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A spatially explicit model of inverse colony formation of cellulolytic biofilms

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ABSTRACT

We propose a spatially explicit mathematical model for the formation of cellulolytic biofilms. It consists of a highly nonlinear degenerate coupled PDE-ODE system for the bacteria that colonise the cellulosic substratum, and for the carbon source that is immobilised in the reactive substratum. Computer simulations show that the model is able to reproduce important features of cellulolytic biofilms that have been reported in the experimental literature. These include the formation of inverse colonies, crater-like colonies, degradation of paper chads at constant speed (which correspond to a traveling wave solution of the model), and temporal CO_2 production pattern.

1. Introduction

Cellulosic ethanol is a liquid fuel that can be implemented in the current transportation infrastructure. It is primarily produced from non-edible, renewable, lignocellulosic plant material, such as woody biomass, grasses and crop wastes with a current estimated annual production of 53 million gallons in US alone [37].

In consolidated bioprocessing (CBP), cellulolytic bacteria perform hydrolysis and fermentation of the sugars in solid lignocellulosic substrates [32]. This poses new conversion kinetics challenges, in a process where concomitant microbial growth, extracellular enzymatic hydrolysis and the metabolic flux of fermentable sugars can all be rate limiting, depending on feedstock preparation, organisms selection and process conditions [25]. Much research has, therefore, focused on improving candidate organisms, often by the metabolic engineering of carbohydrate utilization pathways and electron flux in robust native cellulolytic microorganisms [24,28]. Model organisms such as *Clostridium thermocellum* and *Caldicellulosiruptor obsidiansis*, adhere to cellulose and form bacterial layers, or biofilms, *i.e.* spatially structured, temporally evolving bacterial aggregates, that colonize the cellulose material [4,25,39]. Hence, for cellulose degradation, physical factors such as spatio-temporal dynamics also come into play, in addition to the molecular make-up of bacteria. Indeed, recent literature strongly suggests that cellulolytic biofilms have significantly higher hydrolytic activity on recalcitrant second generation energy-crop feedstock; in particular [29] has shown two-fold improved solubilization of mid-season senesced switchgrass compared to conventional enzymatic methods. While it has been known for several years that sessile biofilms are involved in cellulose hydrolysis [22,24], this is not well understood and only few studies focused on this aspect [4,39], prompting the need for additional research. In such bioconversion systems, where biofilms are the primary catalysts, direct observation of growth on mixed solids is difficult to monitor and experimentally observe. Mathematical models and computer simulations can therefore be useful tools for the development and verification of hypotheses, for prediction, and for the interpretation and explanation of observed phenomena. Furthermore, a mathematical model that captures the spatial limitations to conversion on the biofilm scale can serve as a starting point for a future reactor scale predictive process model.

Our goal is to develop such a mathematical model that, based on few basic assumptions, can describe and explain certain spatio-temporal characteristics of the cellulolytic biofilms that have been reported in the experimental literature. Cellulolytic biofilms differ from the traditional biofilms that are the basis for wastewater treatment and other environmental engineering processes. They tend to not form substantial layers of extracellular polymer substances

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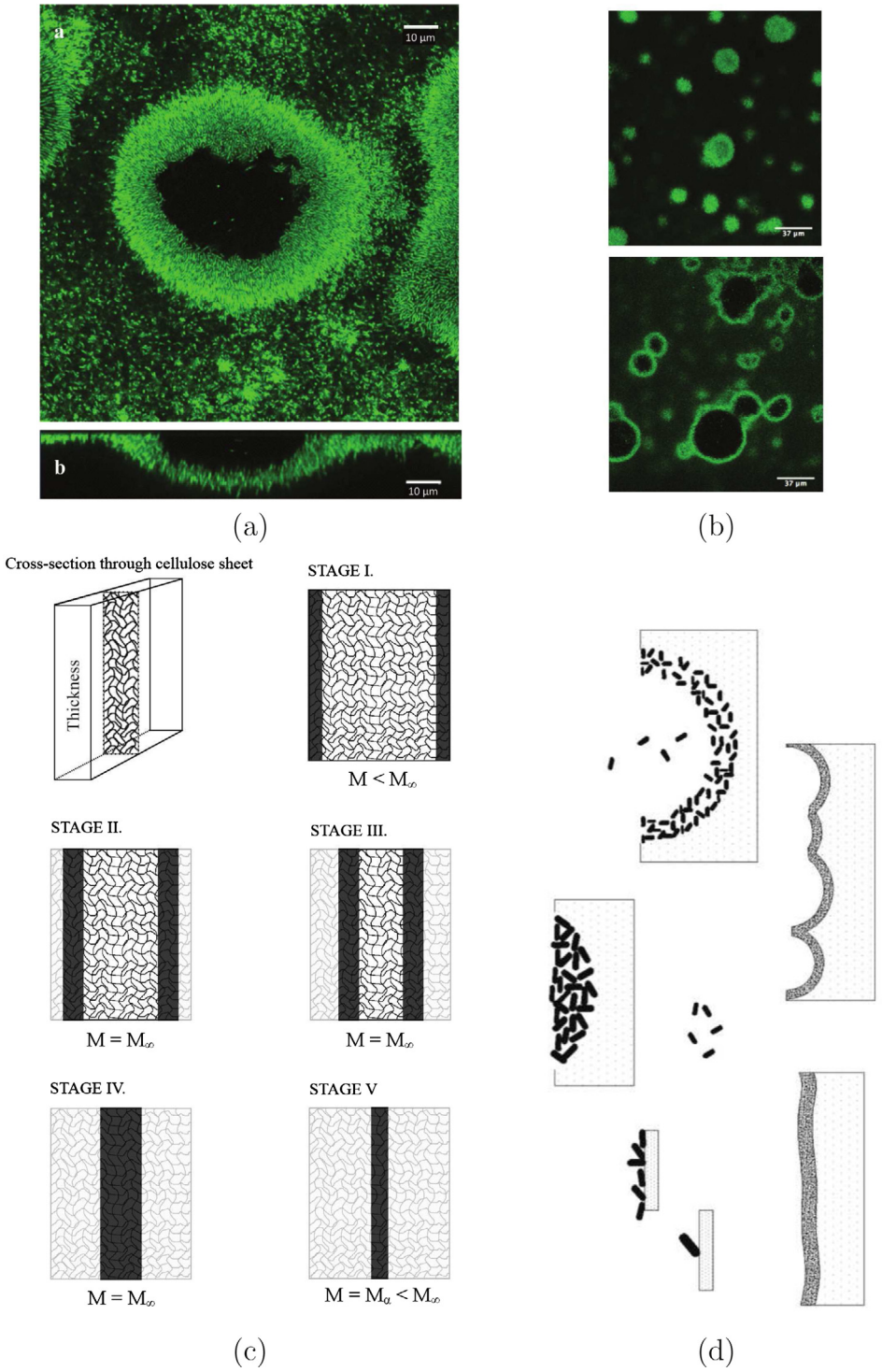

Fig. 1. Confocal laser scanning micrographs of cellulolytic biofilms and schematics explaining cellulolytic biofilm formation: (a) crater like depression formed by a *C. thermocellum* colony, top view (top) and cross-sectional view (bottom) from [39]; (b) colonies of *C. obsidiansis* cells on a cellulose surface, spherical shape 24h into an experiment (top) and merging rings 44h into an experiment (bottom), from [39]; (c) Schematic of penetration of a biofilm front into the cellulose substratum, from [6]; (d) Schematic of inverse colony formation, clockwise starting at bottom from [39]. a,b,d are re-used from [39] under a Creative Commons 2.0 license.

(EPS). This has been reported for *C. thermocellum* [4,39], which is considered a good candidate for consolidated bioprocessing [25], *C. obsidiansis* [39], *Bacteroides succinogenes* [13], *Ruminococcus albus 7* [42], and *Clostridium phytofermentans* [43] until the supporting filter paper fibres lost physical rigidity. Instead, cellulolytic biofilms often consist of bacteria that directly attach to cellulose fibres on which they form single cell layers. Secondly, whereas most biofilms form on an immersed substratum and grow into the aqueous phase, cellulolytic biofilms chew into the substratum that supports them, which has been referred to as inverse colony formation [39]. Thirdly, the nutrients for cellulolytic biofilms are immobilized in the substratum and not replenished from the aqueous phase. Cellulolytic biofilm colonies have been described as crater-like structures (cf. Fig. 1a,b) that can be characterized as layers of bacterial cells that propagate into the substratum and expand. Starting from individual cells or few cells, these structures first