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Automatic Control for the Production of Alginate by Azotobacter Vinelandii

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ABSTRACT Alginates are polymers used in the food and pharmaceutical industries as stabilizing, thickening, gel or film-forming agents. Food coatings formed from alginate have shown to have excellent properties such as flexibility and resistance to tearing. These polysaccharides are extracted from natural brown algae, but they can also be produced by a bio-process. One strategy to produce alginates is through the manipulation of the culture conditions during fermentation using *Azotobacter vinelandii*. This work proposes the development of a PI control strategy to improve the production of alginate from *Azotobacter vinelandii* in a laboratory bio-reactor scale.

INDEX TERMS Alginate production, *Azotobacter vinelandii*, PI control.

I. INTRODUCTION

Alginates are polysaccharides extracted from natural brown algae, but they can also be produced by the *Pseudomonas spp.* and *Azotobacter vinelandii* [1]. These polymers have a wide range of uses in the industry. For example, in the food industry, the alginate has excellent functionality as a thickening agent, gelling agent, emulsifier, stabilizer, texture-improver (for noodles), by improving the quality of food [2], [3]. Based on its unique properties, alginate is added to numerous kinds of food, such as ice cream, jelly, acid milk drinks, dressings, instant noodles, beer, pet food, fish food, etc. Food & Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) certifies alginate in food applications as one of the safest food additives [4].

Alginic acid obtained from alginate is used in several pharmaceutical applications. Alginate forms a gel in the highly-acidic stomach and protects the stomach mucosa [5], [6]. In the textile industry, alginate is used in the color paste substrate when applying patterns to print fabrics, scarves, towels, etc. The use of alginate in the printing of cotton, jute, and rayon is a necessity [7], [8].

One strategy to produce bacterial alginates is through the manipulation of the culture conditions during fermentation using *A. vinelandii* [9], [10]. Studies have found evidence of the factors determining alginate molecular weight, being the most important the oxygen transfer rate (OTR) and oxygen uptake rate (OUR) [11]. These parameters have been used as criteria in the scaling up of the fermentation process.

Other recent findings [12] showed that the application of the OTR as a scale-up criterion was unable to reproduce the alginate molecular weight profile during fermentation. The evidence showed a possible relationship between the molecular weight and the specific oxygen uptake rate (q_{O_2}), rather than OTR, opening the possibility of using q_{O_2} as a scaling criterion to produce alginates with similar molecular weights. Several studies have shown that the molecular weight of alginates produced by *A. vinelandii* is affected by dissolved oxygen tension (DOT) and the oxygen transfer rate (OTR) [10], [13]–[15].

Recently, several studies have been conducted to analyze and understand the role of oxygen in the polymerization of alginate. It is well established that *A. vinelandii* exhibits high respiratory activity [10], [16] and cultures performed

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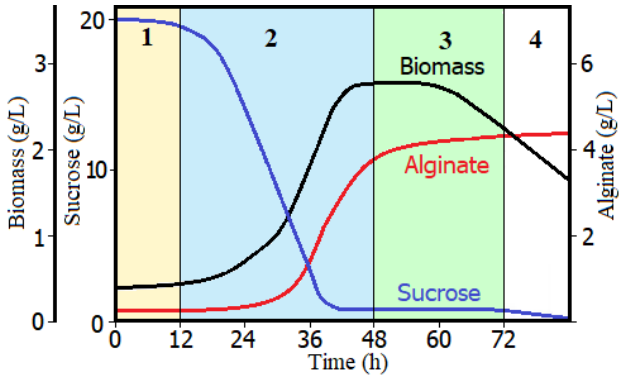

FIGURE 1. Stages of the process.

without DOT-control operate at a DOT close to zero [14], [17]. Under these conditions (i.e. DOT = 0), the cultures are limited by oxygen, which means that OTR can be used to evaluate the growth of the bacteria and alginate production by *A. vinelandii* [10]. It has been documented that cultures limited by oxygen show a characteristic OTR profile [11], [18], [19].

The hypothesis of this work is that can be possible to improve the production of alginate production of *A. vinelandii* by maintaining constant the OTR of a culture. The OTR can be controlled by manipulating the input concentration of O_2 in a bio-reactor. To this end, the objective of this work is to design and to implement a control algorithm to maintain the OTR constant. To his end, the measured variable is the oxygen concentration at the output of the bio-reactor and the manipulated variable is the oxygen concentration at the input of the bio-reactor.

This work is organized as follows: Section II describes the process of production of alginate from the *A. vinelandii* in a scale bio-reactor at the laboratory and describes the mathematical model of the system and its tests. Section III presents the control strategy to improve the production of alginate. Section IV shows the results of this research. Finally, Section V presents the conclusion and future works.

II. ALGINATE PRODUCTION FROM A. VINELANDII

Azotobacter vinelandii is a strict aerobic bacteria that produces alginate and is characterized by its high respiration rate. It also can synthesize at least two molecules of biotechnological interest: alginate and poly (3-hydroxybutyrate) (PHB). An aspect of great importance in the production of microbial polysaccharides is the ability to influence their composition and properties, by controlling the conditions in the culture medium, in addition to the possibility of obtaining alginate of uniform composition [1], [20]. Figure 1 shows the four stages of production of alginate from *A. vinelandii*, relating the growth of the bacteria with the consumption of the substrate (sucrose) and the production of alginate.

The stages of the process are the following: 1) Latency (approx. 12h for this work, yellow region in fig. 1), where the bacteria adapt to the culture medium and the growth is too small; 2) Cellular growth (36h approx. for this work, cyan region in fig. 1), where the bacteria begin to grow consuming the substrate (sucrose), breathing O_2 and producing alginate. The process remains in this state while it has substrate; 3) Stationary phase (24h approx. for this work, green region in fig. 1), where the bacteria stop to grow and the production of alginate decreases; and 4) Dead of the bacteria (white region in fig. 1), where the substrate is over and the bacteria die. The most important stages are the second one and the third where the bacteria are growing and producing alginate while consuming O_2 and substrate. Some reports have studied the elements that affect the production of the alginate produced by *A. vinelandii* [14], [18], [21]–[24]. For example, the concentration of the alginate and other properties like its molecular weight are affected by the agitation rate, dissolved oxygen tension, dilution rate and OTR. Figure 2 shows the elements involved in alginate production by *A. vinelandii*.

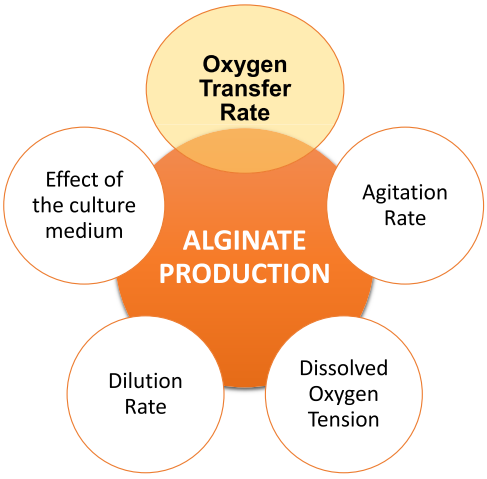

FIGURE 2. Components involved in the production of bacterial alginate.

FIGURE 3. Bioreactor in the laboratory.

A. ALGINATE PRODUCTION IN THE LABORATORY

In our lab, we have a 3-L bio-reactor Applikon (Schiedam, Netherlands) and it can be operated under different agitation rates (between 300 and 700 rpm) [14], [18], [22]. Figure 3 shows the bio-reactor in the laboratory.

The fermentor, which was equipped with two Rushton turbines, was aerated at 2 L/min (1.0 vvm). The DOT was measured using a polarographic oxygen probe (Ingold, Mettler-Toledo), and it was not controlled. Samples of cultures (20 or 30 ml) were withdrawn from the bio-reactor

TABLE 1. PI controller parameters.

Property	Value
Culture Volume	1.5L
Carbon Source	sucrose
Agitation Rate	500 rpm
Air Flow	1.5 L/min
Temperature	30°C
Culture Time	72h
DOT	free
pH	7.0

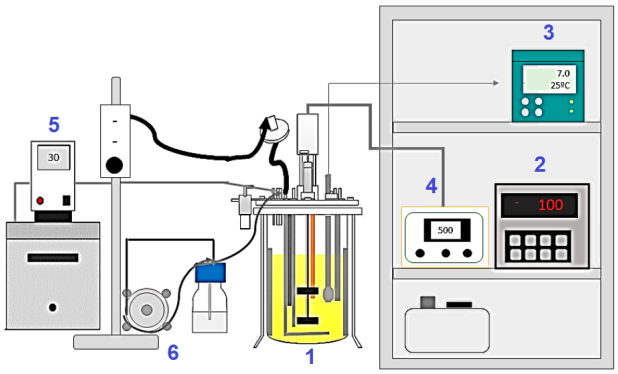

FIGURE 4. Schematic description of the bio-reactor in the lab.

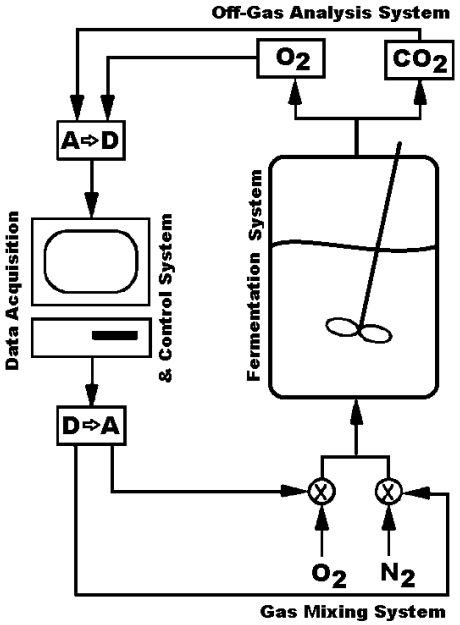

FIGURE 5. Schematic of the process.

for analytical measurements. All of the experiments were conducted in triplicate using 20 g/L of sucrose. The cultures were carried out using wild-type Azotobacter vinelandii ATCC 9046. The cultures were conducted under nitrogen fixation (diazotrophic condition). The bacterium was grown in a medium with the composition described in [22]. Table 1 shows some of the main properties of the batch cultures in the bio-reactor Applikon.

Figure 4 shows the schematic of the set-up in our lab. Its components are the following: 1) Applikon bio-reactor; 2) TOD meter; 3) pH meter; 4) Agitation controller; 5) Thermo-regulator; and 6) NaOH pump.

B. MODEL DESIGN AND TEST

According to the studies presented, it is clear that OTR affects alginate production. As was mentioned before the main goal of this process is the alginate production based in batch culture of the *A. vinelandii*. This process takes place in a bio-reactor where the initial defined conditions of the environment (pH and temperature) have to be controlled. The culture is made by batch, which consists of an initial load of the substrate that the bacteria consume during its growth. This growth is limited to the amount of substrate supplied to the bio-reactor at the start of the process.

That is why it is very important to control the O_2 consumption. Figure 5 shows the schematic of this process [25], which is a bio-reactor composed of four subsystems: the Data Acquisition, Gas Analysis, Gas Mixing, and Fermentation.

Note that this work has been carried out using batch cultures, which presents different features to the continuous cultures. In continuous cultures, the metabolic conditions are different and not can be comparable to batch cultures modality. Because it is possible to reach a steady-state, which is not the stationary phase observed in batch cultures. Thus, during the steady-state (in continuous culture) the growth of the cells is at a specific growth rate, on the contrary, during the stationary phase (in batch culture) the cells do not grow.

The process is continuously receiving a mix of gases (N_2 and O_2) through the gas mixing system. Note that the air sterilization is carried out using an air filter sterile Millexz-FG of 0,20 μm PTFE hydrophobe, 50 mm.

The O_2 is consumed by the bacteria in liquid form, which means that it must be transferred from gas to liquid. The outputs of the systems are a mix of gases (O_2 and CO_2). The behavior of oxygen consumption is estimated by calculating the OTR as shown in Equation (1) [14], [26].

$$OTR = M\left(X_{O_2}^{in} - X_{O_2}^{out}\left(\frac{1 - X_{O_2}^{in} - X_{CO_2}^{in}}{1 - X_{O_2}^{out} - X_{CO_2}^{out}}\right)\right), \quad (1)$$

where M is

$$\frac{M_{O_2} * F_G^{in}}{V_R * V_M}, \quad (2)$$

$M_{O_2} * F_G^{in}$ is the flow of the mixture of gases at the input of the bio-reactor (same for CO_2); $X_{O_2}^{in}$ is the molar fraction of oxygen in the input gas mixture (same for CO_2); $X_{O_2}^{out}$ is the molar fraction of oxygen in the output gas mixture (same for CO_2); V_M is the molar volume of the ideal gas in standard conditions; V_R is the volume of the bio-reactor. Note that the CO_2 input gas is zero and N_2 is not considered in the equation (1) because it is not consumed.

The control objective for this process is going to work with average OTR to enhance alginate production (made it by bacterias). OTR control during growing stages must be

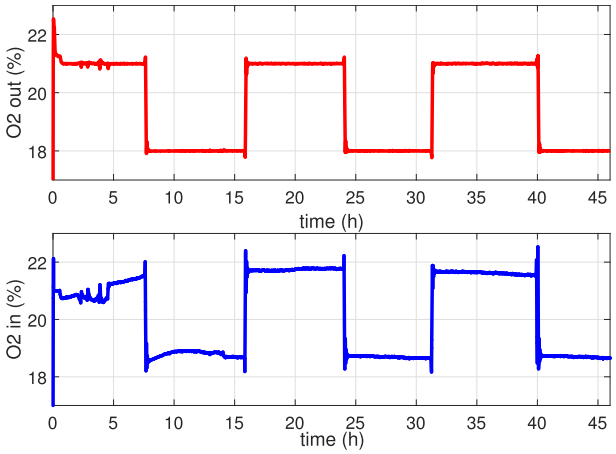

FIGURE 6. The pulse response of the system in closed-loop.

improved. The proposal is to design a closed-loop control between oxygen out concentration (O_2^{out}) and oxygen concentration at the input (O_2^{in}), like a direct relation between system variables.

III. PID CONTROL OF THE OTR

The identification of the system was carried out in closed-loop with real data of the plant [27]. Figure 6 shows the pulse response of the system in closed-loop, which indicates that the system is stable in open-loop. The result of the identification is the direct relationship between the input O_2^{in} and the output O_2^{out}.

With the data of the identification experiment and the System Identification Toolbox of MATLAB, a second-order under-damped model is obtained, with a fit percentage of 95.32%, using equation (3).

$$\frac{O_2^{out}(s)}{O_2^{in}(s)} = \frac{0.97e^{-18s}}{2981s^2 + 87.25s + 1} \tag{3}$$

Figure 7 shows the block diagram of the control closed-loop used to design the PI controller. Where the manipulated variable is the concentration of oxygen at the input of the bio-reactor (O_2^{in}) and the controlled or measured variable is the concentration of oxygen at the output of the bio-reactor (O_2^{out}), which can indicate the result of the oxygen consumption of the bacteria. A PI controller was implemented to eliminate the steady-state error and place overshoot limits in transient response for this process. Note that the primary purpose of this control algorithm is to maintain constant OTR value. But at this time it is not possible to measure directly the OTR value. That is why it has to be controlled indirectly through the O_2^{out} output. The OTR value is calculated with the equation 1 to see if its value remains constant.

The parameters of the PI controller were obtained with the MATLAB Control System Design Toolbox. Obtaining a robust response for 266 seconds of settling time and keeping the behavior less than saturation limits. Note that it was not used derivative action, because of the noisy measurement in

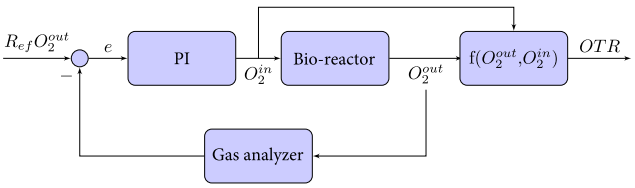

FIGURE 7. Block diagram of the proposed closed-loop.

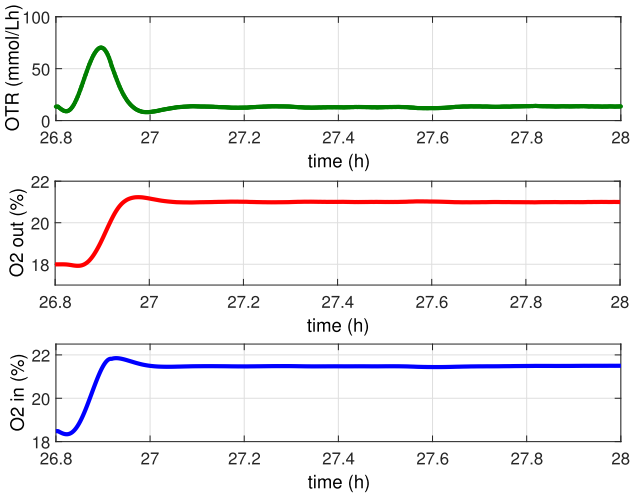

FIGURE 8. Oxygen output, Oxygen input, and OTR vs. time.

the oxygen sensor as the literature normally proposes for PID controllers [28]. The PI controller parameters used were the following: $K_p = 0.32$ and $T_i = 46.77\ sec$.

With these parameters some batch cultures were made by changing the O_2^{out} reference from 21% to 18% every 8 hours, to observe the behavior of the system for these changes. Figure 8 shows one of these step responses of the system.

The red line represents the O_2^{out} vs. time, the blue line represents the O_2^{in} vs. time and the green line represents the OTR vs. time. As can be seen, for a change in the reference (step), the O_2^{in} is stabilized, which means that the system is controlled. As a consequence, the output O_2^{out} is also stabilized.

Note that the OTR remains constant after the stabilization of the input (O_2^{in}) and output (O_2^{out}) of the system. This result shows that the OTR is controlled through the O_2^{in}. The value of OTR can be then calculated by using the oxygen concentration at the output of the bio-reactor (O_2^{out}) as shown in Figure 7.

IV. RESULTS

Figure 9 shows the oxygen concentration at the output of the bio-reactor (mean value and standard deviation of 3 different experiences for both cases). The red line represents the oxygen concentration at the output of the bio-reactor without the control algorithm and the blue line represents the same value but with the control algorithm. Note that the latency stage is 12h long and in this stage, the control is not applied. The OTR is not controlled during this stage because the quantity of biomass for this period is too short, which implies that oxygen

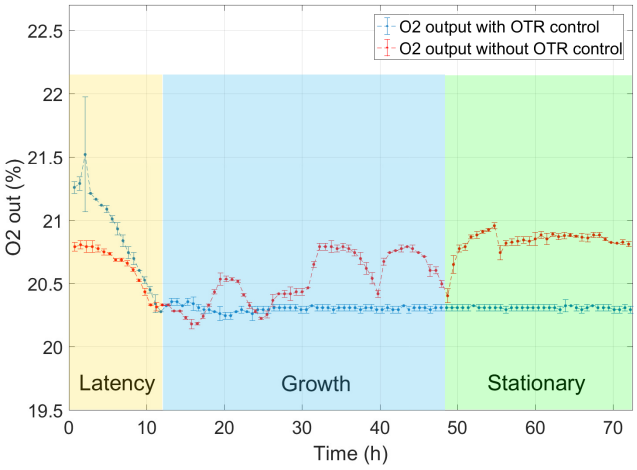

FIGURE 9. Oxygen concentration at the output with and without control vs. time.

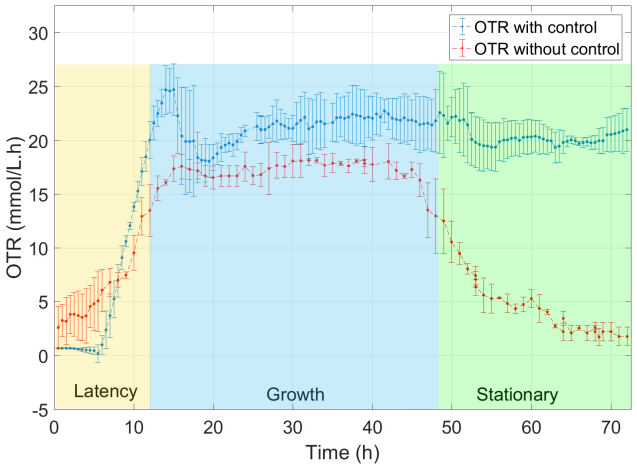

FIGURE 10. Oxygen Transfer Rate with and without control vs. time.

consumption is around 20.9%. That is why the mixture of gases at the input is fixed to 21% of oxygen and 79% of nitrogen. After this first stage, the control is manually started.

For the controlled culture, the reference used for the oxygen concentration at the output of the bio-reactor is the maximum value measured (when the OTR is maximum) for the uncontrolled culture during the growth stage.

As can be seen, in the uncontrolled system the oxygen concentration presents an oscillatory behavior, which implies that the oxygen input is not controlled and the output value is affected by the oxygen consumption of the bacteria. On the other hand, the controlled system shows that the value of oxygen concentration remains constant around the desired value after the first stage. These results can be classified as very good and they support the hypothesis of this work.

Figure 10 shows the result of the OTR calculation of the previous experiments (also the mean value and standard deviation of 3 different experiences for both cases).

The red line represents the results for the experiences without control and the blue line represents the result for the experiments with control. During the latency stage (first

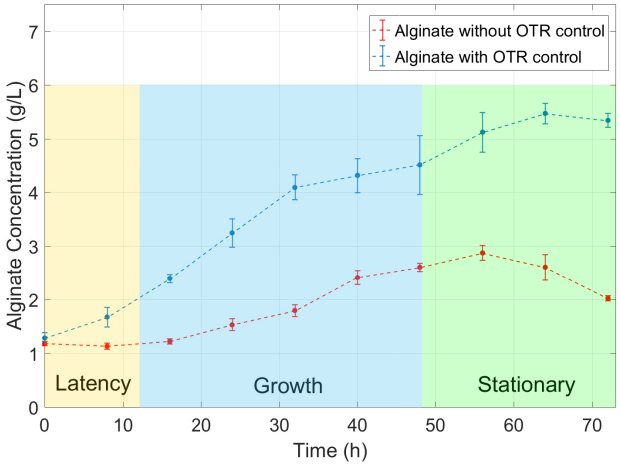

FIGURE 11. Alginate mass concentration vs. time for a controlled and uncontrolled cultures.

12h), when the control algorithm is not working, the behavior of both cultures is similar. At this period the OTR starts to increase its value. After this stage, the control is started manually, when the OTR value is maximum.

As can be seen, for the experience without control, the results are oscillatory and after 45 hours the values dismiss considerably. The maximum value of the three experiences was 19.76 mmol/Lh. On the other hand, the results for the controlled system show that the value of OTR remains in stable value from 12h to 72h, which is one of the goals of this research. In this case, the maximum value of the three experiences was 26.61 mmol/Lh.

Figure 11 represents the concentration of the alginate produced during the experiments (as in the previous cases, mean values of 3 experiences). The red line represents the results of the experiments in a culture without control of OTR and the blue line represents the production of alginate in a controlled culture.

As can be seen, the results of the alginate production are much more efficient in the controlled culture than without control. The maximum value of alginate mass concentration for the three experiences with control is 5.47 ± 0.2 g/L and for the three experiences without control, the maximum value is 2.86 ± 0.1 g/L. Moreover, during the stationary stage, the alginate molecular weight was 328 ± 42 g/mol under OTR control and 415 ± 54 g/mol without OTR control.

These results confirm our hypothesis at the beginning of this work. It is possible to produce more alginate by controlling the oxygen concentration at the input of the bio-reactor. By using a control algorithm to maintain the OTR constant during the experiment with the oxygen concentration at the output of the bio-reactor as a measured variable.

Note that the decrease in the alginate concentration in the cultures without OTR control can be due to an increase in the activity of alginate lyase. It is documented that lyase alginate affecting the molecular weight of alginate [13], which could affect also the concentration. However, other studies will permit verify this possibility.

V. CONCLUSION

This article proposes a PI control algorithm of the OTR in batch cultures to improve the production of alginate from *A. vinelandii*. The idea is that if the oxygen concentration at the output is constant, the OTR also maintains its value constant.

The results demonstrate that with the OTR constant, the production of alginate is improved. The quantity of alginate produced in a controlled culture showed higher values than the system without control of OTR.

Note that, the OTR of a batch culture can not be controlled directly because it can not be measured during the experiment. That is why it is necessary to control it by measuring the oxygen concentration at the output of the bio-reactor and by manipulating the oxygen concentration at the input. Another circumstance is that the OTR is not controlled during the latency stage (first 12 hours) because the quantity of bio-mass for this period is too short, which implies that the oxygen consumption is around 20.9%. That is why the mixture of gases at the input is fixed to 21% of oxygen and 79% of nitrogen.

In general, these results are significant in the improvement of alginate production from *A. vinelandii*. Further work will involve the control of this bioprocess in a larger bio-reactor.

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