

*Full Length Research Paper*

# **Modeling the growth dynamics of *Lactobacillus lactis* CWBI B-1410: Effect of changes in glucose and nitrogenized matter in the Man Rogosa Sharpe (MRS) culture media**

**Modou DIENG<sup>1\*</sup>, Ndèye Adiara NDIAYE<sup>2</sup>, Mouhamed NDONG<sup>3</sup>, Ndèye Coumba TOURE/KANE<sup>4</sup>**

<sup>1</sup>Laboratoire d'analyses et Essais, École Supérieure Polytechnique / University Cheikh Anta DIOP University in Dakar, Sénégal.

<sup>2</sup>Laboratoire de Microbiologie, École Supérieure Polytechnique / University Cheikh Anta DIOP in Dakar, Sénégal.

<sup>3</sup>Canadian Rivers Institute, Department of Civil Engineering, University New Brunswick, Fredericton, NB E3B 5E1, Canada.

<sup>4</sup>Faculté de Médecine / University Cheikh Anta DIOP in Dakar, Sénégal.

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Food preservation is a big challenge for the industries. The mechanisms of preservation involved microorganisms such as lactic acid bacteria. Modeling the micro-organisms growth is a very useful information to well-understand their behavior and to propose the best conditions for food preservation. In this way, this study was to propose a mathematical model of the dynamical growth of *Lactococcus lactis* CWBI-B1410 which play a main role for the bio-conservation of fish. This study is conducted by varying the concentration of glucose and nitrogenized matter. The results showed that the mathematical model predicted the growth of *L. lactis* CWBI-B1410. The curves that are predicted are exactly those that can be observed experimentally. The results showed that the production of biomass by *L. lactis* CWBI-B1410 is obtained on the MRS medium composed of 15 g of glucose per liter and 40 g of nitrogenous material per liter. From the obtained results, it could be clearly advocated that for two tests with the same amount of nitrogenous matter, it is possible to achieve economies of scale in glucose while maintaining the same biomass performance.

**Key words:** Mathematical models, lactic bacteria, *Lactococcus lactis* CWBI-B1410, glucose, nitrogenous matter.

## **INTRODUCTION**

Quality control and microbiological safety of fish products are a permanent challenge for fishery industry. They always

\*Corresponding author. E-mail: [modouabsadieng@gmail.com](mailto:modouabsadieng@gmail.com).

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consider fish products degradation due to conservation issues (Fabinyi et al., 2017). Fish conservation is very sensitive to environmental conditions that can increase the risk to undesirable micro-organisms growth under certain conditions and then constitute a threat for public health. This system for food production and distribution in fishery industries has to ensure that undesirable microorganisms growth is controlled. Then, understanding the conditions leading to micro-organisms growth is important (Nikolaev, 2010). Different optimization approaches to determining appropriate levels of metabolic enzymes have been used in a number of purely simulation-based studies for various aspects of microbial metabolism, including the production of serine (Nikolaev, 2010), tryptophan (Chen and Zeng, 2017), and bacteriocin produced by a strain of *Streptococcus gallolyticus* (Abdu et al., 2020). The simultaneous production of serine and tryptophan in *E. coli* has also been considered using a multi-objective optimization strategy (Lee et al., 2010). According to Alfredo et al. (2020), silage technology, with the use of lactic acid bacteria, could help extend the shelf-life of orange residue by up to 10 days by keeping it fresh. In order to describe the behavior of microorganisms in foods and particularly their growth based on key ecological food characteristics, many mathematical models have been proposed in the literature during the recent years (Alfredo et al., 2020). Predictive microbiology based on mathematical models is very useful and it helps to understand microbial hazards in a food chain. It has been showed that it is relatively easy and inexpensive to assess and to validate theoretical models that can be used to predict and to quantify the effects of experimental conditions on microorganism growth (Delhalle et al., 2012). These modeling activities result to link the growth parameters to the environmental factors through mathematical equations (Maoura et al., 2006). Comparing the adjustment of primary models on laboratory data has been the subject of several studies (Pal et al., 2008). Currently, it is not possible to select a particular model to represent the most appropriate bacterial growth. The simplest models can often be sufficient to properly represent the basic parameters of growth. The first growth model in predictive microbiology described by Buchanan (1918) described the exponential phase, but it does not take into account the latent phase or the stationary phase. However, Buchanan's model can be used in a first approach to assess the evolution bacteria population and growth rate. An alternative exponential model has been proposed by Brilllet et al. (2016). This exponential model takes into account the reaction time and the deceleration phase. Delhalle et al. (2012) described the exponential growth phase which appeared in the form of a linear portion when the evolution of microbial population is represented by the time. For a given bacterium, the value of this maximum

growth rate depended on the characteristics of the medium culture (Kyu et al., 2009). When the microbial concentrations are expressed in semi logarithmic mathematical form the maximum specific growth rate can be determined by calculating (Fahimi, 2012). Senegal is known today for its high level of production of sea products (Diop et al., 2008). However, their fishery industry is not efficient due to the weak conservation methods (Diei-Ouadi, 2005). The insufficient safety and toxic substances affected the organoleptic quality and health products reducing the fish production (Diop et al., 2008). Through their work's, Diop et al. (2008) developed a bio-conservation method for fishery industry by using a strain of *L. lactis* CWBI-B1410 isolated from cereals in Senegal. This important discover is very important for fishery industry to increase their productivity by improving the conservation process. However, the biological characteristic of *L. lactis* CWBI-B1410 expressed into mathematical growth model is still not available in the literature. Then, the main objective of this study was to develop a mathematical model to predict the growth of the strain of lactic acid bacterium *L. lactis* CWBI-B1410 in two components of MRS culture medium, glucose and nitrogenous matter.

## METHODOLOGY

This study is conducted in the Analysis and Testing Laboratory at École Supérieure Polytechnique de Dakar (University Cheikh Anta Diop), Senegal.

### Materials

Two types of substrates were used: glucose and nitrogenous matter. The nitrogenized matter was provided by the culture medium composed of casein peptone (Sharlau, Spain).

The laboratory equipment's were glassware oven at 30°C, Petri dishes, a spectrophotometer Helios TM Gamma UV-VIS, Thermo Fisher Scientific, (UVD 134502, England).

### Preparation of the culture medium

The culture medium used in this work was the MRS agar (Man Rogosa Sharpe) with different concentrations of glucose and nitrogenous matter. The pH of the culture medium was adjusted to 6.6 by adding hydrochloric acid (1 N) in order to obtain an optimal pH for growth of *L. lactis* CWBI-B1410. The medium was stored into tubes slants (10 ml/tube). These tubes containing the medium were put in a autoclave for 15 min at 120°C.

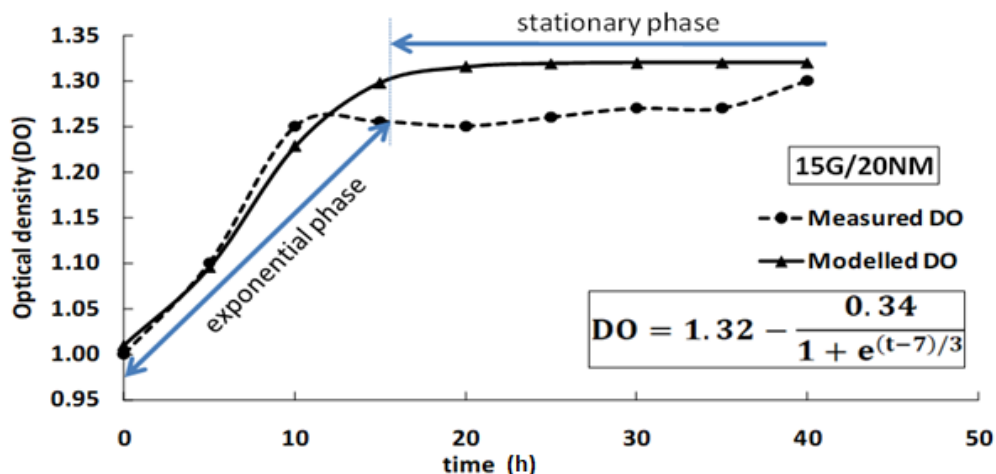
### Preparation of the inoculum

The strain used in this study was *L. lactis* CWBI-B1410. It is a strain of the laboratory collection in Belgium (the Bio-industries Wallonia Center of Industrial Biology). It was revived by two subcultures Man Rogosa Sharpe liquid medium and stored at a temperature of 4°C in conservation tubes containing MRS agar substrate.

The re-activation of the strain was done by extracting some

**Table 1.** Various combinations of concentrations of glucose (G) and nitrogenous matter (N.M.).

Glucose (G) g/l MRS	Nitrogenous matter (N.M.) g/l MRS
15	20
20	20
20	40
15	40

**Figure 1.** Evolution of biomass as a function of time with 15 g of glucose and 20 g of nitrogenous matter per liter.

colonies from these subcultures and transferred into 10 ml tube containing MRS medium liquid. This tube was then incubated at 30°C during 24 h to get our pre-culture.

After 24 h of incubation, 1 ml of this pre-culture was introduced into an Erlenmeyer flask with a capacity of 250 ml containing 100 ml of MRS liquid medium and incubated at 30°C for 40 hours under constant mixing condition by agitation. The all septical conditions were respected to avoid any external contamination.

#### Determination methods

The total biomass concentration of *L. lactis* CWBI-B1410 is determined by measuring the optical density (OD) by using a spectrophotometer Helios TM Gamma UV-VIS at 620 nm.

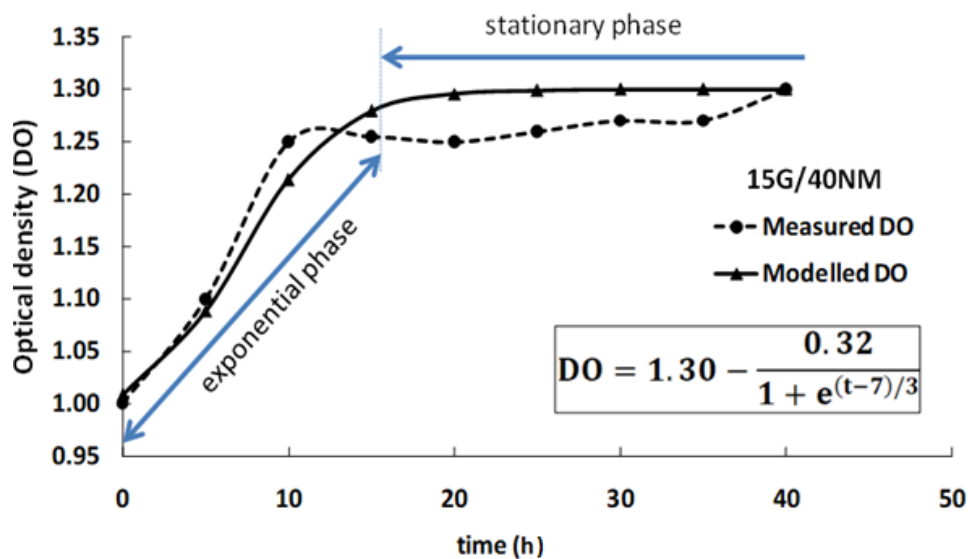
Dieng et al. (2013) showed that the best growth of *L. lactis* CWBI-B1410 are obtained with combinations of different concentrations of glucose and nitrogenized substrate as shown in Table 1.

These associations of glucose (G) and nitrogenous matter (NM) were then tested and mathematical models were proposed by determining the equation for each association. These mathematical models allowed a variation of the biomass according to the quantity of glucose and the nitrogenous matter.

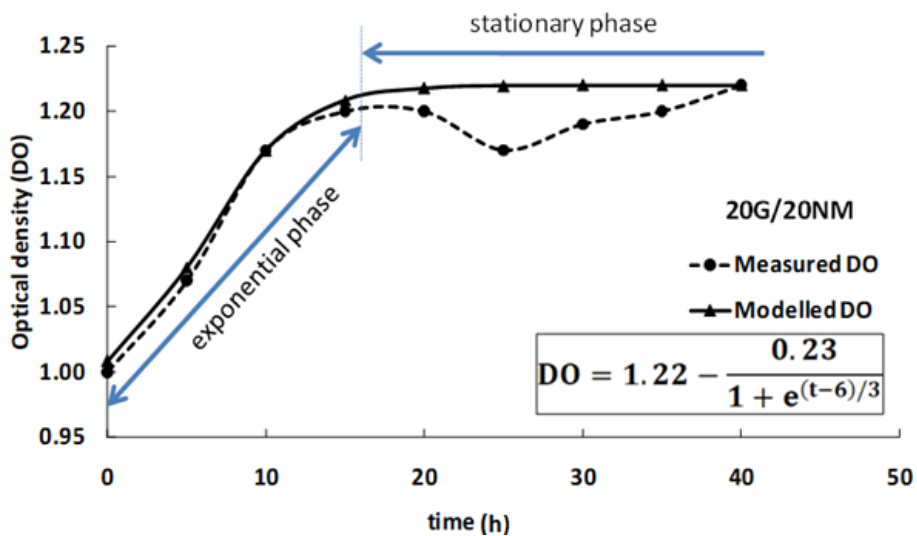
The evolution of the biomass concentration was evaluated by measuring the optical density at 620 nm as a function of time. The data was analyzed with the help of the Excel software. To understand biological phenomena more precisely, the equation parameters have been identified after many simulations in excel to get the best results.

## RESULTS

The effect of different combinations of glucose and nitrogenous matter on the growth of *L. lactis* CWBI-B1410 was evaluated and to study if the concentrations of glucose and nitrogenous material vary, number of bacteria varies from one association (Table 1) to another. The biomass measurements performed showed very similar trends for different situations. Figure 1 clearly shows a startup culture at the fifth hour. The four different biomass obtained have almost super imposed curves. It was also noted that the MRS media with a concentration of 40 g/l crude protein have a higher biomass compared with the media that contain 20 g/l. At the 10<sup>th</sup> hour, the curves corresponding to associations 15 G / 20 N.M. and 20 G / 20 N.M. had OD<sub>600nm</sub> close to 1.2 while the curves corresponding to 15 g/40 N.M. and 20 G / 40 N.M., which look better, have an OD<sub>600nm</sub> of about 1.30 despite the fact that all cultures are seeded with cells in the same physiological state and at the same time. Moreover, from the 10<sup>th</sup> hour of culture, bacteria *L. lactis* CWBI-B1410 seemed to start the stationary phase for all tests. At the 40<sup>th</sup> hour, Figure 1 shows that the best growth is presented by the MRS medium 15G/40 N.M. followed by MRS medium 20 G/40 N.M. reaching respectively an



**Figure 2.** Evolution of biomass as a function of time with 15 g of glucose and 40 g of nitrogenous matter per liter.



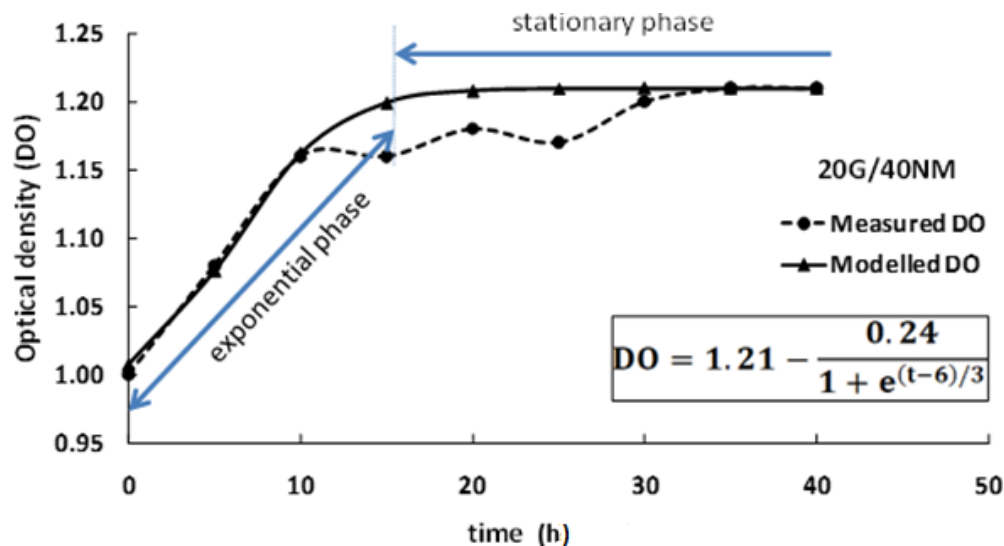
**Figure 3.** Evolution of biomass as a function of time with 20 g of glucose and 20 g of nitrogenous matter per liter.

OD<sub>600nm</sub> value of 1.32 and 1.30.

**DISCUSSION**

These findings are consistent with those of Yaov et al. (2019), which validated a mathematical approach using different experiments with *Escherichia coli*. The approach in this study provided an effective way to predict growth of *L. lactis* CWBI B-1410 by the variation of glucose and

nitrogen. The results also shows that for two tests with the same quantity of nitrogenous matter (Figure 1), it is possible to achieve economies of glucose while maintaining the same performance biomass (Figures 2, 3 and 4),. Mataragas et al. (2003) showed that continuous presence of carbon in the MRS medium during culture is necessary but the amount of glucose is not proportional to the amount of biomass in the culture medium. Moreover, increasing the amount of glucose does not necessarily produce a high level of bacteriocin



**Figure 4.** Evolution of biomass as a function of time with 20 g of glucose and 40 g of nitrogenous matter per liter.

(Abbasiliasi et al., 2017). The study showed that the variation in the amount of glucose and nitrogen influenced the growth of *L. lactis*. This is consistent with the results of Thiago et al. (2019), which showed that the biosynthesis of bacteriocins can be influenced by various culture conditions, such as the composition of the medium, pH, temperature and growth kinetics of the microorganisms.

These results are in agreement with the work of Pemmaraju et al. (2016) who showed that *Candida albicans* formed more biofilms in the presence of an important carbon source. Moryl et al. (2013) have been shown that the composition and quantity of the polysaccharide matrix of a biofilm depend on a carbon source such as glucose. According to studies, stressors (salt, oxygen, microbial competition, etc.) can positively influence the production of bacteriocin in lactic acid bacteria grown in MRS broth (Neysens and DE-Vuyst, 2005). The kinetics of growth and the increase in nitrogenous matter present a best fit by a single function called Boltzmann function with the correlation coefficient of  $R^2 \geq 0.97$ . This value expresses a little error between reality and experimentation. According to the results, it was concluded that there was biomass production by *L. lactis* CWBI-B1410 on the MRS 15 G / 40 N.M.

## Conclusion

The main objective of this study was to develop a mathematical model able to predict the evolution of a strain of lactic acid bacterium *Lactococcus lactis* CWBI-B1410 by varying two components of MRS culture

medium, glucose and nitrogenous substrate. It was found, according to the type of MRS medium used (standard MRS and modified MRS), from the 1<sup>st</sup> to 6<sup>th</sup> hour of the tests showed that the average number of bacteria varies considerably from one medium to another. The application of negative binomial regression showed that the model is globally significant. The model shows a significant statistical difference between the standard MRS medium and the modified MRS medium. This approach has also stimulated the interest of studying a mathematical model for modeling the growth dynamics *L. lactis* CWBI-B1410. To increase the interest of such a model, the effect of pH conditions should be integrated. In future work, the significance of the effect of inoculum size on the length of the lag phase will be also assessed. Finally, for economic reasons, it is important to consider the use of another less expensive source of nitrogenous material such as certain wastes from the food industry as constituents of culture media for the reformulation of the MRS medium in the production of bacteriocins.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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