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Research article

Effect of synthetic and natural media on lipid production from *Fusarium oxysporum*

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ABSTRACT

Background: Dependence on fossil resources, for the production of fuels and energy, has resulted in environmental and financial problems, which require our immediate action in order to reverse the situation. Use of renewable sources for the production of fuels and energy is an important alternative with biodiesel remains as one of the promising options. Aim of this work is to evaluate the fungus *Fusarium oxysporum* for its potentials to accumulate microbial lipids when grown on synthetic media and saccharified sweet sorghum stalks.
Results: The effect of different carbon sources, nitrogen sources and C/N ratio on the lipid production was initially examined, which resulted in a lipid concentration of 4.4 g/L, with lipid content of 42.6% *w*/*w*. Sweet sorghum stalks were able to support growth and lipid production of the fungus, both as carbon source and as nitrogen source. It was also shown that saccharification of the dried stalks is an important step to increase lipid production. Removal of the remaining stalk solids enabled the lipid production during cultivation in increased initial solids of up to 16 *w*/*w*. This resulted in a lipid production of 3.81 g/L.
Conclusions: It was demonstrated that *F. oxysporum* can be used as an efficient oleaginous microorganism, with sweet sorghum serving as an excellent raw material for the cultivation of the fungus. The lipids obtained during this work were also found to have a fatty acid profile with good potentials to be used for biodiesel production.

1. Introduction

Our society strongly depends on fossil fuels, with 81% of world's energy derived from fossil fuels (cruel oil, coal and natural gas); with oil being the most consumed fuel in the world and used primarily for transportation [1]. The drawbacks of using fossil sources are that they are being depleted and their usage contributes to greenhouse gas (GHG) emission, which leaves a great negative impact on environment. Moreover, the need for importation of fossil fuels results in energy insecurity. Progressive decrease of fossil fuels, environmental problems, and increased energy consumption lead to a demand for more alternative and renewable energy sources [2]. Among many alternatives, biofuels have been showed to be a promising solution to replace nonrenewable fuels. They have attracted much attention due to their renewability, biodegradability and improved quality of exhaust gases [3]. Biofuels can be categorized into liquid, gas and solid fuels that are mainly produced from biomass [4]. In the last decade, biodiesel has attracted attention as a renewable and environmentally friendly fuel that can replace petroleum derived fuels [5].

Biodiesel is a mixture of fatty acid methyl esters (FAMEs) produced by transesterification of triacylglycerols (TAGs) in the presence of an alcohol (the most commonly used is methanol) and an acidic or basic catalyst, with vegetable oil often serving as the source of TAGs [6,7]. Biodiesel is a renewable, non-toxic, biodegradable, nonflammable, environmentally friendly fuel and does not contain sulfur or aromatic compounds [8]. Moreover, as biodiesel contains higher oxygen content compared to conventional diesel, when used in diesel engine the exhaust emissions have lower concentration of particles, carbon monoxide, sulfur, polyaromatics, hydrocarbons and smoke. Finally, the use of fuels derived from vegetable oils can be considered as "carbon neutral", as their burning does not contribute to the net production ofatmospheric CO_2 since the plants capture atmospheric CO_2 via photosynthesis [3,9]. Different sources of TAGs can be utilized forbiodiesel production. Most common sources are vegetable oils (e.g. sunflower oil, soybean oil) and animal oils (e.g. beef and sheep tallow and poultry oil) [4]. However, using edible raw materials for biodiesel production is controversial and it raised a lot of criticism as it is the main cause of increased global food market prices. The main factor affecting the economic viability of biodiesel market is the price of the

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feedstock [10]. In order to make biodiesel more competitive and at the same time avoid the fuel vs food dilemma, utilization of non-food crops, agro-industrial wastes and other non-edible renewable resources is needed [10,11].

One of the alternative sources of TAGs is microbial oils or single cell oils (SCOs). SCOs are produced by microorganisms with the ability to accumulate lipids more than about 20% (*w*/*w*) of its total dry biomass weight and are considered as oleaginous [12]. Microbial sources of TAGs present a promising feedstock for biodiesel, because of the short production time, little labor required and the potentials so scale up the process [13]. Storage of lipids in cells of oleaginous microorganisms occurs during secondary metabolic growth, under conditions where carbon is in excess and another essential nutrient (most often nitrogen) is limiting [14]. Oleaginous microorganisms can be found in bacteria, yeasts, algae and fungi genera [15], with some filamentous fungi able to accumulate lipids as high as 80% of their cell biomass [16]. Fungi present some positive characteristics such as short life cycles, no need of light energy, they are easily scalable and can use a wide range of carbon sources, such as lignocellulosic material, agro-industrial residues and wastewater [14]. There are several filamentous fungi that can accumulate lipids such as *Aspergillus oryzae*, *Claviceps purpurea*, *Humicola lanuginose*, *Mortierella isabellina*, *Mortierella vinacea* and *Mucor circinelloides* [10,15]. Some fungi strains are also capable of accumulating polyunsaturated fatty acids such as: docosahexaenoic acid (DHA), γ-linolenic acid (GLA), eicosapentaenoic acid (EPA) and arachidonic acid (ARA) [13].

Fusarium oxysporum is a filamentous fungus that is capable of excreting cellulases and hemicellulases, making it an ideal candidate to be cultivated on different lignocellulosic materials [17]. *F. oxysporum* has been extensively evaluated for the production of cellulolytic and hemicellulolytic enzymes [18,19,20,21], and as a fermenting microorganism for the production of ethanol from various lignocellulosic raw materials [17,22,23,24]. Apart from ethanol, *F. oxysporum* was also evaluated as an oleaginous microorganism [25,26, 27,28] although not as extensively as in ethanol. A potential advantage of using *F. oxysporum* as oleaginous microorganism is the ability of the fungus to secrete cellulolytic and hemicellulolytic enzymes. These enzymes can facilitate the hydrolysis of cellulose and hemicellulose of lignocellulosic raw materials and in turn reduce the amount of enzymes required for the preparation of lignocellulosic hydrolysates. Lower enzyme loads are beneficial for reducing the production cost of the process.

One of the main challenges in the commercialization of SCOs production is the high cost of the feedstock for the cultivation of the oleaginous microorganisms [29]. It is estimated that using commercial glucose for the cultivation of oleaginous microorganisms can account for the 80% of the total material cost, which is equal to 35% of the overall biodiesel production cost [11]. To avoid high production costs, utilization of low-cost material such as lignocellulosic material, which do not compete with food production, has been employed [29]. Among the different options the use of energy crops, such as sweet sorghum, offers a sustainable solution. Sweet sorghum (*Sorghum bicolor* L. Moench) is a C4 crop in the grass family that can grow to heights from 120 cm to above 400 cm [30]. Sweet sorghum possesses an efficient photosynthetic system, is rich in soluble sugars, can grow rapidly, has great water- and nitrogen- use efficiency, requires little chemical fertilizers, can tolerate harsh environments and can adapt to marginal lands [31,32,33]. One of the challenges of using sweet sorghum is that, due to the high concentration of sugars present in sweet sorghum, the stalks can easily get contaminated if stored at room temperature. To prevent microbial contamination and also to reduce the total volume of the stalks, a drying step, under mild conditions, was previously proposed [34]. Another challenge of using sweet sorghum for the cultivation of oleaginous microorganisms is the relatively low carbon to nitrogen (C/N) ratio which was estimated to be approximately 60–65 [35]. Increase of the concentration of carbon and subsequent of the C/N can be achieved by partially hydrolyzing the insoluble carbohydrates (such as cellulose) that are present in the sweet sorghum stalks.

The aim of this work is to examine the use of the fungus *F. oxysporum* F3 as an oleaginous microorganism. Initially, a study of the potential of the fungus for accumulating lipids took place on synthetic media mimicking the composition of sweet sorghum to examine and optimize different cultivation parameters. Finally, the ability of the fungus to grow on saccharified sweet sorghum stalks was evaluated in terms of optimizing the accumulation of lipids and the results were compared with other oleaginous fungi growing on lignocellulosic raw materials.

2. Materials and methods

2.1. Raw material, microorganism and its maintenance

Sweet sorghum (*S. bicolor* (L.) Moench) belonging to the Keller cultivar was used in the current work and was kindly provided by Prof. George Skarakis (Department of Crop Science, Agricultural University of Athens). Sweet sorghum stalks were stored at -20°C, after the leaves and seeds were removed by hand. Dried stalks were prepared as previously described [34] and were milled to 0.75 mm particles. The carbohydrate content of dried sweet sorghum stalks was as follows (% *w*/*w*): cellulose, 19.6; fructose, 8.1; glucose, 8.2; hemicellulose, 15.2 and sucrose, 34.4 [34]. The fungus strain used in this work was *F. oxysporum* F3, which was previously isolated from cumin [36]. The fungus was maintained on agar plate with following composition: 39 g/L potato dextrose agar and 2 g/L yeast extract.

2.2. Pre-culture media

Prior to each experiment, the fungus was inoculated into 250 mL Erlenmeyer flasks containing 50 mL of pre-culture broth with following composition: $CaCl_2 \cdot 2H_2O$, 0.3 g/L; KH_2PO_4, 1 g/L; $MgSO_4 \cdot 7H_2O$,0.3 g/L; $NaH_2PO_4 \cdot 2H_2O$, 6.94 g/L; $Na_2HPO_4 \cdot 2H_2O$, 9.52 g/L; glucose, 20 g/L; $(NH_4)_2HPO_4$, 10 g/L [17]. The pH of the pre-culture broth was adjusted to 6, followed by sterilization at 121°C for 20 min. Inoculation was done using a loop containing cells from agar plates and incubation of the pre-culture media was carried out at 30°C and 160 rpm for 48 h.

2.3. Cultivation of F. oxysporum on synthetic media

Cultivation of the fungus on synthetic media to evaluate its ability to accumulate lipids took place in 1 L Erlenmeyer flasks containing 200 mL of cultivation broth with a medium of the following composition: $CaCl_2 \cdot 2H_2O$, 0.3 g/L; $MgSO_4 \cdot 7H_2O$, 0.3 g/L; $NaH_2PO_4 \cdot 2H_2O$, 6.94 g/L; $Na_2HPO_4 \cdot 2H_2O$, 9.52 g/L. The pH of the cultivation broth was adjusted to 6 and sterilized at 121°C for 20 min. To determine the effect of the sugars present in sweet sorghum stalks on the growth and lipid accumulation of the fungus, preliminary tests were done using glucose, fructose and sucrose alone or in a combination similar to the one found in sweet sorghum stalks. The initial sugar concentration was 40 g/L and a mixture of yeast extract and ammonium sulfate was used as nitrogen source, at a concentration resulting in a C/N ratio of 100. For the trials determining the effect of nitrogen source and of the C/N ratio on the fungus growth and lipid accumulation ability, the mixture of the sugars was used as a carbon source at a concentration of 40 g/L, whereas the concentration of the nitrogen source varied in order to achieve the desired C/N ratio, which, during the study of the nitrogen source effect, was set as 100. Inoculation took place with 5% v/v of pre-culture media. All the cultivations were performed in duplicates.