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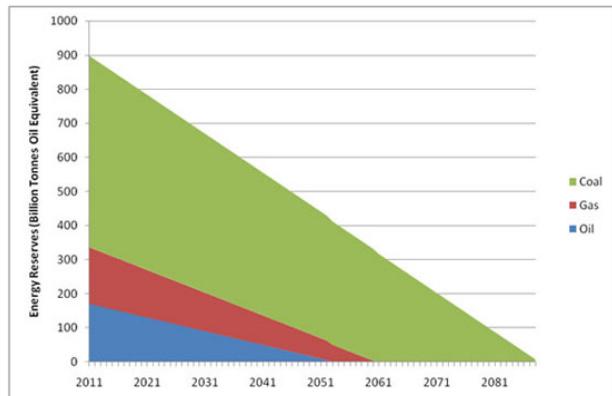
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Esterifying FFA's and Phospholipids to Increase the Yield of BioDiesel from Feedstock

Abstract : Petrol and diesel are finite natural resources, and soon will be exhausted. The search for synthetic or alternative fuels is an ongoing area of research, to reduce our dependency on petroleum, and its derivatives. Algae are photosynthetic organisms capable of high growth rates in culture media, and are capable of accumulating huge amounts of lipids, making them a suitable candidate for BioDiesel Feedstock.

My work emphasises on drastically increasing the yield from perfectly viable feedstock - FFA's and Phospholipids, which is otherwise wasted by the current production processes.

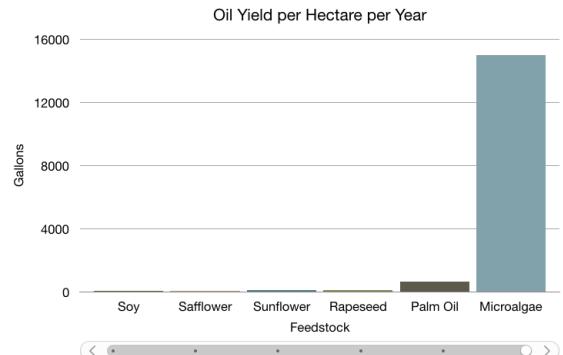
Introduction : Fossil Fuels are fast depleting and are projected to run out by year 2052. There is a pressing need to find alternative energy sources to meet the ever increasing demand. One such viable alternative is Biodiesel.



Ref : CIA World Factbook

Biodiesel is a variety of ester-based oxygenated fuels derived from natural renewable biological sources such as vegetable oil. Through a process termed transesterification, biodiesel is produced from oilseeds and plant and animal matter. More research is being geared towards finding an alternative renewable fuel through biological means

because of its positive environmental benefits (Bridgewater, 2006 and Arthe et al., 2008). Recent research(Michael et al., 2010) has shown that algae is viable as biodiesel feedstock.



Two approaches for transesterification of vegetable oils for the production of biodiesel have been suggested(Haas et al., 2002). The first is a chemical one in which alcoholysis of oil by methyl or ethyl alcohol in the presence of a strong acid or base produces biodiesel and glycerol (Fukuda et al., 2001). Chemical transesterification is efficient in terms of reaction time; however, the chemical approach to synthesize biodiesel from triglycerides has drawbacks, such as difficulty in the recovery of glycerol and the energy intensive nature of the process. The second approach is the enzymatic one, in which lipase-catalyzed transesterification is carried out in non-aqueous environments.

Phospholipids are rejected from the process by degumming(Greg et al.,2010), as they are non-transesterifiable, and are corrosive to the engine. FFA's also act as contaminants and must be neutralised. They result in wastage. By introducing methods to recover this wasted material, we can increase yield of biodiesel.

Process of Lipid Extraction: The lipids first need to be extracted from the microalga*(Chlorella sp.)*. *Chlorella sp.* is a fairly common species of green algae capable of accumulating huge amounts of lipids(Feng et al.,2011) in their cell body. They are good candidates for feedstock of biodiesel.

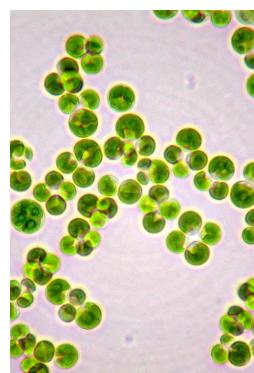


Image under Microscope - *Chlorella sp.*

Various solvents or solvent combinations have been suggested as extractants, but I used chloroform-methanol (2:1 by volume) as suggested by Folch.

The extract was shaken and equilibrated with one fourth its volume of a saline solution, and mixture partitioned into two layers, of which the lower was composed of chloroform-methanol-water contained all of the lipids, while the upper phase consisted of the solvents and contained much of the non-lipid contaminants.

Tissue was homogenised with chloroform:methanol (2:1) to a final dilution 20 times the volume of the tissue sample. Homogenate was filtered through a suitable paper into a glass-stoppered bottle. Crude extract was washed with 0.2 of its volume of water solution. Solution was allowed to separate into two phases. The volumes of the upper and lower phases were 40 and 60% of the total volume respectively. Upper layer was removed by siphoning. Interface was rinsed three times with pure 'upper phase', i.e. the chloroform:methanol:water 3:48:47 so that the lower phase is not disturbed. Finally methanol was added so that the lower phase and the rinsing liquid form one phase.

The extract was now ready. This contained all the lipids including phospholipids, as compared to major constituent of extract being triglycerides in the general extraction method, leaving out **20-50%** of lipid content(Y Li et al.,2014).

Table 12.3. Range of lipid levels found in different classes of algae

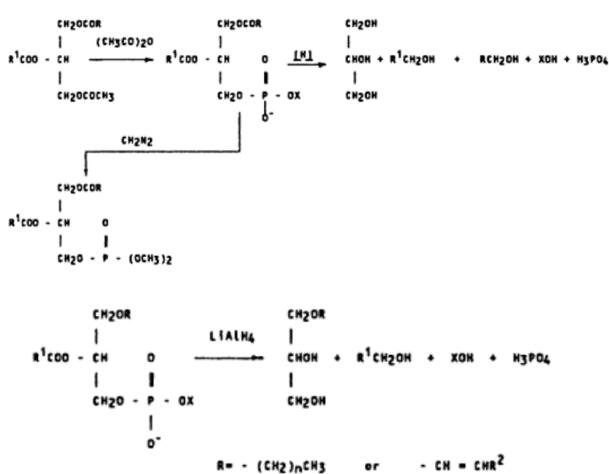
| Algal class | Total lipids (% of dry matter) | Percentage of total lipids | | |
|-------------------|-----------------------------------|----------------------------|-------------|---------------|
| | | Neutral | Glycolipids | Phospholipids |
| Cyanobacteria | 2-23 | 11-68 | 12-41 | 16-50 |
| Chlorophyceae | 1-70 | 21-66 | 6-26 | 17-53 |
| Crysophyceae | 12-72 | — | — | — |
| Prymnesiophyceae | 5-48 | — | — | — |
| Cryptophyceae | 3-17 | — | — | — |
| Xanthophyceae | 6-16 | 44 | 17 | 39 |
| Rhodophyceae | 1-14 | 41-58 | 42-59 | — |
| Bacillariophyceae | 1-39 | 14-60 | 13-44 | 10-47 |

Source: After Borowitzka, 1988.

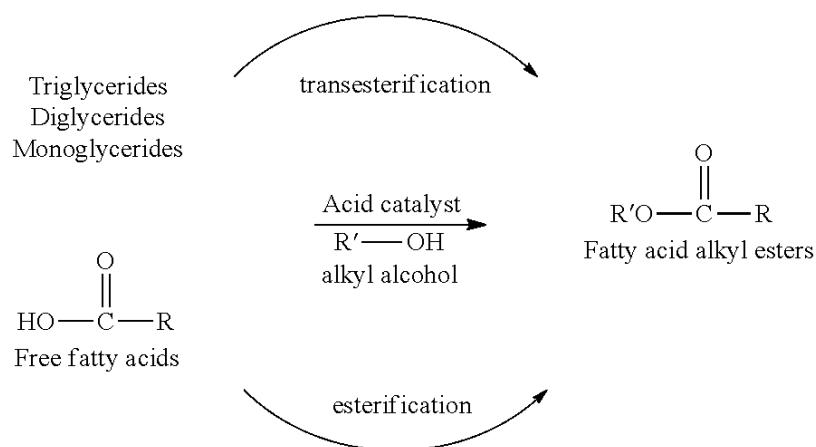
Transesterification : The transesterification process is catalyzed by alkalis, acids and enzymes. The most common alkaline catalysts are sodium hydroxide (NaOH) and potassium hydroxide (KOH) (Schuchardt et al., 1998; Marchetti et al., 2008; Robles et al., 2009). These chemicals proved to be the most economic because of higher conversion rate of esters under a low temperature and pressure environment and short reaction time (Bacovsky et al., 2007).

Acid catalysts slow down the process(Leung et al.,2010), that is why they are not generally used to transesterify. Conversely, basic catalysts reduce yield, as they neutralise FFA's, greatly reducing yield. Therefore, a method need to be developed, that doesn't reduce yield, yet doesn't slow down the process.

Also, Phospholipids are removed during degumming(Silva et al.,2014) as they corrode engine parts and aren't transesterified. Thus, the normal degumming was done, but, following that, as the phospholipids precipitate, they were separated by centrifugation, then reduced with LiAlH₄. They were reduced to their respective alcohols, and monoglycerides. After treatment with Methanoic acid, having H₂SO₄ as catalyst, they were converted to methyl esters(BioDiesel).



Thus, Phospholipids were successfully converted into BioDiesel, increasing yield by about 20-50%.



The extract, devoid of phospholipids was treated with methanol (20:1 methanol-FFA molar ratio) and sulphuric acid (5% weight relative to FFA), if it contained 15-35% FFA. If oil had contained ~5% FFA, the extract would have been treated with methanol (40:1 methanol-FFA molar ratio) and sulphuric acid (10% weight relative to FFA). The mixture was agitated and heated to 60C.

This ensured good yield, in less time, as there was little or no soap formed during the transesterification process (due to lack of FFA), therefore the layers settled faster. Also, the methanol and the sulphuric acid present need not be removed, as the methanol was used up in transesterification, and sulphuric acid was neutralised by the basic catalyst.

The Free Fatty Acids instantly underwent esterification, with H_2SO_4 acting as the catalyst. Now the same methanol was used as reactant in the transesterification. Thus, a base like NaOH was added to the mixture to neutralise the acid as well as act as the

catalyst for transesterification. This provides the best yield, as the FFA's aren't wasted, as well as the TG's are also completely utilised in transesterification. Also the Phospholipids are converted into their derivative methyl esters, ensuring max. yield.

Overall, this results in a massive increase in biodiesel yield over the current methods used to produce biodiesel.

Purification : Any remaining impurities were removed by adding water to the transesterified oil, followed by distillation to boil off the oil, which was condensed to produce the final BioDiesel.



Conclusions : By esterifying FFA's and Phospholipids, I have been able to increase yield of phospholipids from same amount of feedstock. Thus, making BioDiesel more economical and affordable by optimising the production process. I hope this can make it competitive with ordinary Diesel, encouraging people to make the shift to BioDiesel, thus decreasing CO₂ emissions, leading to a cleaner environment.