

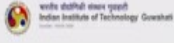
Biomass Conversion and Biorefinery
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Module 02
Lecture-05
Oil Crops and Microalgae

Good morning students, this is lecture 3 under module 2.

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Module	Module name	Lecture	Content
02	Biomass	03	Oil crops and their biorefinery potential
			Microalgae as feedstock for biofuels and biochemical.

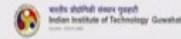


In today's lecture we will discuss about the dedicated oil crops and their biorefinery potential one by one. And then later we will discuss about microalgae. How micro algae can be used as a feedstock for biofuels and biochemicals under a bio refinery platform.

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Oil crops and their biorefinery potential

- Interest in the use of biofuels worldwide has grown strongly in recent years due to the limited oil reserves, concerns about climate change from greenhouse gas emissions and the desire to promote domestic rural economies.
- The term biofuel is as such referred to solid, liquid, or gaseous fuels that are produced from plant matter and residues, agricultural crops, municipal wastes, and agricultural and forestry by-products.
- Biodiesel can be derived from a variety of sources including vegetable oils, animal fats, and waste cooking oil.
- Vegetable oils, also known as triglycerides, are chemically an ester in which three fatty acid groups are attached to one glycerol molecule.



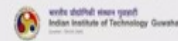
So, interest in the use of biofuels worldwide has grown strongly in recent years due to the limited oil reserves, concerns about climate change from greenhouse gas emissions and desire to promote domestic rural economies. The term biofuel is as such referred to solid, liquid or gaseous fuels that are produced from plant matter and residues, agricultural crops, municipal wastes and agricultural as well as forestry by-products.

Biodiesel can be derived from a variety of sources, including vegetable oils, animal fats and waste cooking oil. So, waste cooking oil has been tried for biodiesel production since almost a decade and it has been quite successful. Vegetable oils, also known as triglycerides, are chemically an ester in which three fatty acid groups are attached to one glycerol molecule.

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Oil crops and their biorefinery potential

- Vegetable oils from renewable oil seeds can be used as alternate to diesel fuels.
- The advantages of vegetable oils as diesel fuel are their portability, ready availability, renewability, higher heat content (about 88% of no. 2 Diesel fuel), lower sulfur content, lower aromatic content, and biodegradability.
- The main disadvantages of vegetable oils are higher viscosity, higher cost, lower volatility, and the reactivity of unsaturated hydrocarbon chains.
- The vegetable oils are all extremely viscous, with viscosities ranging 10–20 times greater than no. 2 Diesel fuel.
- Blending of vegetable oils with diesel, however, reduces the viscosity drastically and the fuel handling system of the engine can handle vegetable oil–diesel blends without any problems.



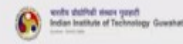
Vegetable oils from renewable oil seeds can be used as alternate to diesel fuels. The advantages of vegetable oils as diesel fuel are their portability, ready availability, renewability, higher heat content (almost about 88% of number 2 diesel fuel), lower sulfur content, lower aromatic content and biodegradability. However, the main disadvantages are the higher viscosity, higher cost, lower volatility and the reactivity of unsaturated hydrocarbon chains.

The vegetable oils are all extremely viscous, with viscosities ranging almost 10 to 20 times greater than number 2 diesel fuel. Blending of vegetable oils with diesel, however, reduces the viscosity drastically. And the fuel handling system of the engine can handle vegetable oil - diesel blends, without any problem. Initially when the blending was started, it was almost 5%. Then gradually it can be increased. So, now government is desiring for a 10% blend in number 2 diesel as well as in our petroleum, gasoline.

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Oil crops and their biorefinery potential

- Over the last 30 years, the oil crop production in the world increased by 240%, while the increase in area and in yield was 82% and 48%, respectively.
- The main oilseed produced in the world is the *soybean* whereby it represents more than 50% of total oil crop production in the world.
- A 40% increase of growing area and an over 100% increase in total crop yield was observed from 1989 to 2008.
- The expansion was brought about by a 150% increase in oil palm acreage and additional increases in rapeseed, soybean, and sunflower acreages by 75%, 65%, and 64%, respectively.



So, over the last 30 years, the oil crop production in the world increased by 240%, while the increase in area and in the yield was 82% and 48% respectively. The main oilseed produced in the world is soybean whereby it represents more than 50% of total oil crop production in the entire world. A 40% increase of growing area and an over 100% increase in total crop yield was observed from 1989 to 2008.

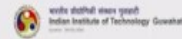
The expansion was brought about by a 150% increase in oil palm acreage and additional increases in rapeseed, soybean and sunflower acreages by 75%, 65% and 64% respectively. Now, these data or statistics basically indicate that, there has been a huge upsurge in the plantation as well as production of the different vegetable oils; whether it is sunflower, whether it is soybean, whether it is rapeseed. In India mustard has also taken a significant space.

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Oil crops and their biorefinery potential

- Annual as well as perennial oil crops was grown on a worldwide acreage of over 261 million hectares of agricultural land in total (2008 data; FAOSTAT, 2009).
- The overall yield achieved from oil crop production (fruit or seed yield, respectively) was about 72 million tons for the 2008 season; due to high difference in oil concentration between the various crop species this translates into an estimated vegetable oil yield of 157 million tons (production quantity of the primary oil crops aggregate; FAOSTAT,2009)
- On the level of plant species, 38% of the total oil crops acreage is planted with soybean, whereas cottonseed and oilseed rape are grown on 12% each, groundnuts and sunflower on 10% each, and oil palm on 6% of the acreage.

FAOSTAT: Food and Agriculture Organization Corporate Statistical Database, United Nations

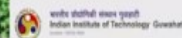


Now annual as well as perennial oil crops was grown on a worldwide acreage of over 261 million hectares of agricultural land in total. The overall yield achieved from oil crop production was about 72 million tons for the 2008 season; due to high difference in oil concentration between the various crop species this translates into an estimated vegetable oil yield of 157 million tons. So, on the level of plant species, 38% of the total oil crops acreage is planted with soybean, whereas, cottonseed and oilseed rape are grown on 12% each, followed by groundnuts and sunflower and later on comes the oil palm (6%).

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Oil crops and their biorefinery potential

- In terms of production, 32% of the total crop yield is made up by soybean, whereas about 28% is from oil palm, which is due to the high annual fruit yield of that perennial species.
- In the past, vegetable oils and fats were predominantly used for food and livestock feeding purposes, whereas nonfood utilization of vegetable oils in oleochemistry applications mainly focused on particular crop species such as *oilseed rape (canola), linseed, cotton, or castor*.
- This situation is currently changing due to the growing need of oils as biofuel feedstocks.
- Between marketing years 2005 and 2007, biofuel use of vegetable oils increased from 4.1% to 8.5%, and by the year 2017, over 15% of worldwide vegetable oil production was used as biofuel feedstock.



So, in terms of production, 32% of total crop yield is made up by soybean, whereas, about 28% is from oil palm which is due to the high annual fruit of that perennial species. In the past,

vegetable oils and fats were predominantly used for food and livestock feeding purposes, whereas, nonfood utilization of vegetable oils in oleochemistry applications mainly focused on particular crop species such as oilseed rape, such as canola, linseed, cotton or castor.

So, you can see these are the four pictures or images of these crops. The first one is the canola, the second one is the linseed, third one is the cotton and fourth is the Castor. So, this situation is currently changing due to the growing need of oil as biofuel feedstocks. Between marketing years of 2005 and 2007, biofuel use of vegetable oils increased from 4.1% to 8.5% (almost double) and by the year 2017, over 15% of the worldwide vegetable oil production was used as biofuel feedstock.

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Oil crops and their biorefinery potential

□ Traditional Oil Crops

- Most of the biodiesel is currently made from soybean, rapeseed, sunflower, and palm oils.
- New plant oils that are under consideration include mustard seed, peanut, sunflower, and cotton seed.
- Soybean oil is commonly used in the United States and rapeseed oil is used in many European countries for biodiesel production, whereas, coconut oil and palm oils are used in Malaysia and Indonesia for biodiesel production.




Figure 1. A: Oil palm, B: Soybean, C: Rape, D: Sunflower.

Source: Influence on process parameters in transesterification of vegetable oil waste oil. A review by Moha et al.

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So, let us look for the traditional oil crops. Most of the biodiesel is currently made from soybean, rapeseed, sunflower and palm oils. Now having said that, kindly note that in India we are not doing so. As I told you in the last class that whether it is in India or developing countries, when we talk about vegetable oil to biodiesel, it comes directly under the food versus feed problem, so we are not doing so. But having said that you have to understand that these are being done in some of the countries in which the production is huge. And they have a huge problem of storing the oils. So, new plant oils that are under consideration include mustard seed, peanut, sunflower and cottonseed. Soybean oil is commonly used in the United States and rapeseed oil is used in

many European countries for biodiesel production, whereas coconut oil and palm oils are used in Malaysia and Indonesia for biodiesel production.

So, it is all about the supply and the production; basically, how much we are producing and how much we are consuming. If the production of any such crops is much higher than the requirement of a particular country, then, they can think of converting those to biofuels otherwise it is not possible.

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- About 80% of the European Union's total biofuel production is comprised of biodiesel produced from rapeseed and sunflower seeds.
- Soybean oil accounts for approximately 90% of the biodiesel produced in the United States.
- Rapeseed oil has 59% of total global biodiesel raw material sources, followed by soybean (25%), palm oil (10%), sunflower oil (5%), and other (1%).

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So, about 80% of the European Union's total biofuel production is comprised of biodiesel produced from rapeseed and sunflower seeds (because they can afford to do that). So, soybean oil accounts for approximately 90% of the biodiesel produced in the United States, rapeseed oil has a 59% of total global biodiesel raw material sources followed by soybean, palm oil, sunflower and others.

Now another thing I want you to know, that whenever we are directly showing this type of statistics 59% of these or 28% of that, please understand that it is not only the oil that is getting produced from that particular species but also its waste. So, let us say bagasse, the stalk, the kernels, the husk etc. So, those are also being added to that particular statistics to produce different types of biofuels not only biodiesel but also bio alcohols.

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Oil crops and their biorefinery potential

Table 1: Fuel-related properties of selected vegetable oils.

Oil/A	Iodine value	CN ^a	CP ^b (K)	PP ^c (K)	FP ^d (K)	Viscosity ^e (mm ² /s)	References
<i>A. indica</i>	82–98	–	286.0	280.0	–	35.83	Aransiola et al. (2014)
<i>J. curcas</i>	101	23	283.0 ^f	267.0	459	35.4	Singh and Padhi (2009)
<i>M. indica</i>	65	–	287.0	288.0	499	25.0	Padhi and Singh (2010)
<i>P. pinnata</i>	87	42	276.5	279.0	498	40.2	Bobade and Khyade (2012)
<i>R. communis</i>	84.4	42.0	274.0	259.0	>533	240.1	Deligiannis et al. (2009)
Rapeseed	94–120	37.6	269.1	241.3	519	37.0	Knothe et al. (1997)
Soybean	117–143	37.9	269.1	260.8	527	32.6	Knothe et al. (1997)
Sunflower	110–143	37.1	280.2	258.0	547	37.1	Knothe et al. (1997)

^aCN: cetane number; ^bCP: cloud point; ^cPP: pour point; ^dFP: flash point; ^emeasured at 313 K; ^fcloud point combined from Rashid et al. (2010).

So, this table will make you understand different fuel related properties of the selected vegetable oils. So, you can see that, the first one is the different types of oil, *A. indica* is *Azadirachta indica*, that is neem's oil, then *Jatropha*, then *Mahua* that is *Madhuca indica*, then *Pongamia pinnata* that is karanja and then there are other seeds. So, you can see that iodine values are very good. However, you can (also) see that the viscosity of these oils are very high.

Especially the *R. communis* is extremely high, thereby, making its direct use in an engine more difficult. And you can see there are other properties like cetane number, cloud point, pour point, flash point all these details.

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Oil crops and their biorefinery potential

- The major obstacle for commercialization of biodiesel is its cost as approximately 70%–90% of biodiesel cost is arises from the cost of raw materials.
- Therefore, biodiesel produced from edible vegetable oils is currently not economically feasible.
- **Non-edible oil plants** are easily available in all the countries and are very economical comparable to edible plant oils.
- Extensive use of edible oils may cause other significant problems such as starvation in developing countries. There are concerns that biodiesel feedstock may compete with food supply in the long term.
- Biodiesel produced from **non-edible vegetable oil** has good potential as an alternative diesel fuel.

So, the major obstacle for commercialization of biodiesel is its cost as approximately 70% to 90% of the biodiesel production cost arises from the cost of the raw materials. Therefore, biodiesel produced from edible vegetable oils is currently not economically feasible. Now that is what I was just mentioning to you about, that whether it is a developing country or developed country let us tell it in a sustainable way.

So, in a sustainable way producing this is not so feasible. Let us understand that in a particular year, sunflower has been produced so large in quantities, that storing is a big problem. Now please understand, that may not happen in the next year or next to next year. Because most of the countries are still depending upon the season or the climate for the agricultural purposes whether it is in India, or it is any developing country.

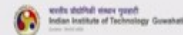
And there are other factors which also govern the yield and the mass production of the crops. So, in a sustainable way, it is very difficult to do that; whether it is in developing country or developed country. So, now later has moved from the edible to non edible oil seeds. So, non edible oil plants are easily available in all the countries and are very economical compared to the edible plant oils.

Now the biggest thing about this non edible oil plants is that this do not come under the food versus feed problem. And then extensive use of edible oils may cause other significant problems such as starvation in developing countries (this is what I was mentioning). There are concerns that biodiesel feedstock may compete with food supply in the long term. So, the sustainability always comes into picture and economics also has to be taken care of. So, biodiesel produced from non edible vegetable oil has a good potential as an alternative diesel fuel.

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Oil crops and their biorefinery potential

- The use of non-edible plant oils when compared with edible plant oils is very significant because of the tremendous demand for edible oils for food, and they are far too expensive to be used as fuel at present.
- Non-edible oil plants *can be grown in waste lands* that are not suitable for food crops and the *cost of cultivation is much lower* because these plants can still sustain reasonably high yield without intensive care.
- There are many examples for non-edible oilseed crops such as jatropha (*Jatropha curcas*), karanja (*Pongamia pinnata*), mahua (*Madhuca indica*), castor bean seed (*Ricinus communis*), neem (*Azadirachta indica*), rubber seed tree (*Hevea brasiliensis*), tobacco seed (*Nicotiana tabacum*), rice bran, etc.



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The use of non-edible plant oils when compared with edible plant oils is very significant because of the tremendous demand for the edible oils for food and they are far too expensive to be used as fuel at present. Now non edible oil plants can be grown in waste lands (that is another biggest advantage) that are not suitable for food crops and the cost of cultivation is also much lower because these plants can still sustain reasonably high yield without intensive care. So, there are many examples of non-edible oilseed crops such as Jatropha, Mahua, karanja, castor, neem, rubber seed, tobacco seed, rice bran etc.

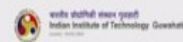
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Oil crops and their biorefinery potential

❖ Non-edible oil seed crops

❑ *Jatropha curcas*

- *Jatropha curcas* is a tall bush or small tree, up to 5–7 m tall, belonging to the *Euphorbiaceae* family.
- Originally from Central America, *Jatropha curcas* is found throughout the tropics including much of Africa and Asia.
- A research study showed that one hectare of *Jatropha curcas* could capture up to 25 tons of CO₂ from the atmosphere every year.
- *Jatropha curcas* seeds have an oil content ranging between 30% and 40%. *Jatropha curcas* oil contains approximately 24.60% of crude protein, 47.25% of crude fat, and 5.54% of moisture content.

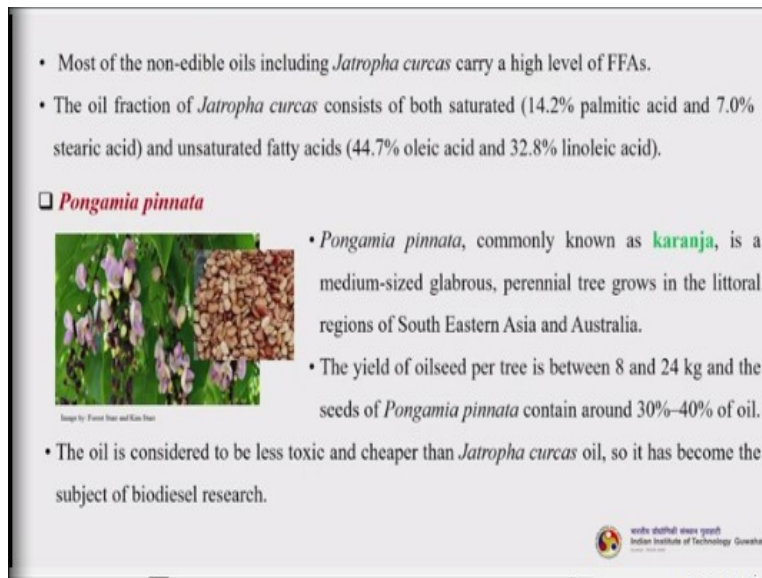


So, now we will see one by one, what are their properties and how they can contribute to this biodiesel production. So, the first one *Jatropha curcas*; we have discussed about Jatropha, I

showed you the *Jatropha* lifecycle in last class. So, it is a tall bush or small tree up to 5 to 7 meter tall, belonging to the *Euphorbiaceae* family. Originally from Central America, *Jatropha curcas* is found throughout the tropics including much of African and Asia.

So, a research study showed that one hectare of *Jatropha curcas* could capture up to 25 tons of carbon dioxide from the atmosphere every year (that is a significant number of course). So, *Jatropha curcas* seeds have an oil content ranging between 30% and 40%. *Jatropha curcas* oil contains approximately 24.6% of crude protein, 47.25% of crude fat and 5.54% of moisture content.


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• Most of the non-edible oils including *Jatropha curcas* carry a high level of FFAs.

• The oil fraction of *Jatropha curcas* consists of both saturated (14.2% palmitic acid and 7.0% stearic acid) and unsaturated fatty acids (44.7% oleic acid and 32.8% linoleic acid).

□ ***Pongamia pinnata***



• *Pongamia pinnata*, commonly known as **karanja**, is a medium-sized glabrous, perennial tree grows in the littoral regions of South Eastern Asia and Australia.

• The yield of oilseed per tree is between 8 and 24 kg and the seeds of *Pongamia pinnata* contain around 30%–40% of oil.

• The oil is considered to be less toxic and cheaper than *Jatropha curcas* oil, so it has become the subject of biodiesel research.

Image by Forest Her and Kiki Her

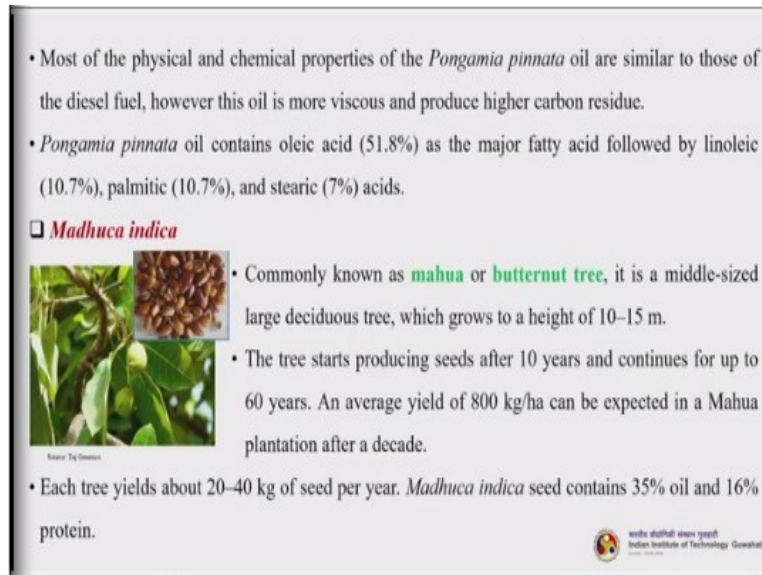
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So, most of the non edible oils including *Jatropha* carry a high level of the free fatty acids. The oil fraction of *Jatropha* consists of both saturated and unsaturated fatty acids.

So, the next one is *Pongamia pinnata*, which is commonly known as karanja. It is a medium sized glabrous, perennial tree that grows in the littoral regions of South Eastern Asia and Australia. India is full of these plants; you can see in many places. The yield of oil seed per tree is between 8 and 24 kg.

And the seeds of *Pongamia pinnata* content about 30 to 40% of oil. The oil is considered to be less toxic and cheaper than *Jatropha curcas* oil, so it has become the subject of biodiesel research.


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• Most of the physical and chemical properties of the *Pongamia pinnata* oil are similar to those of the diesel fuel, however this oil is more viscous and produce higher carbon residue.

• *Pongamia pinnata* oil contains oleic acid (51.8%) as the major fatty acid followed by linoleic (10.7%), palmitic (10.7%), and stearic (7%) acids.

□ ***Madhuca indica***



• Commonly known as **mahua** or **butternut tree**, it is a middle-sized large deciduous tree, which grows to a height of 10–15 m.

• The tree starts producing seeds after 10 years and continues for up to 60 years. An average yield of 800 kg/ha can be expected in a Mahua plantation after a decade.

• Each tree yields about 20–40 kg of seed per year. *Madhuca indica* seed contains 35% oil and 16% protein.

Source: Tq. Ganesan

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Most of the physical and chemical properties of the *Pongamia pinnata* oil as similar to those of the diesel fuel, however, this oil is more viscous and produce higher carbon residue. So, *Pongamia pinnata* oil contents oleic acid (51.8%) as the major fatty acid followed by linoleic, palmitic and stearic acid.

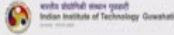
So, the next is also very famous tree in India which is called *Madhuca indica*. It is commonly known as Mahua or butternut tree. It is a middle sized large deciduous tree which grows to a height of 10 to 15 meter. The tree starts producing seeds after 10 years and continues for up to 60 years. An average yield of 800 kg per hectare can be expected in a mahua plantation after a decade. So, each tree yields about 20 to 40 kg of seeds per year, mahua seed contains 35% of oil and 16% of protein.

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- Fresh Mahua oil from properly stored seeds is yellow, while commercial oils are generally greenish yellow with an offensive odor and disagreeable taste.
- Mahua oil contains a high level of FFAs (up to 20%) and a proper procedure for converting this oil to biodiesel is very much required.

□ ***Ricinus communis***

- *Ricinus communis*, commonly known as **castor oil plant**, is a plant species of the family *Euphorbiaceae*.
- It originates in Africa but it is found in both wild and cultivated states in all the tropical and subtropical countries of the world.
- It is a small wooden tree that can reach a height of about 6 m. The comparative advantage of *Ricinus communis* is that its growing period is much shorter than that of *Jatropha* and *Pongamia*.



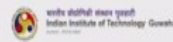
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Fresh Mahua oil from properly stored seeds is yellow, while commercial oils are generally greenish yellow with an offensive odour and disagreeable taste. Mahua oil contains the high level of free fatty acids (almost up to 20%) and a proper procedure for converting this oil to biodiesel is very much required.

The next is *Ricinus communis*, which is popularly known as castor oil plant and belongs to the family *Euphorbiaceae*. It originates in Africa but it is found in both wild and cultivated states in all the tropical and subtropical countries of the world. In India also we have huge castor oil plantation. It is a small wooden tree that can reach a height of about 6 meters. The comparative advantage of this plant is that, its growing period is much shorter than that of the *Jatropha* and *pongamia*.

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- Castor seed is an ideal candidate for production of high value, industrial oil feedstocks because of the very high oil content (48%–60%) of the seed, and the extremely high levels of potential oil production (500–1000 L of oil/acre).
- The main constituent of castor oil is ricinoleic acid (90%), which contains 18 carbon atoms with a hydroxyl group position 12.
- Castor oil contains more oxygen than other oils and, therefore, castor oil and its derivatives are more soluble in alcohols during the transesterification reactions.
- The main disadvantage of castor oil is its high viscosity (240.12 mm²/s at 313 K). The high viscosity of this oil lead to its poor atomization of the fuel, incomplete combustion, choking of the fuel injectors, and ring carbonization.




So, Castor seed is an ideal candidate for production of high value industrial oil feedstocks because of the very high oil content (almost 48 to 60% of the seed) depending upon the species, and the extremely high levels of potential oil production (500 to 1000 liters of oil per acre) which is a very good yield. So, the main constituent of castor oil is ricinoleic acid (which is 90%), which contains 18 carbon atoms with a hydroxyl group position at 12.

Castor oil contains more oxygen than other oils and therefore castor oil and its derivatives are more soluble in alcohols during the transesterification reaction, thereby yielding a higher biodiesel, after the reaction. So, the main disadvantage of castor oil is its high viscosity, the high viscosity of this oil leads to its poor atomization of the fuel, incomplete combustion, choking of the fuel injectors and ring carbonization.

So, in India we have huge plantations of all such things, whether it is Castor, whether it is Jatropha, whether it is Mahua. You will see Mahua and Castor especially in the eastern side of the country. So, huge plantation is there in Odisha, Jharkhand, Bengal and Bihar. Jatropha was planted in huge quantities. But as I told you in the last class, its sustainability has become a big problem. Therefore, most of the Jatropha plantation has been discontinued.

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□ *Azadirachta indica*



- *Azadirachta indica* (commonly known as “**Neem tree**”), a member of the family *Meliaceae*, is a majestic, evergreen, tropical forest tree, with a broad crown and a height of approximately 25 m.
- It is well established plant in at least 30 countries worldwide, in Asia, Africa, and Central and South America.
- *Azadirachta indica* seeds contain about 45% of a brownish-yellow fixed oil mainly constituted by oleic acid (34.01%), palmitic acid (12.1%), stearic acid (12.95%), linoleic acid (38.26%).
- Traditionally, neem oil has been used as fuel in lamps for lighting purpose in rural areas and it is used on an industrial scale for manufacturing of soaps, cosmetics, pharmaceuticals, and other nonedible products.

Image by Jiffrey


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So, next is *Azadirachta indica* which is the neem tree. It is a member of the family *Meliaceae*. It is a majestic, evergreen, tropical forest tree with a broad crown and a height of approximately 25 meters. So, it is a well established plant in at least 30 countries worldwide in Asia, Africa and Central and South America. India is full of these trees, anywhere you go; and due to its environmental benefits such as purifying oxygen/air, it has been deliberately planted in the roadsides.

So, neem seeds contain about 45% of the brownish yellow fixed oil, mainly constituted by the oleic acid, palmitic acid and stearic acid followed by linoleic acid. Traditionally, neem oil has been used as a fuel in lamps for lighting purposes in rural areas. And it is used on an industrial scale for manufacturing of soaps, cosmetics, pharmaceuticals and some non edible products. And one more important thing is that neem oil also has certain medicinal properties.

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□ ***Hevea brasiliensis***



• *Hevea brasiliensis*, commonly known as **rubber tree**, is a fast growing tree that belongs to the family *Euphorbiaceae*.

• It is the major source of natural rubber and is native to the Amazon forests and is now widely cultivated in tropics across the world.

- Normal seed production yields vary from 70 to 500 kg per hectare per year while the annual seed production potential in India is about 150 kg per hectare.
- Rubber seed contain approximately about 40% of kernel with 20%–25% of moisture.
- Apart from its use in latex production for foreign exchange, rubber tree produces oil-bearing seed whose oil content in dried kernel varies from 35% to 45%.
- *Hevea brasiliensis* oil does not contain any unusual fatty acids, and its rich source of essential fatty acids C18:2 and C18:3 that make up 52% of its total FFA composition.

Image by: Supriyong Witteerakul

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So, next is *Hevea brasiliensis*, commonly known as the rubber tree. It is a fast growing tree that belongs to the family *Euphorbiaceae*. It is the major source of natural rubber and is native to the Amazon forests and is now widely cultivated in tropics across the world. In India also we have huge rubber plantation in states like Kerala, Karnataka and many southern states.

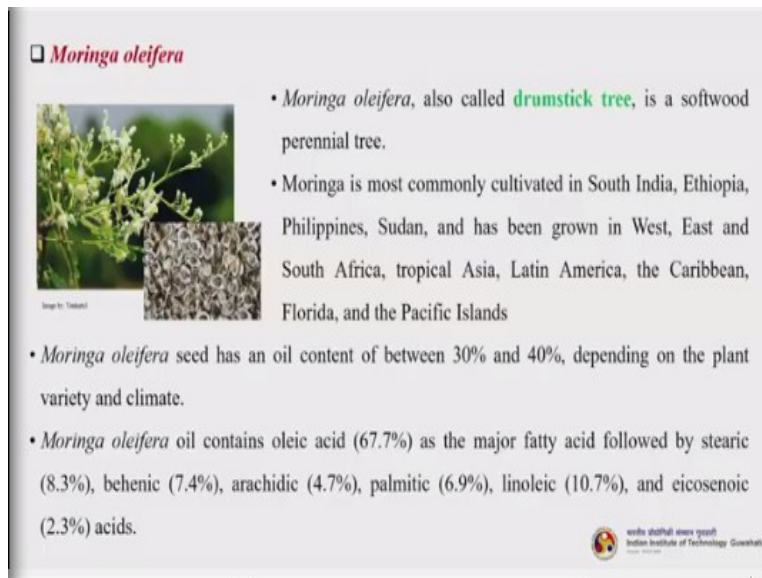
So, normal seed production yields vary from 70 to 500 kg per hectare per year, while the annual seed production potential in India is about 150 kg per hectare. Rubber seed contain approximately about 40% of kernel with 20% to 25% of moisture. Apart from its use in latex production for foreign exchange, rubber tree produces oil bearing seed whose oil content in dried kernel varies from 35% to 45%. Now rubber oil does not contain any unusual fatty acids and its rich source of essential fatty acids, C18:2 and C18:3, that makes up almost 52% of its total free fatty acid composition.

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Asia, making it the most consumed cereal grain. Rice bran is the low value co product of rice milling which contains approximately 15 to 23% of oil. The oil fraction of rice bran consists of both saturated and unsaturated fatty acids, cultivated in countries like China and India.

Very little research has been done to utilize this oil as a replacement for the mineral diesel. I used to tell you that the CSIR Institute of Chemical Technology or CSIR ICT, Hyderabad. So, they have developed an excellent process for converting this rice bran to the vegetable oil for the human consumption. And it is in the market and has been consumed by a big number of people in India as well as in the world.

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□ *Moringa oleifera*

Moringa oleifera, also called **drumstick tree**, is a softwood perennial tree.

Moringa is most commonly cultivated in South India, Ethiopia, Philippines, Sudan, and has been grown in West, East and South Africa, tropical Asia, Latin America, the Caribbean, Florida, and the Pacific Islands

• *Moringa oleifera* seed has an oil content of between 30% and 40%, depending on the plant variety and climate.

• *Moringa oleifera* oil contains oleic acid (67.7%) as the major fatty acid followed by stearic (8.3%), behenic (7.4%), arachidic (4.7%), palmitic (6.9%), linoleic (10.7%), and eicosenoic (2.3%) acids.

Image by: Tishani

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So, the next is *Moringa oleifera* which is also called as drumstick tree. So, its fruit has been consumed in India in huge quantities in South India as well as East and West India. So, *Moringa* is most commonly cultivated in South India, Ethiopia, Philippines, Sudan and has been grown in West, East and South Africa, tropical Asia, Latin America, Caribbean, Florida and Pacific Islands.

So, *Moringa* seed has an oil content of between 30% to 40% depending upon the plant variety and climate. *Moringa* oil contains oleic acid as the major fatty acid followed by stearic acid, behenic acid, arachidic acid, palmitic acid, linoleic and eicosenoic acid.

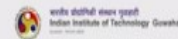
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❑ *Calophyllum inophyllum*



Image by Forest and Kim Ber

- *Calophyllum inophyllum*, commonly known as **polanga or honne**, is a large evergreen, belongs to the *Clusiaceae* family, widespread in East Africa, India, South East Asia, and Australia.
- It is a medium and large-sized evergreen sub-maritime tree that averages 8- 20 m in height with a broad spreading crown of irregular branches.
- The nut kernel contains 50%–70% oil and the mature tree may produce 1–10 kg of oil per year depending upon the productivity of the tree and the efficiency of extraction process.
- Traditionally, polanga oil has been used in medicinal applications, soap, lamp oil, hair grease, and cosmetics in different parts of the world.



The next is *Calophyllum inophyllum*. It is commonly known as polanga or hone; it is a large evergreen tree and belongs to the *Clusiaceae* family, widespread in East Africa, India, Southeast Asia and Australia. So, it is a medium and large sized evergreen sub maritime tree that averages 8 to 20 meter in height, with a broad spreading crown of irregular branches. The nut kernel contains 50% to 70% of oil and the mature tree may produce 1 to 10 kg of oil per year depending upon the productivity of the tree as well as the efficiency of the extraction process.

Traditionally, polanga oil has been used in medicinal applications, soap, lamp oil, hair grease and cosmetics in different parts of the world.

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❑ *Simmondsia chinensis*



Image by: Lantingqin

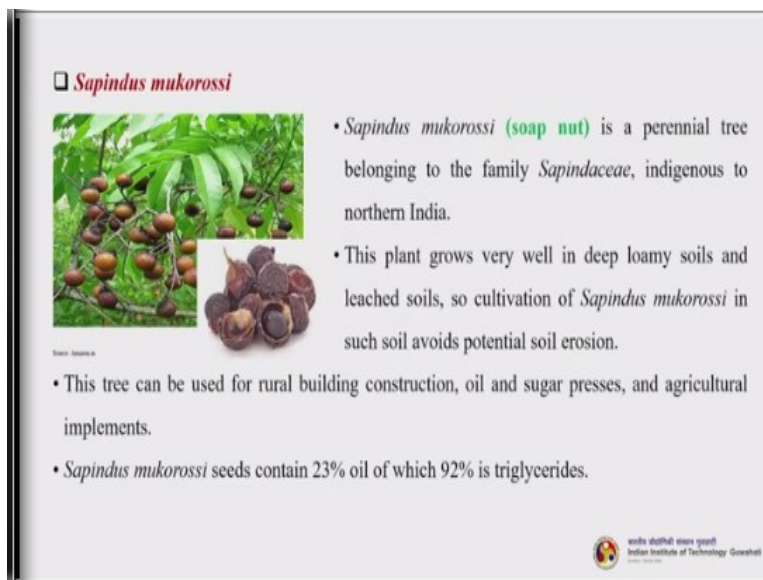
- *Simmondsia chinensis*, commonly known as **jojoba**, is a perennial shrub belonging to the *Simmondsiaceae* family.
- This plant is native to the Mojave and Sonoran deserts of Mexico, California, and Arizona.
- A 10-year old tree yields on an average 1 kg of seeds per year.
- It is unique among plants in the fact that its seeds contain about 50% of oil by weight, which is more than the amount in soybean and somewhat more than in most oil seed crops.
- Jojoba oil is practically colorless and odorless and it is composed mainly of straight chain monoesters of C20 and C22 acids and alcohols with two double bonds.




Then *Simmondsia chinensis*; so this is commonly known as jojoba. It is a perennial shrub belonging to the familiar *Simmondsiaceae*. This plant is native to Mojave and Sonoran deserts of Mexico, California and Arizona. A 10-year-old tree yields on an average of 1 kg seeds per year. It is unique among plants in the fact that its seeds contain about 50% of oil by weight, which is more than the amount in soybean and somewhat more than in most of the oil seed crops.

Jojoba oil is practically colorless and odorless and it is composed mainly of straight chain monoesters of C20 and C22 acids and alcohols with two double bonds.

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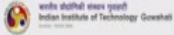
□ *Sapindus mukorossi*



- *Sapindus mukorossi* (soap nut) is a perennial tree belonging to the family *Sapindaceae*, indigenous to northern India.
- This plant grows very well in deep loamy soils and leached soils, so cultivation of *Sapindus mukorossi* in such soil avoids potential soil erosion.

Source: Internet

- This tree can be used for rural building construction, oil and sugar presses, and agricultural implements.
- *Sapindus mukorossi* seeds contain 23% oil of which 92% is triglycerides.




The next is *Sapindus mukorossi*; so *Sapindus mukorossi* is a well known as soap nut tree. It is a perennial tree belonging to the family *Sapindaceae*, indigenous to northern India. Now this plant grows very well in deep loamy soils and leached soils. So, cultivation of *Sapindus mukorossi* in such soil avoids potential soil erosion; it has been deliberately planted in most of the places to restrict the soil erosion.

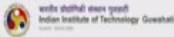
This tree can be used for rural building construction, oil and sugar presses, agricultural implements. *Sapindus mukorossi* seed contains about 23% of oil out of which 92% is triglycerides.

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❑ **Melia azedarach**



- *Melia azedarach* (**syringa**) is a deciduous tree that grows between 7 and 12 m in height in the mahogany family of *Meliaceae* that is native to India, South-East Asia, and Australia.
- The oil content of dried syringa berries is around 10 wt%.
- *Melia azedarach* oil is characterized by a high percentage of unsaturated fatty acids such as oleic (21.8%) and linoleic (64.1%) acids.
- Other constituents that are present in greater than 1% are saturated species such as palmitic (10.1%) and stearic (3.5%) acids.



Then *Melia azedarach*; so *Melia azedarach* which is also known as syringa, is a deciduous tree that grows between 7 to 12 meter in height in the mahogany family of *Meliaceae* that is native to India, Southeast Asia and Australia. The oil content of dried syringa berries is around 10 wt%. *Melia azedarach* oil is characterized by a high percentage of unsaturated fatty acids, such as oleic and linoleic acids. Other constituents that are present in greater than 1% are saturated species such as palmitic and stearic acid.

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❑ **Vernicia fordii**



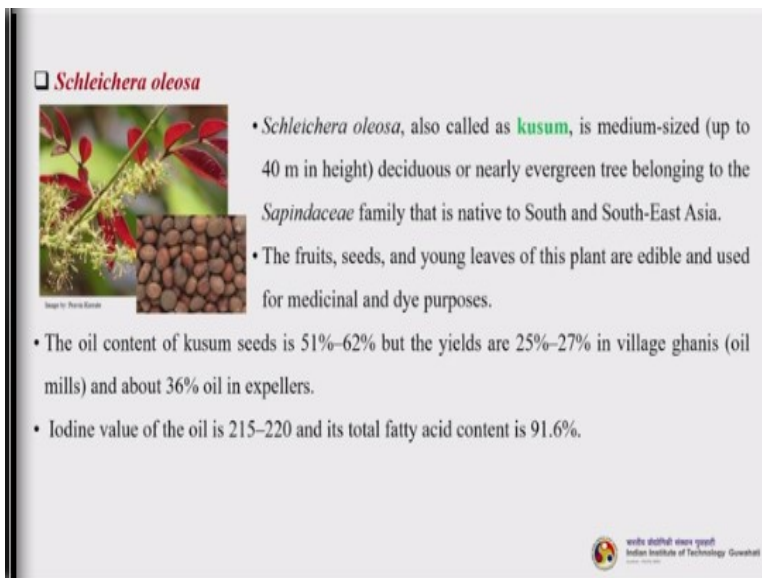
- *Vernicia fordii*, commonly known as **tung tree**, is an oil-bearing woody plant belonging to the *Euphorbeceae* family that is native to China, Burma, and Vietnam.
- The oil content of tung seeds and whole nuts is approximately 21 and 41 wt%, respectively and the average yield of oil is about 450 – 600 kg per hectare.
- Its seed oil had been conventionally used in lamps for lighting, as well as an ingredient for wood paint and varnish.
- Tung oil principally contains the unusual conjugated fatty acid, eleostearic acid (9,11,13-octadecatrienoic acid; 63.8%), with linoleic (11.5%), oleic (8.6%), and behenic (8.4%) acids also present in significant quantities.




Vernicia fordii, it is commonly known as tung tree, is an oil bearing woody plant belonging to *Euphorbeceae* family that is native to China, Burma and Vietnam. The oil content of tung seeds and the whole nuts is approximately 21 and 41 wt% respectively and the average oil yield is

about 450 to 600 kg per hectare. It is a good oil yield basically from this species. Its seed oil had been conventionally used in lamps for lighting, as well as an ingredient for wood paints and varnish. Tung oil principally contains unusual conjugated fatty acids, eleostearic acid, octadecatrienoic acid; with linoleic, oleic, behenic acids also present in significant quantities.

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Schleicheria oleosa



- *Schleicheria oleosa*, also called as **kusum**, is medium-sized (up to 40 m in height) deciduous or nearly evergreen tree belonging to the *Sapindaceae* family that is native to South and South-East Asia.
- The fruits, seeds, and young leaves of this plant are edible and used for medicinal and dye purposes.
- The oil content of kusum seeds is 51%–62% but the yields are 25%–27% in village ghanis (oil mills) and about 36% oil in expellers.
- Iodine value of the oil is 215–220 and its total fatty acid content is 91.6%.

Image by: Pankaj Datta

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Then *Schleicheria oleosa*, it is also known as kusum. So, it is a medium sized up to almost 40 meter in height, deciduous or nearly evergreen tree belonging to the *Sapindaceae* family that is native to South and South-East Asia. So, the fruits, seeds and young leaves of this plant are edible and used for medicinal and dye purposes. The oil content of kusum seeds is 51% to 62% but the yields are 25% to 27% in village ghanis (the oil mills) and about 36% in the expellers.

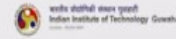
So, of course when you do a better processing or extraction technology, the yield of oil that will come from the same seed will be much higher. Iodine value of the oil is almost 215 to 220 and its total fatty acid content is 91.6%.

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Oil crops and their biorefinery potential

Table 2: The oil content in seeds and kernels of some non-edible plants.

Botanical name	Plant type	Oil content		References
		wt% Seed	Kernel	
<i>Azadirachta indica</i> (neem)	Tree	25–45	30–50	Anya et al. (2012); Djenontin et al. (2012)
<i>Calophyllum inophyllum</i> (polanga)	Tree	60–75	50–70	Azam et al. (2005); Chavan et al. (2013)
<i>Hevea brasiliensis</i> (rubber)	Tree	40–50	35–45	Fadleyibi and Osunde (2012); Kant et al. (2011)
<i>Jatropha curcas</i>	Tree	36	56	Karaj and Müller (2010)
<i>Madhuca indica</i> (mahua)	Tree	30–45	20–50	Jha et al. (2013); Puhan et al. (2005)
<i>Melia azedarach</i> (syringa)	Tree/shrub	10–45	–	Atabani et al. (2013)
<i>Moringa oleifera</i> (drumstick)	Tree	30–40	–	Mohammed et al. (2003)
<i>Nicotiana tabacum</i> (tobacco)	Herb	33–40	17	Bankovic-lic et al. (2012); Khan et al. (2014)
<i>Pongamia pinnata</i> (karanja)	Tree	27–39	30–40	Rahman et al. (2011); Armah et al. (2011)
<i>Ricinus communis</i> (castor)	Tree/shrub	48–60	–	
<i>Sapindus mukorossi</i> (soapnut)	Tree	51	–	Chigozie et al. (2014)
<i>Simmondsia chinensis</i> (jojoba)	Shrub	45–55	–	Borugadda and Goud (2012)



So, this table will make you understand the oil content in seeds and kernels of some of the non-edible plants. So, you can see the listed neem, polanga, rubber, mahua, syringa, drumstick, tobacco, karanja, castor, soap nut, jojoba. All those what we have actually discussed, you can see, in most of trees (these are plants and some are shrubs), you can see this wt% seed and wt% kernels. Let us focus on the seed only. You see that mostly they are comparative or complementing each other (almost 20 to 30 to 40% in that range), which emphasizes that most of these seeds can produce a huge amount of oil. And again I am telling you that extraction of oil is a tedious job. If you are going for the traditional extraction then you may end up in getting almost 60 to 70% or even less than that.

When you talk about chemical based or some other supercritical based extraction, then you may go up to 80% yield. Depending upon seed, oil and in which type of soil it has been grown and under what climatic conditions it has been grown; so many things actually affect the final yield of the oil.

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Oil crops and their biorefinery potential

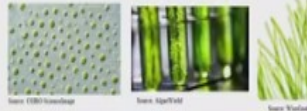
Table 3: The yields of various non-edible oil feedstocks.

Energy crop	Kg per hectare	References
<i>Azadirachta indica</i> (neem)	2670	Azam et al. (2005)
<i>Calophyllum inophyllum</i> (polanga)	4680	Atabani et al. (2013)
<i>Cynara cardunculus</i> (cardo)	500-750	Razon (2009)
<i>Hevea brasiliensis</i> (rubber)	150	Reksowardojo et al. (2011)
<i>Jatropha curcas</i>	2500	Katwal and Soni (2003)
<i>Moringa oleifera</i> (drumstick)	3000	Mohammed et al. (2003)
<i>Nicotiana tabacum</i> (tobacco)	1171	Zdremnan and Zdremnan (2006)
<i>Pongamia pinnata</i> (karanja)	900-9000	Karmee and Chadha (2005)
<i>Ricinus communis</i> (castor)	450	Razon (2009)
<i>Simmondsia chinensis</i> (jojoba)	1125-2250	Razon (2009)
<i>Vernicia fordii</i> (tung)	450-600	Zhuang et al. (2011)

So, this table will again make you understand the yields of various non edible feedstocks. It is given in kg per hectare. You can see that polanga, followed by drumstick, then jatropha and neem, so these are high yield varieties.

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Microalgae as feedstock for biofuels

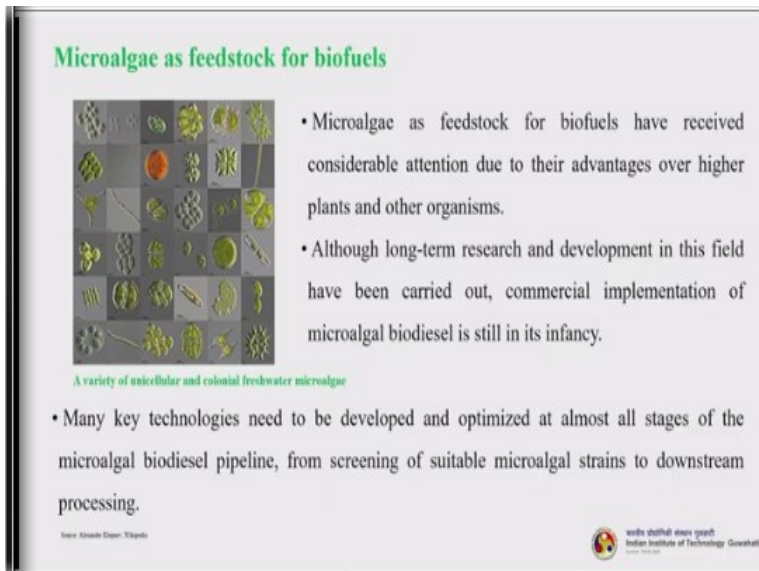


- Although oil crops are renewable resources, biodiesel production from oil crops in large quantities has been deemed unsustainable.
- Production of crop derived biodiesel will require large amounts of arable land, which has to compete with the cultivation of food crops.
- This has led to the controversy of “food versus fuel”.
- The increasing criticism of the sustainability of many first-generation biofuels has stimulated the interest in developing second-generation biofuels produced from non-food feedstocks, such as lignocellulose.

Now we will discuss about microalgae. So microalgae as a feedstock for biofuels production, not only biodiesel but how we can use microalgae in a bio refinery concept, to produce various other biofuels apart from biodiesel as well as other platform chemicals.

Although oil crops are renewable resources, biodiesel production from oil crops in large quantities has been deemed unsustainable. Production of crop derived biodiesel will require large amount of arable land, which has to compete with the cultivation of food crops. Now this has led to the controversy of “food versus fuel”. The increasing criticism of the sustainability of many first generation biofuels has stimulated the interest in developing second generation biofuels which are being produced from non-food feedstocks such as lignocellulosic biomass.

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Microalgae as feedstock for biofuels

- Microalgae as feedstock for biofuels have received considerable attention due to their advantages over higher plants and other organisms.
- Although long-term research and development in this field have been carried out, commercial implementation of microalgal biodiesel is still in its infancy.

A variety of unicellular and colonial freshwater microalgae

- Many key technologies need to be developed and optimized at almost all stages of the microalgal biodiesel pipeline, from screening of suitable microalgal strains to downstream processing.

Source: Alkandak, Özgen, Tripathi

स्वास्थ्य शिक्षण संस्था गुणवत्ता
Indian Institute of Technology Guwahati
2015-2016

Now microalgae as a feedstock for biofuels has received considerable attention due to their advantages over higher plants and other organisms. Although long term research and development in this field have been carried out, commercial implementation of microalgal biodiesel is still in it is infancy (there are various reasons for that). Many key technologies need to be developed and optimized at almost all stages of microalgal biodiesel pipeline, from screening of suitable microbial strains to downstream processing.

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Advantages of microalgae as biodiesel feedstock

Microalgae appear to be the only promising alternative to biofuel crop plants because of the following facts:

- a) Microalgae grow rapidly and many species contain high amount of lipids which can provide sufficient feedstock for large-scale biodiesel production.
 - b) Non-requirement of arable land for microalgal culture makes their growth without conflict with food production.
- ✓ According to an estimate, meeting only half the existing U.S. transport fuel needs by biodiesel would require 24% of the total cropland to grow oil palm with the highest oil productivity (5,950 l ha⁻¹).

Algal Blooms



So, microalgae appear to be the only promising alternative to biofuel crop plants because of the following facts:

- (a) it grows rapidly and many species contain high amounts of lipids (basically the oils) which can provide sufficient feedstock for large scale biodiesel production;
 - (b) Non-requirement of arable land for microalgal culture makes their growth without conflict with the food production.
- ✓ According to an estimate, meeting only half of the existing U.S transport fuel needs by biodiesel would require 24% of the total cropland to grow oil palm with the highest oil productivity. (So, you can see these are some of the algal blooms or green algal blooms).

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- ✓ On the other hand, only 2.5% of existing cropping area would be required for cultivation of microalgae with 30% oil in biomass, which can also produce equivalent biodiesel.
- ✓ The percent of cropping area required can be still lower, because 30% oil content in the biomass can be achieved easily for many oleaginous microalgae.
- c) Microalgal cells have photosynthetic mechanism similar to those of higher plants to fix CO₂ in air and convert the C to carbohydrates and lipids, with some species accumulating large amounts of triacylglycerides (TAGs), which are suitable for biodiesel production.
- ✓ The photosynthetic mechanism of microalgae is cost-effective compared with oil-producing heterotrophic microorganisms that utilize glucose and other organic carbon sources.



Algal Blooms

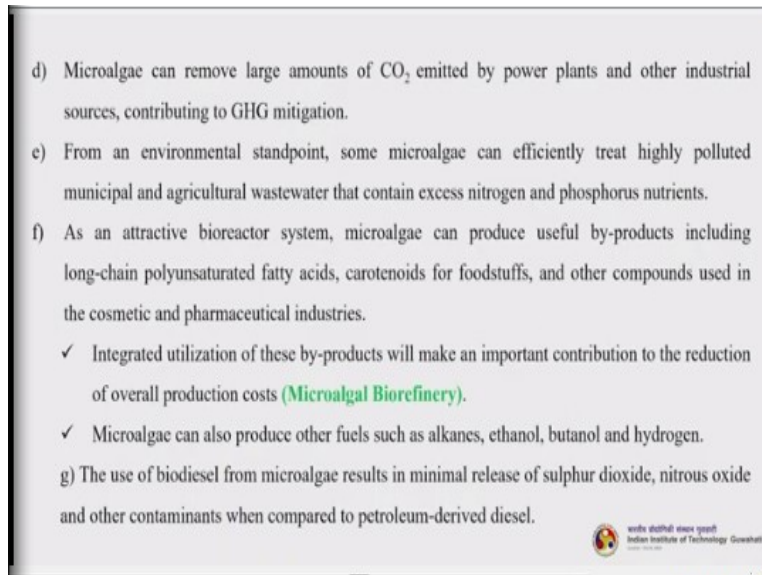
(A brown algal bloom is being shown here)

- ✓ On the other hand, only 2.5% of existing cropping area would be required for cultivation of microalgae with 30% oil in biomass, which can also produce equivalent biodiesel.
- ✓ The percentage of cropping area required can still be lower, because 30% oil content in the biomass can be achieved easily for many oleaginous microalgae.

(c) The next advantage is that, microalgal cells have photosynthetic mechanism similar to those of higher plants to fix carbon dioxide in air and convert the carbon to carbohydrates and lipids, with some species accumulating large amounts of triacylglycerides (TAGs - these are also triglycerides), which are suitable for biodiesel production.

- ✓ The photosynthetic mechanism of microalgae is cost effective compared with oil producing heterotrophic microorganisms that utilize glucose and other organic carbon sources.

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(d) So, the next advantage is that microalgae can remove large amounts of carbon dioxide emitted by power plants and other industrial sources contributing significantly to the greenhouse gas mitigation.

(e) From an environmental standpoint, some microalgae can efficiently treat highly polluted municipal and agricultural wastewater that contain excess nitrogen and phosphorus nutrients.

(f) As an attractive bioreactor system, microalgae can produce useful byproducts including long-chain polyunsaturated fatty acids, carotenoids for foodstuffs, and other compounds used in the cosmetic and pharmaceutical industries.


- ✓ Integrated utilization of these byproducts will make an important contribution to the reduction of overall production costs. Now this is what I will just explain you briefly. Now please note that, microalgae is something, each and every part of that particular organism is being useful, it is just like a banana plant. So, the fruits we eat, the flowers are also being eaten in India and many parts of the South East Asia. The leaves are being used traditionally for various purposes, the trunk is also eatable. So, it is an endless thing. Similarly, for algae also every part is being utilized. What we are talking about in these particular slides, is about the biodiesel from microalgae; but, it is not the end of the story. See, once you extract the biodiesel, that means the oil is extracted or lipid is extracted and there is something left out solid, which is called lipid extracted biomass, microalgal biomass. This will have so many other valuable things present such as a huge amount of carbohydrates. It may have pigments, it may have other important valuable products, such as astaxanthin, vitamins so on. So, what I mean to say is that once you extract the lipid from the microalgae, it is not the end of the story. So, then we are going to work on the leftover part, the solid part. So, depending upon its component analysis we can convert it; if there is huge carbohydrate, we can go for hydrolysis followed by fermentation thereby producing bio alcohol. We can also produce bio butanol, following the Abe fermentation. If we see that, it has good chlorophyll content, we can extract that chlorophyll; if it has good astaxanthin content or some different pigments, that can also be extracted. So, vitamins also can be extracted, so we can play with it depending upon what species it is and what is that component analysis after the oil is being extracted. So, if we look into that perspective, in a complete biorefinery perspective, then it will become sustainable. Otherwise, if you only talk about microalgae to biodiesel, it is not going to be a sustainable process.
- ✓ Microalgae also produce other fuels such as alkanes, ethanol, butanol and hydrogen in a more bio refinery platform.

(g) The use of biodiesel from microalgae results in minimal release of sulfur dioxide, nitrous oxide and other contaminants when compared to petroleum derived diesel.

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Biodiversity of microalgae lipid properties

- Microalgae comprise several groups of unicellular, colonial or filamentous, photosynthetic or heterotrophic microorganisms containing chlorophyll and other pigments.
- Microalgae can grow autotrophically or heterotrophically, with a wide range of tolerance to different temperature, salinity, pH and nutrient availabilities.
- More than 40,000 microalgal species have been classified as prokaryotes (cyanobacteria) and several eukaryotes including green algae, diatoms, yellow-green algae, golden algae, red algae, brown algae, dinoflagellates and others.



Courtesy: European Algae Biomass Association

वर्ल्ड स्टॉफ़्थू सेव्ज स्प्रेड
Indian Institute of Technology Guwahati

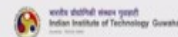
Now let us understand the biodiversity of microalgal lipid properties. Please look at this particular slide; you can see there are so many different types of species that has been shown here. The picture has been taken from the European algae biomass association, you can see the different types of algae, please do understand that algae is only green, it is not so. There are blue green algae, there is brown algae (it is just like a plant).

So, you do not usually understand if you see with the naked eye that it is an algae. So, it is very diverse basically. Microalgae comprise several groups of unicellular, colonial or filamentous, photosynthetic and heterotrophic microorganisms containing chlorophyll and other pigments. So, microalgae can grow autotrophically or heterotopically with a wide range of tolerance to different temperature, salinity, pH and nutrient availabilities.

More than 40,000 microalgal species have been classified as prokaryotes (cyanobacteria) and several eukaryotes including green algae, diatoms, yellow green algae, golden algae, red algae, brown algae, dinoflagellates and others.

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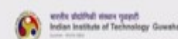
- Many different classes of lipids can be produced in microalgal cells.
- Based on chemical structures and polarity, these lipids are divided into *polar* and *neutral* lipids.
- In most cases, polar lipids function as membrane structure components, which commonly include:
 - a) *phospholipids* (e.g. phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine)
 - b) *glycolipids* (e.g. monogalactosyldiacylglycerol and digalactosyldiacylglycerol)
- Neutral lipids include tri-, di- and mono-acylglycerols, waxes and isoprenoid-type lipids (e.g. carotenoids), among which triacylglycerols (TAGs) are frequently found to be accumulated as energy storage under various stress conditions.



Many different classes of lipids can be produced in microalgal cells. Based on chemical structures and polarity, these lipids are divided into polar and neutral lipids. In most cases, polar lipids function as a membrane structure component, which commonly include phospholipids and glycolipids. So, neutral lipids include tri, di and mono acyl glycerols, waxes, isoprenoid type lipids (for example, carotenoids), among which triacylglycerols (TAGs) are frequently found to be accumulated as energy storage under various stress condition. These TAGs will be eventually converted to biodiesel by the transesterification pathway.

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- Although almost all types of microalgal lipids can be extracted, only TAGs are easily transesterified into biodiesel by traditional methods.
- Analysis of thousands of microalgal species have shown tremendous difference in lipid content among different strains, ranging from 1% to approximately 85% of dry cell weight (DCW).
- Microalgae produce a wide variety of fatty acids with chain length from C10 to C24, depending on species or strains.
- For example, the filamentous cyanobacterium *Trichodesmium erythraeum* can synthesize C10 fatty acid accounting for almost 50% of total fatty acids; whereas the dinoflagellate *Cryptocodinium cohnii* can produce docosahexaenoic acid (DHA) as high as 30–50% of total fatty acids.
- Moreover, for any one microalgal strain, the lipid content, lipid class and fatty acid composition fluctuate under different culture conditions.

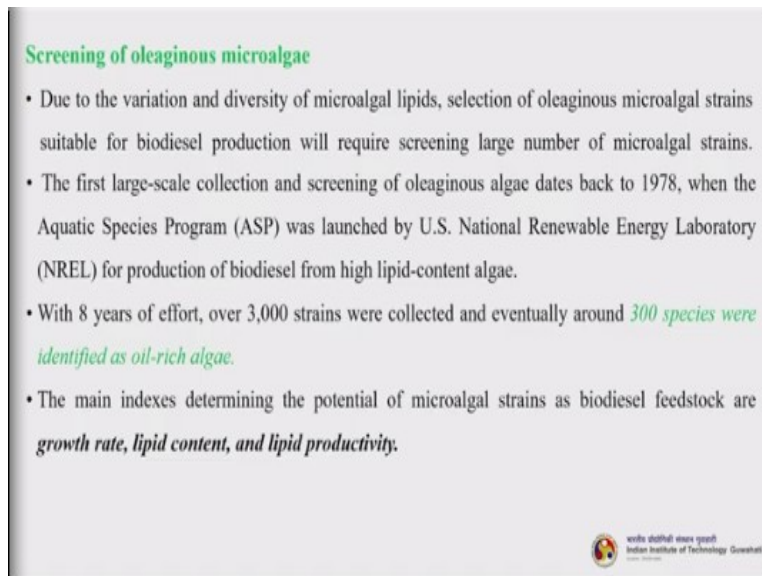


So, although almost all types of microalgal lipids can be extracted, only TAGs are easily transesterified into biodiesel by traditional methods. Analysis of thousands of microalgal species

have shown tremendous difference in lipid content among different strains ranging from 1% approximately to 85% of that dry cell weight. Microalgae produce a wide variety of fatty acids with chain length from C10 to C24 depending on species or strains.

For example, the filamentous cyanobacterium, that is the *Trichodesmium erythraeum* can synthesize C10 fatty acid accounting for almost 50% of total fatty acids. Whereas dinoflagellate *Cryptothecodinium cohnii* can produce docosahexaenoic acid (DHA) as high as 30 to 50% of the total fatty acids. Moreover, for any one microalgal strain the lipid content, lipid class and fatty acid composition fluctuate under different culture conditions.

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Screening of oleaginous microalgae

- Due to the variation and diversity of microalgal lipids, selection of oleaginous microalgal strains suitable for biodiesel production will require screening large number of microalgal strains.
- The first large-scale collection and screening of oleaginous algae dates back to 1978, when the Aquatic Species Program (ASP) was launched by U.S. National Renewable Energy Laboratory (NREL) for production of biodiesel from high lipid-content algae.
- With 8 years of effort, over 3,000 strains were collected and eventually around *300 species were identified as oil-rich algae.*
- The main indexes determining the potential of microalgal strains as biodiesel feedstock are *growth rate, lipid content, and lipid productivity.*

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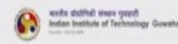
With 8 years of effort about 3000 strains were collected and eventually around 300 species were identified as oil rich algae. The main indexes determining the potential of microalgal strains as biodiesel feedstock are growth rate, lipid content and lipid productivity.

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Table 4: Some microalgal species with relatively high lipid content and productivities

Microalgae species	Class	Lipid content (% w/w)	Lipid productivity (mg l ⁻¹ day ⁻¹)
<i>C. protothecoides</i>	Chlorophyceae	15–58	1214
<i>Chlorococcum</i> sp.	Chlorophyceae	19	54
<i>Chlorella sorokiniana</i>	Chlorophyceae	19–22	45
<i>D. salina</i>	<i>D. salina</i>	6–25	116
<i>Ellipsoidion</i> sp.	Eustigmatophyceae	27	47
<i>Nannochloropsis</i> sp.	Eustigmatophyceae	21–36	38–61
<i>Nannochloropsis oculata</i>	Eustigmatophyceae	22–30	84–142
<i>Neochloris oleabundans</i>	Chlorophyceae	29–65	90–134
<i>Pavlova salina</i>	Prymnesiophyceae	31	49
<i>Pavlova lutheri</i>	Prymnesiophyceae	36	50
<i>Phaeodactylum tricornutum</i>	Bacillariophyceae	18–57	45
<i>Scenedesmus</i> sp.	Chlorophyceae	20–21	41–54

Source: *Strained production with microalgae in Berkeley*. from *microalgal biofuels*. Elsevier Ltd (2011): 11-128-129.

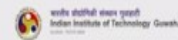


This table will make you understand about different microalgal species with relatively high lipid content and productivity. So, you see that these are some of the microalgal species, here the lipid content is given and that is the lipid productivity. You can see mostly they are complementing to each other whereas this *Pavlova lutheri* giving us the highest in this particular species that is being reported here, so followed by *Neochloris* sp. as well as *Nannochloropsis oculata*.

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Microalgae as feedstock for biochemicals

- Microalgae (both microalgae and cyanobacteria) are considered as potential source of high-value nutrients such as **pigments, proteins, carbohydrates and lipid molecules**.
- Industrial scale production of microalgae has evolved worldwide due to human consumption of microalgae as nutritional supplements.
- Apart from biomass, microalgae produces variety of pigments molecules like chlorophyll, carotenoids and β -carotene that are used as colorants in cosmetic and food industry.
- Algae strains, *Chlorella* sp., *Dunaliella* sp., and *Scenedesmus* sp., and cyanobacterial strains (*Spirulina* sp. and *Nostoc* sp.) are used as sources of fine chemicals and nutrient rich food supplements.

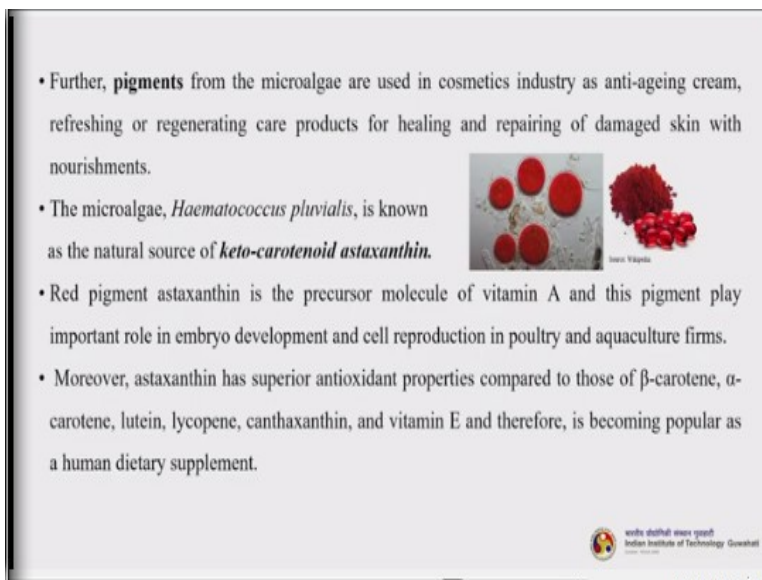


Both microalgae and cyanobacteria are considered as potential source of high value nutrients, such as pigments, proteins, carbohydrate and lipid molecules. And this is what I was mentioning about in the broad bio-refinery concept. It is not only about the lipid molecules that is being getting extracted for biodiesel but we can play with all these things pigments, proteins,


carbohydrates, there are vitamins and there are certain other chemicals which also can be purified and made into the platform chemicals.

So, industrial scale production of microalgae has evolved worldwide due to human consumption of microalgae as nutritional supplements. Apart from biomass, microalgae produce variety of pigment molecules like chlorophyll, carotenoids, beta carotene, that are used as colorants in cosmetic and food industry. Algae strains *Chlorella* sp., *Dunaliella* sp., *Scenedesmus* sp. and cyanobacterial strains such as *Spirulina* sp. and *Nostoc* sp. are used as sources of fine chemicals and nutrient rich foods supplements.

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- Further, **pigments** from the microalgae are used in cosmetics industry as anti-ageing cream, refreshing or regenerating care products for healing and repairing of damaged skin with nourishments.
- The microalgae, *Haematococcus pluvialis*, is known as the natural source of *keto-carotenoid astaxanthin*.
- Red pigment astaxanthin is the precursor molecule of vitamin A and this pigment play important role in embryo development and cell reproduction in poultry and aquaculture firms.
- Moreover, astaxanthin has superior antioxidant properties compared to those of β -carotene, α -carotene, lutein, lycopene, canthaxanthin, and vitamin E and therefore, is becoming popular as a human dietary supplement.



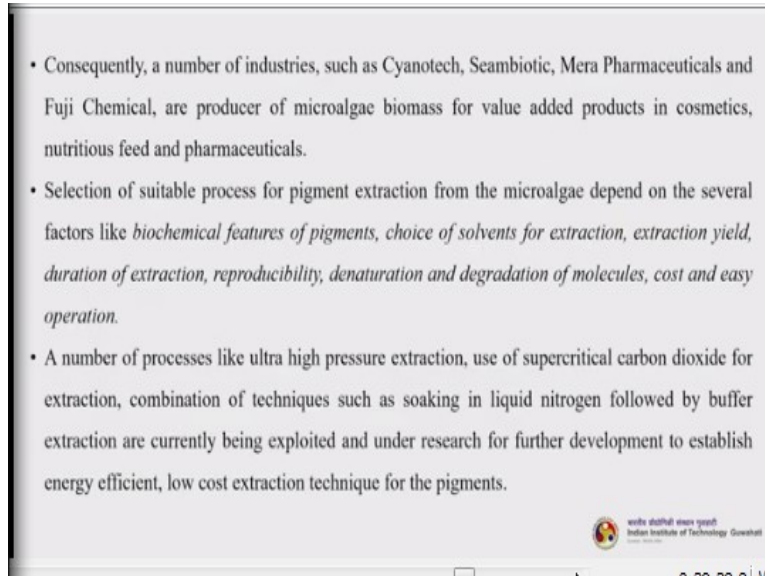
Microscopic view of *Haematococcus pluvialis* cells, showing red pigments (astaxanthin) within the cells.

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Further pigments; pigments are interesting class of chemicals, which can be purified from the microalgal species. So, they are used in cosmetic industry as anti-ageing cream, refreshing or regenerating care products for healing and repairing of damaged skin with nourishments. The microalgae *Haematococcus pluvialis* (which is shown in this particular slide you can see), is known as the natural source for the keto-carotenoid astaxanthin. Astaxanthin is one such pigment which is of course having a lot of commercial value. Red pigment astaxanthin is the precursor molecule for vitamin A and this pigment play important role in embryo development and cell production in poultry as well as aquaculture firms. Moreover, astaxanthin has superior antioxidant properties compared to those of beta carotene, alpha carotene, lutein, lycopene, canthaxanthin and vitamin E, and therefore is becoming as popular as a human dietary

supplement. That is what I was just mentioning about; that there is a huge commercial value of this particular pigment.

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Consequently, a number of industries such as Cyanotech, Seambiotic, Mera Pharmaceuticals and Fuji chemical, are the producers of microalgae biomass for high value added products in cosmetics, nutritious feed and pharmaceuticals. Selection of suitable process for pigment extraction from the microalgae depend on several factors like biochemical features of pigments, choice of solvents for extraction, extraction yield, duration of extraction, reproducibility, denaturation and degradation of molecules, cost and easy operation. Now all these factors will eventually determine how much pigments we are able to extract from a particular microalgal species.

So, a number of processes like ultra high pressure extraction, use of supercritical carbon dioxide for extraction, combination of techniques such as soaking in liquid nitrogen followed by buffer extraction are currently being exploited and under research for further development to establish energy efficient, low cost extraction technique for the pigments.

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- Microalgae also considered as reliable rich source of vegetable **protein**.
- Nutritional studies on different microalgae have demonstrated that microalgae produced high amount and high quality proteins, which are the source of essential amino acids.
- Certain algae species like red algae (*Rhodophyta*) and cyanobacterial strain produce a group of accessory photosynthetic pigment protein complexes for light harvesting purpose are called phyco-biliproteins.
- These proteins have high demand in pharmaceutical industries and specific application in the biological field as fluorophore.
- Protein extraction from microalgae is done using aqueous, acidic, and alkaline methods, followed by centrifugation, ultrafiltration, precipitation, or chromatography techniques for the recovery of the protein molecules.



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So, microalgae are also considered as reliable rich source of the vegetable protein. Nutritional studies on different microalgae has demonstrated that microalgae produced high amount and high quality proteins which are the source of essential amino acids. You can see there is a particular picture there, that picture is of the red algae *Rhodophyta*. These *Rhodophyta* and other cyanobacterial strains produce a group of accessory photosynthetic pigment protein complexes, for light harvesting purpose are also called phyco-billiproteins, these are high value products.

So, these proteins have a high demand in pharmaceutical industries and specific application in the biological field as fluorophores. So, fluorophore are chemical compounds, which are essentially responsible for emitting light. So, protein extraction from microalgae is done using aqueous, acidic and alkaline methods followed by centrifugation, ultrafiltration, precipitation, chromatography techniques for the recovery of the protein molecules.

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- Industrial scale extraction and purification of proteins from microalgae is not studied widely and scalable downstream processes of microalgae for efficient extraction of proteins are still in very high demand.
- Diversity in microalgal species, variation in the cell structure, variation in the intracellular protein content, release of protein degrading (protease) enzymes from the cells are major obstructions for up-scaling of the protein extraction process.
- Some novel extraction techniques such as pulsed electric field, microwave-assisted extraction and ultrasound-assisted extraction are employed for successful extraction of proteins from microalgae.

So, industrial scale extraction and purification of proteins from microalgae is not studied widely and scalable downstream processes of microalgae for efficient extraction of proteins are still in very high demand. So, diversity in microalgal species, variation in the cell structure, variation in the intracellular protein content, release of protein degrading enzymes (proteases) from the cells are major obstructions for up-scaling of the protein extraction process.

Some novel extraction techniques such as pulsed electric field, microwave assisted extraction and ultrasound assisted extraction are employed for successful extraction of proteins from microalgae. So, please note that the downstream processing cost usually constitute almost 40 to 50% of the entire product cost. So, that particular cost has to be brought down to a certain level, so that the cost of the product eventually decreases.

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- Manipulation in growth conditions can enrich microalgae with high amount of **carbohydrate** or **polysaccharide molecules**.
- Major components of the cell wall of algae are cellulose and hemicellulose. Other than the cell wall, algae also store polysaccharide molecules in the cytoplasm.
- Marine algae produce complex sulfated cell wall polysaccharides, which have many biomedical applications.
- Some cyanobacterial strains (e.g. *Nostoc sp.*, *Spirulina sp.*, *Porphyridium sp.*) are surrounded by a matrix of polymeric substance mainly constituted by polysaccharides, which form a protective layer between the cell and the immediate environment.



Various cyanobacterial strains, source: U. S. Hall, Encyclopedia of Astrobiology, 2011.

So, manipulation in growth conditions can enrich microalgae with high amount of carbohydrate or polysaccharide molecules. Major components of the cell wall of algae are cellulose and hemicellulose. Other than the cell wall algae also store polysaccharide molecules in the cytoplasm. Marine algae produce complex sulfated cell wall polysaccharides, which have many biomedical applications.

Some cyanobacterial strains (you see some of these are shown in this slide), are surrounded by a matrix of polymeric substance mainly constituted by polysaccharides, which form a protective layer between the cell and the intermediate environment.

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- Biotechnological potential of the cyanobacterial extracellular polymeric matrix are attracting increasing attention to the pharmaceutical, bio-plastic and food industries.
- Novel extraction technologies such as enzyme-assisted extraction, microwave assisted extraction, ultrasound assisted extraction, supercritical fluid extraction, and pressurized liquid extraction are currently being applied for the extraction of bioactive molecules from microalgae.
- These extraction technologies are attracting interest from the industries because of its advantages (higher yield, reduced treatment time, and lower cost) compared to the conventional solvent extraction techniques.





Various cyanobacterial strains, source: U. S. Hall, Encyclopedia of Astrobiology, 2011.


Biotechnological potential of the cyanobacterial extracellular polymeric matrix are attracting increasing attention to the pharmaceutical, bio-plastic as well as food industries. Novel extraction technologies such as enzyme assisted extraction, microwave assisted extraction, ultrasound assisted extraction, supercritical fluid extraction and pressurized liquid extraction are currently being applied for the extraction of bioactive molecules from microalgae.

These extraction technologies are attracting interest from the industries because of its advantages (such as higher yield, reduced treatment time and lower cost) compare to that of the conventional solvent extraction techniques. A huge scope is still available for developing the downstream processing part.

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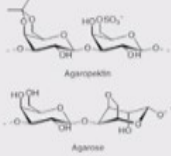
Table 5: Industrial products from microalgae

Product category	Product name	Source	Structure	Application
Pigment	β -Carotene	<i>Spirulina platensis</i> , <i>Caulerpa taxifolia</i> .		β -Carotene is found to be useful in the prevention against cancer and heart disease, to slow the progression of cataracts, to boost immunity, to protect the skin against sunburn, asthma and depression.
	Chlorophyll	Green algae	 Structure of Chlorophyll A	In the food industry, chlorophyll is used as a natural pigment ingredient in processed foods. Have antioxidant activity.

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So, we will quickly go through some of the industrial products from microalgae, please see this pigment. The product name is beta carotene, chlorophyll (chlorophyll is from green algae); this is the structure, it is being used in the food industries, it is a natural pigment ingredient. Similarly, beta carotene from *Spirulina* and, *Caulerpa* species; that is the structure of beta carotene and it is found to be useful in the prevention against certain type of cancer and heart diseases.

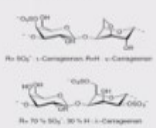
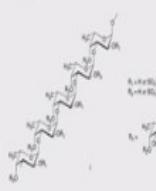
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Product category	Product name	Source	Structure	Application
Protein	Protein powder or tablets	Microalgae. Exp: <i>Chlorella</i> sp. and Cyanobacteria. Exp: <i>Anthrospira</i> sp.	 <p>Spirulina powder at 400x, unstained wet mount.</p>	Nutritional benefits. Used as feedstock for animals and poultry.
Carbohydrate	Agar	<i>Rhodophyta</i> (red algae) Exp.: <i>Gelidium</i> , <i>Gracilaria</i> and <i>Pterocladia</i> sp.	<p>Consists of 70% agarose and 30% agarpectin</p>  <p>Agarpectin</p> <p>Agarose</p>	Best known application of agar is the preparation of culture media in Petri dishes for the growth of micro-organisms. In the food industry agar is used as thickening agent and can be applied as substitute for pectin, gelatin and starch.

The next is protein; so protein powder or tablets is the form. There are different species such as *Chlorella* and other cyanobacteria species such as *Anthrospira*. And this is the Spirulina powder how it looks like, this is a SEM image. It has so much of nutritional benefits, used as a feedstock for animal and poultry. Then carbohydrate; the product name is agar, so *Rhodophyta* and red algae and there are many other species.


This is how it almost consist of 70% of agarose and 30% of the agarpectin, so this is the structure of agarpectin and agarose. So, best known application of agar is the preparation of culture media and in petri dishes, huge application in the research lab for the growth of the microorganisms.

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Product category	Product name	Source	Structure	Application
Carbohydrate	Carrageenan	Red seaweeds Exp.: <i>Chondrus crispus</i> and <i>Mastocarpus stellatus</i> .	Generally two types found  Fucoidans are sulfated polysaccharides mainly consisting of L-fucose with molecular weights ranging from 13 to 950 kDa	Common food additives due to their thickening, gelling and emulsion stabilising properties in the food industry. Application in the pharmaceutical and cosmetic industry.
	Fucoidan	Brown seaweed Exp.: <i>Fucus vesiculosus</i> , <i>Peletia canaliculata</i>		Exhibit anticoagulant abilities by enhancing the heparin cofactor II, so they may become an alternative to heparin due to their herbal origin. Antitumor efficacy, owing to their ability to inhibit proliferation and to induce apoptosis via caspase and extra-cellular signal regulated kinase pathways.

The next is carbohydrate again, Carrageenan, Fucoidan. So Carrageenan can come from the red seaweeds. Now these are the structures, it is common food additives due to their thickening, gelling and emulsion stabilizing properties. Fucoidan, this is the structure; it exhibits anticoagulant abilities by enhancing the heparin cofactor II. So, they may become an alternative to heparin due to their herbal origin.

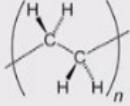
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Product category	Product name	Source	Structure	Application
Carbohydrate	Alginate	Algae, Exp.: <i>Macrocystis pyrifera</i> <i>Ascophyllum nodosum</i> <i>Laminaria sp.</i> <i>Ecklonia sp.</i>	Alginate is a linear polysaccharide consisting of two types of anionic monomers. β -D-Mannuronic acid (M) and α -L-galuronic acid (G) are linked via 1,4-glycosidic bonds in homopolymeric MM- and GG- blocks respectively 	Widely applied as a stabilising, thickening or emulsifying agent in the food, cosmetic, paper and dye industries. In medical fields alginates are used for example as dental impression materials, for tissue engineering applications or as cell encapsulation material

So, the next is Alginate, carbohydrate in the form of Alginate. Different species such as *Macrocystis pyrifera*, *Ascophyllum nodosum*, these are the structures. Alginate is a linear polysaccharide consisting of two types of monomers. It is widely applied as a stabilizing,

thickening or emulsifying agent in the food, cosmetic, paper and dye industries, it has so much of medicinal applications also.

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Product category	Product name	Source	Structure	Application
Organic Plastic	Biopolymers (PLA, bio polyethylene etc.)	<i>Nostoc</i> sp., <i>Phormidium mucicola</i> , <i>Chlorella stigmaaphora</i> , <i>Chlorella vulgaris</i>	Repeating group of polyethylene which can be derived from algal biomass 	Thickening agents for mobility control in water flood oil recovery, food additives, flocculants useful in waste water treatment, Soil conditioning, drilling mud extenders, pet food, farm feed stabilisers.

And the last one is the organic plastic, in the form of biopolymers (PLA or poly lactic acid, bio polyethylene etc.). So, *Nostoc* sp., *Phormidium mucicola*, then *Chlorella stigmaaphora* and then *Chlorella vulgaris*. *Chlorella vulgaris* is a well-known species. This is the structure, it is a monomer and the repeating unit. So thickening agents for mobility control in water flood oil recovery, food additive, flocculants useful in the wastewater treatment, soil conditioning, drilling mud extenders, pet food and farm feed stabilizers. So, this is all about microalgae and I windup today's lecture.

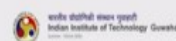
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(Overview of next lecture)

Module	Module name	Lecture	Title of lecture
02	Biomass	04	Enhancing biomass properties for biofuels, challenges in conversion

Thank you

For queries, feel free to contact at: kmohanty@iitg.ac.in



So, in the next lecture we will discuss about how to enhance the biomass properties for biofuels and what are the challenges in conversion. So, thank you very much for listening; if you have any query please feel free to drop mail to me at kmohanty@iitg.ac.in also post your query in the Swayam portal, thank you.