RESEARCH CENTER FOR BIOTECHNOLOGY

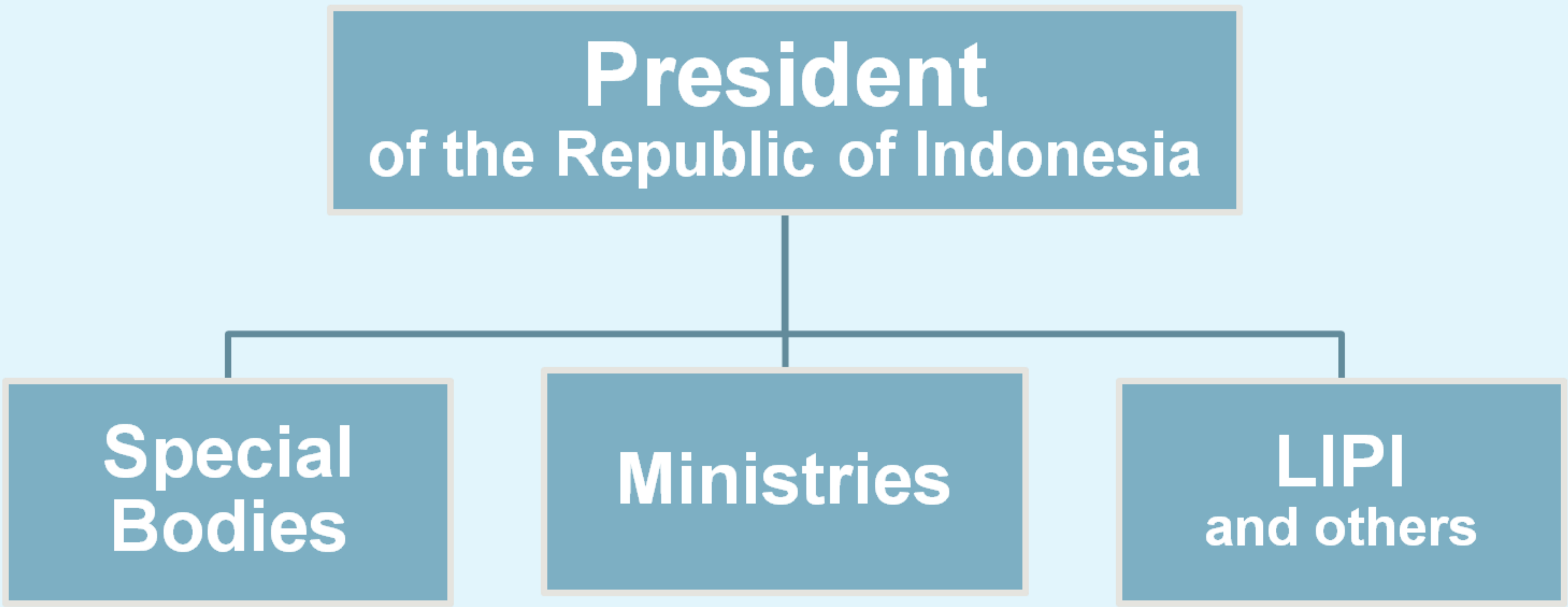
LIPI

Indonesian Institute of Sciences

Utilization of Liquid Waste from Palm Oil Industry in Sumatra and Java Islands, Indonesia for Hydrogen bioproduction.

DWI SUSILANINGSIH, Ph.D.

National Hierarchy



World Class Research Institution

- **Oldest research institution**
- Established in 1967
- Its history is much older, rooted from colonial era, in 1817 (Center for Plant Conservation/ Bogor Botanical Garden)

- **Largest research Institution**
- **Top 5% world wide (Webometrics)**
- **Leading patent contributor in Indonesia**

Largest Research Institution

- 26 Research Centers
- 16 Technical Implementation Units
- 4 Administrative Bureaus
- 2 International Center
- 4 Botanical Gardens
- 4648 employees (1543 researchers)
- Located in 11 provinces

World Class Facilities
For Biodiversity and Life Sciences

- **Herbarium Bogoriense**
- Third largest herbarium in flora reference collections
- Established in 1817

- **Zoologicum Bogoriense Museum**
- Top ten largest fauna specimen reference collections

- **4 Botanical Gardens**
- Bogor (est. 1817), Cibodas (est. 1861)
- Bali and Purwodadi (1941)

- **Indonesian Culture Collection (2014)**

Other Facilities

LIPI

- **2 Marine Research Vessel**
- Baruna Jaya VII and VIII

- **National Centre for Scientific Documentation and Information**

- **Center for Measurement and Assesment Standards serves as National Reference for Measurement and Testing**

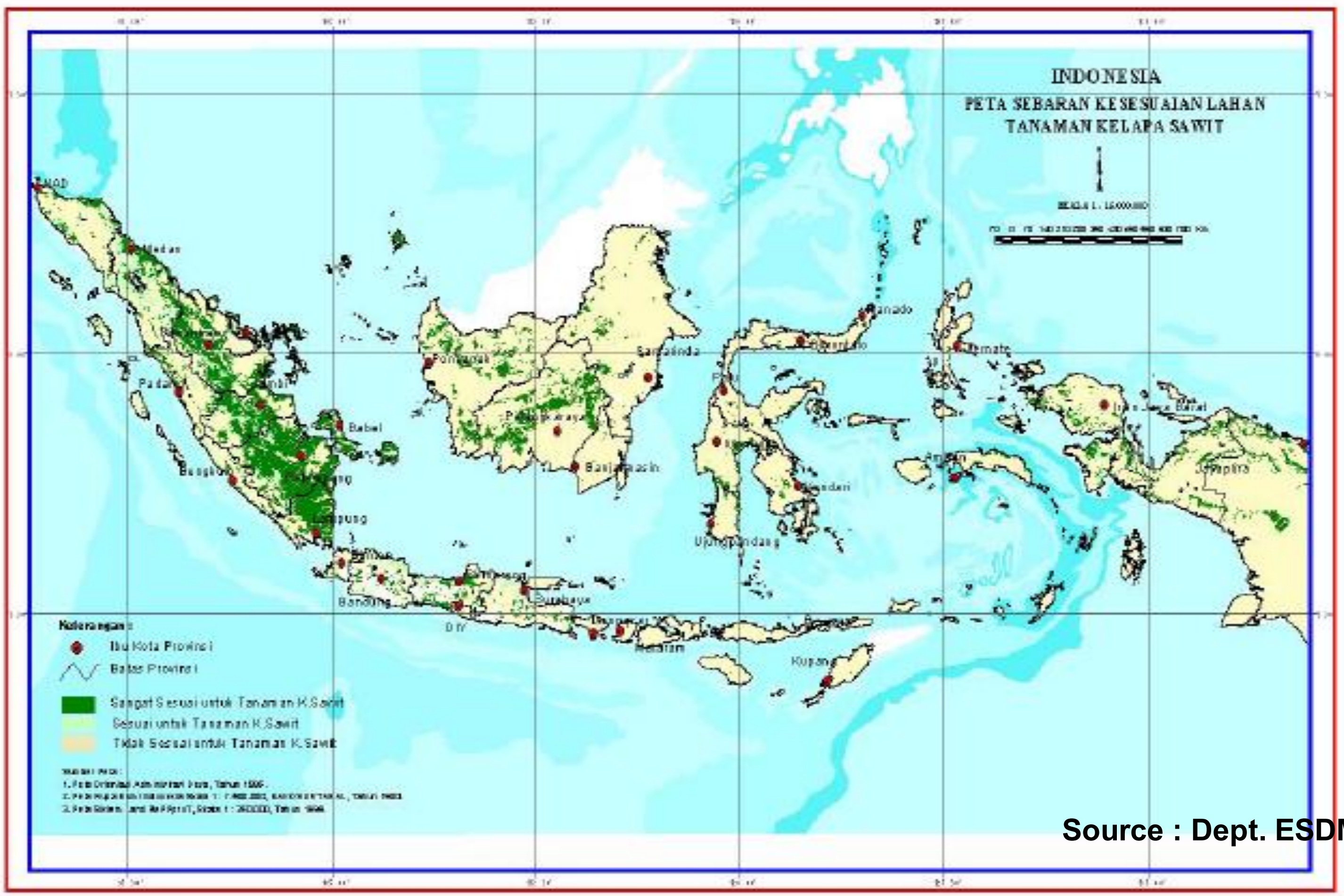
Presentation Organization

- Introduction
- Status of the POME waste in Sumatra and Java
- Strategies of the research
- Bioproduction hydrogen & gas from POME
- Conclusion

INTRODUCTION

LAND AND CLIMATE COMPATIBILITY MAP FOR PALM

(3 million hectares)



Source : Dept. ESDM

Existing Palm Oil plantation ~2014

Riau, Sumatera Utara dan Kalimantan adalah provinsi dengan lahan sawit terluas

Sumber: BPS 2014

Palm Oil plantation In SUMATERA & JAVA

LIPI

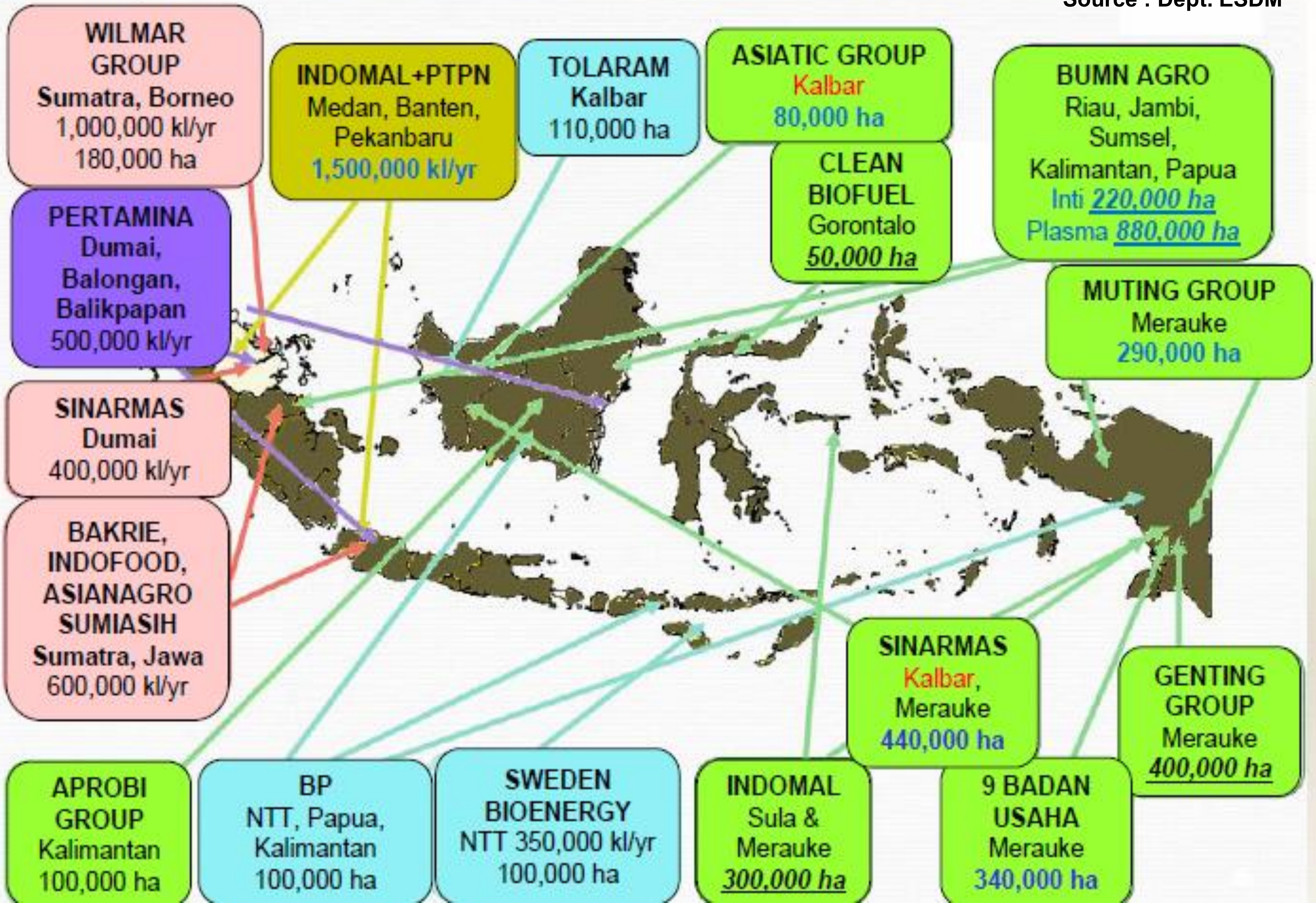
Table Area and Palm Oil Of National Private Estate and Foreign Private Estate By Province and Tree Crop Classification, 2015 **)

No.	Provinsi/*Province*	Luas Areal/*Area (Ha)*				Produksi *Production* (Ton)	Produktivitas/ *Productivity* (Kg/Ha)	Jumlah Tenaga Kerja (TK)
		TBM/ *Immature*	TM/ *Mature*	TTM/TR/ *Damaged*	Jumlah/ *Total*			
1.	ACEH	36.640	132.435	4.677	173.752	442.807	3.344	52.652
2.	SUMATERA UTARA	97.996	578.947	6.156	683.099	2.516.430	4.347	207.000
3.	SUMATERA BARAT	28.329	157.656	520	186.505	636.384	4.037	56.517
4.	R I A U	92.597	743.764	6.635	842.996	3.031.972	4.077	255.453
5.	KEPULAUAN RIAU	730	18.753	166	19.649	39.941	2.130	5.954
6.	J A M B I	26.855	219.708	1.795	248.358	786.756	3.581	75.260
7.	SUMATERA SELATAN	91.819	430.446	4.389	526.654	1.589.113	3.692	159.592
8.	KEP. BANGKA BELITUNG	24.493	132.562	18	157.073	463.209	3.494	47.598
9.	BENGKULU	18.268	83.007	431	101.706	340.926	4.107	30.820
10.	LAMPUNG	6.600	51.841	153	58.594	210.549	4.061	17.756
WILAYAH SUMATERA		424.327	2.549.119	24.940	2.998.386	10.058.088	3.946	908.602
11.	DKI. JAKARTA	-	-	-	-	-	-	-
12.	JAWA BARAT	1.548	1.925	-	3.473	5.431	2.821	1.052
13.	BANTEN	2.254	525	-	2.779	1.146	2.183	842
14.	JAWA TENGAH	-	-	-	-	-	-	-
15.	D.I. YOGYAKARTA	-	-	-	-	-	-	-
16.	JAWA TIMUR	-	-	-	-	-	-	-
WILAYAH JAWA		3.802	2.450	-	6.252	6.577	2.684	1.895

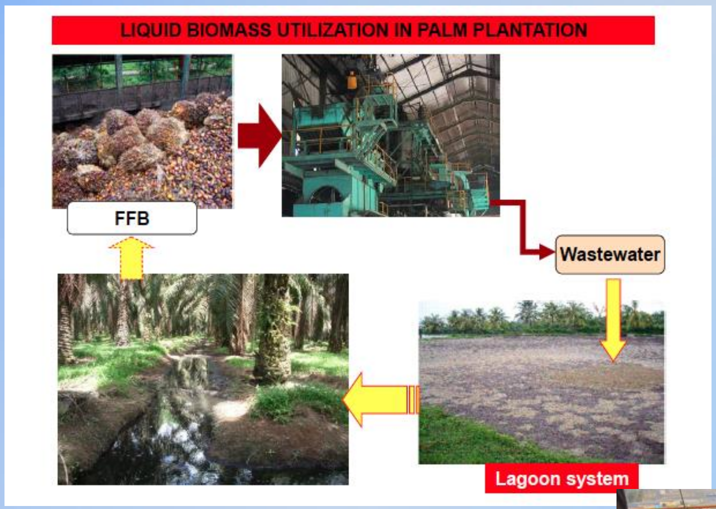
Statistik Perkebunan Indonesia Komoditas Kelapa Sawit 2013 - 2015

ADDITIONAL PRODUCTION OF BIODIESEL : 2 million kL/year (3,6 million ha): 2007 – 2011

Source : Dept. ESDM



Status of POME



Liquid waste

↓

Separation Liquid waste
Water and sludge

↓

An-aerobic treatment pond

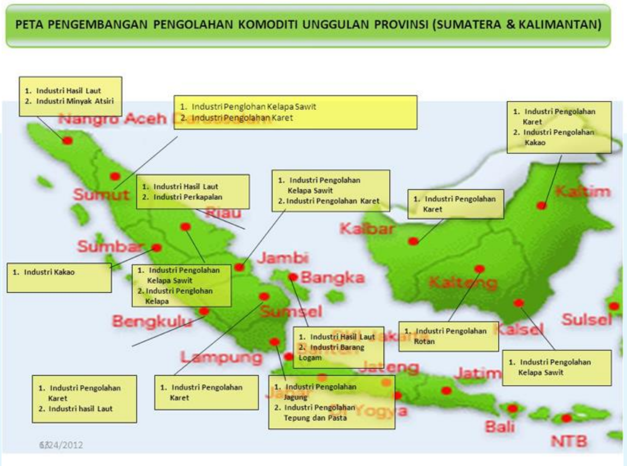
↓

Oxidation treatment pond
[active sludge degrader]

Land Application

River

Sumatera Palm Oil Industry



Luas Areal Kelapa Sawit Perkebunan Besar Swasta

Wilayah	Luas Lahan (Hektare)		
	2013	2014*	2015 **
Sumatera	2.558.113	2.693.034	2.830.123
Jawa	5.652	5.950	6.252
Nusa Tenggara & Bali	-	-	-
Kalimantan	2.428.344	2.556.419	2.686.555
Sulawesi	155.529	163.732	172.067
Papua & Maluku	65.265	68.707	72.205
Total Indonesia	5.212.903	5.487.842	5.767.202

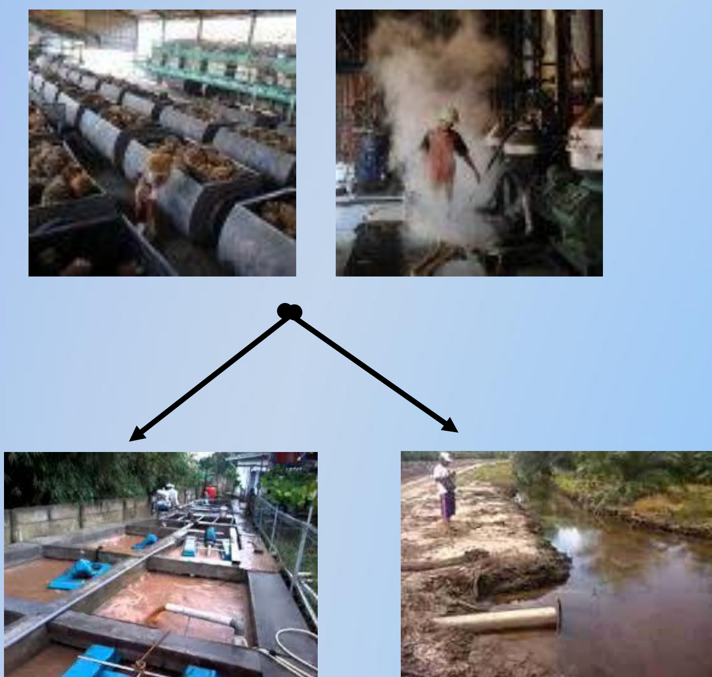
Catatan: * Data Sementara ** Estimasi
Sumber: Statistik Perkebunan Kelapa Sawit Indonesia – Direktorat Jenderal Perkebunan (2015)

Aceh	0.45 Million Ha	0.8 Million tons CPO
Sumatera Utara	1.34 Million Ha	4.7 Million tons CPO
Sumatera Selatan	1.1 Million Ha	2.85 Million tons CPO
Riau	2.29 Million Ha	7.1 Million tons CPO
Jambi	0.68 Million Ha	1.8 Million tons CPO

POME

Palm Oil Factory at Java Island

Palm oil plantation: Java,
PT Jaya Agra Wattie Tbk



- 3.1 million Ha (total area 5.1 million Ha)
- Malingping, Banten, West Java
- 3.6-7.5 tons/Ha
- 45% farmers, 7% Government, 48% private companies

http://m.tempo.co/read/news/2015/02/13/

- Summary 1: Handling of the POME waste

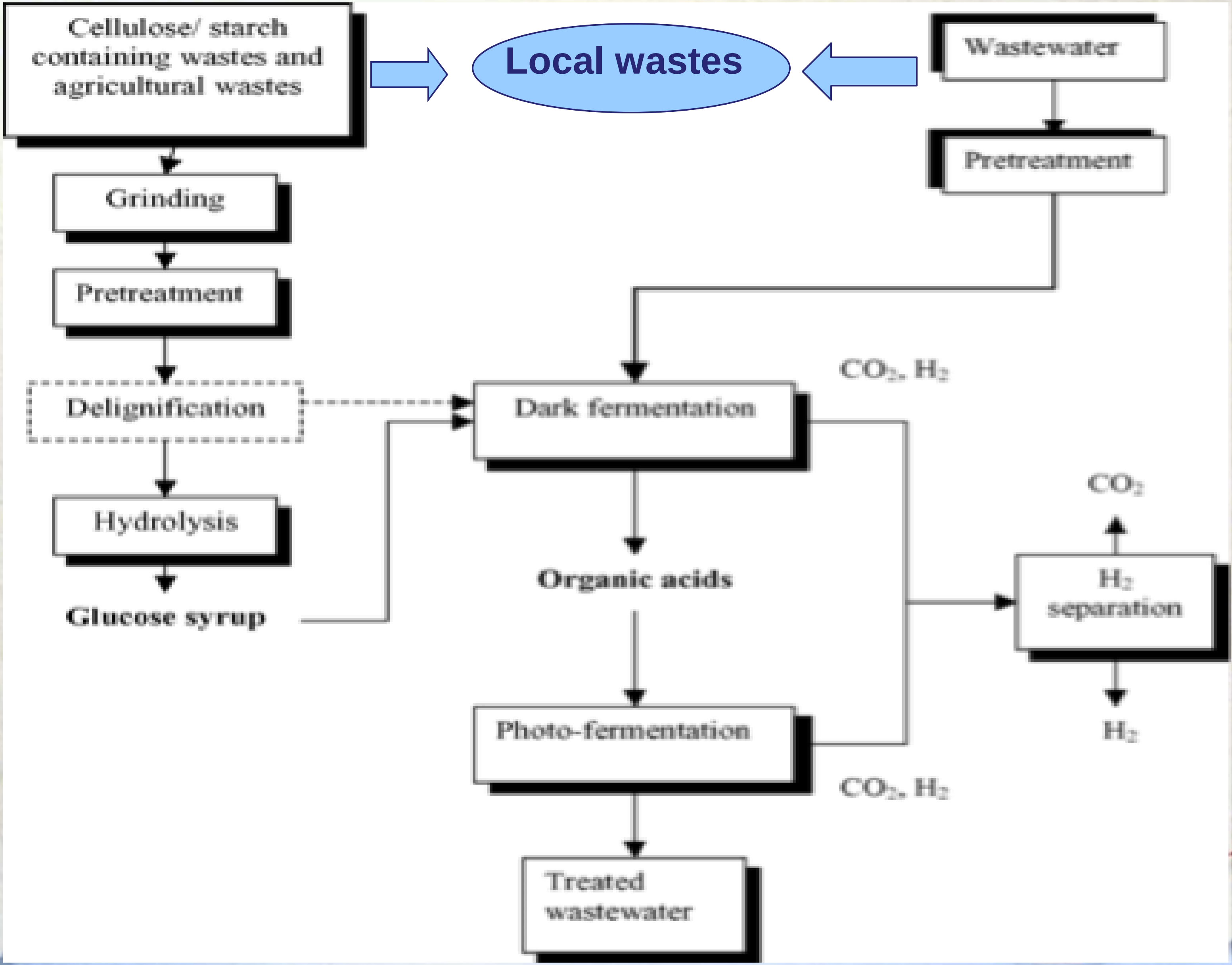
- Solid waste: briquette charcoal, concrete-block
- Liquid waste: Waste Water Treatment, Fermentation media, lagoon, dispose to the river

Strategies of The Researches

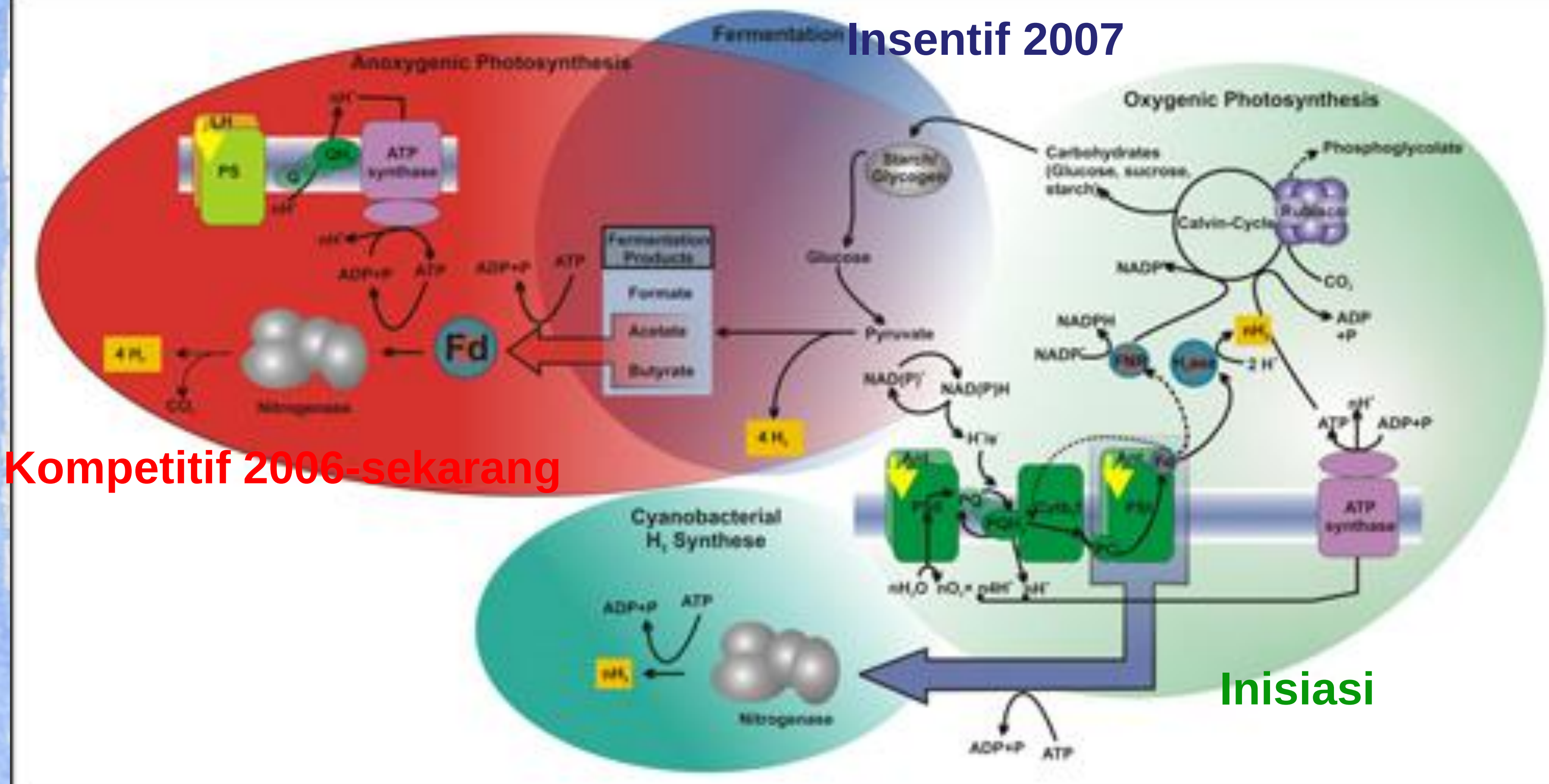
- Objectives
  - Utilization of INACC MICROBES for remedy liquid waste
  - Conversion of liquid waste to be energies
- Goal
  - Microbes potency for remediation liquid waste [in particular POME]
  - Information about conversion the liquid waste to be energy carrier by biotechnology approaches.

Schemes of the biohydrogen from wastes

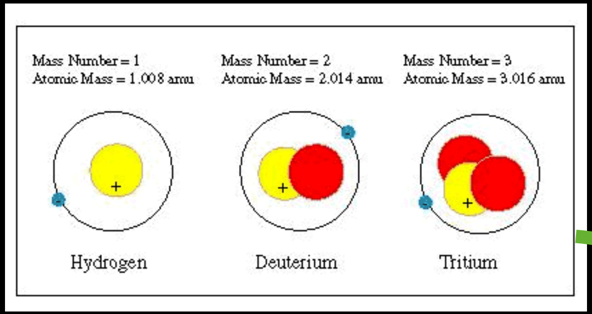
DIDANAI KOMPETITIF LIPI 2006-2008



DIDANAI OLEH KNRT 2007-X

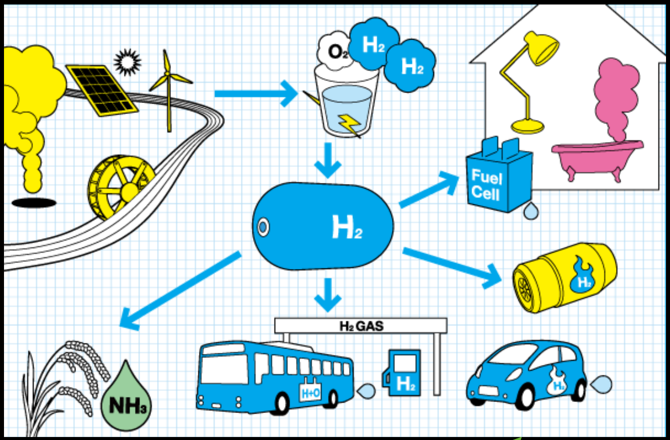

Insentif 2007
Fermentation
Anoxygenic Photosynthesis
Oxygenic Photosynthesis
Kompetitif 2006-sekarang
Cyanobacterial
H2 Synthese
Inisiasi
ATP synthase
Fd
Fermentation Products
Formate
Acetate
Butyrate
Glucose
Pyruvate
Carbohydrates (Glucose, sucrose, starch)
Phosphoglycolate
Calvin-Cycle
NADP+
NADPH
CO2
ADP+P
ATP
4 H2
Nitrogenase
nH2

Hydrogen & gas from POME



H2

[Griffith University](#), Aus.



First element in the periodic table. In normal conditions it's a colourless, odourless and insipid gas, formed by diatomic molecules, H_2. The hydrogen atom, symbol H, is formed by a nucleus with one unit of positive charge and one electron. Its atomic number is 1 and its atomic weight 1,00797 g/mol. It's one of the main compounds of water and of all organic matter, and it's widely spread not only in The Earth but also in the entire Universe. There are three hydrogen isotopes: protium, mass 1, found in more than 99,985% of the natural element; deuterium, mass 2, found in nature in 0.015% approximately, and tritium, mass 3, which appears in small quantities in nature, but can be artificially produced by various nuclear reactions.

Read more: http://www.lenntech.com/periodic/elements/h.htm#ixzz3E7fxYQlY

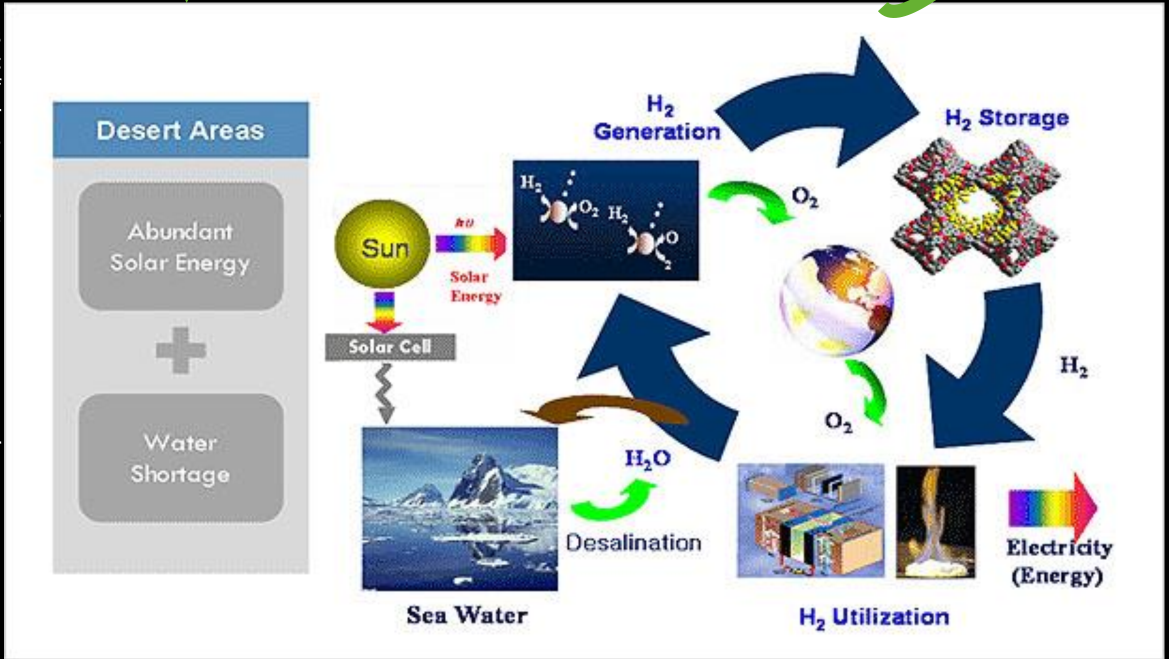


Figure 1. Interrelation of Sustainable Hydrogen with Water and Solar energy

http://eewseng.kaist.ac.kr/eng/

BIOHYDROGEN from AGROFORESTRY WASTES

Characterization of the wastes

Cellulosic biomass	Celluloses	Hemicelluloses	Lignin	Ash
Bagasse	40-45	24	18	2
Rice Straw	30-35	30-35	4-6	8
Empty fruit bunch of Palm oil tree	40-55	20-36	24-31	0.1-2.0
(%) of dry weight (average)				

Delignification Process

Methods	Yield of degradation (% of dry biomass weight)	Highest result
CHEMICALLY Diluted acid	5 – 30 %	BAGASSE (30 %)
Concentrated acid	70 -90 %	HUSK (90 %)
BIOLOGICALLY Consortia fungus	10 – 50%	BAGASSE (50 %)
Single fungi	1-10 %	HUSK STERILIZED (10 %)

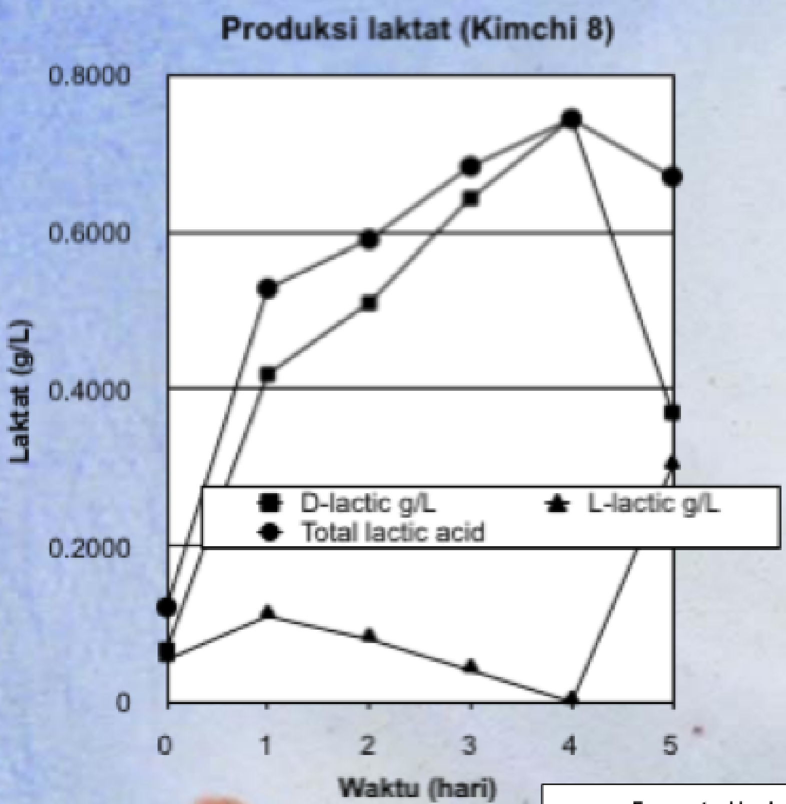


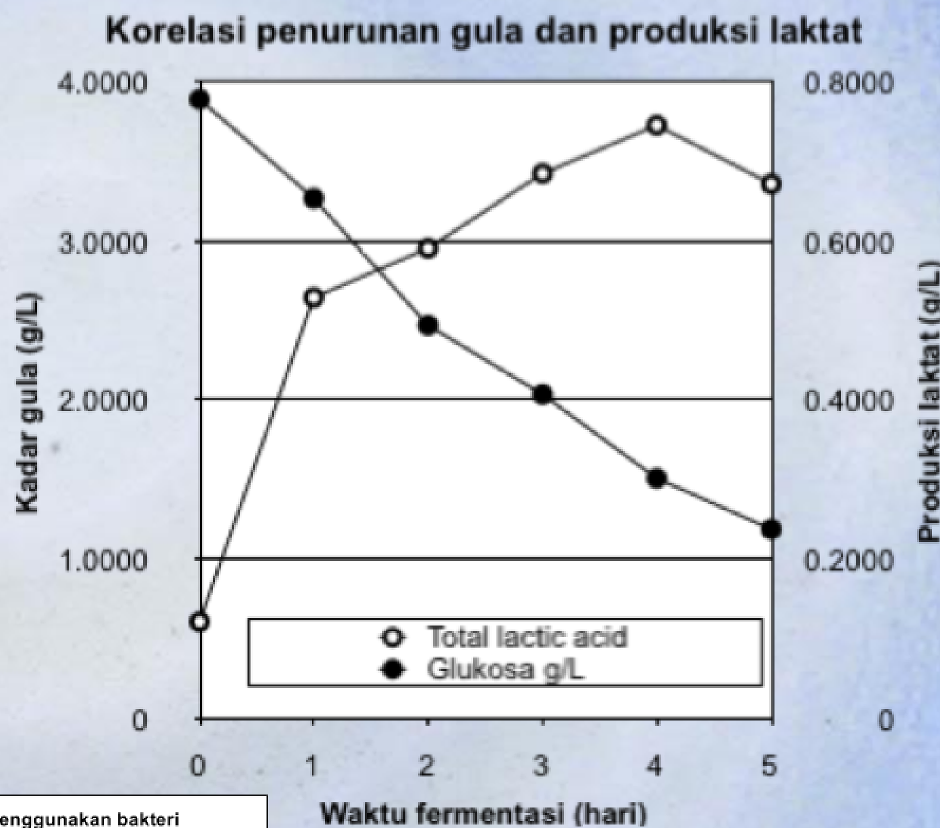


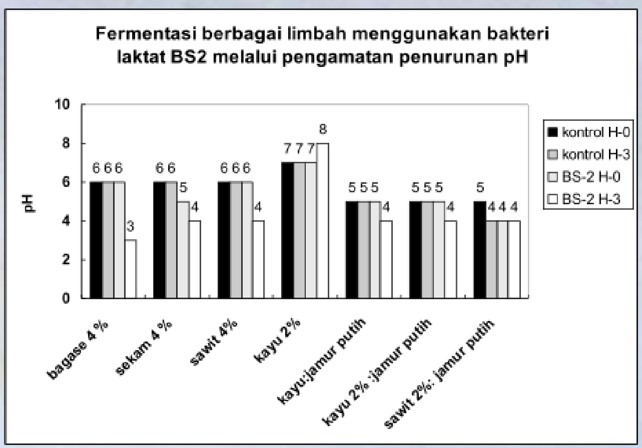
BIOLOGICALLY LIGNIFICATION BY FUNGUS CONSORTIA

Biomasses	Dry weight (g)	Origin total sugar (g)	Total sugar after hydrolisis (g)	Efficiency (%)
Wood	0.057	0.0018	0.013	19.65
Bagasse	0.052	0.0016	0.027	48.85
Husk	0.051	0.004	0.018	27.45

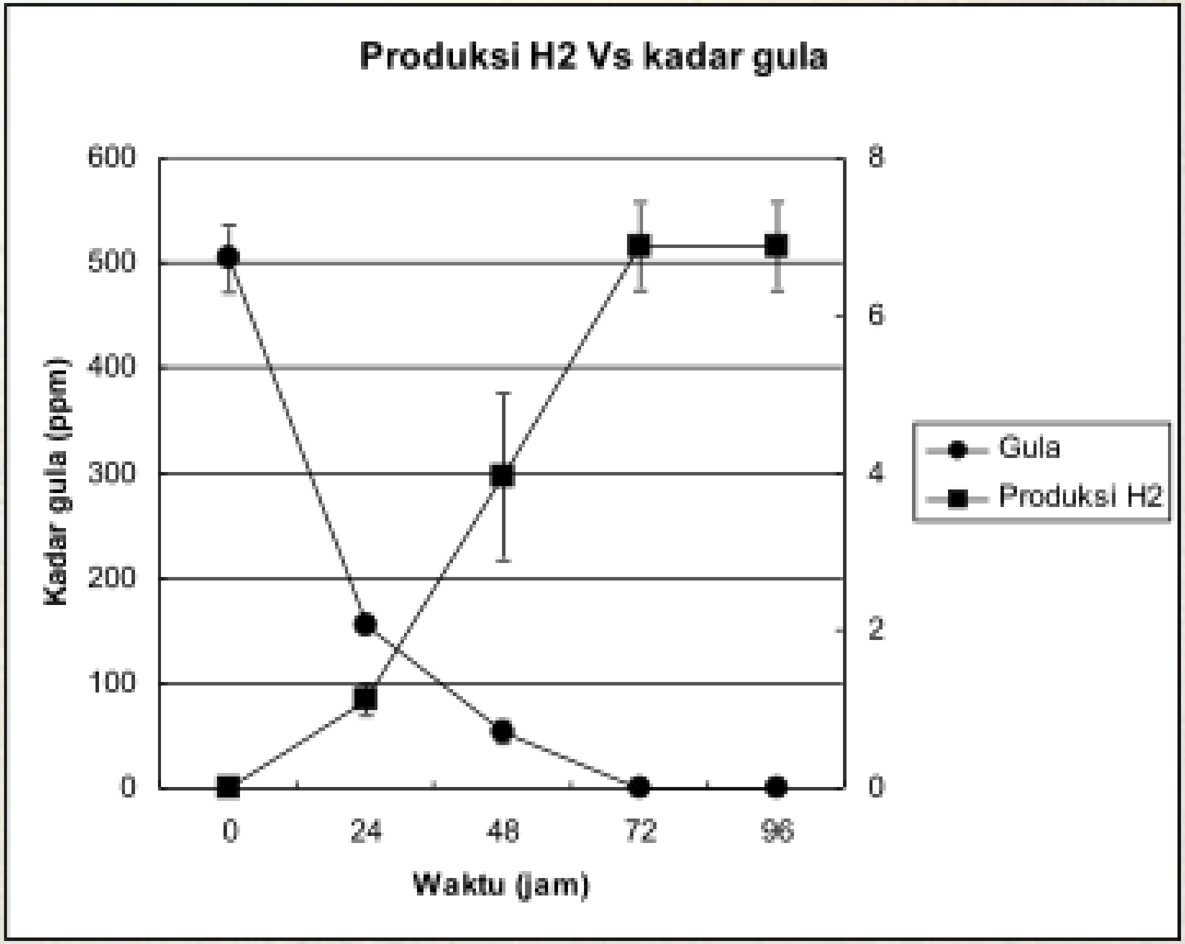
Organic acid production from the Saccharification result compounds







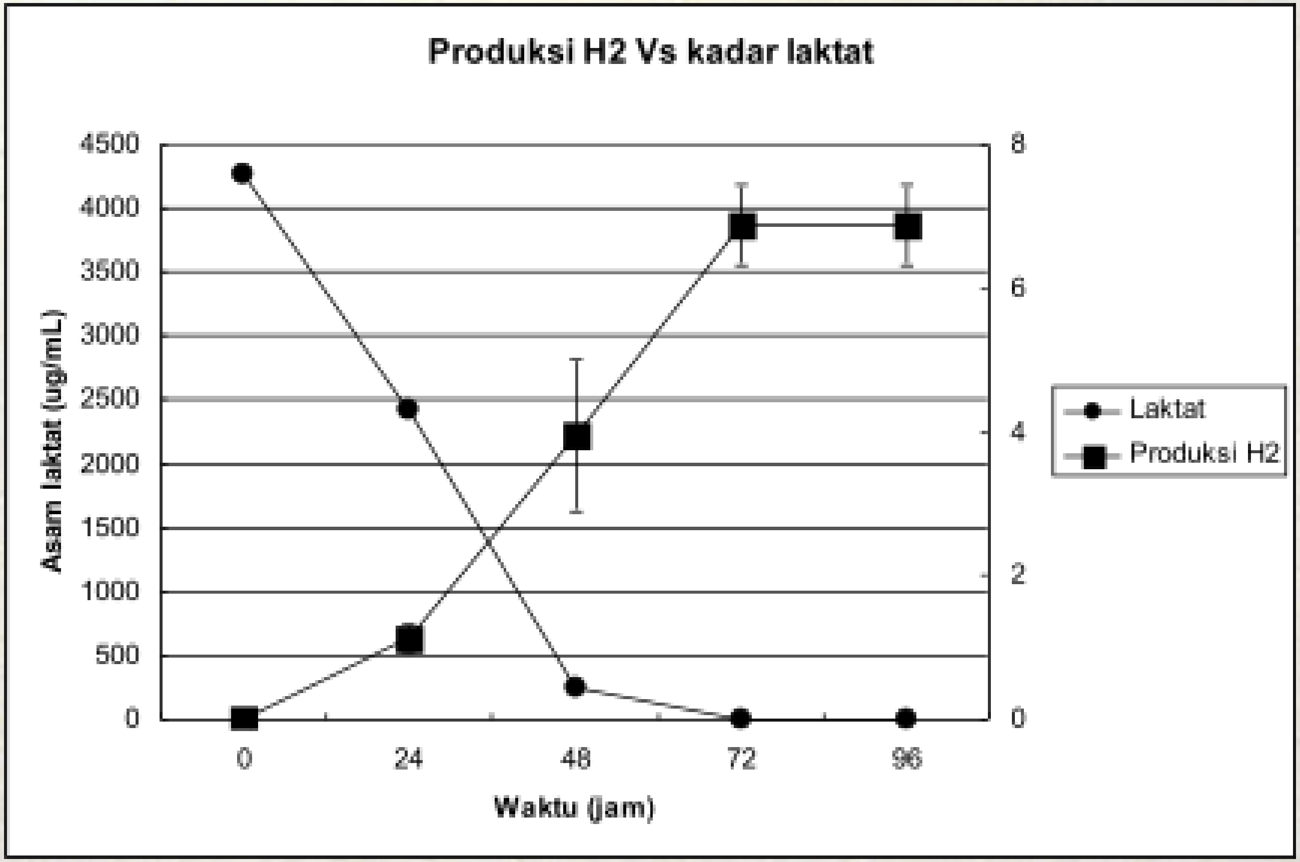
Correlation of the total sugar content and hydrogen production during fermentation



● = Kadar gula (ppm)
■ =Produksi Hidrogen (%)

100% Hidrogen (Teledyne 2240)= 10.000 ppm

Correlation of consumption of lactates and hydrogen production during fermentation



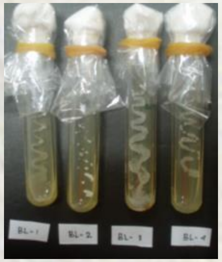
● = Kadar asam laktat (ppm)
■ =Produksi Hidrogen (%)

100% Hidrogen (Teledyne 2240)= 10.000 ppm

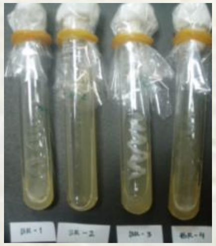
ISOLATION AND SCREENING of MICROBES WHICH INVOLVED IN BIOHYDROGEN PRODUCTION

Screening process for woody degrading microbes

Delignification agent
From decay materials



Belimbing



Brem

Bisbul

Jeruk

Sale pisang

Lactic acid bacteria
From fruits

Photosynthetic bacteria

Source; Seashore and garbage

IDENTIFICATION RESULTS AND THEIR ACTIVITIES

a. 4 RALS F=Scopulariopsis sp
b. 4RALS G=Penicillium spp.
c. BGS-BPPG=Fusarium spp. 1
d. K+t1=Fusarium spp. 2
e. SKM2 CKIT=Fusarium spp. 1
f. SKM2 Orange=Fusarium spp. 1

Prelemenary assay for enzymes specific activity

KAPANG	XYLANASE	MANNANASE	CELULASE	HUSK	BAGASSE	GRASS
sawit 1 (b)	+++	+	++	+	+	-
sawit 2 (a)	+	+	-	+	+	+
Kayulapuk (d)	++	+	-	+	+	+
Sayuran (new)		+			69 +	

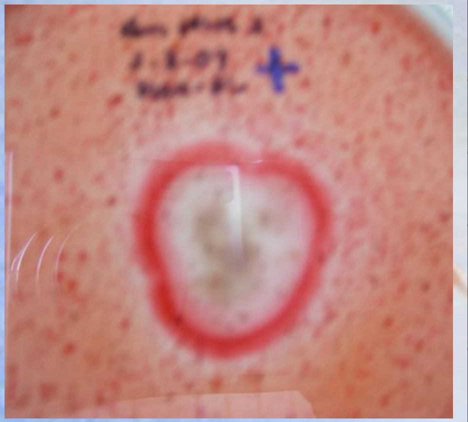
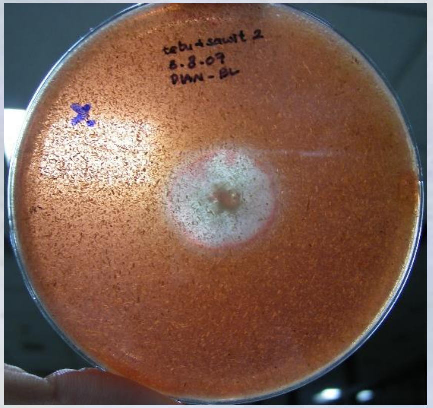


Plate assay

Hasil uji coba aktifitas enzim secara kualitatif

Metode plate assay

KAPANG	XYLANASE	MANNANASE	SELULASE	SEKAM	TEBU	RUMPUT
sawit 1	+++	+	++	+	+	-
sawit 2	+	+	-	+	+	+
kayulapuk	++	+	-	+	+	+
sayuran		+			+	

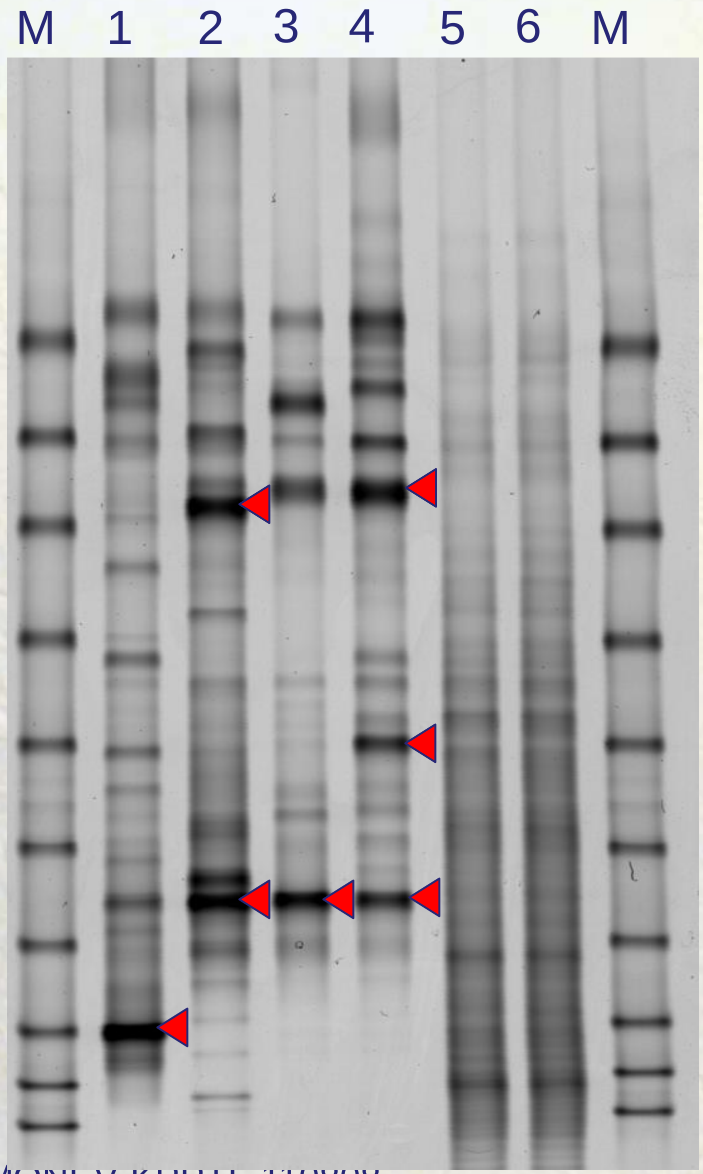


Determination results Lactobacillus

IDENTIFIKASI LAB

No dan sample	gram	bentuk bakeri	catalase	pH	OD	glu (%)	degradasi glu (%)
1. as 1	+	diplo coccus	-				
2. as 5	+	Batang?	-	4			
3. as 6	+	streptocoocus	-	4			
4. as 8	+	streptocoocus	-	4			
5. bk 5	+	streptocoocus	+	8		0.0011	95.30
6. bs 2 (Leuconostoc)	+	streptocoocus	-	4	0.4472	0.0143	40.60
7. jm 3	+	streptocoocus	-	4	0.7306	0.0195	19.06
8. jm 7	+	streptocoocus	-	4	0.3592	0.0162	32.56
9. kim 1	+	streptocoocus	-				
10. kim 10	+	streptocoocus	-				
11. kim 11	+	streptocoocus	-				
12. kim 12	+	streptocoocus	-				
13. kim 3	+	Bacil/Lactobacillus	-				
14. kim 4	+	Bacil/Lactobacillus	-	4	0.7137	0.0206	14.53
15. kim 5	+	streptocoocus	-				
16. kim 6	+	streptocoocus	-	4	0.6535	0.0222	7.61
17. kim 7	+	streptocoocus	-				
19. kim 8 (L. plantarum)	+	bacil/Lactobacillus	-	4	1.2506	0.0116	51.79
20. kim 9	+	bacil/Lactobacillus	-	4	0.8566	0.0210	12.56
21. mg 3	+	streptocoocus	-	4	0.7370	0.0186	22.91
kontrol/media				6.5	0.0157	0.0241	

Analisis molekuler pada konsorsium mikroba fotosintetik yang terlibat dalam konversi limbah menjadi gas hidrogen



16S rDNA PCR/DGGE, Dwi sample(15 April 2008)

1. **DW-1 photosynthetic bacteria (Sanur)**
2. **DW-2 photosynthetic bacteria (Amed)**
3. **DW-3 photosynthetic bacteria (Candidasa 1)**
4. **DW-4 photosynthetic bacteria (Candidasa 2)**
5. **DW-5 soil (treatment 105 ^{o}C)**
6. **DW-6 soil (without treatment)**

M. Marker (DGGE Marker II, Nippon gene Co.,Ltd)

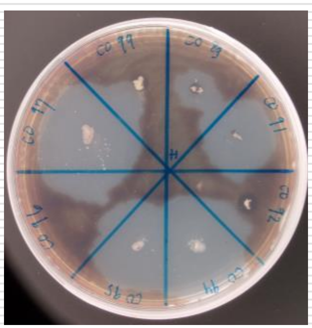
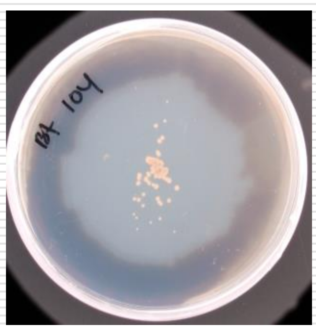
Red marks(◀) point to bands that will be further excised and sequenced.

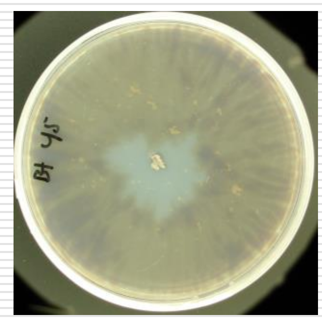
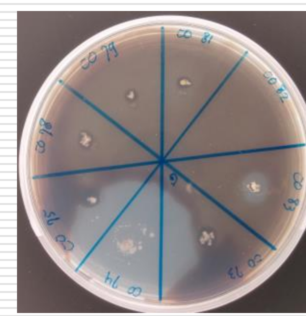
Screening surfactant producing bacteria by Plate Assay

RC Biology [114]
RC Oceanography [99]
RC Biotechnology [250]

PA → 66 isolates

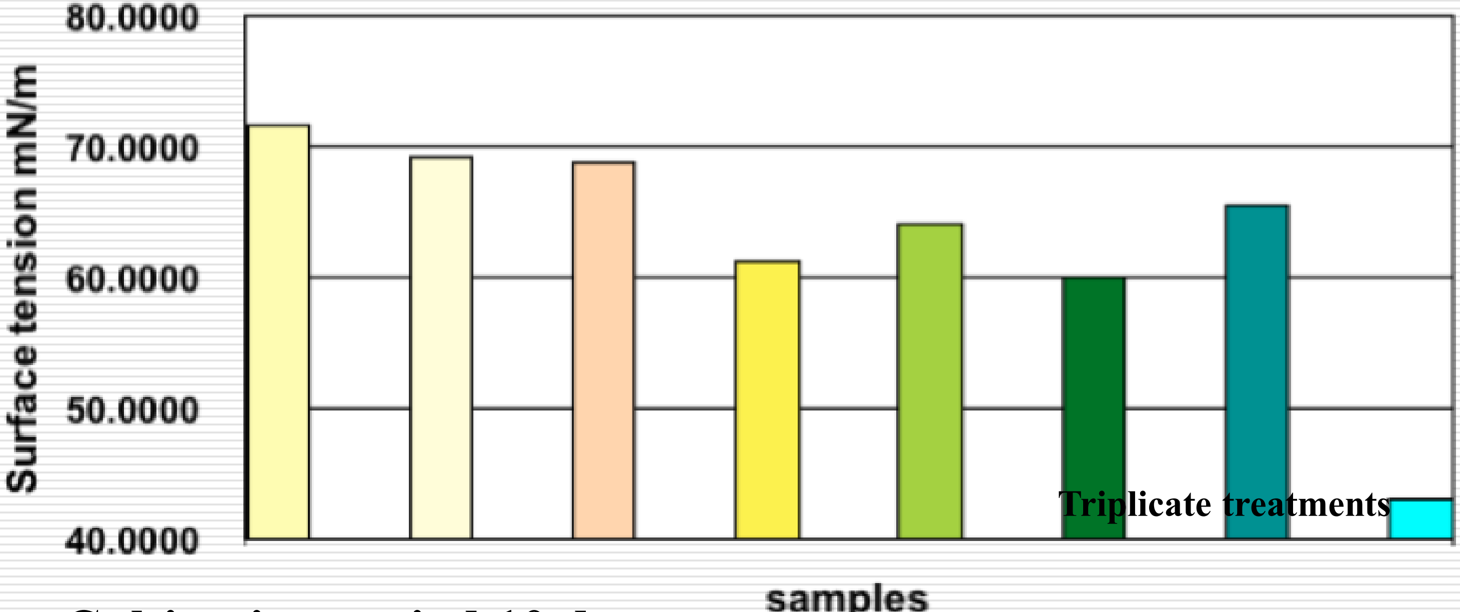
ST → Candidate: 22 isolates

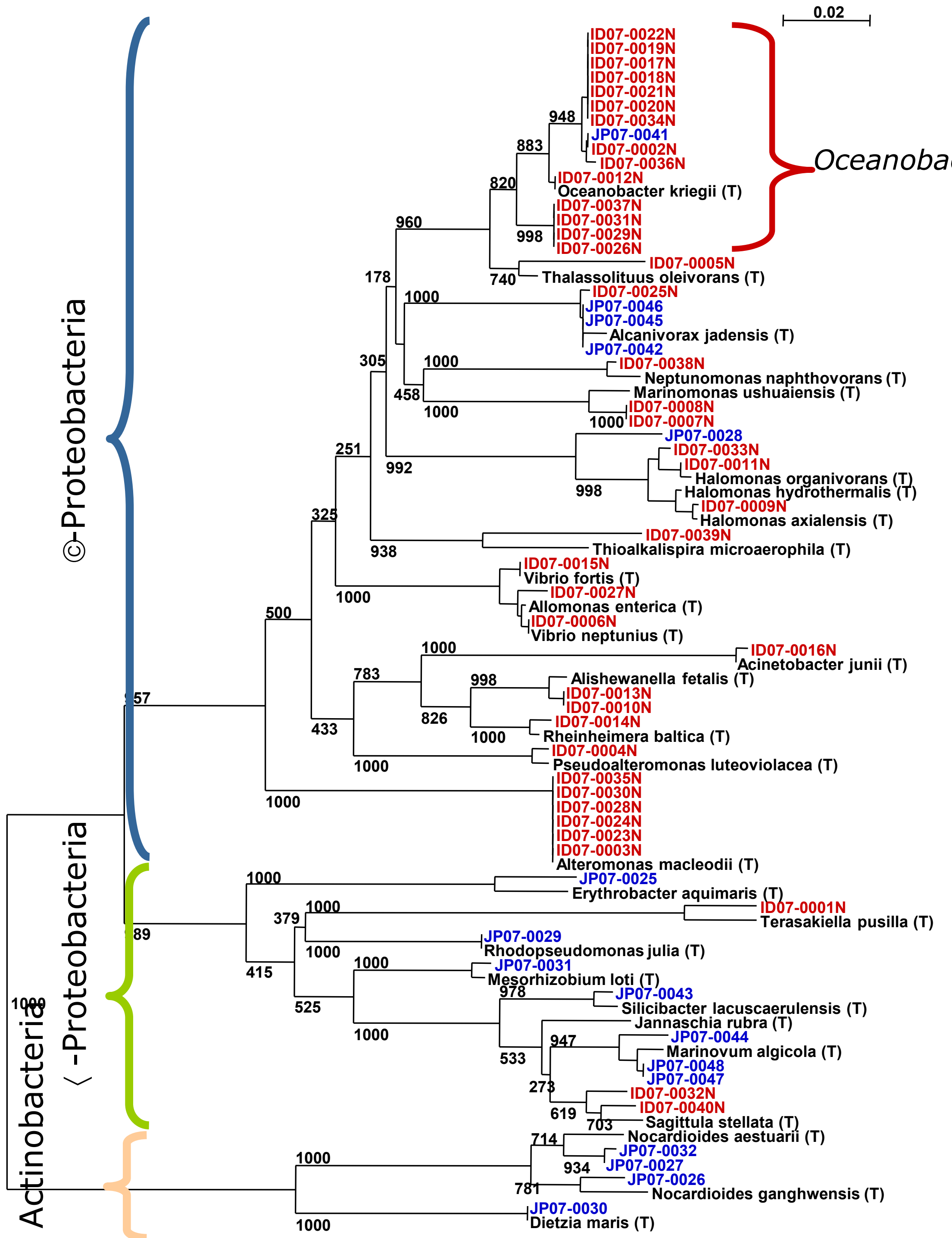



By: Kitamura & Dwi

Surfactant activities assay results



Cultivation period 10 days



Putative petroleum-hydrocarbon degrading bacteria isolated from native seawater by direct plating method

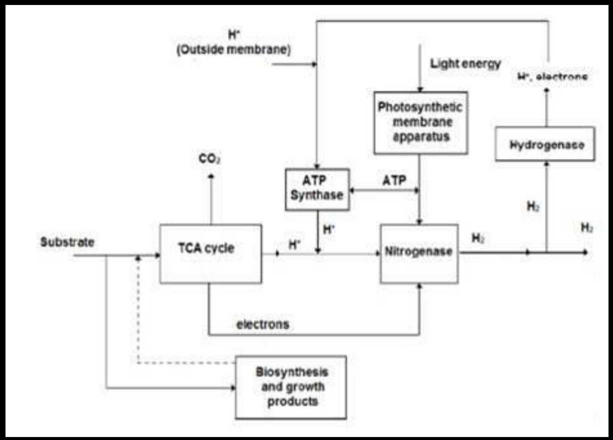
From Indonesia

From Japan

Hydrogen from Biomass

Biomass (waste)	Dry Biomass (g)	Sugar (g) before treatment	Sugar (g) after bio-hydrolysis treatment	Lactate production (g/L)	Hydrogen production (mmol)
Timber	0.057	0.0018	0.048	0.54	1.823
Husk	0.052	0.0016	0.043	0.48	3.605
Palm oil wood	0.051	0.0040	0.049	0.63	4.657
Liquid POME	-	0.0021	0.0021	-	6.543

Electricity production from biological hydrogen

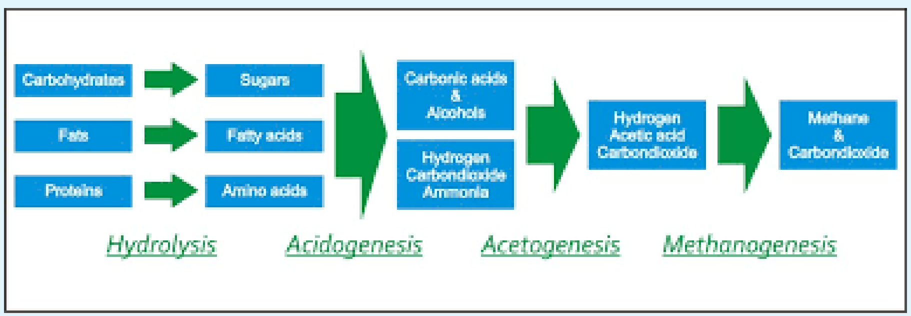


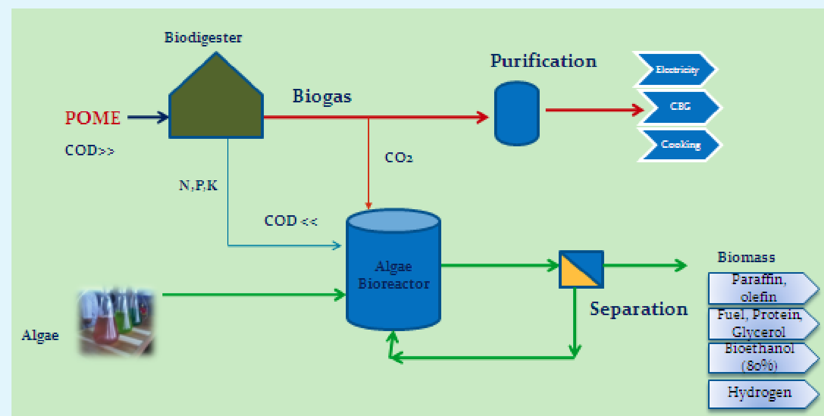
Waste	**Amount of waste (L)/kWatt electricity/Hours**
Milk	53/1/1
Soy sauce	52/1/1
Sugarcane	5/1/1
POME	3.4/l/l

* Assumption: 1 kilowatt per hour of electricity is created from 650 litres of hydrogen at an efficiency of 47%.

Conversion the waste in the field

Integrated Bio-energy Facility in Riau, Sumatra





- http://www.stcresources.com/completed-projects/integrated-bio-energy-facility-riau-sumatra/

Conversion the POME into electricity source using bioprocess in Sumatera (Lampung)

ika-usu-jkt.org

Tabel 1.
Potensi listrik dari limbah cair kelapa sawit berdasarkan kapasitas pabrik

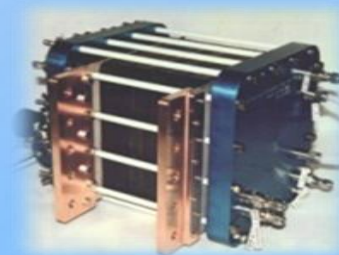
Parameter	30 tons/ hari	45 tons/ hari	60 tons/ hari
Desain aliran POME	15 m^3/ hari	23 m^3/ hari	30 m^3/ hari
Efisiensi penghilangan COD	905	90%	90%
Produksi biogas	380 - 450 m^3/hari	565-670 m^3/hari	755-895 m^3/hari
Tangkapan gas metana	55-65%	55-65%	55-65%
Produksi energi	7,400-8,750 MJ/hari	11,110-13,130 MJ/hari	14,810-17,510 MJ/hari
Potensi kapasita	+750 kWc	+1 MWc	+2 MWc

Hybrid Energy System Base on Wind and Solar energy at Malimping, Lebak, Banten

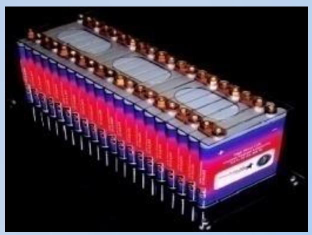
WIND TURBIN
5 kW

Elektrolisa

Gas H2

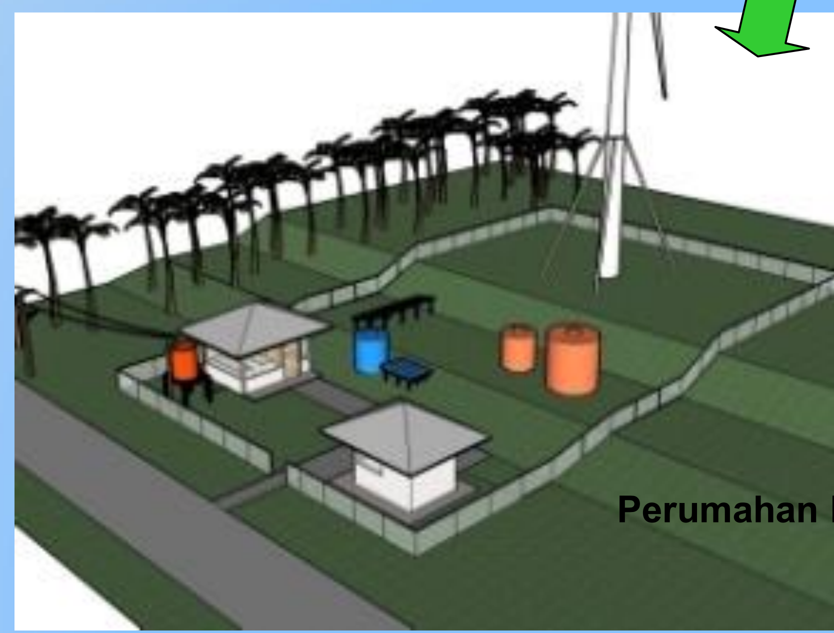


Fuel-Cell 3 kW



Baterei

SOLAR CELL
1 kW



Perumahan Penduduk

Microbial consortium – converting organic substances to biogas for coocking and electricity with a good quality of organic fertilizers as by-product

With 2 cattles, could provide a farmer family with a 2 hours continuous supply of coocking energy and 7 hours of 0.5 to 1.0 k W of electricity ....

Some Advantages:

- Improve quality of environment (biogas instalation a better way in managing organic waste)
- Improve quality of agricultural land by using organic fertilizer, a by-product of biogas fermention
- Preventing further degradation of forest (wood fire no longer required)
- Provide more time for productive live by reducing time for collecting wood fire
- Improve quality and prosverity of life of the poor

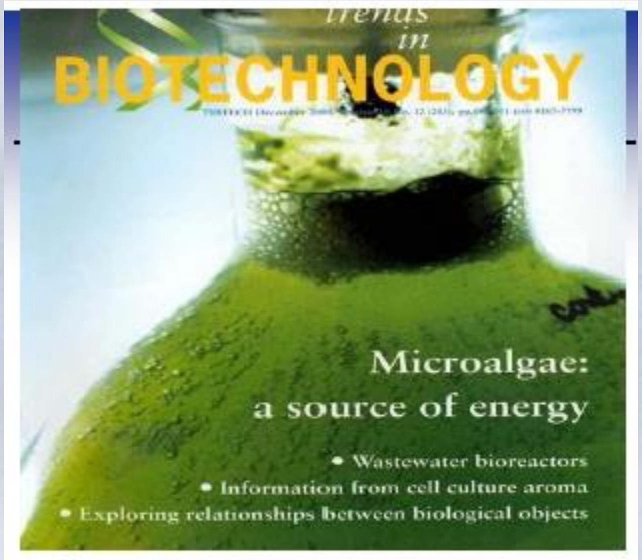
LIPI Pilot project of 10,000 Watt Electricity

CONCLUSION

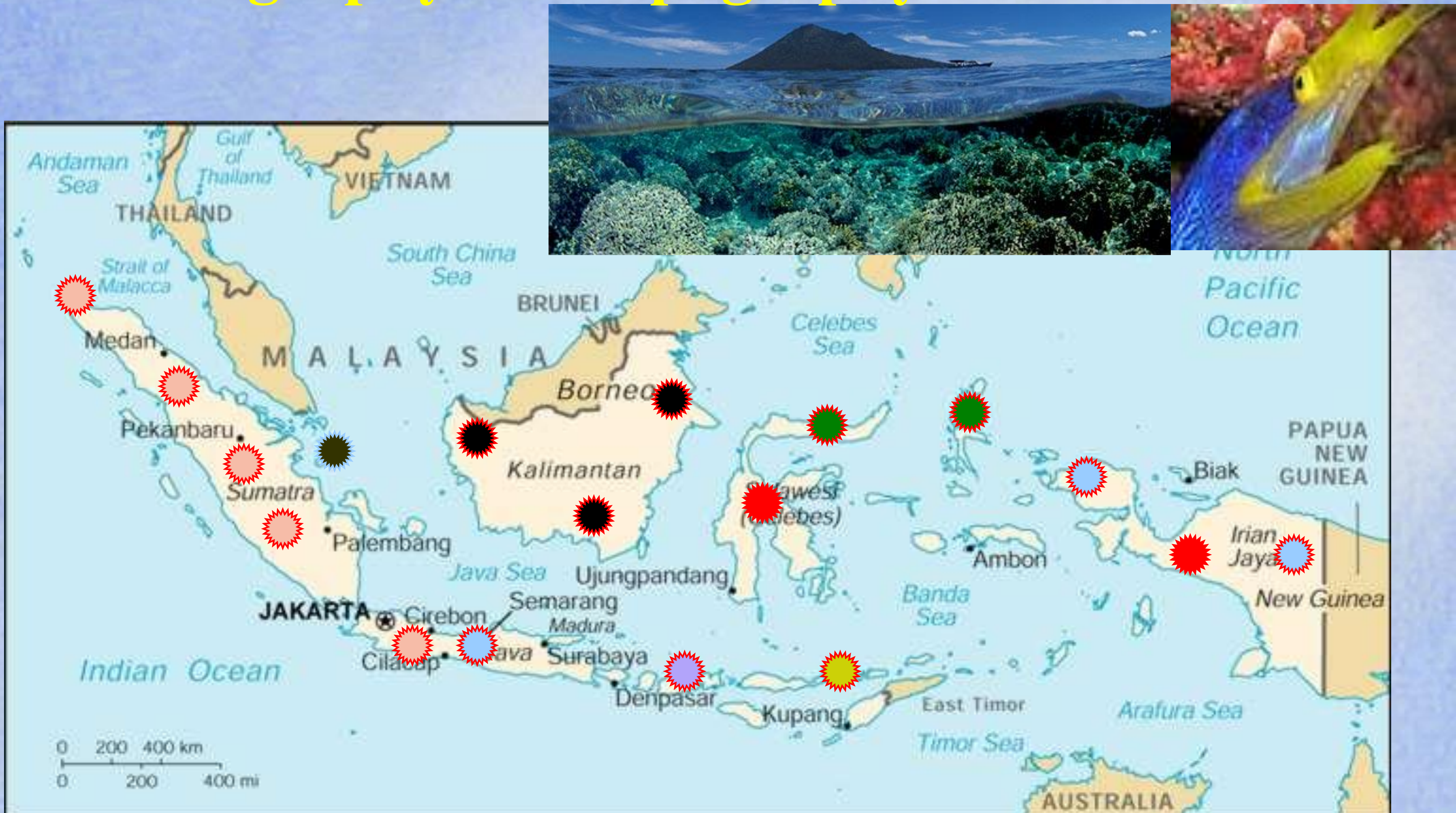
- Energy generate from POME waste: briquette, methane gas, hydrogen gas.
- Integrated refinery POME wastes should be applied for economic and reliable products.
- Social researches must be embedded for handling the POME wastes.

2. Microalgal-oil production Research Activities

Objectives: exploration of Indonesian microalgal biodiversity which rich of hydrocarbon content



Geography and topography of Indonesia



Vulcano area

Swamp area

Polluted by oil spill

High mineral content area

Polluted area by gold mining Industry

Arid land

Beautiful coral reef

Potencies of Microalgae

- First chain of marine or waters food chain.
- Waters carbon sources.
- Sources of bio-actives compound (energy, pharmaceutical compounds, nutrition's).
- Biological agents for waters environmental balances and bioremediation.

Bio-diesel agent from marine microalgae

- **Why**

- Microalgae deposit the hydrocarbon with C17-C40.
- Easy to maintain (growth and harvesting).
- Simple bioprocess (Extraction and esterification).
- Recycles system, No pollutant and no by products

Alga versus plant (Sazdanov, 2006)

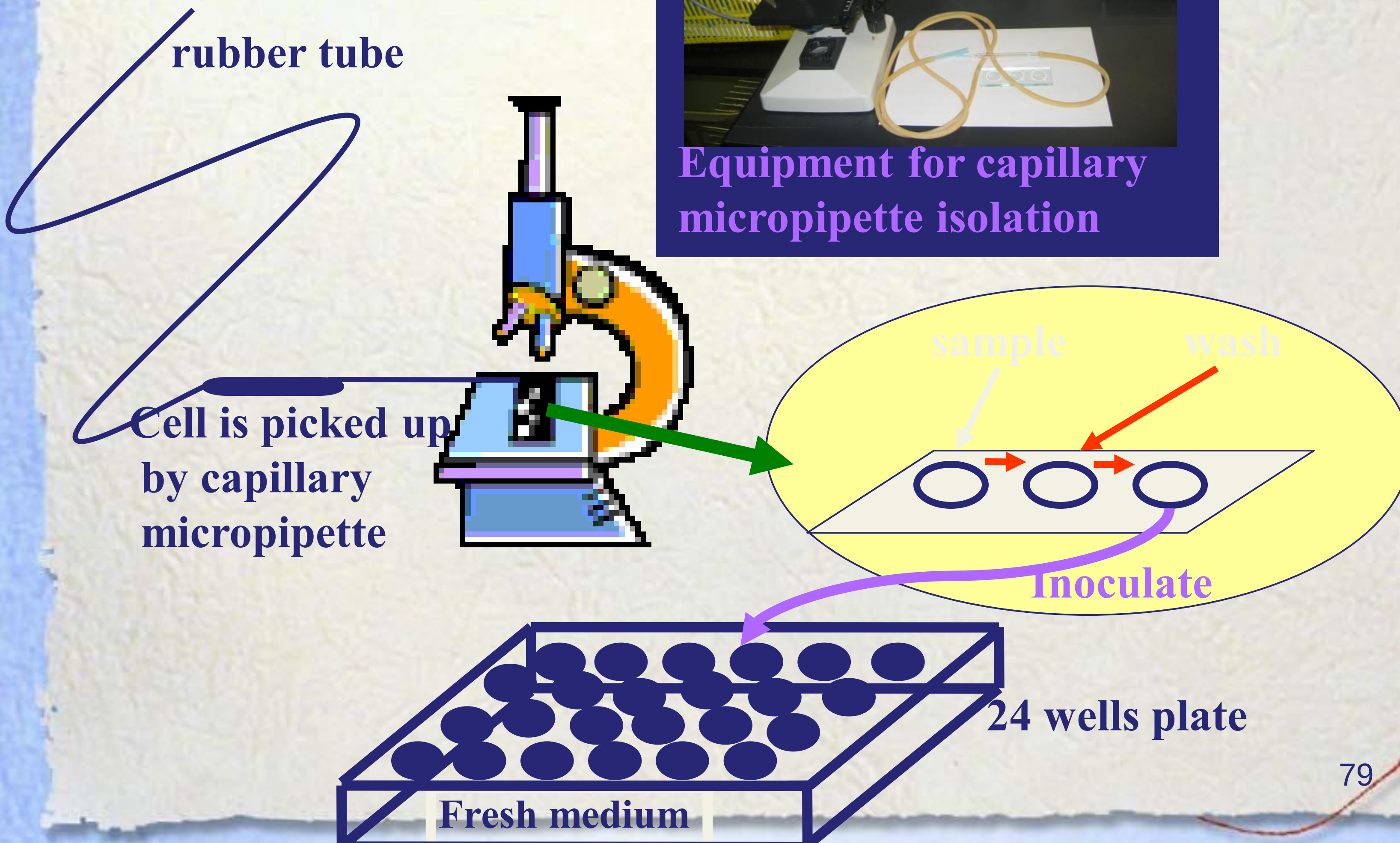
Materials	Oil production (lb.oil)/ acre	Biodiesel (galon/acre)
Algae	***6.757***	***700***
Coconut	**2.070**	**285**
Jathropa	**1.460**	**201**
Rapeseed	**915**	**126**
Peanut	**815**	**112**
Sunflower	**720**	**99**
Soybean	**450**	**62**

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**

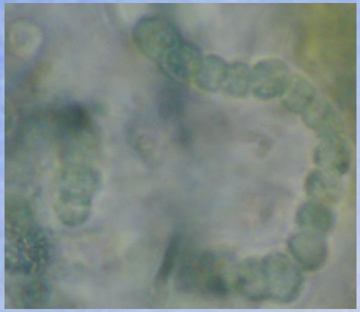
Isolation technique: by capillary micropipette

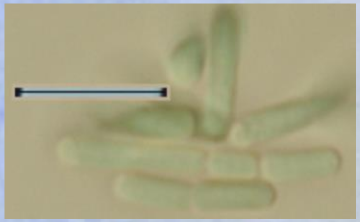
Equipment for capillary micropipette isolation

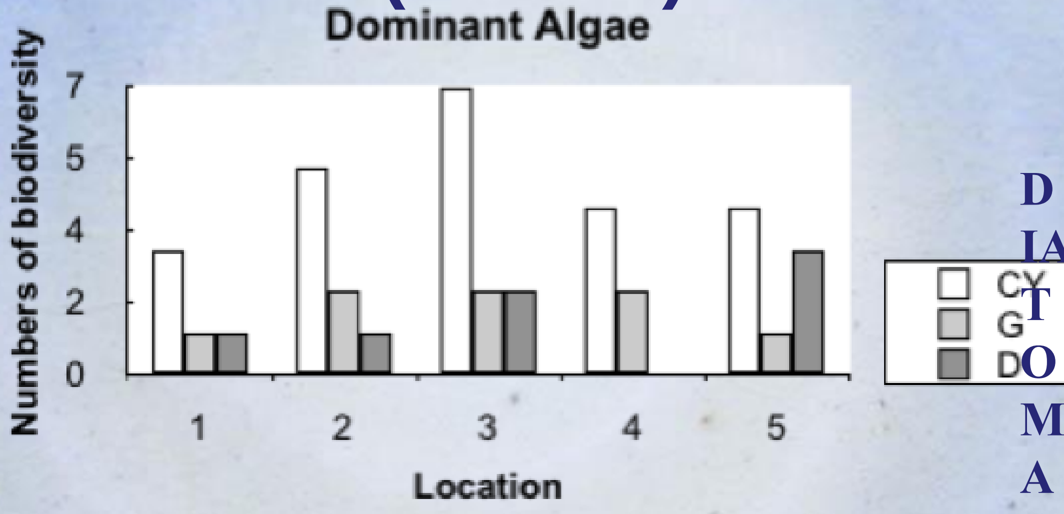


They call Beauty, we call Biodiversity algae (BATAM)

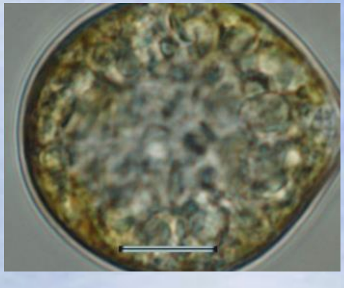
CYANOBACTERIA

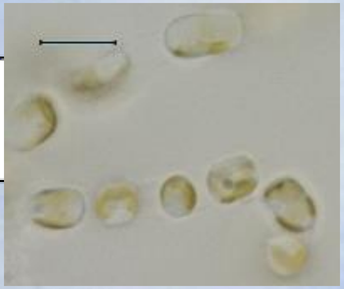




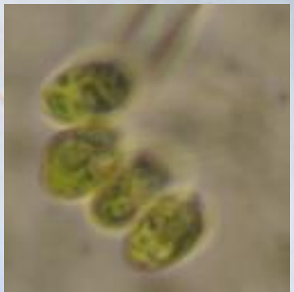


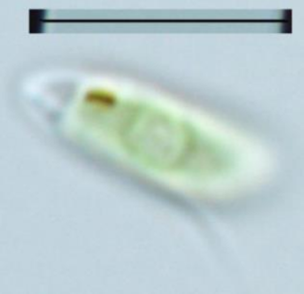
DIATOMAE





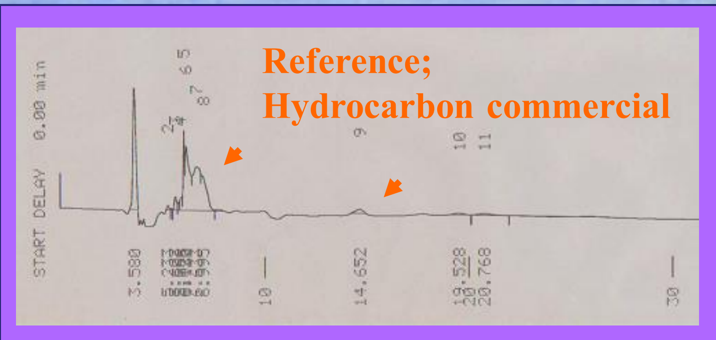
CHLOROPHYTES

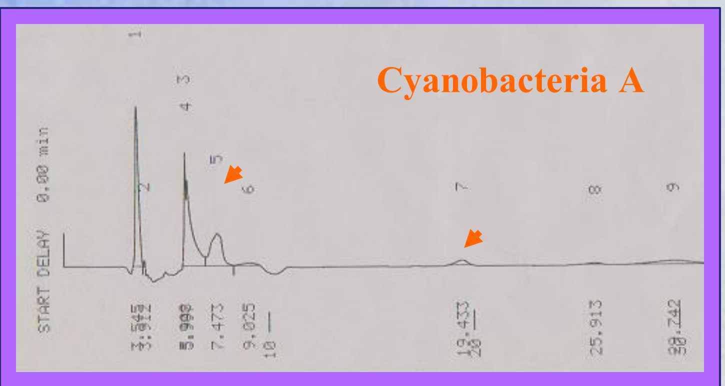




Scale bar : 10 ⌠m

Hydrocarbons analyses by HPLC





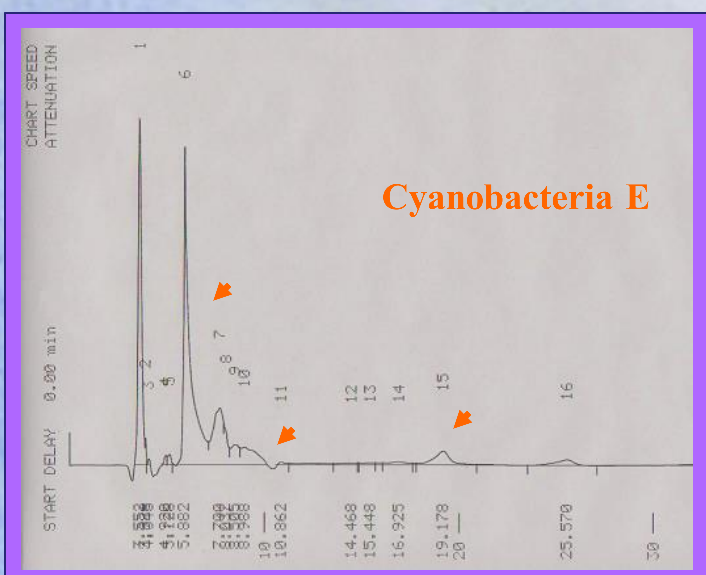


Table of Hyrocarbon analysis from selected microalgae

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

Consensus base on peak area of HPLC detection

Summaries

- Deliginification/ saccharafication process were resulted efficiencies of 10-90% base on dry weight of biomasses.
- The fermentation of organic acid from hydrolysates compounds process resulted the yield of 0.2-2% base on molarities of the lactic acid.
- Hydrogen production from the waste are 1-7% of formed gases (by H_2 sensor).
- Around 11 strains microalgae are pass the screening process for the hydrocarbon depositor.

Thank you very much