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MINI-REVIEW

Perspectives of microbial oils for biodiesel production

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Abstract Biodiesel has become more attractive recently because of its environmental benefits, and the fact that it is made from renewable resources. Generally speaking, biodiesel is prepared through transesterification of vegetable oils or animal fats with short chain alcohols. However, the lack of oil feedstocks limits the large-scale development of biodiesel to some extent. Recently, much attention has been paid to the development of microbial, oils and it has been found that many microorganisms, such as algae, yeast, bacteria, and fungi, have the ability to accumulate oils under some special cultivation conditions. Compared to other plant oils, microbial oils have many advantages, such as short life cycle, less labor required, less affection by venue, season and climate, and easier to scale up. With the rapid expansion of biodiesel, microbial oils might become one of potential oil feedstocks for biodiesel production in the future, though there are many works associated with microorganisms producing oils need to be carried out further. This review is covering the related research about different oleaginous microorganisms producing oils, and the prospects of such microbial oils used for biodiesel production are also discussed.

Keywords Biodiesel · Oleaginous microorganisms · Microbial oil · Sustainability

Introduction

Environmental protection and depleting crude oil supplies are becoming the overriding challenge faced by oil industries. Biodiesel, which is derived from triglycerides or free fatty acids by transesterification or esterification with short chain alcohols (Fig. 1), has attracted considerable attention during the past decade as a renewable, biodegradable, and nontoxic fuel.

The global biodiesel industry has grown significantly over the past decade. The European Union (EU) has arguably been the global leader in biodiesel production. The overall biodiesel production in EU has increased from 3.2 million tons in 2005 to nearly 4.9 million tons in 2006. This represents a 54% yearly growth for EU biodiesel production, which follows a 65% record high growth in the previous year 2005. As a result, the EU biodiesel production has more than doubled in the last 2 years, rising from 1.9 million in 2004 to the 4.9 million in 2006, marking a further acceleration in the continuous expansion of the European biodiesel sector.

Biodiesel production in the United States also increased dramatically in the past few years. The United States has increased its production from two million gallons in 2000 to 250 million gallons in 2006 and an estimated of 450 million gallons in 2007. While 250 million gallons is smaller than the EU production, it represents significant growth. The trend has recently accelerated, and production grew at a pace of 113 million gallons per year between 2004 and 2006. According to the National Biodiesel Board, there are 105 plants in operation as of early 2007 with an annual production capacity of 864 million gallons. An additional of 1.7 billion gallons of capacity may come online if current plants in construction are completed (Carriquiry 2007; US National Biodiesel Board 2008).

A rapid expansion in biodiesel production capacity is being observed not only in developed countries but also in developing countries such as China, Brazil, Argentina, Indonesia, and Malaysia. The global biodiesel market is estimated to reach 37 billion gallons by 2016, growing at an average annual growth of 42%. Europe will continue as the major Biodiesel market for the next decade or so, closely followed by the US market (Sims 2007).

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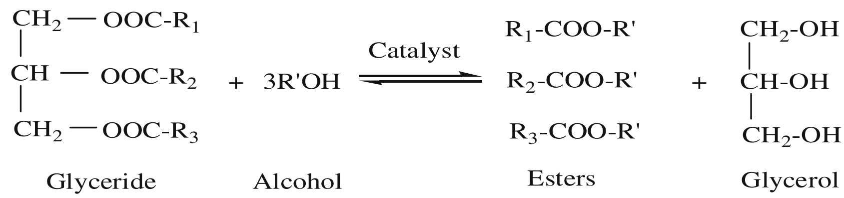

Fig. 1 Biodiesel production by alcoholysis

Currently varied vegetable oils and animal fats such as soybean oils, rapeseed oils, palm oils, and waste cooking oils are usually adopted as feedstocks for biodiesel production (Felizardo et al. 2006; Kulkarni and Dalai 2006). To meet the demand of rapid growth of biodiesel production, other oil sources, especially nonedible oils need to be explored. In recent years, much attention has been paid to the exploration of microbial oils, which might become one of potential oil sources for biodiesel production in the future.

Microbial oils, also called single cell oils, are produced by some oleaginous microorganisms, such as yeast, fungi, bacteria, and microalgae (Ma et al. 2006). It has been demonstrated that such microbial oils can be used as feedstocks for biodiesel production, and compared to other vegetable oils and animal fats, the production of microbial oil has many advantages: short life cycle, less labor required, less affection by venue, season and climate, and easier to scale up (Li and Wang 1997). Therefore, microbial oils might become one of potential oil feedstocks for biodiesel production in the future, though there are many works associated with microorganism-producing oils that need to be carried out further. This review is covering the related research about different oleaginous microorganism-producing oils, and the prospects of such microbial oils used for biodiesel production are also discussed.

Table 1 Oil accumulation produced by different microalgaes

Species	Oil content (% dry cell weight)	Reference
C. vulgaris	40	Illman et al. (2000)
C. emersonii	63	Illman et al. (2000)
C. protothecoides	23	Illman et al. (2000)
C. sorokiniana	22	Illman et al. (2000)
C. minutissima	57	Illman et al. (2000)
C. vulgaris	56.6	Liu et al. (2007)
N. oleaabundans	54	Metting (1996)
P. incisa	62	Solovchenko et al. (2008)
M. subterraneus	39.3	Khozin-Goldberg and Cohen (2006)
N. laevis	69.1	Chen et al. (2008)

Autotrophic microalgaes for oil production

Autotrophic microalgaes can utilize carbon dioxide as the carbon sources and sunlight as the energy for oil accumulation under some special conditions. It has been found that many autotrophic microalgaes, such as *Chlorella vulgaris*, *Botryococcus braunii*, *Navicula pelliculosa*, *Scenedsmus acutus*, *Crypthecodinium cohnii*, *Dunaliella primolecta*, *Monallanthus salina*, *Neochloris oleoabundans*, *Phaeodactylum tricornutum*, and *Tetraselmis sueica* can accumulate oils (Chisti 2007; Liang et al. 2006). Different species of microalgaes had varied ability for oil production, and Table 1 gave the related information.

Apart from algae species influencing oil accumulation, cultivation parameters, such as temperature, light intensity, pH, salinity, mineral, and nitrogen sources, also had influence on oil production. Illman reported that the limitation of nitrogen could increases the oil content in all *Chlorella* strains (Illman et al. 2000); Liu et al. found that Fe^{3+} concentration and its adding time affected oil accumu-

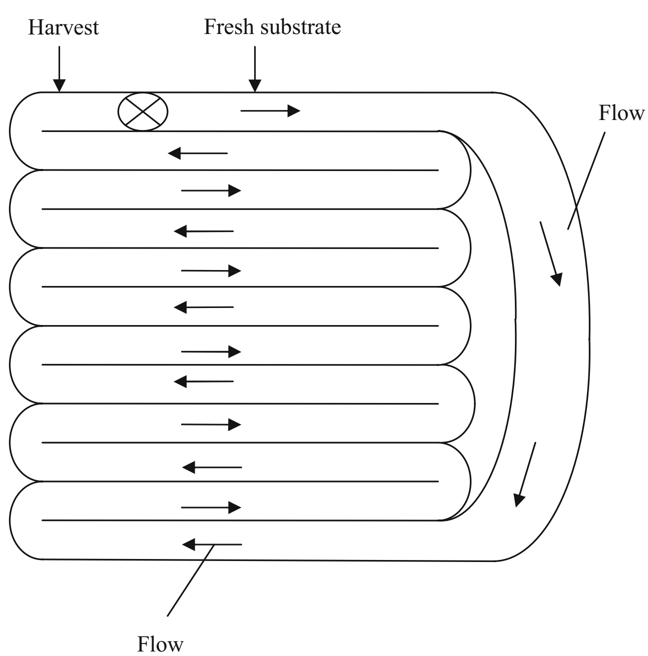

Fig. 2 Outline of a raceway pond

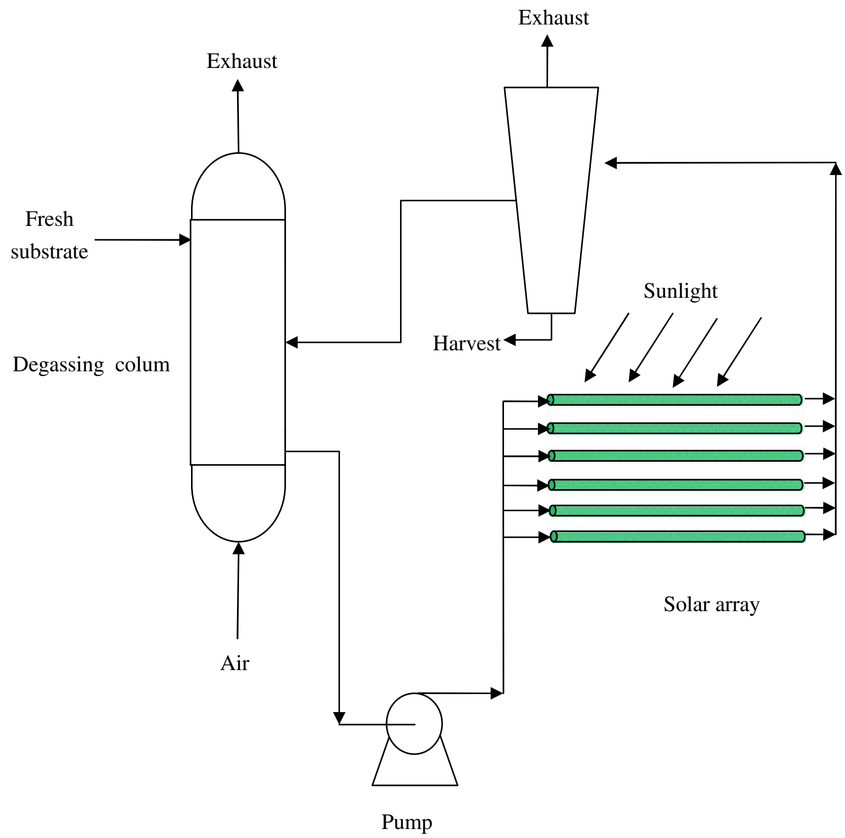

Fig. 3 Typical outline of tubular photobioreactors

lation to some extent (Liu et al. 2007; Chisti 2008; Li et al. 2007c). Solovchenko A. E. reported that highlight stress and nitrogen deficiency are key factors for oil accumulation (Solovchenko et al. 2008).

Scaling up of autotrophic microalgaes for oil accumulation

One of the major differences between autotrophic microalgaes and heterotrophic microorganisms is that the scaling up for autotrophic microalgaes is more complicated, since light is needed during the cultivation process. To minimize the cost, microbial oil production must rely on available free sunlight, despite daily and seasonal variations in light levels (Chisti 2007).

Currently, raceway ponds and tubular photobioreactors are usually adopted for large-scale cultivation of autotrophic microalgaes. Raceway ponds are widely used as an open pond system since it is easy to operate. Generally, the structure of raceway ponds is almost the same as the sewage treatment system (Fig. 2). Production of microalgae biomass has been extensively evaluated in raceway ponds sponsored by the United States Department of Energy (Sheehan et al. 1998).

Closed photobioreactors were also recommended for scaling up of autotrophic microalgaes since this kind of bioreactor could save water, energy, and chemicals compared to some other open cultivation systems (Peer et al. 2008). Most closed photobioreactors are designed as tubular reactors, plate reactors, or bubble column reactors (Pulz 2001). A tubular photobioreactor is usually equipped with fencelike solar collectors. Microalgae broth is continuously pumped through the solar array, where sunlight is absorbed. Fresh culture medium is fed continuously to the degassing column during daylight, and an equal quantity of the broth is harvested at the same time. The degassing

Table 2 Oil production by different yeast

Species	Lipid yield (g/l)	Lipid coefficient (%)	Reference
R. toruloides	13.8	22.7	Li et al. (2006)
L. starkeyi	5.9	20.4	Liu et al. (2000)
L. starkeyi	9.99	14	Kong et al. (2007)
L. starkeyi	6.89	11	Kong et al. (2007)
R. glutinis	7.19	13	Shi et al. (1997)
T. fermentans	5.32	8.42	Kazuyoshi et al. (2006)
C. curvatus	37.1	–	Mainul et al. (1996)

Table 3 The fatty acid composition produced by different yeast (%)

Species	Palmitic acid (C16:0)	Palmitoleic acid (C16:1)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)	Linolenic acid (C18:3)
L. starkeyi	33.0	4.8	4.7	55.1	1.6	n.d.
R. toruloides	24.3	1.1	7.7	54.6	2.1	n.d
C. lbidus	16	1	3	56	n.d.	3
L. lipofera	37	4	7	48	3	n.d
R. gutinis	18	1	6	60	12	2
T. pullula	15	n.d.	2	57	24	1
C. lbidun	25	n.d.	10	57	7	n.d.
Y. lipolytica	11	6	1	28	51	1

n.d. Not determined

column is continuously aerated to remove the oxygen accumulated during photosynthesis, and the oxygen-rich exhaust gas is expelled from the degassing column. Usually, a typical tubular photobioreactor is designed as Fig. 3.

Generally speaking, raceway ponds are perceived to be less expensive than photobioreactors while closed photobioreactors provide a controlled environment that can be tailored to attain a consistently good annual yield of oils. If more modification can be made on current photobioreactors to reduce its operating cost significantly, the prospect of photobioreactors used for large-scale cultivation of autotrophobic microalgaes is quite promising.

Apart from cultivation system influencing oil production, availability of carbon dioxide and water supply also need to be taken into consideration. Usually, it is recommended that scaling up of autotrophic microalgaes is better to be operated close to a power plant and near a sea.

Heterotrophic microalgaes for oil production

Through changing cultivating conditions or using genetic engineering modification, some autotrophic microalgaes can be converted to heterotrophic microalgaes and such heterotrophic microalgaes also can accumulate oils with organic carbon as the carbon sources instead of sunlight. For example, *Chlorella potothecoides*, usually working as autotrophic microalgae, could use organic carbon sources for oil production, and oil content was about four times than that in the corresponding autotrophic cells (Miao and Wu 2004). Some heterotrophic microalgaes could even use cheap organic carbon sources for oil accumulation (Han et al. 2006).

Generally speaking, heterotrophic microalgaes are easily cultivated and controlled in normal fermenters. But, it requires organic carbon sources for oil accumulation, which might limit the application of such microalgaes used for oil production for biodiesel production to some extent.

Yeasts for oil production

Many yeast species, such as *Cryptococcus albidus*, *Lipomyces lipofera*, *Lipomyces starkeyi*, *Rhodosporidium toruloides*, *Rhodotorula glutinis*, *Trichosporon pullulan*, and *Yarrowia lipolytica*, were found to be able to accumulate oils under some cultivation conditions, and it was reported that different yeast species led to different oil accumulation (Table 2; Liu et al. 2000; Liang et al. 2006).

The lipid component of yeast oils were analyzed further and listed in Table 3 (Ma 2006; Liu et al. 2007; Li et al. 2007b). From Table 3, it could be observed that the main fatty acids in yeast oils were myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid. It has been reported that such yeast oils can be used as oil feedstocks for biodiesel production with the catalysis either by lipase or chemical catalyst (Li et al. 2007b; Liu and Zhao 2007).

Table 4 Different carbon source used for yeast oil production

Species	Carbon source	Lipid content% (*w*/*w*)	Reference
L. starkeyi	Xylose	52.6	Kong et al. (2007)
C. potothecoides	Starch hydrolysate	46.13	Han et al. (2006)
T. cutaneum	Spartina anglica hydrolysate	46.3	Shen et al. (2007)
C. curvatus	Glycerol	25	Meesters et al. (1996)
C. echinula	Sweet potato starch processing waste	37.6	Du et al. (2007)
L. starkeyi	Sewage sludge	50.8	Angerbauer et al. (2008)

Table 5 Lipids accumulation by different molds

Species	Lipid yield g/l	Lipid content % (*w/w*)	GLA content %	Reference
M. rouxii	1.0	7	32.4	Hannson et al. (1989)
C. ehinulata	8.0	27	12.1	Chen and Chang (1996)
M. mucedo	12.0	62	3.4	Certik et al. (1997)
C. ehinulata	10.6	58	3.8	Certik et al. (1997)
C. ehinulata	11.5	30	11.7	Chen and Liu (1997)
M. ranmanianna	31.3	50	17.6	Hiruta et al. (1997)
C. ehinulata	4.4	49	16.4	Gema et al. (2002)
M. isabellina	18.1	50	4.4	Seraphim et al. (2004)

Influence of cultivation parameters on yeast oil accumulation

It has been reported extensively that cultivation conditions such as C/N ratio, nitrogen resources, temperature, pH, oxygen, and concentration of trace elements and inorganic salt would have varied influence on oil accumulation. Generally speaking, the more nitrogen compounds contained in medium, the less oil contained in cells. It was reported that when C/N ratio was increased from 25 to 70, oil content increased from 18% to 46% (Mainul et al. 1996). Different nitrogen sources also had varied influence on oil production. Both inorganic nitrogen sources and organic nitrogen sources can be used for yeast cultivation with varied influence on oil accumulation (Liu et al. 2000; Shi et al. 1997). Huang reported that inorganic nitrogen sources were good for cell growth but not suitable for oil production, while organic nitrogen sources such as peptone and broth were good for oil production but not suitable for cell growth (Huang et al. 1998).

Trace metal ions also affect oil accumulation to a varied extent (Li et al. 2006; Wang et al. 2005; Mainul et al. 1996). Li reported that biomass and oil content could be improved significantly by the optimization of Mg^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, and Ca^{2+} concentration (Li et al. 2006). Other cultivation parameters such as dissolved oxygen, temperature, and pH also showed varied influence on cell growth and oil accumulation, and generally speaking, dissolved oxygen concentration in the culture medium is positive correlation with the accumulation of oils (Yi and Zheng 2006; Liang et al. 2006; Yan and Chen 2003).

To achieve high oil production, optimization on aforementioned cultivation parameters is very important.

Exploration of cheap carbon sources for yeast oil accumulation

To reduce the cost of microbial oils, exploring other carbon sources instead of glucose is very important especially for such oils applied to biodiesel production. It was reported that xylose, arabinose, mannose, glycerol, and other agricultural and industrial waste could be used as the carbon sources for yeast oil accumulation, and the related information was listed in Table 4.

It was reported that *C. potothecoides* could accumulate oils with starch hydrolysate as the carbon sources, and cell growth in the starch hydrolysate was even much better than that with glucose as the carbon sources (Han et al. 2006). Du et al. found that sweet potato starch processing waste also could be used as the carbon source for yeast oil production, and such industrial waste was always rich in simple sugar, which could be utilized effectively by many microorganisms (Du et al. 2007).

During the process of biodiesel production, byproduct glycerol will be produced. It was reported that some microorganism even had the ability to use glycerol as the

Table 6 Comparison of oil production by different microorganisms

Species	Fermentation reactor	Biomass g/l	Lipid content% (*w/w*)	Lipid yield g/l	Lipid volumetric productivity (kg m^{-3} day^{-1})	Reference
R. toruloides	Flask	18.2	76.1	13.9	2.78	Li et al. (2006)
R. toruloides	Stirred fermenters	106.5	67.5	71.89	12.96	Li et al. (2007a)
L. starkeyi	Flask	19.0	52.6	9.99	2.0	Kong et al. (2007)
M. ramanniana	Stirred fermenters	67.7	55.2	37.4	17.95	Hiruta et al. (1997)
C. potothecoides	Stirred fermenters	27.1	46.13	12.50	1.63	Han et al. (2006)
Autotrophism microalgae	Photobioreactor facility	4.0	70[a]	2.8	1.535	Chisti (2007)
Autotrophism microalgae	Raceway ponds	0.14	70[a]	0.098	0.117	Chisti (2007)

[a] Theoretical data

carbon sources for oil accumulation (Meesters et al. 1996). In the future, with the large-scale development of biodiesel, more and more byproduct glycerol will be produced, and the utilization of crude glycerol for yeast oil production might be another interesting research filed with much prospect. Apart from glycerol, recently, much attention has been paid to the utilization of cellulose hydrolysate as the carbon sources for microbial oil production, but such cellulose hydrolysate usually contains some toxic components, such as acetic acid, formic acid, and furfural, which might have some negative effect on cell growth (Shen et al. 2007). Therefore, before using such cheap carbon sources for oil production, detoxication is very necessary.

Although there are many works such as process optimization and scaling up that need to be carried out further, utilizing cheap carbon sources for yeast oil production opens a new way for oil cost reduction, which is very important for such oils used for biodiesel production in the future.

Fungi for oil production

Apart from algae and yeasts, fungi also can accumulate oils under some special cultivation conditions.

Although some types of fungi have the ability to produce oils (Table 5), most fungi are explored mainly for the production of some special lipids, such as DHA, GLA, EPA. and ARA, and there are few reports on the utilization of fungi oils for biodiesel production (Ma 2006; Du et al. 2007; Yan and Chen 2003).

Bacteria for oil production

Just like fungi, some sort of bacteria also can accumulate oil under some special environment. But usually, the lipid composition produced by bacteria is quite different from other microbial oils. Most bacteria just produce complex lipoid, and only a few bacteria can produce oils which can be used as the feedstock for biodiesel production (Yi and Zheng 2006). Therefore, bacteria are mainly used for some special lipid production, such as polyunsaturated fatty acids and some branched-chain fatty acids (Patnayak and Sree 2005). Recently, Mona K. et al. found that *Gordonia sp.* and *R. opacus* could accumulate oils under some special conditions with maximum oil content of 80%, but the biomass is only 1.88 g/l (Mona et al. 2008).

Compared to other microorganisms, many expressions of genes in fatty acid synthesis are already understood in bacteria (Alexander et al. 2007; Alvarez and Steinbuchel 2002). Therefore, it is relatively easy to use biological engineering technology, genetic engineering, and metabolic engineering to modify bacteria performance to improve its oil accumulation. It was reported that a metabolically engineered *Escherichia coli* could produce biodiesel (fatty acid esters) directly, and the fatty acid esters concentrations of 1.28 g/l was achieved by fed-batch fermentation using renewable carbon sources (Kalscheuer et al. 2006). Although the yield was low, it provided a new idea about the biodiesel production.

Prospects of microbial oils for biodiesel production

Although there are many microorganisms that have the ability to accumulate oils under some special cultivation conditions, they have different prospects for biodiesel production in terms of oil yield, lipid coefficient, and lipid volumetric productivity. Here, more comparison among different microorganisms is made and shown in Table 6.

Among all heterotrophic microorganism, yeast showed its own advantages in terms of its fast growth rate and high oil content. From Table 6, it can be seen that yeast from *R. toruloides* can give the largest biomass, the highest lipid yield, and the highest lipid content. Further optimizing and improving the yeast abilities of using cheap carbon sources for oil accumulation is very important for such oils applied to biodiesel production in the future.

Autotrophic algae also have their advantages since it can use sunlight and carbon dioxide for oil accumulation, which is thought to be the most environmentally, friendly production of lipids. But, how to realize high-density cultivation and use free sunlight will be the main issues that need to be solved for large-scale cultivation of autotrophic microalgaes. And, developing lower-cost photobioreactors to bring down the costs also need to be explored further.

Generally speaking, microbial oil might become one of potential feedstocks for biodiesel production in the future since it has the following advantages: renewability, fast growth rate, and not taking arable land. Further modifications through genetic engineering and metabolic engineering have much potential for the performance improvement of microorganisms producing oils.

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