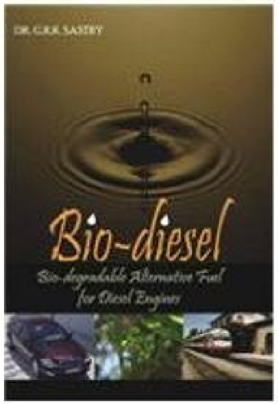
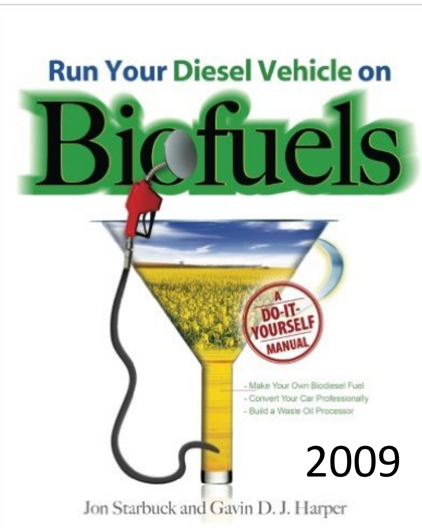
Kusuma School of Biological Sciences

SBL-100: Introductory Biology for Engineers

L-6





Photosynthesis → carbohydrates → Fatty acids → Biofuel

"Synthetic biology is the engineering of biology: the synthesis of complex, biologically based (or inspired) systems which display functions that do not exist in nature. This engineering perspective may be applied at all levels of the hierarchy of biological structures – from individual molecules to whole cells, tissues and organisms. In essence, synthetic biology will enable the design of 'biological systems' in a rational and systematic way." – European Commission – 2014

Operational definition: ***SynBio is the application of science, technology and engineering to facilitate and accelerate the design, manufacture and/or modification of genetic materials in living organisms. - European Commission - 2014***

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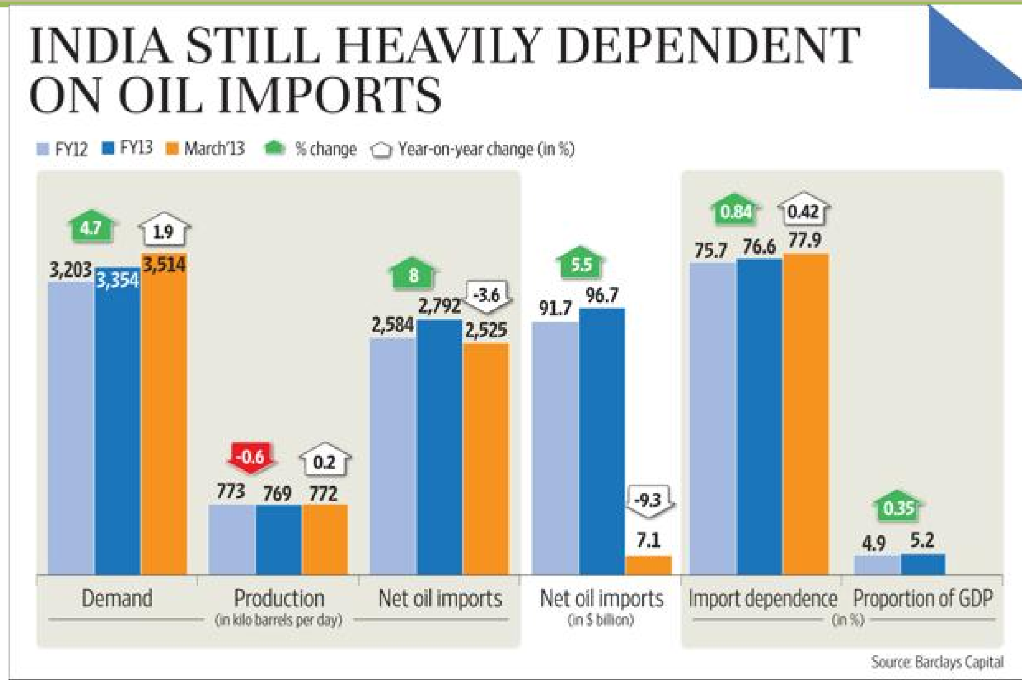


Dec. 9, 2015 – Govt. has estimated India will import 188.23 million tonnes of crude oil in FY16 at a cost of Rs 4,72,932 crores.

Fuels are any materials that can store potential energy in forms that can be practicably released and used as heat energy. More than 60% of electricity generated comes from fossil fuels. Transportation accounts for 25% of fuel demand.

Fossil fuels are not renewable which means they will run out at some point.

Energy Sources (Non-renewable: Oil, natural gas, coal); (Renewable: Solar energy, hydropower, Biofuel, Biomass, Tidal energy, Wind energy, Geothermal, Nuclear energy..)

Biofuel as an alternative to fossil fuels

Any hydrocarbon fuel that is produced from organic matter (living or once living material) in a short period of time (days, weeks or even months) is considered a biofuel.

Energy content of biodiesel is about 90% of petroleum diesel.
Energy content of ethanol is about 50% of gasoline.
Energy content of butanol is about 80% of gasoline.

Biofuels burn cleaner than fossil fuels which means less emission of green house gases, particulate emissions, substances that cause acid rain such as sulfur etc..

Biodiesel produces 80% less CO_2. It is sulfur free. It produces 75% less particulate emissions. It has fewer polycyclic aromatic hydrocarbons linked to cancer.

Biofuels are biodegradable which means less harm in spillages.

Biodiesel flashpoint is ~ 150^{o}C compared to Petroleum diesel (~ 75^{o}C)..safer for transport/storage.

1st generation biofuels. **(Made of sugar, starch, vegetable oil seeds..)**

Vegetable oils (Soybean oil, rapeseed oil, sunflower oil, mustard oil, coconut oil, peanut oil, Palm oil, etc.) → Esterification+ → Biodiesel

Why not vegetable oil directly as fuel? The larger size of vegetable oil molecules means that in cold weather it gels, making it hard to use in an engine. Converting it into biodiesel makes it a smaller molecule, closer to the size of regular diesel, so that it has to get colder than vegetable oil before it starts to gel.

Sugar beet, Sugar cane, corn or even cellulose etc.. → Fermentation+ (through microorganisms, enzymes)→ Bioethanol

2nd generation biofuels (advanced biofuels)..(Starting materials are not for consumption)

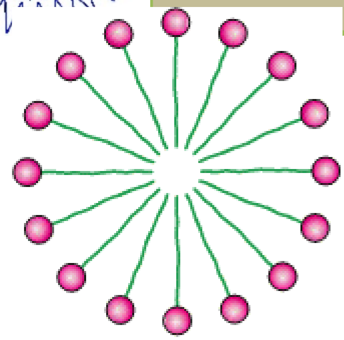
Jatropha seeds have a high oil content.

3rd generation biofuels from Algae (which have a high oil content) and those generated using microbes.

International targets : At least 10% biofuel by 2020.

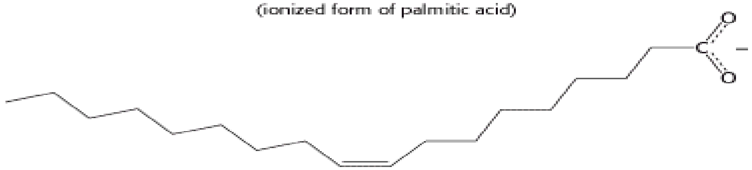
What do all the above oils have in common? Fatty acids & Fats

Fatty Acids



A micelle. Ionized fatty acids readily form such structures

Palmitate
(ionized form of palmitic acid)



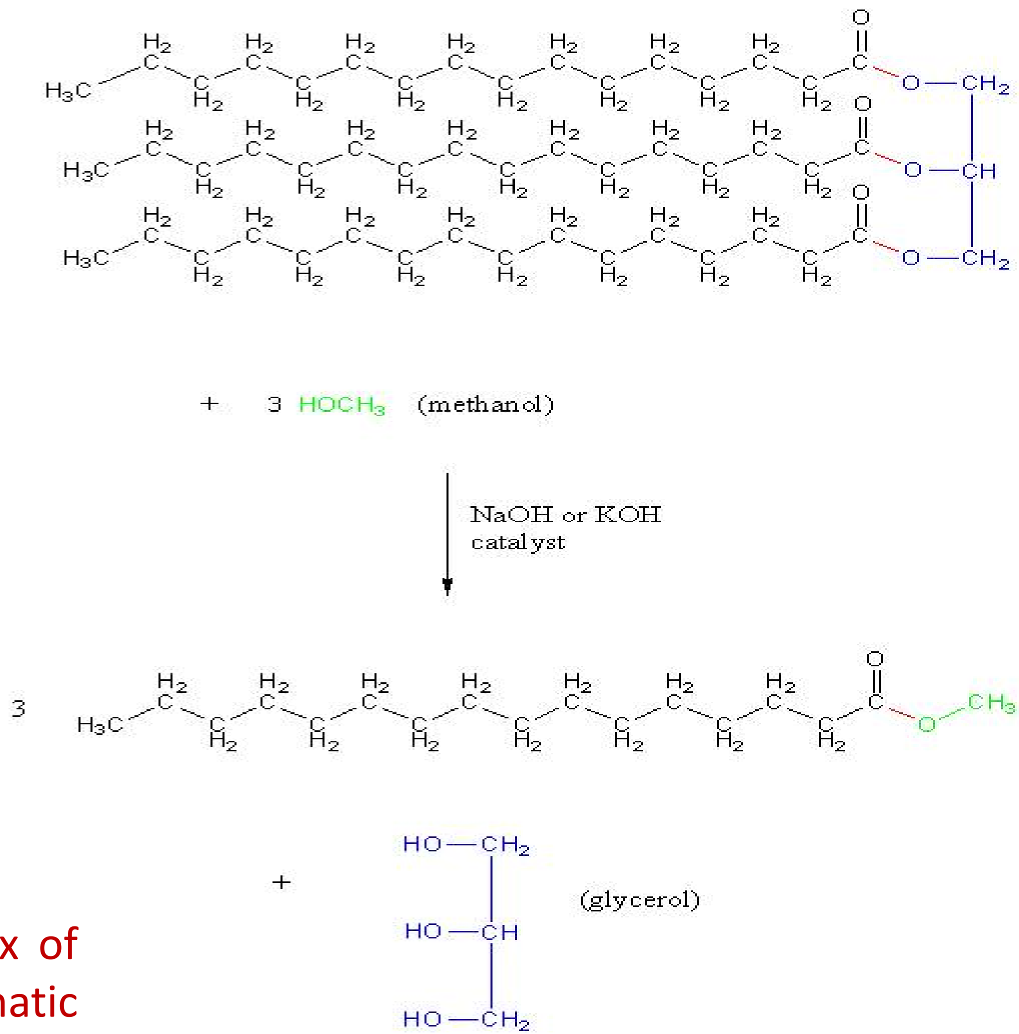
Oleate
(ionized form of oleic acid)

Fatty acids containing one or more double bonds (>C=C<) such as oleic acid are called unsaturated and those which lack a double bond are called saturated. Unsaturated fatty acids (olive oil) melt at lower temperatures than saturated ones (stearic acid, palmitic acid).

Glycerol esters of fatty acids at 20°C are classified as fats (if solid) or oils (if liquid). Waxes are esters of fatty acids with long chain alcohols.

Number of carbons	Number of double bonds	Common name	Systematic name	Formula
12	0	Laurate	n-Dodecanoate	$CH_3(CH_2)_{10}COO^-$
14	0	Myristate	n-Tetradecanoate	$CH_3(CH_2)_{12}COO^-$
16	0	Palmitate	n-Hexadecanoate	$CH_3(CH_2)_{14}COO^-$
18	0	Stearate	n-Octadecanoate	$CH_3(CH_2)_{16}COO^-$
20	0	Arachidate	n-Eicosanoate	$CH_3(CH_2)_{18}COO^-$
22	0	Behenate	n-Docosanoate	$CH_3(CH_2)_{20}COO^-$
24	0	Lignocerate	n-Tetracosanoate	$CH_3(CH_2)_{22}COO^-$
16	1	Palmitoleate	cis-Δ^9-Hexadecenoate	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COO^-$
18	1	Oleate	cis-Δ^9-Octadecenoate	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COO^-$
18	2	Linoleate	cis,cis-Δ^9,Δ^{12}-Octadecadienoate	$CH_3(CH_2)_4(CH{=}CHCH_2)_2(CH_2)_6COO^-$
18	3	Linolenate	all-cis-$\Delta^9,\Delta^{12},\Delta^{15}$-Octadecatrienoate	$CH_3CH_2(CH{=}CHCH_2)_3(CH_2)_6COO^-$
20	4	Arachidonate	all-cis-$\Delta^5,\Delta^8,\Delta^{11},\Delta^{14}$-Eicosatetraenoate	$CH_3(CH_2)_4(CH{=}CHCH_2)_4(CH_2)_2COO^-$

What is biodiesel?



Biodiesel: Methyl, ethyl esters of fatty acids

Remove any traces of water in the oil and reagents so that soap formation (ionized fatty acids) is prevented.

Petroleum Diesel: Mix of aliphatic & aromatic Hydrocarbons.

Avg. formula: $C_{12}H_{23}$

JATROPHA TREE

Common name: Physic Nut, Jatropha, Barbados nut • Hindi: जमाल घोटा Jamal ghota, रतनजोत Ratanjot, जंगली अरंडी Jangli arandi • Manipuri: অৱা কেগে Awa kege • Marathi: Mogli Erand, Maraharalu •Tamil: Kattukkotai • Malayalam: Kattamank, Katalavanakku • Telugu: Nepalam, Adavi amudam •Kannada: Kananeranda • Bengali: Bagbherenda, Bherenda, Sada verenda • Oriya: Jahazigaba, Dhalajahaji • Konkani: Mogli erandi • Assamese: Salika kund, Bongali-botora, Bongali era • Gujarati: Radau-khurung, Jamalgota • Sanskrit: Darvanti • Mizo: Kangdamdawi, Thingthau

Botanical name: *Jatropha curcas* **Family:** *Euphorbiaceae* (Castor family)

Jatropha can be cultivated anywhere along canals, roads, railway tracks etc..even if soil is alkaline, low or high rain fall. Occurs at lower altitudes (0-500 CM), temperature above 20^{o}C, and rain fall 300-1000mm..It is a perennial poisonous shrub.

Jatropha Curcas seed can be used as Biodiesel for any diesel engine without modification.

Some disadvantages of Biofuels: higher production costs, more likely to attract moisture, poor low temperature properties..apart from land, water usage..

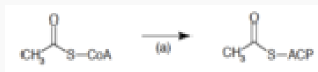
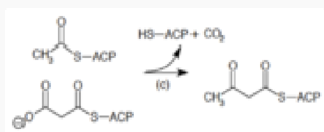
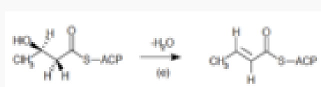
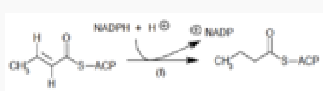
The Future of Biofuels

"Biofuels are not a panacea for all the energy problems of the world. To solve the issue of dwindling fossil fuel reserves, all viable means of harvesting energy should be pursued to their fullest. However, the fact remains that biofuels are a reliable alternative energy resource. With more development and research, it is possible to overcome the disadvantages of biofuels and make them suitable for widespread consumer use. Much of this could rely on the ability of energy producers to discover better plants to raise for fuel that use less water, less land, and grow quickly."

How can genomics era help generate cost effective biofuels?

Biodiesel: Methyl, ethyl esters of fatty acids.

Q. How are oils/ fatty acids synthesized in plants?

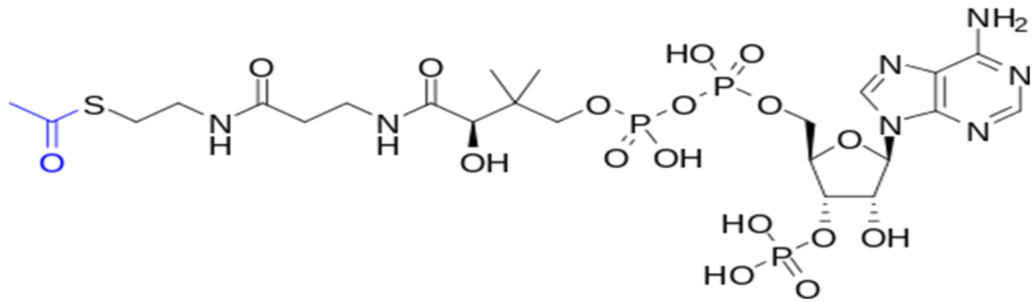
Step	Enzyme	Reaction	Description
(a)	Acetyl CoA:ACP transacylase		Activates acetyl CoA for reaction with malonyl-ACP
(b)	Malonyl CoA:ACP transacylase		Activates malonyl CoA for reaction with acetyl-ACP
(c)	3-ketoacyl-ACP synthase		Reacts priming acetyl-ACP with chain-extending malonyl-ACP.
(d)	3-ketoacyl-ACP reductase		Reduces the carbon 3 ketone to a hydroxyl group
(e)	3-Hydroxyacyl ACP dehydrase		Removes water
(f)	Enoyl-ACP reductase		Reduces the C2-C3 double bond.

Fatty acid biosynthesis occurs via six recurring reactions shown on left, until the 16-carbon palmitic acid is produced facilitated by fatty acid synthase (complex of multiple enzymes).

Need Acetyl-Co A to produce fatty acids. **The cytosolic acetyl-CoA is carboxylated by acetyl CoA carboxylase into malonyl CoA, the first committed step in the synthesis of fatty acids.**

Abbreviations: ACP – Acyl carrier protein, CoA – Coenzyme A, NADP – Nicotinamide adenine dinucleotide phosphate.

Where is acetyl CoA coming from?



Citric acid cycle & Glycolytic end products are used in the conversion of carbohydrates into fatty acids

In humans, fatty acids are formed from carbohydrates predominantly in the liver and adipose tissue, as well as in the mammary glands during lactation. The de novo synthesis of fatty acids in plants occurs in the plastids through the activity of fatty acid synthetase.

The pyruvate produced by glycolysis is an important intermediary in the conversion of carbohydrates into fatty acids and cholesterol. This occurs via the conversion of pyruvate into acetyl-CoA in the mitochondrion. However, this acetyl CoA needs to be transported into cytosol where the synthesis of fatty acids and cholesterol occurs. This cannot occur directly. To obtain cytosolic acetyl-CoA, citrate (produced by the condensation of acetyl CoA with oxaloacetate) is removed from the citric acid cycle and carried across the inner mitochondrial membrane into the cytosol.There it is cleaved by ATP citrate lyase into acetyl-CoA and oxaloacetate. The oxaloacetate can be used for gluconeogenesis (in the liver), or it can be returned into mitochondrion as malate.

Fatty acids are broken down to acetyl-CoA by means of beta oxidation inside the mitochondria, whereas fatty acids are synthesized from acetyl-CoA outside the mitochondrion, in the cytosol.

The **citric acid cycle** – also known as the **tricarboxylic acid** (**TCA**) **cycle** or the **Krebs cycle -** is a series of chemical reactions used by all aerobic organisms to generate energy through the oxidation of acetyl-CoA derived from carbohydrates, fats and proteins into carbon dioxide and chemical energy in the form of guanosine triphosphate (GTP). In addition, the cycle provides precursors of certain amino acids as well as the reducing agent NADH that is used in numerous other biochemical reactions.

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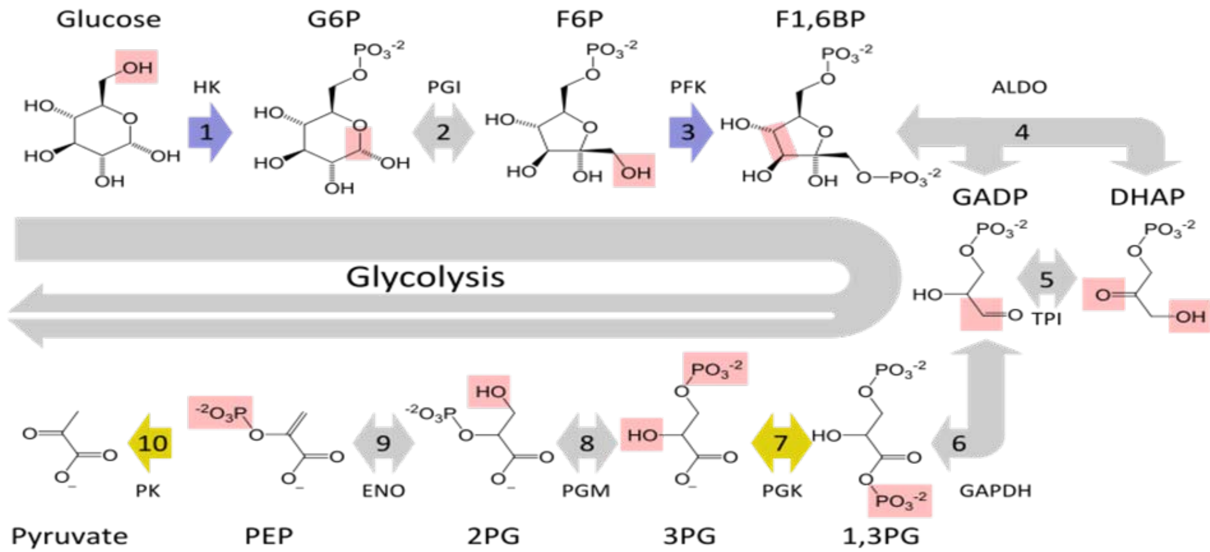

Need pyruvate to produce Acetyl-CoA. Where is pyruvate coming from?

The metabolic pathway of glycolysis converts glucose to pyruvate via a series of intermediate metabolites. Each chemical modification (red box) is performed by a different enzyme. Steps 1 and 3 consume ATP (blue) and steps 7 and 10 produce ATP (yellow). Since steps 6-10 occur twice per glucose molecule, this leads to a net production of ATP.

Where is glucose coming from? Glucose is made during photosynthesis from water and carbon dioxide, using energy from sunlight. The reverse of the photosynthesis reaction, which releases this energy, is a very important source of power for cellular respiration. Glucose is stored as a polymer, in plants as starch and in animals as glycogen, for times when the organism will need it. Glucose circulates in the blood of animals as blood sugar. Glucose can be obtained by hydrolysis of carbohydrates such as milk, cane sugar, maltose, cellulose, glycogen etc. It is however, manufactured by hydrolysis of cornstarch by steaming and diluting acid.

To recapitulate....one of the primary sources of acetyl-CoA is from the breakdown of sugars by glycolysis which yield pyruvate that in turn is decarboxylated by the enzyme pyruvate dehydrogenase generating acetyl-CoA according to the following reaction scheme:

$CH_3C(=O)C(=O)O^-$ (pyruvate) + HSCoA + NAD^+ → **$CH_3C(=O)SCoA$ (acetyl-CoA)** + NADH + CO_2

***Acetyl-CoA* cannot be transported out of the mitochondrion. To obtain cytosolic acetyl-CoA, *citrate* is removed from the citric acid cycle and carried across the inner mitochondrial membrane into the cytosol. There it is cleaved by ATP citrate lyase into acetyl-CoA and oxaloacetate. The oxaloacetate is returned to mitochondrion as *malate* (and then converted back into *oxaloacetate* to transfer more *acetyl-CoA* out of the mitochondrion). The cytosolic acetyl-CoA is used for fatty acid synthesis and the production of cholesterol.**

Summary

Photosynthesis → Carbohydrates → Glucose → Pyruvate → Acetyl CoA → Fatty acids → Biofuels !!

Assignment 11 (2020)

Step 1. Design a synthetic genome (for a plant / algae cell) to carry out just the above pathway.

Step 2. What is the goal? Produce more oil per plant/algae, using less land, water and energy.