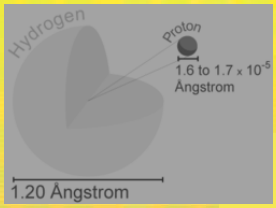
Local Consortium Photosynthetic Marine Bacteria for Hydrogen Production



Dr. Dwi Susilaningsih & Team

Research Center for Biotechnology
Indonesia Institute of Sciences
Jl Raya Bogor Km 46 Cibinong Bogor
Indonesia 16911
Tel. +62-21-8754587 or +62-21-87910507
Fax. +62-21-8754588
Email: dwis002@lipi.go.id
dwisusilaningsih@yahoo.com.sg

1

Bioproduction Green Energy Hydrogen from Lignocellulosic Waste

HYDROGEN

2

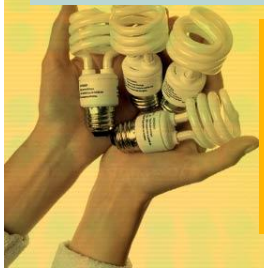
What is Bioenergy and biohydrogen

The term "bioenergy" refers to all types of energy derived from biofuels. Biofuels are fuels derived from matter of a biological origin, or biomass.

FAO categorizes biofuels according to the source of biomass used in production – forest, agriculture or municipal – and the state of the product. Thus, biofuels comprise woodfuels, agrofuels and municipal by-products and each of these groups is divided into solid, liquid and gaseous forms of fuels that can be used for heat or power generation. Taking woodfuels as an example, the following main groups are defined:

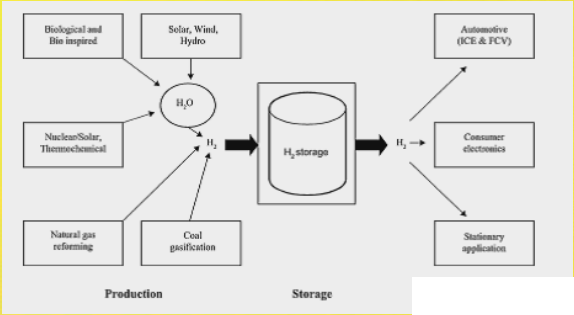
- solid woodfuels – fuelwood (wood in the rough, chips, sawdust and pellets) and charcoal;
- liquid woodfuels – black liquor (a by-product of the woodpulp industry) and ethanol, methanol and pyrolytic oil (from the thermochemical and biochemical breakdown of wood);
- gaseous woodfuels – pyrolitic gas (produced from the gasification of solid and liquid woodfuels).

Hydrogenium: Hydro & genes: water component



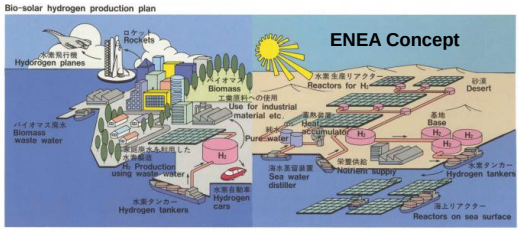
Unsur hidrogen dapat membentuk senyawa dengan hampir seluruh elemen, sebagai contoh adalah air dan senyawa organik. Hidrogen berperan penting dalam kimia asam-basa, banyak reaksi kimia melibatkan pertukaran proton diantara larutan senyawa, dan dari studi energetik dan ikatannya atom hidrogen berperan penting dalam perkembangan mekanik-kuatum. Hidrogen merupakan energi karier yang sangat kuat bahkan bentuk tritiumnya dapat menghasilkan energi ledakan, yang dikenal dengan bom atom (Voet dan Voet, 1995).

Hydrogen and its application



Future Hydrogen Society

Future (Photo)Biological hydrogen production



State of the Art: Research on biohydrogen

Biofuel	Process	Status	Engine application
Biomethanol	Thermochemical/microbial	Pilot plant	[Pure/blend] MTBE/biodiesel
Bioethanol	Microbial	Industrial	Pure/blend
Biobutanol	Microbial	Pilot plant/industrial (until ca 1990)	Pure/blend
ETBE	Chemical/microbial	Industrial	Blend
Biomethane	Microbial	Industrial	Pure/blend
Biohydrogen	Microbial	Laboratory	Bioethanol (Syngas)/pure
Biodiesel	Physical/chemical (enzymatic)	Industrial (laboratory)	Pure/blend

Physical Method for Energy recovery

Waste Water

The combustion energy of the organic compound contained in the waste water from a food factory:

Equal to 0.054 M of Glucose/per 1 Liter

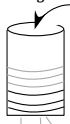
Combustion of 0.054 mol glucose **150 kJ**

Energy to evaporate i Liter of water **2260 kJ**

-)

-2110 kJ

Biological Method for Energy recovery



Utilization of microorganisms *yeast, bacteria*

The combustion energy of biological products per 1 Liter

Conversion to ethanol by yeast **148 kJ**

Conversion to methane gas by methanogen **145 kJ**

Conversion to hydrogen by photosynthetic bacterium **186 kJ**

Miyake et al, 1998

5

Promising substrates: Lignocellulosic Biomass

BIOMASSES INDONESIA→ YESS!!!

- TERESTRIA BIOMASS
- MARINE BIOMASS

1. PALM OIL
2. COCNUT
3. SOYBEAN DAN PEANUTS
4. JATROPHA
5. SUGAR CANE AND GRASSES
6. FOREST BIOMASS
7. WASTES (AGRICULTURE, FORESTRY, DOMESTIC, MUNICIPAL, FEEDSTOCK)
8. MARINE BIOMASS
9. MICROBES

KEY-POINT

Energy Sources

6

Biological Process for Biodelignification

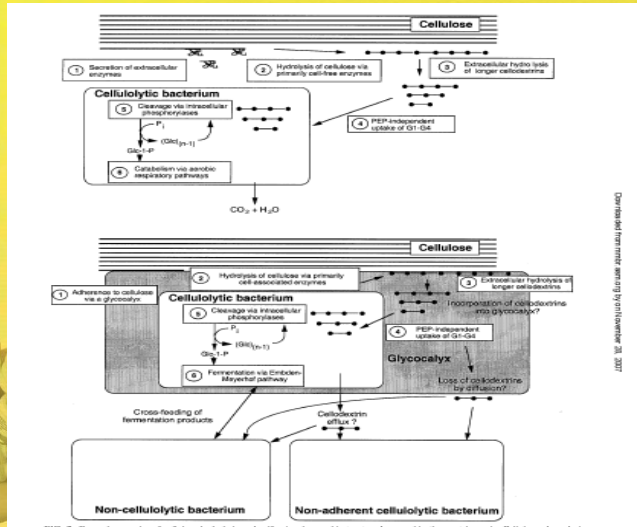
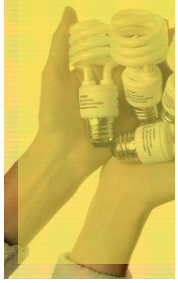


FIG. 2. General strategies of cellulose hydrolysis and utilization by aerobic (top) and anaerobic (bottom) bacteria. Cellulose degradation products and cellular features are not shown to scale. Some features of the alternate strategy type are utilized by one or more species. For example, the cellulose of the anaerobe Clostridium stercorarium is of the noncomplexed type, and members of the facultatively anaerobic Cellulomonas utilize an aerobic-type strategy for hydrolyzing cellulose but perform a mixed-acid fermentative catabolism of the hydrolytic products. Glycocalyces are the dominant means of adhesion among ruminal cellulolytic bacteria, but the importance of such structures in other anaerobic groups has not yet been systematically investigated. Refer to Fig. 1 for a more detailed comparison of complexed and noncomplexed systems at the enzymatic level.



7

The New Industrial Biorefinery

Biomass Feedstock

- Trees
- Grasses
- Agricultural Crops
- Agricultural Residues
- Animal Wastes
- Municipal Solid Waste

Conversion Processes

- Enzymatic Fermentation
- Gas/liquid Fermentation
- Acid Hydrolysis/Fermentation
- Gasification
- Combustion
- Co-firing

USES

Fuels:
- Ethanol
- Renewable Diesel
- Hydrogen

Power:
- Electricity
- Heat

Chemicals
- Plastics
- Solvents
- Chemical Intermediates
- Phenolics
- Adhesives
- Furfural
- Fatty acids
- Acetic Acid
- Carbon black
- Paints
- Dyes, Pigments, and Ink
- Detergents
- Etc.

Food and Feed

PURPOSES AND OBJECTIVES

The works purposes are bioproduction of carrier energy hydrogen from the agriculture and plantation waste

Objectives of the works:

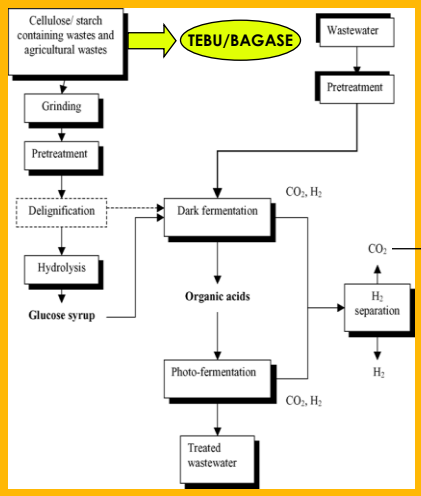
1. Utilization of lignocellulosic biomass for fermentation substrate.
2. Photofermentation strategy for hydrogen conversion from hydrolysate treated lignocellulosic biomass
3. Culture collection of microbes which is involved in bioproduction hydrogen
4. Data base

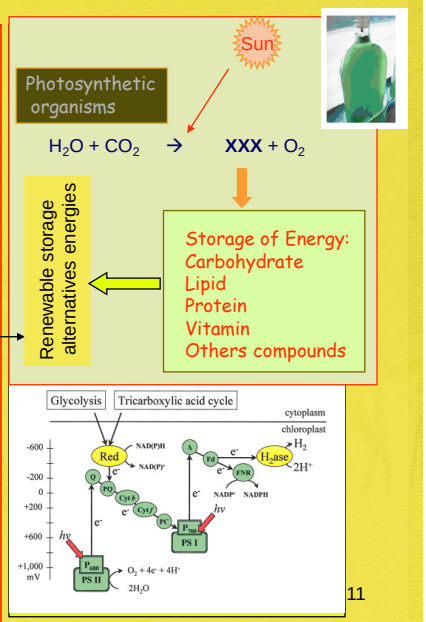
9

LIGNOCELLULOSIC BIOMASS

10

STRATEGIES





11

THREE GROUP OF MICROBES WHICH COULD BE AGENT FOR BIOPRODUCTION HYDROGEN

Photosynthetic organisms:

- **MICROALGAE : *Cyanobacteria* and others**
- **PHOTOSYNTHETIC BACTERIA: *Rhodobium, Rhodobacter, Rhodococcus***

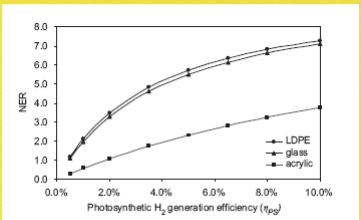


Fig. 1. Net energy ratio (NER) as a function of photosynthetic H_2 generation efficiency (η_{PS}) for a photobioreactor diameter $D = 0.09$ m, for LDPE, glass, and acrylic tube materials. NER values calculated as for Table 4.

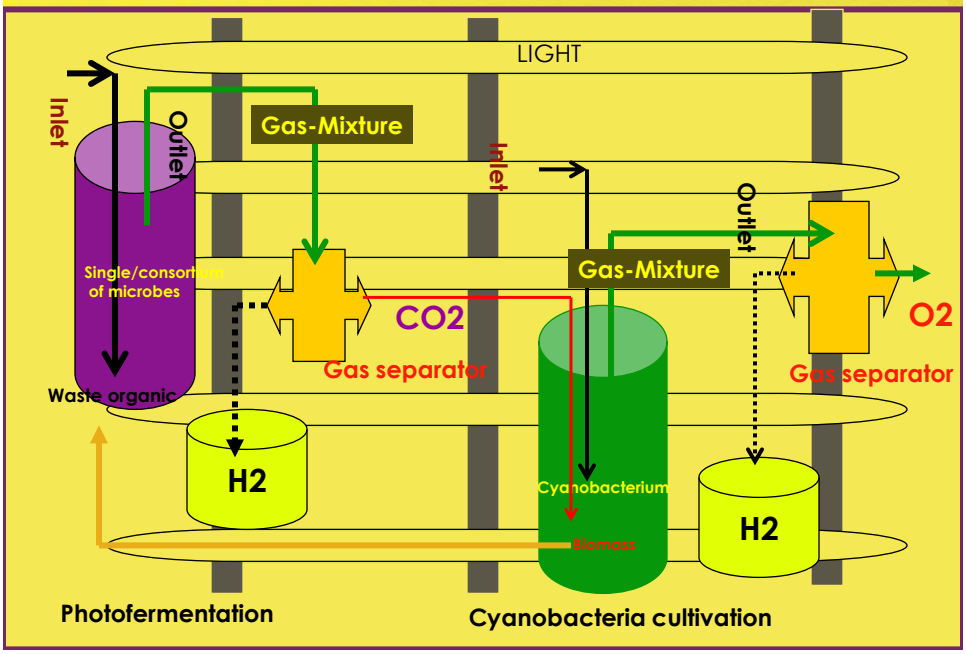
Greg Burgess[a,*], Javier G. Fernández-Velasco[b]
International Journal of Hydrogen Energy 32 (2007) 1225–1234

Non-Photosynthetic organisms:

- ANAEROBIC BACTERIA: *Enterobacter & Clostridium*

12

PROCEDURES



Component of lignocellulosic biomass

Cellulosic biomass	Cellulose	Hemicellulose	Lignin	Ash
Bagass	41	24	18	2
Straw	35	35	6	8
Empty Fruit Bunch Palm Oil	40-55	20-35	25-30	0.2-2.0
(%)				

14

Hydrolyses results of lignocellulosic biomasses

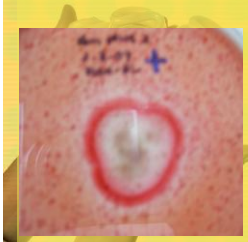
Method	Efficiency	Highest hydrolysate (% total sugar obtained)
Chemically process Dilluted acid	**5 – 30 %**	**BAGASS (30 %)**
High concentration acid	**70 90 %**	**Husk (90 %)**
Biological method Consortium fungus	**10 – 50%**	**BAGASS (50 %)**
Single fungi	**1-10 %**	**Husk sterile (10 %)**

15

Biodeliginification by local fungus

a. 4 RALS F
b. 4RALS G
c. BGS-BPPG
d. K+t1
e. SKM2 CKIT
f. SKM2 Orange

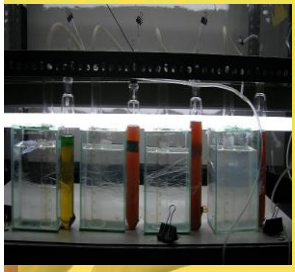
Enzymes activities of the isolated strain

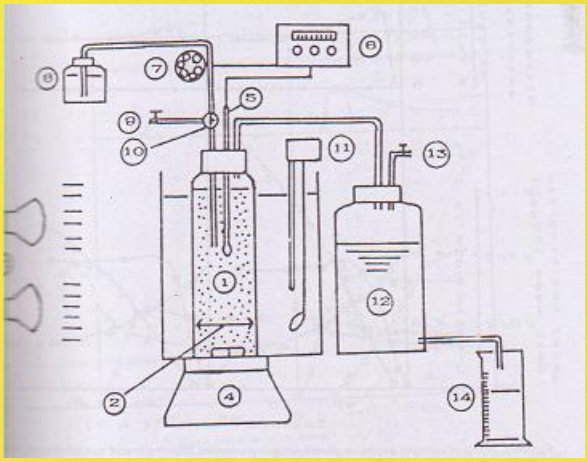


FUNGUS	XYLANASE	MANNANASE	CELLULASE	HUSK	SUGAR CANE	GRASS
Palm oil 1 (b)	+++	+	++	+	+	-
Palm oil 2 (a)	+	+	-	+	+	+
Decay woods(d)	++	+	-	+	+	+
Vegetables		+			+	

16

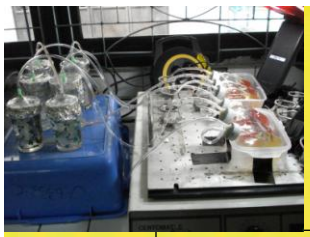
Reactors





Gambar 4. Fermentatif reaktor untuk produksi hidrogen
Keterangan: 1;kultur, 2;bidang cahaya, 3;sumber cahaya, 4; magnet stirer, 5; elektroda pH, 6; kontrol pH, 7; pompa pelistalik, 8; larutan pengontrol pH, 9; tempat sampling, 10; kok 3 arah, 11; kontrol temperatur, 12; NaCl padat, 13; gas outlet, 14; gelas silinder

17



Hydrogen production

Glucose 1%	H2 production (mmol/day/Liter culture)	H2 production (mol/mol glucose)	efficiencies (%)
Rhodobium marinum (Reference)	**1,9244** **0,4800**	**7,8** **1,94**	**64,95** **16,20**
SANUR CONSORTIA (local strains)	**0,1137** **2,0355**	**0,46** **8,2356**	**3,84** **68,70**

18

CONCLUSION REMARKS

- 1. Lignocellulosic biomasses are available for photofermentation substrates of energy production
- 2. Local consortium photosynthetic microbes are capable for hydrogen conversion comparable to the reference bacteria.
- 3. Culture collection for energy purposes

19

WHAT SHOULD DO?

Research on:
- Feedstocks and Co-products
- Cellulosic Technologies
- Sustainable Harvesting

Incentives for:
- Production
- Consumption
- Infrastructure

Education for:
- Policymakers
- Universities
- Farmers
- Environmental Groups
- Health Organizations

20

AKNOWLEGEMENT

- TEAM RESEARCHER
- PROGRAM KOMPETITIVE LIPI
- KRI-JAPAN
- OSAKA UNIVERSITY-JAPAN
- UNUD, UI, UNDIP

21

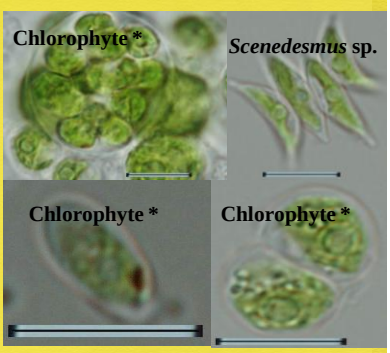
Natural Samples of Marine Microalgae

Scale bars; 10 μm ***unidentified**

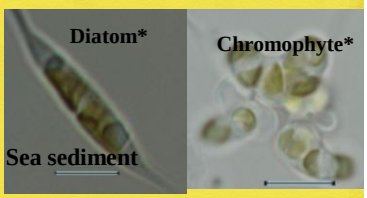
(brackish area)

Port

Swimming Beach



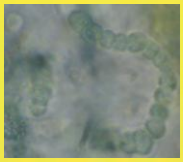
River nearshore area

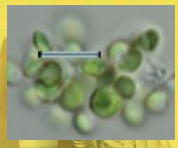


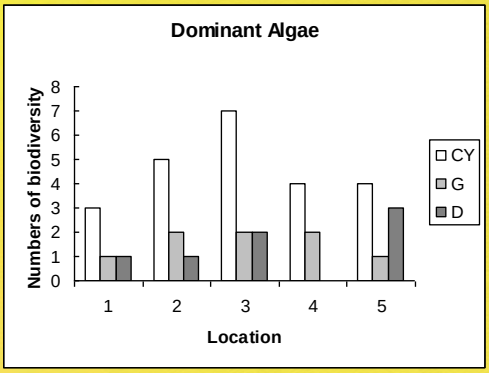
22

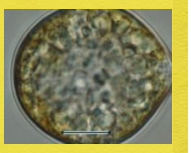
They call Beauty, we call Biodiversity algae

CYANOBACTERIA

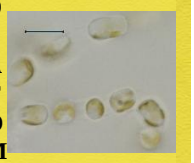




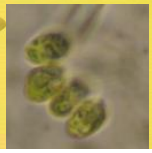




DIATOMAE



CHLOROPHYTES



Scale bar : 10 µm

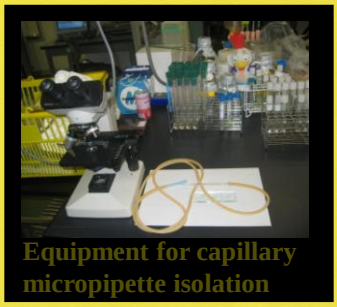
23

Strategies for collecting microalgae from natural environments

- **1. Selection of media (nutrient)**
- **2. Consideration of the sampling area and material**
- **3. Enrich sample**
- **4. Isolation (important and most barrier)**
- **5. Identification**
- **6. Cultivation and maintenance the culture**

24

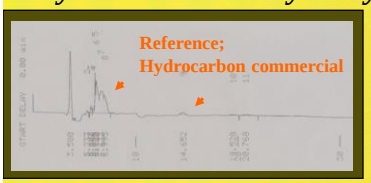
Isolation technique: by capillary micropipette

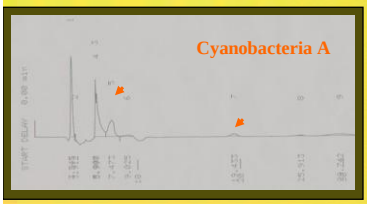


Equipment for capillary micropipette isolation

25

Hydrocarbons analyses by HPLC (MRI, 2008)





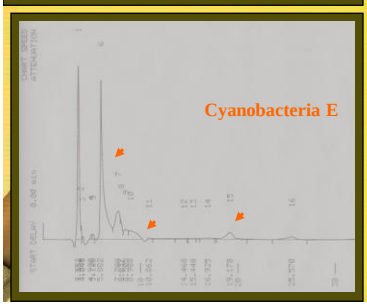


Table of Hydrocarbons analysis from selected microalgae

Isolates	Prediction Carbon content
1. Green algae	C16-C32
2. Cyanobacteria	
A	C18-C24
B	C14-C22
C	C18-C25
D	C14-C18
E	C18-C24
F	C17-C23
G	C18-C24
H	C14-C18
I	C12-C20
J	C16-C22

26

Consensus base on peak area of HPLC detection

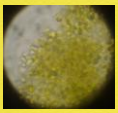
Previous results and LIPI collection

Botryococcus sp.

Reference image dari Broock, 2003

Kandidat mikroalga penghasil hidrokarbon dr laut Indonesia (kep. Seribu)

TORAY FUND 2006



Klorofita/Batam

Kriptofita/Batam

Diatom/Pari

27

LIPI PHOTOSYNTHETIC MICROBES COLLECTION: bacteria and microalgae

28

- **Biodiesel from Indigenous Indonesian Marine Microalgae, *Nannochloropsis* sp.**

  SEAMEO BIOTROP 2008

  FINAL REPORT

Nannochloropsis sp. 60-70% lipid content base on dry weight cells.

0 day

14 days

29