m.k TEKNOLOGI BIOINDUSTRI
TIN 330 (2-3)

Bioethanol Production Technology and Its Prospect to be Developed in Indonesia

Departemen Teknologi Industri Pertanian
FATETA - IPB

What is Bioethanol ?

= Ethanol derived from agricultural sources

(as distinct from petrochemical sources)

Originally, it was produced by the spontaneous fermentation of sugars and was consumed by ancient races for its intoxicating effects. The Arabs and Romans learned to isolate the alcohol and to employ it industrially in the preparation of perfumes, cosmetics, and medicines.

In the current time, the importance of alternative energy source has become even more necessary due to :

1). depletion of limited fossil fuel stock

2). safe and better environment

Replacing fossil fuels with so-called **biofuels**, such as bioethanol, is one way of reducing greenhouse gas emissions from the transport sector, which is responsible for a considerable proportion of total CO_2 emissions

BENEFITS OF BIOETHANOL

- Less dependence on crude oil
- It is a renewable fuel.
- Increase octane number (a standard measure of the performance of motor fuel)
- Reduces air pollution, cleaner environment due to cleaner combustion ➔ lower net carbon dioxide emissions .
- Helping emerge a new market
- Expanded market opportunity in the agricultural field

WHAT ARE THE RAW MATERIALS FOR BIOETHANOL?

There are in general three groups of raw material:

1). Sugar : Beet, Sugar Cane, Sweet Sorghum and Fruits.

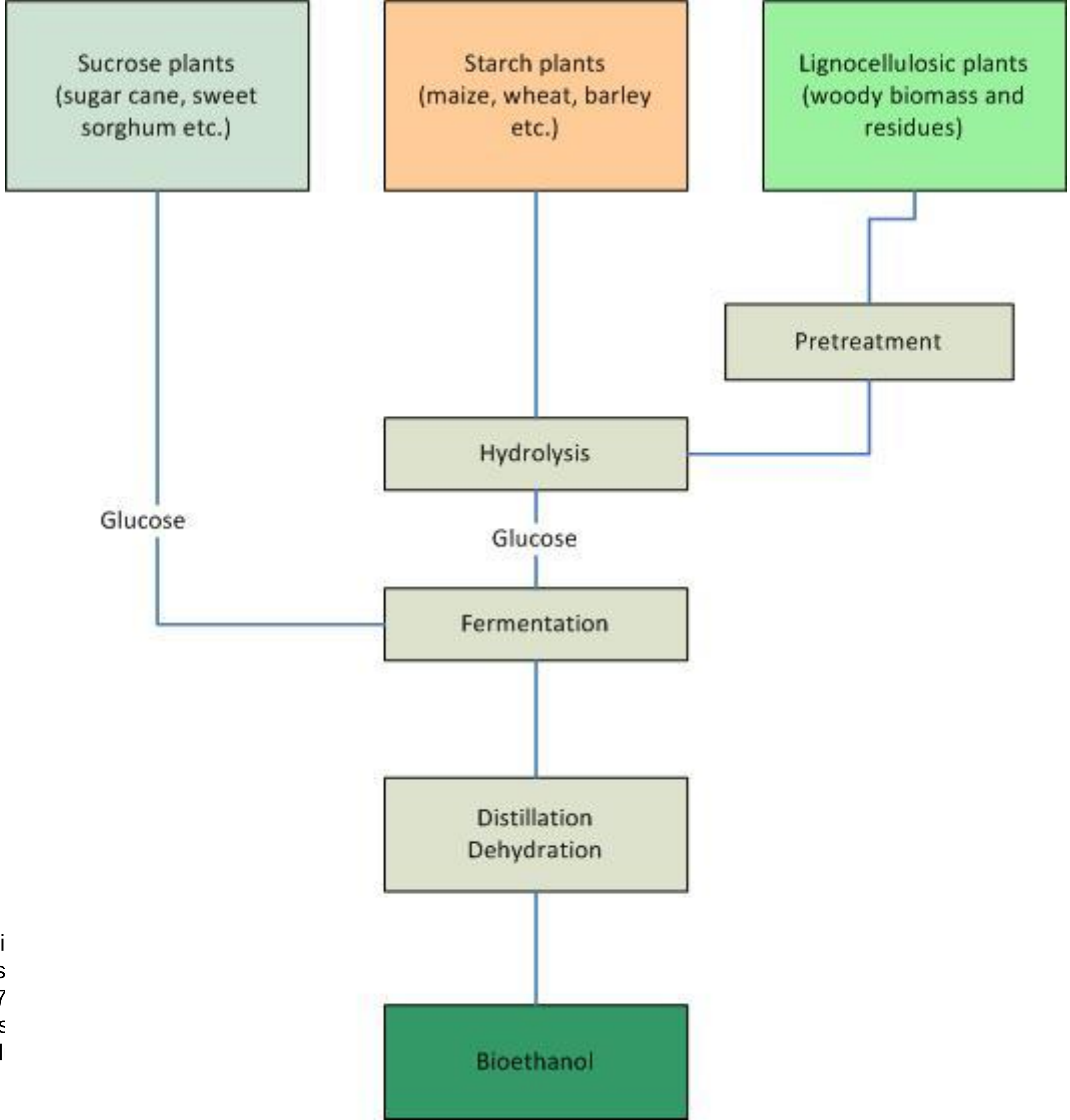
2). Starchy Material such as corn, wheat, rice, potatoes, cassava, sweet potatoes etc.

3). Cellulose materials like wood, used paper, crop residues etc.

STEPS FOR ETHANOL PRODUCTION

- Fermentation Process
- Distillation Process
- Dehydration Process

http://www.zeochem.ch/information/pdfs/ETHANOL%20Process.pdf



http://www.bi
gr/en/articles
oethanol/477
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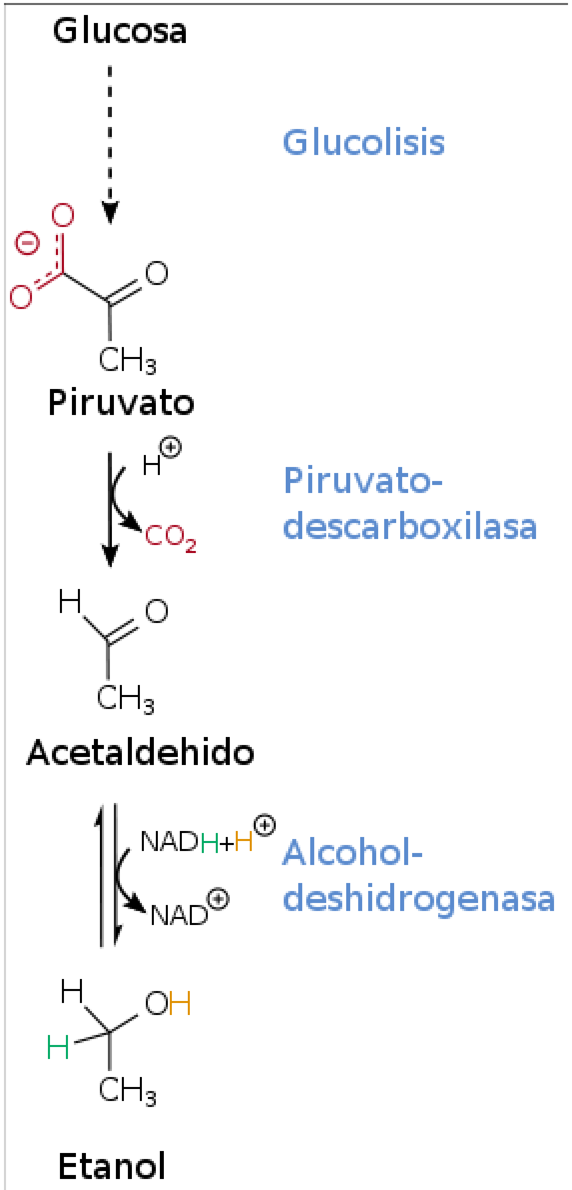
BIOETHANOL PRODUCTION

Ethyl alcohol production is based on two major procedures :

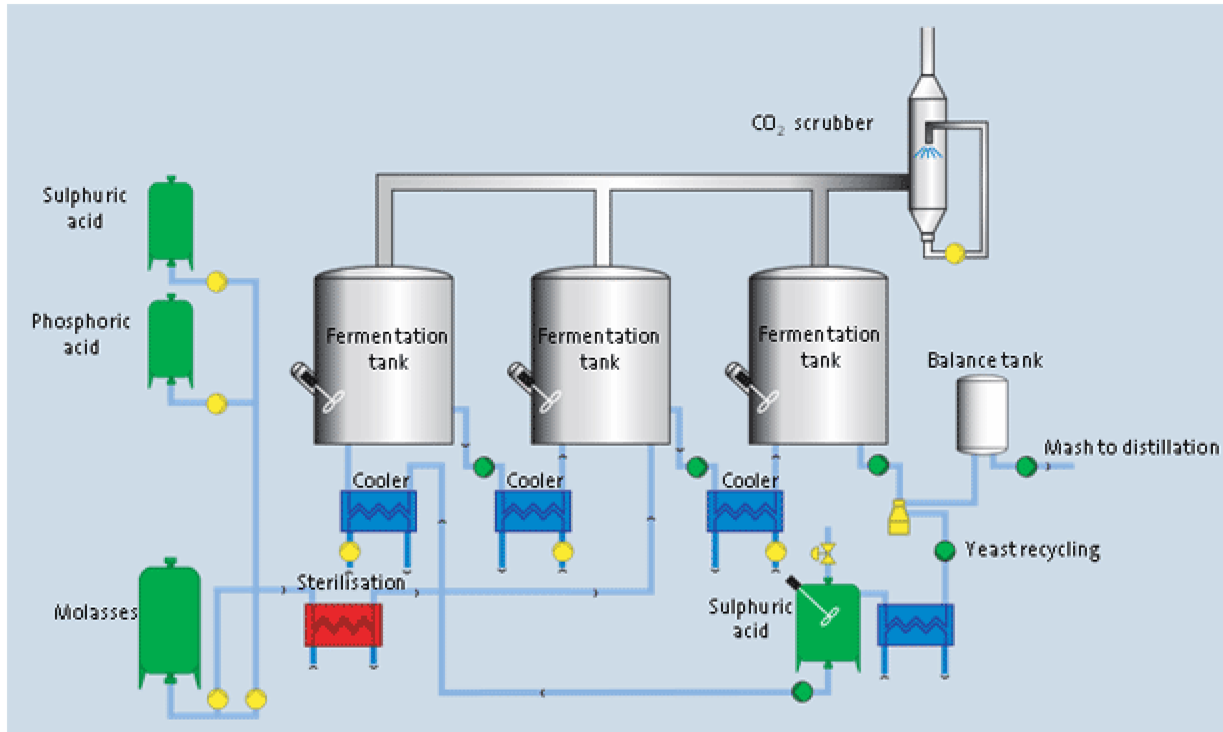
(1). **Fermentation***. Cereal grains (corn, wheat, barley, sorghum, or rye);* sugarcane (molasses); sugar beets; fruit product wastes; other starch crops (potatoes or rice); sulfite liquors (paper pulping); and such high cellulose-containing materials as wood, crop residues, and cultivated fiber crops.

(2). **Chemical synthesis***. Petroleum and natural gas; coal; oil shales; and* tar sands.
Synthetic alcohol is not purer or better quality than fermentation alcohol for industrial use.

Bioethanol Pathway



Bioethanol Production by using Molasses



http://withfriendship.com/user/levis/ethanol-fermentation.php

MICROORGANISMS for BIOETHANOL PRODUCTION

Bacteria	*Zymomonas mobilis* *Bacillus stearothermophilus* *Escherichia coli* *Klebsiella oxytoca*
Yeast	*Saccharomyces cerevisiae* *Pachysolen tannophilus* *Candida shehatae* *Pichia stipitis*

Zymomonas mobilis

- A gram-negative bacteria
- The only bacteria which can use Entner-Doudoroff pathway anaerobically
- Unable to ferment pentose but hexose
- Limitation of using lignocellulose
- Relatively easier to receive and maintain foreign genes
- High ethanol yield

Bacillus stearothermophilus

- Thermophilic organisms fermenting hexose and pentose after being modified
- produces low levels of ethanol following fermentation of glucose at temperatures in excess of 60°C, and a major by-product is l-lactic acid
- Avoid the limitation of high concentration of ethanol harmful to fermentaion

Escherichia coli

- An important vehicle for the cloning and modification of genes
- Ferment hexose and pentose as well with high ethanol yield by recombinant strains
- High glycolytic fluxes
- Reasonable ethanol tolerance

Klebsiella.oxytoca

- Wide sugar utilization
- Form ethanol through the PFL pathway after being modified
- High ethanol yield

Saccharomyces cerevisiae

- The most common and natural fermentative yeast for ethanol
- Only convert glucose to ethanol for wild-type
- Limitation of using lignocellulose
- Relative high ethanol yield
- Can be easily modified by metabolic engineering to ferment pentose

Other Yeast *: Pachysolen tannophilus*, *Candida shehatae*, and *Pichia stipitis*

- Ferment xylose
- Low ethanol yields
- High sensitivity to inhibitors, low pH and high concentration of ethanol

Pichia stipitis

*F*ungus that ferments xylose to ethanol, and degrades lignin and cellulose for the potential conversion of biomass to ethanol, but lack of industrial-grade microorganisms for converting biomass into fuel ethanol. The highest yields for the conversion of biomass to ethanol are expected to come from microorganisms such as *P. stipitis* that can ferment the sugar xylose.

Clostridium thermocellum is an anaerobic bacterium capable of directly converting cellulose from biomass into ethanol.
The degradation of cellulose is carried out by an extracellular cellulase system called the **cellulosome**, and continued research in this area will provide crucial information for better understanding the cellulolytic reaction--a key process in biomass conversion.

http://www.jgi.doe.gov/whoweare/bioenergy/bioenergy_6.html

In recent years, metabolic engineering for microorganisms used in fuel ethanol production has shown significant progress. ➔ *Saccharomyces cerevisiae*, *Zymomonas mobilis* and *Escherichia coli* have been targeted through metabolic engineering for cellulosic ethanol production.

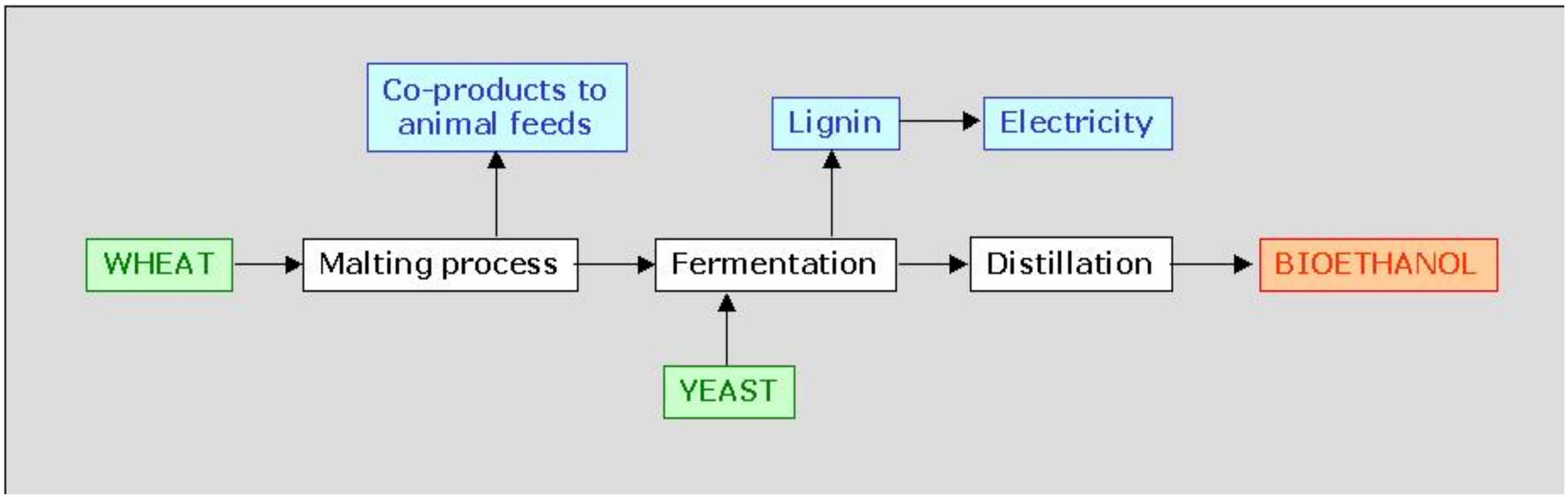
Combined hydrolysis and fermentation

Some species of bacteria capable of direct conversion of a cellulose substrate into ethanol. One example is ***Clostridium thermocellum***, which uses a complex cellulosome to break down cellulose and synthesize ethanol.

However, *C. thermocellum* also produces other products during cellulose metabolism, including acetate and lactate, in addition to ethanol, lowering the efficiency of the process.

Conversion of Starch to sugar and then sugar to ethanol

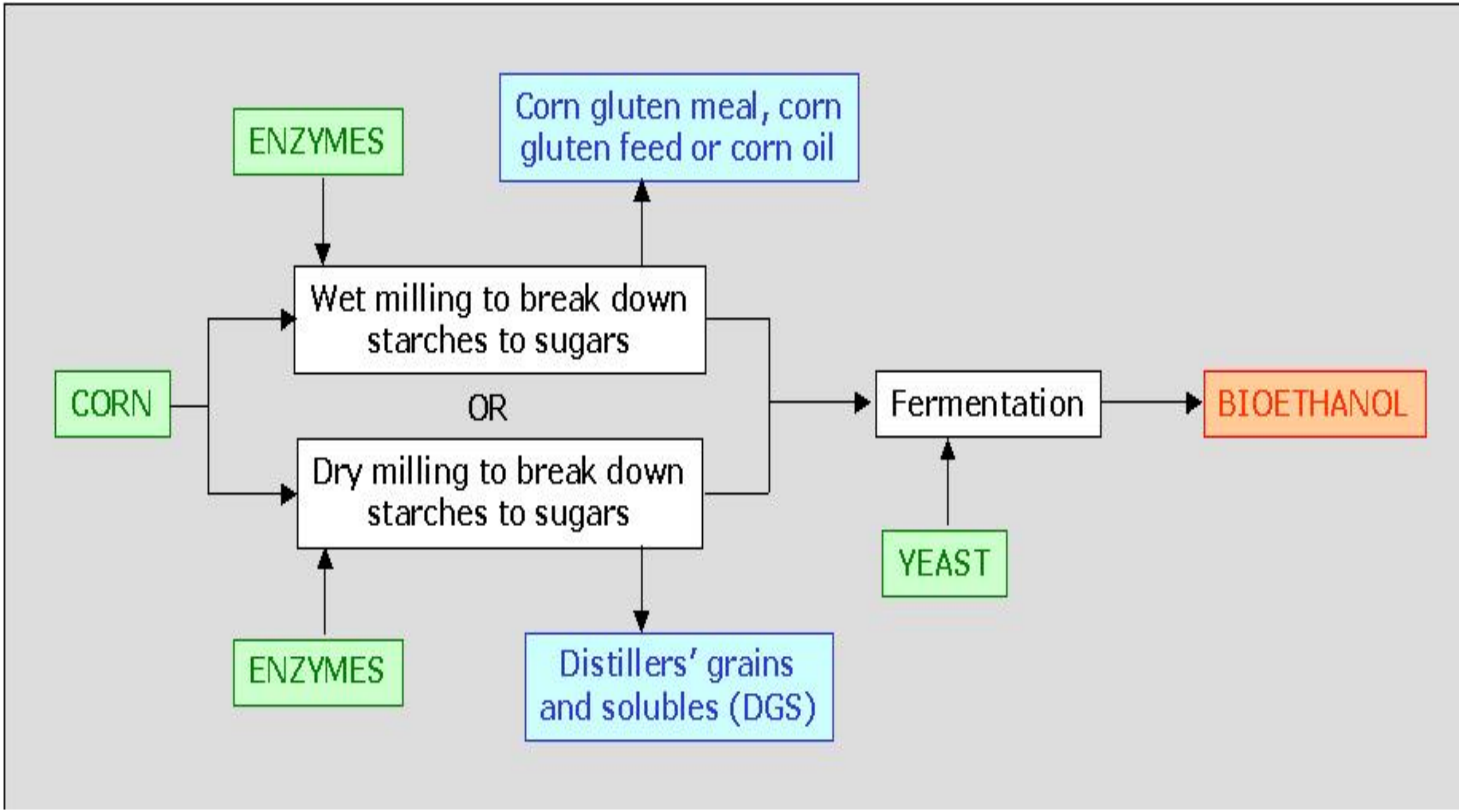
Wheat



- Ethanol is produced at 10-15% concentration and the solution is distilled to produce ethanol at higher concentrations

*Source : sajeewa.wikispaces.com/file/view/bioethanol.**ppt***

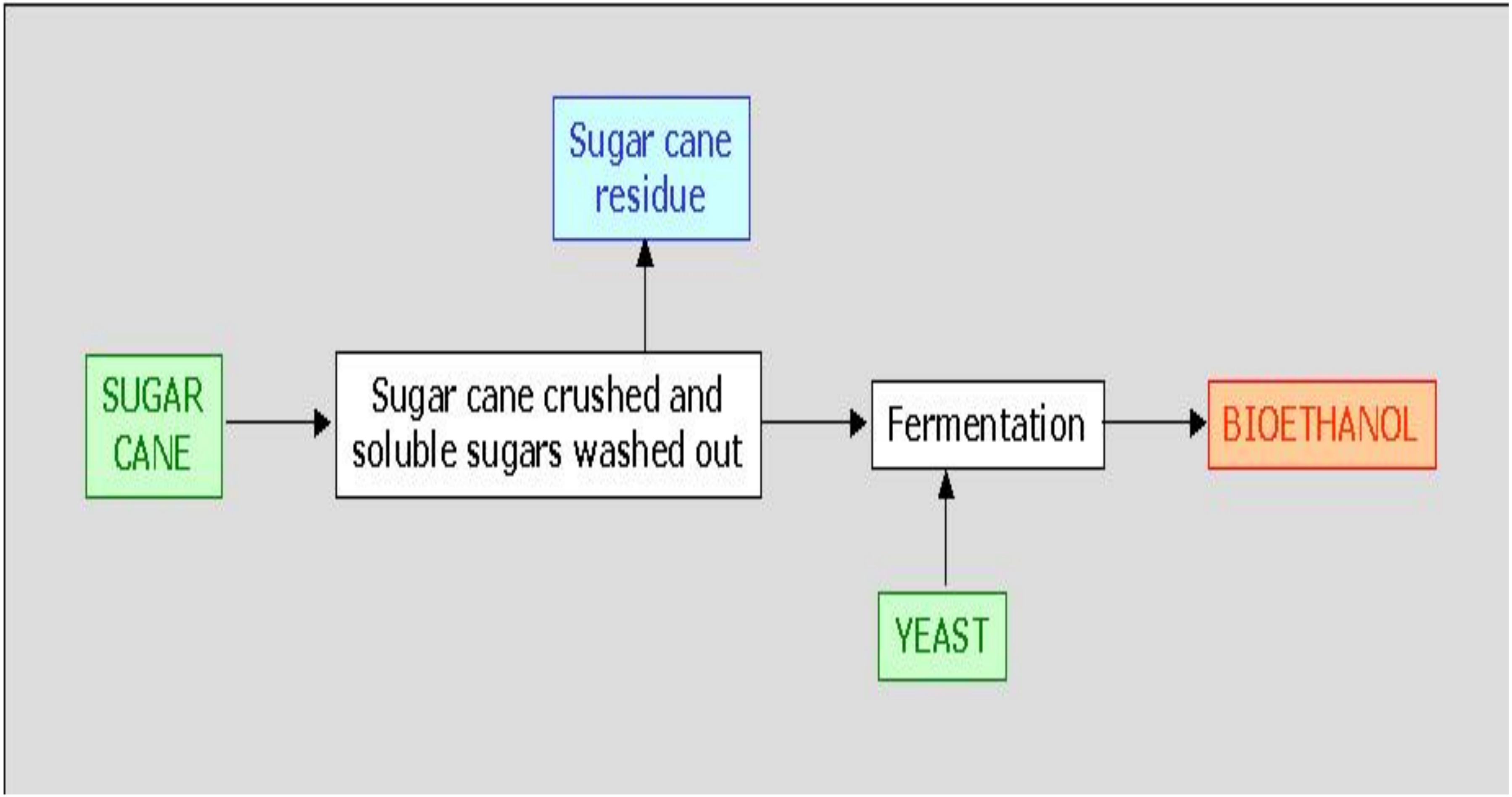
Corn



*Source : sajeewa.wikispaces.com/file/view/bioethanol.**ppt***

http://www.dft.gov.uk/pgr/roads/environment/research.htm

Sugar cane



*Source : sajeewa.wikispaces.com/file/view/bioethanol.**ppt***

http://www.biofuels-platform.ch/en/infos/bioethanol.php

Bioethanol from Molasses

- The molasses is diluted with water until a concentration of 8-10% sugar is obtained in solution.

- To discourage bacterial growth, this is acidified with a little sulphuric acid.

- A nutritive solution of ammonium salts is added.

- The dilute solution obtained as above is taken in big fermentation tanks and some yeast is added (5% by volume).

***Saccharomyces cerevisiae* Yeast** :

Invertase converts sucrose into glucose and fructose.

Zymase, another enzyme present in yeast converts glucose and fructose into ethanol and carbon dioxide.

The carbon dioxide formed is allowed to escape, but air is not allowed to enter. In presence of air ethanol formed would be oxidised to acetic acid.

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow[\text{in yeast}]{\text{Invertase}} \underset{\text{glucose}}{C_6H_{12}O_6} + \underset{\text{fructose}}{C_6H_{12}O_6}$$

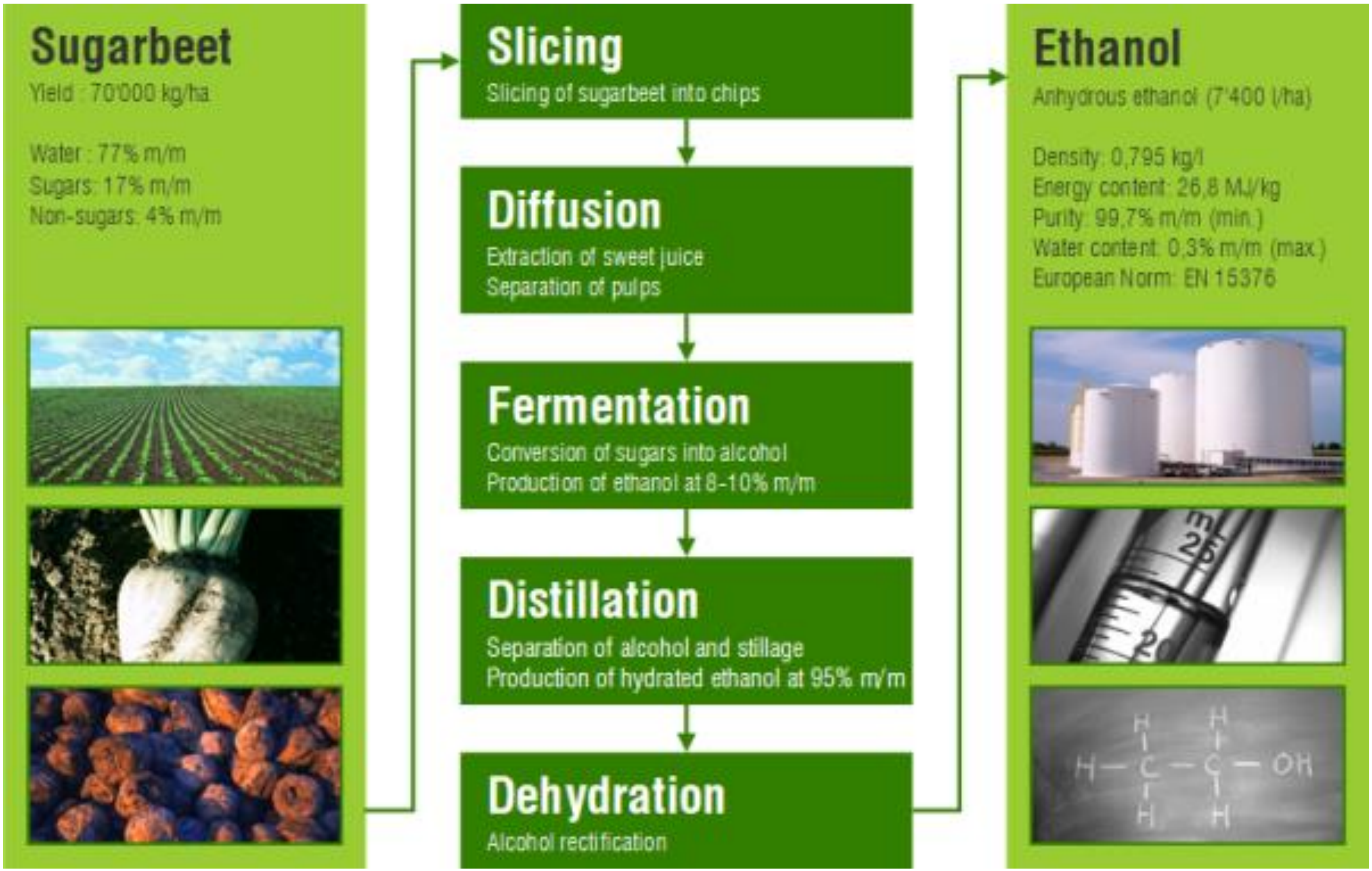
$$\underset{\text{glucose or fructose}}{C_6H_{12}O_6} \xrightarrow[\text{in yeast}]{\text{Zymase}} \underset{\text{ethanol}}{2C_2H_5OH} + \underset{\text{carbon dioxide}}{2CO_2}$$

The fermentation is complete in 3 days.

The carbon dioxide obtained as byproduct is recovered and can be sold.

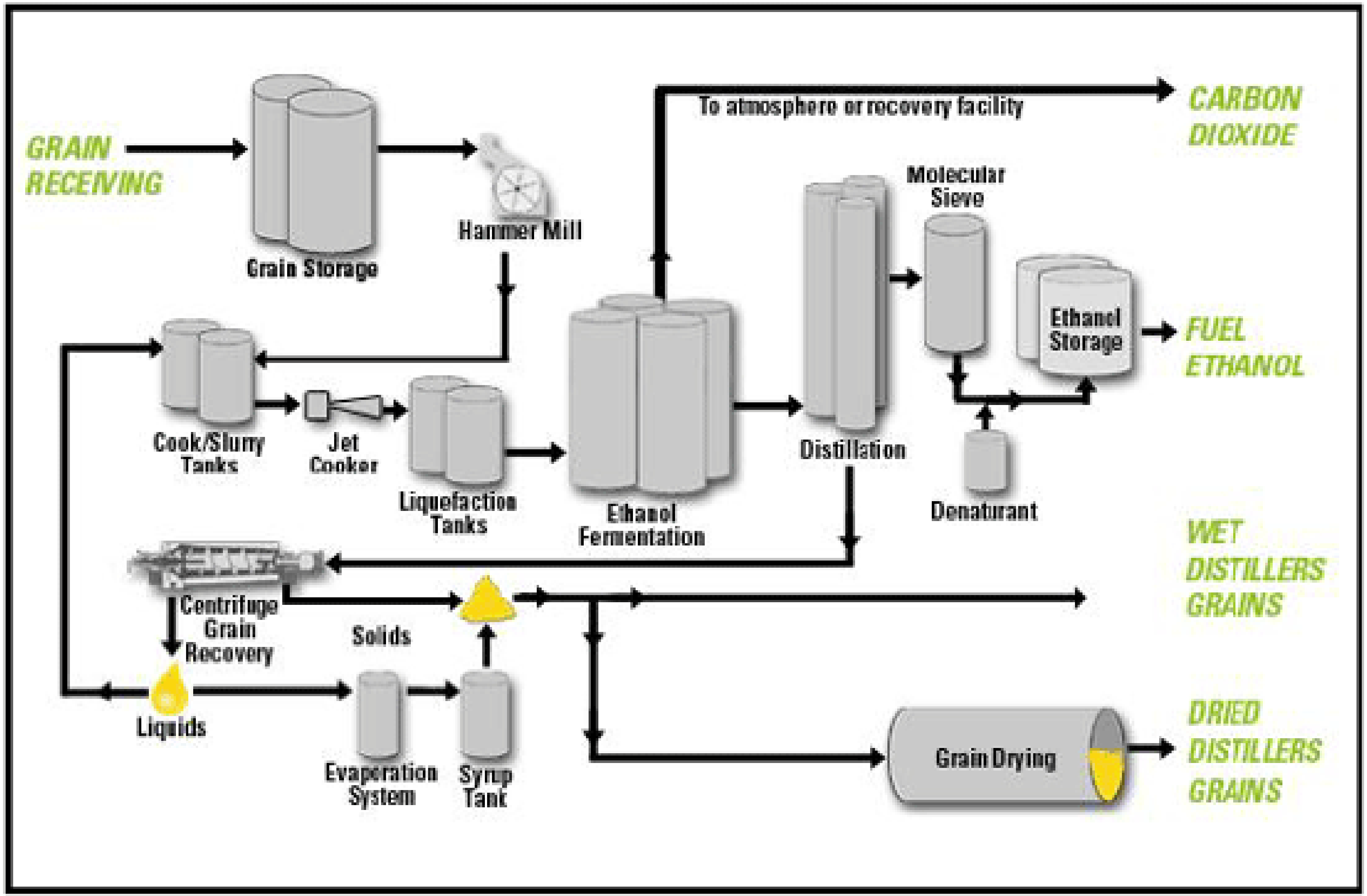
Distillation

- The fermented liquor contains 9-10% of ethanol and is called **wash or wort.** It is distilled to remove water and other impurities.
- The steam condenses and the alcohol vapors escaping near the top are condensed in the condenser.
- The distillate contains about **90% alcohol** and the **residue** left in the still is used as **cattle feed**.



http://www.biofuels-platform.ch/en/infos/bioethanol.php

Grain



http://www.thebioenergysite.com/articles/323/potential-for-improving-carbon-energy-balance-of-bioethanol

Lignocellulosic Biomass Composition

Basically, the lignocellulosic biomass comprises of cellulose, hemicellulose and lignin.

Cellulose is a linear, crystalline homopolymer with a repeating unit of glucose strung together beta-glucosidic linkages. The structure is rigid and harsh treatment is required to break it down. Hemicellulose consists of short, linear and highly branched chains of sugars.

Hemicellulose is a hetero-polymer of D-xylose, D-glucose, D-galactose, D-mannose and L-arabinose.

BIOETHANOL from LIGNOCELLULOSIC MATERIALS_

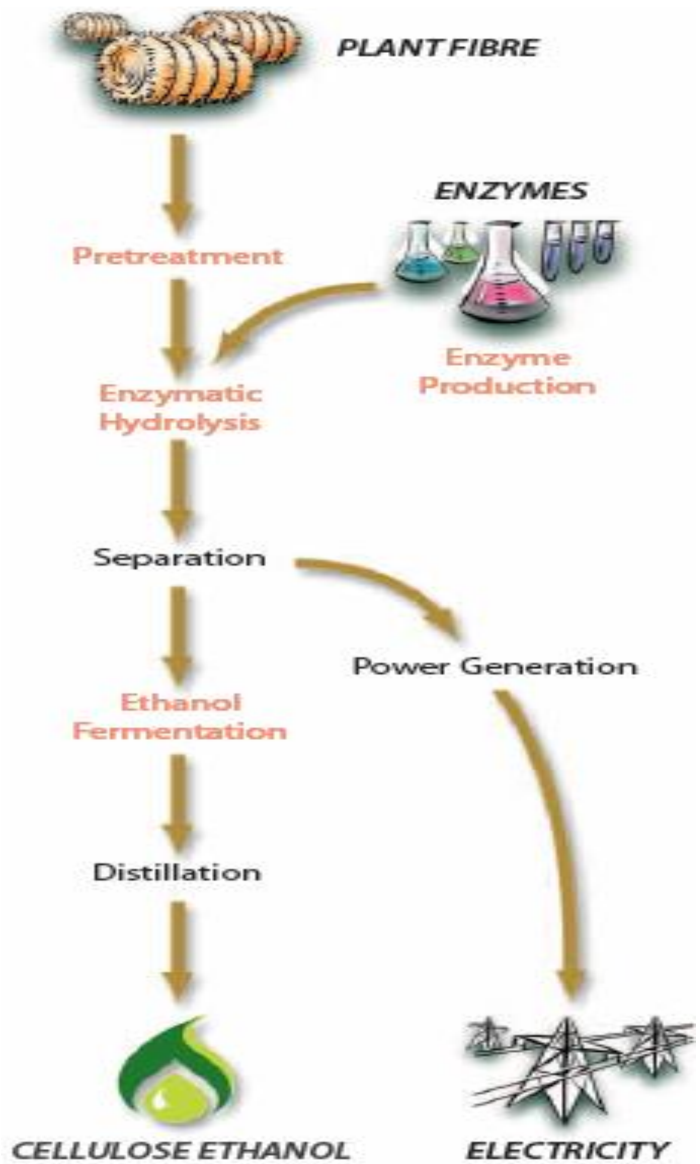
Cellulolysis processes which consist of hydrolysison pretreated lignocellulosic materials, using enzymes to break complex cellulose into simple sugars such as glucose and followed by fermentation and distillation.

Challenge for the future…

Improvement of the **cellulosic ethanol production process.**

Since it is produced from non-edible parts of plants, cellulosic ethanol does not compete with the production of food, resulting in no contribution for the price surge of food.

Overview of the cellulosic ethanol production technology



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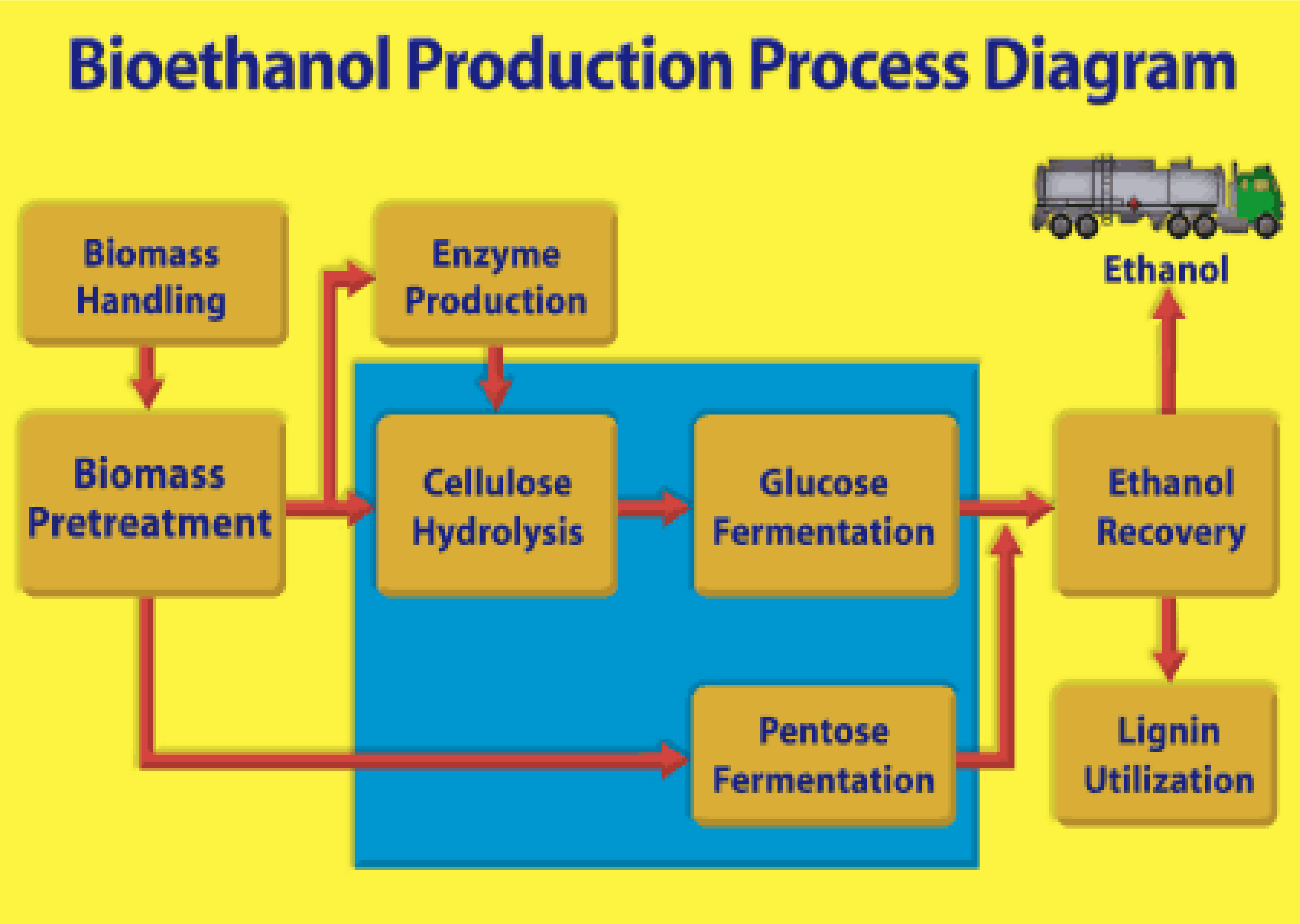


http://www1.eere.energy.gov/biomass/m/abcs_biofuels.html

Steps of conversion of cellulose and hemicellulose to Bioethanol

1. Pretreatment
2. Hydrolysis
3. Fermentation
4. Distillation of the product mixture to separate ethanol

1) Pretreatment

The solubilization and separation of one or more of the four major components of biomass – hemicellulose, cellulose, lignin, and extractives – to make the remaining solid biomass more accessible to further chemical or biological treatment.

2) Hydrolysis

The breaking down of the glycosidic bonds in cellulose and hemicellulose

* **acid hydrolysis**

Sugars made after acid hydrolysis get converted into furfural in the acidic medium which can act as fermentation inhibitors.

- *Reaction should be rapid*
- *Sugars should be rapidly removed*

* **enzymatic hydrolysis**

Enzyme hydrolysis

- Bacteria and fungi are used as sources of cellulases, hemicellulases that could be used for the hydrolysis of pretreated lignocelulosics.

- There are two technological developments.
  - Enzymatic conversion
  - Direct microbial conversion (DMC)

Direct Microbial Conversion (DMC)

- A single microorganism does both hydrolysis and fermentation (cellulose → sugar → bioethanol)

- ✓ Advantage
  - ✓ Cellulose enzyme production or purchase is a significant cost in enzymatic hydrolysis under development → with DMC, a dedicated step for production of cellulase enzyme is not necessary.

- ✓ Disadvantage
  - ✓ Currently available microbes cannot do both processes at the required efficiencies

Applications of Enzymatic Hydrolysis

(a). Simultaneous Saccharification and Fermentation (SSF)

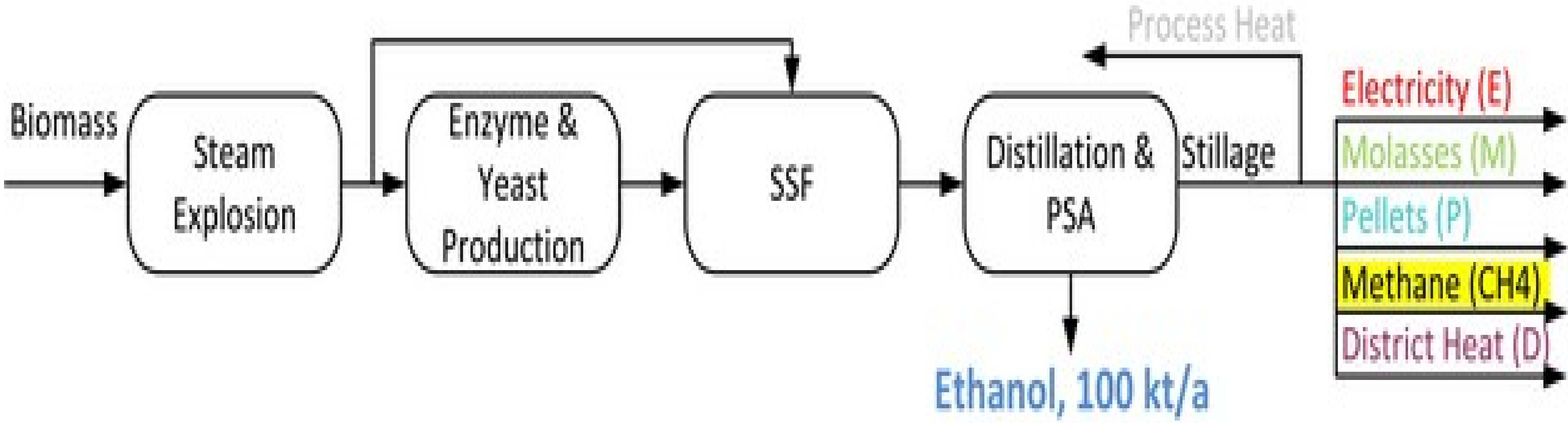
- Cellulase enzymes & fermenting microbes are added to one vessel (hydrolysis and fermentation happen in one reaction vessel).

Advantage :

Minimize accumulation of sugars in the fermenter. ➔ As a result, inhibition of the enzyme β-glucosidase (acts upon β-1,4 bonds linking two glucose or glucose-substituted molecules (i.e., the disaccharide cellobiose) by its product sugars is reduced, and higher hydrolysis rates and yields are possible than for straight saccharification

Disadvantage :

Cellulase enzymes and the fermentation enzymes have to operate under the same conditions - decreases the sugar and ethanol yields.



http://www.vt.tuwien.ac.at/thermal_process_engineering_and_simulation/sustainable_process_engineering_chemometrics/projects/EN/

(b) Sequential Hydrolysis and Fermentation (SHF)

- Hydrolysis and fermentation are done in separate reaction chambers.

- ✓ Advantage→
  - ✓ Enables optimization of conditions for the enzymes.

- ✓ Disadvantage→
  - ✓ Operational and maintenance costs are high.

3). Fermentation

Fermentation of both C_5 and C_6 sugars

Problem

The ability to ferment pentoses along with hexoses is not widespread among microorganisms.

Solution

Develop genetically modified microorganisms using recombinant DNA technology which can ferment both forms of sugars.

e.g. *Zymomonas mobilis*

4) Distillation

This is done to separate ethanol from other products.

PURIFICATION OF BIOETHANOL

Fractional distillation can concentrate ethanol to 95.6% by weight (89.5 mole%). The mixture of 95.6% ethanol and 4.4% water (percentage by weight) is an azeotrope with a boiling point of 78.2 °C, and cannot be further purified by distillation. There are several methods used to further purify ethanol beyond 95.6% :

1. Drying using lime or a salt
2. Addition of an entrainer (The ethanol-water azeotrope can be broken by the addition of a small quantity of benzene or cyclohexane)
3 Molecular sieves (absorb the water from the 95.6% ethanol solution, e.g Zeolite)
4 Membranes (break the water-ethanol azeotrope)
5 Pressure Reduction.(at pressures less than 70 torr (9.333 kPa) , there is no azeotrope)

Production of ethanol from raw cassava starch by a nonconventional fermentation method

Seinosuke Ueda†, Celia T. Zenin, Domingos A. Monteiro‡, Yong K. Park

Article first published online: 18 FEB 2004
Journal of Biotechnology and Bioengineering

Abstract

Raw cassava root starch was transformed into ethanol in a one-step process of fermentation, in which are combined the conventional processes of liquefaction, saccharification, and fermentation to alcohol.

-***Aspergillus awamori*** NRRL 3112 and ***Aspergillus niger*** were cultivated on wheat bran and used as Koji enzymes.

- Commercial *A. niger* amyloglucosidase also used in this experiment.

A raw cassava root homogenate–enzymes–yeast mixture fermented optimally at pH 3.5 and 30°C, for five days and produced ethanol.

Alcohol yields from raw cassava roots were between 82.3 and 99.6%.

Commercially, fuel ethanol is produced by fermentation of sugars (from corn, cane, cheese whey, molasses) by the yeast *Saccharomyces cerevisiae*.

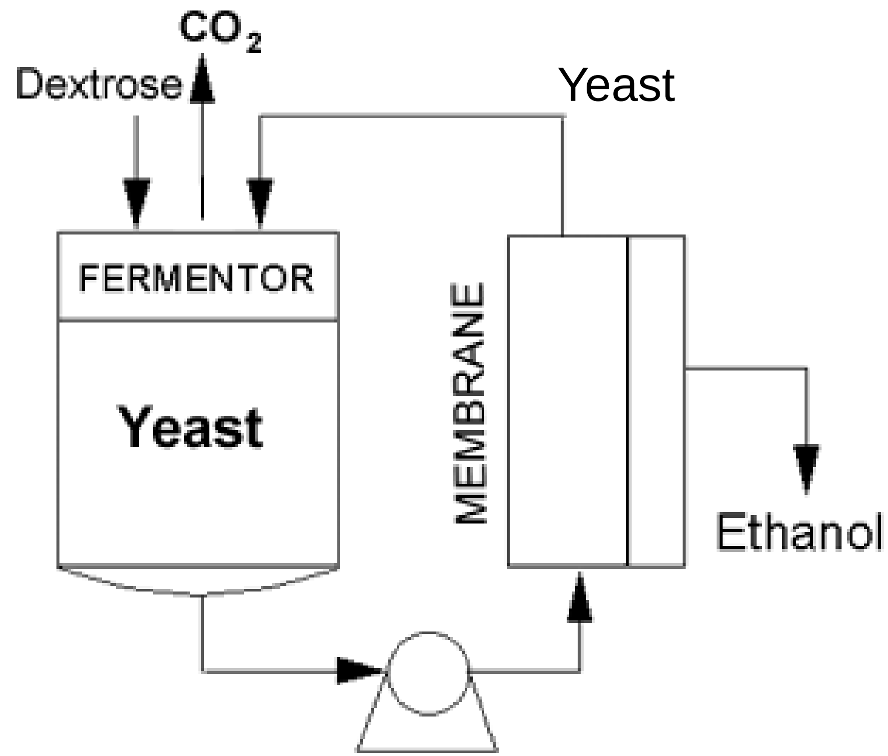
The ethanol obtained from current fermentation technology is relatively expensive. The fermentation-related factors are:

- Most fermenters are opened in a batch or semi-continuous manner.
- Long fermentation times: The process is slow, taking typically 20-60 hours
- High capital costs due to large fermentation volume
- High energy costs for separating and purifying the ethanol to meet fuel-grade standards

Continuous Membrane Bioreactor (CMB)

University of Illinois (UI) developed a high-performance fermentation process that overcomes many of these problems ➔ CMB

CMB use synthetic semi-permeable membranes to separate and recycle the yeast, while simultaneously removing the ethanol as it is formed.



https://netfiles.uiuc.edu/mcheryan/www/ethanol.htm

This has several advantages over current technology:
The continuous separation and recovery of the yeast will reduce yeast costs and the cycle time of the fermenters, since there will be little or no time lost due to start-up and shut down as in present batch fermenters.
The recycle of yeast will allow us to obtain much higher cell densities than currently practiced. Laboratory studies have shown a 1000-fold increase in yeast cell numbers in the CMB during operation. The high concentration allows us to pump the feedstock through the fermenters much faster. "Cell wash-out" is eliminated, thereby allowing operation at dilution rates greater than the specific growth rate of the organism.
The continuous removal of ethanol -- which is inhibitory to the yeast -- allows us to maintain the fermenter at just below the alcohol level which inactivates the yeast. Thus the yeast cells are always viable and producing ethanol.

PROSPEK BIOETANOL DI INDONEESIA

Bahan Baku Potensial :

-Sagu (*Metroxylon* spp)
- Tandan kosong kelapa sawit (TKKS)
- Pati Ganyong (*Canna edulis*)
- Nira Sorgum
- Tetes Tebu (Molase)
- Jerami Padi
- bonggol pisang
- Sigkong karet (singkong gajah)
- Talas (*Colocasia esculenta*)

➔ Bisnis bioetanol di Indonesia mempunyai prospek yang cerah

Sumber : Gusmailina (Pusat Litbang Hasil Hutan, Bogor) (http://indobioethanol.com/sumber_lain.php)

PELUANG DAN PROSPEK BIOETANOL

- Bahan bakar alternatif masa depan yang ramah lingkungan dan bersifat dapat diperbaru (renewable)

- Selain ramah lingkungan, penggunaannya sebagai campuran BBM terbukti dapat mengurangi emisi karbon monoksida dan asap lainnya dari kendaraan.

- Bisa dijadikan pengganti bahan bakar minyak tanah. Selain hemat, pembuatannya bisa dilakukan di rumah dengan mudah, lebih ekonomis dibandingkan menggunakan minyak tanah.

PELUANG DAN PROSPEK

Bioetanol adalah bahan bakar alternatif masa depan yang ramah lingkungan dan bersifat renewable,Bioetanol mempunyai kelebihan selain ramah lingkungan, penggunaannya sebagai campuran BBM terbukti dapat mengurangi emisi karbon monoksida dan asap lainnya dari kendaraan. Saat ini bioethanol juga bisa dijadikan pengganti bahan bakar minyak tanah. Selain hemat, pembuatannya bisa dilakukan di rumah dengan mudah, lebih ekonomis dibandingkan menggunakan minyak tanah. Dengan demikian bisnis bioetanol di Indonesia mempunyai prospek yang cerah karena melimpahnya bahan baku, seperti singkong, tebu, aren, jagung maupun hasil samping pabrik gula (molase). Dari sektor kehutanan bioetanol bisa dihasilkan dari sagu, nipah, dan aren.merupakan salah satu bahan bakar nabati yang saat ini menjadi primadona untuk mengggantikan minyak bumi.

BIOETANOL : PROSPEK & KENDALANYA (H. Darlis MT)

Pengembangan bioetanol sebagai energi alternatif perlu didukung semua pihak, karena :

1. menunjang kemandirian energi,
2. mempunyai “multiplier effect “ terhadap perekonomi dan kesejahteraan sosial.
3. menurunkan polusi udara serta memperbaiki siklus musim hujan dan kemarau sebagai dampak pemanasan global.

Untuk mengatasi kendala dlm pengembangan etanol sehingga menjadi usaha yang menguntungkan :

1. petani perlu dibekali bibit unggul serta diberi penyuluhan dan pelatihan dibidang teknologi budidaya.
2. Diberi jaminan pemasaran, kalau perlu dengan menunjuk perusahaan inti atau instansi tertentu yang bertanggungjawab menampung hasilnya.

3. Pemberian insentif dan kemudahan permodalan thd. perorangan /badan usaha yang ingin mengembangkan tanaman bahan baku etanol dalam skala besar,

4. Pemerintah harus konsisten thd. kebijakan yang telah dikeluarkan dan meninjak-lanjuti dengan aksi konkrit

Ada ide lain ???

REFERENCES

DWIGHT L. MILLER. Ethanol Fermentation and Potential
Northern Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604 - BiotechnoL & Bioeng. SYmp. No.5, 345-352 (197 5). ©1975 by John Wiley & Sons, Inc. (http://ddr.nal.usda.gov/bitstream/10113/25389/1/ENR80000646.pdf)

Chandel *et al*. Economics and environmental impact of bioethanol production technologies: an appraisal . Biotechnology and Molecular Biology Review Vol. 2 (1), pp. 014-032, February 2007

Manufacture of Ethanol by Fermentation
(http://chemistry.tutorvista.com/biochemistry/fermentation.html)

Learn Ethanol Fermentation Process
(*chemistry.tutorvista.com/biochemistry/**fermentation**.html - United States)*

Iwan Sutanto. Bioethanol Prospect in Indonesia (http://www.aist-riss.jp/old/lca/asianetwork/symposium/report/2006/summary/08sutanto.pdf)