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Two stage biodiesel and hydrogen production from molasses by oleaginous fungi and *Clostridium acetobutylicum* ATCC 824

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ABSTRACT

In the present study biodiesel was produced by various fungal species isolated from Egypt using sugarcane molasses as substrate. In the first stage 6 oleaginous fungi, namely, *Alternaria alternata*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, *Fusarium oxysporum*, *Aspergillus parasiticus* and *Emericella nidulans* var. *lata* were used for lipid production. Subsequent to fungal cultivation on sugarcane molasses the cultures were filtered and biodiesel was prepared by direct esterification of dry fungal biomass. Methyl esters of palmitic, stearic, linoleic and elaidic represented the major components while palmitoleic represented a minor component of biodiesel produced from tested oleaginous fungi. In the second stage, the spent medium of fungal culture was used as the fermentation medium for hydrogen production by *Clostridium acetobutylicum* ATCC 824. The maximum total H_2 yield was obtained with the spent medium of *E. nigrum* and *A. alternata*. The results presented in this study suggest a possibility of interlinking the biodiesel production technology by fungi with hydrogen production by *C. acetobutylicum* ATCC 824 to exploit the residual sugars in the spent media and therefore increase the economic feasibility of the biofuel production from molasses.
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1. Introduction

Fossil fuels are not renewable and will be exhausted sooner or later [1]. The increase in prices of petroleum based fuels, future depletion of worldwide petroleum reserves and environmental polices to reduce CO_2 emissions have stimulated research into the development of biotechnology to produce clean energy from renewable resources [2]. Thus, it is necessary to find alternative energy sources that are renewable and environmentally friendly [3–8]. Hydrogen and biodiesel are very promising alternative energy sources and have received more attention all over the world in recent years.

Biodiesel fuel is fatty acid mono alkyl (methyl, propyl or ethyl) esters produced from natural, renewable sources such as vegetable oils or animal fats by transesterification of such triglycerides [9,10]. Biodiesel is rather an attractive alternative to the conventional petroleum diesel fuels because of its biodegradable, nontoxic and clean renewable characteristics [10,11]. One way to increase the world bio oil production that would cause low ecosystem impact is to use lipids from single-cell oil microorganisms [12] for biodiesel production.

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Table 1 – Some constituents of sugarcane molasses.

Constituent	mg/g
Total sugars	388
Total proteins	29
Total nitrogen	4.6
Sodium	0.85
Potassium	24.34
Calcium	5.3
Magnesium	1.9
Phosphorus	0.78
pH	5.5

Oleaginous yeasts and fungi have also been considered as potential oil sources for biodiesel production because they accumulate large amounts of lipids. Among these microorganisms, particular attention has been dedicated to *Epicoccum purpurascens* [13] and *Mortierella isabelina* [14].

Hydrogen is one of the most abundant elements in the universe. It is an odorless, colorless, tasteless and non-poisonous gas. Hydrogen gas has been proposed as the ultimate transport fuel for vehicles and vessels because of its non-polluting characteristics. When hydrogen is used as a fuel, it generates no pollutants but produces water. Hydrogen also enables the use of highly efficient fuel cells to convert chemical energy to electricity [15]. Another major use of hydrogen is in reduction reactions to produce industrial chemicals.

Hydrogen is produced mainly from natural gas, a finite resource, through steam reforming, a process that generates large quantities of carbon dioxide (CO_2) which is a principal cause of global warming. Hydrogen production through dark or photofermentative conversion of organic substrates is of great interest due to its dual function of waste reduction and clean energy production, thereby acting as a promising option for biohydrogen production [16–18]. Anaerobic bacteria capable of hydrogen production can use various renewable biomass and numerous agriculture, municipal and food processing waste and wastewater sources [19,20].

Molasses is the by-product from sugar industry and is considered as one of the most promising feedstocks in the production of biofuel. Black strap molasses is another byproduct of the sugar industry, obtained during sugarcane refining and, like beet molasses, has a high sugar content, around 50% (36% sucrose, 6% fructose, 3% glucose), making it a promising substrate for biohydrogen production [21]. Sugarcane molasses is also an important organic waste due to its high sugar content (55%) and high volume of production. It is even more viscous than beet molasses and the total sugar content is higher. The availability and cost of sugarcane molasses make it an attractive feedstock for use in many countries.

The generation of lipid by fermentative oleaginous fungi accompanies the formation of organic acids as metabolic products [22]. The accumulation of these acid results in a sharp drop in culture pH and subsequent inhibition of fungal growth. But, it is somewhat difficult to achieve the complete utilization of molasses sugar to lipid through fungal fermentation. This appears to be one of the major bottlenecks in the molasses fermentation process for lipid production. So, the process outlined in the present paper takes into consideration the non-utilization of sugar and the acids produced therein (spent media). Combining fermentative oleaginous fungi with hydrogen producing *Clostridium* could provide an integrated system for maximizing the biofuel yield from sugarcane molasses. In such a system, the fermentation of spent medium containing residual sugar and organic acids generated by fungi, which are then converted into hydrogen by *Clostridium* in the second step in a bioreactor. In the present study, sugarcane molasses was considered as a preliminary substrate for biodiesel production by various fungal species isolated from Egypt and the spent medium from this process was used as a substrate for hydrogen production by the strictly anaerobic *Clostridium acetobutylicum* ATCC 824 that also

Table 2 – Lipid production by various species of *Aspergillus* grown on sugarcane molasses.

Fungal isolates	Dry mass (g/25 ml culture)	Lipid content (mg/g)	% Lipid content	Yield (mg/25 ml culture)
Aspergillus aculeatus	0.445 ± 0.178	146 ± 24.3	14.6	64.97
A. aegyptiacus	0.439 ± 0.085	135.2 ± 26.1	13.52	59.35
A. awamorii	0.475 ± 0.021	81.8 ± 23.7	8.18	38.86
A. candidus	0.448 ± 0.08	128 ± 27.9	12.8	57.34
A. carneus	0.341 ± 0.05	119.3 ± 28	11.93	40.68
A. clavatus	0.449 ± 0.007	106.1 ± 11.4	10.61	47.64
A. flavus	0.449 ± 0.018	130.2 ± 28.9	13.02	58.46
A. flavus var. *columnaris*	0.547 ± 0.045	48.8 ± 7.9	4.88	26.69
A. fumigatus	0.324 ± 0.009	74.5 ± 5.98	7.45	24.14
A. japonicus	0.524 ± 0.029	90.6 ± 10.2	9.06	47.47
A. melleus	0.425 ± 0.013	100.6 ± 26.3	10.06	42.76
A. niger	0.619 ± 0.083	145 ± 7	14.5	89.76
A. ochraceus	0.359 ± 0.008	143.9 ± 24.5	14.39	51.66
A. oryzae	0.535 ± 0.015	139.4 ± 7	13.94	74.58
A. parasiticus	0.486 ± 0.005	282.8 ± 70	28.28	137.44
A. sydowii	0.41 ± 0.049	141 ± 33.1	14.1	57.81
A. tamari	0.564 ± 0.1	160.8 ± 11.4	16.08	90.69
A. terreus	0.435 ± 0.087	157.1 ± 26.9	15.71	68.34
A. terricola	0.495 ± 0.137	107.7 ± 16.5	10.77	53.31
A. wentii	0.459 ± 0.068	153.1 ± 4.4	15.31	70.27

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