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SOYBEAN MOLASSES AS SUBSTRATE FOR THE PRODUCTION OF SINGLE CELL OILS BY FILAMENTOUS FUNGI

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ABSTRACT

Alternative low-cost carbon and nutrient sources, as agro-industrial by-products, are essential for a feasible industrialization of microbial oils production. Here, soybean molasses was evaluated as a low-cost substrate for Mucor circinelloides URM 4182 growth and lipid accumulation. Three culture media based in soybean molasses were analyzed, including the utilization of synthetic growth factors and corn steep liquor as added nutrients. Robust biomass growth (up to 9.6 g L^{-1}) and satisfactory lipid content (18-26 wt.%) were obtained for all tested media, although addition of extra nutrients did not represent a valuable strategy to increase lipid productivity. Fatty acid profile showed major composition of palmitic acid (C16:0) and suitable characteristics for biofuel and nutraceutical applications. Results indicated soybean molasses is a promising alternative low-cost carbon and nutrient sources for microbial oil production.

1. INTRODUCTION

Soybean molasses is a by-product generated from soy protein concentrate manufacturing that not only has high carbohydrate content (mostly sucrose and raffinose-family oligosaccharides), but also organic nitrogen and other nutrients which can support microbial growth, as amino acids and minerals. Several studies have addressed the use of soybean molasses as a carbon and nutrient sources for microorganism growth and production of surfactants, antimicrobial agents, enzymes, organic acids and ethanol (Cheng et al., 2017; Al Loman; Ju, 2016).

The use of agro-industrial by-products and wastes as feedstock for single cell oil production is highlighted as a promising alternative to the traditional high-cost sugar-based fermentation processes for typical industrial-scale biosynthesis. Single Cell Oil (SCO) are microbial lipids obtained from bacteria, yeast, fungi and microalgae, usually presenting similar composition to vegetable oils, and have been studied as a potential resource for food and fuel applications (Carvalho et al., 2019; Bharathiraja et al., 2017).

Bento et al. (2019) demonstrated the potential usage of sucrose based media as substrate for fungal lipid accumulation and enzymatic microbial biodiesel synthesis using the filamentous fungus *Mucor circinelloides* URM 4182. Herein, soybean molasses was evaluated as a potential substrate for SCO production using this same oleaginous strain (URM 4182), and the properties of the microbial lipids were assessed regarding the fatty acid composition to elucidate their suitability for industrial applications.

2. MATERIAL AND METHODS

2.1. Microorganism, Culture Media and Bioreactor Operation

M. circinelloides f. *griseo-cyanus* URM 4182 was obtained from the mycology collection (URM) from the Federal University of Pernambuco, Brazil. The strain was maintained in PDA (Potato Dextrose Agar) at 30◦C for 72 h. Soybean molasses was gently supplied by Mellaço de Cana (Aparecida II Unit, located in Saltinho - São Paulo) and used without further treatment. Soybean molasses was used to formulated three culture media containing 40 g L^{-1} of total sugar content and the influence of supplementation was analyzed by addition of Corn Steep Liquor (1 vol.%) or synthetic growth factors (ammonium sulfate, 1.5 g L^{-1}, glutamic acid, 1.5 g L^{-1}, nicotinic acid, 1 mg L^{-1}, thiamine, 1 mg L^{-1} and yeast extract, 0.5 g L^{-1}). Cell cultivations were performed in bioreactor model BioFlo®/CelliGen®115, with 1 L capacity, and carried out aerobically (via air supply set to 1.5 vvm and agitation at 250 rpm) at pH 4.5, 26 °C for 120 h. The initial spore concentration was set at 1×10^5 spores mL^{-1}.

2.2. Lipid Extraction and Fatty Acid Profile Analysis

After fungal growth period (120 h), the biomass was harvested from the culture medium by centrifugation (1520 ×g) for 15 min and filtered. Lipids were extracted from *M. circinelloides* biomass using a microwave-extraction system Model Discover/University-Wave, Cem Corporation as described by Carvalho et al. (2017). The glass vessel containing 1.0 g (dry weight) of wet fungal biomass and 50 mL of ethanol (96%) was heated in microwave reactor at 60 °C for 30 min. The ethanol extract containing lipids was recovered and the solvent was removed in a vacuum rotary evaporator. The flask was subsequently dried at 60 °C until constant weight. Lipid content was calculated as indicated in Equation 1. Fatty acid composition was determined as fatty acid methyl ester (FAME) by gas chromatography according to American Oil Chemists' Society (AOCS) official method Ce 1–62 as described by Carvalho et al. (2019).

$$Lipid\ content\ (\%) = \frac{Lipid\ concentration\ g\ L^{-1}}{Dry\ cell\ concentration\ g\ L^{-1}} \times 100 \quad (1)$$

3. RESULT AND DISCUSSIONS

All three analyzed culture media provided typical cell growth in 120 h (6.5 – 9.6 g L^{-1}) and fungal biomass presented satisfactory lipid content (18-26 wt. %). Results displayed in Table 1 demonstrated that despite higher lipid contents were attained in media supplemented with synthetic nutrients (1.93 g L^{-1}), the simple media containing non-supplemented soybean molasses provided similar lipid concentration (1.69 g L^{-1}) achieving 26% g lipid g^{-1} biomass. These results also showed that lipid productivity can be slightly increased by the addition of nutrients (synthetic or corn steep liquor - CSL). However, the additional expenditures involved in the addition of synthetic nutrients to the process may not be economic interesting, since the values of lipid productivities are similar (0.34 – 0.39 g L^{-1} day^{-1}). On the other hand, fungal biomass productivity was increased by almost 50% when CSL was employed, what may be interesting for the exploitation in animal feed as protein and nutrient source even after oil extraction. The fatty acid profile of the extracted lipids is indicated in Table 2. All samples presented similar fatty acid composition, palmitic acid (C16:0) was the major component (29.92 – 31.20 wt.%) followed by oleic acid (C18:1, ranging from 13.72 to 13.93%).

Table 1. Parameters of *Mucor circinelloides* URM 4182 grown on soybean molasses based media for 120 h at 26 °C and 250 rpm.

	Soybean Molasses	**Soybean Molasses + nutrients**	**Soybean Molasses + CSL**
Biomass, X (g L^{-1})	6.5	8.8	9.6
Lipid (g L^{-1})	1.69	1.93	1.73
% Lipid (g g^{-1} biomass)	26.0	22.0	18.0
Biomass productivity (g L^{-1} day^{-1})	1.30	1.76	1.92
Lipid productivity (g L^{-1} day^{-1})	0.34	0.39	0.35

Table 2. Fatty acid profile of extracted microbial oils obtained from *Mucor circinelloides* URM 4182 grown on soybean molasses based substrates.

Fatty acids	**Soybean Molasses**	**Soybean Molasses + nutrients**	**Soybean Molasses + CSL**
C8:0	11.63	10.06	9.45
C10:0	11.26	9.71	8.99
C12:0	6.53	10.43	8.63
C14:0	4.08	3.53	4.70
C16:0	31.20	30.76	29.92
C16:1	0	2.39	2.95
C18:0	2.10	1.97	1.67
C18:1	13.72	13.85	13.93
C18:2	12.51	9.68	11.58
C18:3	6.97	7.62	8.18

Such composition is highly adequate for biodiesel production, since the mostly founded fatty acids provide desirable characteristics in the final biofuel, as oxidative stability and viscosity as results obtained by Carvalho et al. (2017). All extracted lipids presented high levels of polyunsaturated fatty acids (PUFAs), indicating these oils can be a potential source of essential fatty acids, as linoleic acid (C18:2) and gamma-linolenic acid (C18:3).

4. CONCLUSIONS

The results showed soybean molasses is an interesting carbon and nutrient sources to support oleaginous filamentous fungi growth. It may represent a significant approach in diminishing microbial oil production costs, as it is a highly available low-cost agro-industrial by-product and does not need any extra supplementation to fulfill the culture media requirements. Fatty acid profile of the extracted microbial oils showed a balanced composition of saturated, monounsaturated and polyunsaturated fatty acids, suitable for use in biofuel and nutraceutical industries.

5. REFERENCES

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