

SUPPLEMENTARY MATERIAL

BIOREACTOR CULTIVATION

Experimental setup. For complete process analytics of a decatrienone production process, *F. betulina* was cultivated in a 5 L Minifors 2 bioreactor (Infors HT, Switzerland) equipped with a oxygen probe VisiFerm (Hamilton, Massachusetts, USA), a pH probe EasyFerm (Hamilton, Massachusetts, USA) and off gas analysis BlueInOne Cell (BlueSens gas sensor GmbH, Germany). The reactor was operated with 3 L. As main culture medium minimal medium supplemented with 2 % cabbage cuttings (see Materials and methods - Cultivation conditions) was used. The fermenter was inoculated with 400 mL pre-culture grown in SNL for 14 days. During inoculation the bioreactor was aerated with 0.2 vvm (0.8 L/min) and 120 rpm. During cultivation pH was controlled with 2 M NaOH and 2 M acetic acid at pH 6.0 and the pO₂-value at 20 % (probe was calibrated with 100 % air) *via* increasing aeration (F_G). Twice a day, samples of 10 mL were taken for offline analysis. These samples were used to analyze the glucose concentration and the decatrienone concentration.

Glucose concentrations were analyzed according to the GOPOD assay (Megazyme, Irishtown, Ireland). Therefore 10 µL sample were mixed with 290 µL reagent. Glucose oxidase turned D-glucose to D-gluconate and hydrogen peroxide. The second product is used by a peroxidase to produce a quinone imine dye, which was detected at 510 nm. External standards of D-glucose (0.2 – 1.0 g/L) were used for calibration.

Full process characterization. Immediately after inoculation the pO₂ value declined rapidly within 10 h. When the value of 20 % was reached, the aeration was increased in a linear profile for the following 120 h. In parallel, the exhaust CO₂ increased, and glucose was consumed. After 52 h of cultivation the glucose concentration dropped rapidly, and (5E,7E,9)-decatrien-2-one production was initiated. Product concentration rapidly increased for the following 100 h, whereas glucose concentration declined. A metabolic change of fungal

cultures was observed after 120 h, when the exhaust CO_2 peaked and the slope of aeration increased rapidly to achieve the desired pO_2 of 20 %. It was concluded that this point indicated the change from primary to secondary metabolism. After 130 h, the glucose was fully metabolized, and (5E,7E,9)-decatrien-2-one concentration remained constant at a maximum of 5.5 mg/L. After 175 h the product concentration started to decline. As no further carbon source was available in the medium (130 h), the product formation *via* the polyketide pathway was terminated. Hence, a correlation of fungal growth and production was concluded.

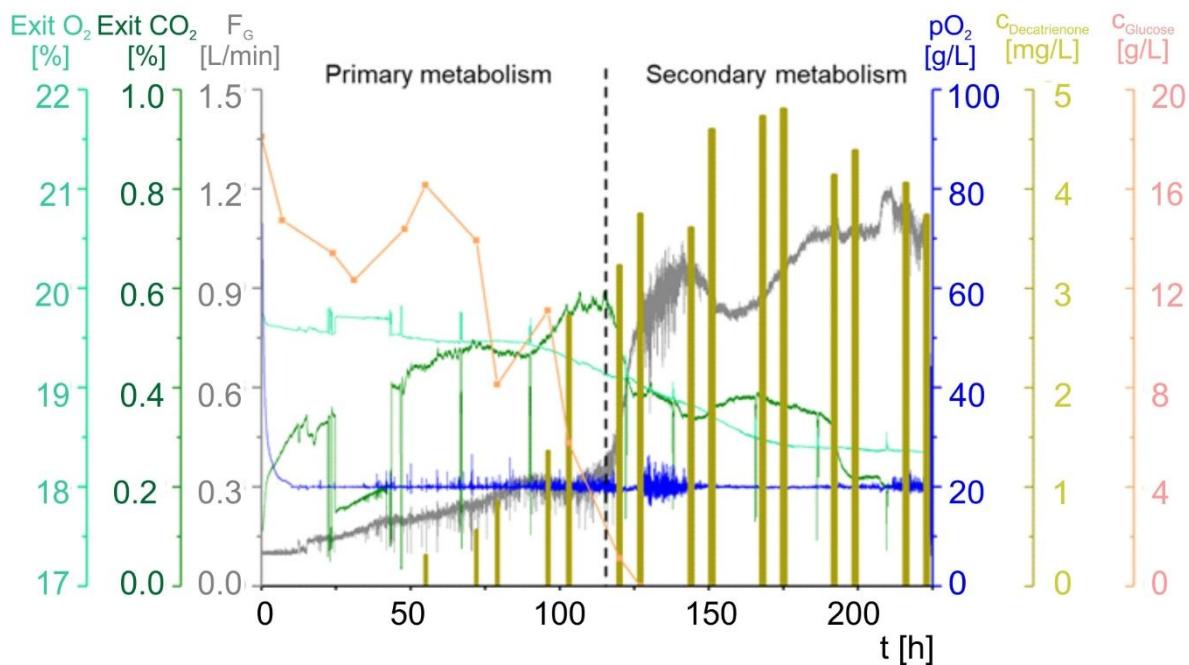


Figure 1: Bioreactor process of *F. betulina* for (5E,7E,9)-decatrien-2-one. Process performed in a Minifors 2 (Infors HT, Switzerland) with 3 L working volume (24°C; 120 rpm, pH 6.0) with a controlled oxygen level of 20 %.

YUZUNONE BIOGENESIS

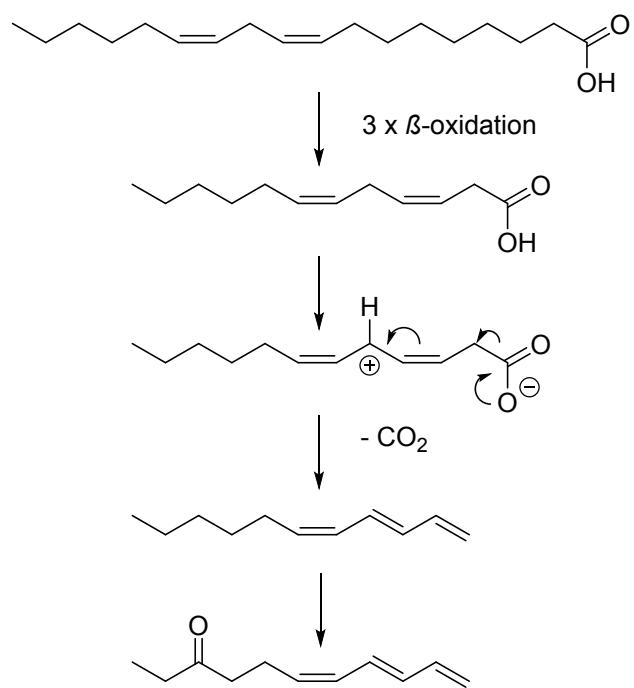


Figure 2: Hypothetical yuzunone biosynthesis. The β -oxidation of the polyunsaturated fatty acid linoleic acid followed by a decarboxylation may result in the desired ethyl ketone (6Z,8E,10)-undecatrien-3-one. Experimental proof is still missing.