ELSEVIER

Biochemical Engineering Journal 35 (2007) 29–36

Biochemical Engineering Journal

www.elsevier.com/locate/bej

Simulation of the cellular anabolic activity within biofilms: Where a new immobilized cell will preferably be born?

P. Gikas *, A.G. Livingston

Department of Chemical Engineering and Chemical Technology, Imperial College, SW7 2BY London, UK

Received 8 March 2006; received in revised form 15 December 2006; accepted 19 December 2006

Abstract

A mathematical model, based on structured growth kinetics, is presented to simulate the profile of assimilated radioactive sulphur by a biofilm, after the release of calculated amounts of $^{35}SO_4^{2-}$ in the biofilm environment. The radiolabelled sulphur (^{35}S) stands as a biomass growth indicator, thus allowing the estimation of the cellular anabolic activity (growth) profile inside the biofilm. The model predicts that the maximum rate of biomass synthesis occurs at the external layer of the biofilm. A good correlation between the model predictions and previously published experimental data is reported, indicating the applicability of the structured model to describe microbial growth in immobilized cell systems.

Keywords: Biofilms; Modelling; Structured model; Radiolabelling; Immobilised cells; Attached biomass; Microbial growth; Air lift bioreactors

1. Introduction

Mathematical modelling of biofilms have undergone a substantial evolution of increasing complexity [1], since the early modelling attempts [2,3], which described biofilms as a uniform single-species film with simple biochemical reactions and with mass transport in one dimension. Numerous studies have been published the last decade in an effort to describe with accuracy the immobilized cell growth, employing two- and three-dimensional modelling (most of witch are approached with cellular automaton methods), incorporating a range of transport processes, advanced biofilm growth kinetics and detachment mechanisms to various extents [4–10]. Recently, individual based modelling has been used to describe biofilm performance, based on the description of the behaviour of individual cells, which are treated as fundamental entities [11,12]. However, despite the mathematical integrity and the advanced computational methods employed to simulate immobilized cell growth, relatively little attention has been given on the microbial growth kinetics which are used to describe immobilised cell growth. It has been found that immobilized cells may perform differently to an equivalent mass of freely suspended cells [13–16], and thus, biomass quality/activity should also be taken into account when kinetic data obtained in freely suspended cell systems are used to describe biofilm growth. This may be achieved by using structured modelling for describing immobilized cell growth. Skowlund [17], Wanner et al. [18] and Monbouquette and Ollis [19,20], have presented mathematical models which accounted for the formation of inert biomass. Hsieh et al. [21] and Wood and Whitaker [22], have presented more sophisticated structured models to simulate immobilized cell growth, using a large number of input parameters. Most of the recently presented biofilm models account with one or the other way for the above phenomena, but in most of the cases the microbial growth kinetics have been directly adopted from suspended cell systems, without previous investigation of the applicability of the growth kinetics in immobilized cell systems. Furthermore, the high mathematical complexity and the need for the estimation of a large number of input parameters, make many of the more "advanced" models extremely difficult to be used. Noguera et al. [1] have successfully noted that "*the real challenge to the modeller is not to create models that include as many parameters as possible, but rather to determine the level of significance of these parameters and their importance in the description of the different biofilm processes*".

Gikas and Livingston [23,24] have proposed a relatively easy to use structured model for suspended cell growth, based on

* Corresponding author. Present address: Ministry of Environmental Planning and Public Works, General Secretariat of Public Works, Special Service of Public Works for Greater Athens Sewerage and Sewage Treatment, Varvaki 12, Athens 11474, Greece. Tel.: +44 20 75945582 (UK)/+30 210 6444410 (Greece); fax: +44 20 75945604 (UK)/+30 210 6452753 (Greece).
E-mail address: petrosgikas@gmail.com (P. Gikas).

1369-703X/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bej.2006.12.018

Nomenclature

C_s	substrate concentration (kg m^{-3})
C_{Sb}	bulk liquid substrate concentration (kg m^{-3})
D	dilution rate (s^{-1})
D_s	diffusion coefficient for *n*-propanol in the biofilm (m^2 s^{-1})
g_λ	radial flux of λ (m^{-2})
k_s	liquid–solid mass transfer coefficient for *n*-propanol in water (m^2 s^{-1})
K_s	half velocity coefficient (kg m^{-3})
r	radius (m)
R_F	total support particle-biofilm diameter (m)
R_p	support particle diameter (m)
s	substrate concentration
s_b	bulk liquid substrate concentration
t	time (s)
TPALB	three phase air lift bioreactor
u	radial velocity (m s^{-1})
v	radial velocity
x	biofilm thickness
Y	ζ-compartment/substrate yield (kg (ζ-compartment) kg^{-1} (substrate))

Greek letters

γ	radioactive substrate concentration
Γ	radioactive substrate concentration (Bq mol^{-1} (SO_4^{2-}))
δ	biofilm thickness (m)
ζ	active fraction of cell
θ	inert fraction of cell
λ	radioactivity level of biomass
Λ	radioactivity level of biomass (Bq g^{-1} (dry biomass))
$\lambda(1)$	radioactivity level of the external layer of the biofilm
λ_s	radioactivity level of suspended biomass
Λ_s	radioactivity level of suspended biomass (Bq g^{-1} (dry biomass))
μ	specific growth rate (s^{-1})
μ_{max}	maximum specific growth rate (s^{-1})
ρ	immobilized biomass density (kg m^{-3})
$\Sigma\lambda$	aggregate immobilized biomass radioactivity level
$\Sigma\Lambda$	aggregate immobilized biomass radioactivity level (Bq g^{-1} (dry biomass))
$\Sigma\lambda_m$	model predicted aggregate immobilized biomass radioactivity
τ	time
τ_o	time that a certain action takes place
φ	constant for ζ conversion into θ
Φ	constant for ζ conversion into θ (s^{-1})
ψ	constant for ζ conversion into products
Ψ	constant for ζ conversion into products (s^{-1})
Ω	constant for substrate conversion into ζ (s^{-1})

the estimation biomass quality/activity by measuring the specific ATP (adenosine 5'-triphosphate) (SATP) concentration and the specific oxygen uptake rate (SOUR). Based on the above structured microbial growth kinetics, they proposed a mathematical model to describe immobilized cell growth, accounting for immobilised biomass quality/activity [25]. They tested the validity of the model experimentally, against the growth of a monoculture growing, under sterile conditions, immobilized on diatomaceous earth support particles, in a three phase air lift bioreactor (TPALB), by measuring immobilised and suspended biomass values of SATP and SOUR. This type of bioreactor set up (TPALB) has the advantage of allowing the formation of biofilms on support particles which are homogeneously suspended in the mixed liqueur. Even if there exist different shear zones in the above type of reactor, each particle is subject to the equivalent detachment forces and substrate loading over time, and thus a representative sample can be easily obtained [9]. Based on the above structured model, Gikas and Livingston [26,27] proposed an integrated model to calculate the biofilm thickness and the bulk liquid substrate concentration in a TPALB using as impute parameters only the inlet flowrate and the inlet substrate concentration. All the models mentioned above in this paragraph, describe immobilized cell growth under steady state conditions, and the experiments designed to validate the relative models were also carried out under steady state. Recently, Gikas and Livingston [28] have shown experimentally, using radiolabelled sulphur in a TPALB, that the biomass exhibits higher anabolic activity (growth) close to the external layer of the biofilm, and that attrition (and not sloughing) is the primary biomass detachment mechanism. The above experiments had been carried out at dynamic conditions with respect to radioactive biomass growth, but at steady state conditions with respect to carbonaceous biomass growth (the sole experimental variable was the concentration of radioactive sulphate ($^{35}SO_4^{2-}$) in the inlet).

The aim of this work is (i) to verify the validity of the structured growth kinetics proposed earlier by Gikas and Livingston [23–25] in describing dynamic immobilized growth situations, and (ii) to provide a mathematical description of the pattern of the cellular anabolic activity within the biofilm, thus predicting where the new immobilized cells will preferably grow. The model is tested against the experimental data obtained by Gikas and Livingston [28].

2. Modelling

2.1. General description

The mathematical model presented below attempts to predict the profile of assimilated radioactive sulphur (^{35}S) in a biofilm, due to variations of the concentration of $^{35}SO_4^{2-}$ in the bulk liquid. SO_4^{2-} stands in the system as the sole sulphur source for microbial growth. During the anabolic process, sulphur is stored up in the cell tissues as organic sulphur, thus the specific ^{35}S concentration (Bq g^{-1} (dry biomass)) can be used as indicator of the newly formed biomass. Finally, the model calculates the aggregate radioactivity hold-up in the biofilm by mathematical integration.