

BIOTECHNOLOGICAL APPROACH TO INCREASE THE YIELD OF BIO-DIESEL AS A SOURCE OF RENEWABLE ENERGY

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ABSTRACT

Fossil fuels are the chief contributors to urban air pollution and a major source of greenhouse gases. Biodiesel is gaining more and more importance as an attractive source of renewable energy due to the depleting fossil fuel resources. This paper discusses scarcity of liquid petroleum and emphasizes the importance of bio-diesel. It briefly narrates the history of biodiesel and how it can be obtained from plants. Chemically biodiesel is monoalkyl esters of long chain fatty acids derived from renewable feedstock like vegetable oils and animal fats through a simple transesterification process. This paper examines the transesterification process that involves mixing of methanol (50% excess) with NaOH (100% excess) at room temperature, then mixing vigorously with vegetable oil and letting the glycerol settle (about 15% of the biodiesel mix). The supernatant is biodiesel and contains a mixture of methylated fatty acids and methanol, the catalyst remaining dissolved in the glycerol fraction. The large vegetable oil molecule is reduced to about 1/3 of its original size, lowering the viscosity making it similar to diesel fuel. The resulting fuel operates similar to diesel fuel in an engine. The reaction produces three molecules of an ester fuel from one molecule of vegetable oil. In this paper approach taken is to increase the production of Palmitic acid (C16) and Oleic acid (C18) content in the oil-seeds employing biotechnology approaches. Four different *E. Coli* cells were grown in laboratory medium at defined conditions. These bacterial strains had different expression vectors cloned in them that express different and in some case, novel fatty acids. Production and transesterification were performed simultaneously for the bacterial strains and estimation / identification of the produced fatty acids were tried employing Gas Chromatography with Flame Ionization and Mass Spectrometer as detectors.

Keywords: Greenhouse Gas, Hydrocarbon Fuels, Methanol, Vegetable Oil, Fatty Acid.

1. INTRODUCTION

Burning of fossil fuels such as oil has proven to be very harmful to our environment. They are the chief contributors to urban air pollution and a major source of greenhouse gases (GHGs) - considered to be the main causes behind the climate change phenomena [1]. Dr. Rudolph Diesel developed a unique engine in 1895. This engine was designed to operate on peanut oil or other vegetable-based fuels. After his mysterious death in 1913, Diesel's engine was adapted to use a by-product of the gasoline refining process. The petroleum industry called it diesel fuel. The use of vegetable oils as engine fuels may seem insignificant today but such oils may become, in the course of time, as important as petroleum and the coal tar products of the present time [2]. Biofuels are liquid fuels made from esters, alcohols, ethers, and

other biomass chemicals. They can be produced in any climate using already developed agricultural practices. Biodiesel is a vegetable oil processed to resemble diesel. It is an ester made from fats or oils fuel [3]. Common biofuels include: ethanol and biodiesel. Ethanol is made from starches or sugars, typically grain or corn. Biofuels are renewable; hence, they can supplement hydrocarbon fuels, assist in their conservation, as well as mitigate their adverse effects on the climate [4].

India imports nearly 70% of its annual crude petroleum requirement. The net oil import bill (import minus exports) was Rs 77,058 crores (Rs 770.58 billion) in 2003-04 as against Rs 74,174 crore (Rs 741.74 billion) the previous year. This expenditure on crude purchase impacts the country's foreign exchange reserves in a big way. The petroleum industry now looks very committed

to the use of ethanol as fuel. It is estimated that 75% of the increase in world demand for oil will come from transport. India's transport sector will consume ever-higher amounts of fuel over the coming years. The combined annual global market for the products derived from bioresources is roughly between US\$ 500 billion and US\$ 800 billion. India is one of the 12 global mega biodiversity centers harbouring approximately 8% of the global biodiversity existing in only 2.4% of the land area[5]. The country is also home to two of the world's 25 hotspots. India has a huge treasure of plant resources with over 45,000 known species representing 11% of earth's flora. In terms of flowering plant diversity alone, India ranks tenth in the world. About 33% of flowering plants and 29% of total plants are endemic to the country. Therefore, the time has come to explore alternatives and tap traditional wisdom. Considering the seriousness of the cost of petroleum products and the pollution caused by the use of these products, many developed and developing countries have ventured into the use of vegetable oils as a better alternative to diesel. Chemically biodiesel is monoalkyl esters of long chain fatty acids derived from renewable feedstock like vegetable oils and animal fats through a simple transesterification process. Suitable initiatives have also been made in India by government agencies, research institutions, and automobile industries. The objective of this investigation is to search out an effective biotechnological to increase the yield of bio-diesel as source of renewable energy

2. IMPORTANCE OF BIODIESEL

Seed oils show promise as fuels, particularly for use in diesel engines [6]. Biomass could be used to replace petroleum and natural gas [7]. A major option for converting photo synthetically produced biochemical energy to form suitable for internal combustion engines in the production of either methanol or ethanol. Either one of these chemicals can be used by itself as a fuel in a suitably designed combustion engine. More commonly, it has been proposed to blend these alcohols in proportion up to 20% with gasoline to give gasohol, a fuel which can be used in existing internal combustion engine with little or no adjustment [8;9] Methanol can also be made from biomass. This is normally accomplished by converting biomass, such as wood, to CO and H₂, and synthesizing methanol from these gases [10] Biodiesel is gaining more and more importance as an attractive fuel due to the depleting fossil fuel resources. Chemically biodiesel is mono-alkyl esters of long chain fatty acids derived from renewable feedstock like vegetable oils and animal fats through a simple trans-esterification process [11]. On the other hand the crops being grown for production of biodiesel to be used as a fuel actually suck out the same amount of carbon dioxide that they will release when in fuel form. As such these renewable fuels do not contribute significantly to global warming. Two major biofuels for the transport sector, bioethanol and biodiesel, are fast becoming popular in many countries

around the world. While bio-ethanol (called ethanol) is produced from raw materials such as molasses, beet, sugarcane juice, grains and tubers, biodiesel is produced from oil (derived from oil-bearing seeds such as *Jatropha curcas*, *Pongamia pinnata* i.e.karanja). A network program has been supported for developing an effective method for various lignocelluloses materials including forest plant residues and crop products for ethanol production. Lab studies on transesterification of *Jatropha*, *Pongamia*, *Madhuca*, *Salvadora* and mixed oils using homogenous alkaline catalyst have been completed at Indian Institute of Petroleum, Dehradun. The biodiesel extract is being used with conventional diesel for test run in normal diesel engine. Further scale up and process engineering for homogenous catalyst process are underway. Glycerol as byproduct obtained in the process is being purified separately at bench/ pilot scale.

Since it is made domestically, it reduces our dependence on the import of foreign oil. Biodiesel is 100% renewable and it is nearly carbon-neutral, meaning *it contributes a most zero emissions to global warming*. Biodiesel also dramatically reduces other emissions fairly dramatically. Studies have shown it reduces engine wear by as much as one half, primarily because it provides excellent lubricity. Biodiesel is much cleaner than fossil-fuel diesel. It can be used in any diesel engine with no need for modifications --in fact diesel engines run better and last longer with biodiesel and it can easily be made from a common waste product --used cooking oil.. Biodiesel substantially reduces unburned hydrocarbons; carbon monoxide and particulate matter in exhaust fumes. It is environmentally friendly: it is renewable, "more biodegradable than sugar and less toxic than table salt"(US National Biodiesel Board).

Jatropha curcas, hitherto considered a wild oilseed plant of the tropics, is now being regarded as a promising biofuel crop ideally suitable for growing in the wastelands of India. This crop is now in great demand even in the international scenario. This study covers the use of *Jatropha* seeds for biofuel production with special emphasis to increase the yield of *Jatropha* seed derived biofuel by applying biotechnology principles. This potential biofuel crop can bring about major economic activity such as providing rural electrification, income, and employment opportunities to the rural communities...The approach taken for the present research project is to increase the production of Palmitic acid (C16) and Oleic acid (C18) content in the oil-seeds employing biotechnology approaches. In the present work, four different *E. Coli* cells were grown in laboratory medium at defined conditions. These bacterial strains had different expression vectors cloned in them, which express different and in some case, novel fatty acids. Production and trans-esterification were performed simultaneously for the bacterial strains and estimation / identification of the produced fatty acids were tried

employing Gas Chromatograph with Flame Ionization and Mass Spectrometer as detectors.

One of the major achievements of biodiesel research in India is the first successful trial run of a passenger train conducted on 31 December 2002, when the Delhi-Amritsar Shatabdi Express used 5% of biodiesel as fuel. Biodiesel will enable the Indian Railways to save on its rising fuel bill while controlling pollution levels. According to the Railways, sulphur and lead emissions were reduced significantly when biodiesel was used. Ultimately, the percentage of biodiesel would go up to 15% in unison with the accepted global norms. Indian Oil Company now testing the new green fuel extracted from the seeds of the jatropha plant in the laboratory. The plant can easily be grown on either side of railway tracks as it grows well in both arid and semi-arid conditions, requiring low fertility and moisture. The other advantages are the fuel's contribution to the national energy pool and the potential for creation of jobs in rural sector.

Advantages of biodiesel

- The higher cetane number of biodiesel compared to petro-diesel indicates the potential for higher engine performance.
- The superior lubricating properties of biodiesel increases the engine efficiency.
- Their higher flash point makes them safer to store.
- The biodiesel molecules are simple hydrocarbon chains, containing no sulphur.
- They contain higher amount of oxygen (up to 10%), which ensures complete combustion of hydrocarbons.
- Biodiesel almost completely eliminates life cycle carbon-dioxide emissions. When compared with petro-diesel, biodiesel reduces emission of particulate matter by 40%, unburned hydrocarbons by 68%, carbon monoxide by 44%, sulphates by 100%, PAHs (polycyclic aromatic hydrocarbons) by 80%, and carcinogenic nitrated PAHs by 90%, on average. The use of biodiesel complements the working of the catalysator and can help a current Euro-I motor attain the Euro-III standards.
- Use of biodiesel will lead to increased energy independence as well as increased economic activity from fuel production and utilization.

Petro-diesel releases 13 pounds of fossil CO₂ released per gallon burned, but biodiesel releases no fossil CO₂. There are over 350 different plant types that can supply the oil. Here are a few:

| Plant types | Lb of oil per acre | Kg of oil per hectare |
|-------------|--------------------|-----------------------|
| Palm oil | 4585 | 5000 |
| Coconut oil | 2070 | 2260 |
| Jatropha | 1460 | 1590 |
| Rapeseed | 915 | 1000 |
| Peanut | 815 | 890 |
| Sunflower | 720 | 800 |
| Safflower | 605 | 655 |
| Soybean | 345 | 375 |
| Hemp | 280 | 305 |
| Corn | 135 | 145 |

Jatropha curcas best suited – Indian climate. It grows in arid and semiarid and wastelands up to the height of 15 to 20 ft. The yields are yields with more water and rainfalls and having life span of 30 to 40 years. It is already growing presently in a big way in many states and its initial investments is between Rs. 12000 – 14000/acre, yields is between Rs. 12000 – 15000/annum/acre, yield of seeds : 800 to 1000 kg/acre, about 2 million hectare land required for 5% blend and total land identified as surplus – 66 million hectares

The additional benefits of jatropha plantations are:

- Fixation of up to 10 tonnes/hectare/year of CO₂ will benefit international carbon trade.
- Production of 1 tonne/ hectare/year of high protein seed cake (60% crude protein) can be potentially used for animal and fish feeds, and organic matter could be used as organic fertilizer particularly in remote areas.
- Utilization of various other products from the plant (leaf, bark and seed extracts) for other industrial and pharmaceutical uses.
- Localized production and availability of quality fuel.
- Restoration of degraded land over a period of time.
- Generation of rural employment.



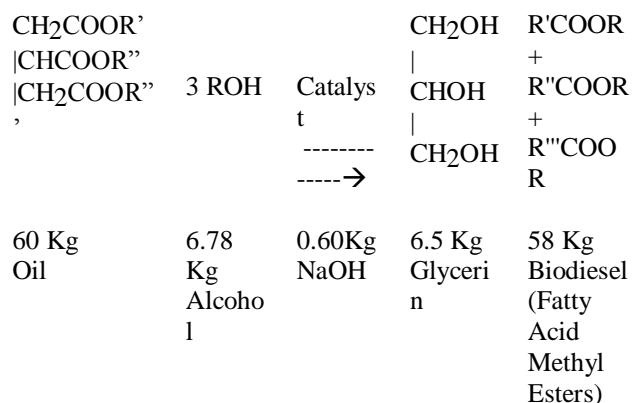
Fig 3. Jatropha or physic nut [12]

| | |
|------------------------|---|
| Botanical name: | <i>Jatropha curcas</i> (Family: uphorbiaceae) |
| English names: | Physic nut, purge nut, pig nut, fig nut, jatropha |
| Name in Malawi: | Nsadsi |
| Names in South Africa: | Mathlapametse (Tswana), Inhlakuva (Zulu), Mafuredonga (Venda), Mbono (Swahili), Purgeerboontjie (Afrikaans) |
| Name in Zambia: | Mutondomoono (Bemba) |
| Name in Zimbabwe: | Shona (Jirimono) |

Typical composition of Jatropha seeds :

| | Possible values | Typical results |
|------------------|-----------------|-----------------|
| Oil content | 16-25% | 18-23% |
| Protein content | 36-50% | 38-45% |
| Fiber content | 3.5-6% | 4.0-5.0% |
| Moisture content | 5-35% | 8-15% |

The process of production of biodiesel is simple to carry out. It involves mixing the oil with methanol and caustic soda and leaving it to stand. Glycerin settles at the bottom of the tank, leaving the methyl ester, or biodiesel, at the top. The glycerin can be used to make a high quality soap, or it can be refined and sold at a range of prices, depending on its purity, to be used in an immense range of products, including cosmetics, toothpaste, embalming fluids, pipe joint cement, cough medicine, and tobacco (as a moistening agent).



The approach taken for the present research project is to increase the production of Palmitic acid (C₁₆) and Oleic acid (C₁₈) content in the oil-seeds.

3.BACTERIAL GENETICS

Biochemistry allows the precise analysis of biological phenomena, but it is typically limited to analyses *in vitro*. Genetic analyses are less precise and direct, but they provide an understanding of the system *in vivo*[13]. The generation and characterization of mutants provides insight into the number of genes involved, their relative location, and their transcriptional organization. Biochemical characterization of mutants that have been genetically characterized and defined as affected in a single gene provides:

- An indication of the various roles that gene product plays *in vivo*;
- Correlation of a gene and its protein product confirms the identity of the actual protein performing a given function *in vivo*; (for example, purifying a protein capable of donating electrons to enzyme X *in vitro* does not prove that it is the *in vivo* electron donor. The isolation of a mutant that fails to reduce enzyme X *in vivo* and the subsequent identification of a protein lacking or altered in that strain would provide a strong argument that the missing protein was the *in vivo* donor.);
- Analysis of metabolites accumulated in mutants provides an indication of metabolic pathways and correlates the mutation (and therefore the affected gene) with a biochemical step. Similarly, analysis of which externally supplied pathway intermediates "bypass" the genetic block confirms pathway characterization and gene-function assignments.

The biochemical characterization of a number of altered versions of a gene product provides insight into the relationship of the structure to the function of the

protein. This is particularly true for proteins whose structure has been determined by X-ray crystallography: the sequence position of the mutation within the gene allows the correlation of the resulting biochemical defect with a position on the 3-D protein structure. There are obvious biotechnological values in the generation of mutants with desirable properties like overproduction of the desired gene products or metabolites, production of novel gene products or metabolites, or the easily regulated production of gene products or metabolites. The specific power of bacterial genetics derives from the possibility of analyzing very large numbers of events (because bacteria are very small) and of performing selections in addition to the fact that bacteria perform a vast number of important biological roles in and of themselves. Genetic information in bacteria and many viruses is encoded in DNA, but some viruses use RNA. Replication of the genome is essential for inheritance of genetically determined traits.

3.1 Genetic information in microbes

Bacteria have few structural or developmental features that can be observed easily, but they have a vast array of biochemical capabilities and patterns of susceptibility to antimicrobial agents or bacteriophages [14]. These latter characteristics are often selected as the inherited traits to be analyzed in studies of bacterial genetics. The Figure 1 shows the structure of DNA represented as a helical ladder. The double helix has a diameter of 2 nm. Each full turn of the double helix contains 10 nucleotide pairs and is 3.4 nm in length. During replication of the bacterial genome, each strand in double-helical DNA serves as a template for synthesis of a new complementary strand. Nucleic acids are large polymers consisting of repeating nucleotide units .

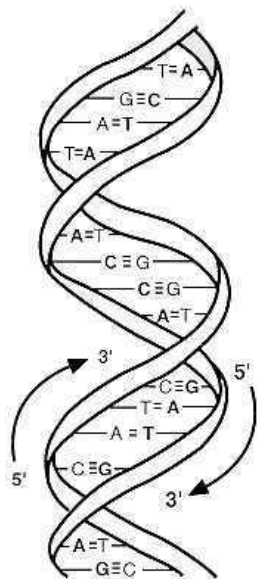


Fig 1. Double helical structure of DNA [15]

Genetic information encoded in DNA is expressed by synthesis of specific RNAs and proteins, and information flows from DNA to RNA to protein as shown in Figure 2.. The DNA-directed synthesis of RNA is called transcription. Because the strands of double-helical DNA are antiparallel and complementary, only one of the two DNA strands can serve as template for synthesis of a specific mRNA molecule. Messenger RNAs (mRNAs) transmit information from DNA, and each mRNA in bacteria functions as the template for synthesis of one or more specific proteins. The process by which the nucleotide sequence of an mRNA molecule determines the primary amino acid sequence of a protein is called translation. Ribosomes, complexes of ribosomal RNAs (rRNAs) and several ribosomal proteins, translate each mRNA into the corresponding polypeptide sequence with the aid of transfer RNAs (tRNAs), amino-acyl tRNA synthetases, initiation factors and elongation factors. All of these components of the apparatus for protein synthesis function in the production of many different proteins. A gene is a DNA sequence that encodes a protein, rRNA, or tRNA molecule (gene product). The genetic code determines how the nucleotides in mRNA specify the amino acids in a polypeptide. Because there are only 4 different nucleotides in mRNA (containing U, A, C and G), single nucleotides do not contain enough information to specify uniquely all 20 of the amino acids. In dinucleotides 16 (4×4) arrangements of the four nucleotides are possible, and in trinucleotides 64 ($4 \times 4 \times 4$) arrangements are possible. Thus, a minimum of three nucleotides is required to provide at least one unique sequence corresponding to each of the 20 amino acids. The "universal" genetic code employed by most organisms is a triplet code in which 61 of the 64 possible trinucleotides (codons) encode specific amino acids, and any of the three remaining codons (UAG, UAA or UGA) results in termination of translation. The chain-terminating codons are also called nonsense codons because they do not specify any amino acids [12](Sambrook et al 1989). The genetic code is described as degenerate, because several codons may be used for a single amino acid, and as nonoverlapping, because adjacent codons do not share any common nucleotides as shown in Table 2.2. Exceptions to the "universal" code include the use of UGA as a tryptophan codon in some species of Mycoplasma and in mitochondrial DNA, and a few additional codon differences in mitochondrial DNAs from yeasts, Drosophila, and mammals. Translation of mRNA is usually initiated at an AUG codon for methionine, and adjacent codons are translated sequentially as the mRNA is read in the 5' to 3' direction.

CENTRAL DOGMA

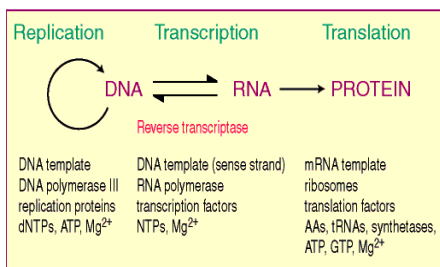


Fig 2. DNA replication sequence [16]

4. MATERIALS AND METHODS

The plant oils usually contain free fatty acids, phospholipids, sterols, water, odorants and other impurities [17]. Because of these, the oil cannot be used as fuel directly. To overcome these problems the oil requires slight chemical modification mainly transesterification, pyrolysis and emulsification. Among these, the transesterification is the key and foremost important step to produce the cleaner and environmentally safe fuel from vegetable oils. Biodiesel is the monoalkyl esters of long chain fatty acids derived from renewable feed stocks, such as vegetable oil or animal fats, for use in compression ignition engine. Biodiesel, which is considered as a possible substitute of conventional diesel fuel is commonly, composed of fatty acid methyl esters that can be prepared from triglycerides in vegetable oils by transesterification with methanol [18]. Transesterification or alcoholysis is the displacement of alcohol from an ester by another in a process similar to hydrolysis, except than alcohol is used instead of water [5]. This process has been widely used to reduce the high viscosity of triglycerides.

To obtain an idea about the chromatographs of various fatty acids extensive laboratory work is done by using standard samples.

1. Chromatograph of n-Hexane, which is to be used as a solvent for the transesterified fatty acids, is obtained.
2. Chromatograph is obtained for Palmitic acid-methyl ester in n-hexane by using standard samples.
3. Chromatograph of fatty acids present in the mustard oil is obtained by following the standard procedure.
4. Chromatographs are obtained for the fatty acids present in the mustard seeds and seism seeds by following the same standard procedure as done in the previous step.

Reagents:

Reagent 1, Saponification: 45g sodium hydroxide is dissolved in 150ml of distilled water after

weighing carefully. Then 150ml methanol is added to this solution and mixed it thoroughly. **Reagent 2,**

Methylation:

325ml of certified 6.0N hydrochloric acid and 275ml methyl alcohol are mixed thoroughly. This drops the pH of the solution below 1.5 and causes Methylation (for the increased volatility in a partially polar column) of the fatty acids. The fatty acid methyl ester is poorly soluble in the aqueous phase at this point. **Reagent 3, Extraction:** 200ml of hexane and 200ml of methyl tertiary-butyl ether are mixed thoroughly. This will extract the fatty acid methyl esters into the organic phase for use with the Gas chromatography. **Reagent 4, Sample cleanup:**

10.8g of sodium hydroxide is dissolved in 900ml of distilled water. This reduces the contamination of the injection port liner, the column and the detector.

4.1 Preparation of Ampicillin solution

This is Ampicillin sodium salt of molecular weight =371.4.

$C_{16}H_{18}N_3O_4SNa$

1.0g of Ampicillin (98%) is added to 50ml of sterilized distilled water. This solution contains 20mg per ml.

This solution is preserved in refrigerator at 4°C.

4.2 Preparation of LB media

10g ----- Bactotrypton
5g ----- Bactoyeast extract
10g ----- Sodium chloride
1000ml ----- Distilled Water.

The constituents are dissolved in distilled water carefully to prevent the formation of froth in the media. Then the media pH is adjusted to 7.5 by adding the NaOH solution. Then the media is sterilized by autoclaving at 121°C and 15lb pressure for 20min. After autoclaving the media is preserved in refrigerator.

4.3 Preparation of IPTG solution

76mg of IPTG is added to 3.2ml of distilled water to get 100mM of IPTG solution.

IPTG---Isopropyl 4-thio-β-D- galactopyranoside

Molecular formula: $C_9H_{18}O_5S$, Molecular weight : 238.3

Chromatography is a separation process that is achieved by distributing the components of a mixture between two phases, a stationary phase and a mobile phase. Those components held preferentially in the stationary phase are retained longer in the system than those that are distributed selectively in the mobile phase. As a consequence, solutes are eluted from the system as local concentrations in the mobile phase in the order of their increasing distribution coefficients with respect to the stationary phase; ipso facto a separation is achieved.

- 45g sodium hydroxide is dissolved in 150ml of distilled water after weighing carefully. Then 150ml methanol is added to this solution and mixed it thoroughly.
- 325ml of certified 6.0N hydrochloric acid and 275ml methyl alcohol are mixed thoroughly. This drops the pH of the solution below 1.5 and causes Methylation (for the increased volatility in a partially polar column) of the fatty acids. The fatty acid methyl ester is poorly soluble in the aqueous phase at this point.
- 200ml of hexane and 200ml of methyl tert-butyl ether are mixed thoroughly. This will extract the fatty acid methyl esters into the organic phase for use with the Gas chromatography.
- 10.8g of sodium hydroxide is dissolved in 900ml of distilled water. This reduces the contamination of the injection port liner, the column and the detector.

5. RESULTS AND DISCUSSION

As per the flowsheet of the experiment to grow the cloned bacteria, we cultured the bacteria three times in the whole project period. After growing the bacteria we have to extract the fatty acids from the culture. There are innumerable procedures to extract the fatty acids from culture. We analyzed the samples obtained from the experiment in GC/ FID with WAX column. The characteristics of the column are as follows:

To obtain an idea about the chromatographs of various fatty acids extensive laboratory work is done by using standard samples.

- Chromatogram of n-Hexane standard is obtained, so that we can apply solvent delay for the good resolution of the components.
- Chromatogram of (n-Hexane + Methyl Palmitate standard) mixture is obtained.
- Chromatograms of Na-salt Musturd Oil and K-salt Musturd Oil before washing and after washing are obtained.
- The bacterial vectors are grown under specified conditions for the analysis.
- Preparation of media and preserving and growing bacterial vectors are learned by me.
- From the grown bacterial vectors the fatty acid methyl esters are extracted by following the standard procedure.
- The all four samples are analysed in GC/FID as well as GC/MS for the determination of the presence of the palmitic acid, oleic acid, linoleic acid and linolenic acid.
- Chromatogram of standard fatty acid mixture is obtained.
- After comparing the chromatograms obtained from the bacterial vector samples with standard

mixture chromatogram it was concluded that the desired fatty acids are developed within the each bacterial vectors.

- It is to be determined the quantity of the desired fatty acids present in the bacterial vectors.
- The way of approach for a research problem and the maintenance of the laboratory was learned by me.

The presence of C(16) and C(18) Compounds in the all four samples was observed during the study. The results from GC/FID were cross checked by the results obtained by GC/MS. It is required to determine the quantity of the desired fatty acids in the bacterial vector samples. For further study one can adopt still much efficient methods to extract the fatty acid methyl esters from the samples.

6. CONCLUSION

The Indian Biotechnology sector is gaining global visibility and is being tracked for emerging investment opportunities (Anon 2004). Human capital is perceived to be the key driver for global competitiveness. Added to this there is a decreasing appetite for risk capital in developed countries, which has led to a decline in the biotechnology sector in these regions where survival lifelines are being provided by the lower cost research environs of the developing world such as India. For a country like India, biotechnology is a powerful enabling technology that can revolutionize agriculture, healthcare, industrial processing and environmental sustainability. The varied cultural diversity across the country as well as a very ancient traditional knowledge system associated with the biodiversity represents added assets. Nonetheless, much of this biodiversity is in peril owing, in the main, to anthropogenic causes. Thus, if the goal of converting our bioresources - animal, plant, microbial and marine - into commercially useful products and processes is to be realized, we need to not only conserve the biodiversity and but also utilize it in a sustainable manner. Genetic erosion is rampant and conservation is a priority. Prospecting of wild plant resources using molecular approaches and mechanism-based screening should be used to identify novel genes (temperature, drought, salinity tolerant) and gene products (therapeutic compounds, dyes, essential oils, biocontrol agents, gums resins and taxmins).

There are potential ornamentals, including foliage - and flower - bearing plants that could be bulked up to be subsequently cultivated on large scale for domestic and international trade. Bioconversion - both cellular and microbial - can be employed to convert intermediates of secondary metabolism into valued added products. Application of genomics, proteomics and metabolomics in carefully selected plants will be very useful. Biotechnology can contribute substantially in providing cost-effective therapeutically active biomolecules through target/mechanism - based screens,

biotransformation, metabolic engineering and transgenic approaches

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