

WET MASS PRODUCTION OF ALGAL BIO-DIESEL

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ABSTRACT

In context of climatic changes and soaring prices per barrel of petroleum, renewable carbon neutral, transport fuels are needed to displace petroleum derived transport fuel, which contribute to global warming and are of limited availability. Biodiesel derived from oil crop is a potential renewable and carbon neutral alternative to petroleum fuel. Unfortunately, biodiesel from oil crop, waste cooking oil and animal fat cannot realistically satisfy even a small fraction of the existing demand for transport fuel. As demonstrated here, biodiesel from microalgae seem to be the most promising renewable biofuel that has the potential to completely displace petroleum-derived transport fuel without adversely affecting supply of food and other crops products. Like plants, microalgae use sunlight to produce oil but they do so more efficiently than crop plants. Oil productivity of many microalgae greatly exceeds the oil productivity of the best producing oil crops. The present review covers the approach for making algal biodiesel more economically and competitive with petro-diesel.

KEYWORDS: Wet Mass Production of Algal Bio-Diesel

INTRODUCTION

Algae have recently received a lot of attention as a new biomass source for the production of renewable energy. Some of the main characteristics which set algae apart from other biomass sources are that algae (can) have a high biomass yield per unit of light and area, can have a high oil or starch content, do not require agricultural land, fresh water is not essential and nutrients can be supplied by waste-water and CO2 by combustion gas. The first distinction that needs to be made is between macroalgae (or seaweed) versus microalgae. Microalgae have many different species with widely varying compositions and live as single cells or colonies without any specialization. Although this makes their cultivation easier and more controllable, their small size makes subsequent harvesting more complicated. Macroalgae are less versatile, there are far fewer options of species to cultivate and there is only one main viable technology for producing renewable energy: anaerobic digestion to produce biogas. Both groups will be considered in this report, but as there is more research, practical experience, culture and there are more fuel options from microalgae, these will have a bigger share in the report. First, existing biofuel sustainability standards are analysed for applicability, followed by a thorough analysis of the opportunities and risks of ABB sustainability. Secondly, sustainability is discussed in the context of potential and threats for developing countries. Microalgae comprise a vast group of photosynthetic, auto/heterotrophic organism which has an extraordinary potential for cultivation as energy crops. These microscopic algae use photosynthetic process similar to that of higher-developed plants. They are veritable miniature biochemical factories, capable of regulating carbon dioxide(CO), just like terrestrial plants .In addition, these micro-organisms are useful in bioremediation applications and as nitrogen fixing biofertilizers. This review article discusses the potential of microalgae for sustainably providing biodiesel for the displacement of petroleum derived transport fuels in India. The need of energy is increasing continuously, because of increase in industrialization as well as human population. The basic sources of this energy are petroleum, natural gas, coal,

hydro and nuclear .The major disadvantage of using petroleum based fuel is atmospheric pollution. Petroleum diesel combustion is a major source of greenhouse gases (GHG). Apart from these emissions, petroleum diesel combustion is also major source of other air contaminants including NOx, SOx, CO, particulate matter and volatile organic compounds which are adversely affecting the environment and causing air pollution. These environmental problems can be eliminated by replacing the petroleum diesel fuel with an efficient renewable and sustainable biofuel. Algal biomass is one of the emerging sources of sustainable energy. The large-scale introduction of biomass could contribute to sustainable development on several fronts, environmentally, socially and economically .The biodiesel generated from biomass is a mixture of mono-alkyl ester, which currently obtained from transesterification of triglycerides and monohydric alcohols produced from various plant and animal oils. But this trend is changing as several companies are attempting to generate large scale algal biomass for commercial production of algal biodiesel.

Biodiesel is non-toxic and biodegradable alternative fuel that is obtained from non-renewable sources. In many countries, biodiesel is produced mainly from soybeans. Other sources of commercial biodiesel include canola oil, animal fat, palm oil, corn oil, waste cooking oil. But the recent research has proved that oil production from microalgae is clearly superior to that of terrestrial plants such as palm, rapeseed, soybeans or jatropha. Important advantage of microalgae is that, unlike other oil crops, they can double their biomass within 24 hr. the biomass doubling time for microalgae during exponential growth can be as short as 3 to 4 hr, which is significantly quicker than the doubling time for oil crops .It is for this reason microalgae are capable of synthesizing more oil per acre than the terrestrial plants which are currently used for the fabrication of biofuels. In the production of energy from micro algal biomass, two basic approaches are employed depending on the particular organism and the hydro-carbon which they produce. The first is simply the biological conversion of nutrients into lipids or hydrocarbons. The second procedure entails the thermo-chemical liquefaction of algal biomass into lipid or hydrocarbons. Lipids and hydrocarbons can normally be found throughout the micro algal biomass. They occur as membrane components, storage products, metabolites and sources of energy for microalgae. Algal strains, diatoms, and cyanobacteria (categorized collectively as microalgae) have been found to contain proportionally high level of lipid (over 30%). These microalgal strains with high lipid content are of great interest in search for sustainable feedstock for production of biodiesel. The global economy literally runs on energy. An economic growth combined with a rising population has led to a steady increase in the global energy demands. If the governments around the world stick to current policies, the world will need almost 60% more energy in 2030 than today, of this 45% will be accounted by China and India together. Transportation is one of the fastest growing sectors using 27% of the primary energy. The continued use of fossil fuels is not sustainable, as they are finite resources, and their combustion will lead to increased energy-related emissions of green house gases (GHG) viz., carbon dioxide (CO2), sulfur dioxide (SO2) and nitrogen oxides (NOx). The future reductions in the ecological footprint of energy generation will reside in a multi-faceted approach that includes nuclear, solar, hydrogen, wind, and fossil fuels (from which carbon is sequestered), and biofuels .Biofuel can be broadly defined as solid, liquid, or gas fuel consisting of, or derived from biomass. Rudolph Diesel first demonstrated the use of biodiesel from a variety of crops in 1900. However, the widespread availability of inexpensive petroleum during the 20th century determined otherwise. Generally, shifting society's dependence away from petroleum to renewable biomass contributes to the development of sustainable industrial society and effective management of GHG. A major criticism often leveled against biomass, particularly against large-scale fuel production, is that it will consume vast swaths of farmland and native habitats, drive up food prices, and result in little reduction in GHG emissions. However, this so-called "food versus fuel" controversy appears to have been exaggerated in many cases. Credible studies show that with plausible technology

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developments, biofuels could supply some 30% of global demand in an environmentally responsible manner without affecting food production. At the moment, only biodiesel and bioethanol are produced on an industrial scale. They are the petroleum replacement for internal combustion engines, and are derived from food crops such as sugarcane, sugar beet, maize (corn), sorghum and wheat, although other forms of biomass can be used, and may be preferable. The most significant concern is the inefficiency and sustainability of these first generation biofuels. In contrast, the second generation biofuels are derived from non-food feedstock. They are extracted from microalgae and other microbial sources, ligno-cellulosic biomass, rice straw and bio-ethers, and are a better option for addressing the food and energy security and environmental concerns. Microalgae, use a photosynthetic process similar to higher plants and can complete an entire growing cycle every few days. In fact, the biomass doubling time for microalgae during exponential growth can be as short as 3.5h. Some microalgae grow heterotrophically on organic carbon source. However, heterotrophic production is not efficient as using photosynthetic microalgae, because the renewable organic carbon source required is ultimately produced by photosynthetic crop plants. Microalgae are veritable miniature biochemical factories, and appear more photosynthetically efficient than terrestrial plants and are efficient CO2 fixers. The ability of algae to fix CO2 has been proposed as a method of removing CO2 from flue gases from power plants, and thus can be used to reduce emission of GHG. Many algae are exceedingly rich in oil, which can be converted to biodiesel. The oil content of some microalgae exceeds 80% of dry weight of algae biomass. The net annual harvest of algal biomass cultivated in subtropical areas can be as high as 40 tons ha-1(dry matter), even higher if CO2 is supplied. It is possible to produce about 100 g m-2 d-1 of algal dry matter in simple cultivation systems .In theory, high oil content algae could produce almost

100 times of soybean per unit area of land [19]. The calculations made by Chisti clearly demonstrate the strong scenario for microalgal biofuels. The use of algae as energy crops has potential, due to their easy adaptability to growth conditions, the possibility of growing either in fresh- or marine waters and avoiding the use of land. Furthermore, two thirds of earth's surface is covered with water, thus algae would truly be renewable option of great potential for global energy needs. This paper aims to analyze and promote integration approaches for sustainable micro-algal biodiesel production, with engine compatibility based on ASTM standards.

TECHNOLOGY

Strains used- Nanochloropsis Occulatta

Chlorella pyrenoidosa

Scenedesmus

Production of Microalgal Biomass

The production of microalgal biodiesel requires large quantities of algal biomass. Most of algal species are obligate phototrophs and thus require light for their growth. Several cultivation technologies that are used for production microalgal biomass have been developed by researchers and commercial producers. The phototropic microalgae are most commonly grown in open ponds and photobioreactors. The open pond cultures are economically more favorable, but raise the issues of land use cost, water availability, and appropriate climatic conditions. Further, there is the problem of contamination by fungi, bacteria and protozoa and competition by other microalgae. Photobioreactors offer a closed culture environment, which is protected from direct fallout, relatively safe from invading microorganisms, where temperatures are controlled with an enhanced CO2 fixation that is bubbled through culture medium. This technology is relatively expensive

compared to the open ponds because of the infrastructure costs. An ideal biomass production system should use the freely available sunlight. It is reported the best annual averaged productivity of open ponds was about 24 g-1 dry weight m-2 d-1. A productivity of 100 g-1 dry weight m-2 d-1 was achieved in simple 300 l culture systems. This level has been viewed as deriving from the light saturation effect. The light requirement coupled with high extinction coefficient of chlorophyll in algae has necessitated the design and development of novel system for large scale growth. Experiments have also elucidated that algal biomass production can be boosted by the flashing light effect, namely by better matching photon input rate to the limiting steps of photosynthesis. Indeed, the best annual averaged productivity has been achieved in closed bioreactors. Tridici has reviewed mass production in photobioreactors. Many different designs of photobioreactor have been developed, but a tubular photobioreactor seems to be most satisfactory for producing algal biomass on the scale needed for biofuel production. Closed, controlled, indoor algal photobioreactors driven by artificial light are already economical for special high-value products such as pharmaceuticals, which can be combined with production of biodiesel to reduce the cost.

CULTIVATION

Biodiesel is an attractive fuel for diesel engines that it can be made from any vegetable oil (edible or non edible oils), used cooking oils, animal fats as well as microalgae oils. It is a clean energy, renewable, non toxic and sustainable alternative to petroleum based fuels, and it is able to reduce toxic emissions when is burned in a diesel engine. The interest of this alternative energy resource is that fatty ester acids, known as biodiesel, have similar characteristics of petro-diesel oil which allows its use in compression motors without any engine modification.

We cultivated the algae in the shake flask as if it is under the submerged fermentation phase. We used the F2 medium for the cultivation of these algae.

METHODOLOGY

We prepared 100ml of the F2 media and autoclaved it to avoid the contamination.

We inoculated the pre-inoculum of the algae into two different flasks.

Further we kept it on the shaker @161rpm with the proper availability of the sunlight.

The media with the culture was allowed to grow for the period of 15days .

Once the growth is dense we scaled it up to 500ml flask with the same parameters.

The OD 0f 0.56 to 0.65 confirms the growth of algae.

Harvesting & Lipid Extraction

This is the very important step in the biodiesel production because it is the process that help in obtaining the dense lipid content & it has to be done at right time. We extracted the lipids from the biomass using the soxhlet extraction method.

METHODOLOGY

The sample was homogenized and weighed accurately.

Mix the sample with anhydrous sodium sulphate with a 4:1ratio.

Take 150ml of hexane in the erlynmeir flask.

Set up the apparatus.

Start boiling the mixture at the boiling point of hexane and use the boiling chips.

Finally the excess water is evaporated and the lipid is obtained.

Lipid Analysis

Once the lipid was obtained we used the Folch protocol to estimate the lipid content.

A 15-ml glass vial containing algal biomass, 2 ml methanol, and 1 ml chloroform were added and kept for 24 h at 25°C. The mixture was agitated in a vortex for 2 min. One milliliter of chloroform was again added and the mixture was shaken vigorously for 1 min. After, 1.8 ml of distilled water was added and the mixture was mixed in a vortex again for 2 min. The layers were separated by centrifugation for 10 min at 2,000 rpm. The lower layer was filtered through Whatman No. 1 filter paper into a previously weighed clean vial (W1). Evaporation was carried on in a water bath and the residue was further dried at 104°C for 30 min. The weight of the vial was again recorded (W2). Lipid content was calculated by subtracting W1 from W2,and was expressed as % dcw. At the end of the experiment we got the lipid content to be 6.3g/250ml which tallies with the standard i.e, 33g/ltr.

Oil Production

Trans-esterification refers to the reaction of an ester group with an alcohol that has a structure different to that of the original alcohol moiety of the ester, such that a new ester group is formed in which the original alcohol moiety is exchanged with that of the reacting alcohol. In the case of the trans-esterification of triglycerides of fatty acids (vegetable oils) with methanol (classical biodiesel production process), the three ester groups of a triglyceride molecule in which three fatty acid moieties

are attached to a single alcohol moiety (i.e. that of glycerol) react with three molecules of methanol to yield three molecules of esters each containing single fatty acid and methanol moieties and one molecule of glycerol (Scheme 1). Therefore, the general chemical name of biodiesel produced by the trans-esterification of vegetable oils is fatty acid methyl esters (i.e. FAME biodiesel).

Methodology

Sodium /potassium methoxide is prepared freshly by pre-determining the quantity of NaOH/KOH usually 1% of the weight.

The lipid and the alkoxide is mixed in a flask with the continuous stirring at a temperature of 65-75degree celcius.

After the completion of the reaction it is carefully transferred to a separating funnel to stand over-night.

Then the oil is drained and collected in flask for FA.

CH2-OOC-R1			R _I -COO-R		CH2–OH
CH-OOC-R ₂	+ 3ROH	Catalyst	R ₂ -COO-R	+	сн–он
 CH2-OOC-R3			R3-COO-R		I CH₂−OH
Triglyceride	Alcohol		Esters		Glycerol

ENGINE TESTING

Туре	TVL Vertical 4 Stroke Cycle, Single Cylinder, High Speed, CI Engine
Serial Number	234/172
BHP	5.2
RPM	1500
Method of starting	Manual crank start
Compression ratio	17.5:1
Coolant	Water
Stroke	110mm
Bore	875mm
Governor type	Mechanical Centrifugl type
Injection time	19/23/27
Injector	3hole of 0.3mm dia
Injection pressure	230bar/2601
Nozzle opening pressure	200-205bar

In IC engine, the thermal energy is released by burning the fuel in the engine cylinder. The combustion of fuel in IC engine is quite fast but the time needed to get a proper air/fuel mixture depends mainly on the nature of fuel and the method of its introduction into the combustion chamber. The combustion process in the cylinder should take as little time as possible with the release of maximum heat energy during the period of operation. Longer operation results in the formation of deposits which in combination with other combustion products may cause excessive wear and corrosion of cylinder, piston and piston rings. The combustion product should not be toxic when exhausted to the atmosphere. These requirements can be satisfied using a number of liquid and gaseous fuels. The biodiesel from non edible sources like Jatropha, Pongamia, Algae etc meets the above engine performance requirement and therefore can offer perfect viable alternative to diesel oil.

Test Plan

When this project was begun, the original plan was to be operational mid-way through the second academic term so that long term reliability testing could be done.. The Superflow 901 dynamometer has the ability to measure many different performance evaluation criteria on an engine. The performance data can either be read manually from the control console, or with a data acquisition system and computer. Due to time constraints and problems with the data acquisition system, readings were taken by hand using the control console. Measurements taken in this experiment were engine speed, torque output, power output and airflow. These can all be read off of the control console. Also, average fuel consumption was measured by weighing the fuel on a digital readout scale before and after extended tests.

Following Conclusions are Made from the Results and Observations

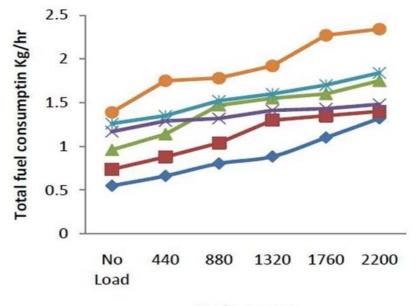
- The bio-diesel is more eco friendly compare to Diesel.
- Bio-diesel is the substitute of Diesel
- Without any modification in Diesel Engine the Bio-diesel can be used as fuel.
- The wear and tear on components is under limit.
- Bio-diesel is more suitable fuel for slow speed Diesel Engine compare to high-speed Diesel Engine.

- Exhaust temperature is higher compare to diesel operation.
- Lubricant oil get early deteriorate compare to diesel due to the glycerin as composition in bio-diesel.
- Specific fuel consumption is Higher (8 to 15%) comparing to diesel without modification in Diesel Engine.
- To have more details study it is suggested to undergo the test as per IS-10000 & 10001 of 1981/11700 of 1985 which consist of 512 hours test (32 cycle-16 hours per day).
- Bio-diesel can be better fuel for future, which is made from agriculture product (Renewable Energy).
- Bio-Oil is also one of the alternative fuel can be use along with Diesel up to 20% without refines.

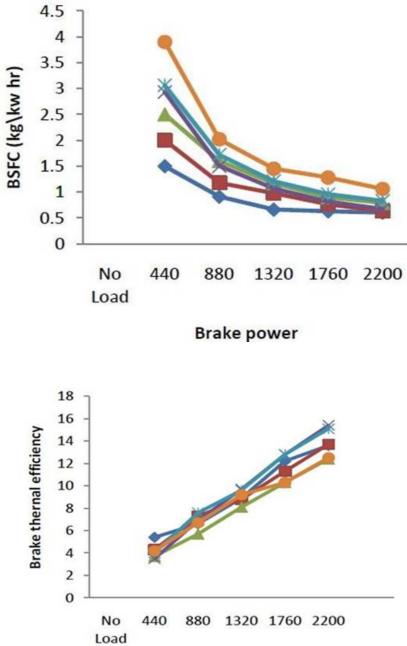
Results and Comparison

Here we compare the bio-diesel characteristics data with the ASTM standards and normal diesel standards.

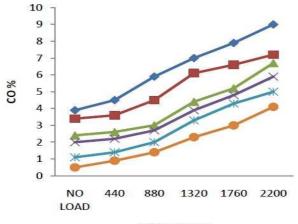
Parameters	ASTM Standards	Diesel	Algal Bio-Diesel
Density	0.86-0.91g/cm3	0.85g/cm3	0.889g/cm3
Viscosity	1.9-6mm2/sec	2.6mm2/sec	4.97mm2/sec
pH value	4.6-6.4	-	5.6
Acid value	2.8-9.16mg.KoH/g	0.4mg.KoH/g	3.97mg.KoH/g
Saponification value	125-244.8mg.KoH/g	-	189.3mg.KoH/g
Flash point	127-221°c	75-110°c	179°c
Fire point	>=150°c	-	201°c
Molecular weight	-	-	933.45g
Iodine value	<=125mg	-	120mg



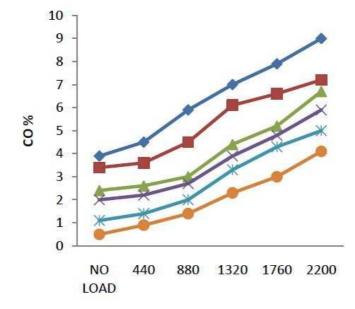
Brake power



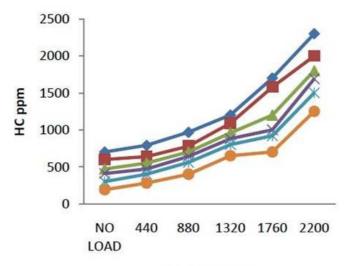
Brake power



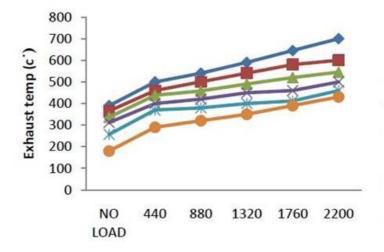
Brake power



Brake power



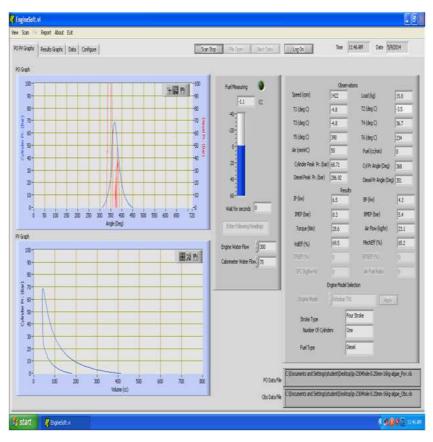
Brake power



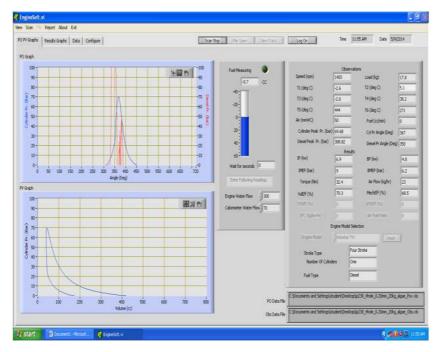
Brake power

Parameters	Diesel	B20	B40
BP	5.15kw	4.97kw	4.9kw
Torque	9425NM	3629.8NM	7259.4NM
Fuel consumption	0.000335kg/s	0.0003426kg/s	0.0004852 Kg/s
BTE	38.5%	32.47%	28.58%
Volumetric Efficiency	88%	78%	80%
IP	8.5kw	7.57kw	7.7kw
FP	3.2kw	2.6kw	2.8ke
Mechanical Efficiency	77.5%	65.7%	63.6%
НС	29.92ppm	21.47ppm	19.8ppm
СО	0.119%	0.102%	0.09%
CO2	4.18%	4.12%	3.79%
NOx	22.5ppm	32.56ppm	39.8ppm
BSFC	0.286	0.328	0.356

ENGINE TESTING RESULTS



Engine Testing Online Software Data Aquisition-80% Load



Engine Testing Online Software Data Aquisition-100% Load

CONCLUSIONS

From the comparison we can say that all the test done and results obtained are in the range defined by the ASTM standards. Hence algal bio-diesel can be considered as a fuel and can be used in automobiles with minor modifications.

Current efforts and business investment are driving attention and marketing efforts on the promises of producing algal biodiesel and superior production systems. A large number of companies are claiming that they are at the forefront of the technology and will be producing algal biodiesel economically within the next few years. However most of these companies have limited technical expertise and few have actually made biodiesel from algae. Producing algal biodiesel requires large-scale cultivation and harvesting systems, with the challenging of reducing the cost per unit area. At a large scale, the algal growth conditions need to be carefully controlled and optimum nurturing environment have to be provided. Such processes are most economical when combined with sequestration of CO2 from flue gas emissions, with wastewater remediation processes, and/or with the extraction of high value compounds for application in other process industries. Current limitations to a more widespread utilization of this feedstock for biodiesel production concern the optimization of the microalgae harvesting, oil extraction processes, and Supply of CO2 for a high efficiency of microalgae production. Also, light, nutrients, temperature, turbulence, CO2 and O2 levels need to be adjusted carefully to provide optimum conditions for oil content and biomass yield. It is therefore clear that a considerable investment in technological development and technical expertise is still needed before algal biodiesel is economically viable and can become a reality. This should be accomplished together with strategic planning and political and economic support.

Further efforts on microalgae production should concentrate in reducing costs in small-scale and large-scale systems. This can be achieved for example by using cheap sources of CO2 for culture enrichment (e.g. from a flue gas), use of nutrient-rich wastewaters, or inexpensive fertilizers, use of cheaper design culture systems

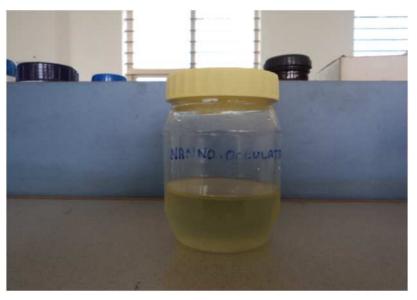
with automated process control and with fewer manual labor, use of greenhouses and heated effluents to increase algal yields. Apart from saving costs of raw-materials (nutrients and fresh water use), these measures will help to reduce GHG emissions, waste amount, and the feed cost by using of nitrogen fertilizers.

Also, will raise the availability of microalgae biomass for different applications (e.g. food, agriculture, medicine, and biofuels, among others) and will contribute to the sustainability and market competitiveness of the microalgae industry.

Photos



Scenedesmus Algal Culture



Nano-Chloropsis Occulatta



Algal Culture Grown at Shake Flask Level



Soxhlet Extraction



ALGAL BIO-DIESEL

Team with Product



REFERENCES

- Halim R, Michael KD, Paul AW (2012) Extraction of Oil from Microalgae for Biodiesel Production: A Review. Biotechnol Adv 30: 709-732.
- Vlysidis A, Michael B, Webb C, Theodoropoulos C (2011) A Techno-Economic Analysis of Biodiesel Biorefineries: Assessment of Integrated Designs for the Co-Production of Fuels and Chemicals. Energy 36: 4671-4683.
- 3. Liu J, Huang J, Fan KW, Jiang Y et al. (2010) Production Potential of Chlorella Zofingienesis as a Feedstock for Biodiesel. Bioresource Technol 101: 8658–8663.
- 4. . Demirbas MF (2011) Biofuels From Algae for Sustainable Development. Appl Energ, 88: 3473–3480.
- 5. Janaun J, Ellis N (2010) Perspectives on Biodiesel as a Sustainable Fuel. Renew Sust Energ Rev 14: 1312–1320.
- 6. Pinzi S, Garcia IL, Lopez-Gimenez FJ, Luque de Castro MD et al. (2009) The Ideal Vegetable Oil-Based Biodiesel Composition: a Review of Social, Economical and Technical Implications. Energy Fuels 23: 25–41.

- B. Baiju, M.K. Naik, L.M. Das, A comparative evaluation of compression ignition engine characteristics using methyl and ethyl esters of Karanja oil, ,Renewable Energy 34 (2009) 1616-1621
- 8. J .Siddarth, "Performance Evaluation Of IC Engine Using Biodiesel from different fuel sources" M. Tech Dessertation June 2008, IIT Roorkee
- 9. G. Dwivedi "Performance Evaluation of DieselEngine Using Biodiesel from Karanja Oil "M.Tech Dessertation June 2011,IITRoorkee
- Gaurav Dwivedi, M.P. Sharma, Siddarth Jain, "Impact of biodiesel and its blends with diesel and methanol on engine performance", International Journal of Energy and Science IJES Vol.1 No.2 2011 PP.105-109
- 11. Gaurav Dwivedi, M.P. Sharma, Siddarth Jain," Pongamia as a source of Biodiesel in India-A review", Smart grid and Renewable Energy, Paper Published (Vol. 2 No. 3) 2011
- Gaurav Dwivedi, M.P. Sharma, Siddarth Jain," Impact Analysis Of Biodiesel On Engine Performance- A Review", Renewable and Sustainable Energy Review, Volume 15, Issue 9, December 2011, Pages 4633-4641
- Sanchez, Miron. A., Ceron, Garcia. M. C., Contreras, Gomez. A., Garcia, Camacho. F., Molina, Grima. E. and Chisti, Y. (2003). Shear stress tolerance and biochemical characterization of in quasi steady-state continuous culture in outdoor photobioreactors., 287-97.
- 14. Matthew, N. Campbell. (2008). Biodiesel: Algae as a Renewable Source for Liquid Fuel. 1916-1107.
- 15. Pulz, O. (2007). Evaluation of GreenFuel's 3D Matrix Algal Growth Engineering Scale Unit: APS Red Hawk Unit AZ, IGV. Institut Fur Getreidevarabeitung GmbH.
- Schneider, D. (2006). Grow your Own:Would theWide Spread Adoption of Biomass-Derived Transportation Fuels Really Help the Environment., 408-409.
- 17. Scott, A. and Bryner, M. (2006). Alternative Fuels: Rolling out Next-Generation Technologies. 20-27
- Sharma, Y.C., Singh, B. and Upadhyay, S.N. (2008). Advancement in development and characterization of biodiesel: A review.2355-2373.
- 19. Zhang, Z., Moo-Young, M. and Chisti, Y. (1996). Plasmid stability in recombinant Saccharomyces cerevisiae., 401-35.
- 20. Shay, E.G. (1993). Diesel fuel from vegetable oils: Status and opportunities., 227-242
- 21. Gavrilescu, M. and Chisti, Y. (2005). Biotechnology-a sustainable alternative for chemical industry., 471-99.
- 22. Lantz, M. (2007). The prospects for an expansion of biogas systems in Sweden-incentives, barriers and potentials., 1830-1843.
- 23. Gokalp, I. and Lebas, E. (2004). Alternative fuels for industrial gas turbines (AFTUR), 1655-1663.
- 24. Alex, H. West., Dusko, Posarac. and Naoko, Ellis. (2008). Assessment of four biodiesel production processes using HYSYS plant., 6587-6601.