Engineering of microalgae for biofuel production

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Abstract

Increasing of the world population along with the economic wealth deepens the energy crises every day. Hence we need to find the new alternative energy sources that will satisfy the energy demand and concomitantly deliver no emission to the environment.

In this particular situation, plants offer us a highly efficient and effective solutions. However use of higher plants for such purposes can cause several problems such as food competition, water shortage, arable land, fertilizer etc. Algae are tiny biological factories that use photosynthesis to transform carbon dioxide and sunlight into energy so efficiently that they can double their weight several times a day. As part of the photosynthesis process algae

produce oil and can generate 15 times more oil per acre than other plants used for biofuels, such as corn and switchgrass. Algae can grow in salt water, freshwater or even contaminated water, at sea or in ponds, and on land not suitable for food production.

We are working on Chlamydomonas reinhardtii and some other algae to increase their biomass and lipid production which can in future stand as an alternative energy source for next generation. We started our research with Chlamydomonas reinhardtii since it was a model organism. Chlamydomonas reinhardtii is a single celled photosynthetic microorganism and produces a kind of lipid which can be easily converted for biofuel production. The process of making biodisel from algae contains a number of seperation steps; separating the algea from the media, separating the oil from the algea and removing the glycerol from the oil to lower the viscosity. Hence our aim is to produce a high biomass algea strain containing a high amount of lipid which can be efficiently used for biodiesel production.

1.INTRODUCTION

Energy is one of the most essential requirements for all living organisms and industry. Developing technologies and increasing world population also deepens this requirement. In order to meet this energy demand, today we heavily depend on the conventional energy sources such as coal, natural gas, oil, firewood etc but estimations show that these energy sources are not both sustaniable and environmental friendly.

They are also a potential threat for the global climate changes since they contribute the highest amount of greenhouse gases. So we need to produce global strategies that must be sustaineble, renewable and environmental friendly [1]. In this particular situation, plants offer us a highly efficient and effective solutions. However, intenting to use higher plants for such purposes can cause several problems such as food competition, water shortage, arable land, fertilizer etc. But lower plants such as microalgae offer us more sustainable and permanent solutions. Algae can grow in salt water, freshwater or even contaminated water, at sea or in ponds, and on land not suitable for food production. Recent researches and estimations also confirmes that algae are one of the most promising biodiesel source that are able to meet the global biofuel demand. Some oil containing foods and crops have been used as a first generation biodiesel source but none of them has proved that they can be a candidate for biodiesel production in comparison with algae. A comparison of some important food crops and algae is given in Table 1 [2].

Table 1

Source	Gallons of oil acre/year
Algae	5000–20,000
Oil palm	635
Coconut	287
Jatropha	207
Rapeseed/Canola	127
Peanut	113
Sunflower	102
Safflower	83
Soybeans	48
Hemp	39
Corn	18

2.Choice of the best microalga species

The word ‘microalgae’ is a general term that does not specify any particular alga(e). When we say as microalgae we mean all algal species’ and strains. Since there are thousands of algal species’ and strains, and their lipid content and productivity vary from one another, at the first step we have to choose the species or strain(s) that is suitable for biodiesel production. Table 2 shows the lipid content and productivity of some of the fresh and marine water algal species. Average lipid contents of the some algae specified in Table 2 vary between 2% and 70% [3].

Table 2

	Microalga species	Lipid content (%, w/wDW)	Lipid productivity (mg L-1 d-1)
Fresh water	Botryococcus sp.	25.0–75.0	-
	Chaetoceros muelleri	33.6	21.8
	Chaetoceros calcitrans	14.6–16.4/39.8	17.6
	Chlorella emersonii	25.0–63.0	10.3–50.0
	Chlorella protothecoides	14.6–57.8	1214
	Chlorella sorokiniana	19.0–22.0	44.7
	Chlorella vulgaris	5.0–58.0	11.2–40.0
	Chlorella sp.	10.0–48.0	42.1
	Chlorella pyrenoidosa	2.0	-
	Chlorella sp.	18.0–57.0	18.7
	Chlorococcum sp.	19.3	53.7
	Ellipsoidion sp.	27.4	47.3
	Haematococcus pluvialis	25.0	-
	Scenedesmus obliquus	11.0–55.0	-
	Scenedesmus quadricauda	1.9–18.4	35.1
	Scenedesmus sp.	19.6–21.1	40.8–53.9
Marine water	Dunaliella salina	6.0–25.0	116.0
	Dunaliella primolecta	23.1	-
	Dunaliella tertiolecta	16.7–71.0	-
	Dunaliella sp.	17.5–67.0	33.5
	Isochrysis galbana	7.0–40.0	-
	Isochrysis sp.	7.1–33	37.8
	Nannochloris sp.	20.0–56.0	60.9–76.5
	Nannochloropsis oculata	22.7–29.7	84.0–142.0
		12.0–53.0	60.9–76.5

Nannochloropsis sp.	29.0–65.0	90.0–134.0
Neochloris oleoabundans	30.9	49.4
Pavlova salina	35.5	40.2
Pavlova lutheri	18.0–57.0	44.8
Phaeodactylum tricornutum	4.0–16.6	-
Spirulina platensis		

Choosing best performing microalgae only by looking at its lipid content must be tricky because not all types of fats are used for biodiesel production. TAGs are only the fats that are used for biodiesel. So at strain selection, multicriterion strategies must be adopted. Lipid content, lipid productivity, growing rate, lipid quantity and quality, response to environmental stimuli such as nutrient, light, temperature etc, nutrient preference, biomass harvesting, lipid extraction and purification must be sepearately evaluated and then strain selection must be done [3]. Since the Chlamydomonas reinhardtii is a model organism, we chose its strains CC400 and CC125 in our research.

2.1.Availibity of algae for biodiesel production

Researches show that algae are able produce 200 times more oil than that of even best-performing plants [4]. Theorically algae can be grown at anywhere as soon as sunlight, CO2 and some macro and micro elements are provided but in practise, we need an optimization process. Algae synthesize lipids for different metabolic purposes such as membrane structure, storage, energy source and other metabolic activities. But only TAGs can be used for biodiesel production so optimization processes and molecular engineerings must be done for improving these TAGs. Today there is no single algae species or strain can be shown that was already optimized for maximum biodiesel production. Optimization processes such as nutrient deficient media and different stress factors, gene insertions, limiting different metabolic pathways etc. are going on researches [5, 6, 7].

3.Methods

3.1 Identification and cultivation of the microalga

Chlamydomonas CC400 and CC125 were used for this study. Both strains were grown in 100 ml erlanmeyer flasks, each containing 50 ml TAP liquid media under the constant illumination with white flourescent light and unlimited CO2. Flasks were shaken at 100 rpm. Temperature was 20 ± 30 C.

3.2.Verification of strains

18S rRNA sequence analysis was done for the verification of strains Chlamydomonas CC400 and CC125. For this, genomic DNA was extracted, primers were prepared for 18S rRNA and then 18S rRNA was amplified and sequenced [8].

3.3 Bioinformatic analysis of 18S rRNA sequence

Sequence alignment analysis was done on the NCBI database by BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Bioinformatic analysis verified that harvesting strains belong to the Chlamydomonas CC400 and CC125.

3.4 Extraction of lipids

Algae (4g) were seperated from the medium by centrifugation. Fresh algal pellets were boiled in 10 ml alcohol to inhibit the lipase activity and then were dried under nitrogen gas. The dried pellet was mixed with chloroform-methanol (1:2) and BHT (antioxidant). After centrifugation, 1.6 ml ddwater, 10 ml chloroform and 1.6 % potassium chloride were added to the supernatant respectively. The mixture was vortexed and homogenized layer was collected, and then concentrated under nitrogen gas [9].

3.5 Esterification of fatty acids

Extracted lipids were disolved in 6 ml ethanol. Sulfuric acid was used as a catalyzer for the esterification of fatty acids [10]. The mixture was refluxed by Dean-Stark apparatus and was washed with saturated hydrogen carbonate solution, and then dried over anhydrous sodium sulfate. To obtain fatty acids, solvent was removed by distillation [11].

3.6 GC/MS analysis

Hewlett–Packard 6890 was used for GC/MS analysis. The gas chromatograph was equipped with a HP-5M capillary column. The oven temperature was programmed from 85 oC (5 min) to 265 oC at the rate of 7 oC/min and finally held at 265 oC for 10 min. The carrier gas was helium with the flow rate of 1.2 mL/min.The mass spectrometer was operated in EI (Electron Ionization) mode at 70 eV. The interfacetemperature was 265 oC and the mass range was 15–650 m/z.The identification of fatty acids was performed, comparing the obtained mass spectra with Wiley (275) libraries [11].

4.Results and discussions

We bought our algae from the microalgal collection center and grew them in convinent media in lab. Genomic DNAs of these algae were isolated for 18S rRNA amplification. 18S rRNA sequence analysis was done on the NCBI database by BLAST search. Both strains were verified that they belong to the Chlamydomonas CC400 and CC125 respectively. Total lipids were extracted by the method specified in 3.4. Extraction of lipids and after esterification process, GS/MS analysis was carried out. Different types of FAMEs were detected.

Their easy handling and fast growing rate paved the way for better understanding of optimization process for different algal species. Since the algae are living organisms, it needs a systematic approach, meaning that different pathways such as carbohydrate pathway, lipid pathway, protein pathway etc must be evaluated together at molecular level by microarray

analysis. Researches show that limiting one pathway for giving more allocation to another is a temporary solution so we should find new systematic and holistic approaches.

5.CONCLUSION

This study shows that microalgae are the best candidate for the biodiesel production although studied strains have lower lipid productivity in comparison with other algal species.

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