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CENTRO TECNOLÓGICO CARTIF

INFLUENCE OF ACETATE AS MAIN CARBON SOURCE FOR LIPID PRODUCTION VIA OLEAGINOUS YEAST FERMENTATION

Sanz Martín J.M.[1], Ramos Andrés M.[1], Piñero Hernanz R.[1], Barriuso Maicas J.[2], García López J.L.[2], Vlaeminck E.[3], Quataert K.[3], Atsonios K.[4]

[1]Centro Tecnológico CARTIF, Av. Francisco Vallés 4, Boecillo, Valladolid, Spain.
[2]Centro de Investigaciones Biológicas (CIB-CSIC), c/ Ramiro de Maeztu 9, Madrid, Spain.
[3]Bio-based Europe Pilot Plant (BBEPP), Rodenhuizekaai 1, Gent, Belgium.
[4]Centre for Research & Technology Hellas /Chemical Process and Energy Resources Institute, 6th km. Charilaou-Thermis, Thermi, Greece.
Corresponding author email: josmar@cartif.es

Concept

Microbial oils are proposed as a suitable alternative to petroleum-based chemistry in terms of environmental preservation. These oils have traditionally been studied using sugar-based feedstock, which implies high costs, substrate limitation, and high contamination risks. In this sense, low-cost carbon sources such as acetate are envisaged as promising building blocks for lipid biosynthesis to produce oil-based bioproducts such as chemicals and drop-in biofuels. Here, we investigated the use of acetate as main carbon source for the production of triacylglycerides (TAGs) using the oleaginous yeast *Yarrowia lipolytica*. In order to enhance TAGs production a genetic strategy was used to obtain recombinant obese strains of *Y. lipolytica* capable of accumulating a high amount of lipids. Batch fermentations and continuous fermentations with cell recycle fed with a diluted acetate solution, were carried out at lab scale using 1.5 L bioreactors. Fermentation conditions were studied at laboratory level to determine the most important parameters influencing lipid accumulation (pH, DO, C/N ratio) as well as alternative carbon sources to increase yield of lipid production.

Objectives

The aim was to evaluate feed rates, growth rates and conversion rates (g/g), titers (g/L), volumetric productivity (g/L/h) and fatty acid composition of produced TAGs using different carbon sources (acetate, acetate+glycerol, glucose).

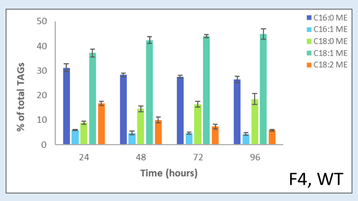
In addition, the performance of the *Y. lipolytica* wild-type (WT) strain and the *Y. lipolytica* modified strain (DGA) have also been compared.

Results

Batch fermentations with acetic acid

A preliminar design of experiments was carried out to study the influence of three parameters: initial acetate concentration, DO and pH. For each parameter, two reference values were selected. Fermentations were performed during 96 hours.

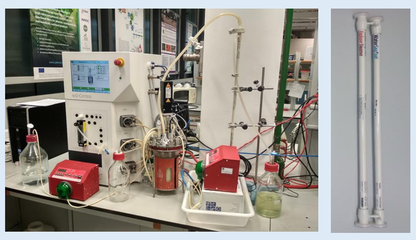
	pH	DO (%)	Acetate (g/L)	CDW (g/L)	TAGs (g/L)	TAGs/CDW (%)	qTAGs (g/L/h)
F1, WT	5.5	20	15	7.93	1.418	19.85	0.014
F2, WT	5.5	20	50	8.53	1.358	16.29	0.014
F3, WT	8	20	15	13	3.04	12.79	0.031
F4, WT	8	20	50	17.4	3.479	19.45	0.036
F5, WT	8	0	15	1.198	0.1	8.55	0.0011

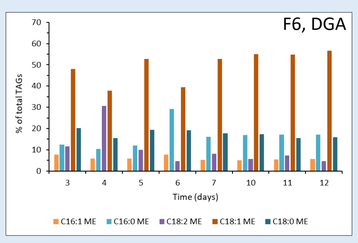


Continuous fermentations with acetic acid

The operation was based on an initial fed-batch stage and a continuous stage. Initial culture volume was 0.5 L. Substrate was fed until 1.5 L was reached. At this point, continuous operation was started, maintaining a constant volume through continuous extraction of cell-free broth stream (tangential filtration with hollow fiber membrane). Fermentations were performed during 6 days at pH 7 and DO 30%.

	Feed 1	Feed 2	Feed (mL/min)	Biomass (g/L)	% TAGs	TAGs (g/L)	TAGs yield (g/g), Prod (g/L/h)
F5, WT	3 % AA 5 g/L AmSulf C/N=13	3 % AA 0.65 g/L AmSulf C/N=102	0.3	7.9	29.1	2.3	0.20
F6, DGA	3 % AA 5 g/L AmSulf C/N=13	3 % AA 0.65 g/L AmSulf C/N=102	0.25	23.0	22.6	5.2	0.14, 0.04
F7, DGA	3 % AA 5 g/L AmSulf C/N=13	3 % AA 0.65 g/L AmSulf C/N=102	0.88-1.6	9.8	25.2	2.5	0.06, 0.03

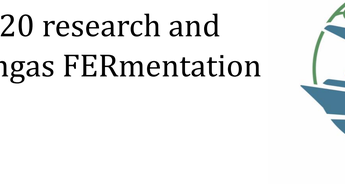




Continuous fermentations with other carbon sources

Effect of glycerol as co-substrate together with acetic acid was studied. Biomass grew to 67.5 g/L. Lipid content was similar to other fermentations, but lipid concentration was high, reaching 16.9 g/L.
Using glucose as sole carbon source, resulting biomass concentration was similar to that obtained with acetic acid. The percentage of TAGs was higher than that obtained so far, reaching 30.8 %.
TAGs profile distributions were similar to other fermentations.

	Feed 1	Feed 2	Feed (mL/min)	DO (%)	pH	Biomass (g/L)	TAGs (%)	TAGs (g/L)	TAGs yield (g/g), Prod (g/L/h)
F8, DGA	3 % AA 3 % glycerol 5 g/L AmSulf C/N=26	3 % AA 3 % glycerol 0.65 g/L AmSulf C/N=204	0.4-0.6	30	7	67.5	25.1	16.9	0.28, 0.10
F9, DGA	6 % AA 6 % glycerol 18 g/L AmSulf C/N=14	6 % AA 6 % glycerol 2.6 g/L AmSulf C/N=100	0.15	30→5	7 to 3 (fast)	26→16.2	12.0	1.9	0.04, 0.03
F10, DGA	3 % AA 3 % glycerol 0.65 g/L AmSulf C/N=204	3 % AA 3 % glycerol C/N = inf	0.09-0.12	30→5	7 to 2.5 (slow)	27.8	24.1	6.7	0.27, 0.06
F11, DGA	(100 g/L glucose in medium)	6 % NaAc 6 % glycerol	0.1	30→10	7	90.5	21.9	19.8	0.177, 0.261
F12, DGA	60 g/L glucose 10 g/L AmSulf C/N=13	60 g/L glucose 1.3 g/L AmSulf C/N=102	0.2	30→5	7 to 2.5 (slow)	22.9	30.8	7	0.11, 0.08



Conclusions

- The use of a continuous fermentation mode with cell recycling using hollow fiber membranes to recirculate the cells while removing exhausted culture medium resulted in increased lipid production.
- Acetate fermentations with modified *Y. lipolytica* strain resulted in a TAGs content of 25% but with low biomass (20-25 g/L) and lipid titer (6.2 g/L).
- Acetate fermentations with modified *Y. lipolytica* strain using glycerol as a co-substrate resulted in similar TAGs accumulation (%) but higher biomass (67.5 g/L) and therefore higher lipid titer (16.9 g/L).
- Glucose fermentation does not provide more TAGs if the nitrogen concentration is not low enough.
- Glucose fermentation with stress conditions such as low DO (5%) and low pH (2.8) during the 2nd stage of the fermentation resulted in higher TAGs accumulation (31%) with no by-product accumulation.

Overall, results obtained show that *Y. lipolytica* is a promising biotechnological tool for lipid generation using low-cost acetate media as substrates

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