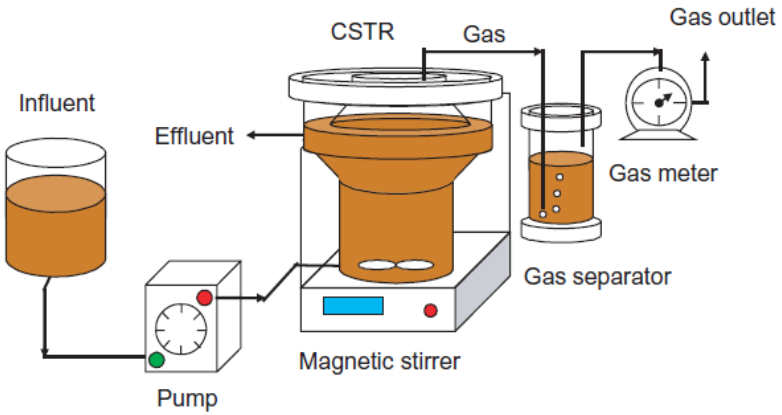
Biohydrogen

In the framework of the postgraduate course “Renewable Energy”



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Introduction

Biohydrogen (BIO-H_2) has significant feasibility since biological processes are much less energy intensive compared with electrolysis and thermo-chemical processes.

It is widely recognized that considerable amounts of hydrogen (H_2) can be produced from renewable resources without using energy from fossil fuels.

Biological processes and mainly bacterial fermentation are considered as the most environmentally friendly alternatives for satisfying future hydrogen demand.

Glucose to BIO-H_2: $C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$

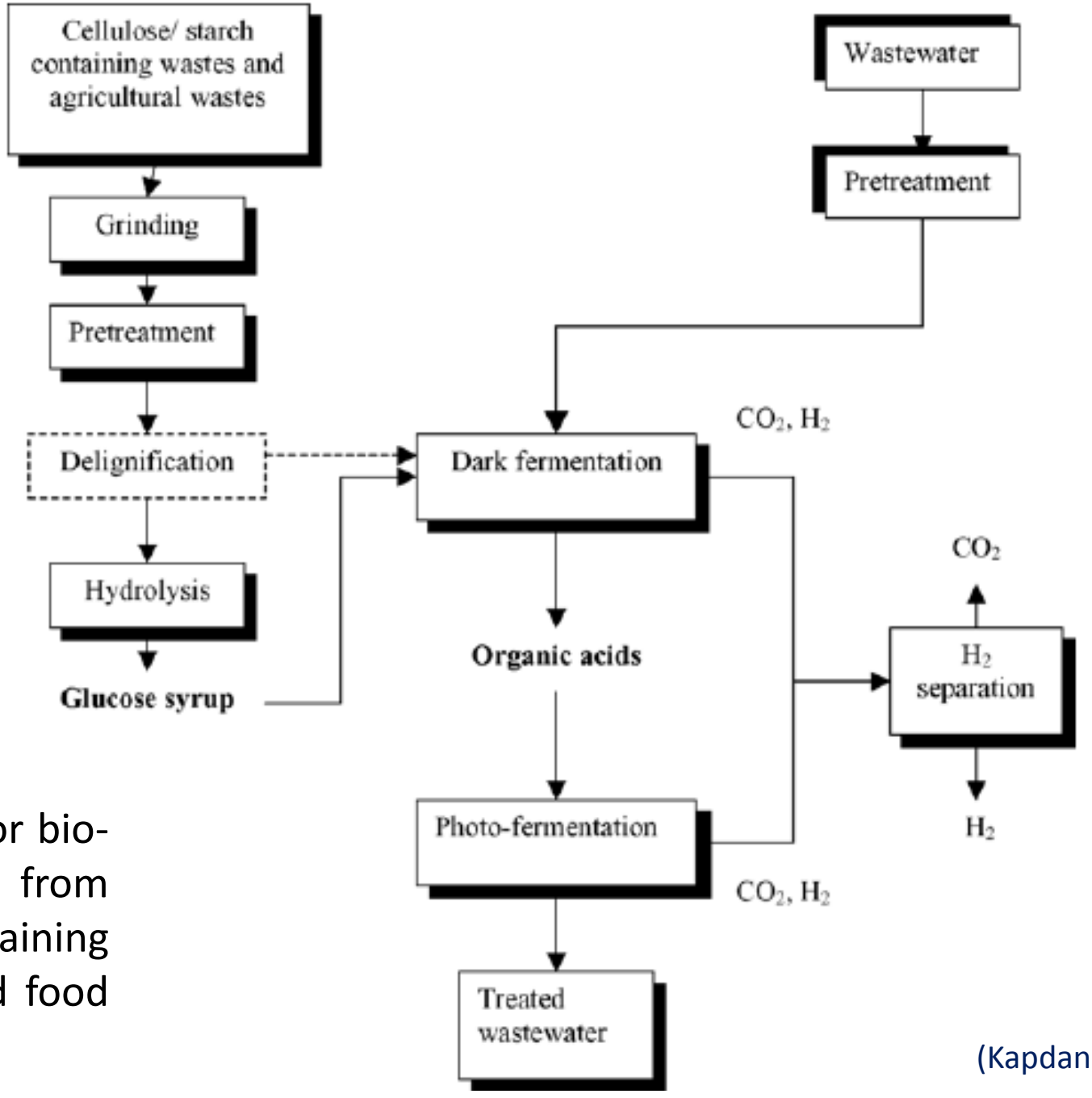
(Ginkel et al., 2005; Azbar and Levin, 2012; Urbaniec and Bakker, 2015)

Biohydrogen production from agricultural and agro-industrial solid waste and wastewater is considered as highly advantageous as materials of this kind are abundant, cheap and biodegradable.

Bacterial fermentation of waste for H_2 production is a family of bioprocesses that can be roughly divided into three groups:

1. Dark fermentation
2. Photofermentation (the availability of light is necessary)
3. Two-stage bioprocesses combining dark fermentation with photofermentation

(Claassen and de Vrije, 2006; Hallenbeck, 2009; Urbaniec and Bakker, 2015)



A schematic diagram for bio-hydrogen production from cellulose/starch containing agricultural wastes and food industry wastewaters

(Kapdan and Kargi, 2006)

Comparison of the Different Biohydrogen Processes.

Process	Production rates (mls H_2/l/h)	Yields	Advantages	Disadvantages	Future prospects
Biophotolysis	2.5–13[a]	≤0.1%[b]	Abundant, inexhaustible substrate (water)	Evolves oxygen, destroying the hydrogen evolving catalyst (hydrogenase)	Near term incremental improvements possible through creation of antenna mutants
			Totally carbon independent pathway	Low photosynthetic conversion efficiencies	
			Simple products, hydrogen and oxygen	Potentially explosive gas mixtures formed	Immobilization might bring some improvement
				Large surface areas required	Creation of an oxygen resistant hydrogenase would be a breakthrough
				Need for inexpensive photobioreactors	Materials science breakthrough
Photofermentation	12–83[c]	≤1%[d], 80%[e]	Uses readily available waste streams	Low volumetric rates of production	Strain improvement through metabolic engineering
			Nearly complete substrate conversion	Low efficiency hydrogen production by nitrogenase	replacement of N_2ase with H_2ase
			Can extract additional hydrogen from dark fermentation effluents	Low photosynthetic conversion efficiencies	Near term improvement possible through creation of antenna mutants
				Need for inexpensive photobioreactors	Materials science breakthrough
				Large surface areas required	
Dark fermentation	10–15 × 10^3	33%[f]	Can use a variety of waste streams	large amount of byproducts	metabolic engineering could achieve breakthrough in metabolic limitations
			Simple reactor technology, non-sterile conditions acceptable	low COD removal	Two stage systems can extract additional energy, decrease COD
			high rates achieved with immobilized mixed cultures	reactor to reactor variation	

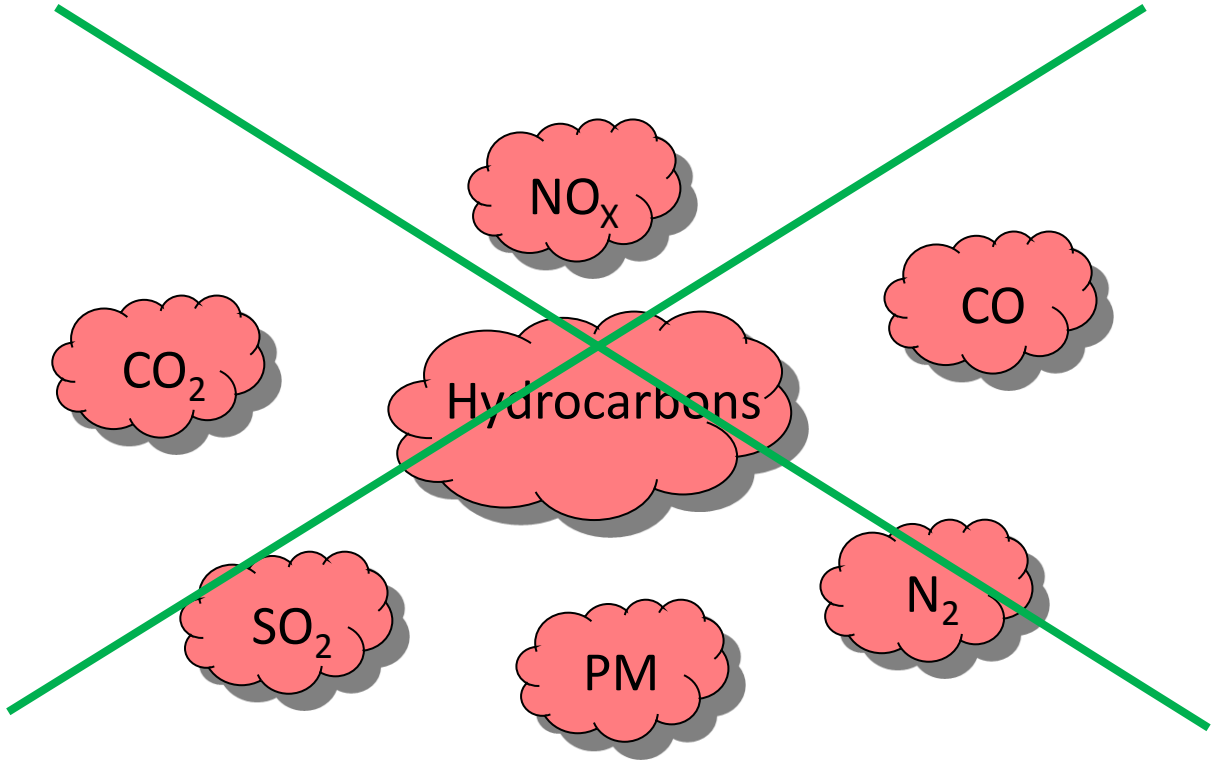
[a] Sulfur-deprived green algae (Laurinavichene et al., 2006) and cyanobacteria (Tsygankov et al., 1998).
[b] Conversion of total incident light energy to hydrogen at full solar power.
[c] (Eroglu et al., 1999; Kim et al., 2006).
[d] At low (relative to full solar) light intensities (Abo-Hashesh et al., 2011b).
[e] Conversion of substrate (organic acid) to hydrogen, does not account for light energy used.
[f] 4 mol of hydrogen per mole of glucose equivalent, theoretically 12 mol are available. There appears to be an inverse relationship between hydrogen production rates and yields, so the high rate reactors giving the quoted high volumetric rates (Lee et al., 2006; Wu et al., 2007) have yields significantly lower than this.

(Hallenbeck et al., 2012)

Environmental Impact

The combustion of hydrogen with oxygen produces water as its only product:

$$2H_2 + O_2 \rightarrow 2H_2O$$



3 Videos

Hydrogen-Producing Bacteria

Hydrogen production can be achieved either through mixed acidogenic microbial cultures, derived from natural environments such as soil, wastewater sludge, and compost, or through pure cultures of selected hydrogen producing bacteria.

Such bacteria can be mesophilic (25–40^{o}C), thermophilic (40–65^{o}C), extreme thermophilic (65–80^{o}C), or even hyperthermophilic (80^{o}C).

The type of used culture can be mixed, pure or co-culture.

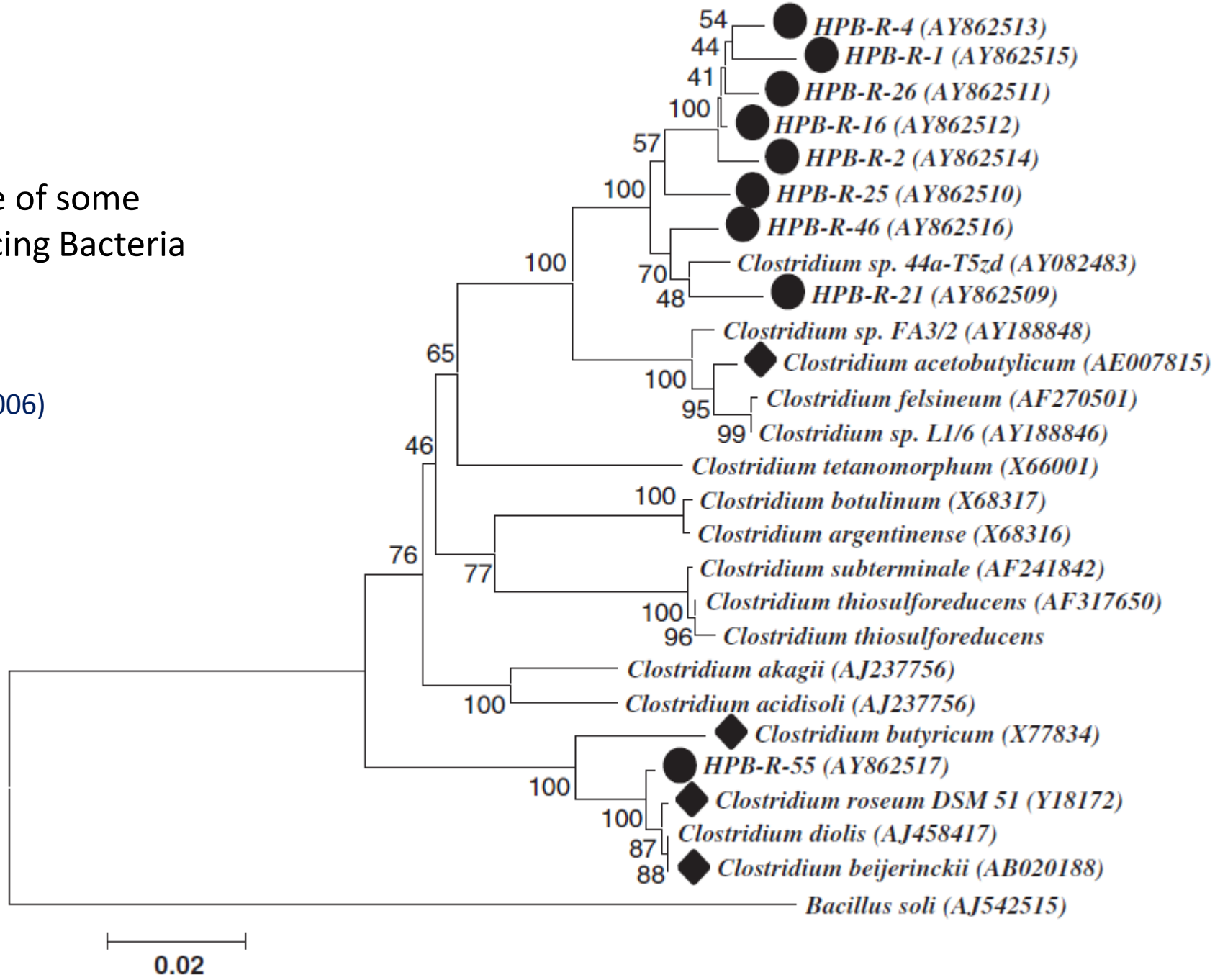
A number of hydrogen-producing bacteria were reported, such as:

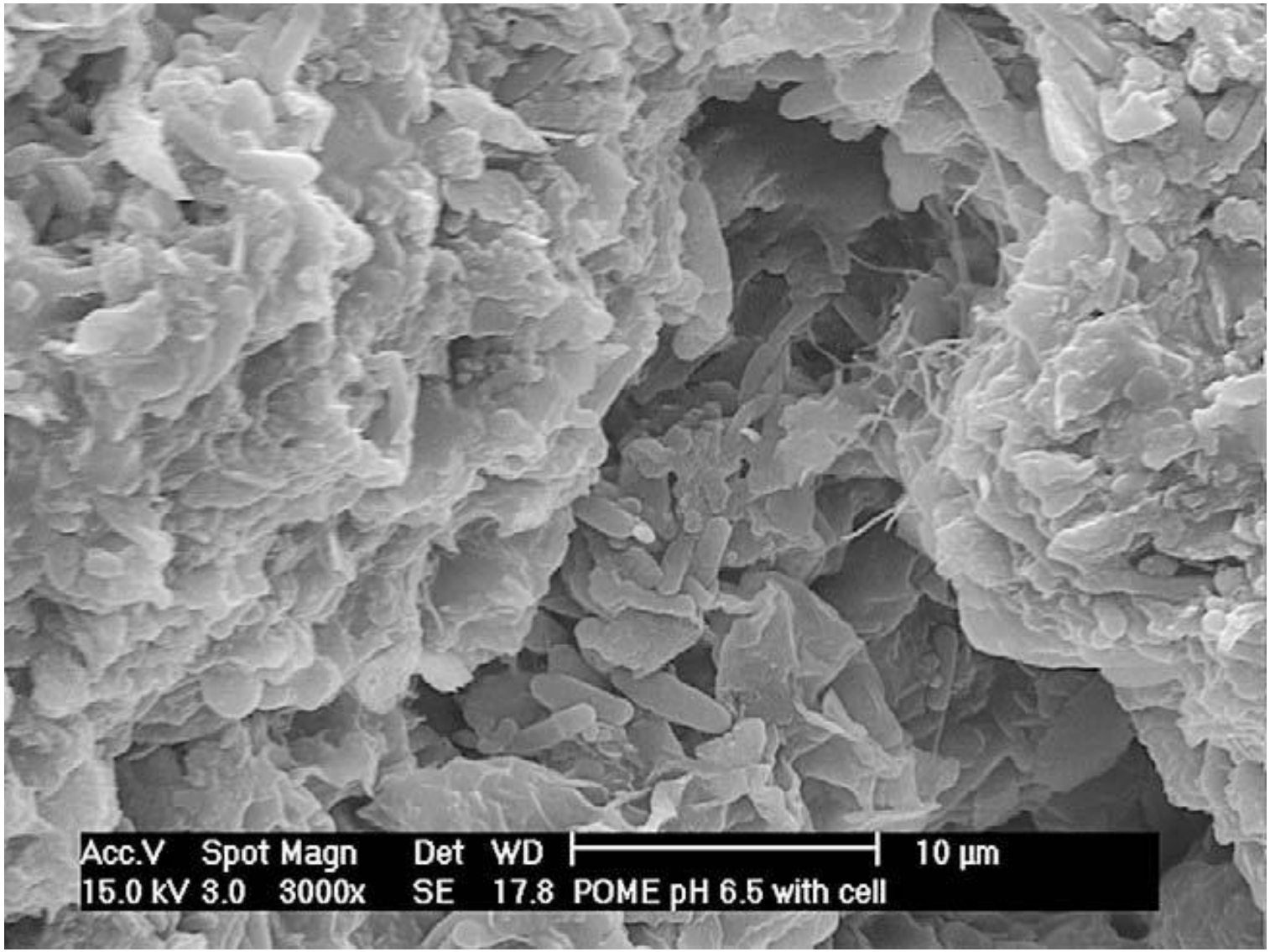
Clostridia* (e.g. *Clostridium butyricum*) and *Enterobacteria

(Fang et al., 2006)

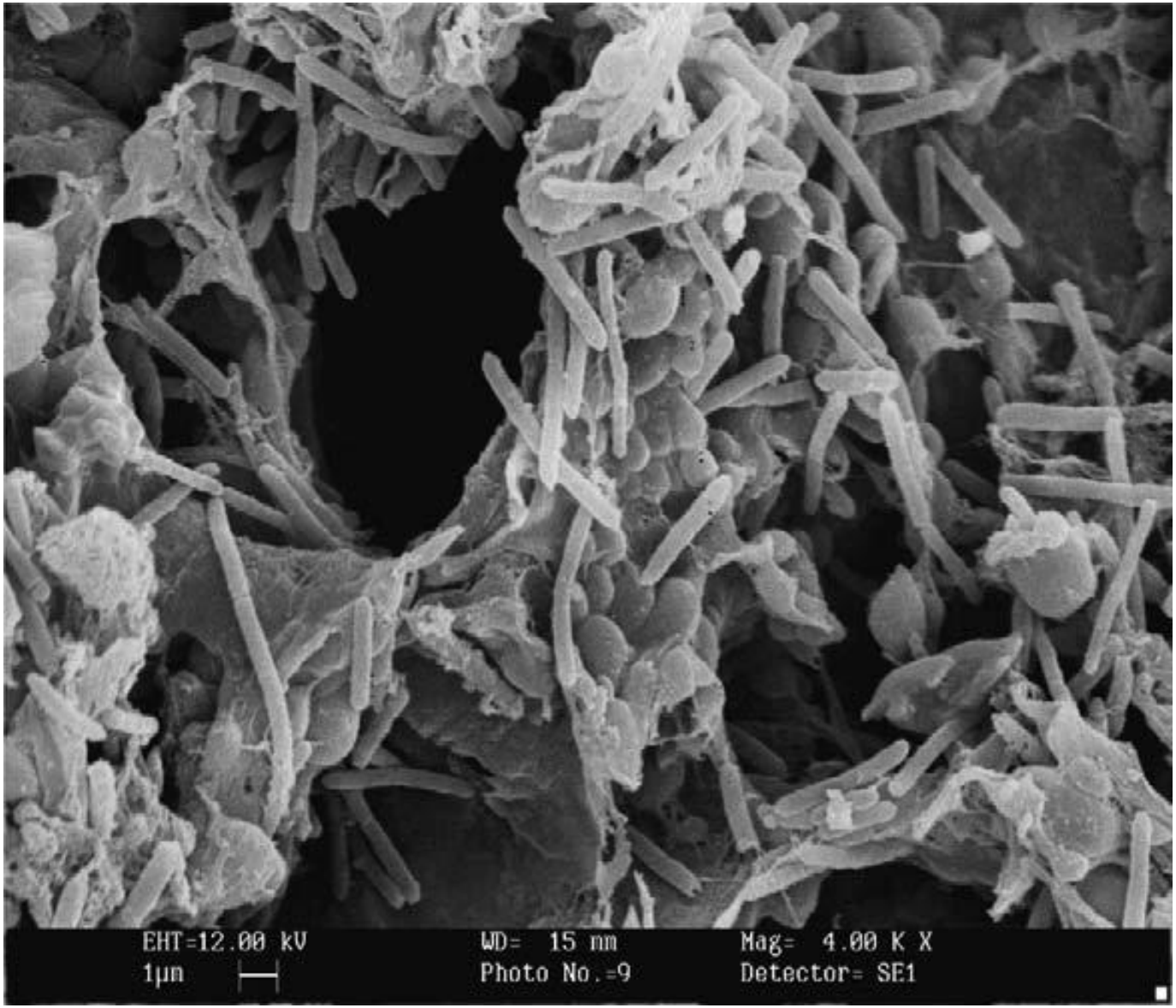
Phylogenetic Tree of some Hydrogen-Producing Bacteria

(Fang et al., 2006)



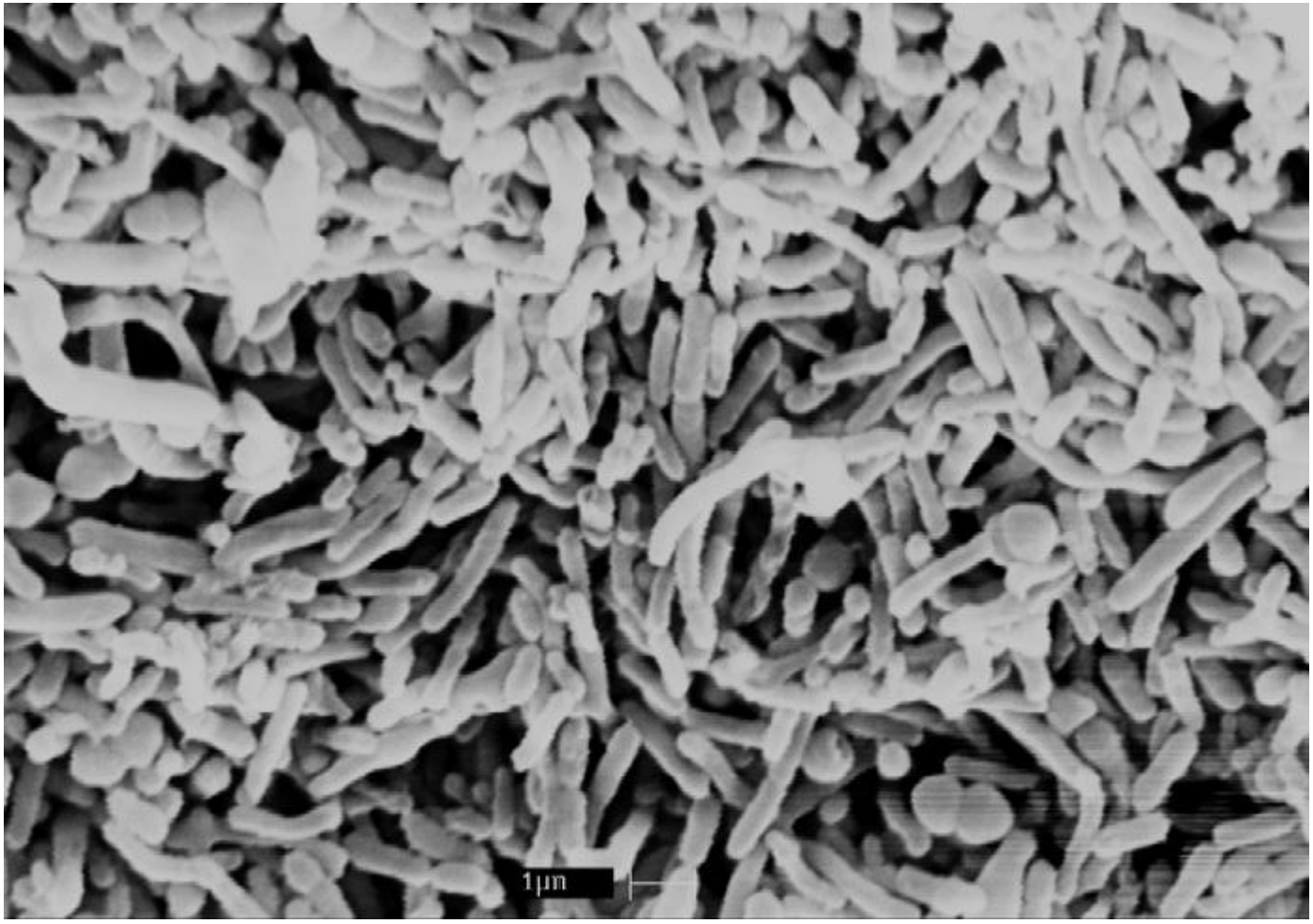


Scanning electron microscopy (SEM) photo of *Clostridium butyricum* EB6, growing in Palm oil mill effluent (POME) at pH 6.5 and 37 °C (Chong et al., 2009a)



SEM image of the hydrogen-producing bacteria at pH 4.5

(Fang et al., 2006)

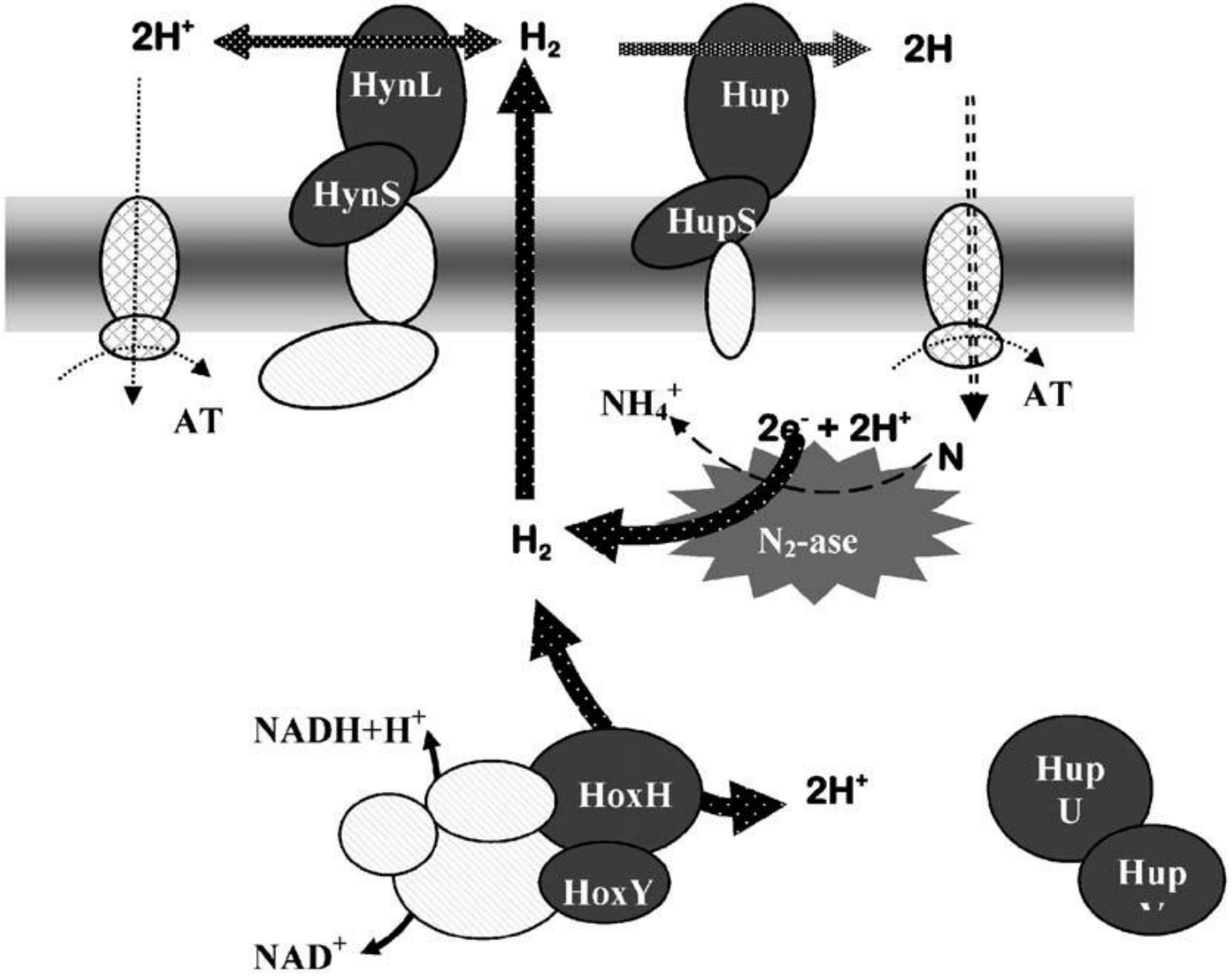


Scanning electron microscopy of the attached bacteria in the GAC-AFBR (magnification: 5000×)

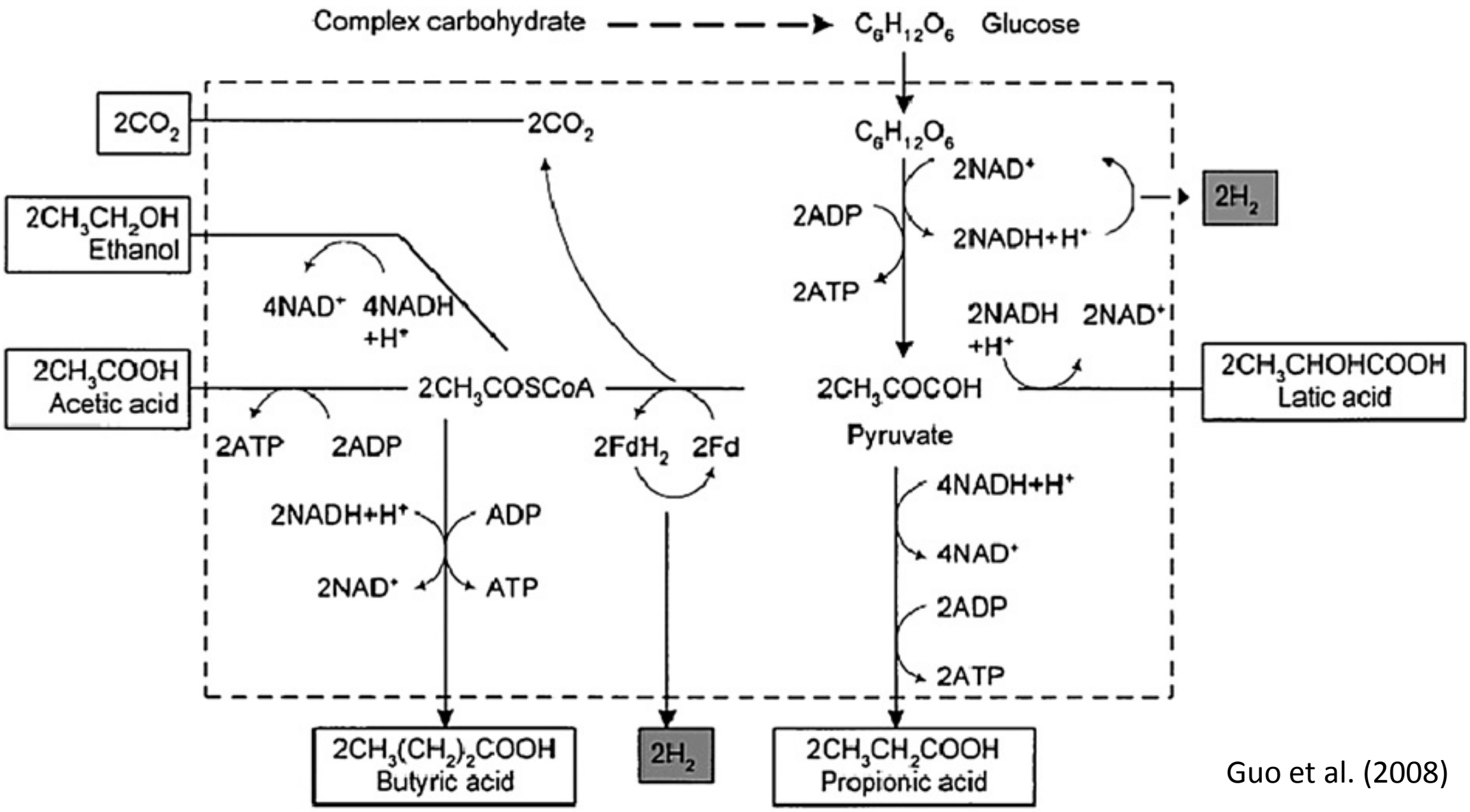
GAC: granular activated carbon
AFBR: anaerobic fluidized bed reactor

Zhang et al. (2007)

Hydrogenases in *Thiocapsa roseopersicina.* Membrane-associated HynSL and HupSL enzymes are in the same orientation in the photosynthetic membrane and in vivo are linked to H_2 uptake. Nitrogenase, the pentameric HoxYH hydrogenase and the putative H_2 sensor HupUV are located in the cytoplasm. The core hydrogenase dimer is indicated by black color, other structural proteins are light.

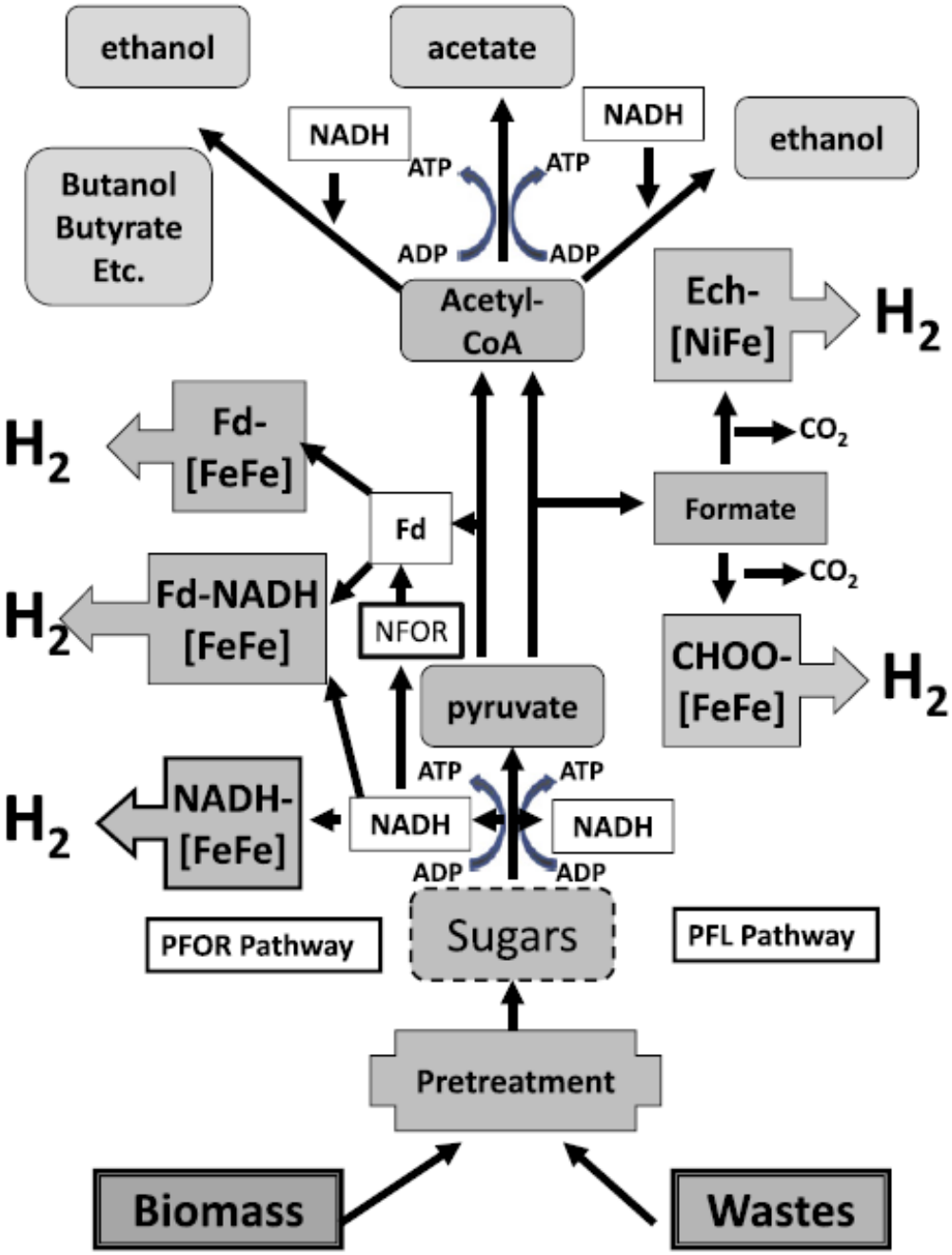


Kovács et al. (2006)



Guo et al. (2008)

Metabolic pathways possible for the carbohydrate fermentation



Dark fermentative biohydrogen production

Hallenbeck et al. (2012)

Hydrogen production by various bacteria

Organism	Substrate	Mode of operation	pH/ temperature	Volumetric H_2 production (L/L med)	Yield (mol H_2/mol substrate)
Anaerobic bacteria					
C. butyricum EB6	POME	Batch	5.5/37 °C	3.2	–
C. butyricum ATCC19398	Glucose (3 g/L)	Batch	7.2/35 °C	0.94	1.8
C. acetobutyricum M121	Glucose (3 g/L)	Batch	7.2/35 °C	0.88	2.29
C. tyrobutyricum FYa102	Glucose (3 g/L)	Batch	7.2/35 °C	0.63	1.47
C. beijerinckii L9	Glucose (3 g/L)	Batch	7.2/35 °C	1.19	2.81
C. thermolacticum	Lactose (10 g/L)	Continuous	7/58 °C	–	3.0
C. thermocellum 27405	Delignified wood fiber	Batch	6.3/60 °C	–	1.6
C. tyrobutyricum	Glucose (5 g/L)	Immobilized	HRT 2 h	7.2 L H_2/L d	223 ml/g hexose
Facultative anaerobic bacteria					
E. aerogenes ATCC29007	Glucose (118.06 mM)	Batch	6.13/38 °C		425.8 ml H_2/g DCW h
Klebsiella oxytoca HP1	Glucose (10 g/L)	Batch	7.0/65 °C	87.5 ml H_2/L h	1.0
Citrobacter sp. Y19	Glucose (10 g/L)	Batch	7.0/36 °C	32.2 mmol H_2/g cell h	2.49
E. asburiae SNU-1	Glucose (25 g/L)	Batch	7.0/30 °C	398 ml H_2/L h	–
Thermophilic bacteria					
T. thermosaccharolyticum PSU-2	Sucrose (10 g/L)	Batch	6.2560 °C	12.12 mmol H_2/L d	2.53
T. saccharolyticum JW/SL-YS485	Xylose (4 g/L)	Batch	6.2/55 °C	–	0.88
T. maritima DSM3109	Glucose (7.5 g/L)	Batch	6.5/65 °C	0.275	1.67
T. neapolitana DSM4359	Glucose (10 g/L)	Batch	7.0/65 °C	0.29	1.84
Caldicellulosiruptor saccharolyticus DSM8903	Sucrose (10 g/L)	Batch	7/70 °C	8.4 mmol H_2/L	5.9

(Chong et al., 2009b)

Microorganism	Substrate	H_2 yield (mol H_2/mol substrate)
C. butyricum	Glucose	1.40–2.30
C. beijerinckii	Glucose and starch	1.20–20
C. acetobutylicum	Glucose	1.97
C. paraputrificum M-21	Glucose	1.40
C. beijerinckii AM21B	Glucose	1.80–2.00
C. cellobioparm	Glucose	2.73
C. pasteurianum	Glucose	1.50
Clostridium sp.	Glucose	0.85
C. beijerinckii	Glucose	2.00
Clostridium. sp. strain No. 2	Glucose	2.36
C. acetolyticum	Glucose	2.00
C. pasterium (dominant)	Sucrose	4.80
Clostridium sp.	Microcrystalline	2.18
Clostridium. sp. strain No. 2	Xylose	1.80–2.10
Hydrogen-producing sludge (dominated by *Clostridium* sp.)	Xylose	1.30
C. uliginosum sp. nov.	Xylose	2.59
C. butyricum CGS5	Xylose	0.68–0.73
C. butyricum	SCB hemicellulose hydrolysate	1.73

Comparison of hydrogen yield in various types of sugar by different *Clostridium* species

Pattra et al. (2008)

Bioenvironmental and Operational Conditions

The working temperature ranges from 30 to 70 °C.

Another important factor is the pH, where the working pH ranges from 4.5 to 7.5

The Hydraulic Retention Time (HRT) ranges from 24 h to 5 days (steady-state must be reached first, which takes 20 days approx.).

Chong et al. (2009)

Cumulative Hydrogen Production

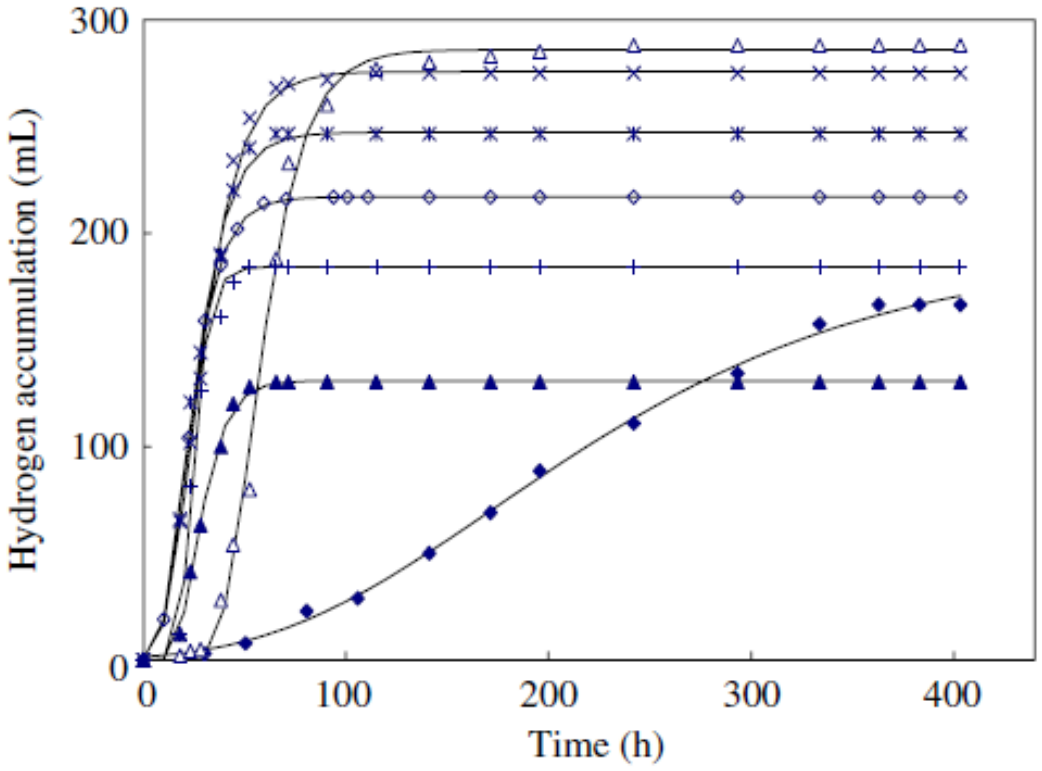


Fig. 1. Cumulative hydrogen production at pH 4.0–7.0: ♦ pH = 4.0; △ pH = 4.5; × pH = 5.0; ∗ pH = 5.5; ◇ pH = 6.0; + pH = 6.5; ▲ pH = 7.0.

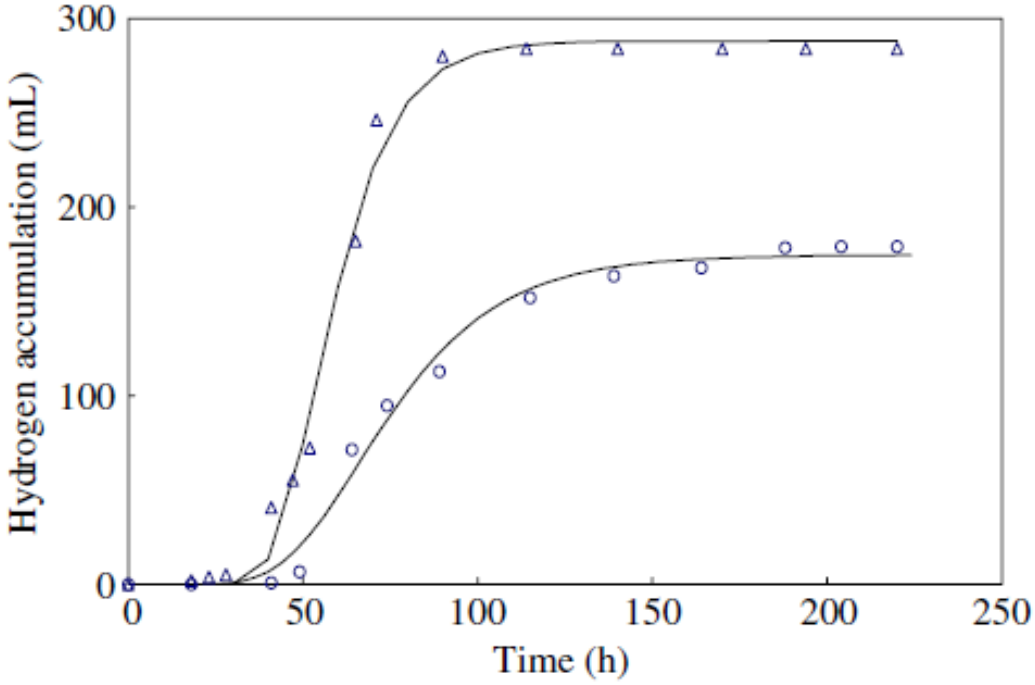
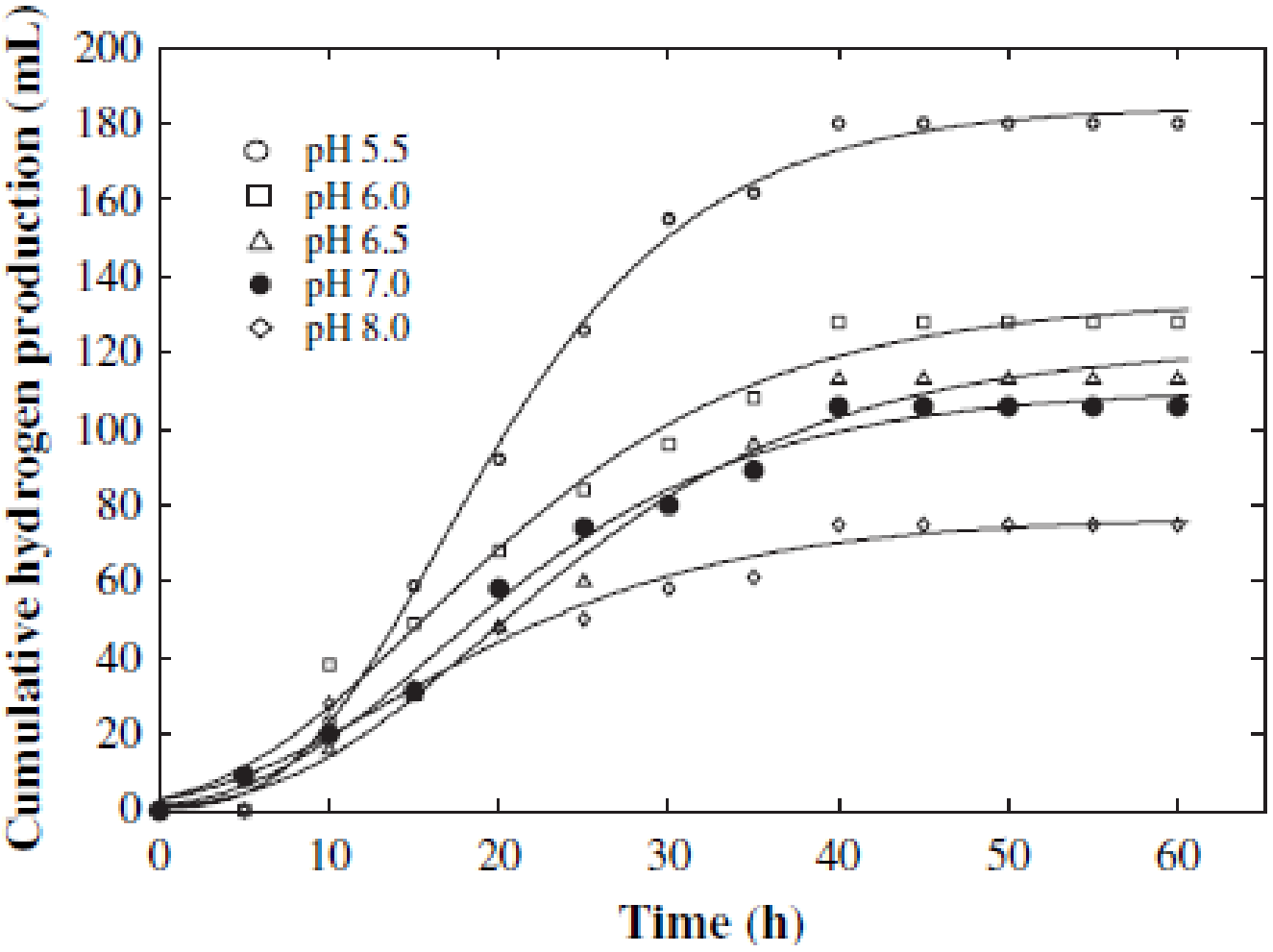


Fig. 2. Cumulative hydrogen production at two temperatures (△ 37 °C; ◯ 55 °C).

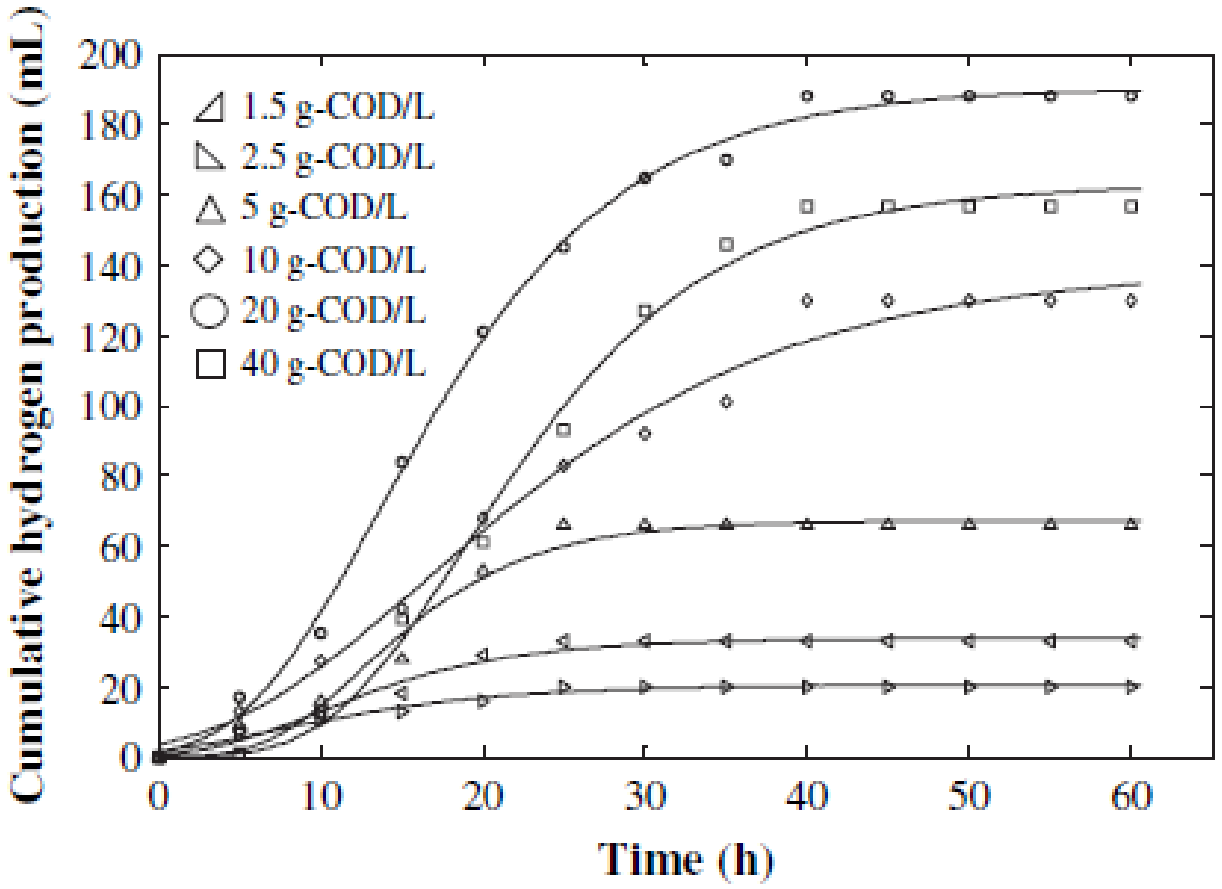
Cumulative hydrogen production

Fang et al. (2006)



– Cumulative hydrogen production profiles for *C. butyricum* at different initial pHs. (Temperature = 37 °C, initial total sugar concentration = 20 g-COD/L; symbols: observed data, curves: prediction with Gompertz equation.)

Pattra et al. (2008)



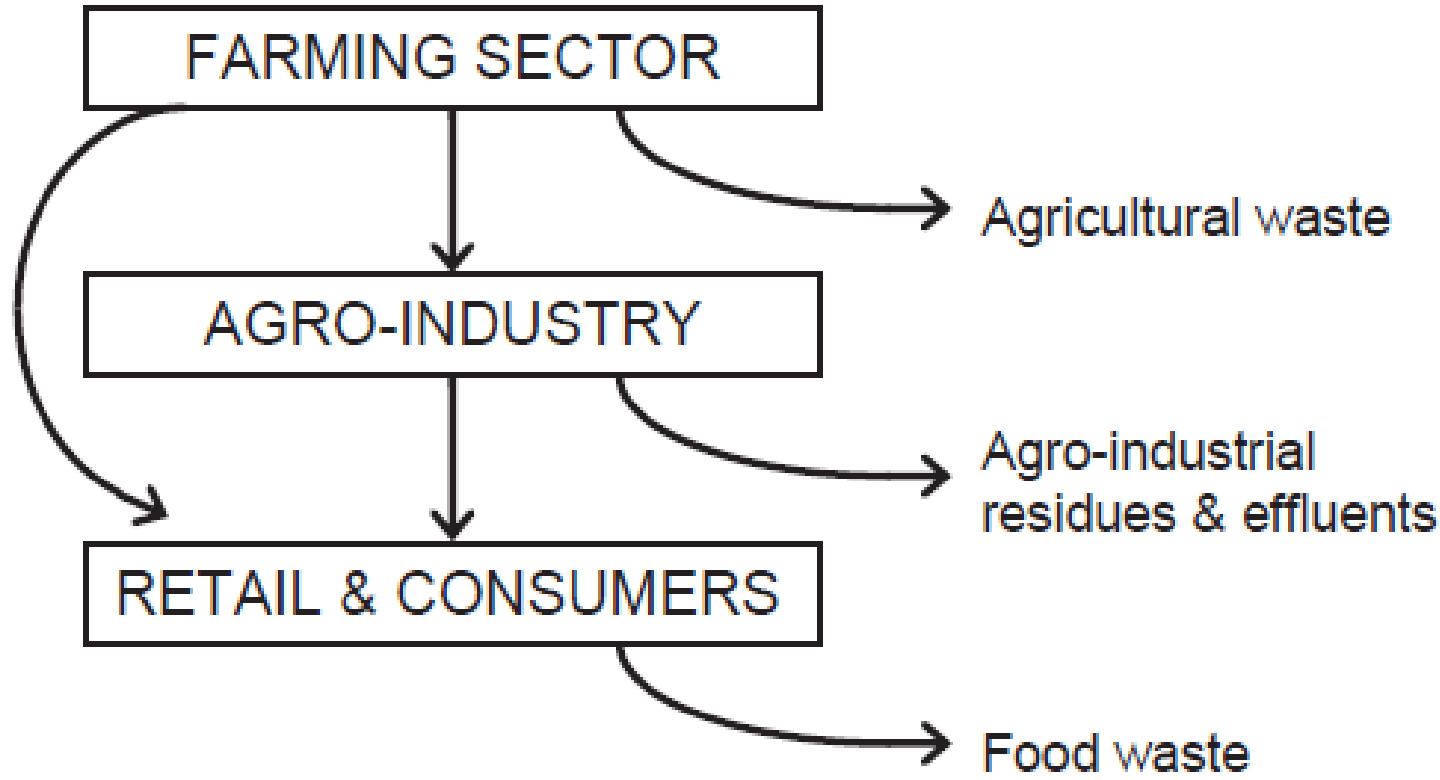
– Cumulative hydrogen production profiles for C. *butyricum* at different initial total sugar concentrations. (Temperature = 37 °C, initial pH = 5.5; symbols: observed data, curves: prediction with Gompertz equation.)

Pattra et al. (2008)

Feedstocks

The following substrates/biowastes are usually used as feedstocks for biohydrogen production:

1. Municipal/Domestic wastewater (sewage)
2. Activated sludge
3. Food processing wastewaters
4. Food waste
5. Cheese whey
6. Molasses
7. Manure
8. Corn stover
9. Sugarcane bagasse
10. Wheat straw
11. Rice slurry
12. Algal biomass
13. Palm oil mill effluent
14. Glycerol
15. Further substrates …etc.



– **Scheme of material flows related to biomass use and generation of biomass waste.**

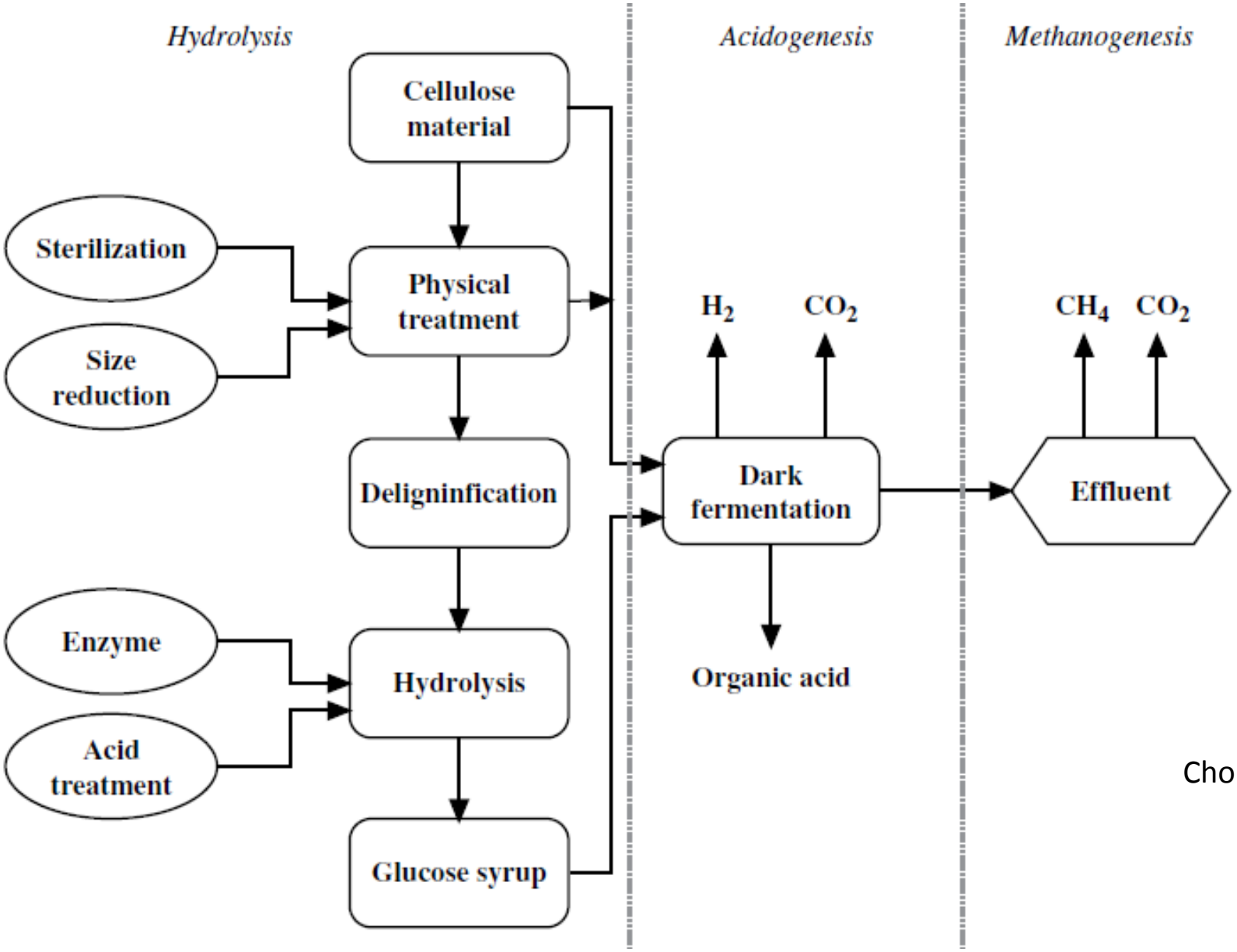
Urbaniec and Bakker (2015)

Selected large streams of by-products and waste from biomass processing industries

Sector	By-product/waste
Wheat milling	Wheat millfeed[a]
Potable & fuel ethanol from grain	Wet distiller's grain
Vegetable oil and biodiesel	Olive cake
	Rapeseed cake
	Sunflower cake
Beet sugar	Beet pulp
Wine	Grape pomace
Juice	Fruit pomace
	Vegetable cake
Beer	Brewer's spent grain
Fruit & vegetable preserves	Fruit and vegetable peelings & discards

[a] Includes wheat bran.

Urbaniec and Bakker (2015)



Chong et al. (2009b)

A schematic diagram for biohydrogen production from cellulose

Characteristics of the food waste (FW), primary sludge (PS) and waste activated sludge (WAS)

	FW	PS	WAS
TS (g/L)	11.4±0.75	30.6±5.6	8.8±1.5
VS (g/L)	10.5±0.78	19.8±3.9	6.5±1.3
Carbohydrates (mg/L)	4480±106	124±44	31±10
Soluble COD (mg/L)	9230±300	4480±2160	240±110
Total COD (mg/L)	19250±1360	35900±12600	10600±2890
Acetic acid (mg/L)	678±205	1140±516	n.d.
Propionic acid (mg/L)	302±156	581±284	n.d.
Butyric acid (mg/L)	65±20	289±183	n.d.
TKN (mg/L)	505±45	1233±350	709±190
PO_4–P (mg/L)	327±30	216±130	n.d.
Ba (mg/L)	0.03	2.41	0.45
Ca (mg/L)	38	418	108
Cu (mg/L)	0.17	3.22	2.22
Fe (mg/L)	1.35	735	558
K (mg/L)	160	70.9	60.3
Mg (mg/L)	12.5	77.9	29.3
Mn (mg/L)	0.12	2.05	1.66
Mo (mg/L)	0.01	0.06	0.14
Na (mg/L)	143	148	109
Zn (mg/L)	0.36	3.43	1.28
Total acidity as $CaCO_3$	340	1972	2200
Total alkalinity as $CaCO_3$	40	1960	840
pH	4.7±0.1	5.9±0.1	6.8±0.1

n.d.—not detectable.
The detect limits for acetic, propionic, butyric acids and PO_4–P were 0.28, 0.64, 0.55 and 0.08 mg/L, respectively.

Zhu et al. (2008)

Biohydrogen production by *Clostridium butyricum* EB6, using raw Palm Oil Mill Effluent (POME) as sole substrate

pH	Temp. (°C)	$FeSO_4 \cdot 6H_2O$ (g/L)	H_2 content (%)	CO_2 content (%)	Total hydrogen production (mL)		Volumetric H_2 production rate (mL/h/L)		Hydrogen yield (mL H_2/g COD)
					Overall[a]	Maximum[b]	Overall[c]	Maximum[b]	
5	37 °C	None	0	0	0	0	0	0	0
5.5			62	38	3345	3195	278.8	1034.7	31.95
6.5			66	34	3062	3022	251.8	790.8	30.2
7.5			70	30	1929	1959	160.8	735.1	19.6
8.5			68	32	587	618	41.3	201.1	6.18
Uncontrolled			65	35	2249	2253	112.5	296.4	22.53
5.5	30 °C	None	64	36	2795	2858	116.5	314.8	26.38
	37 °C		62	38	3345	3195	278.8	1034.7	31.95
	55 °C		0	0	0	0	0	0	0
5.5	37 °C	None	62	38	3345	3195	278.8	1034.7	31.95
		0.25	56	44	2648	2638	189.1	498.9	22.53

a Overall hydrogen production at *i* time.
b Maximum hydrogen production at *i* time and maximum volumetric production rate calculated based on the modified Gompertz equation.
c Overall volumetric hydrogen production rate calculated by dividing the maximum cumulative hydrogen production (V_i) over by the time required to reach a maximum.

Chong et al. (2009a)

Organism	Substrate	Mode of operation	Supplement[a]	pH/ temperature	Yield
Food waste					
Mixed culture	Apple processing wastewater (9 gCOD/L)	Batch	With	6.0/–	0.9 L H_2/L medium (0.1 L H_2/g COD)
Mixed culture	Potato processing wastewater (21 gCOD/L)	Batch	With	6.0/–	2.1 L H_2/L medium (0.1 L H_2/g COD)
Mixed culture	Food waste	Continuous	Without	6.5/35 °C	0.39 L H_2/g COD
Mixed culture	Food waste	batch	Without	5.6/50 °C	57 ml H_2/g VS
Starch-based wastewater					
Mixed culture	Molasses	Continuous	Without	7.0/35 °C	5.57 m^3 H_2/m^3 reactor/d
Mixed culture	Rice slurry (5 gCHO/L)	Batch	With	4.5/37 °C	346 ml H_2/g carbohydrate
Thermoanaerobacterium sp mixed culture	Starch wastewater	Batch	With	6.0/55 °C	92 ml H_2/g starch
Cellulosic waste					
C. acetobutylicum X9 + *Ethanoligenens harbinense* B49	Microcrystalline cellulose	Batch	With	–/37 °C	1.8 L H_2/L-POME
Thermoanaerobacterium-rich sludge	POME	Batch	With	5.5/60 °C	6.33 L H_2/L-POME
Mixed culture	POME	Batch	Without	5.5/60 °C	4.7 L H_2/L-POME
Mixed culture	POME	Repeated batch	Without	5.5/60 °C	2.3 L H_2/L-POME
Mixed culture	POME	HRT 5d	Without	5/–	0.42 L/g COD reduced
Lactose-based wastewater					
C. saccharoperbutylacetonicum ATCC27021	Cheese whey (49.2 g lactose/L)	Batch	Without	6/30 °C	2.7 mol H_2/mol lactose
Mixed culture	Dairy waste (10.4 g COD/L)	HRT 24 h	Without	6/28 °C	1.105 mmol H_2/m^3/min
Mixed culture	Cheese processing wastewater (10 gCOD/L/d)	HRT 24 h	Without	7.5/35–38 °C	2.4 mM H_2/gCOD

a Supplement with vitamins and minerals.

Yield of biohydrogen production from food and starch-based waste

Chong et al. (2009b)

Crop	Microorganism	Operation mode	Maximum H_2 production rate (LH_2/l/day)	Maximum H_2 yield (mol H_2/mol cons. hexose)
Miscanthus (pretreatment: mechanical and NaOH)	*Thermotoga elfii*	Batch	–	1.1[a]
Wheat starch	Mixed mesophilic cultures	Continuous	3	1.26
Sugarbeet juice	Mixed mesophilic cultures	Continuous	2.2[b]	1.9
Corn starch	Mixed mesophilic cultures	Continuous	2.57	0.51
Sweet sorghum extract	Indigenous microbial mesophilic culture	Continuous	8.52	0.86
Sweet sorghum stalks	*Rumicococcus albus*	Batch	–	3.15 (59 l/kg wet biomass)
Sweet sorghum extract	*Rumicococcus albus*	Batch	–	2.61
Ryegrass	Mixed mesophilic cultures	Continuous	6	82[c]
Sweet sorghum	*Caldicellulosiruptor saccharolyticus*	Batch	–	1.75 (30.17 l/kg dry biomass)
Sugar beet extract	*Caldicellulosiruptor saccharolyticus*	Batch	–	–
Barley grains	*Caldicellulosiruptor saccharolyticus*	Batch	–	–
Corn grains	*Caldicellulosiruptor saccharolyticus*	Batch	–	–
Miscanthus (pretreatment: NaOH, $Ca(OH)_2$)	*Thermotoga neapolitana*	Batch	13.1[d]	3.2
Miscanthus (pretreatment: NaOH, $Ca(OH)_2$)	*Caldicellulosiruptor saccharolyticus*	Batch	12.6[d]	3.4

Ntaikou et al. (2010)

[a] mol H_2/mol consumed sugars

[b] ml/min l

[c] ml H_2/g dry mass

[d] mmol H_2/l h

Fermentative hydrogen production from energy crops

Lignocellulosic residue	Pretreatment	Microorganism	Operation mode	H_2 production rate	Maximum H_2 yield (mol/mol cons. hexose)
Wood fibers	Mechanical	*Clostridium thermocellum*	Batch	–	1.47
Corn stover	Steam explosion (90–220°C, 3–5 min)	Mixed mesophilic cultures	Continuous	10.56 mmol/h	3
Sugarcane bagasse hydrolysate	Acid-thermal hydrolysis H_2SO_4 0.27–7(v/v), +121°C, 60 min	*Clostridium butyricum*	Batch	1.611 l/l/day	1.73[b]
Fobber maize juice	Mechanical	Mixed mesophilic cultures	Continuous	–	69.4[c]
Sweet sorghum residues	Mechanical	*Rumicococcus albus*	Batch	–	2.59
Wheat straw	Mechanical	*Caldicellulosiruptor saccharolyticus*	Batch	–	3.8 (44.7 l/kg dry biomass)
Maize leaves	Mechanical	*Caldicellulosiruptor saccharolyticus*	Batch	–	3.6 (81.5 l/kg dry biomass)
Barley straw	Mild acid 1.8% H_2SO_4w/w	*Caldicellulosiruptor saccharolyticus*	Batch	–	–
Corn stalks	Mild acid 1.8% H_2SO_4w/w	*Caldicellulosiruptor saccharolyticus*	Batch	–	–
Bagasse	Alkali-thermal 0.2–4 g/l NaOH, 100°C, 2 h	Mixed thermophilic cultures	Batch	0.28 mmol/h/g TVS	13.39[d]
Corn stover	Acid-thermal hydrolysis H_2SO_4 0.25–4(v/v), +121°C, 30–180 min	*Thermoanaerobacterium thermosaccharolyticum*	Batch	3.305 l/day	2.24

Fermentative hydrogen production from lignocellulosic residues

Ntaikou et al. (2010)

[a] l/kg TVS
[b] mol/mol total sugar
[c] ml H_2/g dry mass
[d] mmol H_2/g TVS

Fermentative hydrogen production from different types of waste and wastewaters

Type of waste/ wastewater	Microorganism	Operation mode	H_2 production rate	Maximum H_2 yield
Sugar factory wastewater	Mixed thermophilic culture	Continuous	4.4 l/l/day	2.6 mol/mol hexose
OFMSW	Mixed mesophilic culture	Batch	0.4 l/g VSS/day	0.15 l/g OFMSW
Rice winery wastewater	Mixed culture	Continuous	9.33 l/g VSS/day 3.81 l/l/day	2.14 mol/mol hexose
Food waste—sewage sludge	Mixed mesophilic culture	Batch	2.67 l/g VSS/day	122.9 ml/g COD carbohydrate
Food waste	Mixed thermophilic culture	Batch	0.288 l/g VSS/day	1.8 mol/mol hexose
Cheese whey	*Clostridium saccharoperbutylacetonicum*	Batch	28.3 ml/h	7.89 mmol/g lactose
Potato processing wastewater	Mixed mesophilic culture	Batch	–	2.8 l/l wastewater
Cheese whey	Mixed mesophilic culture	Batch	–	10 mM/g COD
Dairy wastewater	Mixed mesophilic culture	Continuous	1.59 mmol H_2/l/day	–
Molasses	Mixed mesophilic culture	Continuous	4.8 l/l/day	–
Cheese whey	Mixed mesophilic culture	Batch	8.1 mmol/l/h	5.9 mol/mol lactose
Cheese whey	Mixed mesophilic indigenous microbial culture	Continuous	2.51 l/l/day	0.9 mol/mol hexose
Olive pulp	Mixed mesophilic culture	Continuous	0.26 l/l/day	0.19 mol/kg TS
Olive oil mill wastewater	Mixed mesophilic culture	Continuous	201.6 ml/day	196.2 ml/g hexose
Wastepaper	*Ruminococcus albus*	Batch	–	2.29 mol/mol hexose (282.76 l/kg dry biomass)

Ntaikou et al. (2010)

Yields and rates of bio-hydrogen production from pure carbohydrates by batch dark fermentations

Organism	Carbon source	SHPR	VHPR	H_2 yield	% H_2 yield	H_2 content in gas mixture (%)
Klebsielle oxytoca HP1	Glucose (50 mM)	9.6 mmol/g DW h	87.5 mL/L h	1 mol/mol glucose	16.7	
E. cloacae IIT-BT 08	Glucose (1%)		447 mL/L h	2.2 mol/mol glucose		
E. coli	Glucose (20 g/L)			4.73×10^{-8} mol/mol glucose		
H. alvei	Glucose (10 g/L)			5.87×10^{-8} mol/mol glucose		
Sludge compost	Glucose (10 g/L)		147 mL/L h	2.1 mol/mol glucose		
Mixed culture	Glucose (1 g COD/L)			0.9 mol/mol glucose	23	60
Mixed culture	Sucrose (6 g/L)	9 mL/g VSS h		300 mL/g COD		40
Klebsielle oxytoca HP1	Sucrose (50 mM)	8.0 mmol/g DW h		1.5 mol/mol sucrose	12.3	
C. pasteurium (dominant)	Sucrose (20 g COD/L)	4.58 mmol/g VSS h	270 mmol/L d	4.8 mol/mol sucrose		55
E. cloacae IIT-BT 08	Sucrose (10 g/L)	29.5 mmol/g DW h	660 mL/L h	6 mol/mol sucrose	28	92
Mixed culture	Sucrose (1 g COD/L)			1.8 mol/mol sucrose	23	
Thermoanaerobacterium	Cellulose (5 g/L)	11.9 mL/g VSS h		102 mL/g cellulose	18	
Clostridium sp.	Microcristalline cellulose (25 g/L)	0.46 mmol/VSS d		2.18 mmol/g cellulose		60
E. aerogenes	Starch[a] (20 g glucose/L)	9.68 mmol/g DW h	17.4 mmol/L h	1.09 mol/mol glucose		
Thermoanaerobacterium	Starch (4.6 g/L)	15.2 mL/g VSS h	1.9 mL/h	92 mL/g strach	17	60
C. pasteurium	Starch (24 g/L)	9.9 mL/gVSS h	4.2 mL/h	106 mL/g starch	19	
Mixed culture	Potato starch (1 g COD/L)			0.59 mol/mol starch	15	
Mixed culture	Sugar beet juice			1.7 mol H_2/mol hexose		

[a] Hydrolysate; SHPR, specific hydrogen production rate; VHPR, volumetric hydrogen production rate.

(Kapdan and Kargi, 2006)

Yields and rates of bio-hydrogen production from pure carbohydrates by continuous dark fermentations

Organism	Carbon	SHPR	VHPR	H_2 yield	% H_2 content	Reactor	HRT_t (h)
C. acetobutyricum	Glucose	6 mmol/OD_{600} h L		2 mol/mol glucose	50	Fed-batch	
Mixed culture	Glucose (20 g COD/L)	20 mmol/g VSS h		1.1 mol/mol glucose		CSTR	4
Mixed culture	Glucose (13.7 g/L)		376 mmol/L d	1.2 mol/mol glucose	60	Trickling biofilter	4–12
Clostridia sp.	Glucose (20 g COD/L)	14.2 mmol/g VSS h	359 mmol/L d	1.7 mol/mol glucose	42.6	CSTR	6
Mixed culture	Glucose (7 g/L)	191 mL/g VSS h		2.1 mol/mol glucose	64	CSTR	6
Mixed culture	Glucose (20 g/L)		300 mL/L h		60	UASB	20
Clostridium sp.	Glucose (10 g/L)		640 mL/h		60	AMBR[a]	3.3
E. aerogenes HO39	Glucose (10 g/L)		850 mL/L h			Fixed film	1
Mixed culture	Sucrose (20 g COD/L)		105 mol/h	3.47 mol/mol sucrose		CSTR	8
Mixed culture	Sucrose	340 mL/g VSS h	5.10 L/h L	2.1 mol/mol sucrose	35	CIGSBR[b]	0.5
Mixed culture	Sucrose (20 g COD/L)	2.2 mmol/g VSS h	270 mmol/L d	1.5 mol/mol sucrose	42	UASB	8
Mixed culture	Sucrose (20 g COD/L)	3.7 mmol/gVSS h	470 mmol/L d	2.6 mol/mol glucose	35	SBR	4–12
Klebsiella oxytoca HP1	Sucrose (50 mM)	15.2 mmol/g DW h	350 mL/L h	3.6 mol/mol sucrose		CSTR	5
Mixed culture	Sucrose (20g COD/L)	35 mmol/g VSS h	20.8 L/L d	1.48 mol/mol sucrose	42	CSTR	2
C. butyricum + *E. aerogenes*	Starch (2%)	NA	800 mL/L h	2.5 mol/mol glucose		CSTR	2
C. butyricum + *E. aerogenes*	Starch (2%)	NA	1300 mL/L h	2.6 mol/mol glucose		Immobilized[c]	0.75
Thermococcus kodakaraensis KOD1	Starch (5 g/L)	14.0 mmol/g DW h	9.46 mmol/L h	3.33 mol/mol starch	<10	Gas-lift fermenter	5
Mixed culture	Wheat starch (10 g/L)		131 mL/L h	0.83 mol/mol starch d	50.3	CSTR	12
Mixed culture	Starch (6 kg starch/m^3)	97.5 mL/g VSS h	1497 L/m^3 d	1.29 L/g starch COD	61	CSTR	20
C. termolacticum	Lactose (29 mmol/L)	5.74 mmol/g DW h	2.58 mmol/L h	3 mol/mol lactose	86	CSTR	5–35

[a] Anaerobic membrane bioreactor.
[b] CIGBR, carrier induced granular bed reactor.
[c] Immobilization on porous glass beads; SHPR, specific hydrogen production rate; VHPR, volumetric hydrogen production rate.

(Kapdan and Kargi, 2006)

Yields and rates of bio-hydrogen production from different waste materials by dark fermentation

Organism	Carbon source	SHPR	VHPR	$Y_{P/S}$ yield coefficient	% H_2 content
Mixed culture	OFMSW	16.8 mL/g VSS h	117 mL/g TVS h	150 mL/g OFMSW	66
Thermoanaerobacterium	Food waste (6 gVS/L)	12 mL/g VSS h		1.8 mol/mol hexose	55
Mesophilic mixed culture	Food waste (3% VS)	0.7 mL/g VSS h		0.05 mol/mol hexose	1
Mixed culture	Food waste (3% VS)	111 mL/g VSS h			
Mixed culture	Potato Ind. WW (21 g COD/L)			2.8 L/L WW	60
Mixed culture	Apple (9 g COD/L)			0.9 L/L WW	60
Mixed culture	Domestic WW			0.01 L/L WW	23
E. aerogenes	Molasses (2% sucrose)	36 mmol/L culture h	138 mL/L h	1.5 mol/mol sucrose	60
Mixed culture	Rice winery WW (36 g COD/L)	389 mL/g VSS h	159 mL/L h	2.14 mol/mol hexose	53–61
Mixed culture	Biosolid			1.2 mg/g COD	
Mixed culture	Filtrate			15 mg/g COD	
C. butyricum + *E. aerogenes*	Sweet potato starch residue (0.5%)			2.4 mol/mol glucose	
C. butyricum + *E. aerogenes*	Sweet potato starch residue (2%)			2.7 mol/mol glucose	

OFMSW, organic fraction of solid waste; SHPR, specific hydrogen production rate; VHPR, volumetric hydrogen production rate.

(Kapdan and Kargi, 2006)

Yields and rates of bio-hydrogen production from organic acids by photo-fermentations

Organic acid	Organism	Concentration	Light intensity	Conversion efficiency (%)	LCE[a] (%)	H_2 yield[b]	SHPR	VHPR	Process
Acetate	*Rhodopseudomonas*	22 mM	680 μmol photons/m^2 s	72.8	0.9			25.2 mL H_2/L h	Batch
	R. palustris	22 mM	480 μmol photons/m^2 s	14.8	0.1			2.2 mL H_2/L h	Batch
	R. palustris		2500 lux	60–70		2.8	9.8 mL/g cell h	1.6 mL H_2/L h	Batch
	R. capsulata	4 g/L	200 W/m^2	76.5		1.1	22 mL/g VSS h	0.88 mL/h	Batch
	R. capsulata	1.8 g/L	4170 lux	32.6	4.2		19.07 mL/g DW h		Batch
Lactate	*Rhodopseudomonas*	50 mM	680 μmol photons/m^2 s	9.6	0.4			10.7 mL H_2/L h	Batch
	R. palustris	50 mM	480 μmol photons/m^2 s	12.6	0.5			9.1 mL H_2/L h	Batch
	R. sphaeroides RV	100 mM	3klx	80			75 mL/g DW h	1.5 L/L d	CSTR
	R. capsulatus IR3	30 mmol	120 W	84.8					Batch
	R. sphaeroides GL-1	20 mM	300 W/m^2	86			0.2 mL/mL PU matrix h		[c]
Butyrate	*Rhodopseudomonas*	27 mM	680 μmol photons/m^2 s	8.4	0.3			7.6 mL H_2/L h	Batch
	R. capsulata	1 g/L	200 W/m^2	67.6		2.8	32 mL/g VSS h	1.28 mL/h	Batch
Malate	*Rhodopseudomonas*	15 mM	680 μmol photons/m^2 s	6.6				1.1 mL H_2/L h	Batch
	R. palustris	15 mM	480 μmol photons/m^2 s	36	0.3			5.8 mL H_2/L h	Batch
	R. sphaeroides	15 mM	200 W/m^2				2.4 mL/g DW h	12 mL/L h	Batch
	R. sphaeroides	7.5 mM	150–250 W/m^2	35–45			18 mL/g DW h	5 mL H_2/L h	Batch
PHB[d]	*R. sulfidophilum*		190 W/m^2					33 mL/L h	Batch
Succinate	*R. sulfidophilum*	50 mM	190 W/m^2					26.6 mL/L h	Batch

[a] Light conversion efficiency.
[b] H_2 yield mol/mol substrate.
[c] Immobilized on polyurethane foam.
[d] PHB, poly-hydroxy butyrate; 210 μmol photons/m^2 s = 190 W/m^2.

(Kapdan and Kargi, 2006)

Yields and rates of bio-hydrogen production from food industry wastewaters by photo-fermentations

Wastewater	Dilution (%)	Organism	Light intensity	H_2 yield	HPR	Operation
Sugar refinery effluent + malic acid	20	*R. sphaeroides* OU 001	200 W/m^2	13.44 L/mol C	5 mL/L culture h	Batch
Sugar refinery effluent + malic acid	20	*R. sphaeroides* OU 001	200 W/m^2	11.67 L/mol C	3 mL/L culture h	Continuous
Olive mill WW	2	*R. sphaeroides* OU 001	200 W/m^2		4 mL/L culture h	Batch
Tofu WW	ND[a]	*R. sphaeroides*	8klx	0.24 mL/mg carbohydrate	2.1 L/h m^2 gel	Immobilized
Tofu WW	ND[a]				15.9 mL/L h	Batch
Tofu WW	ND[a]	*R. sphaeroides*	8500 lx		0.393 mL/mg DW h	Immobilized

[a] ND, no dilution.

(Kapdan and Kargi, 2006)

Yields and production rates of bio-hydrogen by the sequential and combined dark–photo fermentations

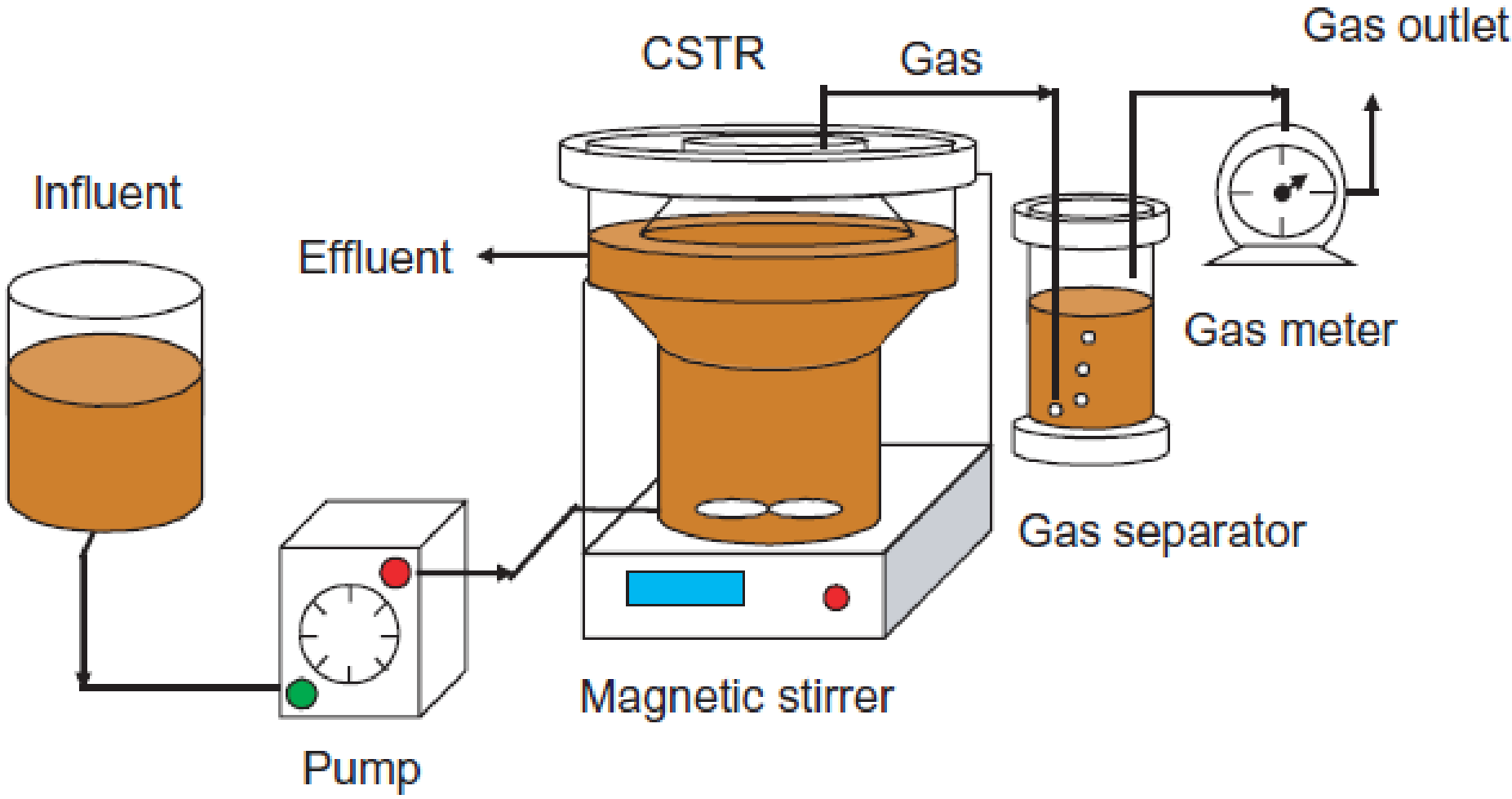
Fermentation type	Organisms	Carbon source	Organic acid	Total H_2 yield (mol/mol glucose)	SHPR
Sequential dark–photo-fermentation	*C. buytricum*, *E. aerogenes*, *Rhodobacter* sp. M-19	Sweet potato starch residue	Acetic, butyric, lactic	7	
	C. buytricum, *E. aerogenes*, *Rhodobacter* sp. M-19	Starch manufacturing wastes	Acetic, butyric, lactic	7.2	
	Lactobacilus amylovorus, *R. marinum* A-501	Algal biomass (*D. tertiolecta*)	Lactic acid		2.47 mmol/L culture h
	Mixed anaerobic culture, *R. sphaeroides* RV	Solid waste	Lactic acid		~110 mL/g DW h
Combined dark–photo-fermentation	*C. buytricum*, *Rhodobacter* sp. M-19	Starch		6.6	
	Lactobacilus amylovorus, *R. marinum* A-501	Algal biomass (*D. tertiolecta*)	Lactic acid		1.55 mmol/L culture h
	V. fluvialis, *R. marinum* A-501	Algal biomass (*C. reindhartii*)	Lactic acid		1.18 mmol/L culture h

(Kapdan and Kargi, 2006)

Bioreactors

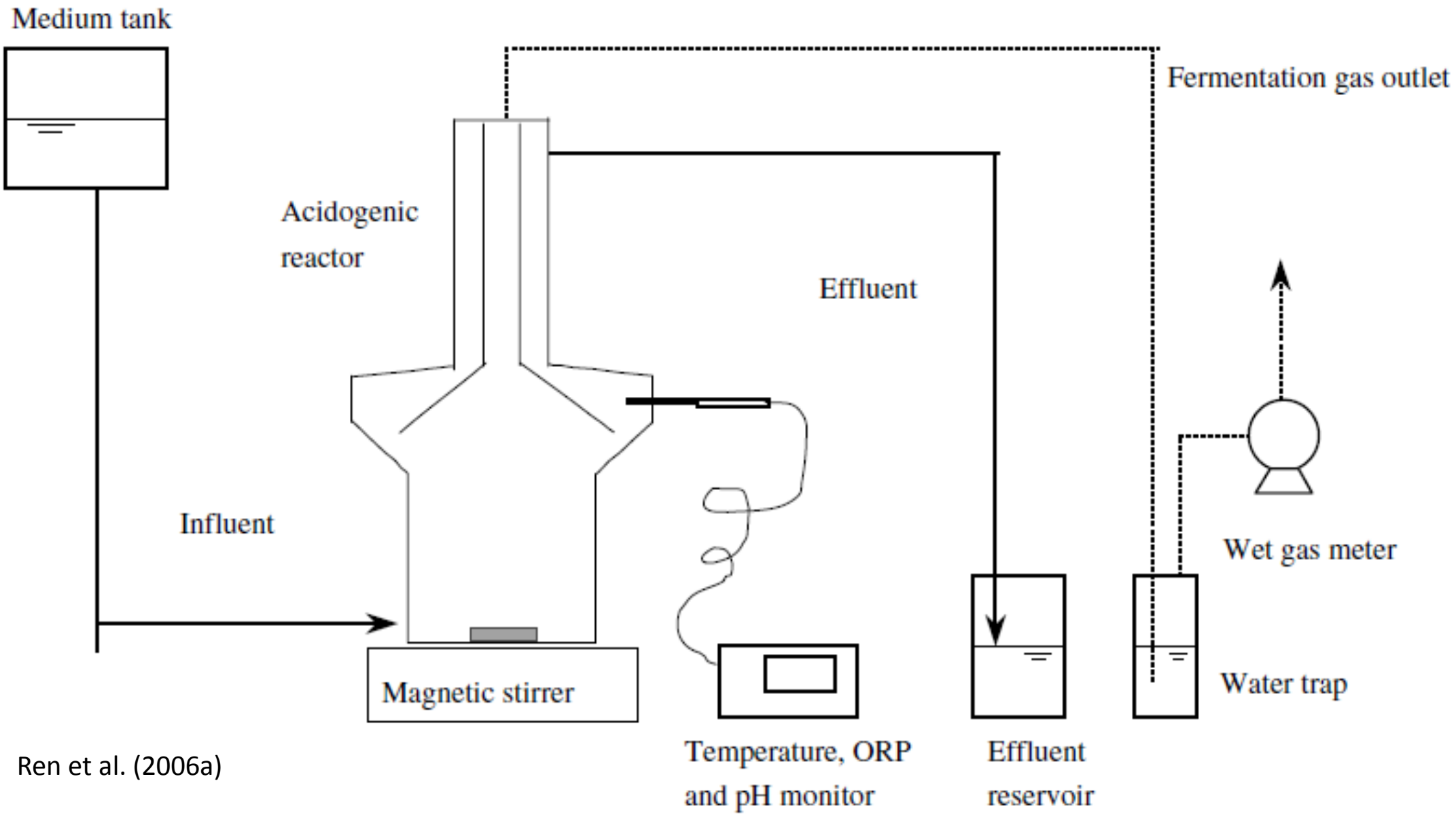
The types of bioreactors used for biohydrogen production are:

1. Batch Flow Reactor
2. Continuous Flow Reactor
3. Continuously Stirred Tank Reactor (CSTR)
4. Upflow Anaerobic Sludge Blanket Reactor (UASB)
5. Packed Bed Reactor (PBR)
6. Anaerobic Sequencing Batch Reactor (ASBR)
7. Fixed Bed Bioreactor with Activated Carbon (FBBAC)
8. Anaerobic Fluidized Bed Reactor (AFBR)
9. Carrier-Induced Granular Sludge Bed (CIGSB)
10. Membrane Bioreactor (MBR)
11. Rhomboidal Reactor



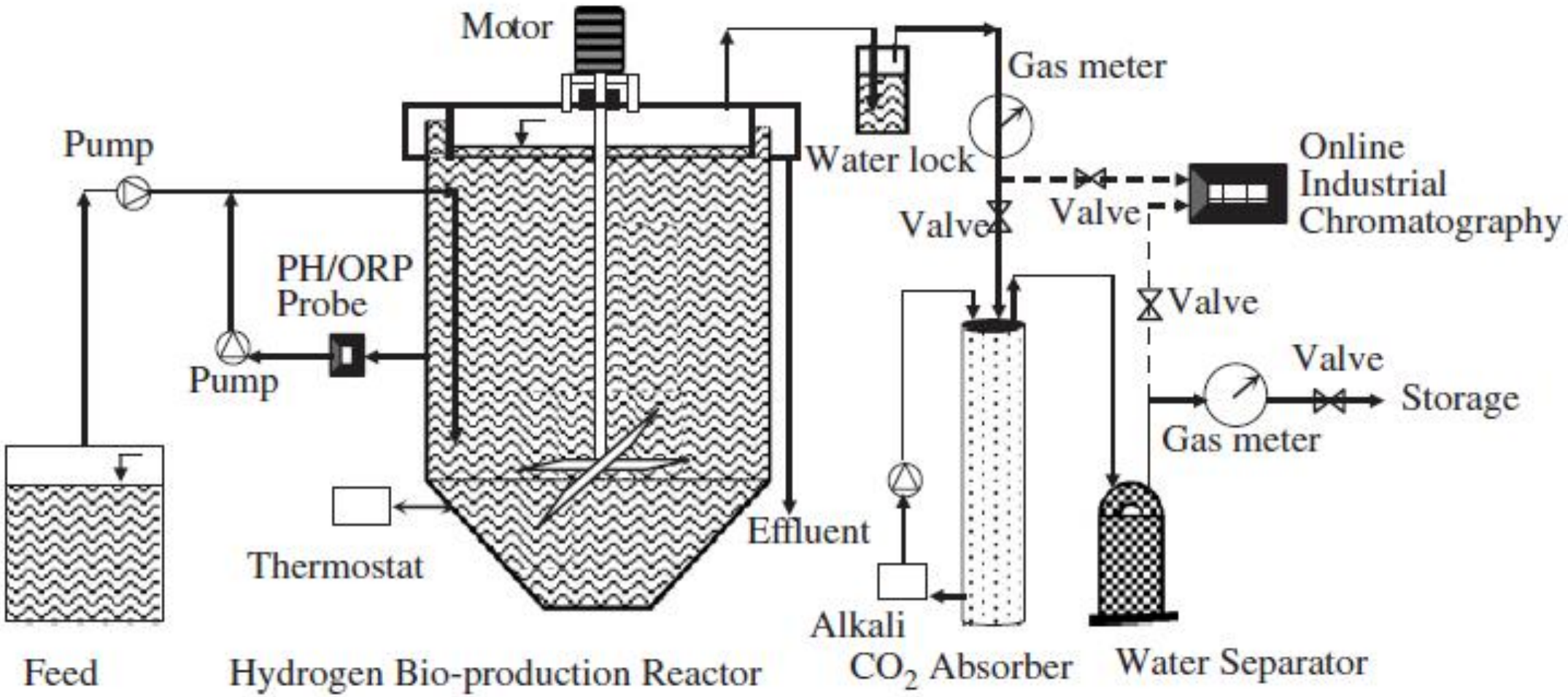
Schematic representation of a continuous stirred-tank reactor (CSTR)

Ren et al. (2007)



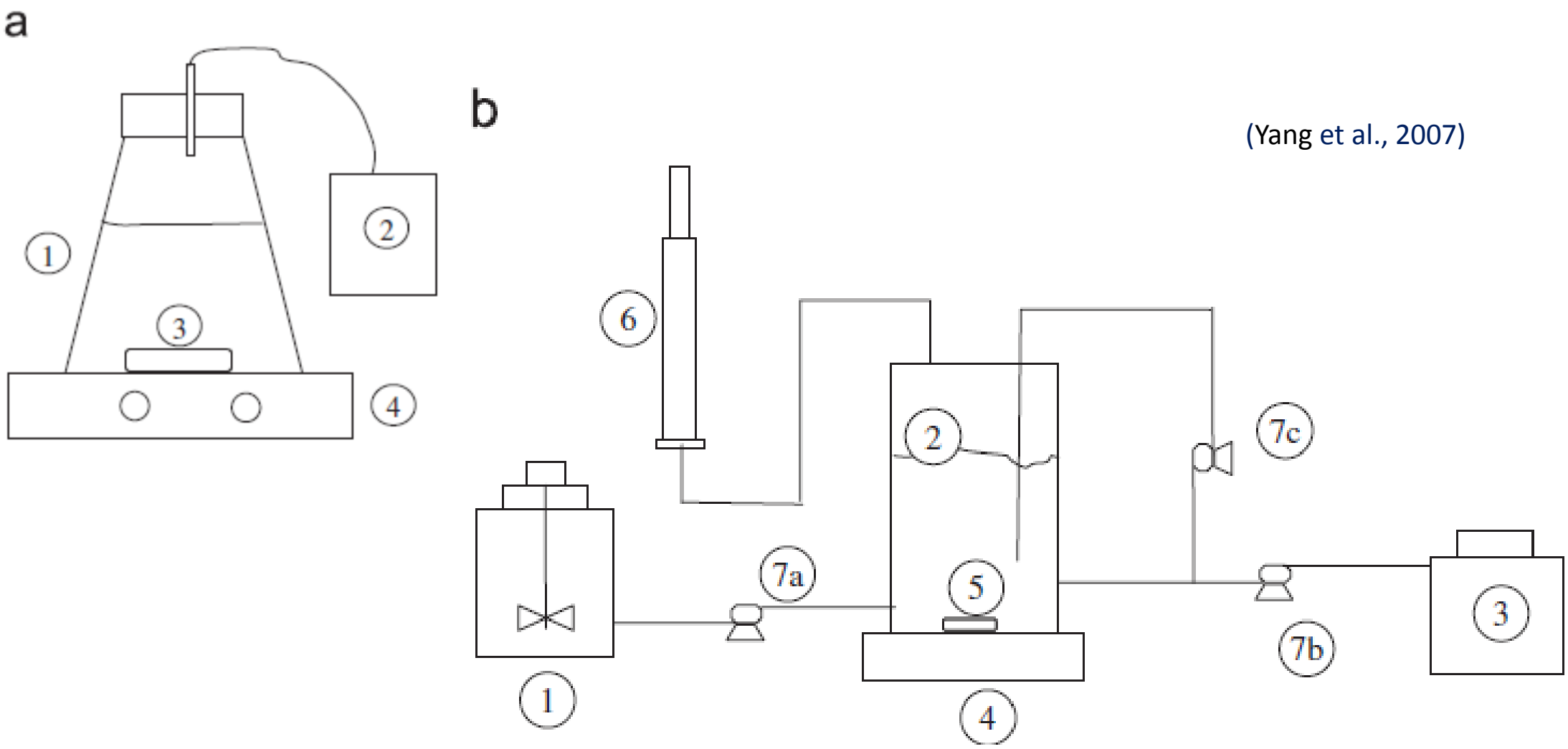
Ren et al. (2006a)

Process schematic of continuous-flow acidogenic reactor with a three-phase separator



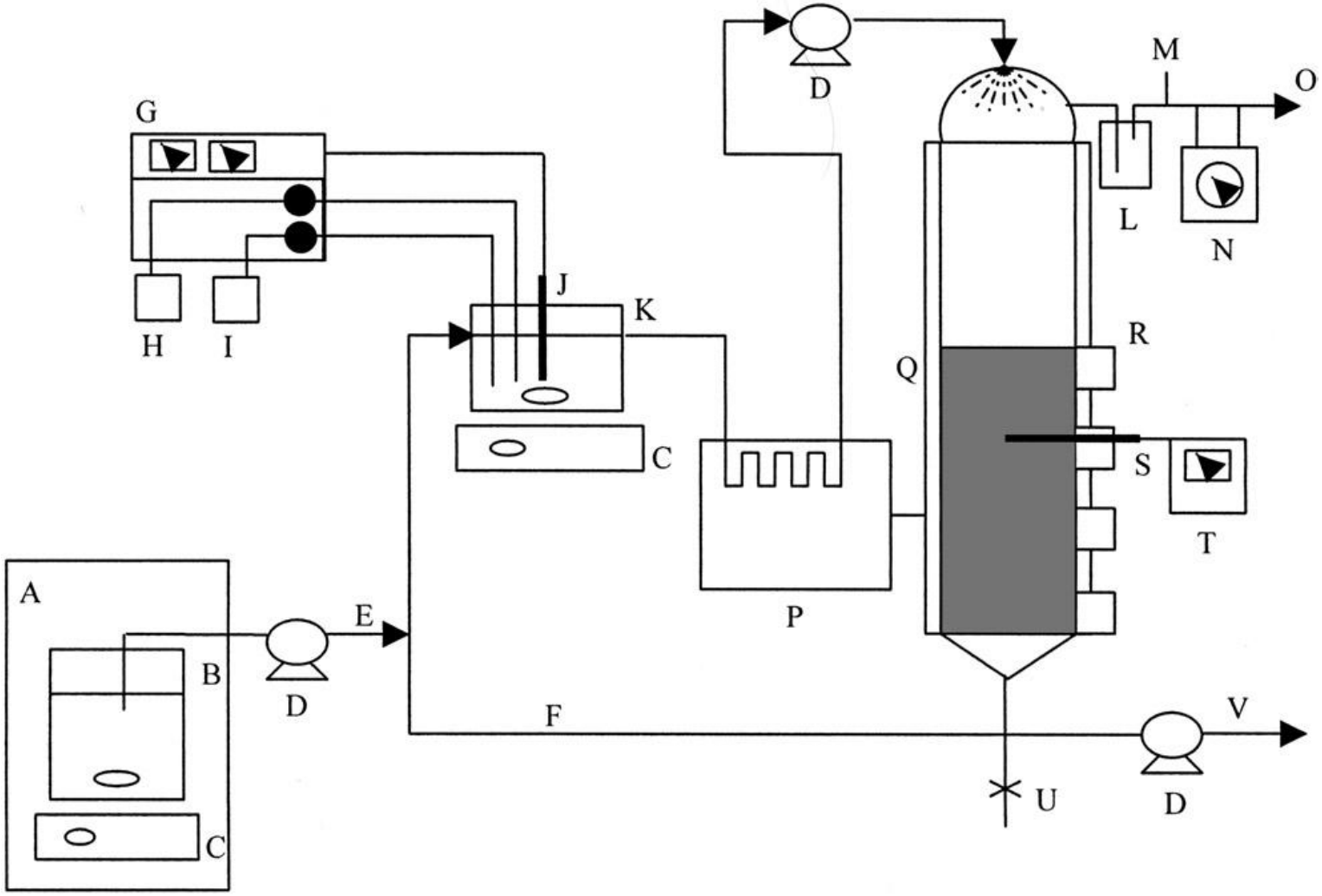
Schematic diagram of a hydrogen bio-producing reactor (HBR) system

Ren et al. (2006b)



(Yang et al., 2007)

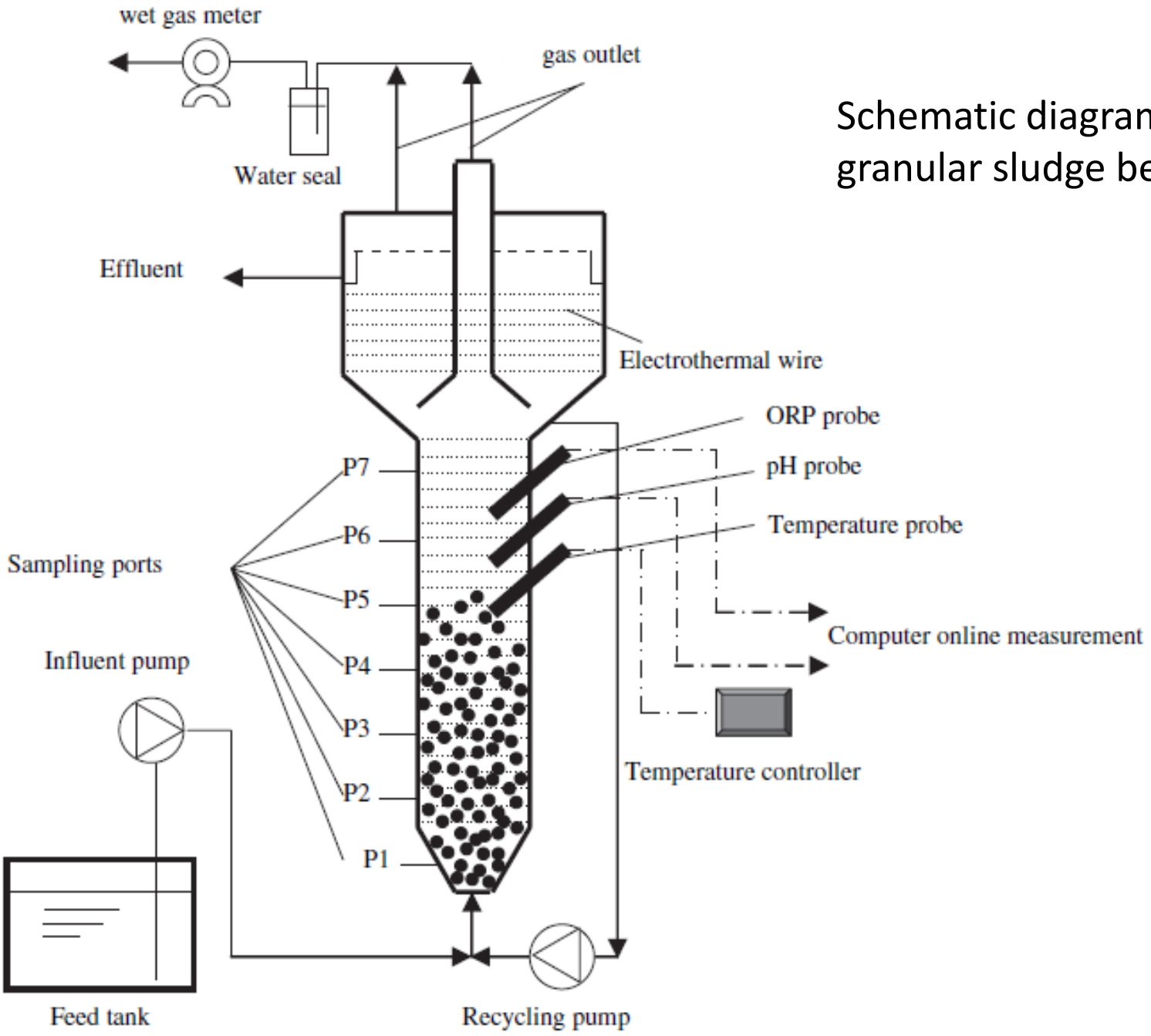
Schematic of H_2 fermentation systems. (a) Batch bioreactor set-up: (1) bioreactor, (2) gas collector, (3) magnetic stirrer, and (4) magnetic plate. (b) Continuous bioreactor system set-up: (1) feed tank, (2) bioreactor, (3) effluent tank, (4) magnetic stir plate, (5) magnetic stirrer, (6) gas meter, and (7) pumps: (a) feed influent, (b) bioreactor effluent, and (c) recirculation.



(Oh et al., 2004)

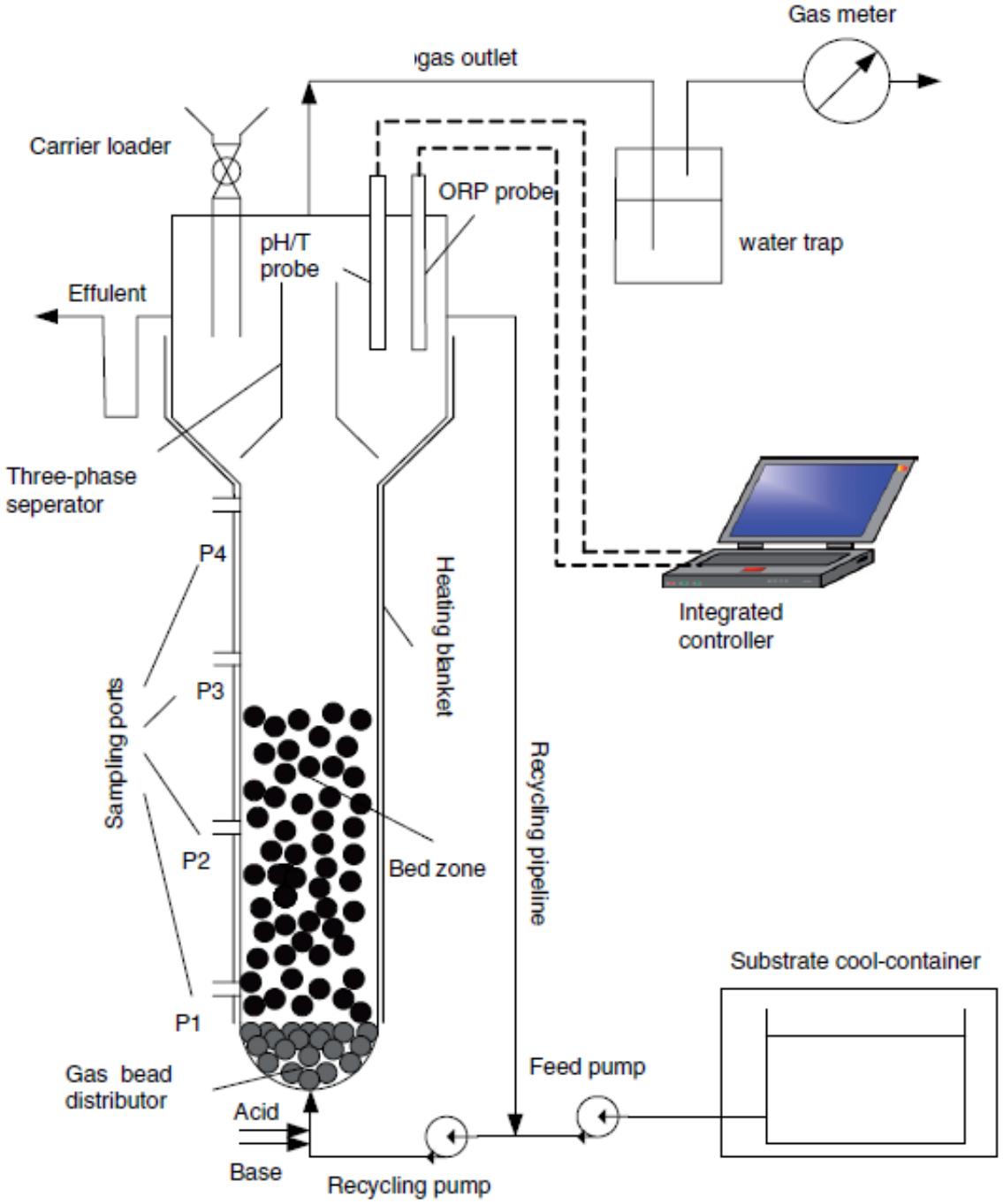
Continuous trickling biofilter reactor (TBR)

Schematic diagram of the TBR system used in the study: (A) 4°C cold chamber; (B) feed tank; (C) magnetic stirrers; (D) peristaltic pumps; (E) influent liquid stream; (F) liquid recirculation circuit; (G) pH control module; (H) 6 N NaOH solution bottle; (I) 4 N HCl solution bottle; (J) pH sensor; (K) pH control vial; (L) liquid trap; (M) gas sampling port; (N) wet gas meter; (O) biogas outlet; (P) water bath circulator; (Q) 'Saran-Lock' packing; (R) media sampling ports; (S) thermocouple probe; (T) thermometer; (U) drain valve; and, (V) liquid waste stream.



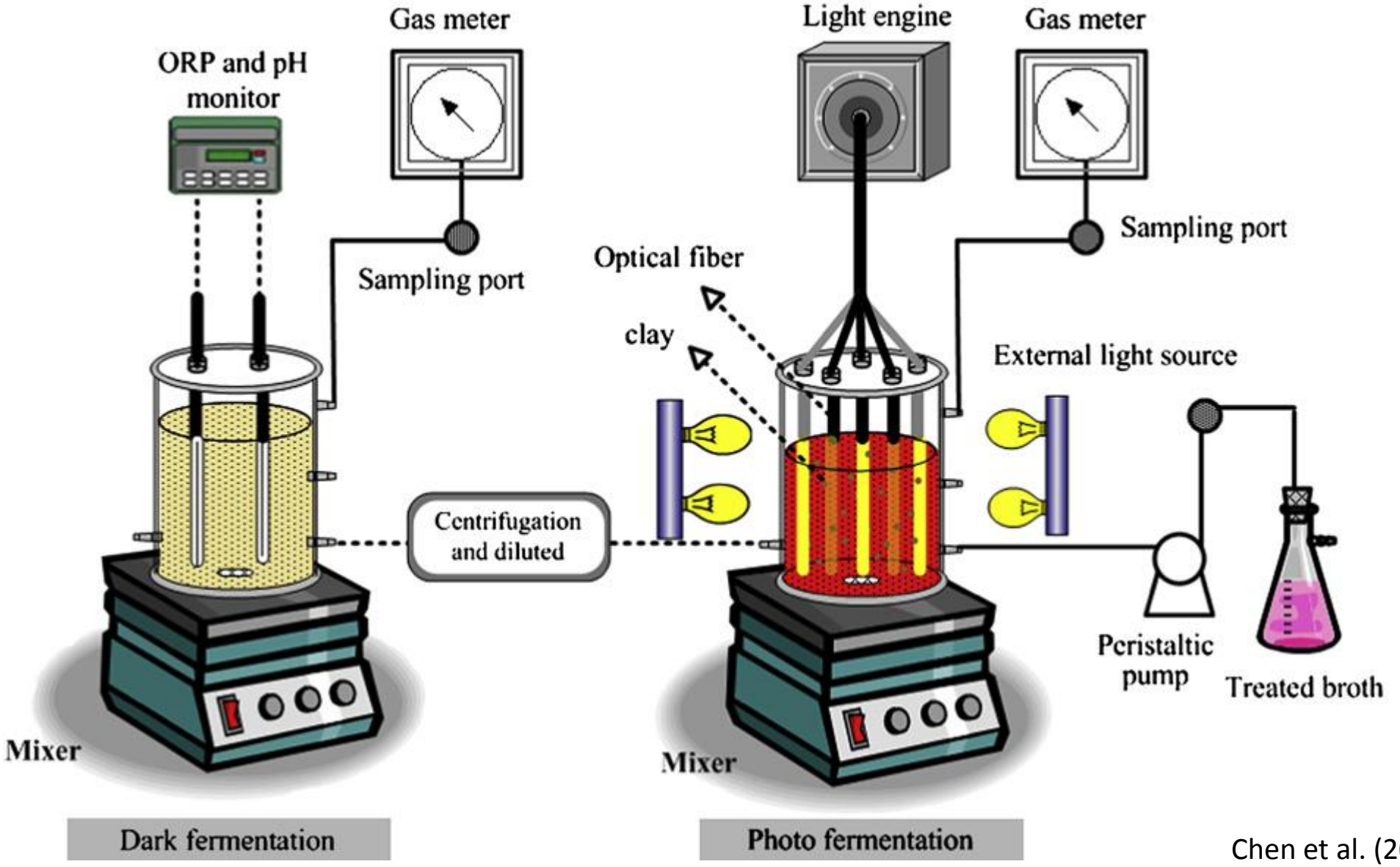
Schematic diagram of the expanded granular sludge bed (EGSB) reactor

Guo et al. (2008)



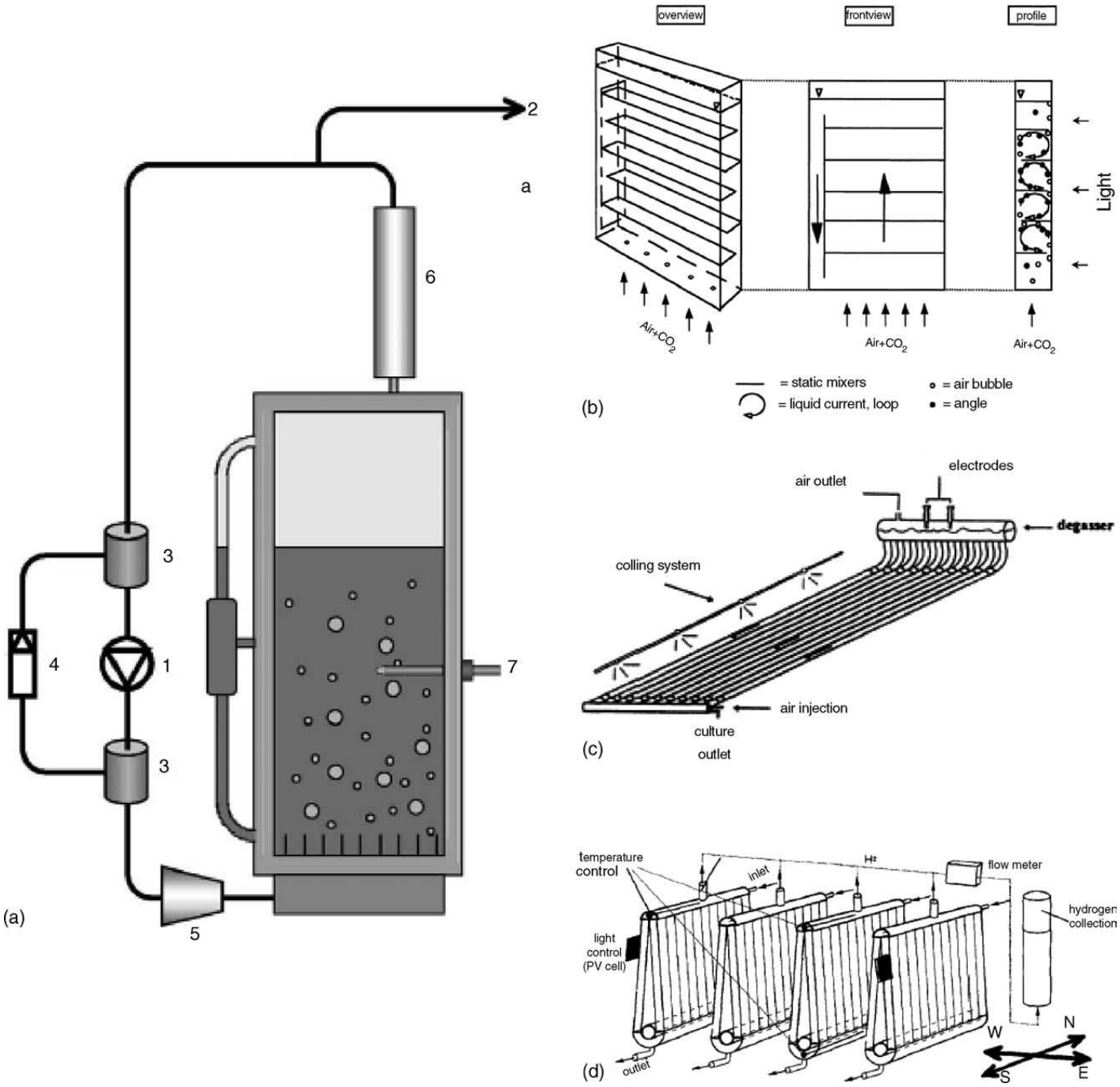
Schematic diagram of the granular activated carbon anaerobic fluidized bed reactor system

Zhang et al. (2007)



Chen et al. (2008)

Schematic description of the two-stage process combining dark and photo fermentation



Some configurations for photo-bioreactors used for biohydrogen production. (a) Photo-bioreactor with gas recirculation: (1) membrane gas pump, (2) gas bag for collection of produced gas, (3) two 1 L pressure vessels, (4) pressure valve, (5) mass flow controller, (6) condenser and (7) pH/redox electrode. (b) Flat panel airlift (FPA) photo-bioreactor. (c) Multi-tubular (Tredici) photo-bioreactor and (d) a modular outdoor photo-bioreactor.

(Kapdan and Kargi, 2006)

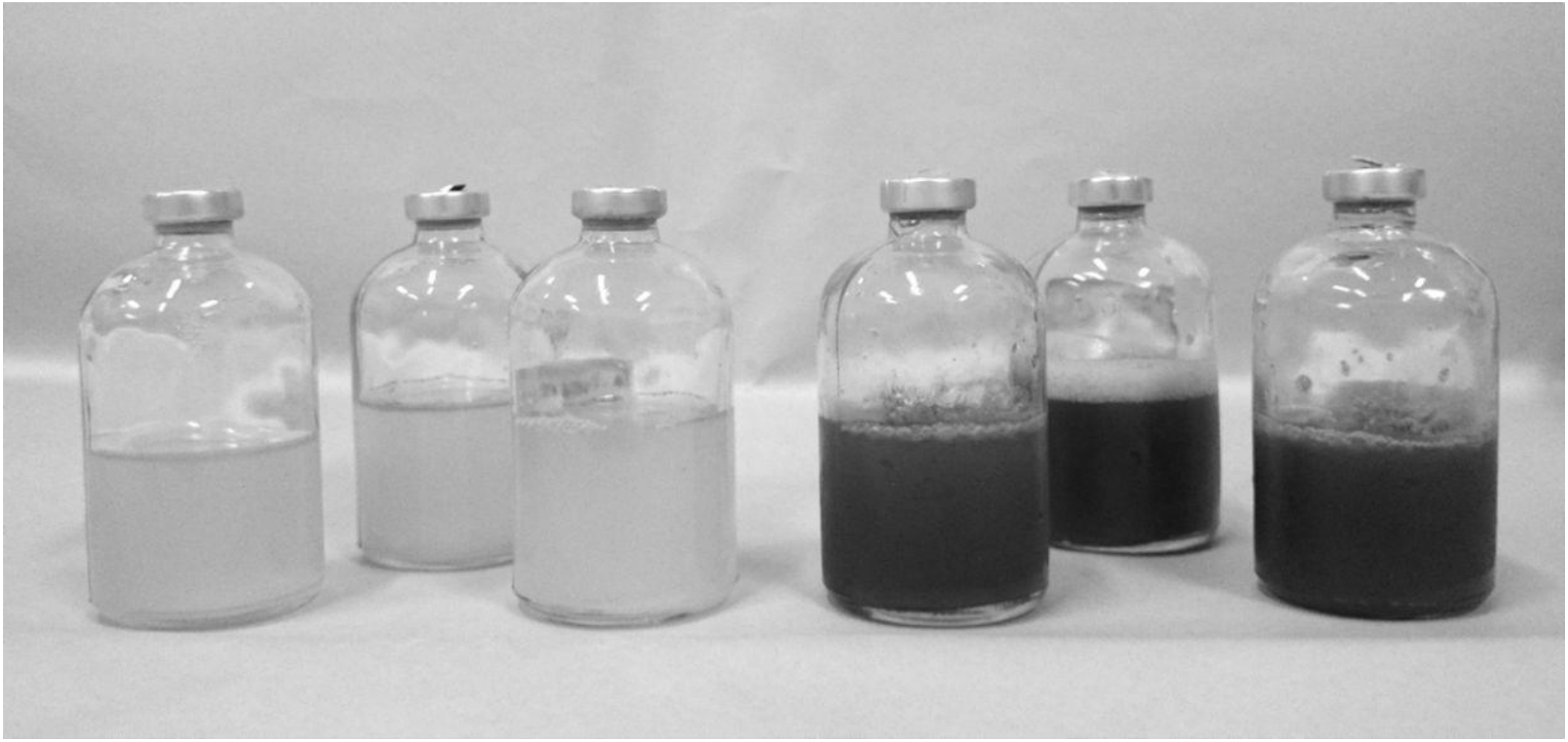
Comparative study on the efficiency of hydrogen fermentation processes

Process	Bacterial growth mode/support medium	Optimal HRT (h)	HPR (L/L h)	Highest biomass conc. (g-VSS/L)
CSTR	Suspension/none	6	0.15	0.8
CSTR	Suspension/none	6	0.58	1.7
AFBR	Entrapment/alginate gel	2	0.93	—
Packed-bed	Flocculation/none	1.5	1.42	17
Packed-bed	Attachment/ligocelluosic agroresidues	1.08	1.85	44
Fixed bed	Attachment/activated carbon	1	1.32	15.8
CIGSB	Flocculation/none	0.5	7.33	26.1
Tricking biofilter	Attachment/fibrous polymeric material	4	1.07	24
GAC-AFBR	Attachment/GAC	1	2.36	21.5

GAC: granular activated carbon
AFBR: anaerobic fluidized bed reactor
CSTR: continuous stirred tank reactor
CIGSB: carrier-induced granular sludge bed
HPR: hydrogen production rate
HRT: hydraulic retention time
VSS: volatile suspended solid

Zhang et al. (2007)

Examples on Bioreactors



Anaerobic batch reactors applied on laboratory scale

Maintinguer et al. (2015)

4 Videos

Calculations

Kinetic modeling

The cumulative hydrogen production in the batch experiments followed the modified Gompertz equation (Fang et al., 2006; Chong et al., 2009a):

$$H = P \exp\left\{-\exp\left[\frac{R_m e}{P}(\lambda - t) + 1\right]\right\}$$

Where,

H: the cumulative hydrogen production (mL)

λ: lag time (h)

P: hydrogen production potential (mL)

R_m: maximum hydrogen production rate (mL/h)

e: 2.718281828

Hydrogen gas production can be calculated from bioreactor headspace measurements of gas composition and the total volume of biogas produced at each time interval using the followed equation (Chong et al., 2009a):

$$V_{H,i} = V_{H,i-1} + C_{H,i}\left(V_{G,i} - V_{G,i-1}\right) + V_H\left(C_{H,i} - C_{H,i-1}\right)$$

$V_{H,i}$ and $V_{H,i\text{-}1}$ are cumulative hydrogen gas volumes at the current (i) and previous (i-1) time intervals, $V_{G,i}$ and $V_{G,i\text{-}1}$ the total biogas volumes in the current and previous time intervals, $C_{H,i}$ and $C_{H,i\text{-}1}$ the fraction of hydrogen gas in the headspace of the bottle measured using gas chromatography in the current and previous intervals, and V_H the total volume of headspace in the bioreactor.

Kinetic parameters for hydrogen production at pH 4.0--7.0

(Fang et al., 2006)

pH	λ (h)	R_m (mL/h)	P (mL)	Maximum specific hydrogen production rate (L/(g-VSS d))	Hydrogen yield (mL/g-carbohydrate)
4.0	40	0.7	175	0.2	212
4.5	36	7.3	286	2.1	346
5.0	12	9.0	277	2.5	336
5.5	12	11.0	248	3.1	300
6.0	11	8.5	220	2.4	264
6.5	18	14.0	185	4.0	223
7.0	18	8.0	132	2.3	160

Kinetic parameters at pH 4.5 and various rice concentrations

(Fang et al., 2006)

Rice concentration (g-carbohydrate/L)	λ (h)	R_m (mL/h)	P (mL)	Maximum specific hydrogen production rate (L/(g-VSS d))	Hydrogen yield (mL/g-carbohydrate)
2.7	38	1.0	115	0.3	278
5.5	36	7.3	286	2.1	346
8.3	36	2.1	302	0.6	244
11.0	40	1.8	291	0.5	176
13.8	16	1.6	325	0.4	157
22.1	12	1.6	510	0.4	154

Comparison of hydrogen yield

Feedstock	pH	Temperature (°C)	Hydrogen yield (mL/g-carbohydrate)	Yield[c] (%)
Rice	4.5	37	346	62.6
Starch	6.0[a]	55	92	16.6
Cellulose	7.0[a]	37	72	13.0
Cellulose	7.0[a]	60	193	34.9
Sucrose[b]	5.5	36	280	53.4
Glucose[b]	5.5	37	261	52.2

[a]Initial pH.
[b]Continuous experiments.
[c]Assuming carbohydrate was totally converted into hydrogen and acetate.

(Fang et al., 2006)

$$\text{Overall } H_2 \text{ production rate} = \frac{\text{Maximum cumulative } H_2 \text{ production (ml)}}{\text{Culturetimefor } H_2 \text{ evolution (h)} \times \text{ Culture volume (l)}}$$

$$H_2 \text{ yield} = \frac{\text{Amount of } H_2 \text{ produced (mol)}}{\text{Amount of substrate (sucrose) consumed (mol)}}$$

Chen et al. (2008)

Table 1 – Effect of photo-H_2 production performance of *Rhodopseudomonas palustris* WP3-5 under different illumination settings using dark fermentation effluent as substrate (containing an initial HBu and HAc concentration of 2900 and 900 mg COD/l, respectively)

Type	Cumulative H_2 production (ml)	Overall H_2 production rate (ml/l/h)	H_2 content (%)	Total COD removal efficiency (%)	Model simulation[a]			
					H_{max} (ml)	R_{max} (ml/h)	λ (h)	r^2
HL/TL[b]	1910	20.5	88.1	72.0	1989	25.2	11.8	0.999
SLOFs/HL/TL[c]	2784	28.3	89.3	86.7	2878	34.7	11.8	0.996
SLOFs/HL/TL-Clay[d]	3170	31.8	88.4	90.3	3186	39.0	12.14	0.996

a Simulation of time-course data by modified Gompertz equation.

b HL/TL means using halogen lamp and tungsten filament lamp as light source, but without the addition of clay carriers into the photobioreactor.

c SLOFs/HL/TL means using combination of halogen lamp (HL), tungsten filament lamp (TL), and side-light optical fibers (SLOFs) as light source, but without the addition of clay carriers into the photobioreactor.

d SLOFs/HL/TL-Clay means using combination of halogen lamp (HL), tungsten filament lamp (TL), and side-light optical fibers (SLOFs) as light source, and with the addition of clay carriers into the photobioreactor.

Chen et al. (2008)

Table 2 – Comparison of the H_2 yield obtained from different two-stage dark/photo fermentation systems reported in the literature

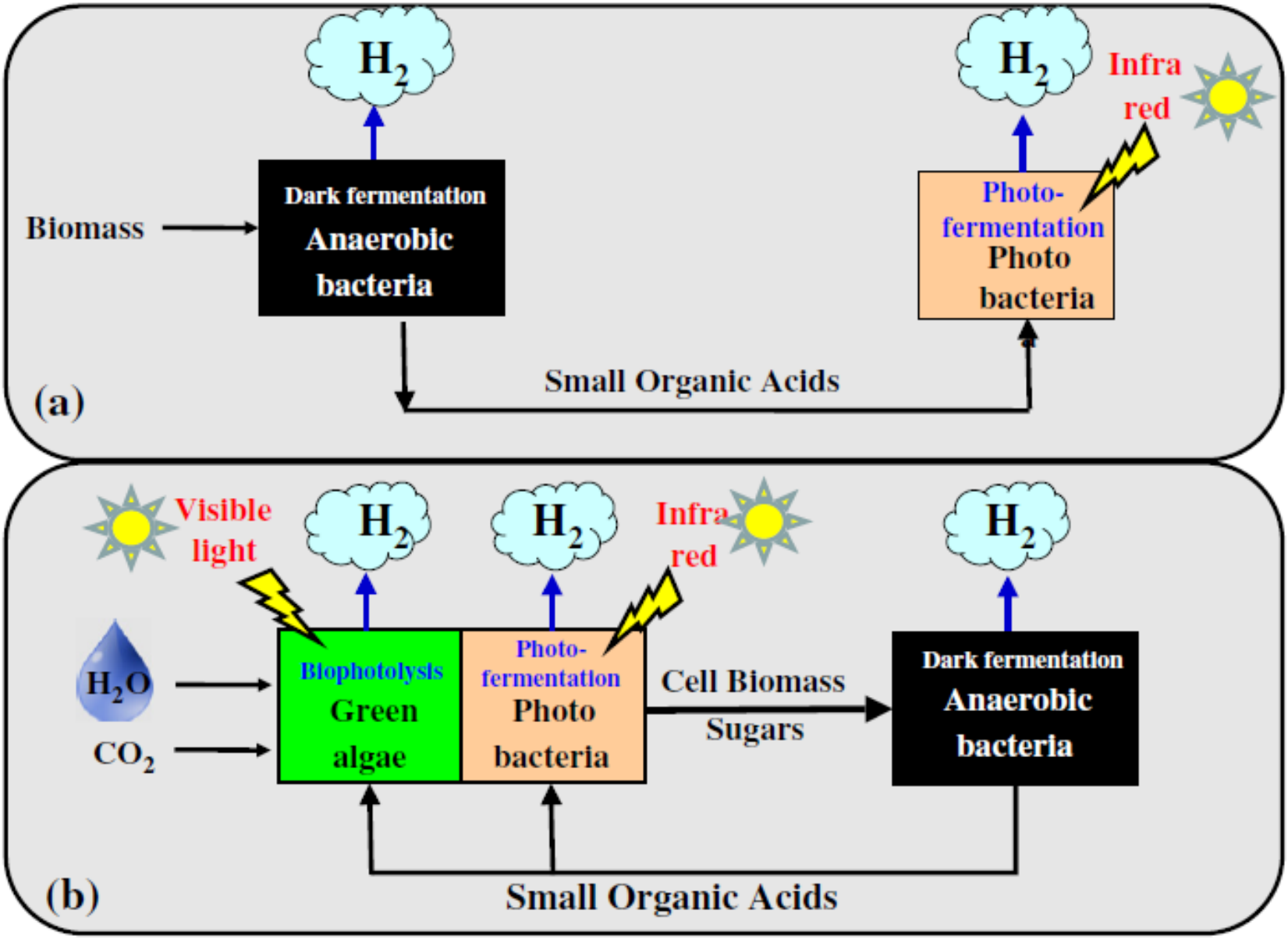
Carbon sources	Microorganism used in dark fermentation	Microorganism used in light fermentation	H_2 yield (mol H_2/mol hexose)	Reference
Glucose	*Escherichia coli* HD701	*Rhodobacter sphaeroides* O.U. 001	2.4	[46]
Glucose	*Lactobacillus delbrueckii* NBRC13953	*Rhodobacter sphaeroides* RV	7.1	[47]
Glucose	*Rhodopseudomonas palustris* P4	*Rhodopseudomonas palustris* P4	4.8–5.6	[21]
Glucose	*Clostridium butyricum*	*Rhodobacter sphaeroides* M-19	7.0	[48]
Glucose	*Clostridium butyricum*	*Rhodobacter* sp. M-19	6.6	[18]
Glucose	*Clostridium butyricum*	*Rhodobacter sphaeroides* and *Rhodobacter capsulatus*	5.6	[26]
Glucose	*Enterobacter cloacae* DM11	*Rhodobacter sphaeroides* O.U. 001	6.61–6.75[a]	[49]
Sucrose	Microflora	*Rhodobacter sphaeroides* SH_2C	3.32	[50]
Sucrose	*Clostridium pasteurianum* CH_4	*Rhodopseudomonas palustris* WP3-5	7.1	This study

a Calculated value based on the reported data.

Chen et al. (2008)

Recent Advancements

Algae for Biohydrogen Production



Simplified schematics for integrated hydrogen production processes:

(a) Dark fermentation followed by photo-fermentation process.

(b) Photosynthetic process (co-cultivated green algae and photo-fermentative bacteria) followed by dark fermentation process.

Melis and Melnicki (2006); Eroglu and Melis (2011)

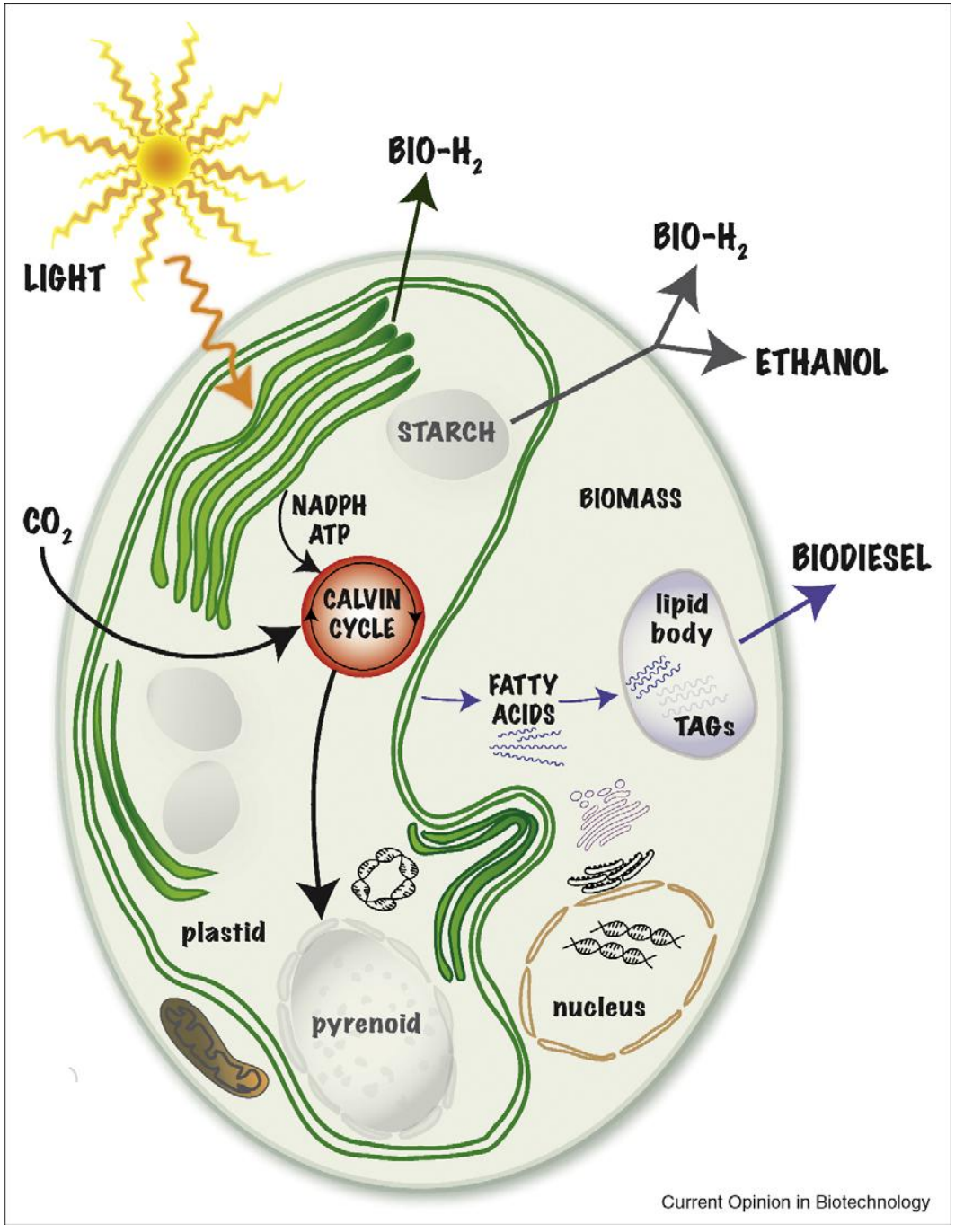
Comparison of H_2 production rates obtained from various photobiological processes

See Table 1

In

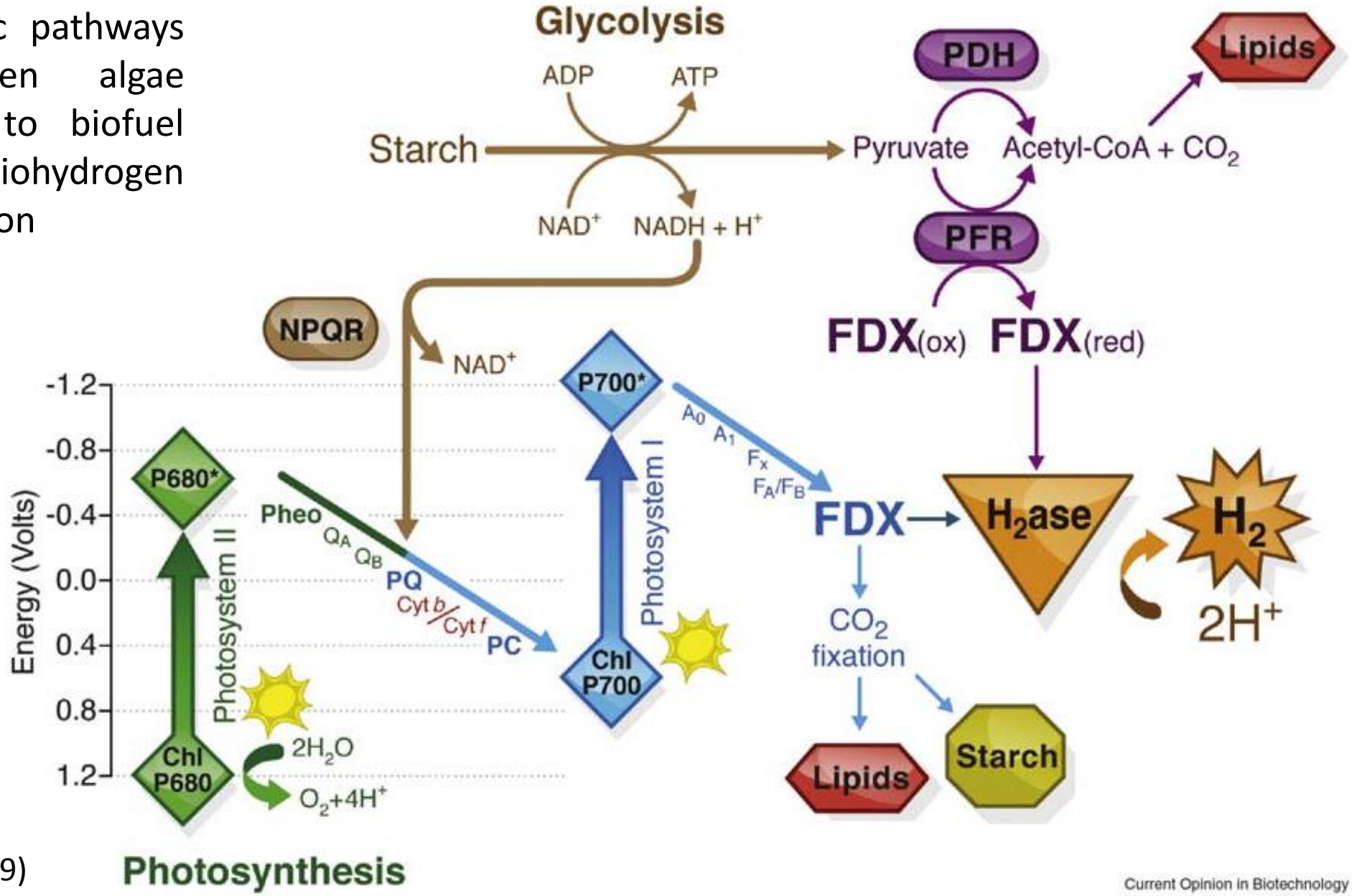
Eroglu and Melis (2011)

Metabolic pathways in green algae related to biofuel and biohydrogen production



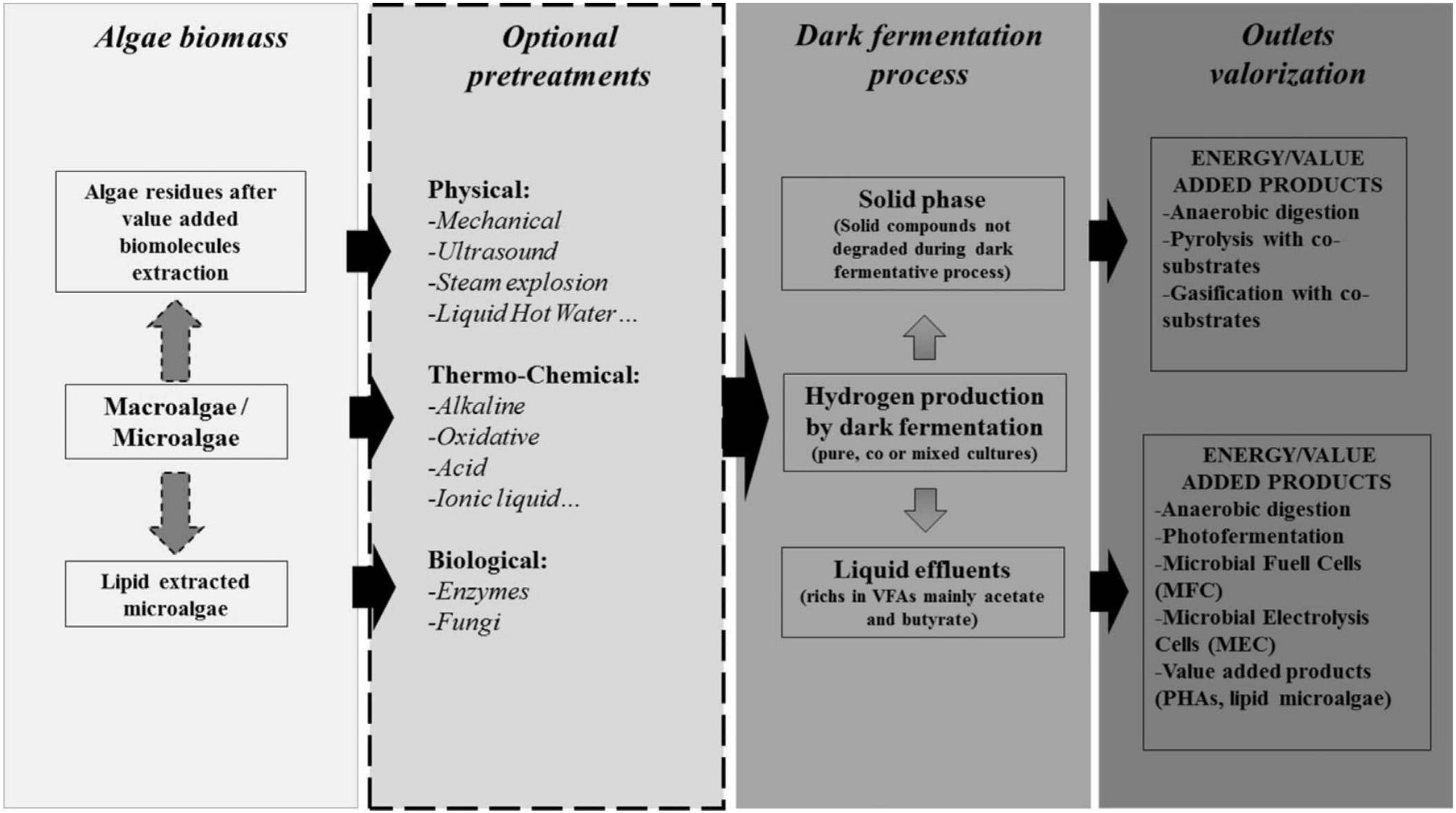
Beer et al. (2009)

Photosynthetic and glycolytic pathways in green algae related to biofuel and biohydrogen production



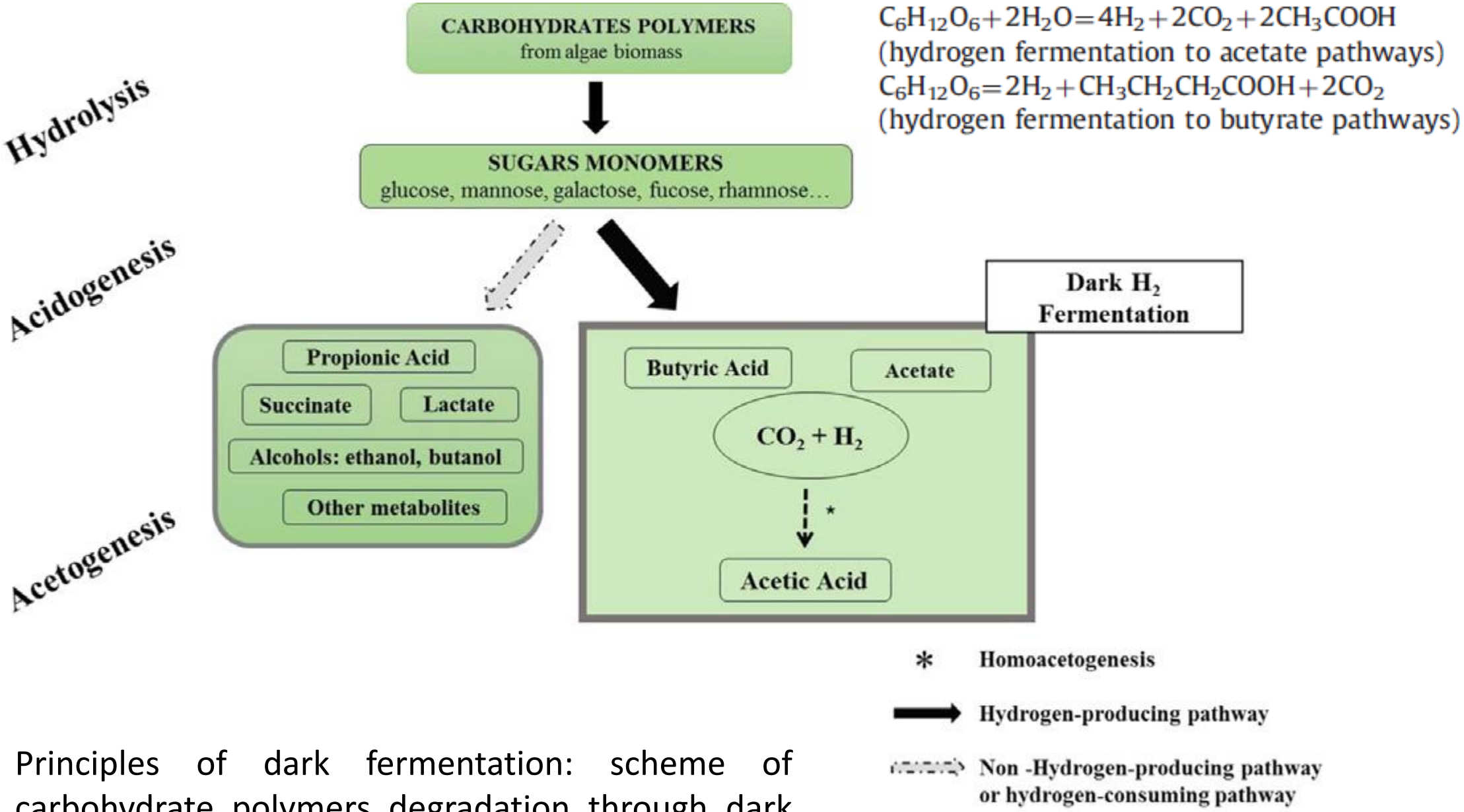
Beer et al. (2009)

Current Opinion in Biotechnology



Algae biomass or co-products conversion into biohydrogen through dark fermentation process integrated in a biorefinery approach concept

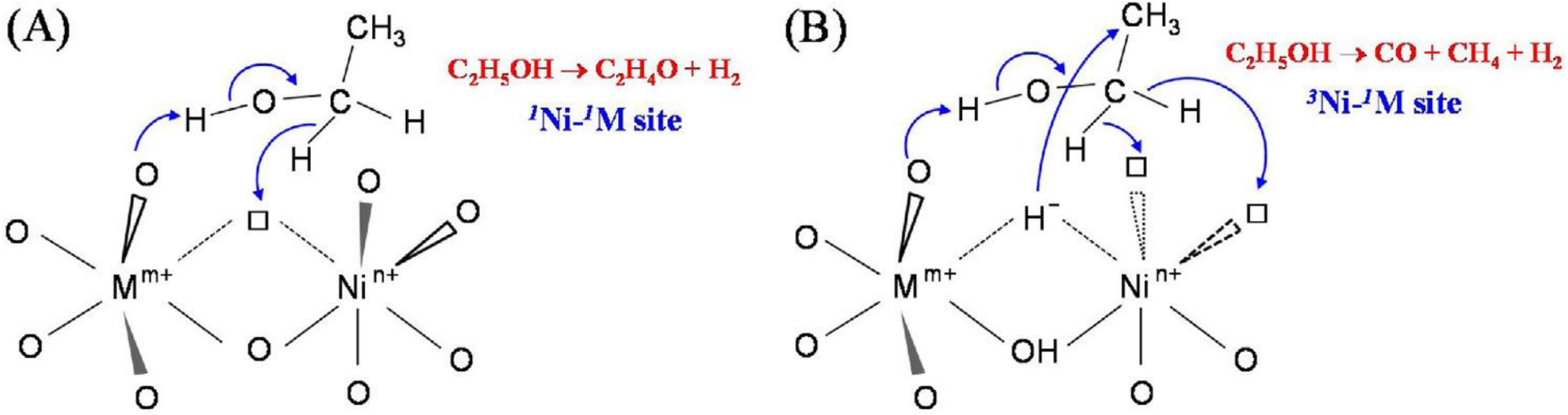
Sambusiti et al. (2015)



Principles of dark fermentation: scheme of carbohydrate polymers degradation through dark fermentation process operated with mixed cultures

(Monlau et al., 2014; Sambusiti et al. 2015)

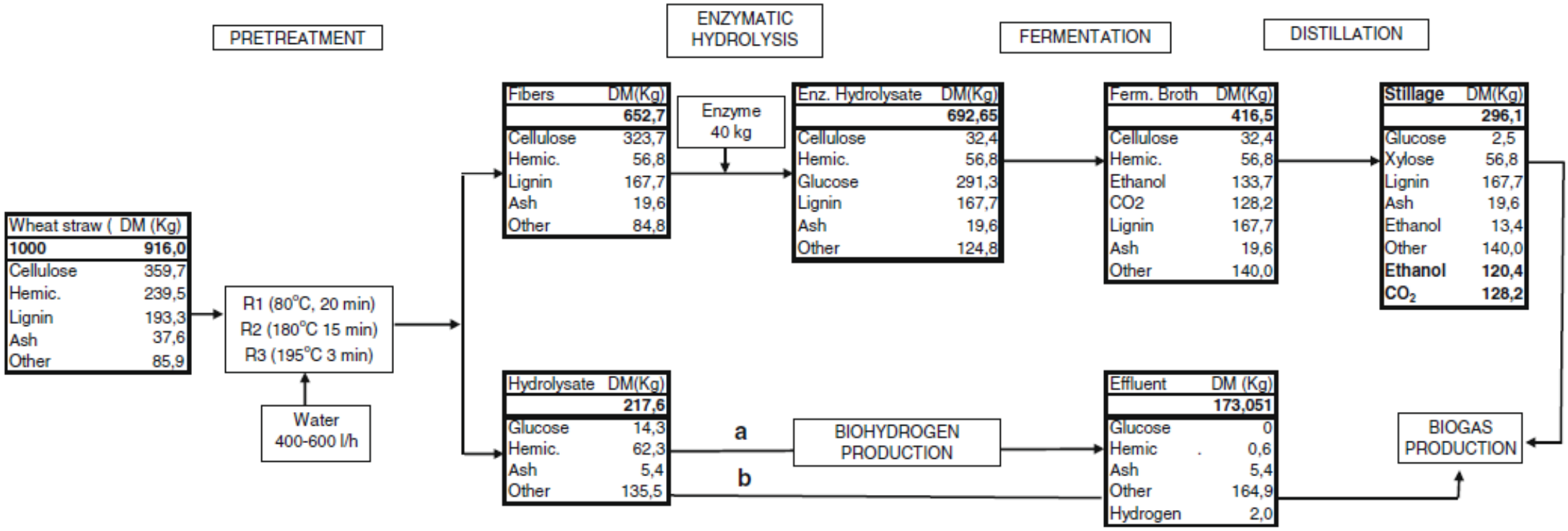
Hydrogen Production from Bioethanol



Hydrogen production from bioethanol using catalysts

Fang et al. (2014, 2015)

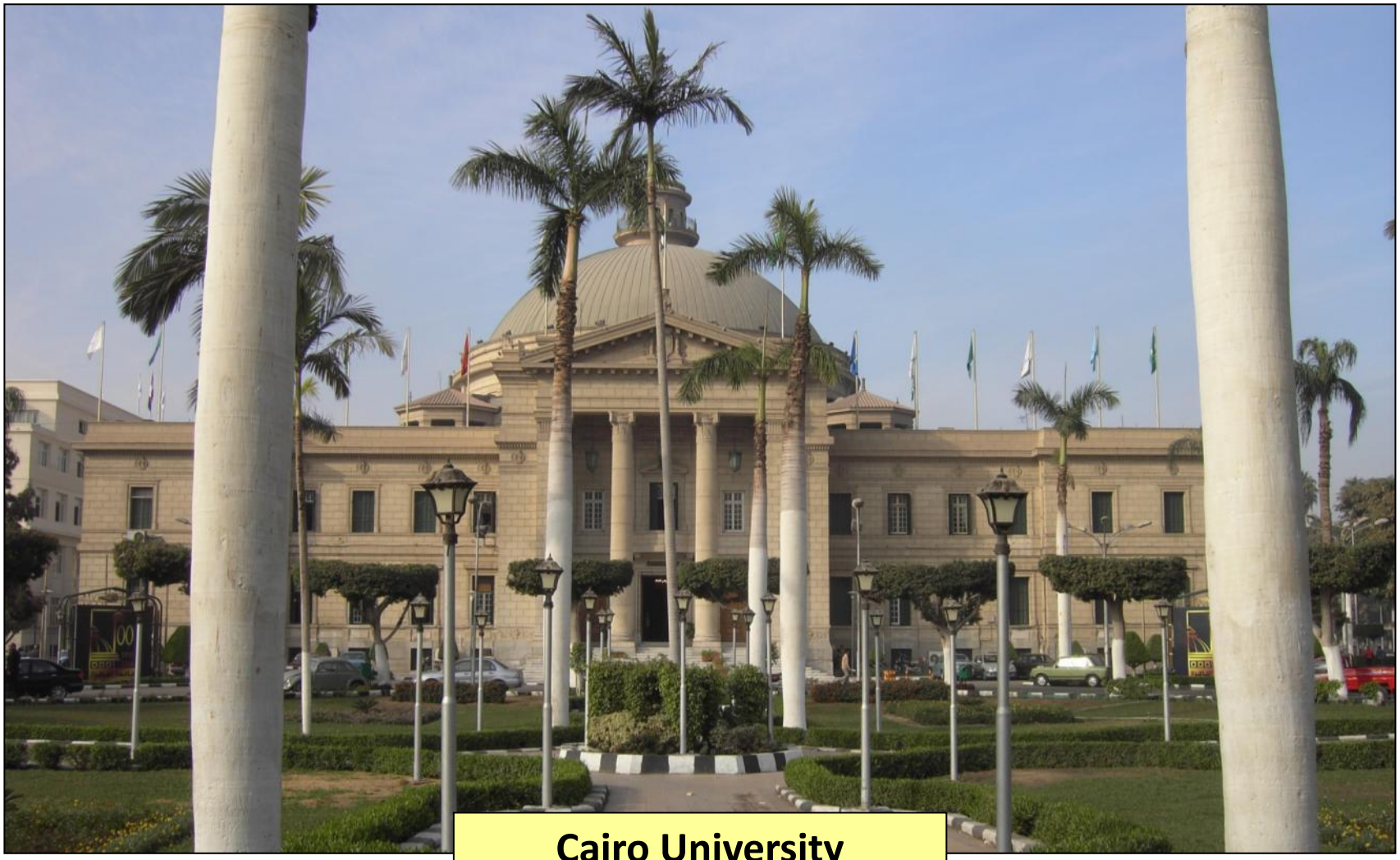
Biohydrogen Production in a Biorefinery Process



Mass flow in a biorefinery process

Kaparaju et al. (2009)

Thank You!



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