

# Production of Pure Microbial Oil (PMO) as a basis for the production of biodiesel with obese micro-organisms.



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## Introduction

Cultivation of “obese” micro-organisms could be an interesting alternative for the production of pure microbial oil (PMO) as a basis for the production of biodiesel. Previous research has shown the potential of PMO production with oil containing yeasts. These micro-organisms has the advantage that they can grow to relatively high densities (50 – 100 g/kg Dry Weight) in a short period of time (2 – 5 days), making them attractive competitors towards other oil producing organisms.

## Results

From previous research a top-6 list of promising strains was selected. These strains were cultivated in batch fermentations under various process conditions (pH and T) to find optimal biomass formation (see Table 1). Strain HBC025 was selected for further studying under fed-batch conditions.

Table 1: Results of batch growth.

| strain no. | optimal pH | optimal T (°C) |
|------------|------------|----------------|
| HBC-07     | 7          | 30             |
| HBC-09     | 4 - 5      | 30             |
| HBC-25     | 3          | 28 - 34        |
| HBC-30     | 7          | 30             |
| HBC-35     | 3          | 34             |
| HBC-36     | 4          | 28 - 34        |

Various fed-batch fermentations were performed with strain HBC025. The process was a batch phase at pH = 3 for 16 hours at a C/N-ratio of 5 to promote biomass production. The batch phase was followed by pH-shift and a stepwise increasing linear feed for 4 days. The culture was fed with glucose to obtain a final C/N-ratio of 75 (see Figures 1 & 2).

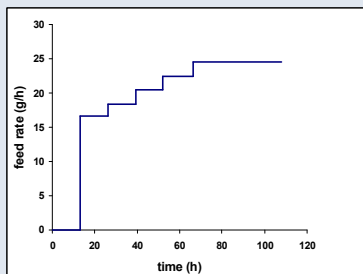


Figure 1: Fed-batch process (feed rate)

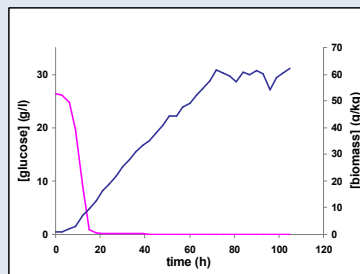


Figure 2: Fed-batch process (biomass and glucose)

PMO accumulation was microscopically detected (see Figure 3) after Oil Red O staining of the cells. EOF cells were harvested, freeze dried and extracted with hexane to extract PMO from the cells. A sample of the hexane extract is shown in Figure 3 containing approximately 80% of PMO (based on GC analysis).

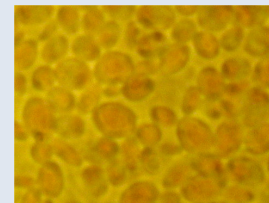


Figure 3: PMO containing yeasts and extracted PMO

## Conclusion / Future Plans

A robust fed-batch fermentation process was obtained, which makes this process suitable for scaling up. PMO was successfully extracted from the cells to a high degree of purity.

Further optimisation and scaling up of PMO production and extraction process.

Detailed analysis of PMO content and quality of other promising yeast strains.

Genetic improvement of HBC025 strain to develop an improved strain for oil production.

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