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# VANILLIN PRODUCTION FROM WHEAT BRAN WITH *Pseudomonas fluorescens* BF13-1P

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## Abstract

Relevant amounts of wheat bran are produced by the Mediterranean Countries, where they are mainly employed in the formulation of animal feeds but also extensively discharged as a waste. Wheat bran contains relevant amounts (5-6 % w/w) of ferulic acid, i.e., a precursor for microbial vanillin production, which can be partially released from the cell wall polysaccharides via selected enzymatic treatments. We recently optimized a batch process for the bioconversion of food-grade ferulic acid into vanillin with resting cells of *Pseudomonas fluorescens* BF13-1p. In this work we investigated the possibility of i) increasing vanillin concentration in a fed-batch process, ii) using the biomass for consecutive bioconversion steps and iii) using ferulic acid from wheat bran hydrolysates as the bioconversion substrate.

*P. fluorescens* BF13-1p cells were grown in a 2-L stirred tank reactor on LB medium at 30°C, pH 6.8 and induced for 1 h with 2.5 mM ferulic acid after 4.30 h of growth. Batch bioconversion experiments were performed in shaken flasks (150 rpm, 30°C) with 6 g (wet weight)/L cells in saline phosphate buffer pH 7.0 or in wheat bran hydrolysate. Fed-batch bioconversions with repeated spikes of food-grade ferulic acid 2.5 mM and 5 mM allowed to obtain vanillin concentrations (3.5 mM and 6.4 mM, respectively) 20% lower than those obtained using the same total amount of ferulic acid in the batch process. Conversely, biomass could be efficiently reused in a second bioconversion process, allowing to obtain vanillin concentration, molar yields and bioconversion selectivity comparable those obtained after the first bioconversion (4.3 mM, 82.8% and 87.5%, respectively), while only 1.1 mM vanillin and 22% molar yield were obtained after the third use of biomass, due to the rapid accumulation of the by-products vanillic acid and vanillyl alcohol. Finally, bioconversions performed using crude bran hydrolyzates as substrates showed unsatisfactory vanillin molar yields (13.6%) and bioconversion selectivity (15%) due to vanillin oxidation to vanillic acid. The process efficiency slightly improved when the hydrolyzate pH was maintained at 7.0, whereas vanillin production comparable to those obtained with food-grade ferulic was obtained when ferulic acid occurring in bran hydrolyzates was purified from sugars on ISOLUTE ENV$^+$ columns.

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