**Production of Mannosylerythritol lipids (MEL) from vegetable oils: Exploring lipase application on substrate pretreatment**

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**Highlights**

* Free Fatty Acids (FFA) and Esters were used as a feed for production of MEL
* All ester-fed cultures, besides butyl esters, performed better than ones fed with oil

Cultures fed with FFAs produced resulted in higher productivity and yields of MEL

**1. Introduction**

Mannosylerythritol lipids (MELs) are a group of extracellular glycolipid biosurfactants, known for their tensioactive versatility. High titers of MELs are obtained using vegetable oils as substrates for *M. antarticus*. However, a significant fraction of the bioproduced MEL is lost during downstream due to difficult separation from triglycerides [1]. Lipases are also produced by *M. antarticus* cultures, which break down the triglycerides that the vegetable oil consists of. Considering these two features of *M. antarticus*, three approaches are explored in this work, with the aim to increase MEL production and enable different downstream routes.: feeding the culture with vegetable oil, using oil partially broken down by lipases produced by *M. antarticus,* or transforming the triglycerides into alcohol esters, and adding them as a carbon source.

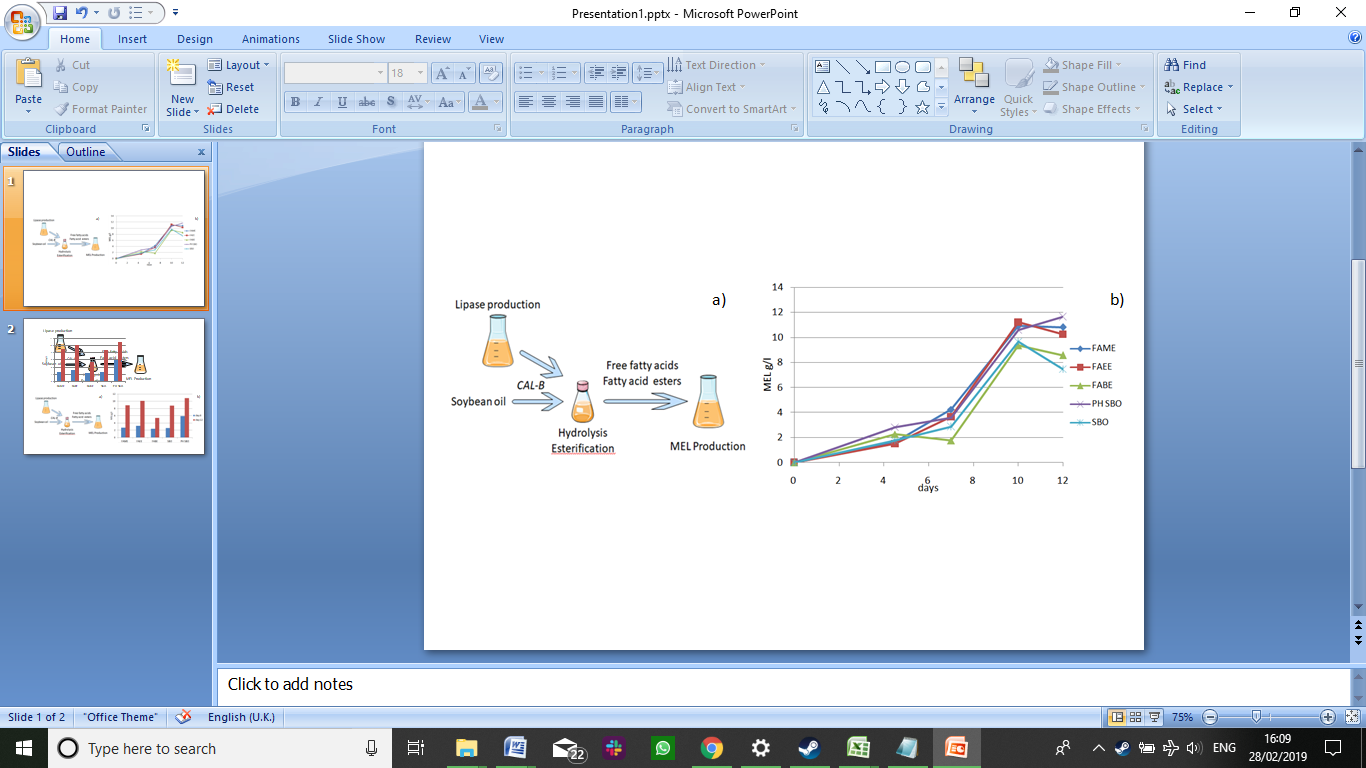
**2. Methods**

Cultures of 50 ml in 250 ml flasks were incubated at 27 °C, 250 rpm for 12 days. Glucose (40 g/l) was used as the initial source of carbon, while soybean oil, pretreated soybean oil and fatty acid alcohol esters (20 g/l) were added on day 4 to some flasks, at the start of the MEL production phase, following the previously established protocol for MEL production by using soybean oil [2]. Oil hydrolysis was performed using lipase-rich supernatant collected from cultures at day 7, and, for proof of concept, esterification was performed with commercially available purified CAL-B, with the addition of methanol, ethanol or butanol. The overview of the experimental plan is presented in Figure 1a.

**3. Results and discussion**

Lipase-rich supernatant (3 U/ml of activity), was mixed with soybean oil, and maintained at different conditions, with a three level variation of parameters, according to a Box-Behnken experimental design. The best performing conditions reached only 39% hydrolysis efficiency. The addition of 0.5% of emulsifiers, Xanthan and MEL, increased FFA concentrations, with the latter achieving 76% hydrolysis efficiency. Coherently, lipase-rich supernatant performed 15% better than the commercial enzyme alone (39%), indicating possible positive contribution of the present MEL as an emulsifier, or the possitive effect of ions or cofactors on lipases [3].

The results show that *M. antaricus* was able to metabolize esters. Whether the cell directly uses esters as a carbon source , or they are hydrolyzed extracellularly, with the resulting FFA and alcohol being used as carbon sources, remains unknown. Higher MEL production was achieved with methyl and ethyl ester feeds compared to oil. Some negative effects of alcohol presence in early stages of the fermentation were observed for methanol and butanol, which corresponds to data found in literature on their toxicity to lipase activity [4][5]. The results for the approach where vegetable oil is partially hydrolyzed prior to fermentation are very promising, since availability of FFA in earlier stages of the fermentation seems to stimulate MEL production. MEL profiles indicate that the prehydrolysis step effectively reduced the fermentation period, and enabled reaching higher MEL concentrations compared to feeds with untreated oil. (Figure 1b)



**Figure 1.** a) Experimental plan overview; b) Preliminary MEL production results. FAME - methyl esters; FAEE - ethyl esters, FABE - butyl esters; SBO - soybean oil; PH SBO - partially hydrolyzed soybean oil.

**4. Conclusions**

Feeds of prehydrolized and esterified oil increased MEL production efficiency by increasing MEL titres, reducing levels of residual tryglicerides in the broth, with the prospect of shortening the fermentation duration. This approach suggests a more efficient and potentially more sustainable alternative for MEL production when compared with the existing processes.

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