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Final report on continuous biocatalytical production of fuel compounds at lab-scale

by
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Publishable Summary

1) *Identification and quantification of key process parameters for growth and excreted fuel production for promising candidates from WP2*

Environomic studies were performed for all project strains. Process variables that affect growth and product excretion (included pH, temperature, light intensity, P-concentration etc.) were evaluated. Following this optimum process conditions for the maximum productivity of product and biomass were identified and quantified for bisabolene, butanol, octanol, octyl acetate and free fatty acids employing chemostat/turbidostat operation (D3.2, D3.9).

2) *Chemostat operation and identification of appropriate methodologies to separate excreted fuels*

A detailed, quantitative evaluation of separation options for bisabolene, butanol, free fatty acids (FFA), octyl acetate and octanol has been provided. In addition, scale-up information, equipment, man-power and advice was given to A4F.

Under optimal conditions and with minimal nutrient levels, the bisabolene strain achieved a growth rate of 317 mg biomass L d^{-1} and a bisabolene productivity of 80 µg L d^{-1}. There appears to be opportunity for higher productivity (>120 µg L d^{-1}) if operated above the minimum nutrient levels.

For the butanol strain, CO_2 perturbations (pH) revealed that a maximum concentration of 2.35 g L^{-1} of butanol can be achieved at pH 7.6. Based on long term chemostat data, biomass productivity was found to be 440 mg L^{-1} d^{-1} and butanol productivity to be 600 mg L^{-1} d^{-1}, meaning that 66% of the total carbon flux can be diverted to butanol production. These experiments were carried out in a 3L continuous culture system. Learnings were successfully transferred to a 120 L PBR, to a point where product was detected and chemostat operation commenced. However, contamination issues prevented longer-term productivity and product separation. (D3,3, D3.9).

Chemostat operation also allowed optimization of light intensity, pH and hold-up for a maximum octanol productivity above 30 mg L^{-1} d^{-1}. Switching to turbidostat operation increased productivity well above 60 mg L^{-1} d^{-1}. Continuous operation could be demonstrated over 165 days. The cumulative extracted octanol concentration could be increased in the extracting solvent (in contact with the medium) to approximately 3750 mg L^{-1} without affecting cell viability.

Similarly to octanol, free fatty acids can also be extracted directly from the algae culture using solvent extraction. Having established optimal conditions for FFA production a gradual increase in hold-up (increasing optical density) led to an increased accumulation of FFA in the culture and solvent.

Continuous culture and production of octyl acetate was demonstrate for 42 days in a chemostat system leading to the accumulation of 485 mg L^{-1} in the extracting solvent and corresponding to a productivity of 13 mg L^{-1} day^{-1}.

3) *Identifying optimal strategies for waste utilisation and recycle.*

A large range of biomass utilisation options were evaluated through a detailed literature survey. Hydrothermal liquefaction (HTL) of algae was identified as a promising way to deal with waste biomass and convert this into valuable biofuel. This process has many advantages compared to other thermal conversion processes such as pyrolysis, gasification or supercritical transesterification. This is mainly due to the fact that HTL can utilise wet algae biomass and this therefore eliminates the cost of dewatering and drying. Since a significant fraction of the carbon flow is diverted to biomass production, it is clear that valorization of this product is directly linked to the efficiency and economic viability of the process. Experiments with a continuous flow HTL reactor demonstrated the efficient conversion of algal biomass to biocrude at short residence times (30 s).

A credible alternative to HTL was for the algal biomass to be subjected to anaerobic fermentation for waste utilisation. Pre-treated algal biomass (from butanol culture) enhanced bioethanol and CO_2 production rates (relative to control). Yeast outperformed *E.coli,* producing higher concentrations of bioethanol and CO_2. Supplementing the medium (BG11 + Biomass) with yeast extract increased biomass-normalised bioethanol yield (D3.6).

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