



## Biotechnology and Energy Conservation

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## 14<sup>th</sup> Lecture Biogas and Biohydrogen

The Aim:

- Students can explain the method of biogas and biohydrogen production
- Students can describe source of raw material and agents (microbes) for producing biogas and biohydrogen

## Biogas

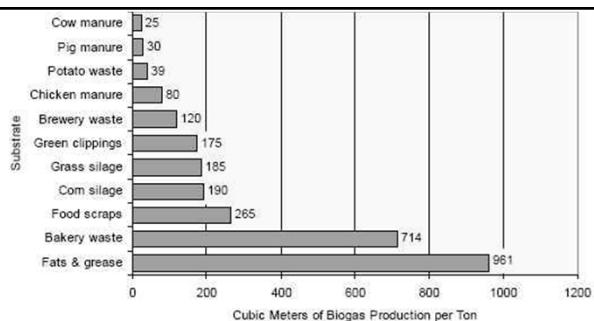
- Biogas is gas produced biologically (fermentation under anaerobic microbes) from bio-organic substances.
- Average concentration of substances composition of raw biogas:

Compositions	Concentration
Methane (CH <sub>4</sub> )	50-57%
Carbon dioxide (CO <sub>2</sub> )	25-45 %-v
Water (H <sub>2</sub> O)	2-7 %-v, (20-40°C)
Hydrogen sulfide (H <sub>2</sub> S)	20-20,000 ppm
Nitrogen (N <sub>2</sub> )	< 2 %-v
Oxygen (O <sub>2</sub> )	< 2 %-v
Hydrogen (H <sub>2</sub> )	<1 %-v

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*Influence of substrates type on production level of biogas*



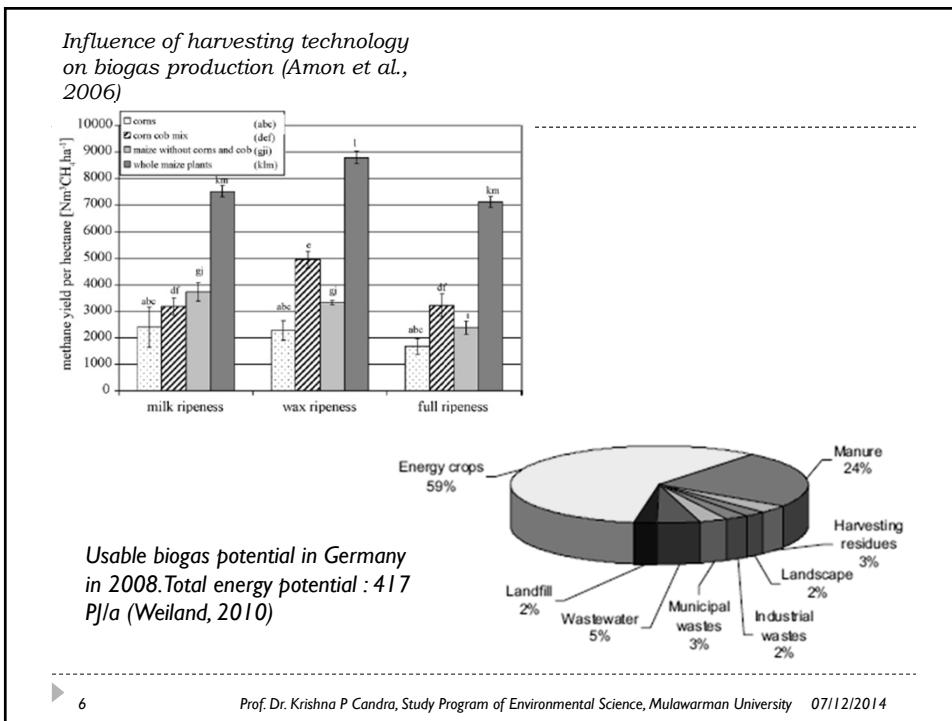
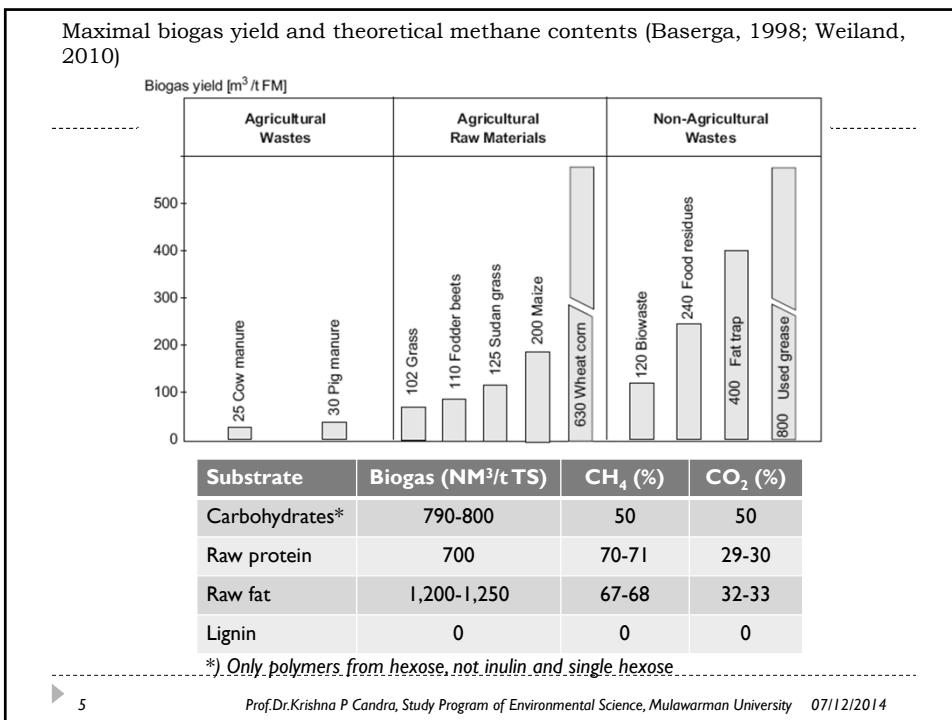
Source: Data derived from [www.biogas-energy.com](http://www.biogas-energy.com). © 2007 Biogas Energy, Inc., translated from: Basisdaten Biogas Deutschland, März 2005; Fachagentur Nachwachsende Rohstoffe e.V.

*Gross crop yield and biogas potential of different crops (Weiland, 2010)*

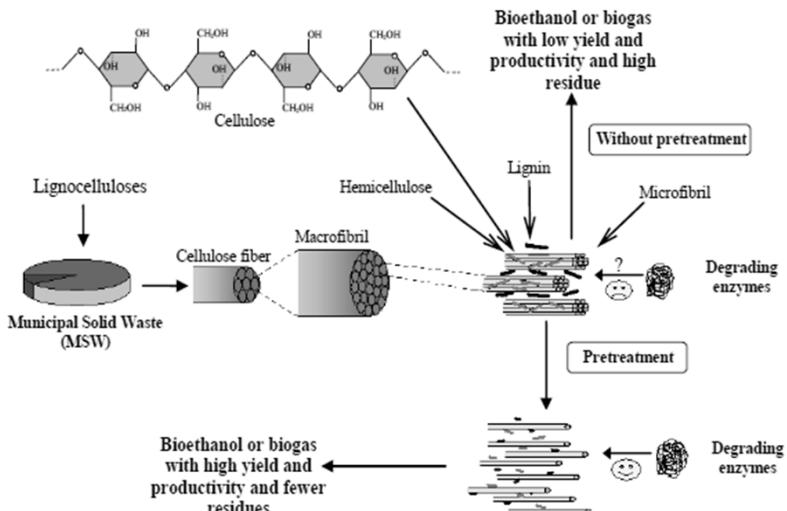
Crop	Crop yield (t FM/ha)	Biogas yield (Nm <sup>3</sup> /(t VS))	Methane content (%)
Sugar beet	40–70	730–770	53
Fodder beet	80–120	750–800	53
Maize	40–60	560–650	52
Corn cob mix	10–15	660–680	53
Wheat	30–50	650–700	54
Triticale	28–33	590–620	54
Sorghum	40–80	520–580	55
Grass	22–31	530–600	54
Red clover	17–25	530–620	56
Sunflower	31–42	420–540	55
Wheat grain	6–10	700–750	53
Rye grain	4–7	560–780	53

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Influence of pre-treatment on bioethanol/biogas production  
(Taherzadeh and Karimi, 2008)



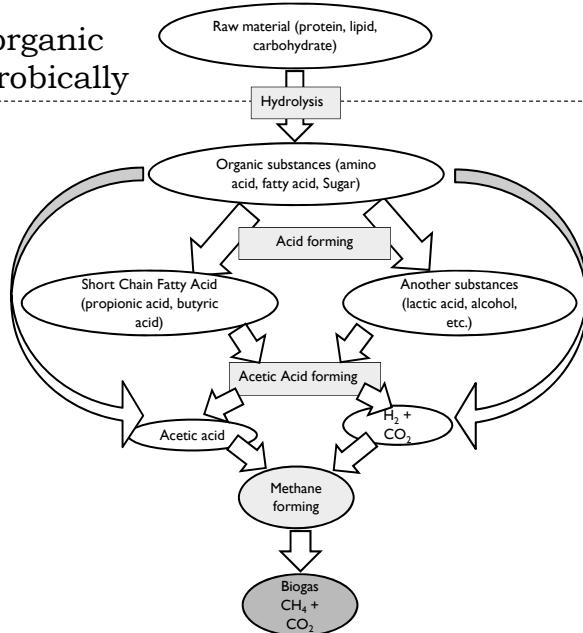
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Digesting of bio-organic substances anaerobically

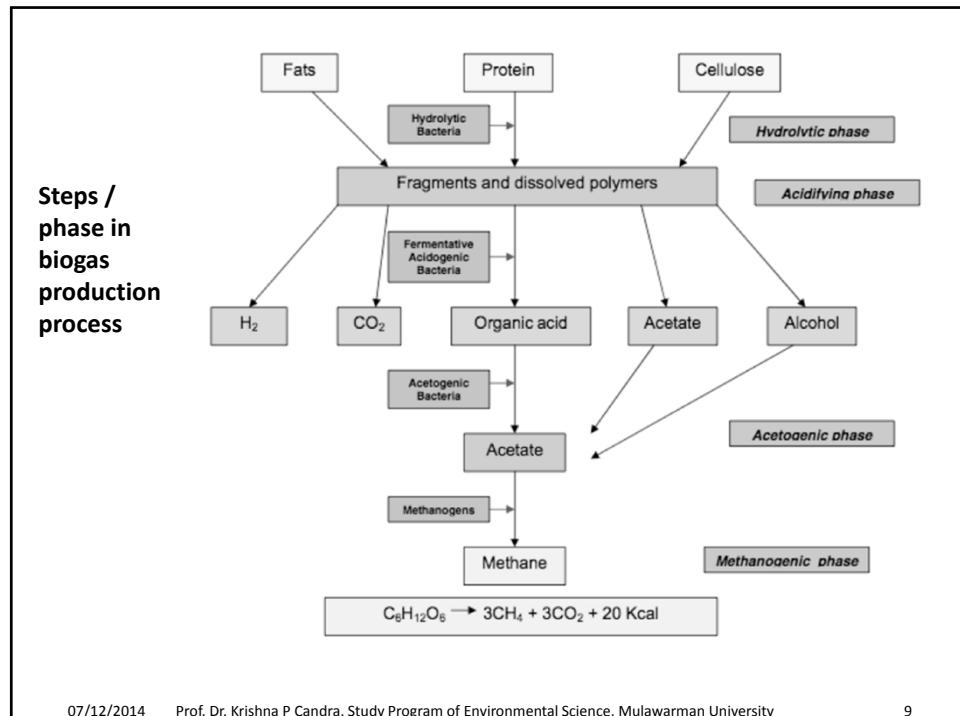
**Digesting process is in liquid state fermentation (LSF) under some requirements:**

- ▶ **Oxygen**
- ▶ **Temperature**
- ▶ **pH**
- ▶ **Substrate availability**
- ▶ **Inhibitor substances**



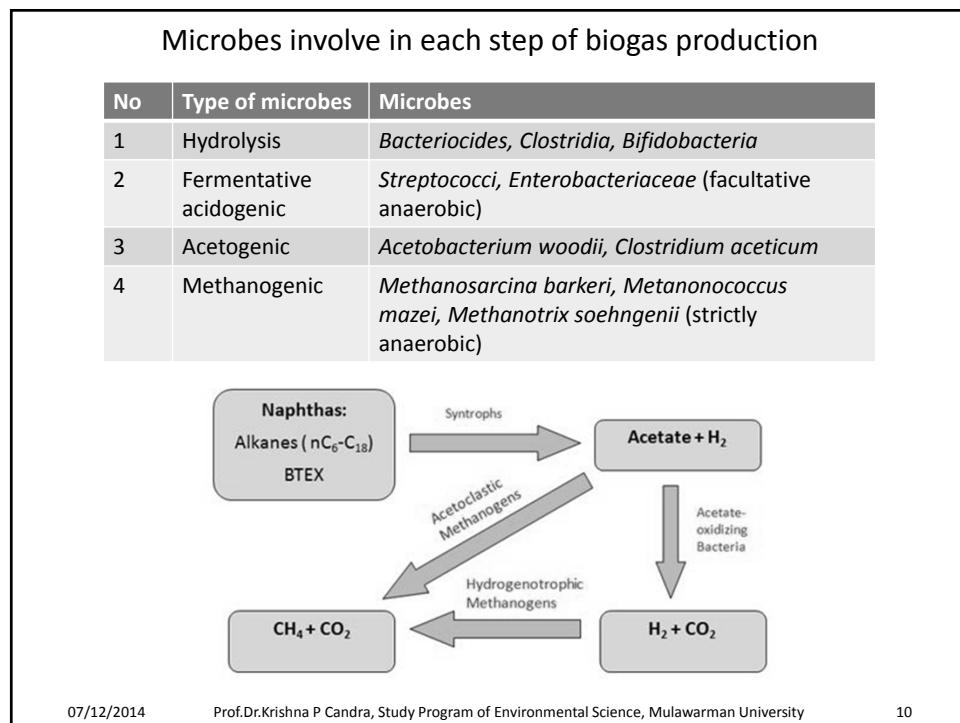
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## Digesting of bio-organic substances anaerobically

Digesting process is in liquid state fermentation (LSF) under some requirements:

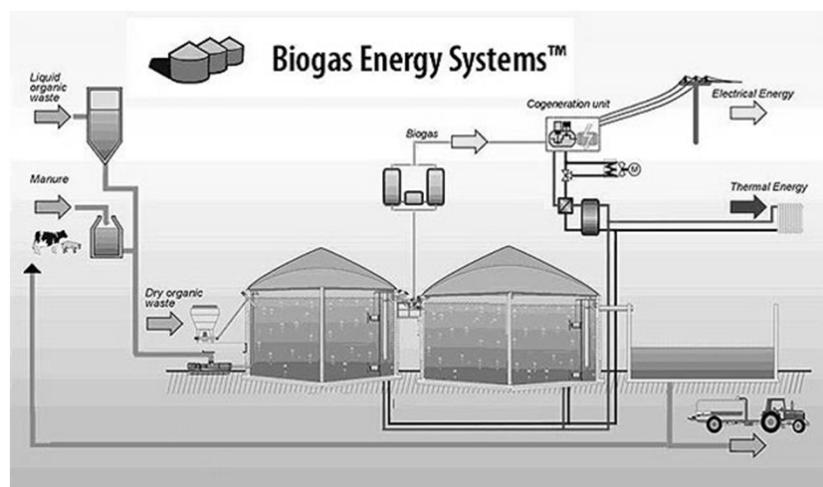
- ▶ **Oxygen** (the oxygen should be very low or in anaerobic condition).
- ▶ **Temperature** (most of methane bacteria is mesophyle, 32-42°C)
- ▶ **pH-value** (hydrolysis and acid forming process at low pH, 4.5-6.3, while acetic acid and methane forming process at neutral pH, 6.8-7.5)
- ▶ **Substrate availability** (C:N:P:S condition is 600:15:5:1)
- ▶ **Inhibitor substances** (some inhibitor substances during fermentation of methane production)

Inhibitors	Concentration
Sodium	6-30 g/L (for some adapt bacteria culture until 60 g/L)
Potassium	Starting at 3 g/L
Calcium	Starting at 2.8 g/L CaCl <sub>2</sub>
Magnesium	Starting at 2.4 g/L MgCl <sub>2</sub>
Ammonium	2.7-10 g/L
Ammonia	Starting at 0.15 g/L
Sulfur	Starting at 50 mg/L H <sub>2</sub> S, 100 mg/L S <sub>2-</sub> , 160 mg/L Na <sub>2</sub> S (for some adapt bacteria culture until 600 mg/L Na <sub>2</sub> S and 1,000 mg/L H <sub>2</sub> S)
Heavy metals	As free ions, starting at 10 mg/L Ni, 40 mg/L Cu, 130 mg/L Cr, 340 mg/L Pb, 400 mg/L Zn As carbonate form, starting at 160 mg/L Zn, 170 mg/L Cu, 180 mg/L Cd, 530 mg/L Cr <sup>3+</sup> , 1,750 mg/L Fe. Heavy metals can be by sulfite sedimented and neutralized
Branch fatty acid	Iso-butyric acid: starting at 50 mg/L

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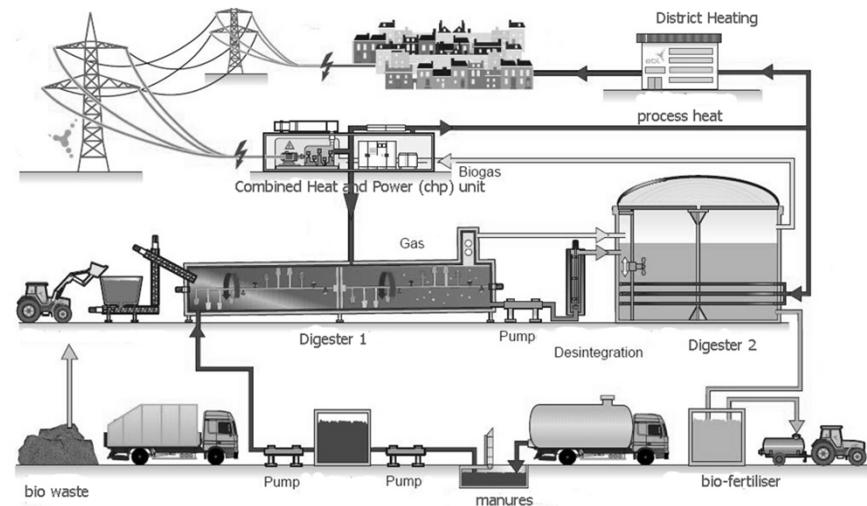
## Flow-storage process of biogas



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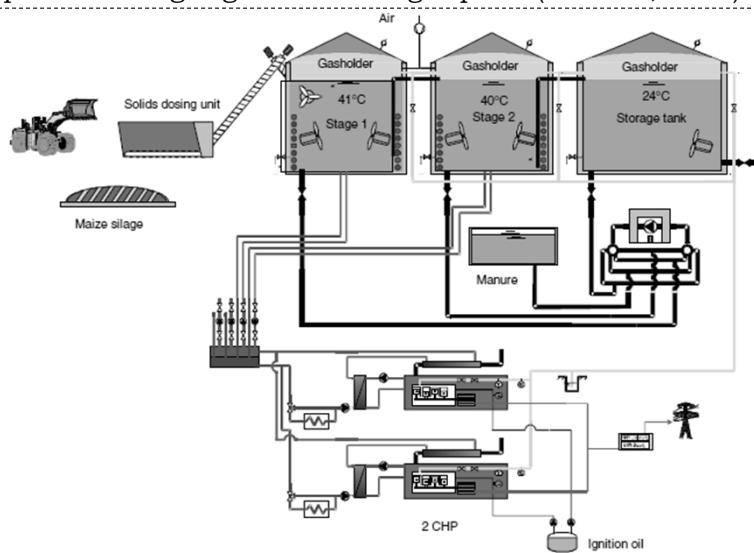
## Flow-storage process of biogas



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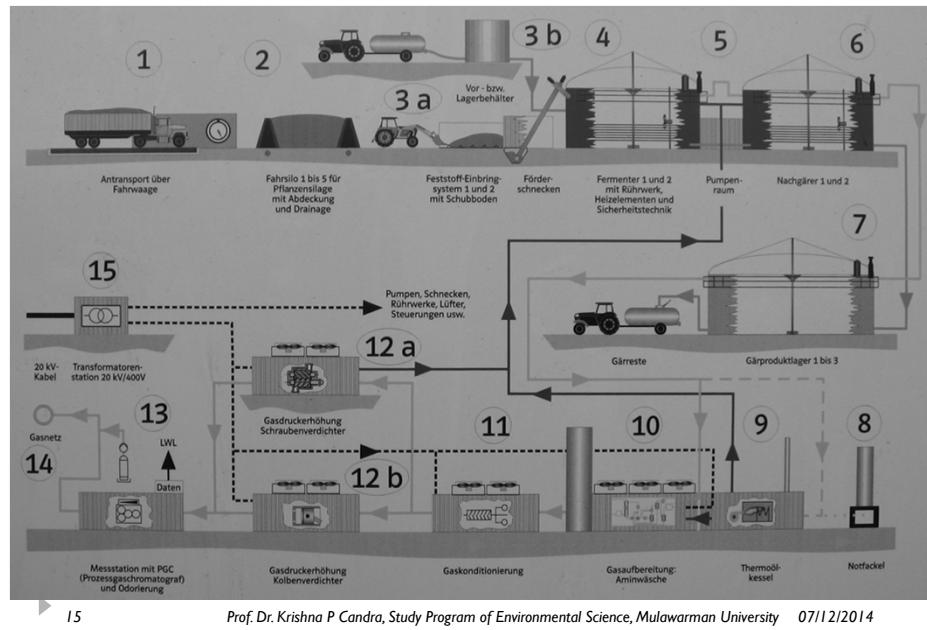
Typical two-stage agricultural biogas plant (Weiland, 2010)



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Flow-storage process of biogas at e-on company near Hannover, Germany

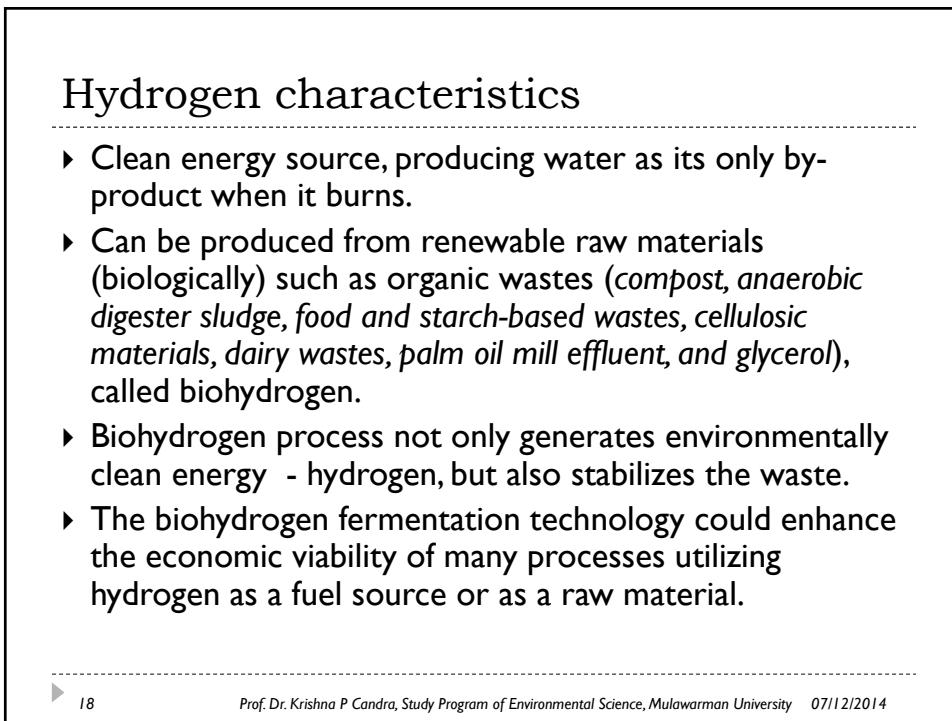
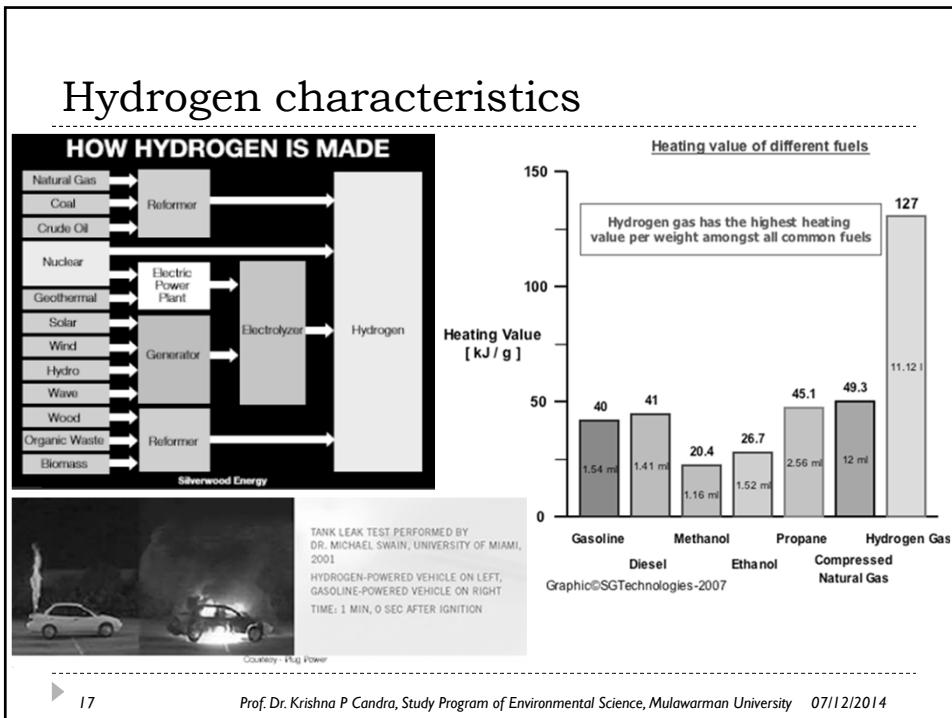


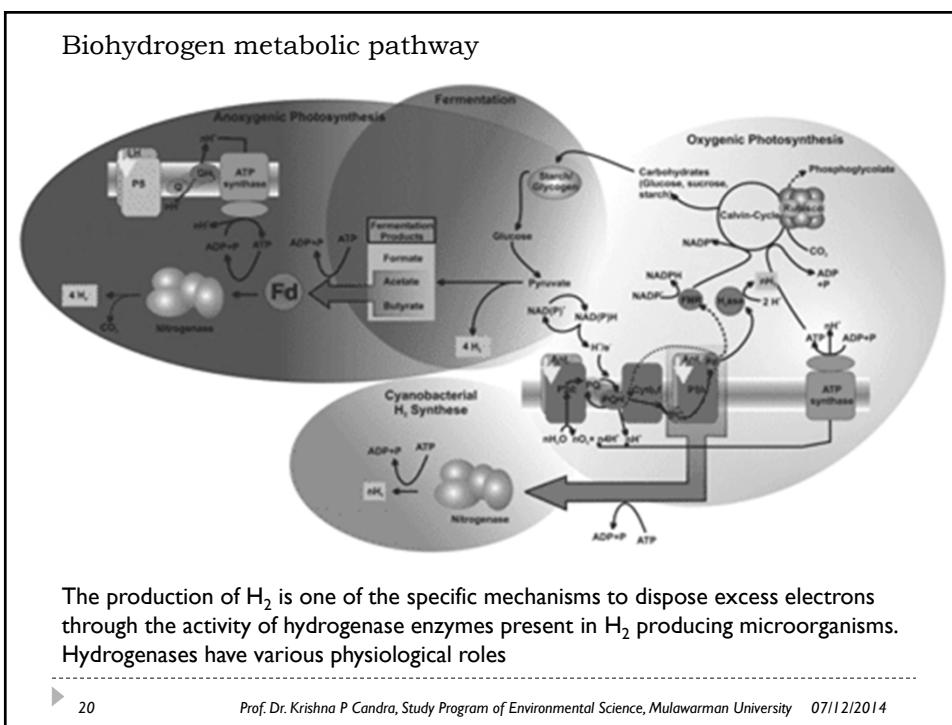
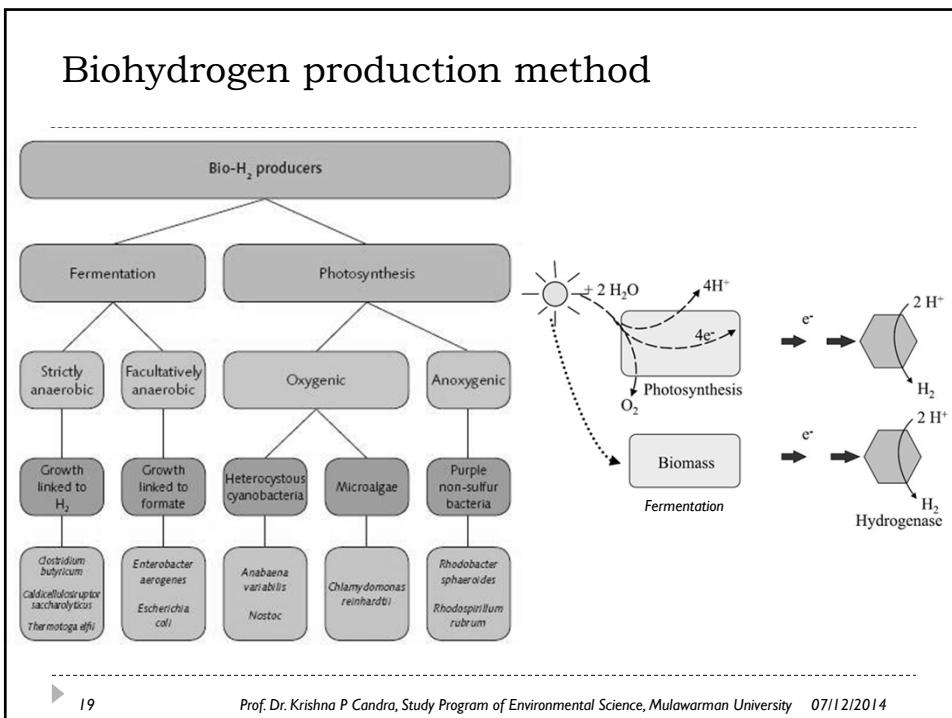
## Biogas Plant

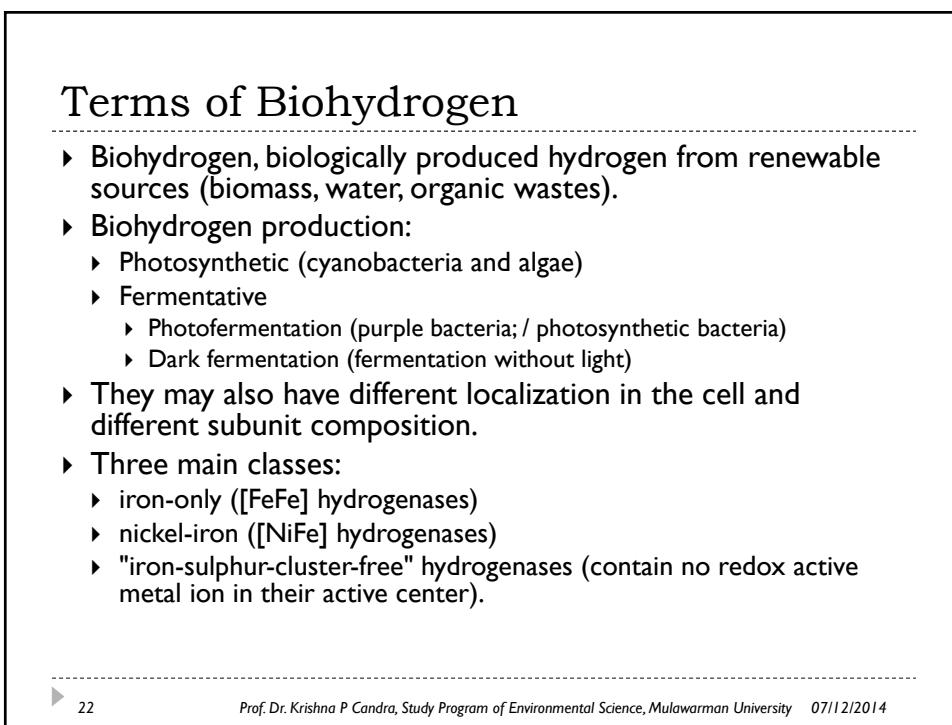
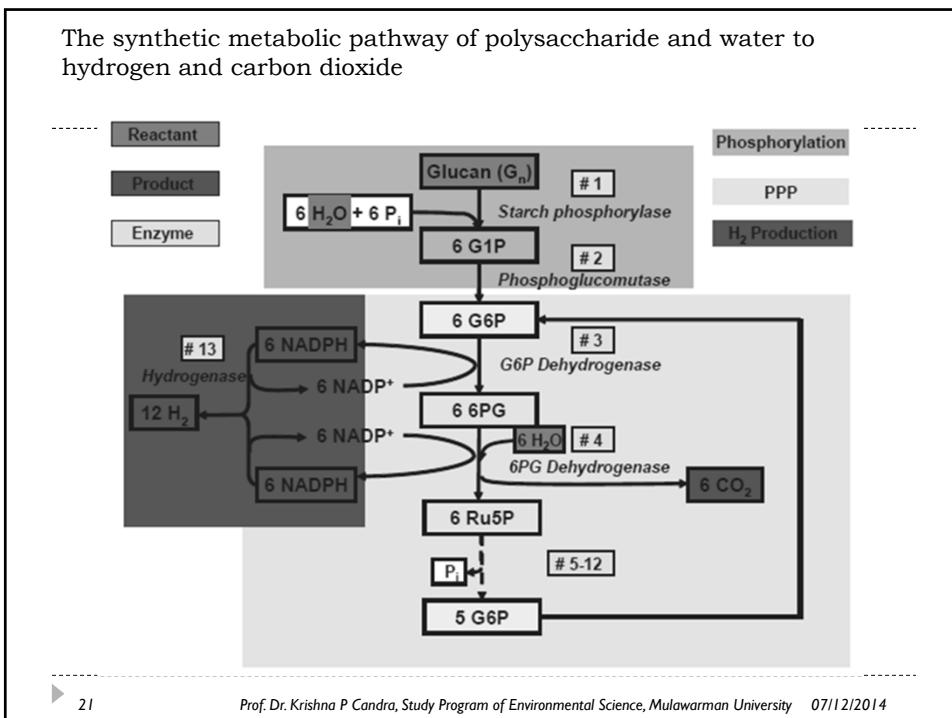


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## Biohydrogen production by dark fermentation

- ▶ Classified into two categories:
  - ▶ Facultative anaerobes (enteric bacteria, e.g. *Escherichia coli*, *Enterobacter*, and *Citrobacter*)
  - ▶ Strict anaerobes (clostridia, methylotrophic methanogens, and rumen bacteria). The extreme thermophile *Caldicellulosiruptor saccharolyticus* is related to clostridia.
- ▶ The genus *Clostridium* has been widely studied for H<sub>2</sub> production, capable of using various organic substrates such as proteins, starch, animal manure and sewage sludge. Some clostridia are both proteolytic and saccharolytic.
- ▶ The fermentative route is a promising method for biohydrogen production due to its high rate of the H<sub>2</sub> evolving [FeFe] hydrogenase and the versatility of the substrates used.

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## Optimization of biohydrogen production of *C. butyricum* EB6

- ▶ Using synthetic medium was done on pH, glucose and iron concentration
- ▶ Optimum production achieved at
  - ▶ pH 5.6,
  - ▶ 15.7 g/L glucose and
  - ▶ 0.39 g/L FeSO<sub>4</sub>
- ▶ Yield of biohydrogen at 2.2 mol H<sub>2</sub>/mol glucose

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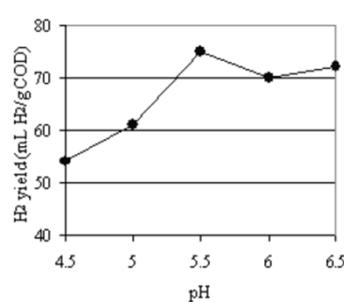
## Improving genetic characteristic of microbe expresse hydrogenase

- ▶ [Fe]-hydrogenase (*hydA*) gene of *C. butyricum* EB6
  - ▶ open reading frames of 1725 bp
  - ▶ encodes *hydA* of 574 amino acids
  - ▶ approximate size of 64 kDaltons
  - ▶ The *hydA* of *C. butyricum* was found 80.5% similar to *hydA* of *C. acetobutylicum* P262 and closely similar to *Clostridia* hydrogenase.
  - ▶ A *hydA*-expressing recombinant EB6 was successfully obtained with higher biohydrogen production of 4.2-4.8 L-H<sub>2</sub>/ L-medium compared to the wild type

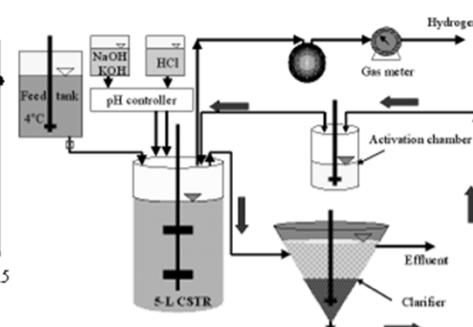
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## Hydrogen production



Effect of pH on hydrogen production in batch studies

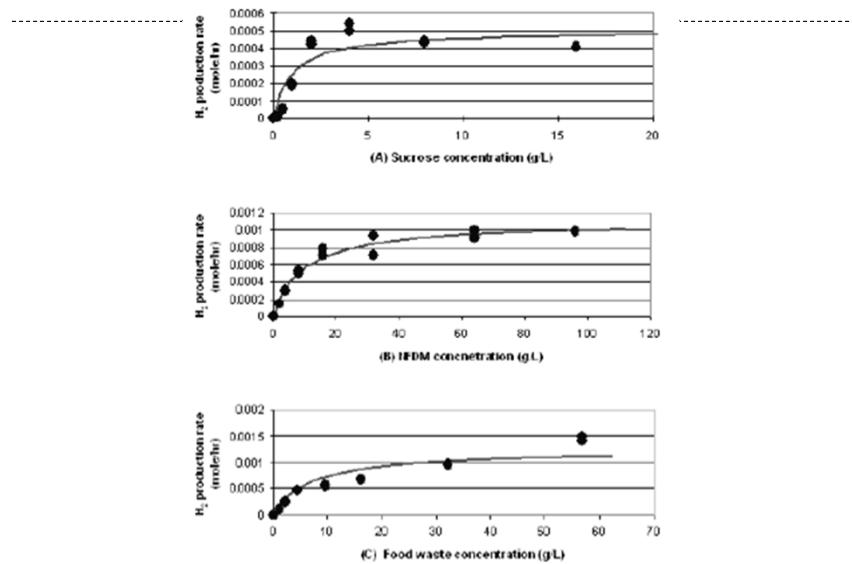


Experimental setup of continuous bioreactor

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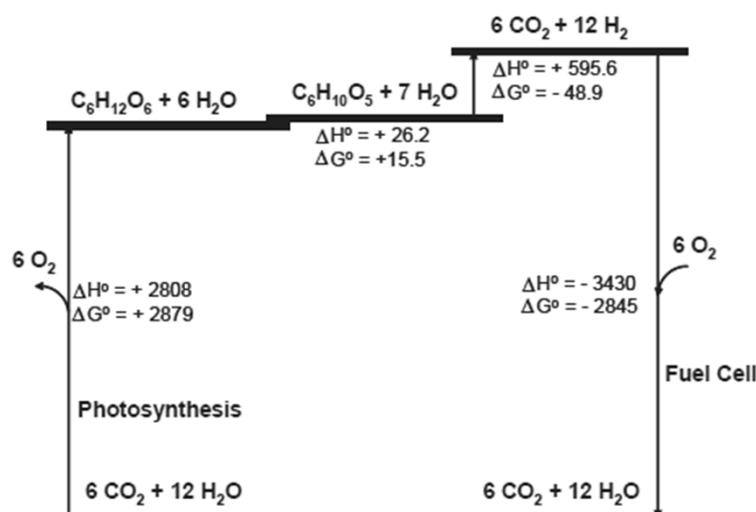
Biohydrogen production rates at various initial substrate concentration  
(A) Sucrose; (B) Non-Fat Dry Milk; (C) Food Waste



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An energy diagram showing the standard enthalpy and free energy changes in kJ/mol for reaction in a renewable energy cycle operating among  $\text{H}_2\text{O}$ ,  $\text{CO}_2$ , glucose, and starch



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The enzymes used for hydrogen production from starch and water, and reaction mechanism, sources, and amounts use in the reaction

E.C.	Enzyme Name	Reaction	Vender	Origin	Unit
24.1.1	glycogen phosphorylase	$(C_6H_{10}O_5)_n + P_i + H_2O \rightarrow (C_6H_{10}O_5)_{n-1} + \text{glucose-1-P}$	Sigma	rabbit muscle	10
5.4.2.2	phosphoglucomutase	G-1-P → G-6-P	Sigma	rabbit muscle	10
1.1.1.49	glucose-6-phosphate dehydrogenase	$G-6-P + NADP^+ \rightarrow 6\text{-phosphogluconate} + NADPH$	Sigma	<i>S. cerevisiae</i>	1
1.1.1.44	6-phosphogluconic dehydrogenase	$6\text{-phosphogluconate} + H_2O + NADP^+ \rightarrow \text{ribulose-5-phosphate} + NADPH + CO_2$	Sigma	<i>S. cerevisiae</i>	1
5.3.1.6	ribose 5-phosphate isomerase	$\text{ribulose-5-phosphate} \rightarrow \text{ribose-5-phosphate}$	Sigma	spinach	1
5.1.3.1	ribulose-5-phosphate 3-epimerase	$\text{ribose-5-phosphate} \rightarrow \text{xylulose-5-phosphate}$	Sigma	<i>S. cerevisiae</i>	1
2.2.1.1	transketolase	$\text{xylulose-5-phosphate} + \text{ribose-5-phosphate} \rightarrow \text{sedoheptulose-7-phosphate} + \text{glyceraldehyde-3-phosphate}$	Sigma	<i>E. coli</i>	1
		$\text{xylulose-5-phosphate} + \text{erythrose-4-phosphate} \rightarrow \text{fructose-6-phosphate} + \text{glyceraldehyde-3-phosphate}$			
2.2.1.2	transaldolase	$\text{sedoheptulose-7-phosphate} + \text{glyceraldehyde-3-phosphate} \rightarrow \text{fructose-6-phosphate} + \text{erythrose-4-phosphate}$	Sigma	<i>S. cerevisiae</i>	1
5.3.1.1	triose-phosphate isomerase	$\text{glyceraldehyde-3-phosphate} \rightarrow \text{dihydroacetone phosphate}$	Sigma	rabbit muscle	1
4.1.2.13	aldolase	$\text{glyceraldehyde-3-phosphate} + \text{dihydroacetone phosphate} \rightarrow \text{fructose-1,6-bisphosphate}$	Sigma	rabbit muscle	1
3.1.3.11	fructose-1,6-bisphosphate	$\text{fructose-1,6-bisphosphate} + H_2O \rightarrow \text{fructose-6-phosphate} + Pi$	[41]	<i>E. coli</i>	1
5.3.1.9	phosphoglucose Isomerase	$\text{fructose-6-phosphate} \rightarrow \text{glucose-6-P}$	Sigma	<i>S. cerevisiae</i>	1
1.12.1.3	<i>P. furiosus</i> hydrogenase I	$NADPH + H^+ \rightarrow NADP^+ + H_2$	[22,42]	<i>P. furiosus</i>	~70