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Design and techno-economic evaluation of microbial oil production as a renewable resource for biodiesel and oleochemical production

Apostolis A. Koutinas [a], Afroditi Chatzifragkou [a], Nikolaos Kopsahelis [a], Seraphim Papanikolaou [a], Ioannis K. Kookos [b,*]

[a] Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece
[b] Department of Chemical Engineering, University of Patras, Rio 26504, Patras, Greece

HIGHLIGHTS

- We develop flowsheets for microbial oil production from renewable feedstocks.
- We develop flowsheets for biodiesel production from renewable feedstocks.
- We perform detailed preliminary economic evaluation of alternative technologies.
- We perform sensitivity analysis of the cost of manufacturing of oil or biodiesel.

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ABSTRACT

Experimental results from the open literature have been employed for the design and techno-economic evaluation of four process flowsheets for the production of microbial oil or biodiesel. The fermentation of glucose-based media using the yeast strain *Rhodosporidium toruloides* has been considered. Biodiesel production was based on the exploitation of either direct transesterification (without extraction of lipids from microbial biomass) or indirect transesterifaction of extracted microbial oil. When glucose-based renewable resources are used as carbon source for an annual production capacity of 10,000 t microbial oil and zero cost of glucose (assuming development of integrated biorefineries in existing industries utilising waste or by-product streams) the estimated unitary cost of purified microbial oil is $3.4/kg. Biodiesel production via indirect transesterification of extracted microbial oil proved more cost-competitive process compared to the direct conversion of dried yeast cells. For a price of glucose of $400/t oil production cost and biodiesel production cost are estimated to be $5.5/kg oil and $5.9/kg biodiesel, correspondingly. Industrial implementation of microbial oil production from oleaginous yeast is strongly dependent on the feedstock used and on the fermentation stage where significantly higher productivities and final microbial oil concentrations should be achieved.

1. Introduction

The versatile application of vegetable oils and fats has led to a constant increase of their worldwide annual production reaching more than 180 Mt in 2012 [1]. Fats and oils are mainly consumed as food (over 80%) [2], as animal feed, as raw material in the chemical industry for oleochemicals production (e.g. surfactants, coatings and lubricants) [3,4] and as raw material for biodiesel production [5]. The development of advanced biorefinery concepts for the production of biodiesel has emerged as current first generation industrial technologies depend almost entirely on natural oils and fats. Current technology employs transesterification of triacylglycerols (provided by various vegetable oils and animal fats) with (predominantly) methanol to produce fatty acid methyl esters (FAMEs). Sunflower, rapeseed, soybean and palm oil seeds are the main oilseeds used for the production of biodiesel [6,7]. In recent years, it has become evident that biofuel production should depend on non-food crops, agro-industrial wastes and renewable resources that do not compete with food and feed production [6].

Recent research initiatives have focused on biodiesel production using microbial oil (MO) that is accumulated by oleaginous microorganisms during fermentation on various renewable resources [8]. When MO is utilised for biodiesel production any sugar-based or similarly metabolized renewable materials (e.g. polysaccharides, glycerol, etc.) can be used as substrates of the oleaginous microorganisms. In this case lipid synthesis is performed at the "imbalanced" growth phase where (in most cases) nitrogen constitutes the limiting substrate and the excess of extra-cellular carbon is directed towards the synthesis of storage lipids (this is the so-called "*de novo*" lipid accumulation process) [8]. In general,

* Corresponding author. Tel.: +30 2610 969 567; fax: +30 2610 990917.
E-mail address: i.kookos@chemeng.upatras.gr (I.K. Kookos).

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a microorganism can be characterized as oleaginous when it accumulates MO to more than 20% of its total cellular dry weight. MO accumulation with similar fatty acid composition to vegetable oils can be achieved under growth limiting conditions by various yeast (e.g. *Rhodosporidium*, *Rhodotorula*, *Lipomyces*, *Trichosporon*) and fungal (e.g. *Mortierella*, *Cunninghamella*) genera [9]. Research on MO production has led to total dry weights up to 185 g/L, MO content in microbial biomass up to 76% (w/w) and productivities up to 1 g/(L h) [10–15]. Fed-batch or continuous fermentations with partial recycling have also been studied. Among different carbon substrates (e.g. glucose, glycerol and lactose) utilized in these studies, the best results were achieved with glucose. The MO produced by yeast contains triglycerides with a fatty acid composition of palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and α-linolenic (C18:3, ω-3) acids [9,12,14–17].

A potential high added value end-use of MO is its utilization as substitute of vegetable oils and animal fats for oleochemical production including, among others, cosmetics, pharmaceuticals, paints, lubricants and polymer additives. Fatty acids and glycerol could be derived from hydrolysis of triglycerides. The most important platform intermediates or end-products that could be derived from fatty acids are fatty acid esters, fatty acid ethoxylates, soaps, fatty amines and fatty alcohols. Glycerol is nowadays considered as an important platform chemical for the future sustainable chemical industry that could be converted through green chemical conversion routes or bioprocessing into a wide range of chemicals, such as dichloropropanol, epichlorohydrin, acrolein, 1,3-propanediol, polyhydroxybutyrate and succinic acid [18]. MOs produced by different oleaginous microorganisms under different cultivation conditions will be composed of diversified fatty acid compositions that could be used either as multi-purpose or case-specific feedstocks [19]. Therefore, MOs that contain common fatty acids (e.g. palmitic, oleic and linoleic acids) could be regarded as multi-purpose feedstocks, whereas MOs that contain uncommon fatty acids with special properties could be regarded as case-specific feedstocks. An important potential utilization of MOs is related to the fact that the price range of naturally occurring oils and fats can vary enormously as the price of individual fatty acids vary from as little as \$0.30/kg to over \$100/kg [15,19]. Identification of microorganisms that could be capable of producing some of the high value oils can result in the successful commercial, large-scale production of the substitutes of these lipids [15,19].

Utilization of oleaginous yeasts for biodiesel or oleochemical production could prove more advantageous than microalgae or vegetable oils. They grow much faster than microalgae as they present duplication times of even lower than 1 h. Cultivation of yeast is not affected by environmental conditions, seasonal production or geographic location as in the case of vegetable oils. There is no competition with food or feed production as oleaginous yeast can grow and accumulate MO on crude renewable resources including agricultural residues, industrial waste streams and non-food crops. Furthermore, scale-up is easier than in the case of microalgae cultivation. For instance, oleaginous yeast can be cultivated in conventional stirred tank or air-lift bioreactor configurations. However, the development of cost efficient biodiesel production from oleaginous yeasts is dependent upon the optimization and advancement of fermentation processes. Since microbial oil accumulation is initiated under nutrient limiting conditions, its production is carried out in fed-batch mode where yeast cell growth is achieved in the first stage and MO accumulation is induced in the second stage under usually nitrogen limiting condition. Therefore, high MO concentration and productivity can only be attained if a high yeast cell density is achieved at high productivity during the growth phase. Needless to say that such advancements should be combined with high carbon source to MO conversion yield.

The main methods that have been employed for MO transesterification are based on either direct transesterification (without extraction of MO from the microbial mass) or indirect transesterification (MO has been extracted from the microbial mass) [17,20,21]. Zhu et al. [17] reported that the microbial oil produced by *Trichosporon fermentans* could be converted into FAME via base catalysis after removal of free fatty acids at a conversion yield of 92% (w/w). Liu and Zhao [20] produced fatty acid methyl esters (FAME) with acceptable CN (56.4–63.5) and lipid to FAME conversion yields higher than 90% (w/w) via direct acid-catalysed transesterification of three oleaginous microorganisms (*Lipomyces starkeyi*, *Rhodotorula toruloides* and *Mortierella isabellina*). The commercial transesterification of MO for biodiesel production is dependent on the development of fermentation processes that achieve high conversion yields, productivities, lipid content in cellular biomass and MO concentration.

The economic evaluation of current technologies that focus on MO production or biodiesel production from MO is essential in order to assess the potential for commercialization. Davies [22] estimated that the production of 1000 t of a refined oil and 1800 t of yeast/whey protein dry mix from the continuous fermentation of 20 m^3/h of whole whey for 250 d/y plant operation time would cost M\$2.8 and provide an internal rate of return of 14% (after tax) if the oil was sold at \$0.7/kg. The oleaginous yeast that was employed in the fermentation stage was *Candida curvata*. It should be stressed that this process aimed at the utilization of MO mainly for food purposes. Ratledge and Cohen [23] reported that the MO production cost from yeast (or fungi) in 2008 would be much greater than \$3/kg. However, there is no detailed study available in the open literature on the estimation of the production cost of MO or biodiesel from MO.

This study focuses on the techno-economic evaluation of MO production and subsequent potential utilisation as raw material for biodiesel production. Four different flowsheets have been evaluated that are based on the production of yeast cells, purified MO and biodiesel produced either by direct or indirect transesterification of MO. A varying cost of glucose has been considered to assume varying upstream processing costs depending on the glucose-based resource available in different plant locations. Thus, in this study the economic potential of MO as raw material for the production of biodiesel or oleochemicals is evaluated.

2. Methodology and process flow diagrams description

2.1. Description of design strategy

Costing studies were based on preliminary economic analysis (accuracy up to ±30%) that were carried out for the estimation of the total capital investment and operating cost for process flowsheets that can be used for the production of MO and biodiesel. The industrial plant is assumed to operate 8300 h/y with an annual production capacity of 10,000 t/y. The material and energy balances were carried out using spreadsheets and validated using SuperPro Designer (Intelligen, Inc.) and UniSim (Honeywell). Equipment sizing was performed using well known procedures and rules of thumb [24–27].

2.2. MO production process

The development of the process flow diagram (PFD) for the MO production process was based on the experimental results provided by Li et al. [14]. The fermentation was carried out in fed-batch mode and the microorganism used was *Rhodosporidium toruloides*. The total dry weight of microbial cells and MO at the end of the fermentation were 106.5 g/L and 71.9 g/L corresponding