Tapping into Indonesia's microbial diversity for conversion of lignocellulosic biomass residue to biofuel and other valuable liquid chemicals

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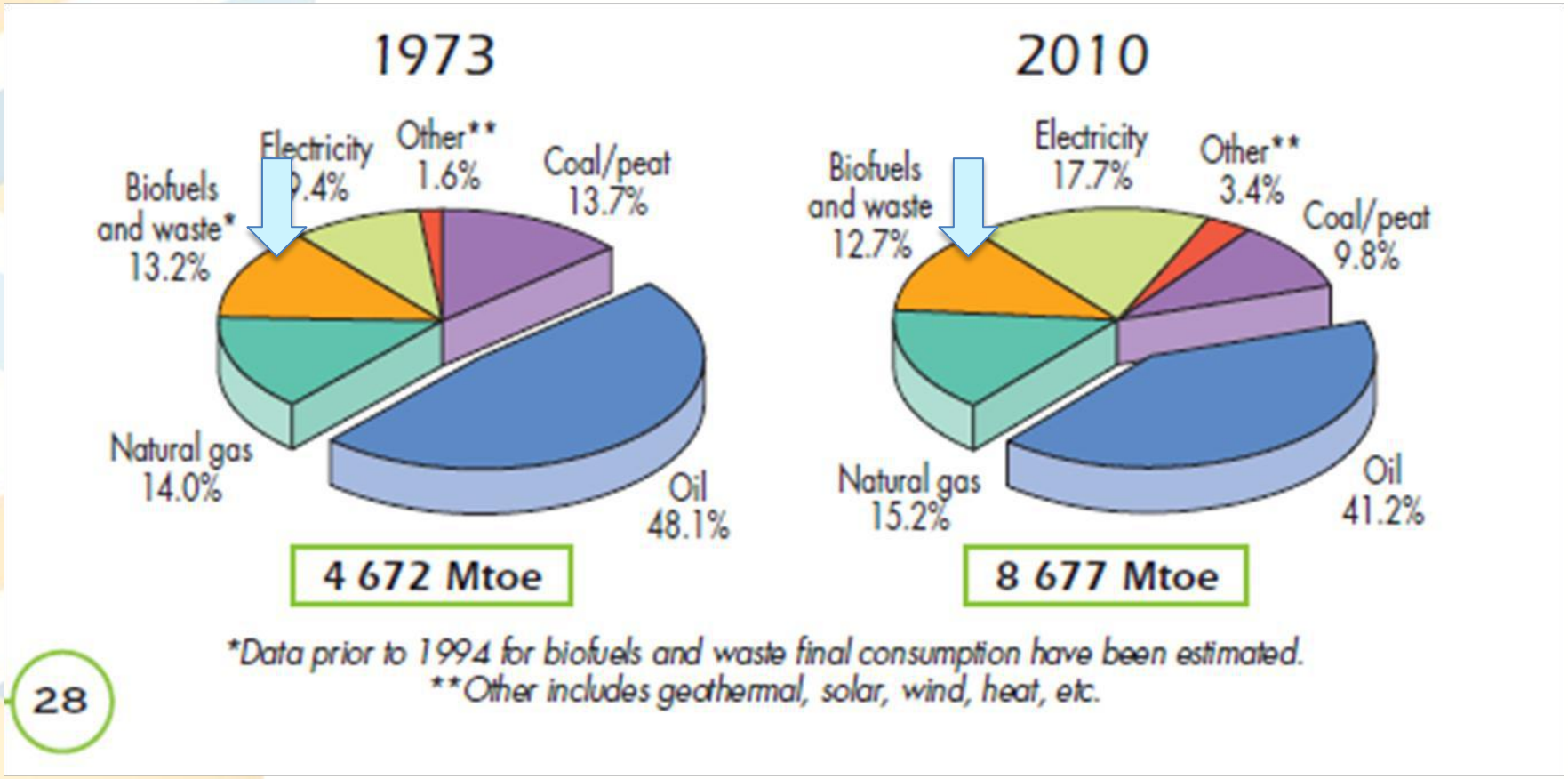
Pojok Iklim, KLHK 26 April 2017

Outline

- World's dependency upon fossil fuel
- Indonesia's richness in biodiversity and biomass residue for sustainable bioenergy
- Bioconversion process and production of liquid chemicals
  - i3L-USAID PEER 4-146 project
  - Yeast for high oleochemical production
- Microbial multi co-products platform for feasible bioeconomy
- Microbial culture collections
- References
- Acknowledgments

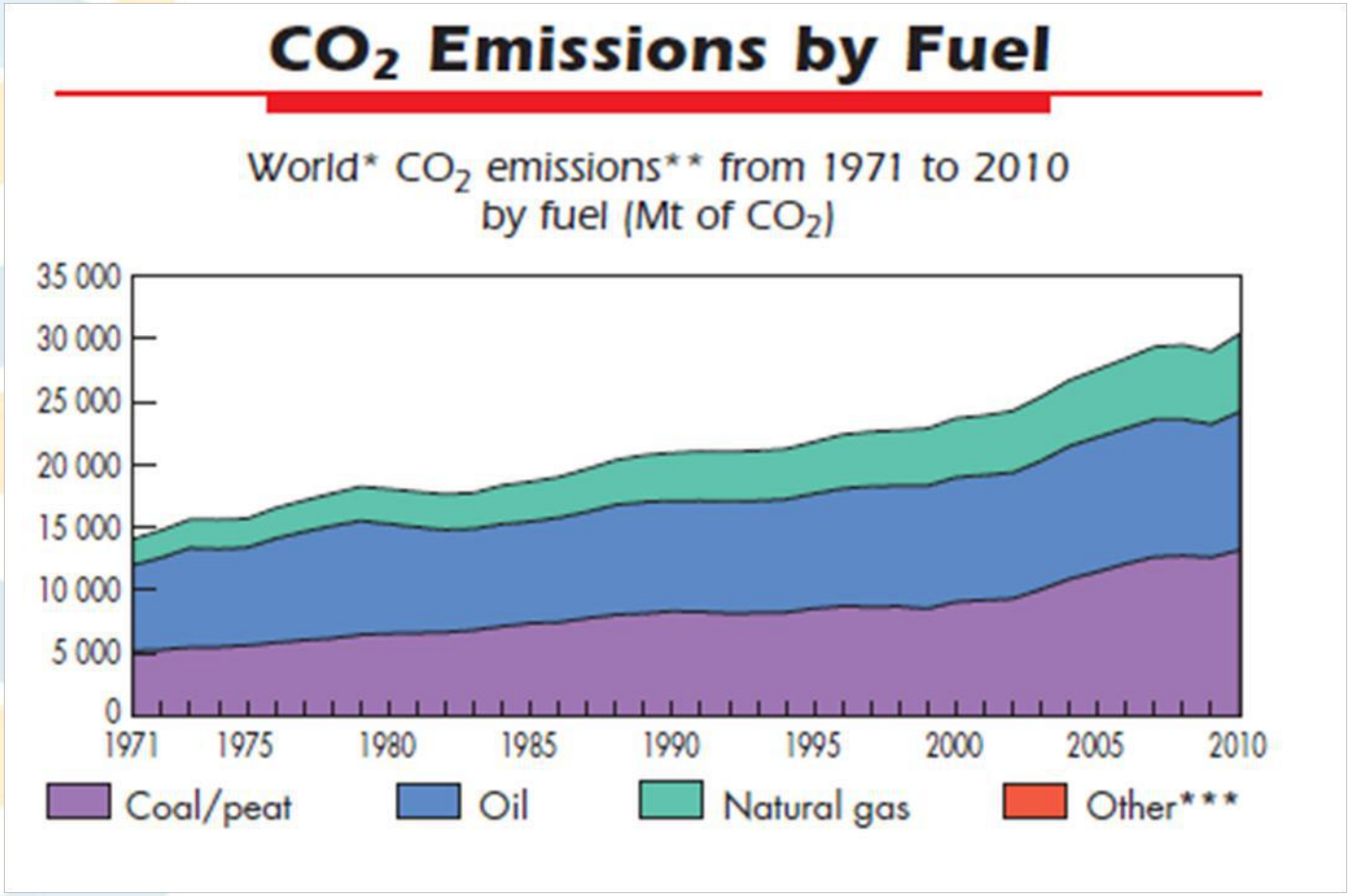
World's fuel consumption



The transport sector completely dominated with 61.5% of the total consumption

(IEA, International Energy Agency, 2012)

CO_2 Emissions by Fuel



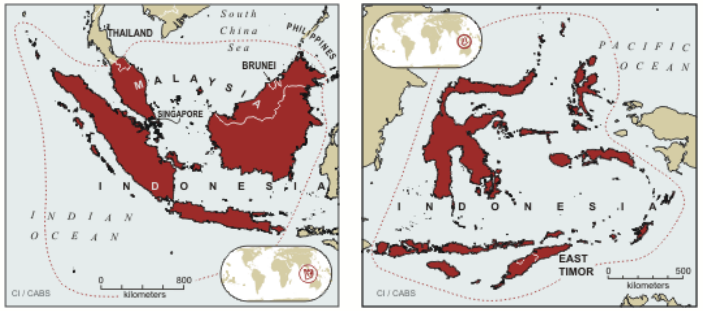
Global warming, fossil fuel depletion & energy security

Renewable and environmentally friendly fuels

(IEA (International Energy Agency), 2012)

Indonesia is rich in biodiversity

- One of the world top mega diverse countries
- 2/25 world biodiversity hotspots
- 1.3% of the world's land
- > 17,000 islands



Sundaland and Wallacea

	Indonesia	US	World
Mammal	515 [1]	428 [2]	5,487 [3]
Birds	1,539 [1]	784 [2]	9,990 [3]
Reptiles	781[5]	311 [2]	8,734 [3]
Amphibians	210[5]	295 [2]	6,515 [3]
Fish	25 - 45% [1,4]	1154 [2]	31,153 [3]
Flowering Plants	25,000 [1]	16499 [2]	268,600 [3]

[1] http://www.ran.org/indonesia_s_rainforests_biodiversity_and_endangered_species

[2] https://www.currentresults.com/Environment-Facts/Plants-Animals/number-of-native-species-in-united-states.php

[3] Chapman, A. D. (2009). *Numbers of Living Species in Australia and the World* (PDF) (2nd ed.). Canberra: Australian Biological Resources Study. pp. 1–80. ISBN 978 0 642 56861 8

[4] https://www.profauna.net/en/facts-about-indonesian-animals#.WKz6FG997IU

[5] Biodiversity Conservation in Indonesia https://blogs.ntu.edu.sg/hp331-2014-03/?page_id=27

Indonesia's Biomass Residue Potential

Forest Residues

135 dry MT/yr

Empty Fruit Bunches

4.6 dry MT/yr

Palm Kernel Shells

1.2 dry MT/yr

Cassava Waste

7.3 dry MT/yr

Sugarcane Baggase

8.1 dry MT/yr

Rice Husk

13.6 dry MT/yr

Coconut Fibre

6.8 dry MT/yr

Coconut Shells

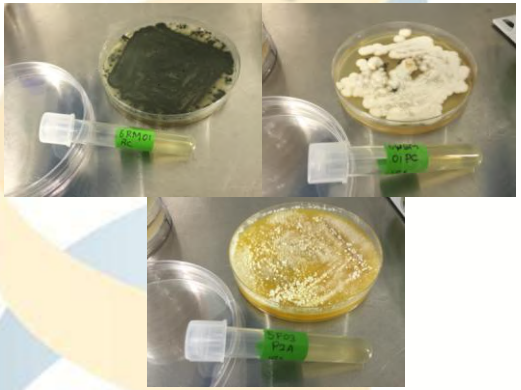
3.0 dry MT/yr

MSW

Farm Waste

Source: *Indonesia – Market Opportunities for Bioenergy*. 2012. NL Agency Ministry of Foreign Affairs.

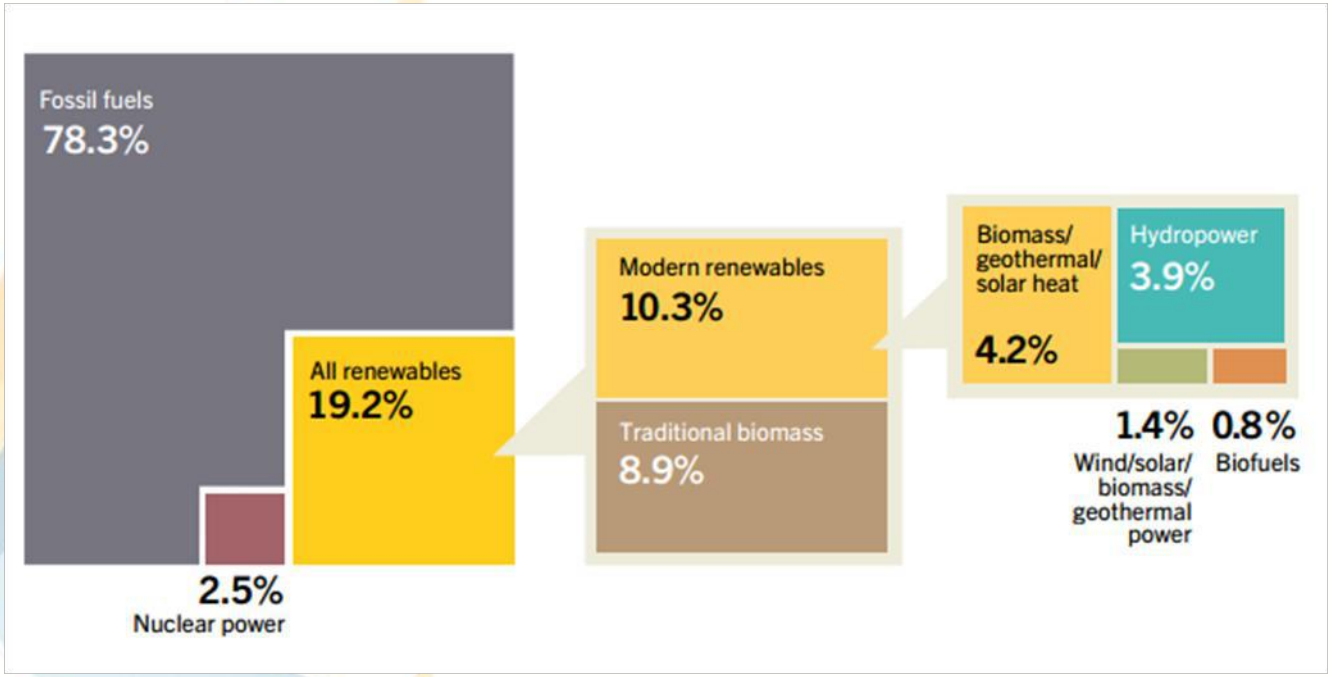
Bio-based Products



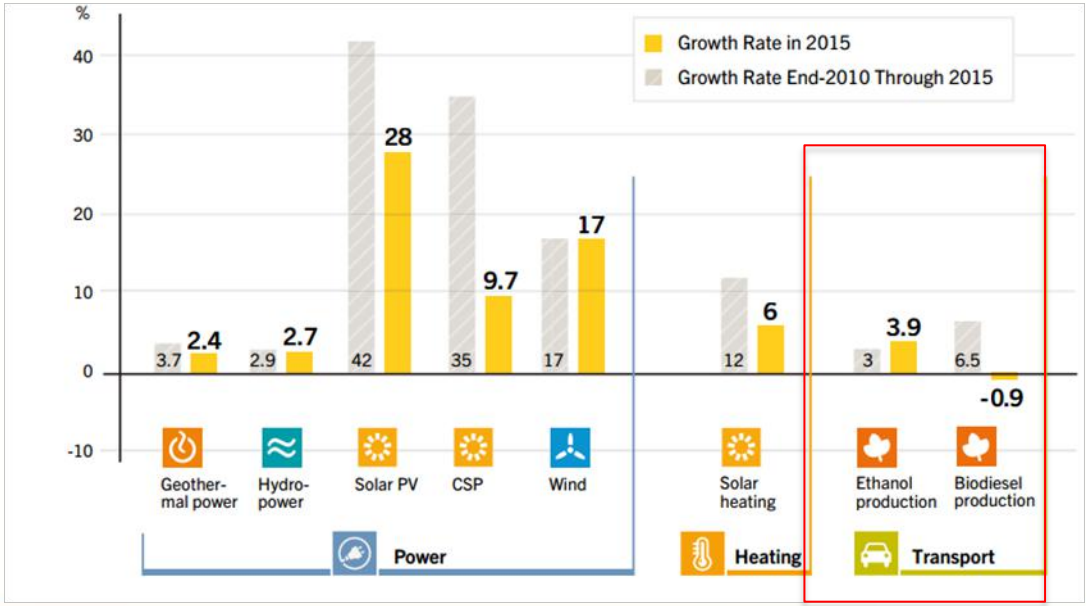
Microbial Diversity

Lignocellulosic biomass

Bioliquid chemicals



- Estimated Renewable Energy Share of Global Final Energy Consumption, 2014



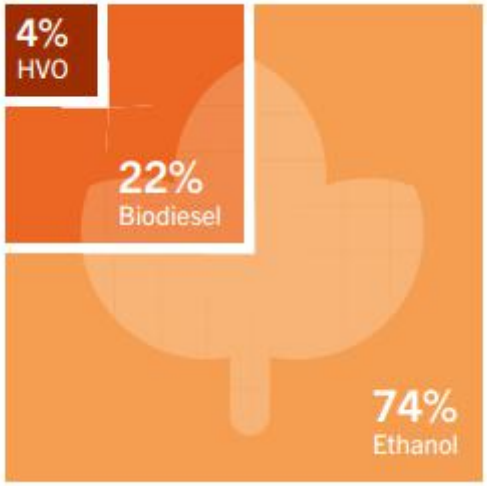
- Average Annual Growth Rates of Renewable Energy Capacity and Biofuels Production, End-2010 to End-2015

(REN21. 2016)

World's leading biofuel players

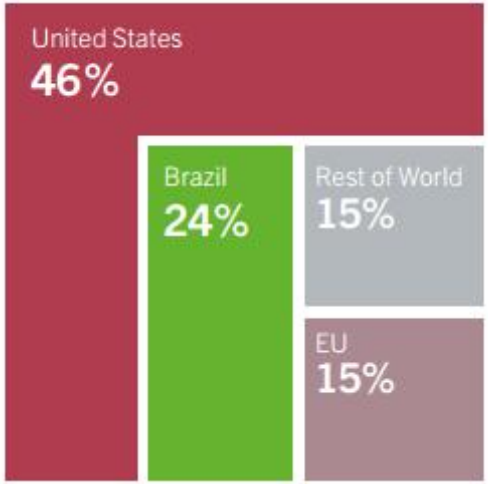
ETHANOL

Country	2015 (Bill. Liter)	Increase fr 2014 (%)
USA	56.1	3.8
Brazil	28.2	10
China	2.8	14
Other	1.7	-1



BIODIESEL

Country	2015 (Bill. Liter)	Increase fr 2014 (%)
USA	4.8	2
Brazil	4.1	20
Argentina	2.1	-30
Europe	11.5	5
Indonesia	1.7	- 40



(REN21. 2016)

Biofuel Generations: Evolving Rapidly

First Generation

- **Starch, sugar**
- **Food *vs* fuel**

Second Generation

- **Residual non food biomass: Switchgrass, corn stover, waste oil, municipal soild waste, etc.**
- **Sustainable and net CO2 emission**

Third Generation:

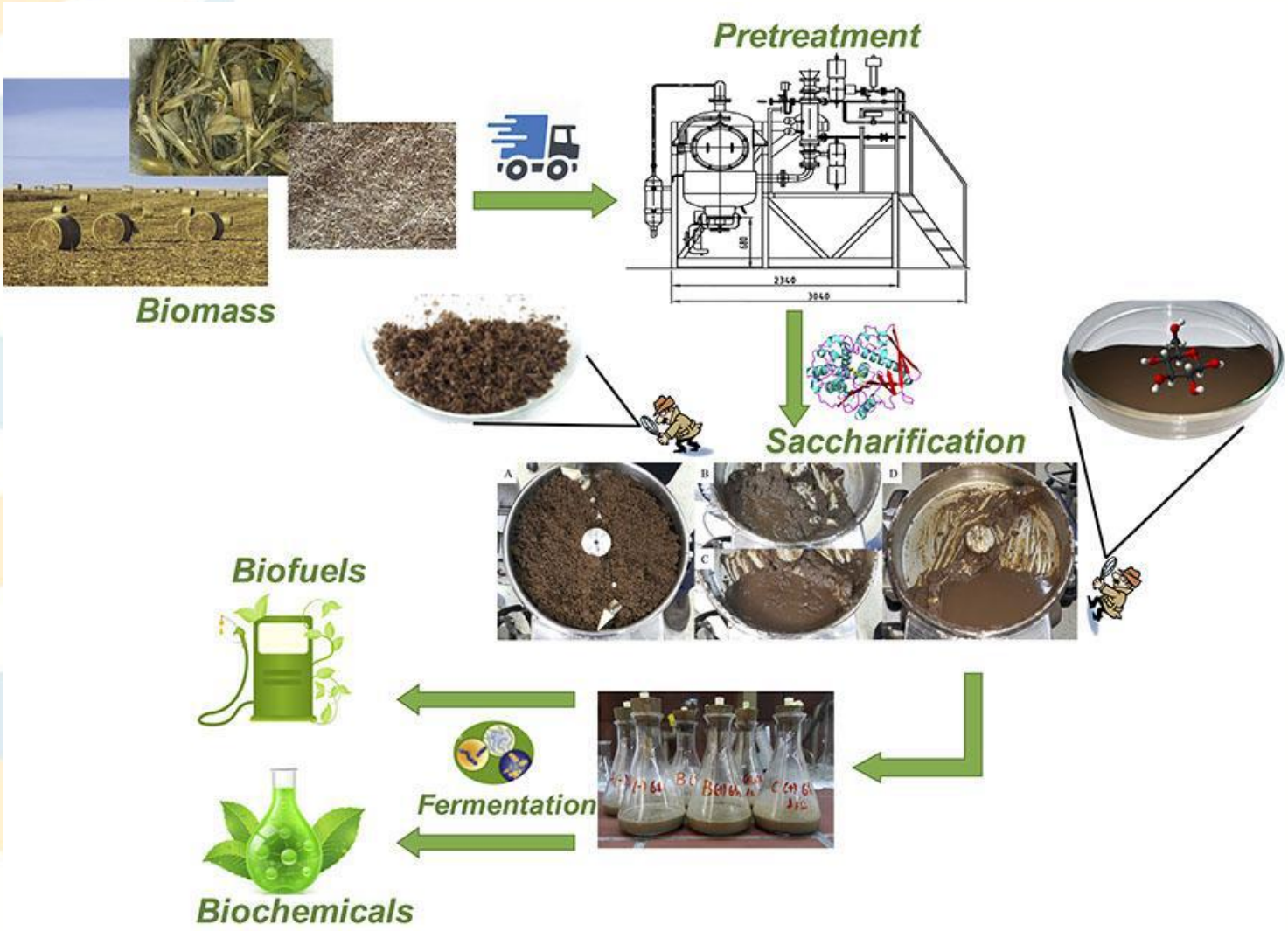
- **Algae as feedstock for** Biodiesel, Butanol, Gasoline, Methane, Ethanol, Vegetable Oil, Jet fuel
- **Drawback:** water, nitrogen and phosphorus requirement
- **Exxon mobil: not viable for now, later in 25 yeasrs**

Fourth generation: super power plant

- **GM crops with increased CO2 storage, low lignin, high biomass, drought tolerant**

http://biofuel.org.uk/third-generation-biofuels.html
http://global.mongabay.com/news/bioenergy/2007/10/quick-look-at-fourth-generation.html

The bioconversion process



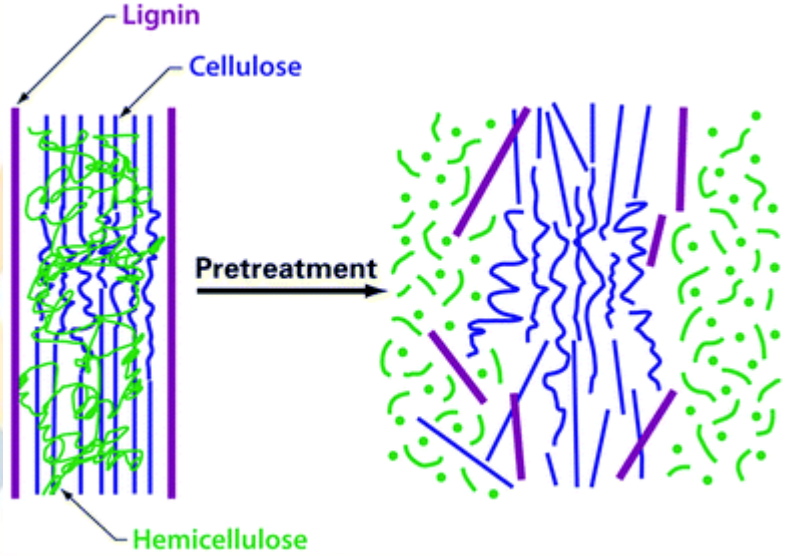
http://www.chemeng.ntua.gr/indubiocat/images/bio.jpg

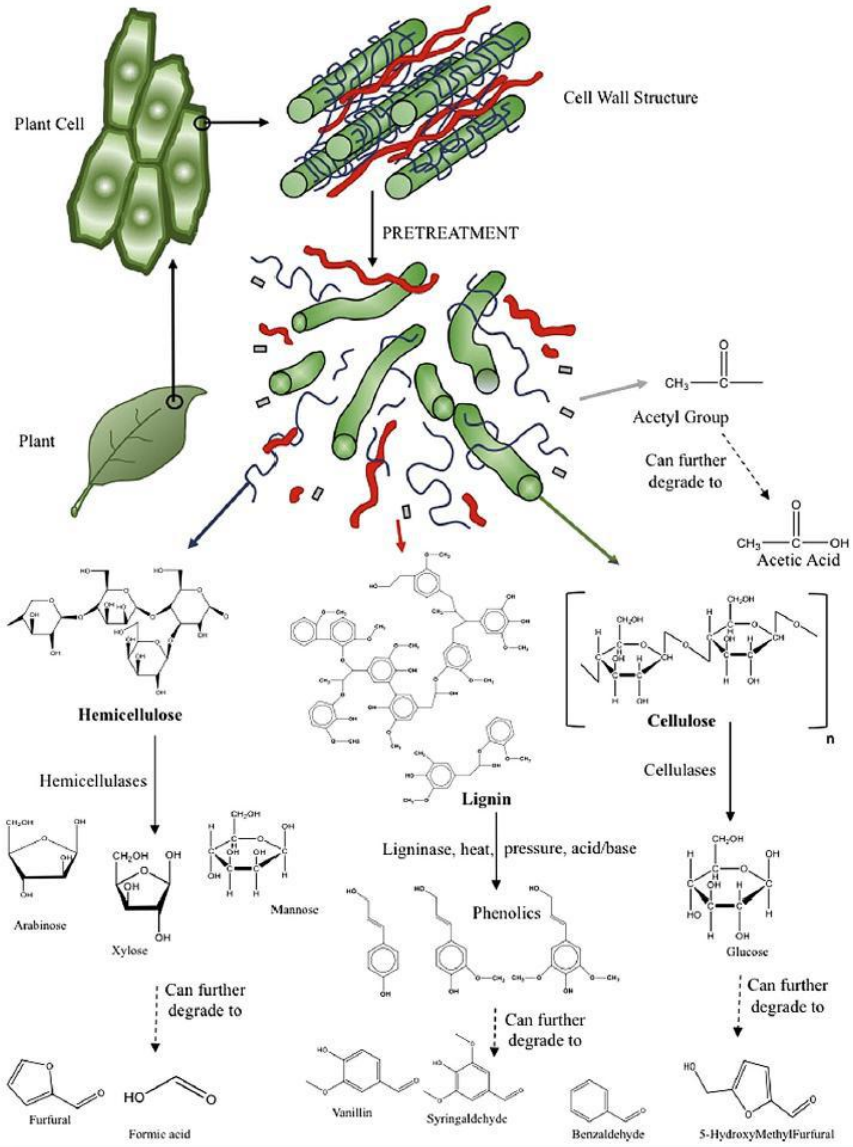
Challenges

- Lignocellulosic biomass is recalcitrant
- Production of cheap and efficient hydrolytic enzymes
- Production of pentose sugar and inhibitors during pretreatment and hydrolysis

Tapping desirable microbes

Lignocellulosic biomass is recalcitrant





Brandt et al., 2013, *Green Chem.*, **15**, 550-583; (Sitepu, Garay et al. 2014)

Composition of lignin, cellulose and hemicellulose varies among plant species

No.	Composition (% Dw)			Reference
	Cellulose	Hemicellulose	Lignin	
EFB	33 -41	20 - 24	21 - 36	Sudayani et al., 2010 Dahnum et al., 2015 Kristiani et al., 2015 Lee et al., 2014
Rice Husk	35	33	23	Johar, Ahmad et al. 2012
Sugarcane Bagasse	42-48	19-25	20-42	Saini JK et al., 2015 Kim M. and Day DF. 2011
Corn Stover	38-40	24-26	7-19	Saini JK et al., 2015 Zhu Y. et al. 2005

Indonesia International Institute for Life Sciences

USAID PEER Cycle 4
Grant ID # 4-146
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Developing a Bioeconomy in Indonesia: Identification of Novel Microorganisms and Microbial Enzymes from Indonesian Peatland and Buffaloes to Improve Bioconversion of Oil Palm Residue

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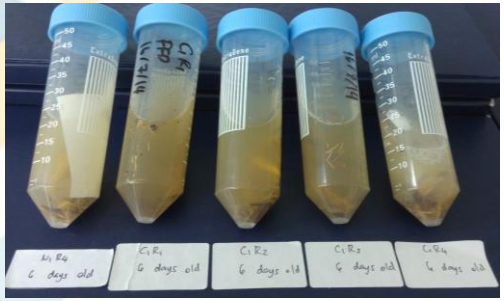
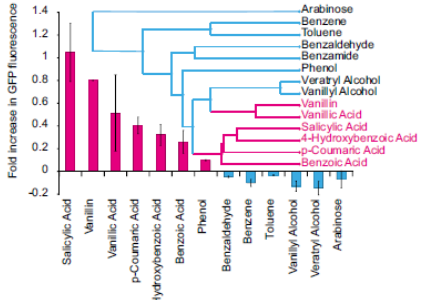
Culturable and non culturable microbes

Sequencing of active enzyme : Novel to science

Recombinant enzyme : Expression and specificity test: substrate, temperature, pH, etc.

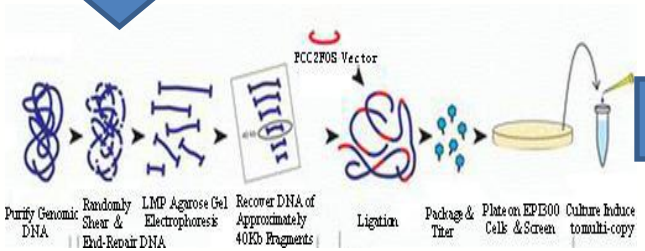


Enrichment study: 50C



Metagenomic:

- phylogenetic analysis → diversity
- cloning fragments of genes



Screening for enzymatic activity using colorimetric assay

Sampling of Peat and Herbivore Manures

Sampling → Microbe Isolation & Identification → Culture Collection

- **8 sites:** oil palm plantation, secondary forest, farms, zoos

32
Peat Samples

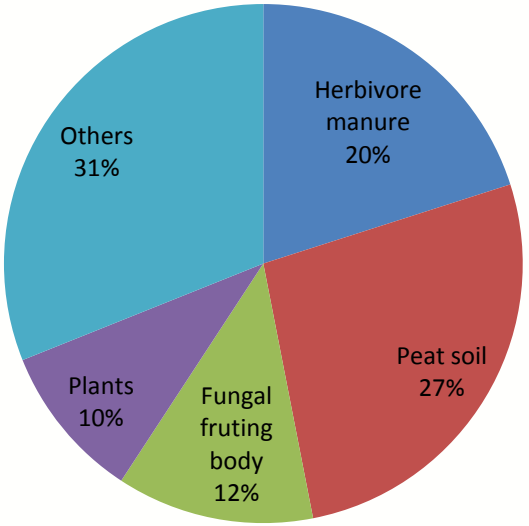
28
Manure Samples

28
Fungi Samples

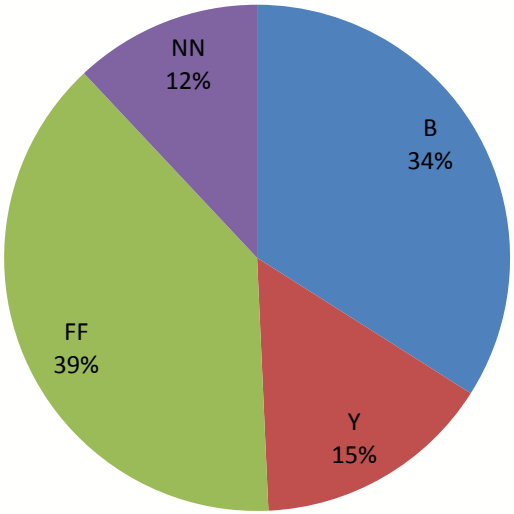
72
Environmental samples

Microbial taxa isolated from herbivore manures, peat and other environmental samples

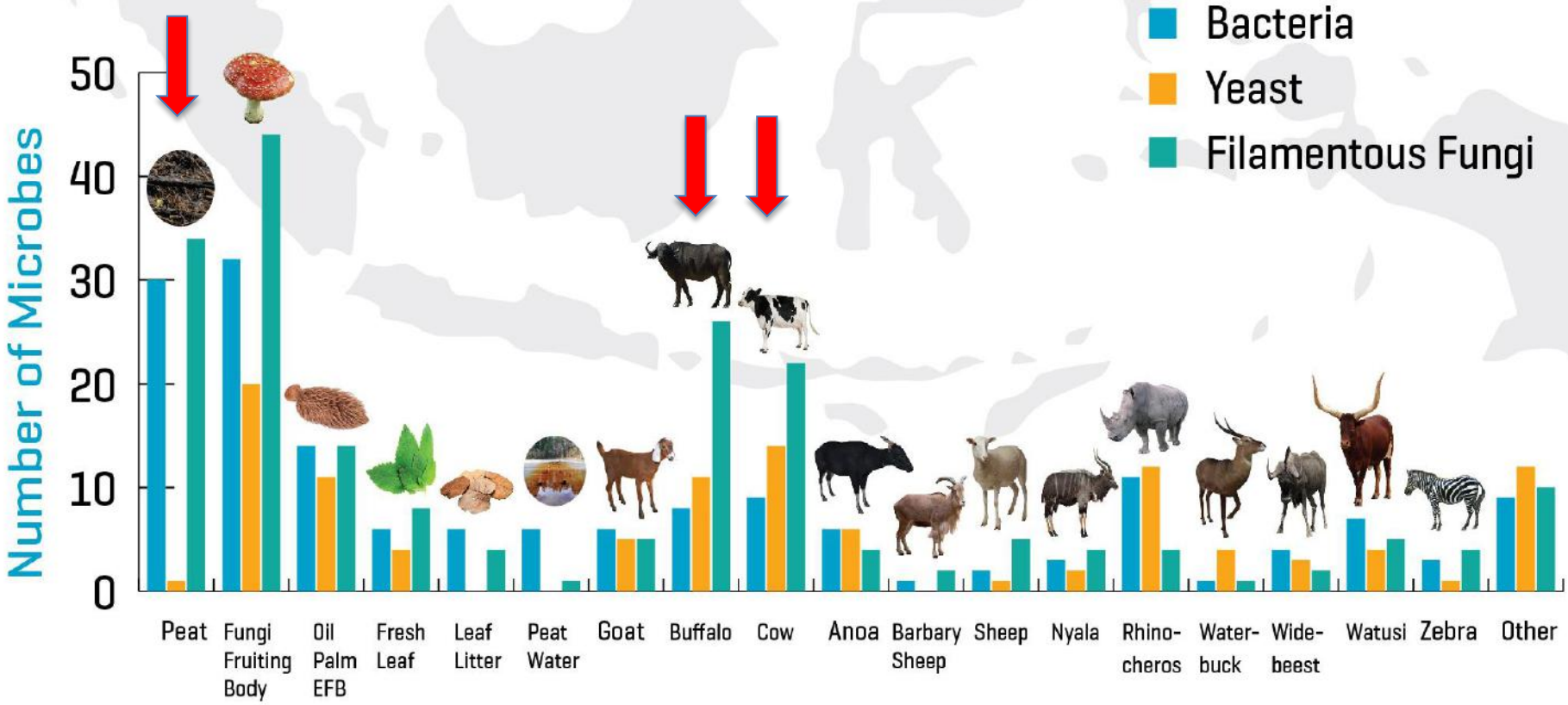
Host	B	Y	FF	NN
Herbivore manure	62	65	94	12
Peat soil	83	29	147	41
Fungal fruiting body	38	14	48	14
Plants	30	17	23	0
Others	96	14	40	42
Total	**309**	**139**	**352**	**109**
Total Strains	**909**			



B: Bacteria
Y: Yeast
FF: Filamentous Fungi



Number and Taxon of Microbes Isolated from Various Environmental Samples



Microbe Identification and Confirmation Study

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Yeast diversity

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Bacteria diversity

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Filamentous fungi diversity

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Filamentous fungi diversity

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Pretreatment of EFB and enrichment of lignocellulosic degrading microbes







- Ongoing

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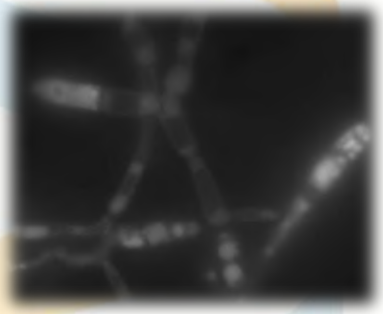
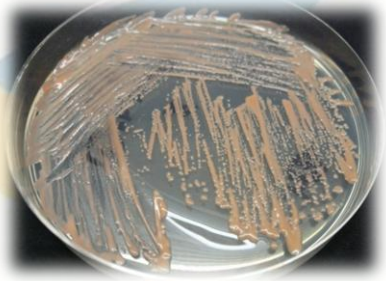
HIGH LIPID PRODUCING YEASTS

Yeast

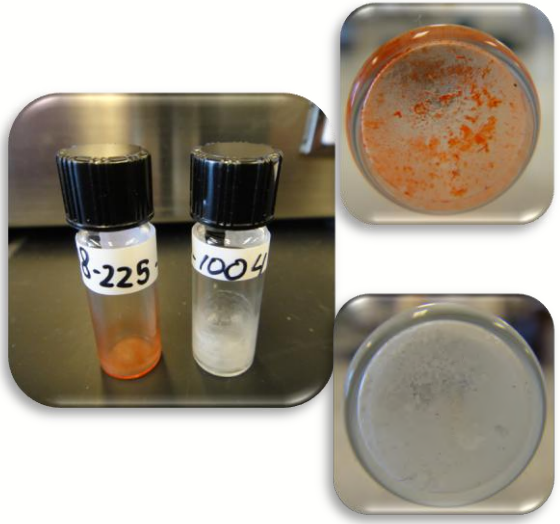


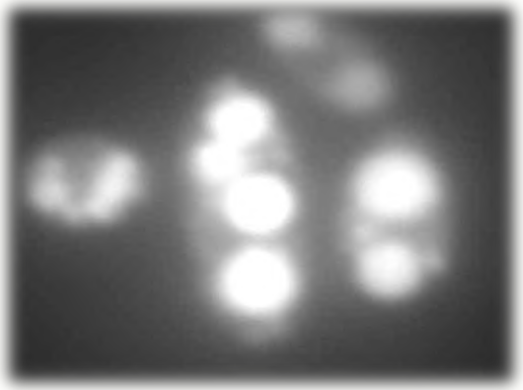


- Single-celled fungi, tolerant to broad pH ranges , can withstand acidic, mostly mesophilic (~25-50C)
- About ~1600 identified species:
  - Increased >2X from 700 (1998)
  - 5% of total yeast
  - Only about 12 species used in industrial scale and 70-80 known to have potential in biotechnology,
- The famous classic yeast species, *Saccharomyces cerevisiae* used in food and fermentation industries
- A single cell factory for production of industrial enzyme, pharmaceuticals, etc.

Yeast for oleochemical production

- Target: Yeast with high lipid content (oleaginous)
- Most yeasts convert excess carbon to stored carbohydrates; very few store lipids
- Yeasts lipid→ biodiesel/other oleochemical





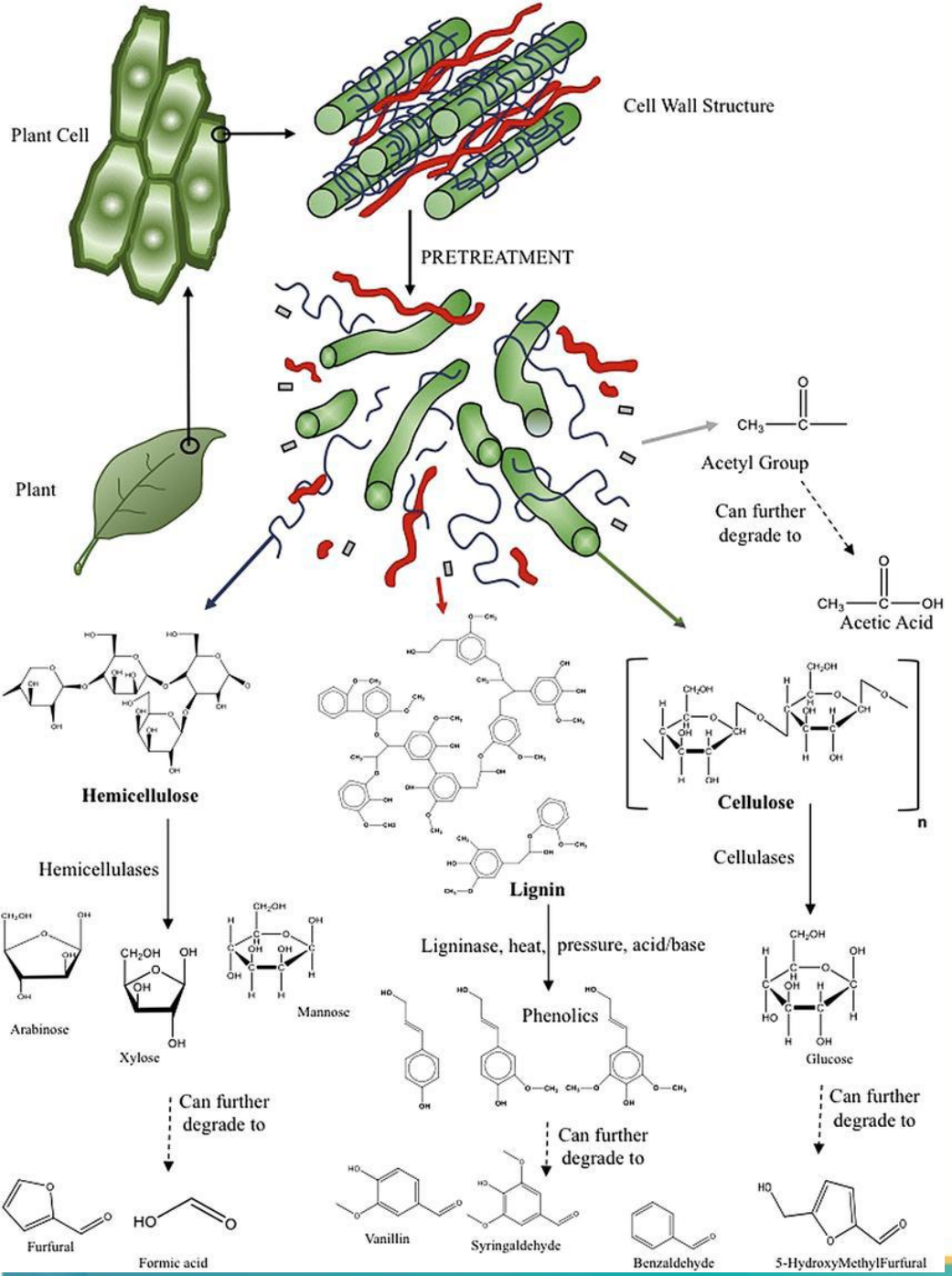
Why Yeast?

- Lipid composition & energy value are similar to plant and animal oils
- Do not compete for food
- Oleaginous yeast accumulate up to 70% oil
- Duplication times $\geq$ 1 hr
- Relatively easy scale-up
- Not subject to seasonal and cyclical weather variations
- Yeast oil has been studied since 1920s
- But, only a few oleaginous species are known and studied
- New oleaginous species may have better properties

Can yeasts convert lignocellulosic waste hydrolysate to biodiesel?

- Synthetic culture media *vs* lignocellulosic hydrolysates
- Biodiesel: triacylglycerols (TAGs) → trans-esterification → biodiesel
- Renewable, sustainable, biodegradable, and non-toxic
- Challenge: Inhibitors in hydrolysates and ability to convert the the released carbon to lipids

Sitepu IR, et al. (2014) Appl Microbiol Biot:1-13

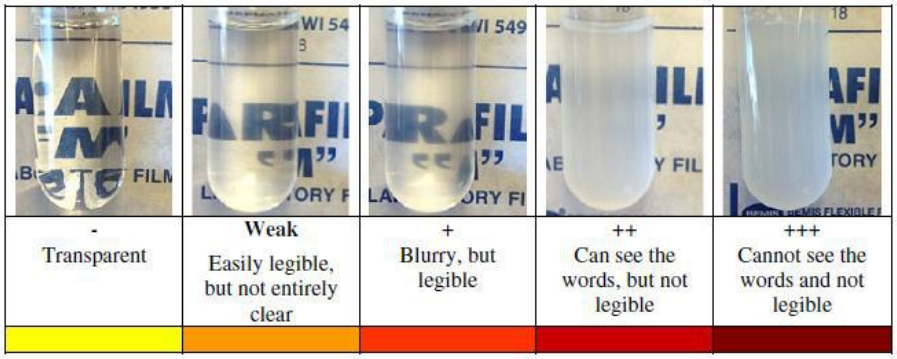


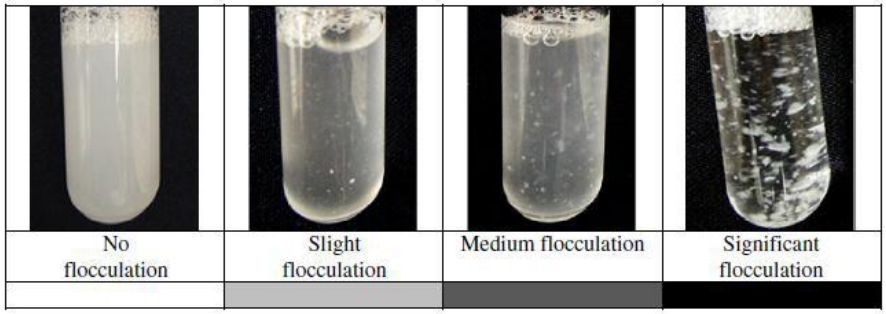
(Sitepu, Garay et al. 2014)

Hydrolysate composition

- Carbon:
  Glucose, D-Xylose, L-Arabinose, D-Arabinose, Cellobiose, Mannose, Galactose, Rhamnose, Sucrose, Galacturonic Acid, Glycerol
- Inhibitors:
  Furfural, acetic acid, HMF, vanillin

Alignment of yeast strain character and composition of lignocellulosic hydrolysate for lipid production is important





- 45 wild species :
  - utilize carbon sources
  - tolerate inhibitors
  - No vitamins

Sitepu IR et al (2014).
J Ind Microbiol Biotechnol:1-10

Range of wild yeasts able to grow on diverse carbon sources and inhibitors

phaff collection UCDAVIS

Species	UCDFST Strain ID#	Flocculation	Carbon Sources											Vitamin Free Media	Inhibitors						
			Glucose	D-Xylose	L-Arabinose	D-Arabinose	Cellobiose	Mannose	Galactose	Rhamnose	Sucrose	Galacturonic Acid	Glycerol		Furfural (0.5g/L)	Furfural (1 g/L)	Acetic Acid (2.5 g/L)	HMF (0.5g/L)	HMF (1g/L)	HMF (2g/L)
Candida aff. *tropicalis*	10-1087																				
Candida diddensiae	10-168																				
Cryptococcus aerius	73-135		D						D		D			D					D	D	
Cryptococcus aff. *taibaiensis*	73-750		D			D					D								D		
Cryptococcus albidus	63-203		D								D		D								
Cryptococcus cf. *aureus*	81-663.4					D							D	D						D	
Cryptococcus curvatus	76-559												D	D						D	
Cryptococcus humicola	10-1004														D						
Cryptococcus humicola	12-717												D		D						
Cryptococcus laurentii	12-803														D					D	
Cryptococcus oeirensis	05-864					D													D		
Cryptococcus ramirezgomezianus	54-11.224												D		D					D	
Cryptococcus terreus	61-443					D			D		D										
Cryptococcus victoriae	10-939								D	D					D						
Cryptococcus wieringae	05-544																				
Cyberlindnera jadinii	76-80		D				D	D			D		D		D	D	D		D	D	
Cyberlindnera saturnus	68-1113														D						
Geotrichum fermentans	89-29				D		D								D						
Hannaella aff. *zeae*	92-112							D		D			D		D				D	D	
Kodamaea ohmeri	54-7					D									D						
Kurtzmaniella cleridarum	76-729.2									D			D							D	
Leucosporidiella creatinivora	62-1032						D		D				D		D					D	
Lipomyces lipofer	78-19		D								D				D					D	
Lipomyces starkeyi	78-23				D	D	D			D	D		D					D	D	D	
Lipomyces tetrasporus	78-28			D	D		D		D		D		D	D					D	D	
Metschnikowia cf. *gruessii*	11-1106																			D	
Metschnikowia cf. *gruessii*	11-1130																			D	
Metschnikowia cf. *pulcherrima*	11-1039																				
Myxozyma melibiosi	52-87					D									D				D	D	
Pseudozyma aphidis	11-1358					D	D			D			D						D	D	
Rhodosporidium paludigenum	09-163								D	D			D							D	
Rhodosporidium babjevae	04-877										D								D		
Rhodosporidium babjevae	05-775																		D	D	
Rhodosporidium diobovatum	04-830													D							
Rhodosporidium fluviale	81-485.4				D	D			D		D			D							D
Rhodosporidium toruloides	68-264				D	D					D		D								
Rhodotorula glutinis	50-309						D				D		D	D							
Rhodotorula graminis	04-862			D			D			D					D						D
Rhodotorula minuta	78-281																		D		
Rhodotorula mucilaginosa	40-129					D			D												D
Schwanniomyces occidentalis	73-1																				
Tremella encephala	68-887.2														D				D	D	
Trichosporon coremiiforme	88-108.4													D	D						
Trichosporon dermatis	63-110												D	D	D						
Trichosporon guehoae	60-59														D						D
Trigonopsis variabilis	75-19																				
Wickerhamomyces ciferrii	04-836						D														
Yarrowia lipolytica	51-30																				

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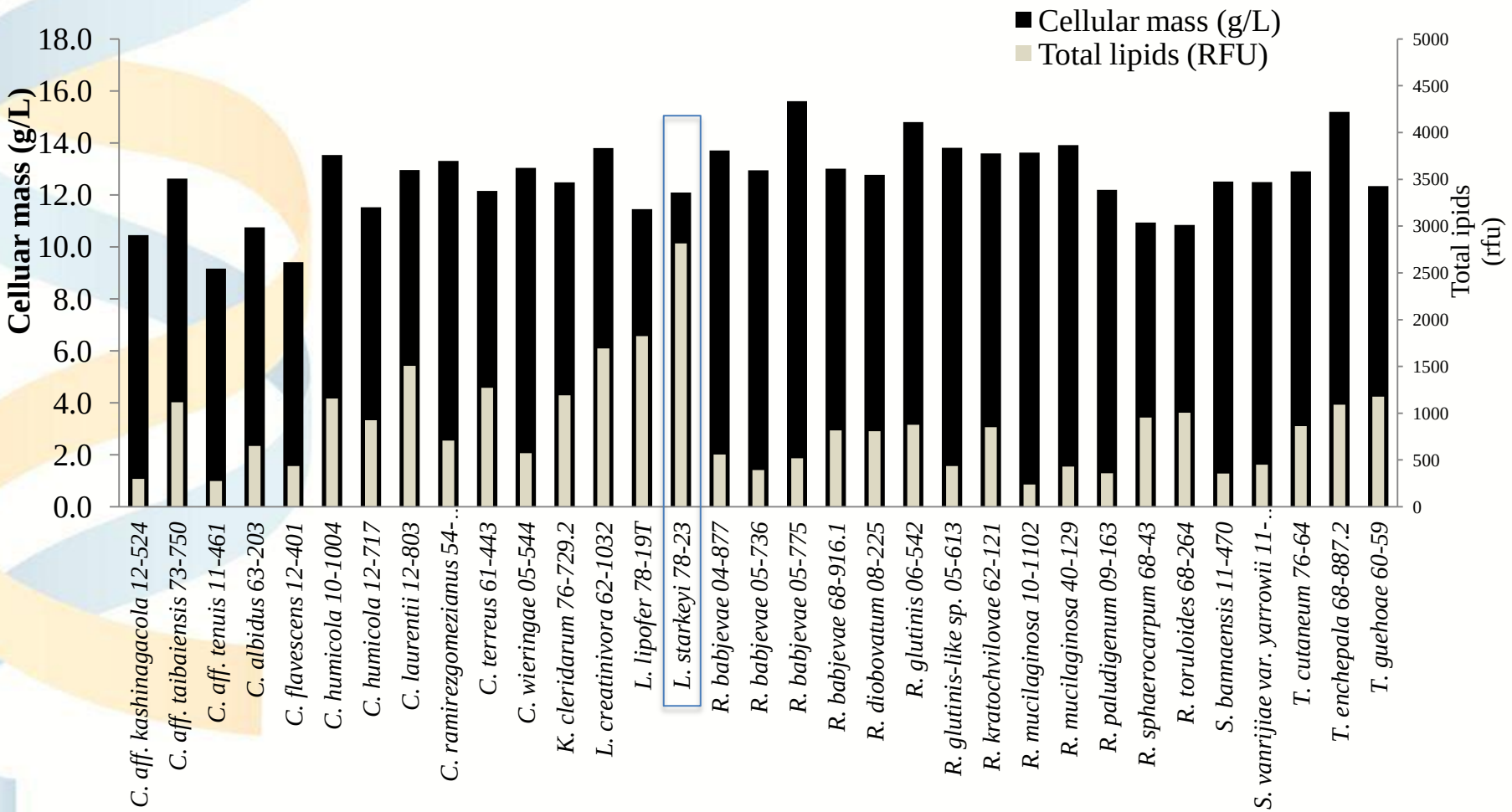
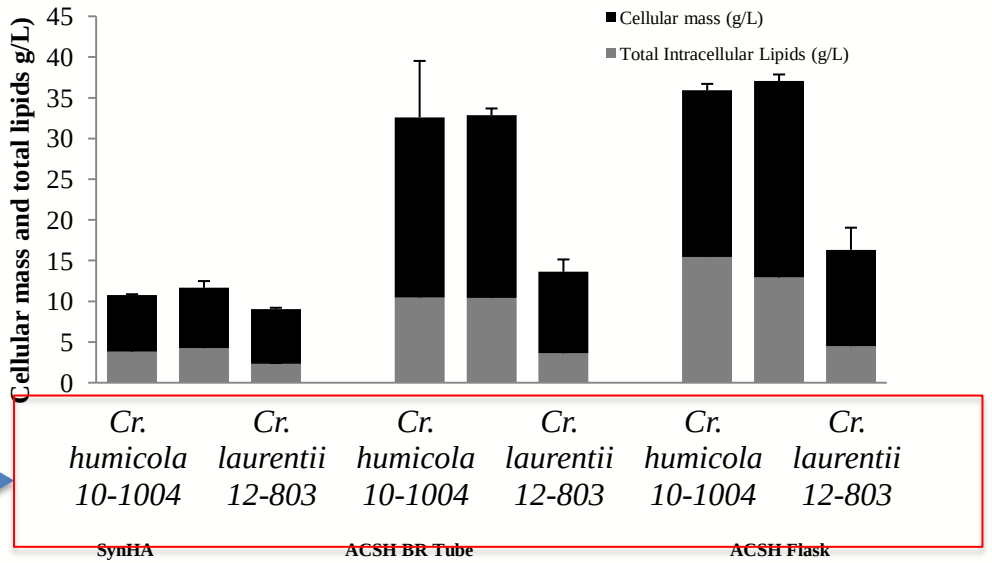
>250 yeast strains tested for growth in different carbon, nitrogen and inhibitr sources

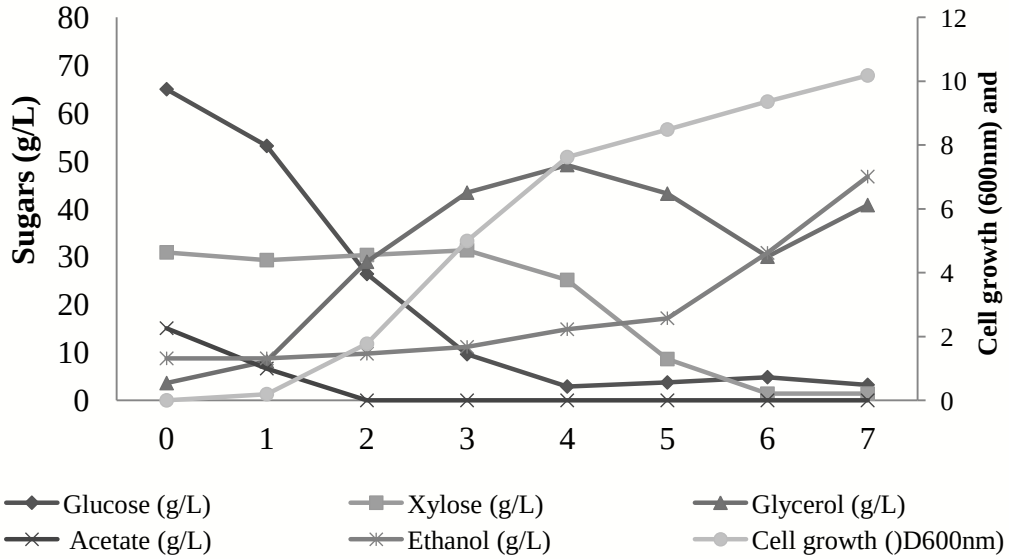
Accumulation of lipid by oleaginous yeast in corn stover hydrolysate



Indonesia's origin



Presence of pentose cannot be utilized by classic yeast

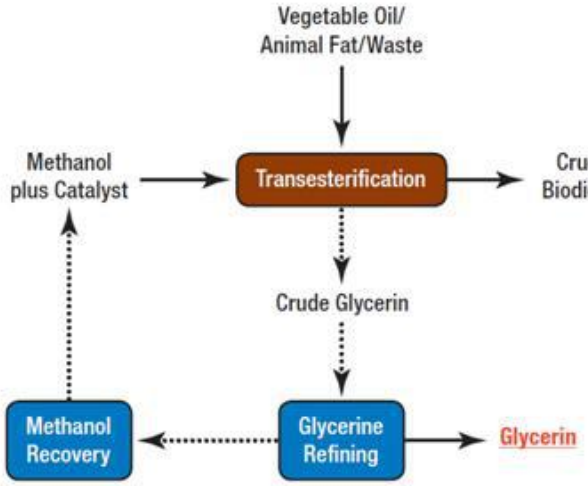


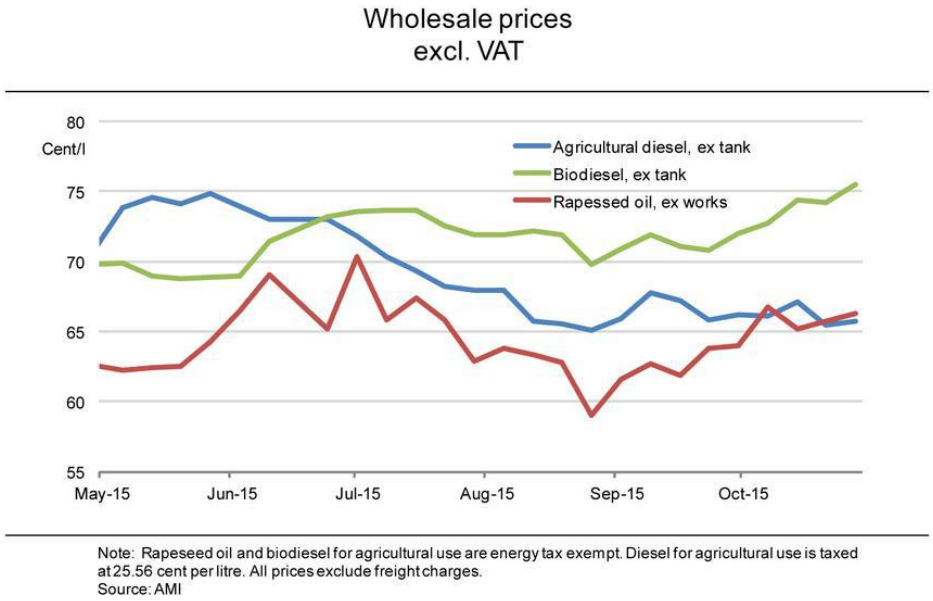
Is it really a feasible thing to do?

http://blog.intercom.io/wp-content/uploads/2012/11/Pick3.jpeg

https://encrypted-tbn0.gstatic.com/images?q=tbn:ANd9GcRAKBe252kIHZv15wqYUariVBWLS7sVZKB8jioPzNiSBnNxa-0wmA

Biodiesel production process





Biodiesel magazine, November 06, 2015

Wholesale price for biodiesel: USD $3.066 per gallon, excl. energy tax *vs* Petroleum diesel : USD $2.6 per gallon (https://www.eia.gov/petroleum/gasdiesel/)

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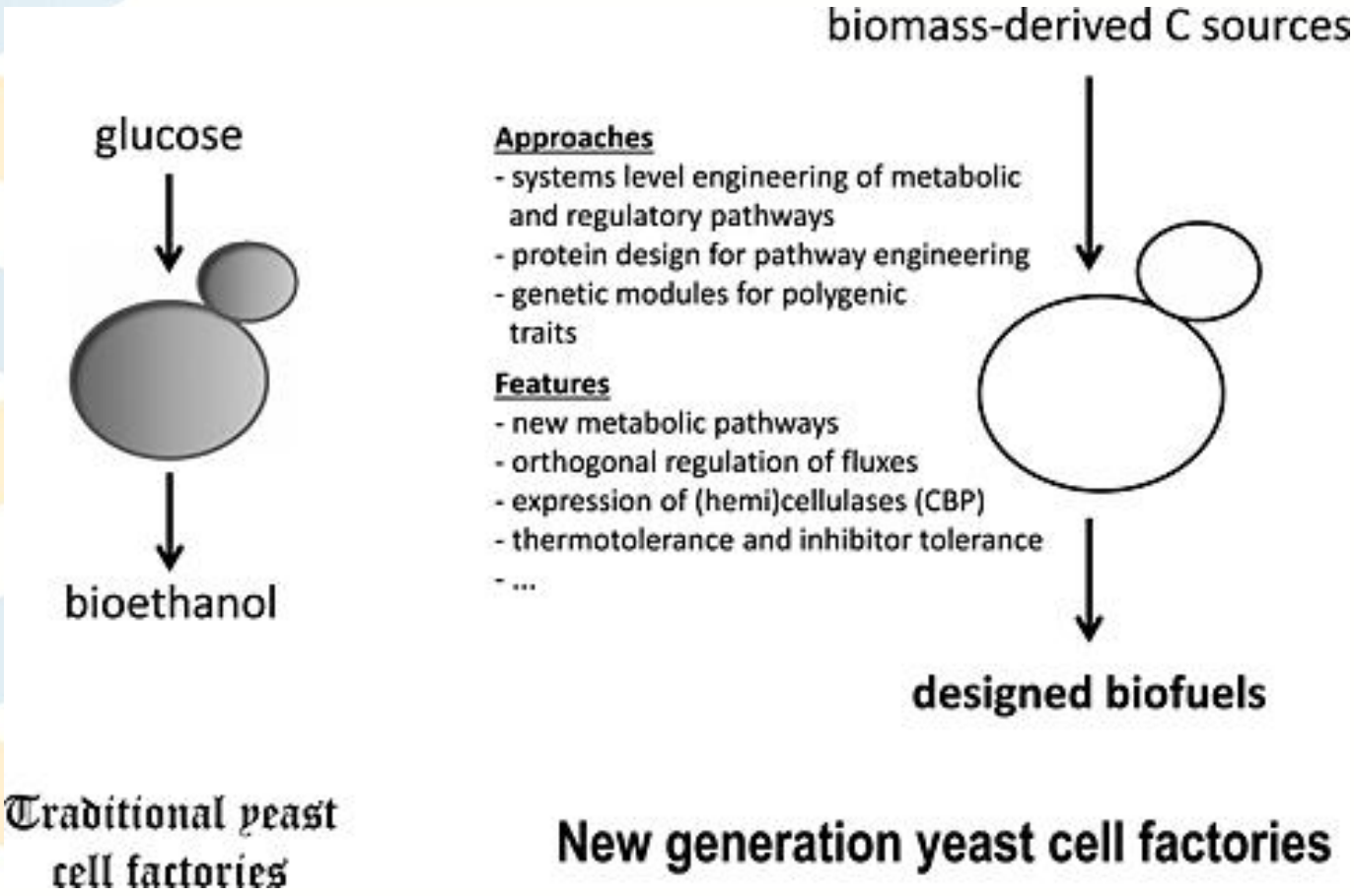
Indonesia International Institute for Life Sciences

MICROBIAL CULTURE COLLECTION

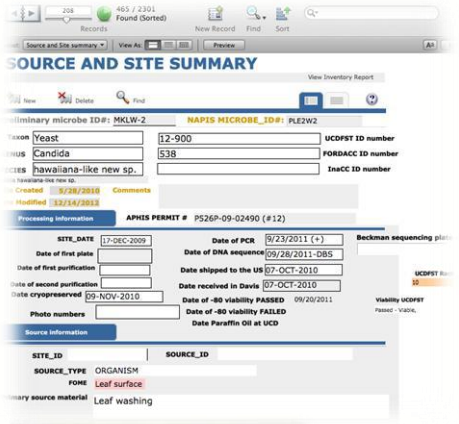
To engineer or not to engineer : Tapping into wild microorganisms diversity



(Petrovic 2015)

Microbial culture collection to facilitate current and future botechnology

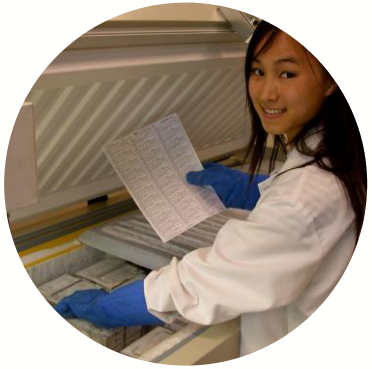
- 20 MCC in Indonesia, 223 in ASIA or 719 in the world
- To rescue and to conserve Indonesia's microbial diversity *ex situ*
- To promote sustainable use of the microbial culture collections including bioprospecting activities
- Our last resort in the future



Microbe collections

UC Davis

- Phaff Yeast Culture Collection
- First isolation 1893
- Fourth largest yeast collection in world
- 7,000 cultures, 800 species of yeasts
- Expertise in screening, especially bioenergy



FORDA

•INTROF CC, since 2000
•>3,000 strains
•Filamentous fungi, bacteria, yeast, soil
•Focus on forestry applications; mycorrhizae growth promoters, biocontrol of tree diseases gaha

i3L CC

Since 2016
>900 microbes (Bacteria, FF, yeast)
Supported by USAID-PEER 4-416

InaCC

- First isolation 1875
- Largest, oldest microbe collection in Indonesia with 3000 strains
- Fungi, bacteria, yeast
- Expertise in taxonomy, ecology, bioprospecting
- Support from Japan to become BRC

Twenty MCCs in Indonesia

Acronym	WDCM Number	Collection	Region
BPPT-ESC	WDCM 627	BPPT Ethanol-Single Cell Protein-Fructose Syrup Technical Unit	Asia
BTCC	WDCM 632	Biotechnology Culture Collection Institution Pusat Penelitian dan Pengembangan Bioteknologi-LIPI	Asia
CAIRCC	WDCM 623	CAIRCC	Asia
DMUIJ	WDCM 472	Department of Microbiology	Asia
FBGMU	WDCM 629	Faculty of Biology Gadjah Mada University	Asia
FNCC	WDCM 755	Food and Nutrition Culture Collection	Asia
i3LCC	WDCM 1137	i3L Culture Collection	Asia
ICBB	WDCM 842	ICBB Culture Collection for Microorganisms and Cell Culture	Asia
InaCC	WDCM 769	Lembaga Ilmu Pengetahuan Indonesia, Indonesian Institute of Sciences	Asia
INTROF-CC	WDCM 943	Indonesian Tropical Forest Culture Collection for Microorganisms	Asia
IPBCC	WDCM 1018	Institut Pertanian Bogor Culture Collection	Asia
ISRI	WDCM 630	Indonesian Sugar Research Institute, Pusat Penelitian Perkebunan Gula Indonesia	Asia
ITBCC	WDCM 44	Institute of Technology Bandung Culture Collection	Asia
PBF	WDCM 633	Perum Bio Farma	Asia
PVF	WDCM 628	Pusat Veterinaria Farma	Asia
RITFC	WDCM 625	Research Institute for Tobacco and Fibre Crops	Asia
SEAMEO	WDCM 626	Seameo-Biotrop	Asia
SPMCC	WDCM 624	Sungei Putih Microbial Culture Collection	Asia
UICC	WDCM 563	University of Indonesia Culture Collection	Asia
UNJCC	WDCM 1145	Universitas Negeri Jakarta Culture Collection	Asia

http://www.wfcc.info/ccinfo/collection/col_by_country/i/62/

Summary

- Indonesia is highly potential for production of sustainable and renewable energy
- Microbial diversity allows discoveries to provide solution to the world problems
- Microbial culture collection facilitates past, present and future biotechnology discoveries

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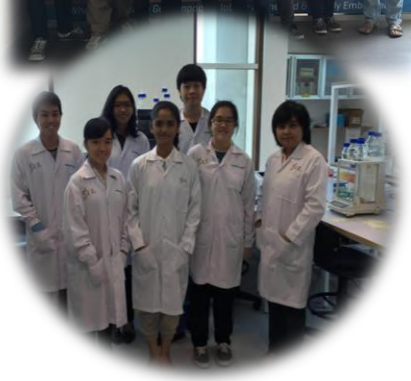

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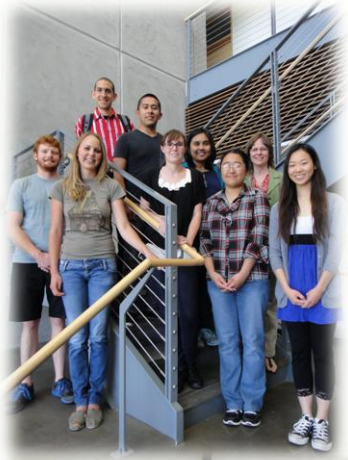

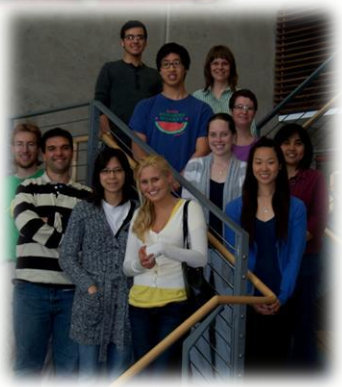

And many more…..

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