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High biomass density promotes density-dependent microbial growth rate

E. Krichen [b,c,*], J. Harmand [a], M. Torrijos [a], J.J. Godon [a], N. Bernet [a], A. Rapaport [b]

[a] LBE, Univ Montpellier, INRA, 11100 Narbonne, France
[b] MISTEA, Univ Montpellier, INRIA, Montpellier Supagro, 34060 Montpellier, France
[c] MARBEC, Univ Montpellier, CNRS, IFREMER, IRD, 34203 Sète, France

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ABSTRACT

Describing the interactions between a population and its resources is a research topic in both microbiology and population ecology. When there are fewer resources for the individuals in a large population, the overcrowding can lead to a density-dependent effect which is reflected by a negative feedback of the organism density on the consumption process. In this paper, we investigate the growth rate of an aerobic microbial ecosystem by two series of experiments performed in continuous agitated cultures. Using a constant dilution rate, but different input substrate concentrations in each experiment, the biomass and substrate concentration were measured at steady state to confront their values with those obtained theoretically from the well-known mathematical model of the chemostat using either resource or density-dependent kinetics. The structures of both flocs and microbial communities were monitored in order to interpret the results. The experiments confirm that density-dependent growth-rate can result either from a high concentration of biomass or from the structuration of this biomass into flocs and we have shown that a new parametrized family of growth functions, that we proposed in this paper, suits better the experimental data than Monod or Contois growth functions.

1. Introduction

The study of predator-prey interactions has been the object of intense researches for several years. As in many subfields of ecology, the science behind predator-prey investigations has been driven by theory, including important advances in mathematical models as tools for understanding and predicting the functioning of ecosystems (cf. [1]. Predator-prey models have been studied mathematically since the publication of the Lotka [2] and Volterra [3] Lotka-Volterra equations in 1920 and 1926 based on the hypothesis of resource (prey)-dependence where the functional response of the predator (*i.e.* number of prey captured per predator per unit of time) is a function of the absolute prey density noted g(N). This hypothesis was questioned by Arditi and Ginzburg in the 1990s [4] (see their recent book on density-dependence [4]), who proposed a specific case of density-dependence, named ratio-dependence, where the prey capture rate is a function of the ratio of the prey density over predator density noted g(N/P).

In microbiology, researchers have often faced similar problems in describing the growth-rate of microorganisms growing on substrates or in the study of competition through resource depletion. The modelling of the functional response, also named the microbial specific growth rate or the reaction kinetics was lifted at the same time in theoretical ecology and in microbial ecology. It is particularly interesting to notice that several models, developed in these two disciplines independently, and thus bearing different names, propose in fact the same growth rate expressions [5]. In other words, the same mathematical functions are used to describe micro as well as macro-organisms growth. The latter being more difficult to handle than microbes, the microbiology has appeared since a few years as a field, particularly suited to study questions of general ecology [6]. If we exclude complex mechanisms such as inhibition, functions describing the growth rate of microorganisms can be classified into two main classes, depending whether they involve only the resource (substrate or nutrient) concentration in the medium containing the culture, as in the case of the Monod model [7] or both substrate and biomass (or predator) densities as in the case of the Contois model [8]. In fact, what is of relative importance with respect to a pure culture (both models have very similar predictions for pure cultures) becomes very important for complex ecosystems in the sense Monod-like models predict extinction of all species in competition on a single substrate, but one (this well-

* Corresponding author at: MISTEA, Univ. Montpellier, INRIA, Montpellier SupAgro, 2 p. Viala 34060 Montpellier, France.
E-mail address: emna.krichen@supagro.inra.fr (E. Krichen).

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Nomenclature

μ_{max}	Maximum specific growth rate, (/day)
CSTR	Continuous stirred tank reactor
D	Dilution rate, (/day)
COD	Chemical oxygen demand, (gO_2L^{-1})
FSD	Floc size distribution
HRT	Hydraulic residence time, (day)
J	Mean square criterion
K_s	Half-saturation constant, (g/L)
PCR	Polymerase chain reaction
Q_{in}	Input flow rate, (l/day)
Q_{out}	Output flow rate, (l/day)
RPM	Rotation per minute
s^*	Substrate concentration at steady state, (g/L)
SE1	First series
SE2	Second series
S_{in}	Input substrate concentration, (g/L)
SS	Suspended solids, (g/L)
SSCP	Single strand conformation polymorphism
V	Reactor volume, (L)
x^*	Biomass concentration at steady state, (g/L)
Y	Yield, (%)

known property is called the competitive exclusion principle and has been studied in ecology from the 1950s, cf. for instance Hardin [9] while Contois-like models allow coexistence of several species (cf. for instance Lobry and Harmand [10].

If we consider Monod functions, for a constant feed rate, the chemostat theory predicts that the equilibrium should only depend on the dilution rate D and be independent of the input substrate concentration S_{in} (on the condition that this latter one is large enough to supply enough resource for the micro-organisms to grow). This prediction was tested by varying dilution rates and influent substrate concentration and letting the chemostat reaching its steady state while measuring the effluent substrate concentration s^* [5]. However, it was only verified for pure cultures. When working with mixed cultures (such as in wastewater treatment or fermentation processes) or using a multicomponent substrate, it is well known that the effluent concentration do not depend only on the dilution rate, but also on the concentration of substrate S_{in} in the influent [11–14]. The independence of the growth rate at steady state with respect to S_{in} in the chemostat has been questioned, following experimental observations since 1959 by Contois, Yoshinori [15] by including the ratio s/x in the expression of the growth rate instead of the absolute value of available substrate and thus emerging an effect of density-dependence. On the latter, the question of the mechanisms at the origin of this phenomenon can be questioned.

In the present work, we investigate whether a high density of biomass can generate density-dependent growth rate as proposed in Harmand and Godon [16], and formalized in Lobry and Harmand [10]. We therefore propose experiments in a chemostat or *CSTR* (Continuous Stirred Tank Reactor) followed by a macroscopic modelling approach and a study of the proposed models to determine what type of growth rate is the most appropriate to explain the experimental data. The novelty with respect to the literature lies in the fact we have followed not only substrate and biomass densities but also monitored microbiology of the complex ecosystem used together with the structure of the biomass. Our results show that density-dependent kinetics may emerge not only from a high density, but also from the structuration of the biomass in flocs.

The paper is organized as follows. We first describe the experiments we performed in chemostat with the different parameters we monitored, we recall the qualitative predictions that can be done from the assumptions on the microbial growth rate at the scale of the whole biomass and we describe the method of the models identification. Then, we show and analyse the results at the light of the monitored parameters and of the modelling approach before some conclusions and perspectives are drawn.

2. Material and methods

2.1. Experimental setup and experiment

The experimental work is divided into two consecutive series of experiments applied in a chemostat device: a first series, named SE1, with increasing substrate step-loads and a second series SE2 where these loads were applied decreasingly. A hydraulic retention time of 24 h was maintained constant throughout the experiments.

All experiments were carried out in the same continuous biological reactor (Fig. 1). The reactor consisted of a glass vessel (noted [1] on Fig. 1) inoculated with constant total volume of 6.8 L of biomass *x* obtained from a return sludge pump of the activated sludge of the treatment plant of Narbonne (handling approximately 60000 EH). The substrate *s* used to feed the reactor is red wine (Bag-In-Box of 5L, Winery: Club des Sommeliers, Grapes: Cabernet Sauvignon, Wine Region: Pays d'Oc, France) whose initial pH (potential of hydrogen) and COD_t (total chemical oxygen demand) are 3.82 and 250.3 $gO2L^{-1}$ respectively. The choice of this substrate is based on the fact that wine is a highly biodegradable substrate. The input substrate concentration S_{in} is daily prepared (7 L), stored in a feed tank [2a] except during weekends where it is stored in a larger tank of 21 L. The reactor was fed continuously and the S_{in} step changes were done by diluting the red wine with water. The *COD/N/P* ratio was adjusted with $NH_4H_2PO_4$ and NH_4Cl in order to equal 200/5/1. The organic loading rate was changed each time the equilibrium was established for a given concentration S_{in}.

The substrate was introduced into the glass vessel by a 16 mm diameter pipe and a pump (5 RPM, type Master flex) [2b] with an input flow rate Q_{in} = 4.6 mL/min. Moreover, the excess of bioreactor liquid was collected in a can [3a], using another pump Q_{out} [3b]. During experiments *SE1*, a continuous pump was used to maintain the useful volume *V* constant in the reactor, which implied Q_{out} to equal the input flow rate. Following technical problems (the tendency of biomass to accumulate in the reactor and the corking of the withdrawal cannula), this pump has been replaced by a programmable pump (type Master flex L/S model 77200-60), early in the second series of experiments. This pump operated in discontinuous mode at the maximum withdrawal rate of 280 mL/min. It was scheduled for a 3 min period for 2.5 h. The withdraw of excess liquid occurred rapidly through a larger diameter pipe.

The reactor was equipped with an aeration system (a series of air diffusers for aquarium [4a] and two vacuum pumps Millivac [4b]) used to send air into the culture medium and to ensure a perfect mixing within the bioreactor. For the series of experiments *SE2*, the bioreactor was also equipped with a pH control system (a pump [5a] allowed a NaOH solution [5b] with a concentration of 5% to circulate in the system, a pH probe [5c] was immersed in the reactor and was connected to a pH controller [5b]). Finally, oxygen and temperature were followed along the experiments using sensors ([6a] & [6b]) allowing on-line measurements. All the measured variables were stored in a computer [7] thanks to the Odin-Silex acquisition and control system.[1] Specific conditions for all experiments are reported in Table 1.

[1] https://team.inria.fr/biocore/fr/software/odin/.