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# A MODEL PREDICTING THE EFFECTS OF OSCILLATING DISSOLVED OXYGEN TENSION ON THE MOLECULAR WEIGHT OF ALGINATE BY *AZOTOBACTER VINELANDII*

# UN MODELO QUE PREDICE LOS EFECTOS DE LAS OSCILACIONES EN LA TENSION DE OXIGENO DISUELTO EN EL PESO MOLECULAR DEL ALGINATO PRODUCIDO POR *AZOTOBACTER VINELANDII*

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**Abstract**

A semiempirical model that describes and predicts the evolution of alginate mean molecular weight (MMW) produced by *Azotobacter vinelandii*, from cultures carried out under oscillating dissolved oxygen tension (DOT), was developed. Data from the literature of cultures carried out at constant DOT was used. The model is able to explain that the main effect on the MMW of alginate is linked with the amplitude of the DOT sinusoidal wave. The model also predicts the weak effect of the oscillating DOT over kinetics of biomass growth, alginate production and sucrose consumption, as compared with cultures conducted at constant DOT.

*Keywords*: mathematical model, alginate, oscillation, DOT, *Azotobacter vinelandii.*

**Resumen**

Se desarrolló un modelo semiempírico que describe y predice la evolución del peso molecular promedio del alginato producido por *Azotobacter vinelandii* en cultivos llevados a cabo bajo condiciones oscilantes de tensión de oxígeno disuelto (TOD). Se usaron datos de la literatura de aquellos cultivos llevados a cabo bajo condiciones constantes de TOD. El modelo explica que el principal efecto sobre el peso molecular promedio del alginato está asociado con la amplitud de la onda sinusoidal de TOD. Además, el modelo predice el débil efecto de la oscilación de la TOD sobre las cinéticas de crecimiento bacterial, producción de alginato y el consumo de sacarosa, comparado con los cultivos llevados a cabo a TOD constante.

*Palabras clave*: modelo matemático, alginato, oscilación, oxígeno, *Azotobacter vinelandii.*

## 1. Introduction

One of the most important issues in aerobic fermentation processes is the availability of dissolved oxygen (DOT) in the bioreactor (Oosterhuis and Kossen, 1984). Fluctuations in DOT and in other nutrients inside bioreactors can be determined either by scale-up effects (*i.e.* inhomogeneities occurring in large tanks (Oosterhuis *et al.*, 1985) or by the high viscosity and the broth rheological complexity, resulting in segregation due to insufficient mixing (Amanullah *et al.* 1998). Alginates are linear copolymers of β-D- mannuronic and their epimer, α-L-guluronic acid, which can be obtained from marine algae and bacteria such as *Azotobater vinelandii* (Sabra *et al*., 2001). From an application point of view, the most important characteristics of the alginate solutions are their viscosity and their capacity as gelling agents. The gel forming properties of alginate depend on the relative content of the two monomers (Clementi *et al*., 1998), whereas the viscosity of alginate solutions,

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which determines their capability as thickener agent, mainly depends on the molecular weight of the polymer (Rhem and Valla, 1997; Peña *et al*., 2000).

In *A. vinelandii* cultures, DOT and agitation speed have a strong effect on the mean molecular weight (MMW) of alginate (Peña *et al.,* 1997; Peña *et al.,* 2000). Efficient production of alginate by *A. vinelandii* is achieved when the culture is conducted under a strict control of DOT between 2 and 5 % (Horan *et al.,* 1983; Peña *et al,*. 2000; Trujillo-Roldán *et al.,* 2003a). Moreover, a recent publication by Trujillo-Roldán *et al.,* (2004) reported that DOT (in controlled culture conditions) determine the molecular weight distributions and the mean molecular weight of the alginate produced by *A. vinelandii*. Trujillo-Roldán *et al.,* (2001) reported that oscillating DOT does not affect culture kinetics but affects significantly the evolution and distribution of the molecular weight of the alginate produced.

There are few papers published in the literature about the modeling of the effect of changing conditions on bioprocess results (Wincure *et al.,* 1995; Pham *et al.,* 1998; Pinchuk *et al.,* 2000). To our knowledge, no information is available regarding the modeling of changing DOT for microbial cultures that produce polysaccharides. The aim of this work was to develop a mathematical model which could describe and predict (from data obtained under constant DOT), the growth kinetics of *A. vinelandii*, alginate production and the evolution of the MMW, of cultures conducted under conditions of oscillating DOT.

## 2. Materials and Methods

### *2.1. Alginate fermentations data*

Data from cultures carried out at constant and oscillating DOT in stirred tank were considered (Peña *et al*., 2000; Trujillo Roldán *et al*., 2001). Analytical determinations (biomass, alginate, sucrose and alginate molecular weight) were conducted as described previously (Peña, *et al*., 1997 and 2000). It is important to point out that all cultures were developed under no-diazotrophic conditions (i.e. in which non nitrogen fixation occurs) and therefore the model is valid for such conditions. In addition, only cultures conducted at 700 rpm were considered. Under these conditions, the cells do not form aggregates and, therefore, it can be ruled out any diffusional limitation (both oxygen or other nutrients) from the bulk liquid to inside the cells.

### *2.2. Model theory*

Fig. 1 shows a flow diagram for solving the developed model. This describes the kinetics of biomass growth, sucrose consumption and alginate production, linked to the evolution of the MMW of alginate. Table 1 summarizes all parameters and equations used in the model and its dependence with DOT in the range of 1 to 7 % of air saturation.

In order to consider the availability of substrate (S) and DOT, a modified logistic equation was used (equation 2 in Fig. 1). The specific growth rate ($\mu$) exhibits a saturation-like dependence on oxygen concentration (Peña *et al.* 2000). The DOT saturation constant ($K_{O2}$) and maximum specific growth rate ($\mu_{max}$) were obtained from data reported previously by our group for cultures carried out at constant DOT of 1, 3 and 5 % (Peña *et al.* 2000). The values of $K_{O2}$ and $\mu_{max}$ were 1.21 % of DOT (0.094 mg/l) and 0.36 $h^{-1}$, respectively. The sucrose saturation constant ($K_S$) was taken from that reported by Kuhla and Oelze (1992). Those authors reported a value of 0.02 g/l, which was calculated from cultures of *A. vinelandii* using sucrose as carbon source and using a range of DOT between 1.0 and 7.0 %.

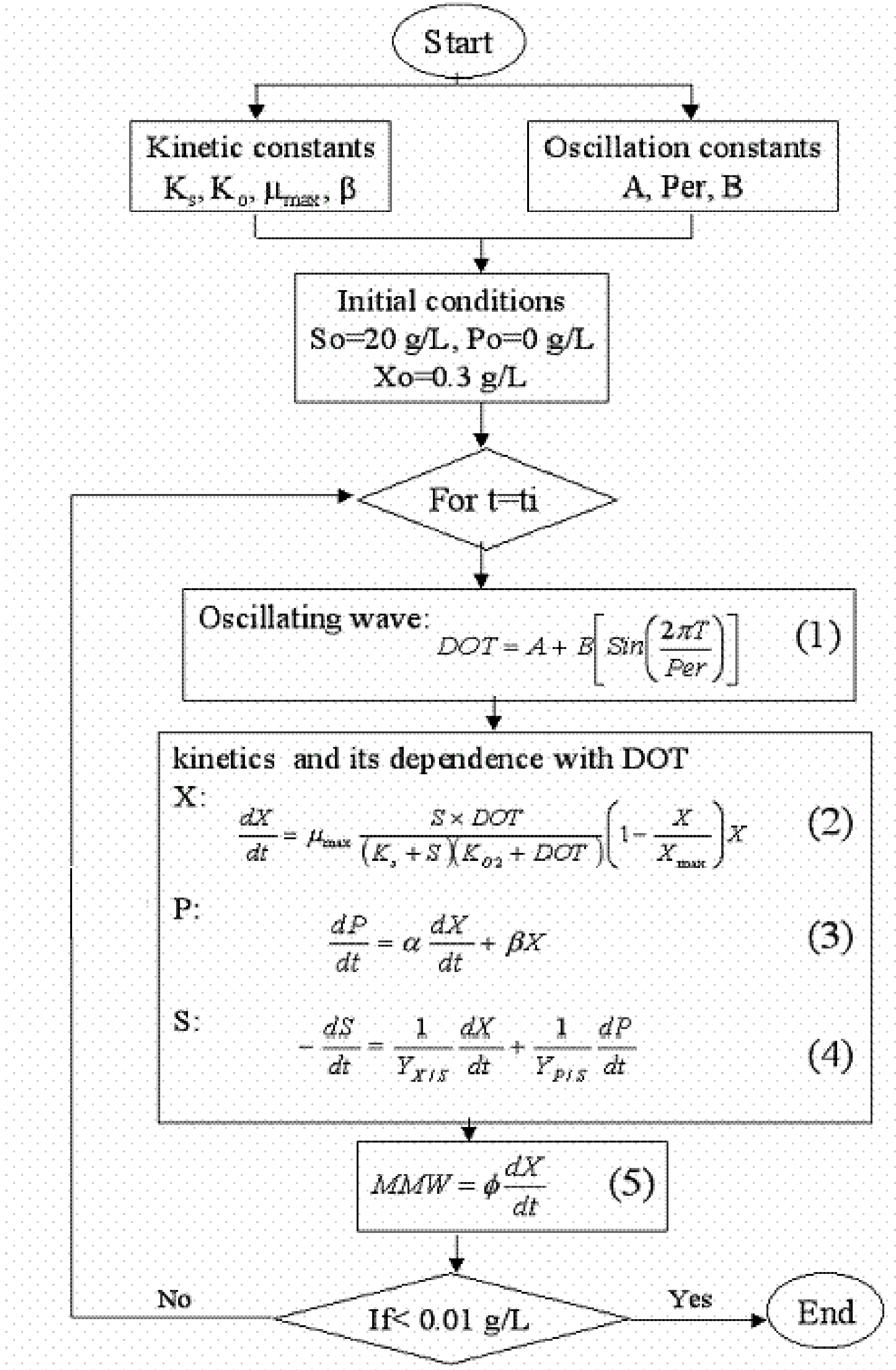


Fig. 1 Flow diagram of the model linking *A. vinelandii* culture kinetics and evolution of alginate mean molecular weight.

Table 1. Parameters and nomenclature used in the modeling, and their dependence with DOT.

| Parameter | Definition | Units | Value taken in the range of 1-7 % DOT | Reference |
|---|---|---|---|---|
| A | Set - point of the DOT sinusoidal wave | % | 3 | Trujillo-Roldán *et al*. (2001) |
| B | Amplitude of the DOT sinusoidal wave | % | 1.0, 1.7, 2.1, 2.2 | Trujillo-Roldán *et al*. (2001) |
| DOT | Dissolved oxygen tension | % | - | - |
| dX/dt | Growth rate | $g_{biom}$ / h | - | - |
| $K_O$ | Dissolved oxygen saturation constant | % | 1.21 | Peña *et al*. (2000) |
| $K_S$ | Substrate (sucrose) saturation constant | g /L | 0.02 | Kuhla and Oelze (1992) |
| MMW | Mean molecular weight | KDa | - | - |
| Per | DOT wave period | s | 1200, 2400, 4000 | Trujillo-Roldán *et al*. (2001) |
| T | Culture time | s | - | - |
| $X_m$ | Maximum biomass concentration | g/L | 6.2 | Trujillo-Roldán *et al*. (2001) |
| $Y_{p/s}$ | Product/substrate yield | $g_{pro}/g_{sub}$ | -0.035DOT+0.36 | This work |
| $Y_{x/s}$ | Biomass/substrate yield | $g_{biom}/g_{sub}$ | -0.048DOT+0.40 | This work |
| $\mu_{max}$ | Maximal specific growth rate | $h^{-1}$ | 0.36 | Peña *et al*. (2000) |
| $\alpha$ | Luedeking-Piret growth associated constant | $g_{pro}/g_{biom}$ | 0.32 DOT + 0.11 | This work |
| $\beta$ | Luedeking-Piret non-growth associated constant | $g_{pro}/g_{biom}$ h | 0.0 | Peña *et al*. (2000) |
| $\phi$ | Proportionality parameter between MMW and dX/dt | KDa h/$g_{biom}$ | $-133DOT^2+870DOT-684$ | This work |
| Subscripts | | | | |
| biom | Biomass | | | |
| Pro | Product (alginate) | | | |
| Sub | Substrate (sucrose) | | | |

For alginate production, a Luedeking-Piret expression (Luedeking and Piret, 1959) was used, as described by equation 3 in Fig. 1. In such equation, , $\alpha$ and $\beta$ are empirical constants for growth and non-growth associated alginate production, respectively. From previously reported data for DOT constant cultures (Peña *et al.,* 2000), it is known that the value of $\alpha$ depends linearly on DOT between 1.0 and 7.0 % and values of $\beta$ were practically zero (table 1). Substrate utilization kinetics was described by a Luedeking-Piret type equation as it is shown in equation 4, Fig. 1. This considers substrate conversion to cell mass and product only. The yields of biomass ($Y_{x/s}$) and product ($Y_{p/s}$) with regard to substrate (data taken from Peña *et al.,* 2000), also depends linearly on DOT (Table 1).

A basic issue for modeling the evolution of MMW was to establish a relationship between kinetic parameters and the molecular properties of the alginate. As very scarce information is available (Trujillo-Roldán *et al.,* 2004) about the biochemical and physiological mechanisms of polymerization and depolymerization of the alginate by *A. vinelandii*, an empirical approach was taken. Using data from cultures carried out under constant DOT (Peña *et al.,* 2000), a linear relationship between the growth rate (dX/dt), and the evolution of the mean molecular weight was established (Fig. 2). This relationship (shown in Ec. 5, Fig. 1) is valid only when the alginate MMW is increasing, a phenomenon occurring during the exponential phase of the culture. The increase in the MMW is due to a combined effect of the activity of the alginate polymerase enzymatic complex and the action of alginate lyase activity (Trujillo-Roldán *et al.,* 2003a; Trujillo-Roldán *et al.,* 2003b; Trujillo-Roldán *et al.,* 2004). A drop in polymer molecular weight occurs towards the end of the culture (Peña *et al.,* 2000), which is known to be caused only by the action of alginate lyase (Trujillo-Roldán *et al.* 2003a; Trujillo-Roldán *et al.,* 2004). However, in the present model, the phase in which the MMW is decreasing was not considered. This was unnecessary, as our main objective was to predict the maximum molecular weight achieved during culture. If the slope of the straight lines depicted in Fig. 2 is taken and then correlated with the DOT, a bell-shaped relationship was found (Fig. 3).

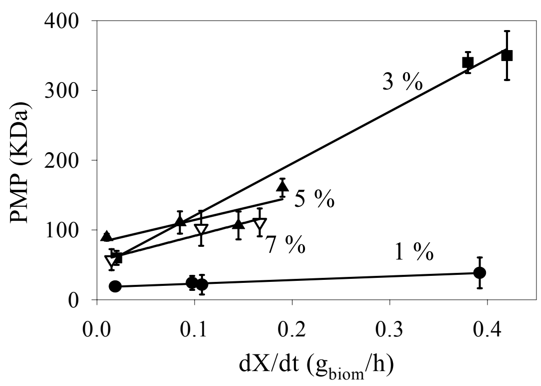


Fig. 2 Relationship between the alginate mean molecular weight and the *A. vinelandii* growth rate (dX/dt), for different values of culture DOT.

For simulation purposes, an empirical second-order polynomial equation ($r^2 > 0.98$) can describe the results in terms of the influence of dX/dt over MMW, as a function of DOT (equation shown in Table 1 for the parameter $\phi$).

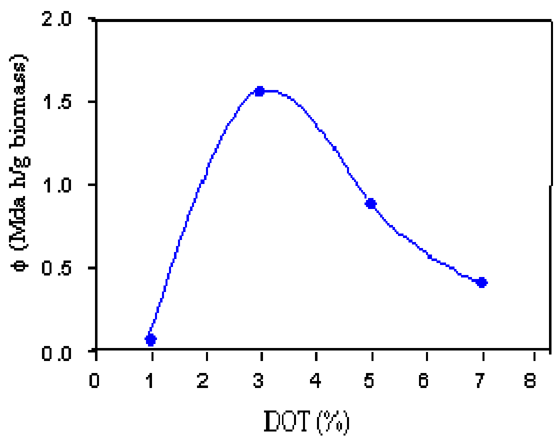


Fig. 3 Relationship between the DOT and slopes obtained from Fig. 2.

Oscillatory DOT simulations were made using a sinusoidal wave equation, as it is shown in equation 1 (Fig. 1). As a set of experimental conditions for oscillation axis (A), amplitude (B) and period (Per) were available (Trujillo-Roldán *et al.* 2001), such values were used to test the model (A= 3 % of DOT, B = 1.0, 1.7, 2.1 and 2.2 % of DOT and Per = 1200, 2400 and 4000 s). The solution of differential equations was performed numerically using the ISIM simulation software (Dunn *et al.,* 1992).

## 4. Results and discussion

Fig. 4 shows that the model could satisfactorily describe and predict the biomass growth, alginate production and sucrose consumption of the oscillating DOT cultures reported by Trujillo-Roldán *et al.,* (2001). It is important to point out that the model only used the data for conditions of constant DOT reported previously by Peña *et* *al*. (2000) for predicting all the oscillations conditions tested. Regardless of the particular oscillatory conditions (wave periods of 1200, 2400 and 4000 s and average amplitudes of 2.2, and 1.0, 2.1, and 1.7 % of DOT, respectively), the model gave practically the same predictions. As the simulations are closely similar, only one prediction is shown in Fig. 4 (solid line). This corresponds for an oscillation period of 1200 s and a wave amplitude of 2.2 % of DOT. The results of the modelling are in agreement with the experimental results, showing the weak effect of the wave amplitude and the period on *A. vinelandii* kinetics. Simulations of bacterial growth carried out at small integration steps suggest an oscillatory growth due to changing DOT conditions (inset of Fig. 4a); however, no experimental verification could be made. Overall, we demonstrated that an integration of the modified logistic and Luedeking-Piret equations could be used to predict the kinetics of cultures of polysaccharide-producing bacteria grown under DOT oscillatory conditions.

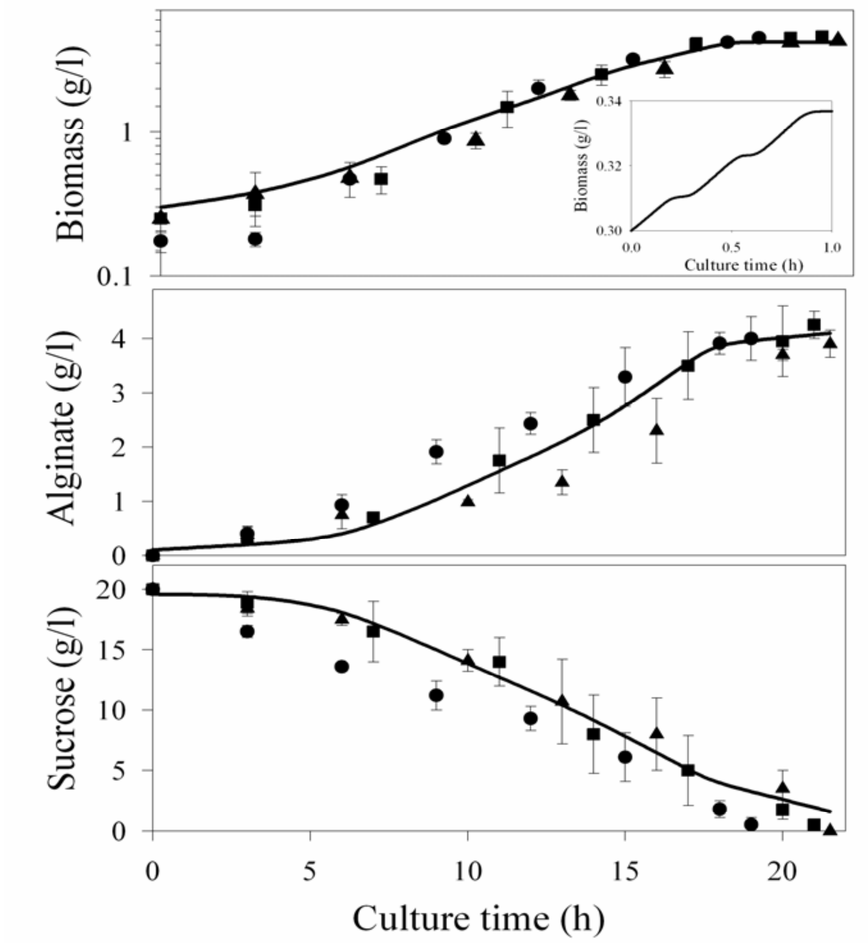


Fig. 4 Modeling (solid line) of the kinetics of *A. vinelandii* growth (a), alginate production (b), and sucrose consumption (c) of oscillating DOT cultures at B = 2.2 %, Per = 1200 s. Experimental data reported by Trujillo-Roldán *et al.* (2001) are shown as: B = 1.0 %, Per = 1200 s (■); B = 2.2 %, Per = 1200 s (□); B = 1.7 %, Per = 4000 s (▲). Inset in (a) shows oscillatory growth modeled by a culture carried out at Per = 1200 s and B = 2.1 %, for a window of 1.0 h.

Fig. 5 shows the MMW evolution of cultures carried out under oscillatory conditions and, for comparison, those conducted at 3 % constant DOT (experimental data from Trujillo-Roldán *et al.,* 2001). The lines show the model simulations for the various conditions tested. The model is able to predict (only using data obtained under constant DOT) the evolution of MMW along cultures for oscillating DOT conditions. The model gives a good description of the effect of the wave period. For example, the simulation of two cultures conducted at wave amplitudes of 1.0 and 2.2 % of DOT (having the same wave period of 1200 s) gave values of $MMW_{max}$ of 230 and 100 KDa, respectively (experimental data were of 260 KDa at ± 1.0 % of DOT and 65 KDa at ± 2.2 % of DOT). On the other hand, for simulations conducted at similar wave amplitudes (2.1 - 2.2 % of DOT), having wave periods of 1200 and 2400 s, the model predicts $MMW_{max}$ values of 100 and 106 KDa, respectively.

Data shown in Fig. 5 suggest that the enzymatic complex responsible of polymerizing the alginate molecule is strongly affected by small (and in this case, oscillatory) changes in DOT. These results are in agreement with those recently reported by Trujillo-Roldán *et al.,* (2004), who demonstrated that DOT directly affects the activity of the polymerase complex as well as the activity of alginate lyase, resulting in relatively important changes in MMW for relatively small changes in DOT (in the range of 1 to 5 % of DOT). Non-constant DOT oscillating conditions have been used to simulate non-ideal mixing conditions in large fermenters (Vandar and Lilly, 1982; Namdev *et al*., 1993). Our model could be adapted to practically any non-constant conditions by simply changing the Ec. that models the oscillation.

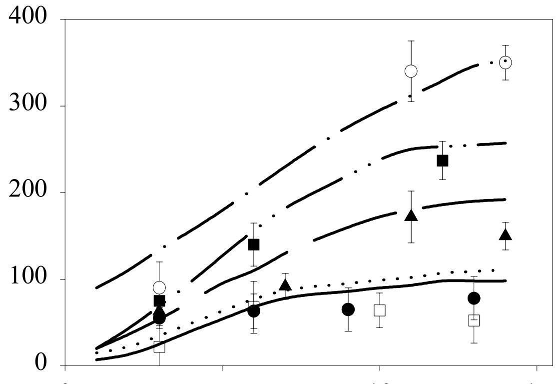


Fig. 5 Modeling (lines) of the evolution of the MMW for *A. vinelandii* cultures carried out at different oscillating conditions of DOT. Experimental data from Trujillo-Roldán *et al.* (2001): B = 2.2 % and Per = 1200 s (□, exp.; ——, model); B = 1.0 % and Per = 1200 s (■, exp.; — ● ● —, model); B = 2.1 % and Per = 2400 s (●, exp.; ● ● ●, model); B = 1.7 % and Per = 4000 s (▲, exp; — —, model); and 3 % constant (○, exp.; — ● —, model).

## Conclusions

A semiempirical, unstructured and unsegregated model was developed and used to describe and predict the effect of oscillating DOT in alginate-producing cultures. The model (based on data taken from constant DOT cultures) satisfactorily predicted the kinetics of cultures under oscillating conditions, as well as the evolution of the mean molecular weight. The model suggests that the enzymatic complexes in charge of polymerizing and depolymerizing the alginate molecules are strongly affected by small (and in this case, oscillatory) changes in DOT. The model can be used, for example, to predict the effects of an inefficient control in DOT during alginate fermentation (due to high viscosity and/or insufficient mixing) and to predict effects on scale-up.

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