**Microbial oil production using hemicellulose hydrolysate as feedstock**

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**Highlights**

* Spent sulphite liquor was used as feedstock for microbial oil production
* Microbial oil derived esters were produced via enzymatic synthesis
* Phospholipids caused inhibition on the transesterification process

**1. Introduction**

Waste valorization through microbial and enzymatic bioprocesses contributes to the growth of bioeconomy and the elimination of the severe environmental impact caused by the petrochemical industry. Spent sulphite liquor (SSL) is the concentrated liquid stream generated by the pulp and paper industry. Valorisation of SSL through fermentation is challenging due to the high content of inhibitory compounds, such as lignosulphonates (LS), phenolics and organic acids. However, the presence of C5 and C6 sugars create an important feedstock for fermentation processes.

Oleaginous fungi of the phylum *Zygomycota* and yeasts (e.g. *Lipomyces starkeyi*) have been reported to effectively produce lipids, when cultivated on lignocellulosic-derived hydrolysates. *Zygomycetes* can produce γ-linolenic acid (GLA) [1]. Microbial oils rich in GLA can be used in the food industry, nutraceuticals and cosmetics [2]. They have also been used for the production of biodiesel and oleochemicals, such as biolubricants and wax esters [3].

The objective of this study was the valorisation of SSL as fermentation feedstock for microbial oil production using oleaginous yeast and fungal strains aiming to the improving fermentation efficiency through the evaluation of different fermentation conditions (carbon to nitrogen ratio and LS concentration) in batch and fed-batch fermentations. In the case of fungal fermentations, the optimum conditions were identified for the production of GLA-rich microbial oil. GLA-rich lipids from *C. echinulata* were subsequently used as feedstock for the production of bio-based esters via enzymatic synthesis in a solvent-free system. The reaction conditions evaluated were temperature, substrate molar ratio and biocatalyst quantity. Under the optimized conditions, the impact of individual lipid classes to esters yield was assessed.

**2. Methods**

The yeast *L. starkeyi* DSM 70296 and 2 fungal strains *C. echinulata* ATHUM 4411 and *Mortierella isabellina* ATHUM 2935 were utilized for the production of microbial lipids. Initially, the effect of carbon to nitrogen ratio (calculated and expressed as carbon to free amino nitrogen ratio - C/FAN ratio) was evaluated for all microbial strains using nanofiltrated SSL (30 g/L total sugars and 5 g/L of LS) in shake flask fermentations. The best C/FAN ratio for each microorganism was applied and the effect of various LS concentrations on lipid production was evaluated using sugar-simulated SSL. Sugar-simulated SSL was prepared using commercial sugars (xylose, glucose, galactose, mannose and arabinose) at the same proportion as in diluted nanofiltrated SSL. Subsequently, *L. starkeyi* and *C. echinulata* were selected for further evaluation in fed-batch fermentations using a bench-top bioreactor. Nanofiltrated SSL was used as fermentation medium. In the case of *L. starkeyi*, experiments were conducted at various C/FAN ratios.

Microbial oil and fractionated lipid classes, including neutral lipids (NL), glycolipids plus sphingolipids (GL+SL) and phospholipids (PL), were used with isopropanol and 2-ethylhexyl alcohol for the enzymatic production of fatty acid esters in a solvent free system catalyzed by Novozyme 435. Transesterifications with microbial oil were studied at various temperatures, substrate molar ratios and enzyme quantity. Transesterifications using NLs were carried out at various molar ratios. The effect of glycolipids plus sphingolipids (GL+SL) and phospholipids (PL) fractions on conversion yield of isopropyl esters was evaluated in mixtures with NL.

**3. Results and discussion**

In fed-batch fermentation, *L. starkeyi* showed the highest lipid production (40.9 g/L) at a C/FAN ratio of 173. In shake flask fermentations of *M. isabellina*, TDW and microbial oil content were highly inhibited when 10 g/L and 50 g/L of LS were applied. In the case of *C. echinulata*, lipid and GLA contents were positively affected with increasing C/FAN ratios leading to the highest values of 60% and 18.8%, respectively. Removal of phenolic compounds (61%) was also observed by the fungal strain. Fed-batch cultures of *C. echinulata* in bioreactor resulted in total dry weight of 12.2 g/L with a lipid content of 56% in media containing 5 g/L LS. Fed-batch fermentations demonstrated that GLA formation was strongly affected probably due to the applied aeration and agitation.

Transesterification reactions were initially optimised and subsequently transesterifications were conducted using NL, GL+SL and PL. The presence of GL+SL and PL fractions negatively affected enzymatic ester synthesis. Transesterification of the NL fraction with isopropanol and 2-ethylhexanol led to the highest conversion yield of 80.2% and 73.8%, respectively.

**4. Conclusions**

The present study showed that *L. starkeyi* and *C. echinulata* could tolerate high LS concentrations. Fed-batch bioreactor cultures of *L. starkeyi* under the optimum C/FAN ratio, resulted in high cell density (81.1 g/L) and high lipid content (50%). *C. echinulata* was able to produce PUFAs-rich microbial oil (60%) with simultaneous phenolics removal in batch fermentation.

The efficient enzymatic conversion of GLA-rich microbial oil into esters using Novozyme 435 was demonstrated. Enzymatic synthesis was mainly affected by temperature and substrate molar ratio. Results showed that the different lipid fractions have a key role in enzymatic activity and conversion yield. Specifically, conversion yield was higher when microbial oil was free of PL and GL+SL fractions.

**References**

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