INTRODUCTION

The lag phase of bacterial cultures has been widely studied for years in an effort to contribute to the improvement of food safety [1]. It is accepted that this lag phase is a complex process affected by environmental factors, preinoculation conditions and species genetics, among others. Although many experiments have been carried out, it is difficult to compare the different results because there is not a holistic understanding of the behaviour of the system. A generic non-specific mathematical analysis may increase the general knowledge of the phenomenon. It may be useful for redfining the experiments to be done and for detecting the parameters with technological relevance.

INDISIM: The mathematical model is tested with INDIVidual DIScrete SIMulation, an Ibm developed by our group to simulate the growth of bacterial cultures [2,3].

THE SIMULATED SYSTEM: We model a batch culture in an agitated liquid medium. The inoculum is taken from the stationary phase of a previous culture. Its cellular mean mass is lower than the mean mass of any sample in the exponential growth stage. The initial number of bacteria is $N_0 = 1000$.

STAGES IN THE BACTERIAL LAG PHASE

Whenever a bacterial inoculum is added into fresh medium, a lag phase in the increase in the number of individuals $N$ is found. Sometimes, a lag in the increase in biomass $B$ is also found. These lags can be split into several stages each

- Initial phase ($P_{\text{IN}}$ or $F_{\text{IN}}$): stage in which the growth rate is negative or equal to 0.
- Transition phase ($P_{\text{IN}}$ or $F_{\text{IN}}$): stage in which the growth rate $\mu$ increases.
- Exponential phase ($P_{\text{EXP}}$ or $F_{\text{EXP}}$): stage in which the maximum growth rate is reached.

When both the biomass and population growth rate ($\mu_b$ and $\mu$) have reached the maximum growth rate in the provided conditions ($\mu_{\text{max}}$, $\mu_{\text{max}}$, $\mu_{\text{max}}$, $\mu_{\text{max}}$), the culture enters the so-called balanced exponential growth phase ($P_{\text{EXP}}$).

The existence and duration of each stage is determined by the changes in the environmental conditions and the state of the inoculum. Any culture lag phase implies, at least, the existence of $P_{\text{IN}}$. At the other extreme is the case where the four stages exist, either consecutively or overlapping.

AN EASY MATHEMATICAL MODEL OF THE TRANSITION PHASE

We assume the hypothesis that $\mu$ increases linearly until it reaches the $\mu_{\text{max}}$ value. Therefore, the logarithmic representation of the transition phase can be modelled as a quadratic curve. If we solve the differential equations for the number of bacteria, we get the following results for each stage:

- $P_{\text{IN}}$: $t_2 < t < t_1$, $\mu(t) = 0$.
- $P_{\text{EXP}}$: $t > t_2$, $\mu(t) = \mu_{\text{max}}$.
- $P_{\text{EXP}}$: $t_1 < t < t_2$, $\mu(t) = \mu_{\text{max}} + a(t - t_2)$.

Lag phase: $\lambda = \frac{P(t_2)}{2} - \frac{t_2}{2}$.

- $t_1$: first division time
- $t_2$: end of the transition phase; it can be easily obtained from the detection time $t_1$ [4]:
  $t_1 = 2t_2 - t_2 - \frac{2}{\mu_{\text{max}}} \ln \frac{N_0}{N_t}$

- $N_t$ and the slope a are:
  $N_t = N_0 e^{\frac{a(t - t_2)}{\mu_{\text{max}}}}$

RESULTS AND CONCLUSIONS

We have fitted a quadratic curve to the transition phases of different cases, both in number of individuals and in biomass (when the transition exists), with quite good results. For the cases presented below, the fitting of this mathematical model to the number of bacteria ($P_{\text{exp}}$) has a high correlation factor ($r_t = 0.9955$, $r_b = 0.9934$).

Actually, the linear increasing of $\mu$ during the transition phase can be observed in both cases.

CASE a: The composition of the new medium does not change, and environmental conditions are similar, so the total biomass increases at its maximum rate ($\mu_{\text{max}}$) from the beginning; there is no lag in the biomass evolution. The lag duration in number of bacteria depends on the biomass distribution conditions of the inoculum. While the mass of the bacteria is too low to allow any bacterial division, $\mu = 0$. Then it increases gradually until $\mu_{\text{max}}$. It is observed that the lag phase in biomass is shorter than the lag phase in the number of individuals.

CASE b: The composition of the new medium is different from the pre-inoculation medium, so the bacteria must carry out a more severe adaptation that usually implies an initial decrease in their biomass [3]. In this case there is a lag phase both in the biomass and population growth. The biomass and population growth rates increase to reach their exponential characteristic value. It is observed that the lag phase in biomass is shorter than the lag phase in the number of individuals.

CASE c: In this figure we show the results of the same simulation, but considering the results that would be obtained with two different experimental techniques:

1. CFU or equivalent techniques
2. Optical methods

CASE γ: The biomass of each individual cell of the inoculum is the same as in the case β plus a constant increment, that is, with a higher initial mean mass. The main difference between the two cases is that all the cells of the inoculum can grow, whereas some cells of the inoculum β can not.

The stages in the bacterial lag phase here presented are a holistic property of the system. These stages reflect the temporal evolution of the distribution among the population of some individual properties. The lag phase in IV strongly depends on the evolution of the biomass distribution. The lag phase in β mainly depends on the evolution of the individual metabolic states. At an individual level, these collective effects are lost. If a single-cell inoculum is studied, these stages can not be directly extrapolated. This case requires a specific treatment that is being carried out for our group at this moment.

REFERENCES: