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When constants are no longer constant: The case of inhibition in bioprocesses

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ABSTRACT

Traditional models describing microorganisms growth assume that changes in the environmental conditions generate immediate responses in microbial population. However, reports are available indicating that in some cases this may not be the case. The existence of a delayed inhibition response (DIR) was studied, exposing a microalgae culture to inhibitory ammonia concentrations. Results revealed the existence of considerable delays in the microalgae response, even when imposing conditions promoting null microalgal activity. Even an extremely unfavourable condition such as 1500 mg L^{-1} of total ammonia nitrogen and pH 9 takes about 24 h to induce complete inhibition. The existence of DIR may cause serious deviations between predictions given by traditional models and real behaviour of a culture. Inclusion of DIR in traditional models may be accomplished by assuming model parameters as variables depending on time. In other words, assuming that inhibition "constant" is no longer constant, but a function of exposure time.

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1. Introduction

Modelling biological systems is a delicate and complex task. There are no laws determining the evolution of microorganisms, and most models in biology depend on empirical laws [1]. The standard mechanistic mathematical models used for microbial growth assume that microbial activity, and therefore the biomass production, are usually a function of the concentration of a single nutrient (typically a carbon source) or product. Normally, mathematical models assume that biological systems respond instantly to changes in environmental conditions. In other words, that microbial activity rate is determined by the concentration of substrate(s) or product(s) in the medium, and that the activity rate adjusts itself instantaneously to changes of such concentrations. This is quite obvious by looking at the most used functions for microbial growth rate such as Monod, Haldane, Contois or logistics.

However, there are some reports indicating that, under some particular conditions, changes in the environmental conditions do not generate immediate responses in the microbial population, and a certain time span is required before changes in the biomass activity can be observed. When the effect of changing an input does not immediately affect an output, we usually say that there is a delay in the system response. Indeed, the delayed growth response (DGR) is a particular aspect of microbial growth that has been already reported [2–6]. The studies that have analysed this issue have been mainly focused in medical applications or theoretical mathematical approaches [7,8]. Indeed, MacDonald [9] identified that delays are a natural phenomenon in dynamical development and growth of biological systems, and explains the phenomena by physical difficulties in transporting nutrients and changes in rate of metabolic reactions due to life cycle of the cells.

Time delays are usually introduced rather qualitatively in growth models, and are not specifically tied to the biological parameters [10]. To the knowledge of the authors, Freedman et al. [11] were the first to incorporate time delay in chemostat models. Jiao et al. [7] and Meng et al. [12] demonstrated that the time delay affects the dynamic behaviour of a chemostat, when perturbations on substrate concentration are performed. Many industrial bioprocesses are performed in non-stationary conditions [13]. Indeed, batch and fed-batch operation are the most common strategies

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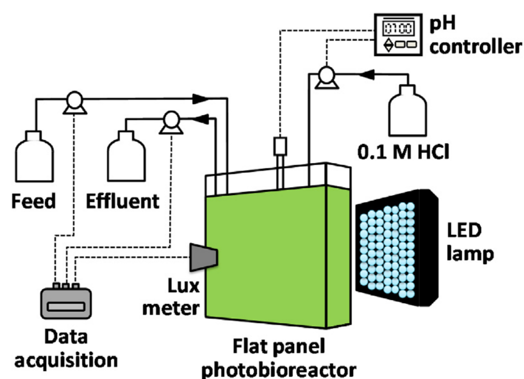

Fig. 1. Schematic representation of lab scale flat panel photobioreactor.

applied in industrial fermentation. Therefore, the existence of these time delays may be of relevance when trying to represent the behaviour of systems where the experimental conditions fluctuate in time, such as in batch, fed-batch and even during the transient periods of continuous systems.

Most reports dealing with delayed microbial growth involve the relation between a substrate and growth. Overall, few reports are available dealing with an eventual delayed response to the presence of inhibitors. The study of *Delayed Inhibition Response* (DIR) may represent a useful contribution to increase the representativeness of microbial growth models. Indeed, many commercial fermentation operations are affected by the action of inhibitory compounds (substrates or products). A good example is microalgae, which is normally inhibited by ammonia (source of nitrogen), compound that would exerts a poisoning effect on the photosynthetic system, causing damage on Photosystem II [14]. Microalgae mass cultivation has experienced a significant growth in the last decade, boosted by their potential as biomass source for the production of biofuels as well as the production and recovery of high added-value products [15,16].

The aim of the present study is to assess the existence of a DIR when considering microalgae growth in the presence of inhibiting concentrations of ammonia. *Chlorella sorokiniana* was used a test alga for this research.

2. Materials and methods

2.1. Microorganisms and culture media

Chlorella sorokiniana CCAP 211/8k was used to conduct this study. It was supplied by the laboratory of CIDERTA-BITAL Group from University of Huelva (Spain). It was cultivated in modified M-8a medium [17]. The composition of the medium was 0.74 $g\,L^{-1}$ KH_2PO_4; 0.26 $g\,L^{-1}$ $Na_2HPO_4{\cdot}2H_2O$; 0.4 $g\,L^{-1}$ $MgSO_4{\cdot}2H_2O$; 0.013 $g\,L^{-1}$ $CaCl_2 \times 2H_2O$; 0.5 $g\,L^{-1}$ NH_4Cl; 0.116 $g\,L^{-1}$ $C_{10}H_{12}N_2NaFeO_8$; 0.0372 $g\,L^{-1}$ $NA_2EDTA{\cdot}2H_2O$; $6.18{\cdot}10^{-5}$ $g\,L^{-1}$ H_3BO_3; $1.3{\cdot}10^{-2}$ $g\,L^{-1}$ $MnCl_2{\cdot}4H_2O$; $3.2{\cdot}10^{-3}$ $g\,L^{-1}$ $ZnSO_4{\cdot}7H_2O$; $1.83{\cdot}10^{-3}$ $g\,L^{-1}$ $CuSO_4{\cdot}5H_2O$. The pH of the medium was adjusted to 7.0 and autoclaved at 121 °C for 20 min before use. When needed, NH_4Cl was further supplemented to the medium in order to provide the reported total ammonia nitrogen (TAN) concentrations.

2.2. Photobioreactor for oximetric assays

The dynamic response of microalgae to inhibitory concentrations of ammonia was studied in a lab-scale flat panel photobioreactor (Fig. 1). Reactor internal dimensions were 26.4 × 24.4 × 2.4 cm. System was operated at 65% of its capacity (useful volume of 1.0 L). Light was provided by a 15 W lamp composed by 240 LED bulbs (Ekoline, IP-65, China). Light intensity at reactor surface was 570 $\mu mol\,m^{-2}\,s^{-1}$ in the PAR range. Photobioreactor was operated as a turbidostat: culture media was continuously fed to the bioreactor in order to keep the transmitted light constant. A lux meter (Rixen, LXT-401A, Taiwan) was used to measure on-line the transmitted light, passing through the culture. Such operation mode was applied to keep the optical density of the culture constant, despite microalgae growth. This ensures that average light intensity that cells receive also remained constant. For that purpose, when feeding culture media, broth was also extracted, using peristaltic pumps. Under the conditions applied, biomass concentration remained at about 0.7 $g\,L^{-1}$, measured as total suspended solids (TSS). Analog signal from lux meter was processed in a data acquisition system (USB 6008, National Instrument) which was also used to control on-line the peristaltic pumps involved in the photobioreactor operation. This device was connected to a PC running LabView (National Instruments). Photobioreactor was run at room temperature (25 ± 3 °C). The reactor was mixed by coarse air bubbling (at a flow of 3 $L\,min^{-1}$), and by using two vertical magnetic stirrers. pH was controlled online using a pH controller (Hanna Instruments 931700-1), which activated a peristaltic pump dosing a 1 M solution of HCl.

Research involved performing several reactors runs, at different values of ammonia concentration. For each new photobioreactor run, fresh microalgae culture was inoculated. In all cases, ammonia concentration in the reactor was kept constant. For that purpose, TAN concentration was periodically measured during reactor operation, and TAN concertation of the influent was modified when necessary.

Table 1
Central composite design to study influence of TAN, pH and exposure time on *C. sorokiniana* oximetric activity.

Factors	Levels		
TAN ($mg\,L^{-1}$)	500	1000	1500
pH	7	8	9
Time (h)	0	24	48

2.3. Effect of ammonium concentration, pH and time of exposure on microalgae activity

The effect of TAN, pH and exposure time was evaluated by running previously described flat plane photobioreactor, at 3 different TAN concentrations and 3 values of pH. Microalgae activity was used as response. Effect of time of exposure to tested conditions was included in the analysis by determining the activity at 3 different times during each operation: 0, 24 a 48 h. Experiments were organized and performed using response surface methodology [18]. A central composite design was used, with 3 factors: TAN, pH and time of exposure (Table 1). Central point was replicated 3 times.

Microalgae activity was determined measuring its volumetric oxygen production rate (OPR). This measurement was performed in the same photobioreactor. In order to do so, dissolved oxygen was striped by bubbling nitrogen, until oxygen concentration decreased below 1 $mg\,L^{-1}$. Then, the increase of dissolved oxygen concentration was measured for 15 min. Observed delays during this research were in the range of several hours, so ORP determination time is considered short enough not to interfere with results interpretation. No aeration was provided during such period. OPR was then determined as a function of the oxygen concentration increasing rate as previously described by Decostere et al. [19].

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