Kinetics of brewing yeast accumulation on the surface of spent grains: A biocatalyst for brewing application

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When cells come into contact with solid surfaces, the physico-chemical and/or special biochemical interactions that occur may lead to biofilm formation. Although the biofilm formation is in many places unwanted, it can also be exploited in biotechnological processes (Characklis and Marshall, 1990). Understanding the biofilm formation kinetics and the physiology of immobilized cells is relevant for increasing the productivity of a biotechnological process taking advantage of microbial biofilms.

The brewing yeast *Saccharomyces pastorianus (carlsbergensis)*, kindly supplied by a brewing company (UNICER – Bebidas de Portugal, S.A., S. Mamede de Infesta, Portugal) were immobilized on acid (HCl) and base (NaOH) treated spent grains (Brányik el al., 2002). Continuous immobilization experiments were carried out in a bubble column reactor with spent grains as carrier at different dilution rates (D). The immobilized cells vitality was monitored through the change of NAD(P)H fluorescence intensity (340/460 nm excitation /emission wavelength) as the response to an aerobic-anaerobic (AA) transition. NAD(P)H fluorescence was considered a good indicator of the redox state of the immobilized cells (Podrazký et al., 2003).

Cell viability may be an important factor in immobilized cell reactor design and performance due to the long periods of time each immobilized cell spends in the reactor. Both the decreasing specific glucose consumption rate by the immobilized cells (q_{im}) and the intracellular NAD(P)H fluorescence changes imply alterations in immobilized cell physiology with time. The changes of the physiological state of the immobilized cells can be ascribed to the aging process to which the immobilized brewing yeasts, eukaryotic cells having a finite replicative lifespan (Baker and Smart, 1996), are exposed in the continuous reactor. Thus, the existence of an actively growing fraction (X_{im}^{act}) of the total immobilized biomass (X_{im}) was hypothesized and its value was estimated at $X_{im}^{act} = 0.12$ g_{IB} g_C^{-1} (g dry immobilized biomass per g dry carrier).

$$\frac{dX_{im}}{dt} = X_{dep}\left(1-\frac{X_{im}}{X_{im}^{\max}}\right)D + \mu_{im}\left(\frac{X_{im}^{act}}{X_{im}^{act}+X_{im}}\right)X_{im} - k_{\det}\left(\frac{X_{im}^{act}+X_{im}}{X_{im}}\right)X_{im} \quad (1)$$

$$\frac{dS}{dt} = D(S_0 - S) - \left(\frac{\mu_{im}}{Y_{X/S}^{im}} + m\right)\left(\frac{X_{im}^{act}}{X_{im}^{act}+X_{im}}\right)\left(\frac{X_{im}P_c}{V_r}\right) - \left(\frac{\mu_{free}}{Y_{X/S}^{free}} + m\right)X_{free} \quad (2)$$

$$\frac{dX_{free}}{dt} = (\mu_{free} - D)X_{free} + k_{\det}\left(\frac{X_{im}^{act}+X_{im}}{X_{im}}\right)\left(\frac{X_{im}P_c}{V_r}\right) - \left(\frac{X_{dep}P_c}{V_r}\right)\left(1-\frac{X_{im}}{X_{im}^{\max}}\right)D \quad (3)$$

$$\mu_{im/free} = \mu_{im/free}^{\max}\frac{S}{S+K_S} \quad (4)$$

$$k_{\det} = k_{\det}^{\max}\frac{S}{S+K_S} \quad (5)$$

A mathematical model was formulated to simulate the development of the immobilized yeast biofilm on the surface of the spent grains carrier (eqs. 1-5). The model was based on three processes believed to govern the rate of brewing yeast immobilization: cell deposition (cell-to-carrier adhesion and cell-to-cell attachment), immobilized cell growth and immobilized biomass detachment (outgrowth, abrasion). The concept of the viable fraction of immobilized cells (X_{im}^{act}) and of the maximum biomass load ($X_{im}^{\max}$) was applied in the mass balance equations. The kinetic model (eqs. 1-5) was used to predict the changes of X_{im}, free biomass (X_{free}) and glucose concentrations (S_{GLUC}) in the continuous immobilized cell reactor. During the induction phase of the biomass accumulation (X_{im} from 0 to 0.03 g_{IB} g_C^{-1}) it was only the cell deposition (1. term of eq. 1) that was taken into account in the modelling of the biomass accumulation process. Starting from X_{im} = 0.03 g_{IB} g_C^{-1} the whole kinetic model was applied and shows a good agreement with the experimental data (Fig. 1).

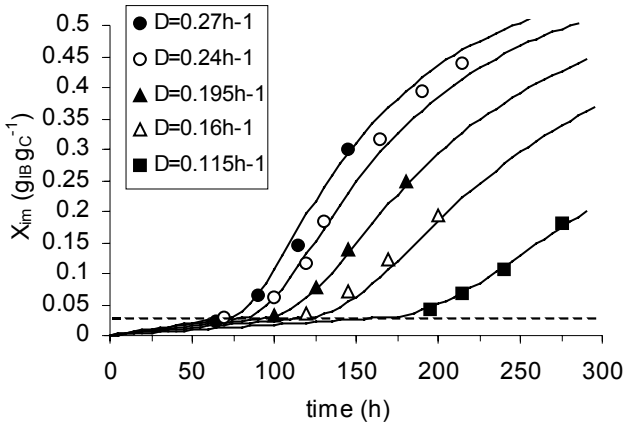

Fig. 1. Immobilized biomass accumulation (X_{im}) on spent grain particles at different dilution rates (D). Markers represent the experimental data, solid lines correspond to model simulations and the dotted line represents the value X_{im} = 0.03 g_{IB} g_C^{-1}.

The proposed model satisfactorily described the yeast biofilm development on the surface of spent grains under constant hydrodynamic conditions in a bubble-column reactor. Using this model, it was possible to predict the rate of the brewing yeast immobilization and the maximum immobilized biomass load at different feed rates (Fig. 1). The finite lifetime of the immobilized biocatalyst has also a practical consequence, namely the need of a regular biocatalyst replacement e.g. during the continuous operation of an immobilized cell bioreactor.

References

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