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Adjustment and Scale-Up Strategy of Pilot Liquid Fermentation Process of *Azotobacter* sp.

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***Abstract*—**The genus *Azotobacter* has been widely used as bio-fertilizer due to its significant effects on the stimulation and promotion of plant growth in various agricultural species of commercial interest. In order to obtain significantly viable cellular concentration, a scale-up strategy for a liquid fermentation process (SmF) with two strains of *A. chroococcum* (named Ac1 and Ac10) was validated and adjusted at laboratory and pilot scale. A batch fermentation process under previously defined conditions was carried out on a biorreactor Infors®, model Minifors of 3.5 L, which served as a baseline for this research. For the purpose of increasing process efficiency, the effect of the reduction of stirring speed was evaluated in combination with a fed-batch-type fermentation laboratory scale. To reproduce the efficiency parameters obtained, a scale-up strategy with geometric and fluid dynamic behavior similarities was evaluated. According to the analysis of variance, this scale-up strategy did not have significant effect on cellular concentration and in laboratory and pilot fermentations (Tukey, $p > 0.05$). Regarding air consumption, fermentation process at pilot scale showed a reduction of 23% versus the baseline. The percentage of reduction related to energy consumption reduction under laboratory and pilot scale conditions was 96.9% compared with baseline.

Keywords*—Azotobacter chroococcum*, scale-up, liquid fermentation, fed-batch process.

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I. Introduction

THE continuous soil degradation caused by cultural practices and excessive use of chemical compounds for fertilization, have led to changes in nutritional composition of soil and low yields at harvest [1], [2]. An alternative to reduce soil degradation, improve agricultural productivity and production quality is the use of bio-based products such as bio-fertilizers instead of fertilizers based on ammonium salts. Bio-based products allow reducing harmful effects of agrochemicals and production costs as well as improving biological balance, soil fertility, and production process [3]. To this aim, many researchers have been working on the manufacture of industrially bio-based products, using either native microbiological species or their metabolites that act as active ingredients for bio-fertilizers, biocontrol or growth promoters under high quality standards. Globally, these products are called bioinputs or bioproducts [4].

In order to develop a biofertilizer, studies of bioprospection on Colombian soil were carried out and two strains of *Azotobacter chroococcum* (Ac1 and Ac10), were isolated and selected for their nitrogen fixation capacity by Soil Laboratory of Corpoica [5]. The rapid growth of the two strains of *A. chroococcum* isolated was the most striking appeal for using them commercially [5]. The species *Azotobacter* sp. represent the main group of heterotrophic bacteria and asymbiotic nitrogen fixers, which can be found free in nature [6]. This genus of bacteria has positive effects on the stimulation and growth promotion of different plant species as consequence of chemical promoters production such as auxins, cytokinins, phenols, and gibberellins [7].

After isolation of the two strains, the Soil Laboratory of Corpoica designed and optimized a liquid culture medium (MBR medium), and determined the appropriate physicochemical conditions for the fermentation process of the two native strains of *A. chroococcum* at laboratory scale [8]. In order to have sufficient quantities of culture broth of the two *A. chroococcum* strains, it was necessary to validate their production in pilot scale bioreactors. Therefore, the aim of this work was to carry out the scale-up of liquid fermentation system (SmF) for the two strains of *A. chroococcum* at pilot scale.

The processes scale-up is one of the most relevant aspects of industrial production, due to the requirement to maintain the same productivity and efficiency characteristics achieved at laboratory scale. Appropriate scale-up criteria must involve known process variables which are related to the physiological effects in the process (oxygen supply, heat and mass transfer, stirring speed, and others) [9]-[11]. For this purpose, different criteria have been developed, such as geometric, temporal or behavior (kinematic, dynamic) similarity [9], which involve the production of dimensionless coefficients with the capacity to remain constant and which are relevant for the process. These scale-up parameters are dimensionless quantities calculated from equations with independent and dependent variables that characterize the system to be scale [9].

The scale-up strategy at pilot level selected in this study was designed based on Chemical Engineering criteria of geometric and fluid dynamic behavior similarities; our purpose was to maintain kinetic and productivity parameters obtained at laboratory scale and reduce energy consumption in the fermentation process.

II. Methodology

A. Bacterial Strain

The microorganisms used in this study were strains Ac1 and Ac10 of *A. chroococcum*. These isolates were provided by Germplasm Bank of Microorganisms with Biofertilizer Potential from Colombian Corporation for Agricultural Research, Mosquera, Colombia.

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B. Bacterial Strains´ Reactivation

The strains maintained at 20 °C were reactivated in LG agar [8], by streak plate method, and incubated for 48 h at 30±2 °C.

C. Inoculum Preparation

Laboratory Scale Fermentation: Each strain of *A. chroococcum* was grown at 30±2 °C for 24 h on LG agar [12]. The biomass was collected by sterile inoculating loop, into an Erlenmeyer flask with 45 mL of sterile saline solution (NaCl 8.5 g/l) and used as inoculum for a 5 L stirred tank bioreactor (STR), Minifors® (Infors HT, Switzerland).

Pilot Scale Fermentation: 4 L of fermentation broth of each strain was produced at bioreactor Minifors® (Infors HT, Switzerland). The fermentation conditions were: A batch culture, a stirring speed of 200 rpm, at 30±0.5 °C for 24 h. The volume produced was used as inoculum for a 70 L STR bioreactor BioLab®, model 50B.

D. Liquid Fermentations

The culture medium for both stains of *A. chroococcum* (Ac1 and Ac10) was designed and optimized in a previous work [8].

Stage 1. Baseline Validation: Fermentation experiments were conducted in a 5 L STR bioreactor, Minifors® (Infors HT, Switzerland) with a working volume of 3.5 L of MBR liquid medium [8]. Bioreactor had stirred speed control and temperature control, as well as pH, dissolved oxygen and temperature sensors. The bioreactor was aerated with a sparger ring and the air flow rate was controlled by means of mass flow controller valve. Homogenization of culture media and dispersion of gas-liquid were generated by two Rushton impellers. The fermentation conditions were: A batch culture, stirring speed of 500 rpm, temperature 30±0.5 °C, air flow rate 1 vvm and final fermentation time of 30 hour and 72 hour.

Stage 2. Process Adjustment and Validation of Scale-Up Strategy at Pilot Scale: Fermentation experiments were carried out in 5 L STR bioreactor described above and a fed-batch feeding strategy was applied. Therefore, the initial working volume was 1.0 L and two feeding pulses of carbon source were evaluated during exponential growth phase, to obtain a final volume of 3.0 L. The other substrates for MBR culture medium were recalculated to assure that the initial volume had the composition of final working volume. The period of time between feedings was half of the doubling time at baseline, and feeding starts when the carbon source concentration was reduced to half of the initial value [13], [14]. For each feeding, the initial carbon source concentration was maintained constant. If, by any chance, a feeding were missed, the amount of carbon source added in the last feeding would have to equal the initial carbon source concentration. In this stage, a decrease of 60% in stirring speed was evaluated, keeping both temperature and air flow rate as constant. Final fermentation time was 30 hours.

Stage 3. Adjustments and Scale-Up Strategy Validation at Pilot Scale: Fermentation experiments were performed in a 70 L STR bioreactor BioLab®, model 50B, which was selected according to geometric scale-up criteria, with a working volume of 50 L. Bioreactor is equipped with temperature and stirring speed control, two Rushton impellers and was aerated with a sparger ring located in the bottom of vessel. pH and dissolved oxygen were measured offline using an electrochemical sensor (Hanna Instruments® model PH211) and a polarographic sensor (Extech Instruments® model DO700), respectively. All fermentations were fed-batch cultures with a unique pulse feeding strategy, and starting with 80% of the total working volume. The fermentation conditions were: Temperature 30±1 °C, air flow rate 1 vvm and stirring speed was adjusted according to fluid dynamic behavior scale-up criteria. Final fermentation time was 26-hour.

E. Kinetic Behavior and Kinetic Parameters

Each two-hour period of fermentation, pH and dissolved oxygen were measured and a sample of 10-15 mL was taken to determine cellular concentration (CFU/mL), applying drop plate technique [15]. In order to do this, an aliquot of 500 µl of cellular broth was dissolved in 4500 µL of sterile saline solution (8.5 g/l). This solution was based on a dilution ratio of 1/10, and decimal dilutions were made up to 10^{-7}, adding 500 µL of the previous dilution to 4500 µL of saline solution. 20 µL of the last three dilutions (10^{-5} to 10^{-7}), were taken and plated as micro-drops in Petri dishes with LG agar. The dishes were incubated under aerobic conditions at 30 ± 2 °C for 48 h. At the end of 48-hour incubation, the colonies formed for each drop were counted. Cellular concentration was quantified by:

$$N = \frac{\bar{X} \cdot 50}{d} \quad (1)$$

$\bar{X}$ is average number of colonies at the dilution selected by accurate counting (CFU); d is the decimal dilution selected.

Fermentation kinetic curves of bacteria growth were made by using SigmaPlot® 6 software. Based on kinetic growth curves for each strain, the time lapse for exponential phase was identified, which followed the mathematic model described in (2) [16]:

$$\ln N = \mu \cdot t + \ln N_0 \quad (2)$$

where: N is cellular concentration at time t (CFU/mL), μ is specific growth rate (h^{-1}), t is fermentation time (h), N_0 is the initial cellular concentration (CFU/mL). Thus, maximum specific growth rates were the slopes calculated from linear regression of experimental dates in the exponential phase.

The doubling time, g, was calculated as [16]:

$$g = \frac{\ln 2}{\mu} \quad (3)$$

F. Efficiency Parameters

Fermentation productivity was expressed as cellular productivity (log CFU/mL·h), P_N, was calculated by (4), where N_{max} is maximum cellular concentration (CFU/mL), and $t_{N_{max}}$ is the fermentation time (h) when maximum cellular concentration was reached.

$$P_N = \frac{N_{max}}{t_{N_{max}}} \quad (4)$$

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G. Air Consumption and Energy Consumption Estimates

The volume of air consumed per hour in batch fermentations and fed-batch, was calculated by:

$$V_c = \frac{\sum_{i=0}^{i=t_f} r_{af} \cdot V_{wi} \cdot t_i}{t_f} \quad (5)$$

V_c is air volume consumed per hour (l/h), r_{af} is air flow rate (L/L·min), V_{wi} is working volume (L) at fermentation time i, t_i is fermentation time i (min), and t_f is final fermentation time (h). Also, the power consumption was calculated with [17]:

$$P = N_p \cdot \rho \cdot N_s^3 \cdot D_i^5 \cdot N_i \quad (6)$$

where: P is power consumption (kW·h), N_p is power number (dimensionless), ρ is fluid density (kg/m^3), N_s is stirring speed (rev/h), D_i is impeller diameter (m), and N_i is the number of impellers. Based on Reynolds Number from each type of impellers, it was established that N_p is 5 [17], [18].

H. Scale-Up Strategy

Scale Factor [11]

For a process scale-up, it is necessary to establish a scale factor that allows developing the process to the desired scale. To determine this factor, the concept of similarity is used and it is calculated with:

$$m' = k \cdot m \quad (7)$$

m' is the variable at model scale (minor scale system), k is scale factor, and m is the variable at prototype scale (major scale system).

Geometric Similarity [19]

Geometric similarity refers to the identity or closeness existing among key geometric relationships of bioreactors at laboratory scale and pilot scale. For STR bioreactors, the key geometric relationship is ratio between height and diameter, calculated with (8). This value is associated with the majority of geometric characteristics of internal components (impellers and baffles).

$$H/D \quad (8)$$

where: H is the internal bioreactor height (m), D is the internal bioreactor diameter (m).

Fluid Dynamic Similarity [17], [20], [21]

With the purpose of maintaining the fluid dynamic similarity (equal fluid density and viscosity), in the two scales, the dimensionless variable, Reynolds number of impeller was used. This variable correlates the inertial forces with viscous forces in a fermentation process. In addition, Reynolds number allows to connect impeller diameter to impeller tip speed (stirring speed, N), as shown in the following equation. Thus, this equation helps to determine stirring speed to be used at prototype scale.

$$Re_i = \frac{\rho \cdot N_s \cdot D_i^2}{\mu_f} \quad (9)$$

Re_i is Reynolds number of impeller, ρ is fluid density (kg/m^3), N_s is stirring speed (rev/s), D_i is impeller diameter (m), and μ_f is fluid viscosity (Pa·s).

To determine stirring speed in the two scales, the following numerical analysis was developed (10):

$$Re_i = \frac{\rho \cdot N_s \cdot D_i^2}{\mu_f} \text{ (Model scale)}$$

$$Re_i' = \frac{\rho' \cdot N_s' \cdot D_i'^2}{\mu_f'} \text{ (Prototype scale).}$$

Assuming: $\rho = \rho'$ and $\mu_f = \mu_f'$ Then: $N_s' \cdot D_i'^2 = N_s \cdot D_i^2$ (10)

N_s is stirring speed at laboratory scale (rev/s), D_i is impeller diameter at model scale (m), N_s' is stirring speed at pilot scale (rev/s), and D_i' is impeller diameter at prototype scale (m).

In this research, all sample determinations of cellular concentrations were done by triplicate. The response variable (N_{max}), were analyzed by ANOVA procedure and then doing a means comparison using Tukey test (α>0.95). This statistical analysis was performed by Statistix® v8 software.

III. Results and Discussion

A. Liquid Fermentations

Stage 1. Baseline Validation

Figs. 1 (a) and (b) show increases pH of more than one unit for both fermentations. Fermentation medium of strain Ac1 was acidified from death phase, while pH culture medium in Ac10 fermentation increased steadily along the process. The pH of the culture medium is directly related to alginate production, when the production of the polymer increases, the medium is acidified [22]. Regarding kinetics of dissolved oxygen concentrations for both mediums, these were constant and very close to saturation concentration (> 95%) (Figs. 1 (a) and (b)). Thus, the bacteria grew without oxygen limitation [23]. Since no negative effects were shown on cellular concentrations, the following fermentations were carried out without pH and dissolved oxygen control.

The kinetics behavior of cellular concentrations for Ac1 and Ac10 strains in 72-hour fermentations (Figs. 1 (a) and (b)) offered the chance to determine that the tested strains had a very short lag phase, and showed that the stationary phase was extended to 48 hours, followed by a 26-hour exponential phase and, subsequently, a well-defined death phase. At 26 hours of fermentation, maximum cellular concentrations were achieved for each strain of *A. chroococcum* with 1.54x10^9 CFU/mL for Ac1 and 2.21x10^9 CFU/mL for Ac10. These concentrations were higher than those reported in previous studies. For example, Damir et al. evaluated two carbon sources, glucose (20.0 g/l) and molasses (40.0 g/l), with fermentation conditions of 180 rpm and 30 ºC. They achieved concentrations of 1.5x10^8 CFU/mL and 3.8x10^8 CFU/mL,

respectively [24]. Pozo et al. worked with wastewater from the olive oil process (this wastage is called Alpechin); the concentration used of Alpechin (as a volume percent) was 60% in water and the fermentation conditions included 100 rpm and 30 °C. For this study the cellular concentration obtained was $5.0x10^8$ CFU/mL [25].

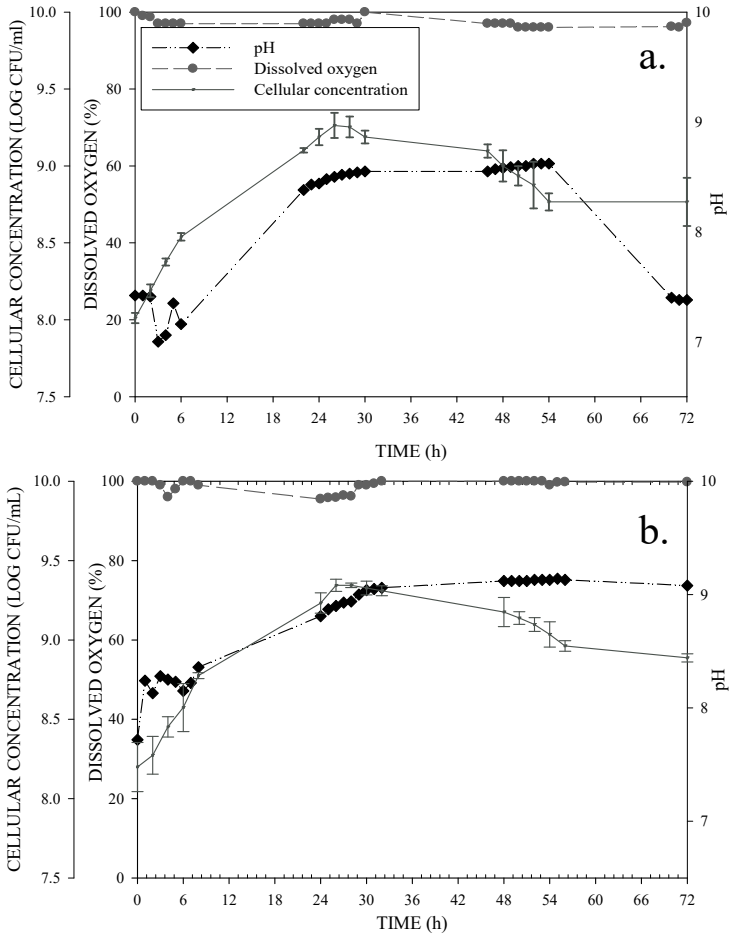

Fig. 1 Growth kinetics of *A. chroococcum* for 72-hour liquid fermentations in Baseline validation Stage: (a) Ac1 strain; (b) Ac10 strain

In order to calculate the maximum specific growth rates and time generation of the strains, linear approximations were made at exponential phase of the kinetic curves (Figs. 1 (a) and (b)). The exponential phase of kinetic growth curves for both strains were adjusted to an equation of order one with R^2>0.95. In a previous study with *A. vinelandii*, a specific growth rate of 0.16 h^{-1} was determined using a bioreactor STR and fermentation conditions of 340 rpm, 29 °C and 0.8 vvm [26]. On the other hand, cultivation of *A. vinelandii* resulted in a specific growth rate of 0.1 h^{-1} at laboratory scale (200 rpm) and 0.13 h^{-1}, at pilot scale [27]. Therefore, the results for present work indicate that the maximum specific growth rates calculated for these fermentations were almost 10 times lower than those reported by other authors (Table I). In addition, low specific growth rates lead to very long doubling times as witnessed in this study.

TABLE I
KINETIC AND EFFICIENT PARAMETERS, ENERGY AND AIR CONSUMPTION ESTIMATES FOR FERMENTATIONS OF BASELINE VALIDATION STAGE AT LABORATORY SCALE

PARAMETERS	Ac1		Ac10	
	72-hour	30-hour	72-hour	30-hour
N_{max} (10^9 CFU/mL)	1.83±0.29	1.71±0.12	2.21±0.16	1.14±0.79
μ (h^{-1})	0.089±0.008	0.071±0.001	0.073±0.015	0.100±0.005
g (h)	7.86±0.70	9.79±0.69	9.77±0.78	6.93±0.31
P_N (log CFU/mL·h)	0.3563±0.0026	0.3551±0.0011	0.3594±0.0012	0.3633±0.0046
Vc (l/h)	210.00			
P (kW·h)	0.1300	0.0541	0.1300	0.0541

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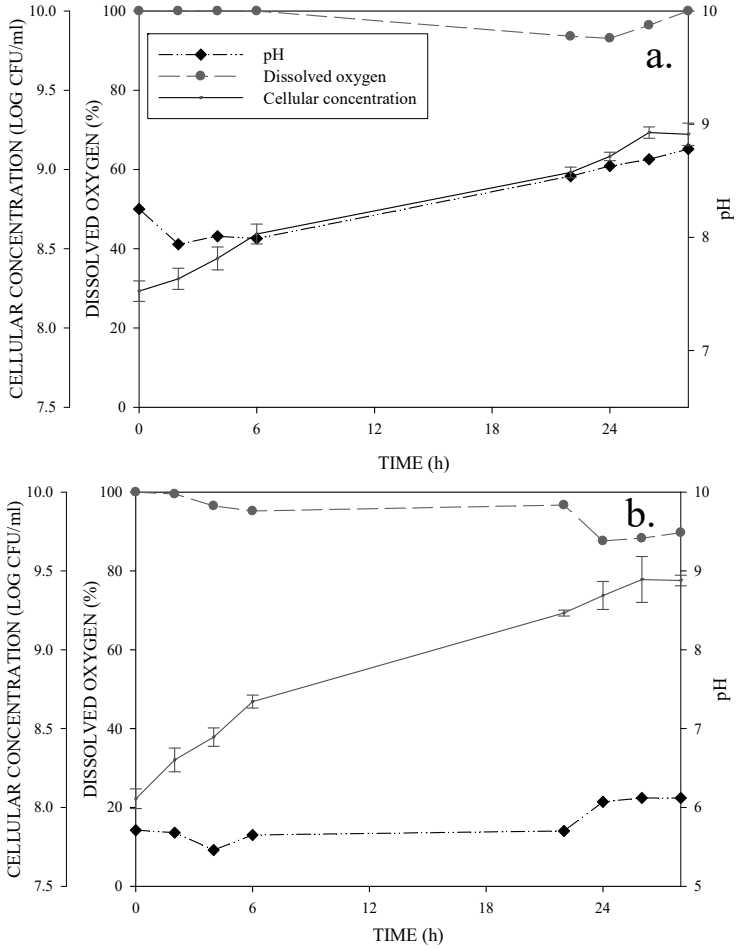

Fig. 2 Growth kinetics of *A. chroococcum* for 30-hour fermentations in Baseline validation Stage: (a) Ac1 strain; (b) Ac10 strain

In order to verify final fermentation time in which maximum cellular concentrations were reached (26 hours), 30-hour fermentations were performed with AC1 and AC10 *A. chroococcum* strains. This would confirm the results found in other reports which claim that the ranges of exponential phase time finalization were between 22 and 36 hours under different conditions of stirring speed at 30 °C [25]-[28].

30-hour fermentations were continued without control of pH and dissolved oxygen (Figs. 2 (a) and (b)) and the behavior of these parameters was similar related to 72-hour fermentations.

Ac 1 and Ac10 fermentations in this stage were reproducible (Figs. 2 (a) and (b)). Also, the maximum cellular concentrations (Table I) were reached after 26 hours with no significant differences from those in 72-hour fermentations (Tukey, $P \leq 0.05$). In relation to fermentation productivities, statistically significant effects were not observed among fermentations with different final time (Tukey, $P \leq 0.05$). The maximum specific growth rates of the strains did not display major numerical differences in comparison with previous fermentations (Tukey, $P \leq 0.05$). However, the maximum specific growth rates obtained for strains of *A. chroococcum* remain lower than the ones found in other studies [26], [27]. Statistically relevant effects were observed on doubling times for 72 and 30-hour fermentations. The doubling time at 30-hours fermentation of Ac1 increases a 24.5%, and for Ac10, decreases a 29.1%.

Stage 2. Process Adjustment at Laboratory Scale

The aim of this stage was to increase maximum cellular concentrations and improve efficiency parameters of the fermentation process of Ac1 and Ac10 strains, based on the results acquired from the previous stage. For this purpose, a fed-batch feeding strategy was adjusted and implemented at laboratory scale. The stirring speed of baseline involves high energy consumption by the impellers associated to the power delivered toward the gassed liquid, and the dissolved oxygen maintained in excess along the process according to experimental kinetics is described above. In order to reduce air and energy consumption, a simultaneously reduction of stirring speed and aeration in fed-batch process were applied.

Figs. 3 (a) and (b) show the evolution of cellular concentration and dissolved oxygen in the adjusted process. Dissolved oxygen concentration decreased during the exponential growth phase (<60%), and it differs in the behavior presented in previous fermentations where the concentrations were above 85%. However, this fact had not generated a negative effect on cellular concentrations of *A.*

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chroococcum strains. Contrary to this, the feeding of carbon source improved cellular concentrations values ($4.25x10^9$ CFU/mL for Ac1, and $4.21x10^9$ CFU/mL for Ac10). As a consequence, a reduction in dissolved oxygen concentration occurred after the addition of fresh medium to the bioreactor and, after a couple of hours, the oxygen regained its previous level (Figs. 3 (a) and (b)). In regard to culture media pH, it has been observed an increase of pH. As shown in Figs. 3 (a) and (b), when a stream of fresh medium was added, the pH decreased due to the fact that the fresh medium had a lower pH (~6.5). Nevertheless, along time it was observed that the system tended to recover the value of pH before the feeding. These behaviors revealed that the fermentation process of Ac1 and Ac10 strains was inherently cushioned and allowed only small changes in pH and dissolved oxygen.

TABLE II
KINETIC AND EFFICIENCY PARAMETERS, ENERGY AND AIR CONSUMPTION ESTIMATES FOR FERMENTATIONS OF PROCESS ADJUSTMENT AT LABORATORY SCALE STAGE

PARAMETERS	Ac1	Ac10
N_{max} (10^9 CFU/mL)	4.25±0.18	4.21±0.59
μ (h^{-1})	0.068±0.012	0.063±0.012
g (h)	10.59±1.90	11.39±1.94
P_N (log CFU/mL·h)	0.3703±.0007	0.3700±0.0025
Vc (l/h)	92.80	
P (kW·h)	0.0017	

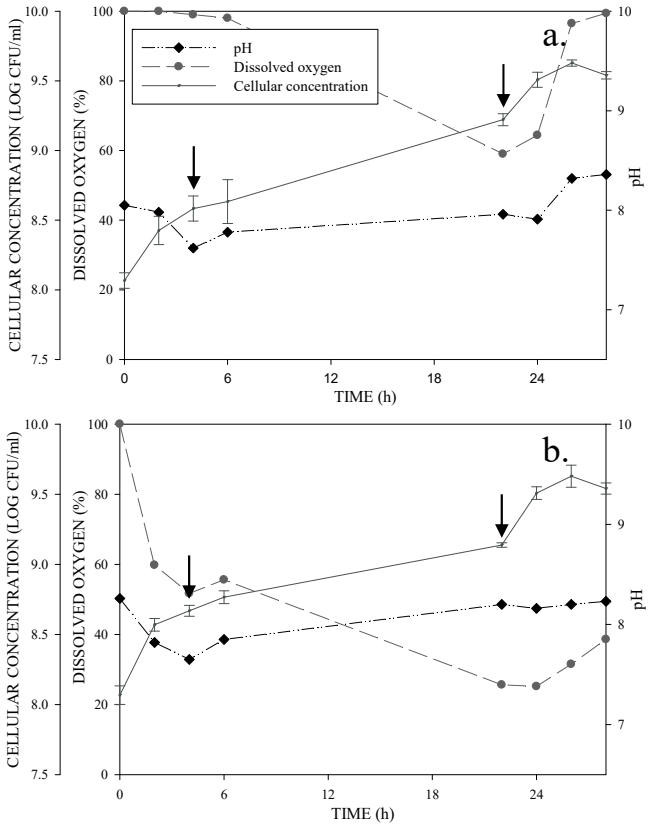

Fig. 3 Growth kinetics of *A. chroococcum* for 28-hour fermentations in Process Adjustment at laboratory scale Stage: a. Ac1 strain; b. Ac10 strain. Note: Arrows symbolize feeding pulse times

The results described in Table II proved that the culture conditions improve maximum cellular concentrations and efficiency parameters for *A. chroococcum* and significant differences for the other parameters from the results of baseline fermentations were not found (Tukey, P <0.05) (Table II). The maximum cellular concentrations were obtained at 26 hours of fermentation and a twofold increase was achieved with respect to the baseline fermentations. Productivity process increased from 2 to 4%, compared to baseline results. Previous studies conducted on fermentations fed-batch using glucose as carbon source achieved productivities of 0.013 log(CFU/mL·h) [24]. This parameter was 30 times lower than those determined in the present work. Specific growth rates and doubling times were similar to those obtained in baseline fermentations; consequently, implementation of the fed-batch feeding strategy and the stirring speed reduction might maintain unchanged strains kinetic behavior (Tukey, P <0.05).

A decrease in energy consumption (0.0017 kW/h as compared to 0.0543 kW/h in baseline fermentations), was obtained when the fed-batch fermentations was performed. It means a reduction of 96.9% with respect to former 30-hour fermentations assays. Regarding air consumption, it decreased from 210.00l/h (baseline) to 92.80l/h (fed-batch), which means a reduction by 55.8%. On the other hand, the feedings of fresh medium were reduced to one at pilot scale (at 22 hours), in order to reduce risks of microbial contamination. This reduction conduced to increase carbon source concentration in the initial culture medium, according to the carbon source concentrations in the feedings between 0 and 22 hours of fermentation.

Stage 3. Process Adjustment and Validation of Scale-Up Strategy at Pilot Scale

Scale Factor

Prior to definition of scale-up strategy, the scale factor to be used was determined. For fermentation process of *A. chroococcum* strains (Ac1 and Ac10), the scale factor used was of volumetric type and was calculated with the characteristics of STR bioreactors available in the Bioproducts Pilot Plant of Corpoica, which were described in methodology section. The maximum design volumes of model and prototype scale bioreactors are: 5L for Minifors® (Infors HT, Switzerland), and 70L for BioLab®, model 50B. The ratio between the two scales can be obtained from (7), as:

$$k = \frac{m\prime}{m} = \frac{70L}{5L} = 14$$

In general, some authors recommend the use of scale factors equal to or higher than 10 [11]. Therefore, the value found is satisfactory and there were sufficient experimental results for scale-up to fermentation of *A. chroococcum* strains.

Geometric Similarity

The first criteria selected to scale-up was geometric similarity, because the selection of the suitable equipment to carry out the scale-up should involve as many similarities as

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possible of geometric relations, in order to ensure similarity between bioreactors. As explained in methodology section, the most important parameter to define geometric similarity is the ratio H/D, which is described for each bioreactor in Table III. The standard range established of the H/D ratio for scale-up is 2.1-3.3 [20], [29]. Both H/D values calculated to the bioreactors used are within the standard range, thus, geometrical similarity between the two systems was verified.

TABLE III
MEASUREMENTS OF SRT BIOREACTORS USED TO SCALE-UP

Geometric Feature	Model Scale Bioreactor	Prototype Scale Bioreactor
Diameter (D)	14.0cm	34.0cm
Height (H)	34.0cm	83.0cm
Total Volume	5.0l	70.0l
Working Volume	3.5L	52.5L
Impeller Type	Rushton	Rushton
No. Impellers	2	2
Impeller Diameter (D_i)	5.9cm	10.5cm
Geometric ratio (H/D)	2.43	2.44

Fluid Dynamic Similarity

For the second scale-up criterion, we use the Reynolds number for impeller (Re_i) to satisfy fluid dynamic behavior similarity in the two scales. Reynolds number was selected as scale-up criterion because it binds characteristic parameters of the inertial forces (fluid speed) and the viscous forces (fluid viscosity, μ), together [21]. Since culture broth of *A. chroococcum* strains corresponds to a Newtonian fluid, with a viscosity between 15.0 and 20.0cP, Reynolds number can be used as a scale-up parameter as clarified by Hewitt [20]. For instance, Maranga et al. [30] used the Reynolds number to define stirring speed in a bioreactor designed to *Spodopetera frugiperda* fermentation. In addition, it is frequently used to describe mixing processes [30].

In order to calculate the prototype-scale stirring speed through (10), experimental data (Impellers diameters) from the two bioreactors (Table III) were used as:

$$N_S' = \frac{N_S \cdot D_i^2}{D_i'^2} = \frac{500rpm \cdot 5.9^2}{10.5^2} = 157.8rpm$$

Liquid Fermentations

The fermentations at pilot scale were carried out in order to validate the results obtained in stage 2 applying all the adjustments selected simultaneously; the production of the inoculum by a seed fermenter (3.5 L Minifors) was also implemented.

The operating variables, pH and dissolved oxygen, show the same behavior observed in fed-batch fermentations at laboratory scale (Fig. 4). Kinetics of pH for these culture media witnesses a trend of increasing during fermentation, varying between 8 and 9 units. In general, during cultivation, dissolved oxygen concentrations of the strains decreased in time but they never reached critical values (OD <20%). As shown Fig. 4, Ac1 strain fermentations had dissolved oxygen concentrations over 70% of saturation. The OD standard deviations, SD, were less than 6% since time 0 to 8 hour of fermentation. However, after this hour, SD was between 19.0% and 23.0%, because dissolved oxygen decreased to 45% since 18 hours of batch fermentation No. 1. On the other hand, OD at Ac10 strain fermentations were over 74% of saturation, and SD were less than 7.35%. In conclusion, for both operating variables it can be said that the aforementioned tendency remains unchanged regardless the scale or the conditions applied.

TABLE IV
KINETIC AND EFFICIENCY PARAMETERS FOR FERMENTATIONS AT PILOT SCALE

Parameters	Ac1	Ac10
N_{max} (10^9 CFU/mL)	3.51±0.67	3.01±1.07
μ (h^{-1})	0.057±0.009	0.048±0.010
g (h)	12.58±2.39	15.25±3.18
P_N (log CFU/mL·h)	0.3667±0.0034	0.3632±0.0070

The fermentations carried out on a pilot plant scale in a bioreactor STR BIOLAB®, 50B model of 70L, served a purpose of validating the fed-batch fermentation conditions adjusted to laboratory level in a STR INFORS® bioreactor, Minifors model of 5L. The kinetics of growth (Fig. 4) permitted verifying the process throughout in relation with the bacteria being maintained steadily for exponential phase of growth. The mean of maximum Ac1 strain cellular concentration was $3.51x10^9$ CFU/mL, with a maximum mean growth rate of 0.057 h^{-1} and a mean generation time of 12.58 hours (Table IV). A mean growth rate of 0.048 h^{-1}, a mean generation time of 15.25 hours and a mean of maximum cellular concentration of $3.01x10^9$ CFU/mL were also observed for Ac10 strain (Table IV). In general, significant differences are not observed in maximum cellular concentrations, among the fermentation batches No. 1, 2, and 3 (Tukey, P <0.05). Comparing these results with those obtained in the fed-batch fermentations at laboratory scale, no significant differences were detected in terms of maximum cellular concentrations (Tukey, P <0.05).

As shown in Table III, efficiency and kinetic parameters of pilot fermentations were similar to those found from fed-batch fermentations of Stage 2, witnessing no important differences (Tukey, p<0.05). There were no variations in magnitude and significant disparity between productivities on the three batches elaborated for *A. chroococcum* strains (Tukey, p<0.05). In a previous study in which a complex medium (molasses) and fed-batch process were performed, they obtained a cellular concentration of $7.6x10^8$ CFU/mL and a productivity of 0.013 log(CFU/mL·h) [24]. Therefore, the fed-batch scale-up and feeding strategies applied in the present work showed better results than those reported (Table III). Also, these results confirmed the reproducibility nature of concentrations and efficiency parameters for pilot scale fermentations compared to those obtained in the laboratory scale.

Some studies with species of *Azotobacter* sp. have been developed with the purpose of producing an exopolysaccharide called Alginate. In these, they used culture medium with high concentrations of carbon sources [13], [14],

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[25]-[28]. However, the pilot-scale batches were carried out in a culture medium with almost 2% of the carbon source concentrations used in those studies; increments in fluid viscosity were observed in the culture media, which indicated the presence of this polymer at high concentrations (about 50%) [26].

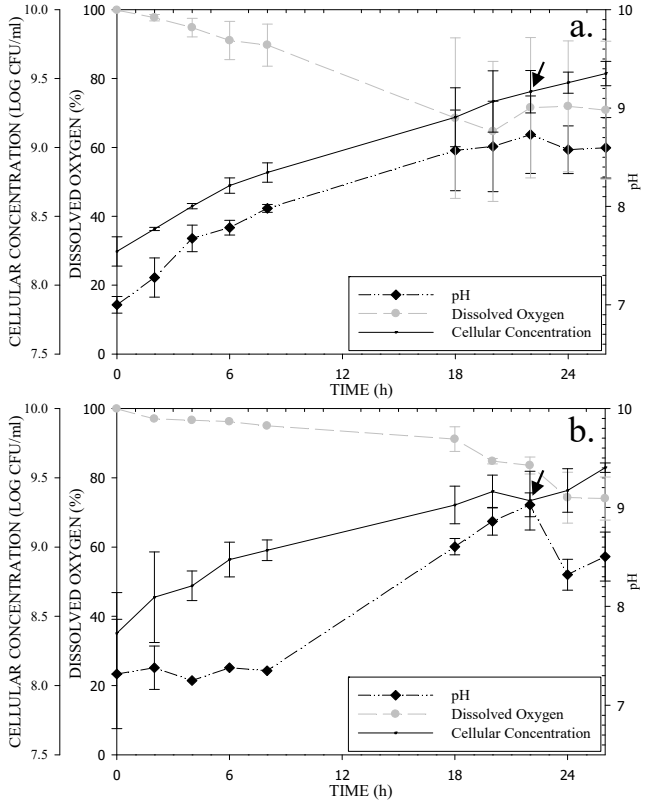

Fig. 4 Growth kinetics of *A. chroococcum* for 26-hour fermentations in Scale-up Stage: a. Ac1 strain; b. Ac10 strain. Note: Arrows symbolize feeding pulse time.

TABLE V
ENERGY AND AIR CONSUMPTION ESTIMATES FOR FERMENTATIONS AT PILOT SCALE

FERMENTATION TYPE	*Vc (l/h)*	*P* (kW·h)
Batch	3000.00	2.419
Fed-batch	2286.23	0.080

The main reason for implementation of fed-batch fermentations were the savings obtained by a reduction on the stirring rate and the initial working volume in the fermentations. In order to quantify those savings, a numerical analysis was performed to predict air and energy consumption for batch fermentations at pilot scale (Table V). For fed-batch fermentation, air consumption savings corresponded to 23.8%, whereas the savings of energy consumption represented a 96.7%. Thus, both scale-up and feeding strategies, proved their effect on cellular production and decreasing cost.

IV. CONCLUSION

Cellular concentration and fermentation productivity for liquid fermentation at laboratory scale for *A. chroococcum* Ac1 and Ac10 strains were improved using a feeding strategy and a reduction of the stirring speed. Also, response variables were reproduced at pilot scale by using a scale-up strategy based in two criteria. At both scales, kinetic parameters were not affected by scale-up and feeding strategies. The adjustments and strategies detailed led to reduce air and power consumption during laboratory and pilot scale fermentations.

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