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Introduction

Continuous depletion of fossil fuel reserves worldwide, soaring petroleum prices as well as increasing greenhouse gas emissions by the utilization of fossil fuel reserves has led to the culmination of research on alternate renewable energy sources [Sitepu *et al*., 2014a]. Amongst the various renewable energy sources, bioenergy sources are particularly promising in providing energy security for the future. Biofuels are the energy sources derived from living organisms or the waste generated by them. The biofuels derived from the plant and biological wastes are the only fuels that are available in liquid, solid or gaseous form and can replace the conventional transport fuels.

Biodiesel is alkyl esters derivative of long-chain fatty acids from plant, microbe or animal-based oils. It can be used directly or as blends with the Petro-diesels in the existing transport vehicles without engine modifications. The microbes belonging to algae, yeast, bacteria, and fungi, which can produce cellular lipids more than 20 percent of their cellular dry weight, are known as oleaginous microbes [Ratledge, 2004].

The single-cell oils (SCO) obtained from these microbes offer an alternative renewable feedstock for biodiesel production, also known as third-generation biodiesel. Oleaginous yeasts can produce lipids up to 75-80 percent (w/w) of their cell dry weight, and hold particular interest among all the oleaginous microbes due to their peculiar nature. These yeasts primarily accumulate lipids as lipid droplets inside the cells. These droplets are composed of triglycerides (TAG) made up of C_{16} and C_{18} fatty acids and few steryl esters. The fatty acid profile of most of the oleaginous yeasts is similar to palm and other vegetable oils [Schilter, 2019]. It makes oleaginous yeast a suitable candidate for the SCO production.

1.1 PURPOSE AND OBJECTIVES OF THE STUDY

Yeasts are ubiquitous, and a broad range of natural environments can be screened for their isolation. Currently, there are more than 1500 yeast species are identified [Kurtzman *et al*., 2011]; out of that, around 70 species are characterized as oleaginous yeasts [Johnson and Echavarri-Erasun, 2011; Sitepu *et al*., 2014a]. Studies indicate that the probability of finding oleaginous yeast in the randomly selected sample is 3-10 % [Sitepu *et al*., 2014a]. Oleaginous yeasts possess immense potential to serve as a source of oils for future fuels, food, feed, and chemicals [Blomqvist *et al*., 2018]. The systematic screening strategies enable the discovery of novel oleaginous yeasts.

Yeasts belonging to genera *Rhodotorula, Cryptococcus, Yarrowia, Lipomyces, Rhodosporidium,* accumulate lipids using pre-treated lignocellulosic or industrial waste, and other renewable feedstock [Leiva-Candia *et al*., 2014]. Considering the consolidated bioprocessing of biomass, it is necessary to find cellulolytic oleaginous yeast. The reports of cellulolytic yeasts are relatively fewer in literature [Baldrian and Vendula, 2008; Strauss *et al*., 2001]. Similarly, lipid isolation and trans-esterification is a cumbersome process that adds substantial industrial cost. In an attempt to generate biodiesel in vivo, yeast capable of producing cellulases, accumulating lipid, and producing an intracellular/extracellular lipase for

in vivo transesterification is searched. Lipase catalyzed transesterification holds a special interest in next-generation biodiesel synthesis [Aguieiras *et al.,* 2015].

Isolation and characterization of a strain must follow the applicability on the real industrial wastes/wastewaters. An ideal strain should be able to utilize industrial/agricultural waste without elaborate pre-treatments, be robust enough to tolerate toxins/inhibitors that may be present in the waste, and give reproducible lipid yields [Martínez *et al*., 2015; Patel *et al*., 2018; Vasconcelos *et al*., 2019].

Keeping all these points in consideration and after reviewing the state of the art, the following objectives are defined for this study:

1. Isolation, identification, and characterization of oleaginous yeast capable of cellulase and lipase production
2. Assessment of the biodiesel potential of the selected yeast using industrial wastewater.
3. Assessment of cell growth and lipid accumulation by yeast in the presence of inhibitors and its ability to grow on agro-industrial wastes.

1.2 BRIEF RESULTS, SCOPE AND FUTURE PROSPECTS OF THE WORK

This thesis is divided into six chapters. The second chapter gives a detailed literature review on the oleaginous yeasts. The limitations and drawbacks of the existing procedures are also discussed. Next three chapters discuss the detailed experimental outcomes which are summarized as follows:

Isolation, identification, and characterization of *Cystobasidium oligophagum* JRC1: A cellulase and lipase producing oleaginous yeast (Chapter 3: published as Vyas and Chhabra, 2017; Bioresource Technology, 223; 250-258)

Oleaginous yeast closely related to *Cystobasidium oligophagum* was isolated from soil rich in cellulosic waste. The yeast was isolated based on its ability to accumulate intracellular lipid, grow on carboxymethylcellulose (CMC), and produce lipase. It could accumulate up to 39.4445 ± 1.1995 % lipids in a glucose medium (12.4533 ± 0.9743 g/L biomass production). It was able to accumulate lipids (36.4615 ± 1.4997%) in the medium containing CMC as the sole substrate. The specific enzyme activities obtained for endoglucanase, exoglucanase, and β-glucosidase were 2.2701 ± 0.0070, 1.2602 ± 0.0400, and 0.9801 ± 0.0400 IU/mg, respectively. The specific enzyme activities obtained for intracellular and extracellular lipase were 2.1637 ± 0.0433 and 2.8835 ± 0.1667 IU/mg, respectively. It could grow and accumulate lipids in substrates including glycerol (42.0403 ± 1.7136%), starch (41.5491 ± 0.3469 %), xylose (36.2410 ± 1.0975 %), maltose (26.3114 ± 1.8228 %), fructose (24.2933 ± 0.9087 %), lactose (21.9134 ± 0.6388 %) and sucrose (21.7229 ± 1.7760 %). The lipid profile of the organism was suitable for obtaining biodiesel with desirable fuel properties.

Assessing oil accumulation in the oleaginous yeast Cystobasidium oligophagum JRC1 using dairy waste cheese whey as a substrate (Chapter 4: Published as Vyas and Chhabra, 2019;3 Biotech, 9;173)

This study assesses the potential for lipid production by the oleaginous yeast *C. oligophagum* JRC1 using the dairy industry waste cheese whey as a substrate. Cheese whey was used either untreated (UCW) or deproteinized (DCW) at different concentrations (25-100 %v/v) to serve as the carbon and energy source. Both UCW and DCW supported high biomass and lipid productivities. The biomass productivity of 0.0760 ± 0.0004 and 0.1240 ± 0.0021 g/L. h, lipid productivity of 0.0335 ± 0.0004 and 0.0272 ± 0.0008 g/L. h and the lipid content of 44.1210 ± 0.8497 and 21.7997 ± 01.0050 % were achieved for 100 % DCW and UCW, respectively. The

soluble chemical oxygen demand (sCOD) removal rate was 8.0490 ± 0.1980 and 10.6103 ± 0.1656 g/L. d (84.9125 ± 0.1553 and 86.8287 ± 0.0679 % removal) for 100 % DCW and UCW respectively. Fatty acid methyl ester (FAME) composition obtained using GC-FID studies revealed the presence of C16 and C18 fatty acid in the lipid extract, and the biodiesel properties were found to be following ASTM and EN standards. The study presents a method for the valorization of cheese whey waste into a feasible feedstock for biodiesel.

Cystobasidium oligophagum JRC1 tolerance assessment on inhibitors released on Lignocellulosic biomass hydrolysis and growth on acid hydrolyzed agro-industrial wastes

Effect of three most common inhibitors found in pretreated lignocellulosic biomass, namely 5-Hydroxymethylfurfural (5-HMF), furfural, and acetic acid on the reducing sugar uptake, growth and lipid accumulation by oleaginous yeast *C. oligophagum* JRC1 was studied. The yeast showed a moderate level of resistance to the 5-HMF and acetic acid. It was highly sensitive to furfural and mixture of all the inhibitors. The yeast could accumulate lipids up to 26.5374 ± 1.7210, 27.4663 ± 2.2252, and 21.0599 ± 2.7903 % (w/w) for 2.0, 1.0, and 1.0 g/L of 5-HMF, acetic acid, and furfural respectively (as compared to 40. 7554 ± 2.8910% for control). The lipid content of 30.7214 ± 1.4616 % was observed when all the inhibitors were provided at 0.25 g/L concentration. The isolate was able to accumulate lipids up to 1.4787 ± 0.2967 g/L (27.3589 ± 2.644 % of CDW) and 1.0161 ± 0.2373 g/L (23.3448 ± 3.2755 % of CDW) on undetoxified industrial sugarcane bagasse and corn cob acid hydrolysates. The lipid extracts were analyzed and compared with control experiments using TLC and NMR and were found suitable as biodiesel feedstocks.

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