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Technology and Engineering of Biodiesel Production: a Comparative Study between Microalgae and Other Non-Photosynthetic Oleaginous Microbes

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Abstract – *Microbial lipids are considered as a promising and sustainable feedstock for biodiesel production due to their fatty acid composition similar to that of vegetable oils. So far, microalgae have attracted more attention as a lipid producer in comparison to other non-photosynthetic oleaginous microbes. Nevertheless, recent studies showed the efficiency of other microorganisms, including bacteria, yeasts, molds which are able to accumulate lipids over 20 % of their dry biomass. Competence of lipid production by those photosynthetic and non-photosynthetic microbes are highly depend on the cost of reactor design, wide range of nutritional substrates, scalability, parasitic energy demand, metabolic function etc. Therefore, integration of biology and engineering is essential for a cost-effective production of microbial lipids. This paper compares microalgae and non-photosynthetic microbes as regards the factors affecting the techno-economical feasibility of the microbial oil production.* ***Copyright © 2012 Praise Worthy Prize S.r.l. - All rights reserved.***

Keywords: *Microbial Lipids, Microalgae, Yeasts, Moulds, Biodiesel*

I. Introduction

In the last decade the production of biodiesel from algae has been an area of considerable interest [1], [2]. It offers some exclusive advantages, namely: (1) algae have higher productivities than land plants, with some species having doubling times of a few hours; (2) some species can accumulate larger amounts of triacylglycerides (TAGs) and (3) fertile agricultural land is not required to grow the biomass. On the other hand, non-photosynthetic microbes can be cultured in a wide range of nutritional substrates, with easier culture methods. In addition, they do not require photo-bioreactor, and their cultures are not affected by seasons or by climates. However, several challenges need to be tackled to allow commercial production of diesel from oleaginous microorganisms at a scale sufficient to offer a significant contribution to our transport energy needs.

II. Traditional Source of Biodiesel and their Limitations

To deal with deteriorated situation of the whole world energy supply, energy environment and energy security, renewable biofuels are receiving considerable attention as substitute [3]. One of the most prominent renewable energy resources is biodiesel, which is produced from renewable biomass by transesterification of triacylglycerols, yielding monoalkyl esters of long-chain fatty acids with short-chain alcohols, for example, fatty acid methyl esters (FAMEs) and fatty acid ethyl esters (FAEEs). Traditionally biodiesel is obtained from vegetable oils like soybean oil [4], [5], jatropha oil [6], rapeseed oil, palm oil, sunflower oil, corn oil, peanut oil, canola oil and cottonseed oil [7]. Apart from vegetable oils, biodiesel can also be produced from other sources like animal fat (beef tallow, lard), waste cooking oil, greases (trap grease, float grease) and algae . In South East Asia, Europe, United States and China, palm oil, rapeseed oil, transgenic soybeans and wasting oil were used to produce biodiesel, respectively. However, all these plant oil materials require energy and acreage for sufficient production of oilseed crops. Likewise, animal fat oils need to feed these animals. In spite of the favorable impacts that its commercialization could provide, the economic aspect of biodiesel production has been restricted by the cost of oil raw materials. If plant oil was used for biodiesel production, the cost of source has account to 70–85% of the whole production cost [2]. Therefore, taking into account of these inhibition factors, exploring ways to reduce the high cost of biodiesel is of much interest in recent research, especially for those methods concentrating on lowering the cost of oil raw material. Microorganisms (microalgae, yeast) have often been considered for the production of oils and fats as an alternative to agricultural and animal sources. Synthesis of microbial lipids or single cell oil (SCO) have some unique advantages over vegetable oils, such as they can be cultivated on degraded and nonagricultural lands that avoid use of high-value lands and crop producing areas and Can utilize salt and waste water streams, thereby greatly reducing freshwater use [8]. Apart from these conditions, a comparison between I-, II- and III-generation biodiesel is presented in the Table I.

III. Cultivation Systems

The most common procedure for cultivation of Chlorophyta (green microalgae) is autotrophic growth. Under autotrophic cultivation, the cells harvest light energy and use CO_2 as a carbon source. Large open outdoor pond cultivation for mass algal production of single-cell protein, health food, and β-carotene is one of the oldest industrial systems.

These cultivation systems present relatively low construction and operating costs and the large ones can be constructed on degraded and nonagricultural lands that avoid use of high-value lands and crop producing areas.

All these benefits notwithstanding, open ponds have several inherent disadvantages: (1) Poor light diffusion inside the pond, decreasing with depth. (2) Mono-cultivation of the desired microalgae is difficult to maintain for most microalgae species because of constant airborne contamination, except for extremophile species; (3) Environmental growth parameters of cultivation rely primarily on local weather conditions, which may not be controlled and make production seasonal; (4) Harvesting is laborious, costly, and sometimes limited by low cell densities; (5) Continuous and clean water is needed; and (6) Production of pharmaceutical or food ingredients is not feasible or is very limited.

An economical process of algae mass culture for lipid production depends strongly on high biomass productivity and high lipid content, but this cannot be achieved invariably. A feasible alternative for phototrophic cultures in photo-bioreactors (PBRs), but restricted to a few microalgal species, is the use of their heterotrophic growth capacity in the absence of light, replacing the fixation of atmospheric CO_2 of autotrophic cultures with organic carbon sources dissolved in the culture media. Heterotrophy is defined as the use of organic compounds for growth.

The heterotrophic growth is the dark supported by a carbon source replacing the traditional support of light energy. This unique ability of essentially photosynthetic microorganisms is shared by several species of microalgae. Where possible, heterotrophic growth overcomes major limitations of producing useful products from microalgae: dependency on light which significantly complicates the process, increase costs, and reduced production of potentially useful products. As a general role, and in most cases, heterotrophic cultivation is far cheaper, simpler to construct facilities, and easier than autotrophic cultivation to maintain on a large scale. This capacity allows expansion of useful applications from diverse species that is now very limited as a result of elevated costs of autotrophy; consequently, exploitation of microalgae is restricted to small volume of high-value products. Heterotrophic cultivation may allow large volume applications such as wastewater treatment combined, or separated, with production of biofuels.

TABLE I
REVIEW OF THE SPECIFICATION OF I, II AND III-GENERATION BIOFUELS

Fuel generation	**Feedstock**	**Limitations**	**Advantages**
I-generation Produced directly from food crops	grains, sugar cane, wheat and vegetable oils, animal fats [9], [10]	- effects the environment and climate change -Changes ecological balance and biodiversity - suppressed the food production -releasing more carbon in their production than their feedstock's capture in their growth [10], [11]	-can offer some CO_2 benefits - can help to improve domestic energy security.[10]
II-generation Produced from non-food crops	wood, organic waste, food crop waste and specific biomass crops, oil and sugar crops such as *Jatropha*, cassava or *Miscanthus*; [9]	-requires costly technologies -involves pre-treatment with special enzymes, [9] -burning the crop residues means decreasing of future organic matter in agricultural soils [11]	-do not compete with food production. -significantly reduce CO_2 production, - more comparable with standard petrol, diesel, and would be most cost effective route to renewable, low carbon energy for road transport. [9], [10]
III-generation Produced from microorganisms	Algae, Yeast, Seaweeds, microbes.[9]	-require high-oil content oil species, which is usually associated with lower yields and is more prone to contamination -dewatering or drying is energy intensive -easily being contaminated	-no arable land use - not depend on climate or season - able to produce 15–300 times more oil than traditional crops on an area basis - microalgae have a very short harvesting cycle (≈1– 10 days depending on the process) [9]

Although microalgae are high lipid microbial, they need a larger acreage to culture algaes and long fermentation period than bacteria. Most of the bacteria accumulate lower lipid than microalgaes, the average oil content is about 20–40% of dry biomass, such as *Arthrobacter sp*. and *Acinetobacter calcoaceticus*, the oil content is 40% and 38%, respectively. Bacteria have a superiority in the production of biodiesel with highest growth rate (reach huge biomass only need 12–24 h) and easy culture method. But very few literatures were found on the study of bacteria for recommending biodiesel production. Most bacteria just produce complex lipoid, and only a few bacteria can produce oils which can be used as the feedstock for biodiesel production [12]. Another reason may be very low biomass yield productivity of bacteria compared to other oleaginous microbes (Table II).

Yeasts and fungi (especially molds) are considered as favourable oleaginous microorganisms since 1980s [13]. Some yeast strains, such as *Rhodosporidium sp*., *Rhodotorula sp.* and *Lipomyces sp*. can accumulate intracellular lipids as high as 70% of their biomass dry weight. Recently, report showed that it can reach a dry biomass and cellular lipid content of 151.5 g L and 48.0% (w/w), respectively, in flask fed-batch cultures run for 25 days [14]. A filamentous fungus – *Mortierella alliacea* Strain YN-15, accumulated arachidonic acid (AA, C20:4n-6) mainly in the form of triglyceride in its mycelia, which yielded 46.1 g/L dry cell weight, 19.5 g/L total fatty acid, and 7.1 g/L AA by 7-d cultivation in a 50-L jar fermenter [15]. Based on these data, oleaginous yeasts and fungi are all potential alternative oil resources for biodiesel production [16], [17].

IV. Lipid Content

Although several kinds of microorganism may accumulate oils, such as microalgae, bacillus, fungi and yeast, not all of them are suitable for biodiesel production (Table III).

Microalgae offer high lipid contents, but they need a larger acreage to be cultured and longer fermentation periods than bacteria. Although bacteria accumulate lower lipid than microalgae but bacteria offer higher growth rates (only 12–24 h are needed for the maximum biomass concentration to be reached) and easy culture methods. Besides this, the most efficient oleaginous yeasts *Cryptococcus curvatus* can accumulate storage lipids up to >60% on a dry weight basis. In addition, when the *C. curvatus* grow under N-limiting conditions, these lipids usually consist of single oil cell- SOC 90% w/w triacylglycerol with a percentage of saturated fatty acids (% SFA) of about 44% which is similar to many plant seed oils [13].

V. Reactors

Microalgae culture needs sophisticated environment (Table IV) like photobioreactor, controlled CO_2 concentration, temperature etc. But, in comparison to microalgae, cultural conditions of yeast and moulds are easy and simple.

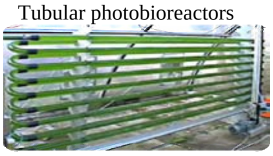
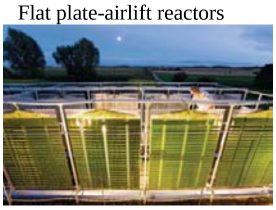
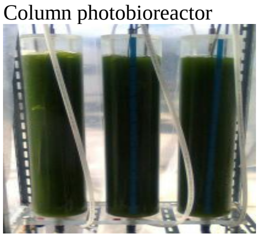
TABLE II
GROWTH PERIOD AND BIOMASS YIELD OF DIFFERENT CULTIVATION PROCESS

Photosensitivity	Microbes	Cultivation process	Strain	Maturity time, hour	Biomass growth, g/L	References
Photosynthetic	Microalgae	Autotrophic	*Chlorella pyrenoidosa*	~100h	14g/L	[18]
		Heterotrophic	*Chlorella protothecoids*	184h	15.5g/L	[19]
		Mixotrophic	Chlorella pyrenoidosa	115h	17g/L	[18]
Non-photosynthetic	Yeast	Submerge fermentation	Lipomyces starkey	240h	10.4 g/L	[20]
			Rhodosporidium toruloides	120h	18.2 g/L	[21]
	Fungi	Solid state fermentation	*Mortierella isabellina*	212h	maximum	[22]
			Microsphaeropsis sp.	216h	78.6%	[23]
			Mortierella isabellina	100h	maximum	[24]
	Bacteria	Submerge fermentation	-	12-24 h		[25]
			Gordonia sp.	36h	1.88 g/L	[16]

TABLE III
LIPID CONTENT OF DIFFERENT CLASSES OF MICROORGANISMS [25]

Microorganisms	Lipid content, % dry wt	Microorganisms	Lipid content, % dry wt
Microalgae		Yeast	
Botryococcus braunii	25–75	*Candida curvata*	58
Crypthecodinium cohnii	16-37	*Cryptococcus albidus*	65
Nitzschia sp.	45–47	*Lipomyces starkeyi*	64
Schizochytrium sp.	50–77	*Rhodotorula glutinis*	72
Bacterium		Fungi	
Arthrobacter sp.	>40	*Aspergillus oryzae*	57
Acinetobacter calcoaceticus	27–38	*Mortierella isabellina*	86
Rhodococcus opacus	24–25	*Humicola lanuginosa*	75
Bacillus alcalophilus	18–24	*Mortierella vinacea*	66

TABLE IV
REACTORS AND THEIR SPECIFICATION [9], [26]

Microorganism	Reactor type	Advantages	Limitations
Micrioalgae	Open ponds photobioreactors 	-Relatively cheap -Easy to clean -Utilises non-agricultural land -Low energy inputs -Easy maintenance -Good for mass cultivation	-Poor biomass productivity -Large area of land required -Poor mixing, light and CO2 utilisation - Cultures are easily contaminated -Difficulty in growing algal cultures for long periods
	Closed photo-bioreactors: Tubular photobioreactors 	-Large illumination surface area -Suitable for outdoor cultures -Relatively cheap -Good biomass productivities	-Some degree of wall growth -Fouling -Requires large land space -Gradients of pH, dissolved oxygen and CO_2 along the tubes
	Closed photo-bioreactors: Flat plate-airlift reactors 	-High biomass productivities -Easy to sterilize -Low oxygen build-up -Readily tempered -Good light path -Relatively cheap -Easy to clean up -Good for immobilization of algae -Large illumination surface area -Suitable for outdoor cultures	- require many compartments and support materials -Difficult temperature control -Small degree of hydrodynamic stress -Some degree of wall growth
	Closed photo-bioreactors: Column photobioreactor 	-Compact -High mass transfer -Low energy consumption -Good mixing with low shear stress -Easy to sterilize -High potentials for scalability -Readily tempered -Good for immobilization of algae -Reduced photoinhibition and photo oxidation	-Small illumination area -Expensive compared to open ponds -Shear stress -Sophisticated construction -Decrease of illumination surface area upon scale-up
Non-photosynthetic microbes (yeast, mould)	Lab scale -Shake flask (yet not developed in specific size and shape)	-Light source not required -Less possibility of contamination	-Need continuous shaking

VI. Conclusion

Microbial lipids would be the best solution to produce biodiesel, to overcome the conflict between food and fuel.

In spite of having many advantages of microbial oils compared to other plant oils such as short life cycle, less labor required, less affection by venue, season and climate, and easier to scale up, till now neither bioethanol nor biodiesel are competitive with fossil fuels. They could compete with conventional fuels only by improving the technology and increasing both the biomass and the lipid yields. Consequently, the development of microorganisms strains offering high lipid content for biodiesel production is of critical importance for an effective industrial application of the III generation biodiesel. Though, microalgae technology is in advanced stage, but nonphotosynthetic microbes are also very promising to be alternative of complicated microalgae culture.

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