The Scientific Temper
Vol. 2; No.1&2 : Jan.-July, 2011 : pp57-60
Email: letmepublish@rediffmail.com

ISSN 0976 8653
e-ISSN 2231 6396

BIODIESEL FROM MICROBIAL LIPIDS BY RHODOTORULA Sp: HOPE FOR A BETTER TOMORROW

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ABSTRACT

In the pursuit of finding an alternative for the fossil fuel which is continuously being depleted, biodiesel has attracted a lot of attention as a renewable source. But the biodiesel production from plant oils is not very cost-effective and it has led to explore the possibility of the potential of microbial lipids for the same. *Rhodotorula sp.* has been a target of increasing attention for the producing microbial lipids as raw material for the biodiesel production. *Rhodotorula glutinis* has been found to be most promising as modulated culture conditions have shown that it can be exploited for increased lipid production which can be used in biodiesel technnology.

KEY WORDS: Biodiesel, microbial lipids, *Rhodotorula glutinis*.

INTRODUCTION

Rising prices of energy fuel, awareness of environment security at global scenario, concerns about petroleum supplies are drawing considerable attention to find renewable biofuels. Biodiesel, a mixture of fatty acid methyl esters derived from animal fats or vegetable oils, is rapidly aquiring the status as an alternative source of energy. However, biodiesel derived from conventional petrol or from oilseeds or animal fat cannot meet realistic need, and can only be used for a small fraction of existing demand for transport fuels and moreover they are not cost effective.

Microbial lipids as a raw material for biodiesel has become a target of increasing attention for exploring its use and one of the prime areas of the current research interests in the microbial lipid by *Rhodotorula sp*. (Antoni et al.,2007, Liu and Zhao,2007, Li Q et al.,2008, Alberto A. et al, 2010) Most of the studies have been carried out on *Rhodoturula glutinis.* Studies of Chao C. et al.(2007) explored a strategy to convert agricultural and forestry residues into microbial lipid, which could be further transformed into biodiesel. After biodiesel was produced by transesterification, the fatty acid esters were analyzed by GC-MS. The results revealed that the composition of biodiesel was quite similar to biodiesel from vegetable oil. Therefore *R.glutinis* could be considered as a potential strain to convert lignocellulosic hydrolysates into a raw material for biodiesel production.

Lignocellulosic biomass can be utilized to produce biodiesel as oleaginous microorganism can use both glucose and pentose. Theoretically corn straw can produce biodiesel 233kg and glycerol 22.8 kg per ton. (Dai et al., 2007). As a result the energy conversion efficiency is about 55%.The isolated *R.glutinis* has shown great promise for industrial application because it could assimilate a wide range of sugars (xylose as the main component) present in the hydrolysate, and withstand the inhibitors produced by the hydrolysis procedure.

Biodiesel production is the process of producing the biofuel, biodiesel, through either transesterification or alcoholysis of lipids. It has been demonstrated that the new process which combines bioengineering and transesterfication is feasible and efficient for the production of high quality and low cost biodiesel from microbial oil.The author in this review proposed that *Rhodoturula* has potential to greatly reduce the price of lipid production, which can be used to produce biodiesel.

OPTIMUM GROWTH CONDITION OF *RHODOTURULA*

In the effort of optimizing the growth conditions, the culture media used contained a source of carbon, nitrogen, phosphorus, potassium, magnesium, zinc and iron in most of the studies and it was found that the optimum growth temperature condition for R.glutinis is 22 ^{o}C and 30 ^{o}C and Lipid production is best at acidic pH. (Alberto A et al, 2002, Dai et al, 2007, Feiyan Xue et al, 2007, Khalid M et al., 1990) In order to determine modulations in culture conditions of *Rhodoturula* for enhanced lipid production different factors of culture media were taken in to account by various researchers.

EFFECT OF TEMPERATURE:

To find out an adequate temperature for the growth of

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yeast under conditions, assays were carried out using culture temperature of 20, 25, 28, and 37 °C. In the experiments the specific growth rates of *R.glutinis* L-1816 was monitored at the temperature indicated using the basal media without peptone for 72 hrs with 2%wt: vol glucose as a carbon source. The result suggested that 28 °C is the optimum temperature of growth with a specific growth rate value of 0.198 ±0.006 per hour. (Claudio M. et al., 2006)

Alberto A. et al., 2002 found that when *R. glacialis* AS 4.7, an oleaginous psychrophilic yeast which was isolated from glacial environments, was cultured batch wise at different temperature, it grew abundantly at all the temperature in range between -3 to 20 °C. Despite its origin, the strain abundantly grew and accumulated lipids up to 20 °C. Similarly to other psychrophilic oleaginous yeasts (Rossi et al, 2009), the growth temperature did not influence the yield coefficient of both biomass and lipid production, but had significant effect on growth rate and thus on volumetric productivity of lipid. Based on both the highest growth rate and volumetric productivity, the optimal temperature for lipid production was 15 °C. The unsaturation index (UI) progressively increased from 0.81 to 1.49 as the growth temperature decreased from 20 to -3 °C mostly due to á –linolenic acid which was absent at 20 °C and accounted for 29% at -3 °C. These observations are in agreement with previous studies on oleaginous species demonstrating that decreasing growth temperature caused an assimilatory response and resulted in a higher length and degree of unsaturation of fatty acid.

EFFECT OF pH:

In the experiment conducted by Claudio M. et al.,2006 found that *R. glutinis* L-1816 showed activity of lipid production at acidic pH . The effects of the acid culture media on the growth of the yeast strain, with an initial pH of 4.0, 5.2, and 7.0 were also studied. Cho et al. showed an important biomass production at an initial pH of between 4.0 and 7.0. It was analyzed the effect growth of maintaining the pH stable during the whole growth period using phosphate citrate pH 5.2 as buffers. Their result showed that there are no statistical differences in the specific growth rate of the yeast using media at a constant pH of 5.2. Furthermore the biomass concentration value at the end of the growth period showed differences of less than 6%. Since growth of this yeast is effected by neutral pH and these values are observed in our studies in batch cultures, it is therefore important to control this parameter in continuous culture.

EFFECT OF C/N RATIO AND C/P RATIO:

In the studies by Alberto A. et al (2002) it was observed that the oleaginous strain *R. glacialis* AS 4.7 accumulates high amount of lipids with in lipid culture when it is cultured in medium with high C: N ratio. Most of lipid accumulation occurred when *R. glacialis* AS4.7 exhausted a nutrient from the medium, but glucose still remained. Glucose continued to be assimilated by the cells and was converted into TAG at approximately. The same rate at which lipid was synthesized during the balance phase of growth .The extent of the carbon excess has major positive effects on lipid production. The extent of carbon excess has major positive effect on lipid production. The lipid content of biomass glucose conversion into lipids, lipid concentration and lipid productivity were all maximum with 120 g/L g glucose (68%, 16%, 19g/L AND 0.054g/L/hr)

Siguo Wu et al., 2010 observed that Lipid accumulation by *Rhodosporidium toruloids* Y4 was directly linked to the carbon to phosphorus (C/P) molar ratios of the culture media. Moreover such lipid accumulations were effective regardless of the presence of high amounts of nitrogen sources. Thus cellular lipid content and lipid yield were 62.2% and .205g/g glucose, respectively, using a medium with carbon to nitrogen (C/N) molar ratio of 6.1 and a C/P molar ratio of 9552. This work suggested that phosphorus limitation can be equally effective and efficient to mediate lipid accumulation which in turn provides opportunities to produce microbial lipid more economically using natural or waste materials with high nitrogen content.

EFFECT OF DIFFERENT SUPPLEMENTS IN CULTURE MEDIA

The effect of different supplementation of the fermentation media was investigated in various studies in order to optimize conditions for enhanced lipid production from *Rhodotorula.*

EFFECTS OF SALTS:

The effect of exogenous supply of different concentration of salts on the growth and lipid production by *Rhodotorula glutinis* was studied and it was found that the amounts of lipid production were markedly affected by the nitrogen level in the medium, reaching its maximum at 5g/L$(NH_4)_2SO_4$ concentration. The lipid

productivity was decreased with increase in the nitrogen level in the medium. Several yeasts could accumulate large amounts of lipid when grown with an excess of carbon and a deficiency of nitrogen.

Weak growth and low lipid yields were maintained upon using medium formulation free from NaH_2PO_4 . The addition of 0.5g/L of the salt allowed the best fermentation yields. On the other hands varying level of the following salts K_2SO_4, $ZnSO_4.7H_2O$.and $FeCl_3$ in the cultivation medium of the experimental yeast have almost no effect on the fermentation studies. (Khalid M. et al. ,1990)

Influence of cultural conditions on lipid production by mutant strain of Rhodotorula glutinis MTCC 1151Mutant of was studied and it was observed it produces high level of lipid (63.6% of biomass) as compared to parent strain (56.7% biomass) .Maximum lipid yield was found with 5% of glucose using ammonium sulphate (0.2%) as a nitrogen source under shake flask conditions after 4 days of incubation at 28 ^oC. The ability of ammonium sulphate to replace comparatively very costly yeast extract is highly appreciable and recommended. **(**Pathak R and Yadav NK, 1997)

EFFECT OF SOME NATURAL AND OIL ADDITIVES:

In this study, the response of the experimental yeast to some natural additives has been examined. Corn steep solid (CSS) was superior in lipid accumulation by the tested yeast (62.4 % lipid on dry weight basis) as compared with the lipid content of the cells grown in absence of additives (37%). The supplementation of the fermentation medium with cotton seed oil (CSO) at a level of 10g/L supported maximum yeast activities. The simulation of lipid production in several fungi due to the addition of some lipid materials has also been recorded. (Khalid M.et al. ,1990)

EFFECT OF MONOSODIUM GLUTAMATE WASTE WATER:

Microbial lipid as a raw material for biodiesel can be produced by *R.glutinis* with the monosodium glutamate waste water as control and the effect of adding glucose to the MSD wastewater on the lipid production was studied by Feiyan X. et al., 2007. Three different strategies, including initial addition, fed batch addition, and glucose fed back addition were attempted. The result showed that the addition of glucose was found favorable not only for cell growth but also for lipid synthesis. Of the three addition method glucose fed batch addition was most efficient one. About 25g/l of biomass, 20% of lipid content and 45% of COD degradation were obtained respectively.

EFFECT OF GLYCEROL:

During the transesterification The ester bond in the TAG are broken leaving behind two products fatty acid methyl ester and glycerol that could provide an inexpensive carbon source to grow oleaginous yeast *R.glutinis*. experiments were conducted to examine the effects of different growth substrates on TAG accumulation and fatty acid production by *R.glutinis*. yeast cultured 24hrs on medium containing dextrose, xylose, glycerol, dextrose and xylose, xylose and glycerol or dextrose and glycerol accumulated 16, 12, 25, 10, 21, and 34% TAG on a dry weight basis respectively. Lipids were extracted from *R.glutinis* and transesterified to fatty acid methyl esters. The result showed a difference in degree of saturation for the carbon source tested. It was observed that the cells cultivated on glycerol alone have the highest degree of unsaturated fatty acids at 53% while xylose has lowest at 25%. *R.glutinis* can be cultivated on all sugars tested as single carbon substrates or in mixtures. Thus it was suggested that glycerol may be used as secondary or primary carbon substrate. (Emily R. et al., 2008)

EXTRACTION OF LIPID

Various solvents and solvents combination have been suggested as extractants, The common method of determining lipids in microorganism is by extraction with organic solvents such as a mixture of chloroform and methanol. Most lipid analysis used chloroform-methanol (2:1 volume) as suggested by Folch et al. 1957. The endogenous water in the tissue is a ternary component of the system. It is not always recognized that how important it is that the proportion of chloroform, methanol and water in the combined phases should be as close as possible to 8:4:3 (by volume) otherwise selective loses of lipid may occur. The Bligh and Dyer method: chloroform –methanol may be used as a best lipid extractant ,but it is certainly not the safest from environment and health standpoints .Ethyl acetate/ ethanol mixtures have been suggested also. Biochemists must be aware of the extraction should carried out immediately after the removal of tissues from living organism. Plants are best extracted with isopropanol to kill off lipolytic enzymes. (Bligh E.G. and Dyer W.J.,1959)

The importance of sonication has not been mentioned in yeast lipid extraction by earlier investigations. Although it was found that sonication was satisfactory when handling a small number of samples. Perchloric acid disruption was superior for the pretreatment of large number of samples. (Wang L.W. et al., 1993)

ANALYSIS OF LIPIDS

There are various methods for analyzing lipids and a combination of these methods has been used and the results were compared. A short extraction period is followed by separation and evaporation of the solvent phase and estimation of the extracted lipid gravimetrically (Nair et.al.,1989). This procedure requires relatively large amounts of sample. A recent method of estimating lipid is by using low resolution nuclear magnetic resonance and enzymatic glycerol estimations was used by Moreton (1989) to determine

lipids in a lipid accumulating yeast, *Apiotrichum curvatum*. This alternative procedure requires less than 1% of the sample used by the conventional gravimetric method and returns similar results. The spectrophotometric method has the additional advantage of being sensitive only to ester groups and hence non-lipid impurities are ignored.The results were obtained from spectroscopic method correlated well with those of the gravimetric method. (Wang L.W. et al., 1993)

FUTURE PROSPECTS

Biodiesel has become more attractive recently because of its environmental benefits, and the fact that it is made from renewable resources. With the rapid expansion of biodiesel, microbial oils might become one of the potential oil feedstocks for biodiesel production in the future. Use of oleaginous yeast *Rhodotorula* as a prospective alternate source of microbial lipid production that can be converted into a technology for producing biodiesel promise a great future. In the past researches much work has been done on production of lipids from *Rhodotorula glutinis* using different economical substrates like industrial products, byproducts and agro waste as substrates e.g. beet molasses, MSD wastewater, lignocellulosic waste. Thus there exist a possibility of using other wastes also and varying growth conditions that can quantitatively and qualitatively enhance the lipid production. Keeping in mind that in the present times the oil prices are increasing every day and air-pollution has risen to alarming rates, there is more and more awareness for using biodiesel. Also mutagenic studies on *Rhodotorula glutinis* can be done to explore possibilities of mutant strains for enhanced lipid production that can be converted into a technology for producing biodiesel. Therefore the microbial lipids by *Rhodotorula glutinis* still remain a valuable tool in optimising its potential for biodiesel production.

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