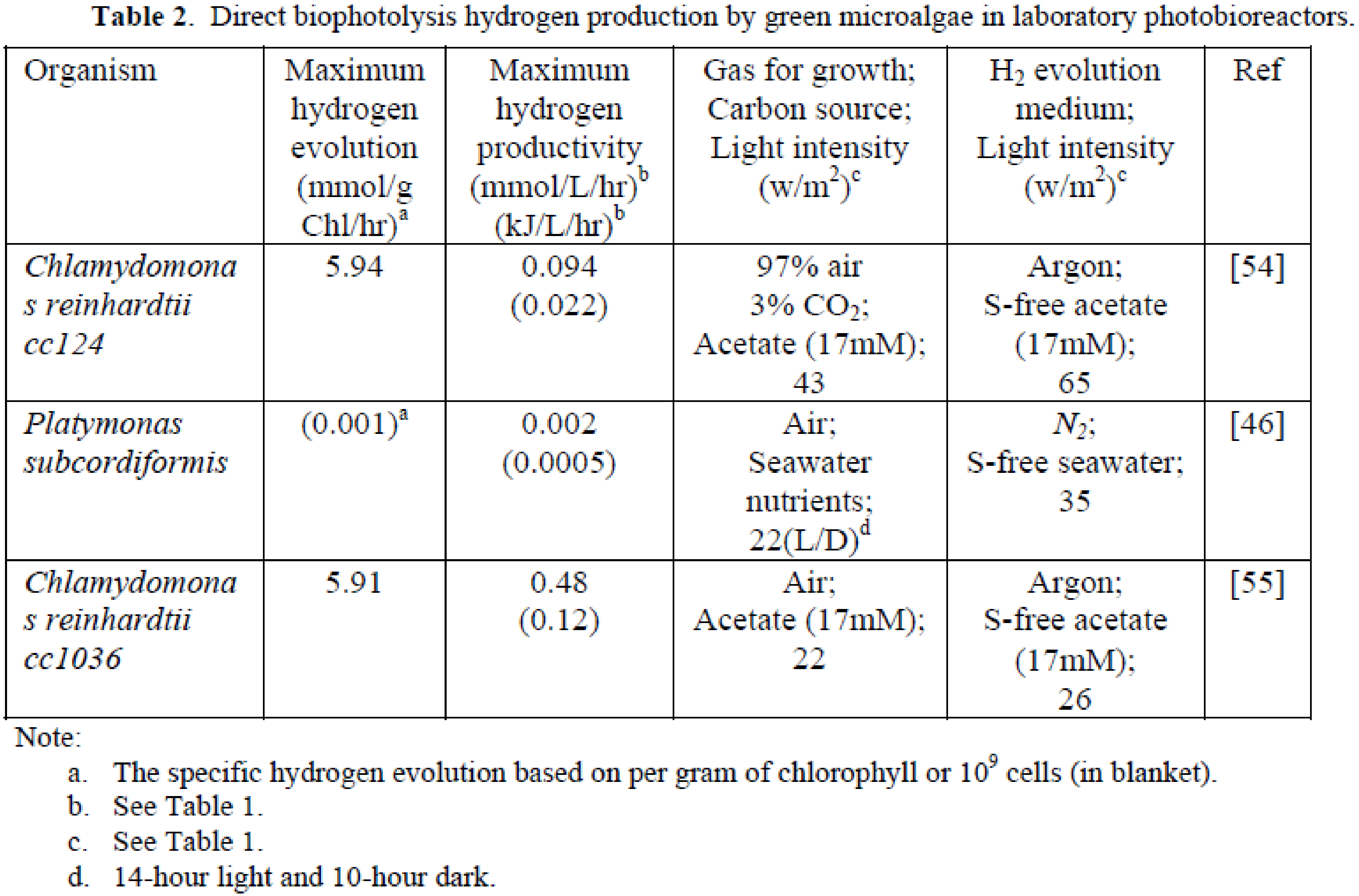


Table 2. Direct biophotolysis hydrogen production by green microalgae in laboratory photobioreactors.

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/m^2)[c]	H_2 evolution medium; Light intensity (w/m^2)[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.

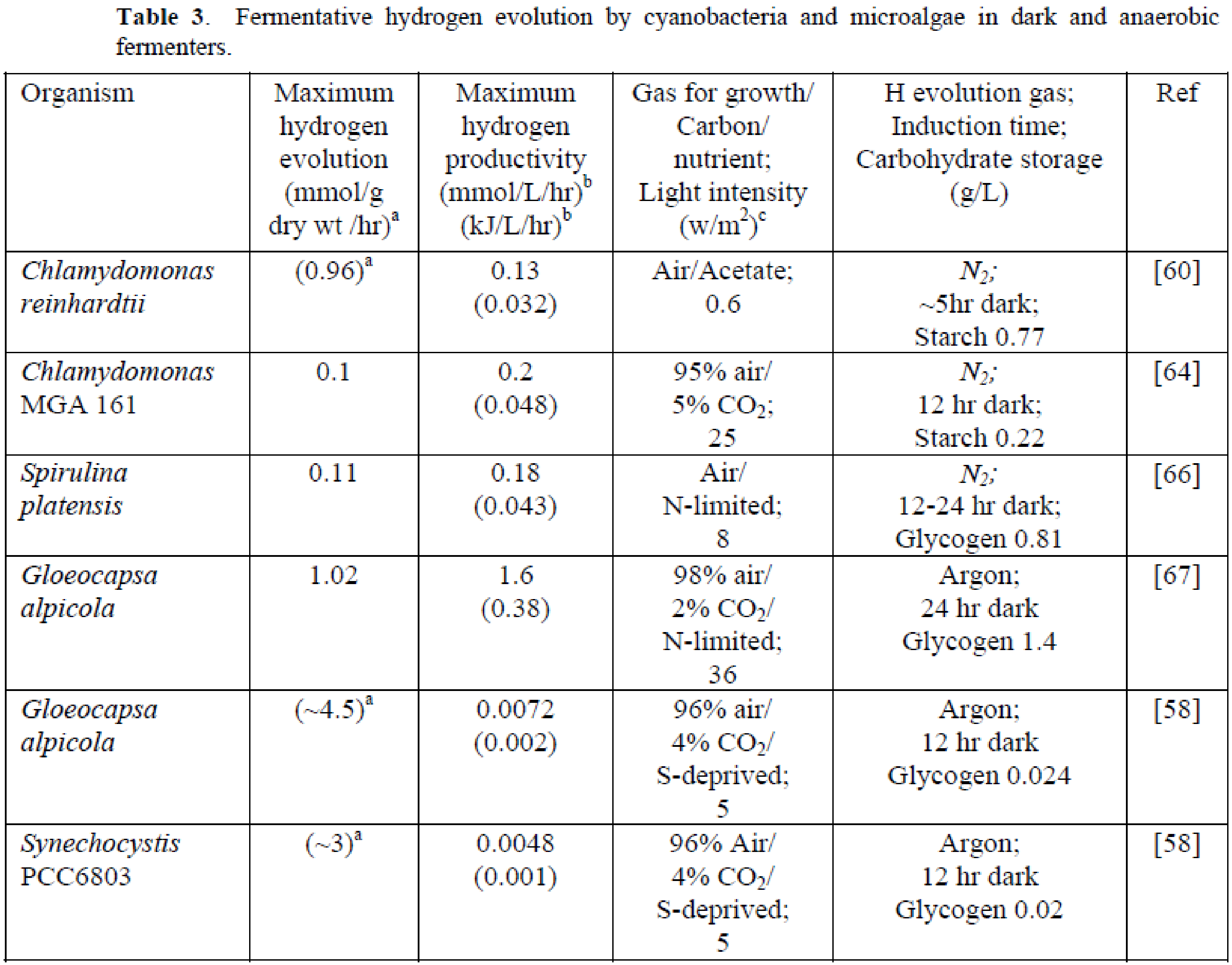


Table 3. Fermentative hydrogen evolution by cyanobacteria and microalgae in dark and anaerobic fermenters.

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/m^2)^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/m^2	[87]
Fruit juice industry wastewater	Hydrogen (DF)	1.4 mol H_2/mol hexose	Electricity (DF)	0.55 W/m^2	[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/m^2	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]

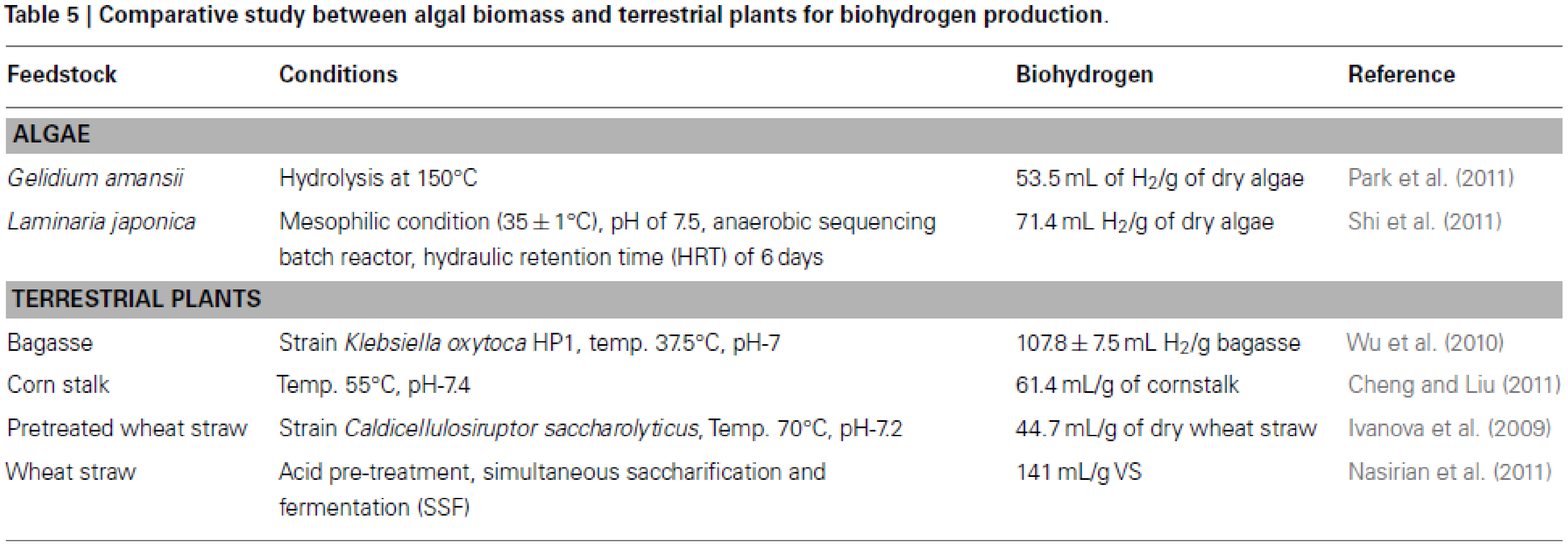
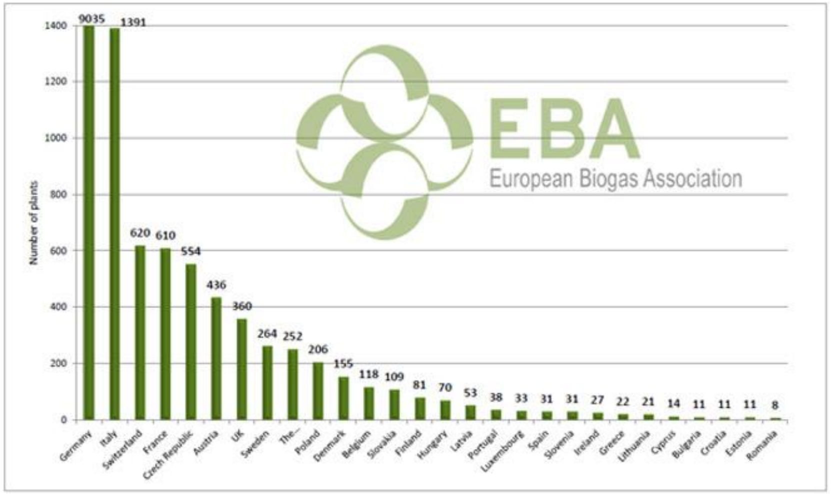


Table 5 | Comparative study between algal biomass and terrestrial plants for biohydrogen production.

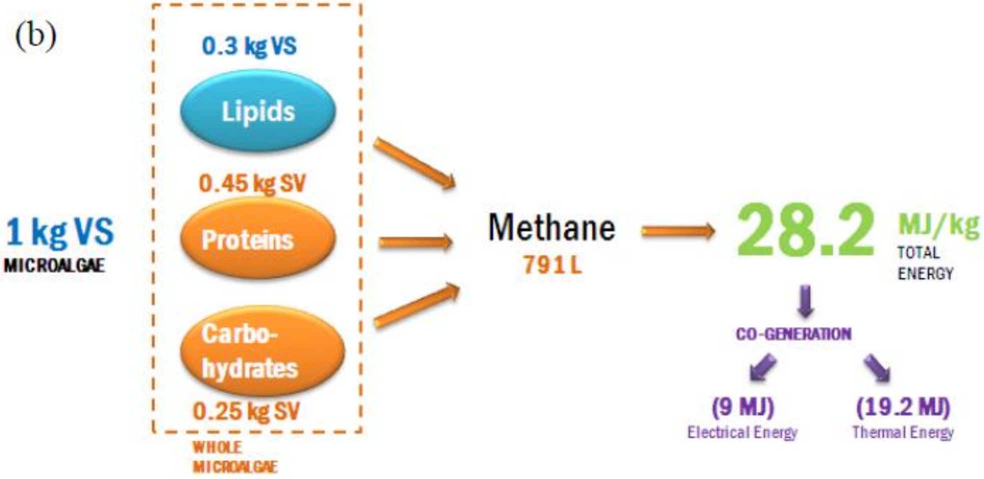
Feedstock	Conditions	Biohydrogen	Reference
ALGAE			
Gelidium amansii	Hydrolysis at 150°C	53.5 mL of H_2/g of dry algae	Park et al. (2011)
Laminaria japonica	Mesophilic condition (35 ± 1°C), pH of 7.5, anaerobic sequencing batch reactor, hydraulic retention time (HRT) of 6 days	71.4 mL H_2/g of dry algae	Shi et al. (2011)
TERRESTRIAL PLANTS			
Bagasse	Strain *Klebsiella oxytoca* HP1, temp. 37.5°C, pH-7	107.8 ± 7.5 mL H_2/g bagasse	Wu et al. (2010)
Corn stalk	Temp. 55°C, pH-7.4	61.4 mL/g of cornstalk	Cheng and Liu (2011)
Pretreated wheat straw	Strain *Caldicellulosiruptor saccharolyticus*, Temp. 70°C, pH-7.2	44.7 mL/g of dry wheat straw	Ivanova et al. (2009)
Wheat straw	Acid pre-treatment, simultaneous saccharification and fermentation (SSF)	141 mL/g VS	Nasirian et al. (2011)

Biogas

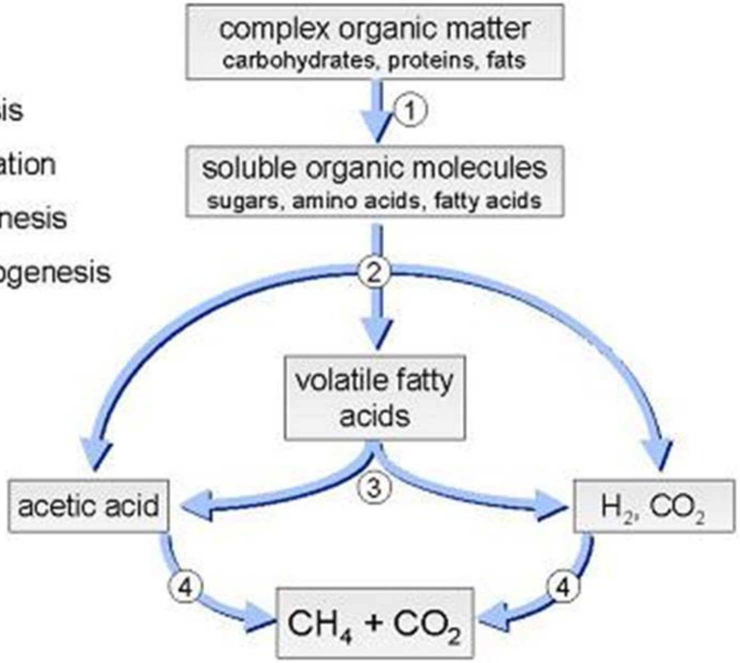


14 563 biogas plants in Europe with total installed capacity of 7 857 MWel (2013)

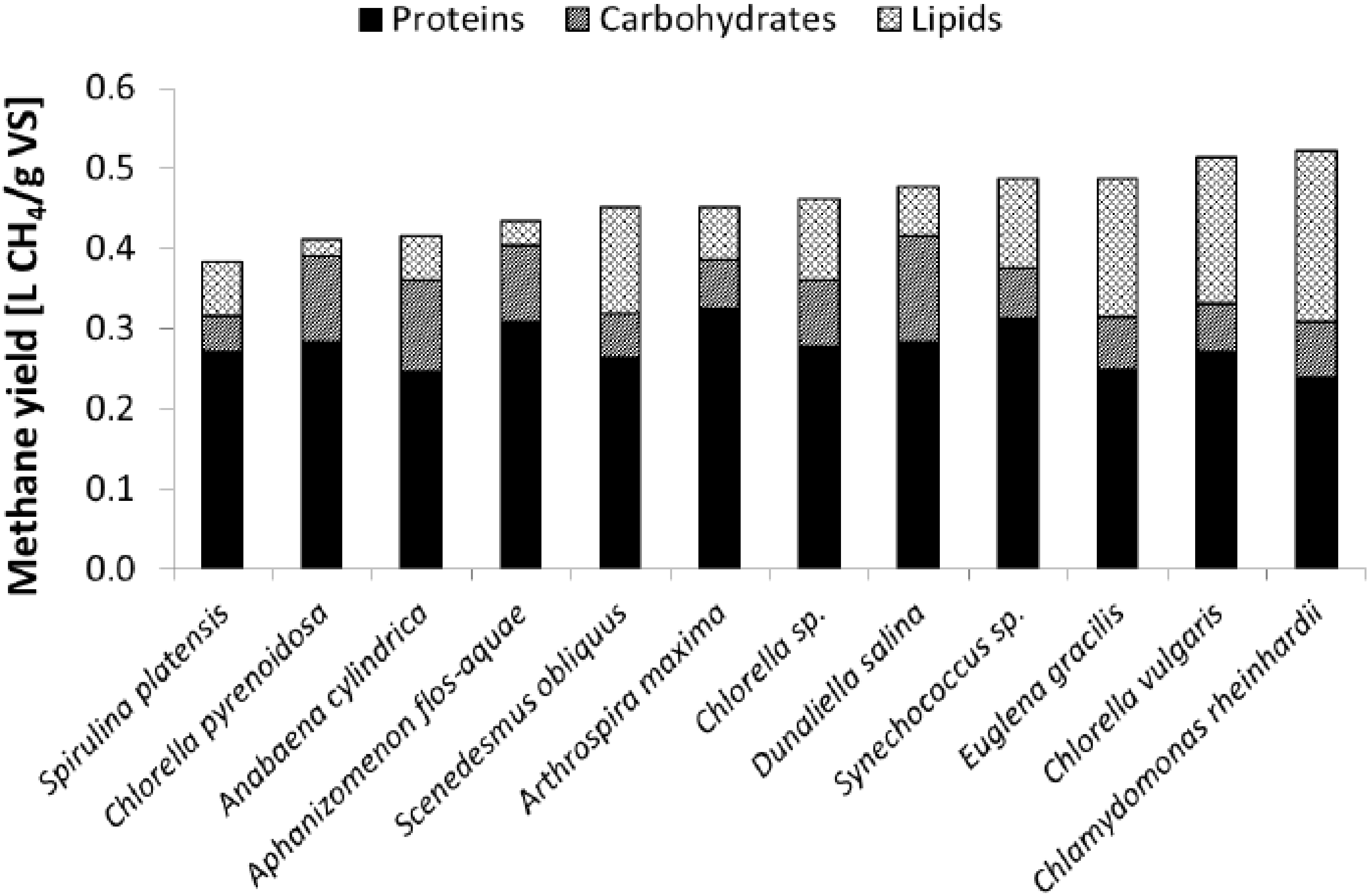
(b)



① hydrolysis
② fermentation
③ acetogenesis
④ methanogenesis

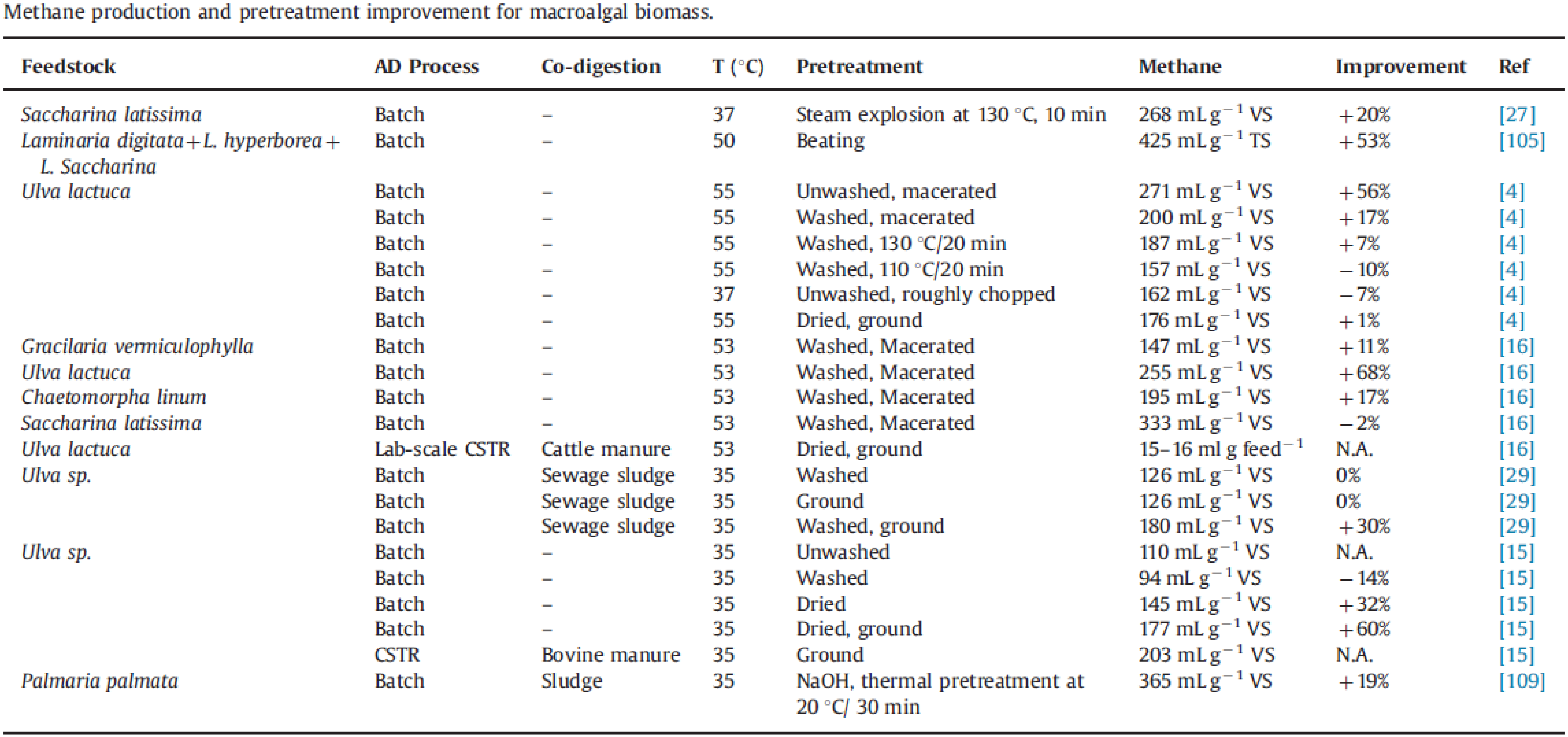


Microalgae	**Cell Wall**	**Cell Wall composition (%)**			**References**
	(% w/w)	Carbohydrates	Protein	n.d.*	
Chlorella vulgaris (F)	20.0	30.00	2.46	67.54	(Abo-Shady *et al.* 1993)
Chlorella vulgaris (S)	26.0	35.00	1.73	63.27	(Abo-Shady *et al.* 1993)
Kirchneriella lunaris	23.0	75.00	3.96	21.04	(Abo-Shady *et al.* 1993)
Klebsormidium flaccidum	36.7	38.00	22.60	39.40	(Domozych *et al.* 1980)
Ulothrix belkae	25.0	39.00	24.00	37.00	(Domozych *et al.* 1980)
Pleurastrum terrestre	41.0	31.50	37.30	31.20	(Domozych *et al.* 1980)
Pseudendoclonium basiliense	12.8	30.00	20.00	50.00	(Domozych *et al.* 1980)
Chlorella Saccharophila	-	54.00	1.70	44,30	(Blumreisinger *et al.* 1983)
Chlorella fusca	-	68.00	11.00	20.00	(Blumreisinger *et al.* 1983)
Chlorella fusca	-	80.00	7.00	13.00	(Loos & Meindl 1982)
Monoraphidium braunii	-	47.00	16.00	37.00	(Blumreisinger *et al.* 1983)
Ankistrodesmus densus	-	32.00	14.00	54.00	(Blumreisinger *et al.* 1983)
Scenedesmus obliquos	-	39.00	15.00	46.00	(Blumreisinger *et al.* 1983)


■ Proteins
▩ Carbohydrates
▨ Lipids
Methane yield [L CH_4/g VS]
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Spirulina platensis
Chlorella pyrenoidosa
Anabaena cylindrica
Aphanizomenon flos-aquae
Scenedesmus obliquus
Arthrospira maxima
Chlorella sp.
Dunaliella salina
Synechococcus sp.
Euglena gracilis
Chlorella vulgaris
Chlamydomonas rheinhardii

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	Ref
Saccharina latissima	Batch	–	37	Steam explosion at 130 °C, 10 min	268 mL g^{-1} VS	+20%	[27]
Laminaria digitata + L. hyperborea + L. Saccharina	Batch	–	50	Beating	425 mL g^{-1} TS	+53%	[105]
Ulva lactuca	Batch	–	55	Unwashed, macerated	271 mL g^{-1} VS	+56%	[4]
	Batch	–	55	Washed, macerated	200 mL g^{-1} VS	+17%	[4]
	Batch	–	55	Washed, 130 °C/20 min	187 mL g^{-1} VS	+7%	[4]
	Batch	–	55	Washed, 110 °C/20 min	157 mL g^{-1} VS	−10%	[4]
	Batch	–	37	Unwashed, roughly chopped	162 mL g^{-1} VS	−7%	[4]
	Batch	–	55	Dried, ground	176 mL g^{-1} VS	+1%	[4]
Gracilaria vermiculophylla	Batch	–	53	Washed, Macerated	147 mL g^{-1} VS	+11%	[16]
Ulva lactuca	Batch	–	53	Washed, Macerated	255 mL g^{-1} VS	+68%	[16]
Chaetomorpha linum	Batch	–	53	Washed, Macerated	195 mL g^{-1} VS	+17%	[16]
Saccharina latissima	Batch	–	53	Washed, Macerated	333 mL g^{-1} VS	−2%	[16]
Ulva lactuca	Lab-scale CSTR	Cattle manure	53	Dried, ground	15–16 ml g feed^{-1}	N.A.	[16]
Ulva sp.	Batch	Sewage sludge	35	Washed	126 mL g^{-1} VS	0%	[29]
	Batch	Sewage sludge	35	Ground	126 mL g^{-1} VS	0%	[29]
	Batch	Sewage sludge	35	Washed, ground	180 mL g^{-1} VS	+30%	[29]
Ulva sp.	Batch	–	35	Unwashed	110 mL g^{-1} VS	N.A.	[15]
	Batch	–	35	Washed	94 mL g^{-1} VS	−14%	[15]
	Batch	–	35	Dried	145 mL g^{-1} VS	+32%	[15]
	Batch	–	35	Dried, ground	177 mL g^{-1} VS	+60%	[15]
	CSTR	Bovine manure	35	Ground	203 mL g^{-1} VS	N.A.	[15]
Palmaria palmata	Batch	Sludge	35	NaOH, thermal pretreatment at 20 °C/ 30 min	365 mL g^{-1} VS	+19%	[109]

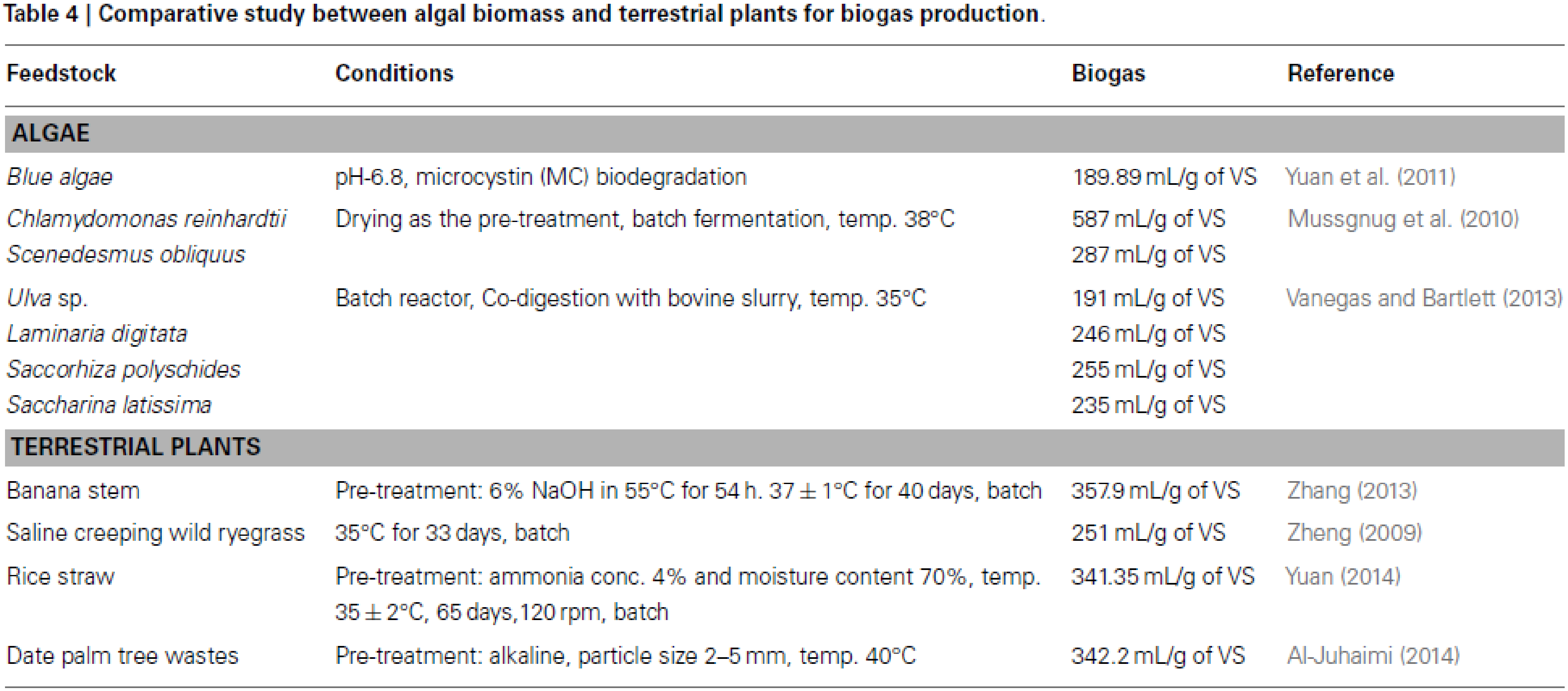


Table 4 | Comparative study between algal biomass and terrestrial plants for biogas production.

Feedstock	Conditions	Biogas	Reference
ALGAE			
Blue algae	pH-6.8, microcystin (MC) biodegradation	189.89 mL/g of VS	Yuan et al. (2011)
Chlamydomonas reinhardtii	Drying as the pre-treatment, batch fermentation, temp. 38°C	587 mL/g of VS	Mussgnug et al. (2010)
Scenedesmus obliquus		287 mL/g of VS	
Ulva sp.	Batch reactor, Co-digestion with bovine slurry, temp. 35°C	191 mL/g of VS	Vanegas and Bartlett (2013)
Laminaria digitata		246 mL/g of VS	
Saccorhiza polyschides		255 mL/g of VS	
Saccharina latissima		235 mL/g of VS	
TERRESTRIAL PLANTS			
Banana stem	Pre-treatment: 6% NaOH in 55°C for 54 h. 37 ± 1°C for 40 days, batch	357.9 mL/g of VS	Zhang (2013)
Saline creeping wild ryegrass	35°C for 33 days, batch	251 mL/g of VS	Zheng (2009)
Rice straw	Pre-treatment: ammonia conc. 4% and moisture content 70%, temp. 35 ± 2°C, 65 days,120 rpm, batch	341.35 mL/g of VS	Yuan (2014)
Date palm tree wastes	Pre-treatment: alkaline, particle size 2–5 mm, temp. 40°C	342.2 mL/g of VS	Al-Juhaimi (2014)