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Evaluation of a Semi-Continuous Disk-Stack Centrifuge for the Harvest of Tsukamurella Paurometabola C-924 Bacterium

Evaluación de una centrífuga de discos semicontinua para la cosecha de la bacteria Tsukamurella Paurometabola cepa c-924

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Abstract

The disk stack centrifuge is an equipment widely used in the current biotechnological industry because of the multiple advantages it offers, the most important being its high operational flexibility, robustness, and processing speed. The objective of this research was to evaluate whether a semi-continuous disk stack centrifuge can be used to harvest *Tsukamurella paurometabola* strain C-924 cells, the active ingredient of the ecological bionematicide HeberNem®, to replace the tubular centrifuges currently used. The methodology used consisted of the development of a type 2^3 factorial statistical design, in which three input parameters were considered: feed rate [Q_{alim}], wet weight of the diluted cell suspension to be centrifuged [PH_{alim}], and time between discharges [t_{desc}]. The output parameters considered were wet weight of concentrated biomass [PH_{bio}] and recovery percentage [%Rec], which must have values greater than 600 g/L and 95 %, respectively, to obtain a corresponding final yield with the quality standards established for this biotechnological product. The results obtained were that the average values for PH_{bio} and %Rec were 657.28 g/L and 97.43 %, respectively, which met the quality standards for this stage. The experimental design was optimized to determine the optimal values for the three input parameters, thus obtaining the following values: 64 L/h for Q_{alim}, 176 g/L for PH_{alim}, and a t_{desc} of 5 min. The semi-continuous disk stack centrifuge evaluated can be successfully implemented in the harvest step of the HeberNem® production process, thus replacing the tubular centrifuges currently employed. The statistical-mathematical programs Statgraphics Centurion® XV.II, Microsoft Excel®, and MATLAB® v7.0.1 were used for data and results processing.

Keywords

Bioengineering, industry applications, materials processing, performance evaluation, production equipment.

Resumen

La centrífuga de discos constituye un equipo ampliamente utilizado en la industria biotecnológica actual, a causa de las múltiples ventajas que ofrece, siendo las más importantes su elevada flexibilidad operacional, robustez y velocidad de procesamiento. El objetivo de esta investigación fue el de evaluar si una centrífuga de discos del tipo semicontinua puede ser empleada para cosechar células de *Tsukamurella paurometabola* cepa C-924. principio activo del bionematicida ecológico HeberNem-S®, en sustitución de las centrífugas tubulares utilizadas en la actualidad. La metodología usada consistió en el desarrollo de un diseño estadístico factorial del tipo 2^3, en el cual se tomaron en cuenta tres parámetros de entrada: caudal de alimentación [Q_{alim}], peso húmedo de la suspensión celular diluida a centrifugar [PH_{alim}], y tiempo entre descargas [t_{desc}]. Los parámetros de salida tomados en cuenta fueron: peso húmedo de la biomasa concentrada [PH_{bio}] y el porcentaje de recobrado [%Rec], los cuales deben presentar valores superiores a 600 g/L y 95 %, respectivamente, para obtener un rendimiento final acorde con las normas de calidad establecidas para este producto biotecnológico. Los resultados obtenidos fueron que los valores promedios para PH_{bio} y %Rec fueron de 657.28 g/L y 97.43 %, respectivamente, los cuales cumplieron con las normas de calidad para esta etapa. El diseño experimental se optimizó para determinar los valores óptimos para los 3 parámetros de entrada, obteniéndose los siguientes valores: 64 L/h para Q_{alim}, 176 g/L para PH_{alim} y 5 min de t_{desc}. La centrífuga de discos semicontinua evaluada puede ser implementada satisfactoriamente en la etapa de cosecha del proceso de producción de HeberNem®, reemplazando por tanto las centrífugas tubulares actualmente empleadas. Se emplearon los programas estadístico-matemáticos Statgraphics Centurion® XV.II, Microsoft Excel® y MATLAB® v. 7.0.1 para el procesamiento de los datos y resultados.

Palabras clave

Bioingeniería, aplicaciones industriales, procesamiento de materiales, evaluación del desempeño, equipamiento de producción.

1. INTRODUCTION

In the current biotechnology industry, the harvesting stage plays a very important role both in the overall economics of the process and in obtaining products with the desired quality parameters [1]. According to [2], the cost of the harvesting stage can reach up to 20-30 % of the total manufacturing cost of a given biotechnology product. In recent years, the centrifugation operation has expanded exponentially within the biotechnology industry due to its multiple advantages, among which we can mention its high flexibility to process different types of products, the ease of cleaning and sterilization, and having relatively high separation efficiency.

It is important to note that the efficiency of the centrifuge during the harvesting stage directly affects the efficiency, quality, and performance of subsequent separation/purification operations (microfiltration, ultrafiltration, etc.), which translates into an increment or reduction in the quantity and quality of the final product, as well as the costs involved in the overall process [3].

For this reason, it is imperative to characterize and evaluate the main operational and process parameters of a centrifuge before using it in a particular application. Currently, the most commonly used types of centrifuges in the biotechnology industry are the disk-stack centrifuge and the tubular centrifuge [4]. The disk-stack centrifuge is of great importance in this type of industry and is currently used in a large number of applications, the most common being the separation of biomass and the recovery of single-cell proteins, enzymes, and antibiotics. Among the advantages that the disk-stack centrifuge presents over its tubular counterparts are its high speed and flexibility of operation, high separation efficiency and the occupation of small space within the plant, as well as the possibility of providing aseptic conditions. However, among the disadvantages of this type of centrifuge are its high cost and high maintenance expenditure.

A disk-stack centrifuge efficiently separates solids and one or two liquid phases in a single, uninterrupted process by generating exceptionally high centrifugal forces. These forces push the heavier solids toward the rotor's inner surface, directing them into a designated specific settling zone, from which they can be extracted either continuously or intermittently through ejection.

The clarified, lower-density liquid phase, meanwhile, forms concentric inner layers and is removed close to the axis of rotation of the equipment in an area at the top of the rotor. The

insertion of special plates (disks) provides a high settling surface, which contributes to considerably speeding up the separation process. It is the special configuration, shape and design of the disks that allows a disk-stack centrifuge to be used in a wide variety of industrial applications, from biomass concentration and enzyme recovery in the biotechnology industry, to the separation of oils and fats in the petrochemical industry.

The intermittent discharge imparted by a disk-stack centrifuge allows for the efficient removal of cells (biomass) and large cellular debris in a semi-continuous operation. Nevertheless, the main drawback of this centrifuge is that, in many designs, the cells enter the centrifuge through a feeding area where high levels of shear stresses exist, which can damage shear-sensitive mammalian cells, leading to the production of sub-micron particles that are transported to the supernatant [5].

Several studies have been carried out to determine the feasibility of using a disk-stack centrifuge in a given industrial application. For example, in [6] a disk-stack centrifuge was evaluated for the separation of mammalian cell cultures in 80 L and 2 000 L fermentations, concluding that the most successful use of a disk-stack centrifuge for this specific application is the clarification of high cell density cultures prior to tangential flow filtration, in order to ensure the efficient operation of the filtration process. These authors recommended that the centrifuge should perform intermittent discharges of the solid phase, followed by a small capacity ultrafiltration system to filter the supernatant obtained.

Also, in [7] the influence of using a disk-stack centrifuge on the main stability parameters of both the yeast used and the final product obtained (beer) during the beer production process was investigated. In this study, a commercial strain of yeast was subjected to different operational conditions, resulting in the decrease of both the cell viability and intracellular pH due to the processing conditions provided by the disk-stack centrifuge evaluated. In this study it was concluded that processing yeast cells through a disk-stack centrifuge operating at high centrifugal forces adversely influences yeast physiology and the physical stability of beer.

Also, in [8] the validation of a disk-stack centrifuge with respect to sterility and aseptic processing was carried out, and a methodology for evaluating sterility and aseptic conditions was developed to be applied in additional equipment that can be based on designs similar to those of the centrifuge.

Likewise, in [9] the use of a batch disk-stack centrifuge during the primary clarification of fermentation broths containing mammalian cells was evaluated. In this study, the influence of certain operating parameters of the centrifuge such as feed flowrate and time between discharges, on various output variables such as viable, non-viable and total cell density, as well as on the turbidity of the supernatant, was evaluated, obtaining satisfactory results in accordance with the quality standards established for this process.

In addition, in [10] computational fluid dynamics modeling was carried out to harvest a monoclonal antibody contained in a culture broth of mammalian cells, where the stress environment within the disk-stack centrifuge was modeled based on various operating conditions of several variables such as rotational speed and feed volumetric flowrate.

Other authors [11] proposed a mathematical model to calculate the overall separation efficiency of a disk-stack centrifuge taking into account the variation of centrifugal force with a position on the disk, with the aim of predicting the performance of an industrial clarifier and providing a simple alternative to the Sigma concept. Similarly, in [12] two disk-stack centrifuges were used to evaluate their performance in harvesting the green algae *Chlamydomonas reinhardtii* strain TN72. which expresses a protein-based antibiotic.

In [13] it was selected for the first time an industrial centrifuge with optimized geometry and operating parameters, through the implementation of computational fluid dynamics simulation and experimental methods, to carry out the efficient separation of *Corynebacterium* bacterial cellular debris from the culture medium and the harvesting of the purified bacterial toxin. In this study, both an industrial-scale tubular centrifuge and a disk-stack centrifuge with different sizes were assessed to compute its intricate hydrodynamics.

In reference [14], the feasibility of using a Kytero 500 disk-stack separator for the separation of Chinese hamster ovary cells that produce monoclonal antibodies was explored. The study not only assessed the separation efficiency by measuring turbidity but also evaluated key quality parameters—including concentrations of host cell proteins, DNA, and lactate dehydrogenase—to determine the extent of cell damage during the centrifugation process.

In [15] the separation process inside a disk-stack centrifuge was visualized by means of a transparent top bowl using a high-speed camera, obtaining as the main result that the ratio of unused clarification area is significant.

Lastly, in [16], a technique was introduced for quickly establishing optimal operating conditions for recovering high cell density fermentation broths through centrifugation. This approach included a predictive framework for assessing the levels of clarification and dehydration attainable with different types of high-speed centrifuges, such as semi-continuous disk-stack centrifuges.

HeberNem® is a biological product effective in the control of nematodes of different species and genera (*Meloidogyne* spp, *Radopholus similis* and *Pratylenchus* spp) through the production of hydrogen sulfur and chitinases, which affects the eggs and larvae of the nematodes. In case of the eggs, it debilitates its external layer, thus originating vacuoles inside the eggs that alter the embryogenesis process, while in larvae similar effects occur regarding the formation of internal gaseous vacuoles, together with damages in the cuticle. The active ingredient of HeberNem® is the Gram-positive bacterium *Tsukamurella paurometabola* strain C-924 isolated from the soil.

A study confirmed that this bacterium promotes the colonization of lettuce roots, and thus the lettuce growth, together with the arbuscular mycorrhizal fungi *Glomus fasciculatum* and *Glomus clarum* [17]. Also, it was demonstrated that the interaction between *T. paurometabola* C-924 and *Rhizobium leguminosarum* biovar *phaseoli* CFH improved the number of leaves and the germination of seeds in bean plants [18]. Similarly, another research [19] revealed that inoculating soil with *T. paurometabola* C-924 enhanced shoot elongation, shoot diameter, and the number of leaves in maize plants cultivated in a greenhouse setting. *In vitro* tests indicated that this bacterium has the capability to produce indole acetic acid, solubilize phosphate, secrete lytic enzymes, and prevent the development of phytopathogenic fungi such as *Sarocladium oryzae*, *Alternaria longipes*, *Pestalotia palmarum*, and *Pythium debaryanum*.

At present, *Tsukamurella paurometabola* C-924 bacterium is produced and formulated so that the microorganism retains its viability and nematicidal properties, while the bionematicide HeberNem® is currently produced in two presentations, liquid (HeberNem-L®) and solid (HeberNem-S®) through a submerged diauxic fermentation process using a substrate rich in yeast extract and sucrose [20].

The production process for the HeberNem-S® bionematicide has an intermediate harvest-recovery stage in which two tubular centrifuges (CEPA®, model Z81) are currently used to separate and concentrate the cells of the *Tsukamurella paurometabola* bacterium contained in the cell broth after the fermentation stage has finished. However, to centrifuge an average fermentation volume of 180 L of cell culture, a work shift of approximately 12-18 h must be carried out, using two operators.

There is currently an ongoing investment process which consists of the erection of a new HeberNem-S® production plant where the final fermentation volume will be 1 000 L of cell culture per batch. Under these conditions, the centrifugation operation, if the 2 tubular centrifuges were used, would have a total time of around 68 to 78 h using at least 3 work shifts with 2 operators each, which is inadequate for the process both from the economic point of view and for the quality and integrity of the final product.

During the design of the new plant, the possibility of using a disk-stack centrifuge to carry out the harvesting-recovery stage was studied and proposed, which could replace its tubular counterparts in order to obtain a more appropriate operating regime in accordance with the increment of production capacity and the real productive characteristics of the new industrial plant, that is, to increase the speed and efficiency of this important stage. Therefore, there is a

need to replace these tubular centrifuges with faster, flexible, robust centrifugation technology like the disk-stack centrifuge.

Previous studies carried out at pilot scale related to the centrifugation of the bacterium *Tsukamurella paurometabola* strain C-924 by means of a disk-stack centrifuge, yielded satisfactory results in terms of percentage recovery, final concentration of concentrated biomass and total centrifugation time, indicating the real feasibility of using disk-stack centrifugation technology during the harvest-recovery stage of the HeberNem-S® production process.

In this context, in this work a semi-continuous, clarifying disk-stack centrifuge was evaluated in order to know if it can be used during the industrial-scale harvest-recovery stage of the *Tsukamurella paurometabola* strain C-924 bacterium, corresponding to the production process of the ecological bionematicide HeberNem-S®, with the aim of replacing the tubular centrifuges currently used and without affecting the main performance parameters of the process, that is, wet weight of the concentrated biomass and percentage recovery, whose values must be higher than 600 g/L and 95 % respectively, in accordance with the quality standards established for this production process. The performance results obtained in this study for this disk-stack centrifuge will enable its introduction or not in the new HeberNem-S® industrial-capacity production plant, perhaps bringing with it a reduction in harvest time, higher yields of the final product and an increase in the operational flexibility and robustness of the process.

2. MATERIALS AND METHODS

2.1 Operational characteristics of the evaluated disk-stack centrifuge

The evaluated disk-stack centrifuge (Alfa Laval®, model BTPX 205SGD-34CDP-60) is a semi-continuous and clarifying type, whose main operational and construction characteristics are shown in Table 1.

Table 1. Main operational characteristics of the disk-stack centrifuge evaluated. Source: created by the authors.

Parameter	Value
Maximum feed flowrate	12 000 L/h
Maximum feed flowrate during discharges	100 – 500 L/h
Outlet flowrate of concentrated solids for a feed flowrate of 150 L/h and a time between discharges of 2 to 10 min.	~ 1 L/discharge
Water consumption for rotor washing	0.5 – 1 L
Total volume of rotor	3.1 L
Total volume for solids sedimentation	1.2 L
Angle of disks	40°
Number of disks	80
Motor power consumption	6.5 kW
Rotational speed of motor	3 000 rpm
Rotational speed of rotor	9 650 rpm
Water consumption (discharge)	0.5 L/discharge
Water consumption (replacement)	< 3 L/h
Water consumption (axial seal)	60 – 100 L/h

2.2 Operational parameters considered, and experimental process applied

To evaluate the operational performance of the proposed disk-stack centrifuge, the following independent parameters were taken into account:

- Feed flow rate to the disk centrifuge [Q_{feed}] (L/h).
- Wet weight of the diluted cell culture to be centrifuged (feed) [PH_{feed}] (g/L).
- Time between discharges [t_{dis}] (min).

The experimental process applied in this work consisted of the elaboration of a 2^3 factorial design involving the three independent parameters described above [Q_{feed}, PH_{feed} and t_{dis}] while two output parameters were assessed: wet weight of the concentrated biomass [PH_{bio}] and recovery percentage [%Rec]. Firstly, the peristaltic feed pump was calibrated. Next, the values of the three independent variables were applied to the disk-stack centrifuge depending on the factorial design implemented, and the two output parameters PH_{bio} and %Rec were measured. Besides, the volume of concentrated biomass per discharge was also computed. Afterward, the values obtained for the two output parameters were statistically processed and analyzed, while two equations were obtained which related the output parameters PH_{bio} and %Rec with the three independent variables. Lastly, the statistical optimization of the operational input parameters was carried out to know its projected values in order to maximize PH_{bio} and %Rec.

2.3 Factorial design

The selected factorial design was of type 23, where eight experimental runs were obtained by randomly alternating the values of each of the three independent parameters selected. In general, the feed flow ranged between 30 - 66.6 L/h, the wet weight between 114.75 - 203 g/L, while the time between discharges was 4 or 5 min. These ranges were taken from recommendations made in previous studies carried out on a pilot scale consisting of the harvesting of the same bacteria by means of a disk-stack centrifuge. Table 2 shows the values generated by the chosen factorial design, as well as the order followed by each of the experimental runs.

Table 2. Proposal of the random statistical factorial design of 2^3 type. Source: created by the authors.

Run	Feed flowrate [Q_{feed}] (L/h)	Feed wet weight [PH_{feed}] (g/L)	Time between discharges [t_{dis}] (min)
1	30.6	203.00	5
2	52.4	175.00	4
3	52.4	176.25	5
4	66.6	146.25	4
5	48.8	135.00	5
6	54.8	187.00	5
7	40.0	158.25	4
8	42.4	114.75	4

2.4 Product used

Certain quantities of the final product HeberNem-S® powder were used, which was rejected due to low viability. It was resuspended in purified water until reaching the desired PH_{feed} values according to the values proposed by the established experimental design.

2.5 Inlet flowrate to the disk-stack centrifuge

The diluted cell broth was fed to the disk-stack centrifuge using a peristaltic pump (Watson-Marlow®, model 604 U/R), and the discharge flow was calibrated as follows: A silicone hose was placed on the head of the peristaltic pump, securing it to the inlet and outlet closures of the equipment. Next, the suction end of the hose was placed inside a 2 L beaker containing this volume of process water. The potentiometer of the peristaltic pump was then moved from the "O" position to the chosen rpm position, in order to have a continuous discharge flowrate. The end of the hose was then placed inside a 1 L plastic test tube, and the volume of process water collected in this test tube at the outlet of the peristaltic pump was recorded over the course of 1 min. This procedure was executed in triplicate, while the positions of the peristaltic pump potentiometer were selected from 15 rpm to 40 rpm with an incremental scale of 5 rpm. Finally, the discharge flowrate values were averaged for each point of the selected potentiometer, and the data were graphed using MATLAB® 7.0.1 software, where the values of rpm applied according to the position of the potentiometer established in the peristaltic pump are on the abscissa axis, while the obtained discharge flowrate values are placed on the ordinate axis. Figure 1 shows the calibration curve obtained for the discharge flowrate of the evaluated peristaltic pump, also presenting the adjusted polynomial equation of 3rd order.

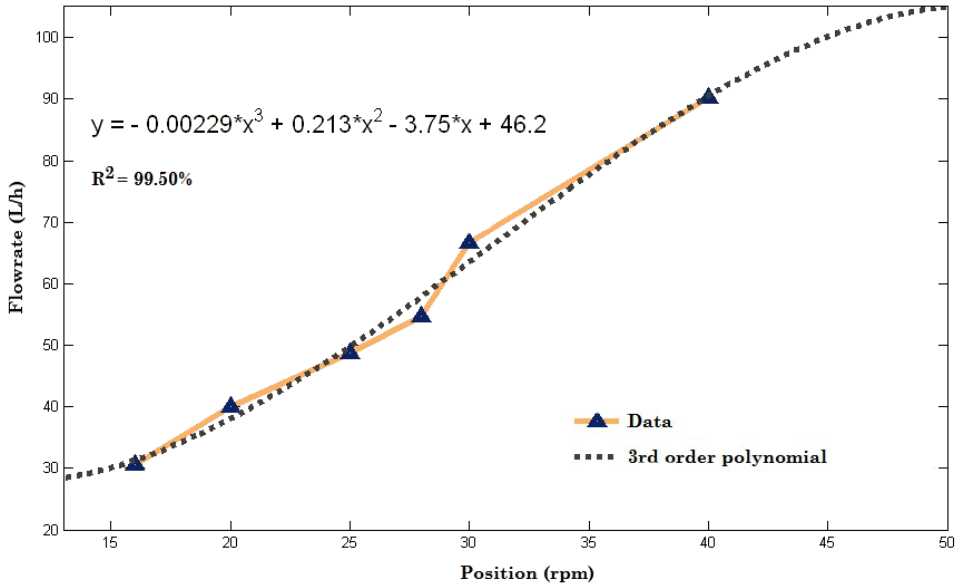


Figure 1. Calibration curve of the feed flowrate to the disk-stack centrifuge evaluated, using a Watson-Marlow® peristaltic pump, model 604 U/R. Source: created by the authors.

2.6 Determination of the wet weight of the process streams

The cell concentration (in g/L) of the main streams involved in the centrifugation process, i.e., feed culture and concentrated biomass, was determined using the wet weight method, which is described below. Four 1.5 mL plastic vials were taken, identified with different digits or letters, and weighed on an analytical scale (Sartorius®) to the last decimal place, recording the weight value of each one in a document prepared for this purpose. 1 mL of the sample of the stream to be analyzed were taken with a P-1000 micropipette (Gilson Pipetman®) and poured into each vial. The four vials containing the sample were then placed in a laboratory centrifuge (Eppendorf®) and centrifuged for 10 min at 14 000 rpm. Once the centrifugation time elapsed, the vials were carefully removed from the centrifuge, uncapped, and the supernatant was decanted into a liquid biological waste container, which was treated according to the approved

procedure. The interior walls of each vial containing the sedimented biomass were then dried using small strips of filter paper, and then the four vials were weighed on an analytical scale (Sartorius®), recording the weighing result in a document provided for this purpose. The difference in weight between the vial after centrifugation and the empty vial was determined using (1). The wet weight of the sample taken was calculated by means of (2).

$$Difference\ in\ weight = Weight\ of\ vial\ with\ biomass - Weight\ empty\ vial \quad (1)$$

$$Wet\ weight\ sample = \frac{[(DW1 + DW2 + DW3 + DW4) \cdot 1\,000]}{4} \quad (2)$$

In this wet weight determination procedure, it is indicated that, when performing wet weight calculations, a subtraction is made between the highest and lowest values calculated using (2), in order to accept or reject the calculations as shown in Table 3:

Table 3. Acceptance or rejection criteria for the wet weight determination procedure. Source: created by the authors.

Wet weights (g/L)	Acceptance limits
≤ 50 g/L	Up to 5 g/L of difference
Between 50 g/L y 200 g/L	Up to 10 g/L of difference
> 200 g/L	Up to 15 g /L of difference

If the difference between the determinations is outside the acceptance limits set out in Table 3, the determination test is repeated, recording the values of the new wet weight determination in the document provided for this purpose.

2.7 Determination of the volume of a concentrated biomass per discharge

To determine the volume of concentrated biomass per discharge (V_{bio}/dis), a graduated cylinder with a total volume of 2 L was used, with a precision of 1 mL, which was inserted into the outlet of the concentrated biomass discharge pipe of the disk-stack centrifuge (See Figure 2). Five samples of V_{bio}/dis were taken for each experimental run performed, which were then averaged to obtain the final value of this parameter.

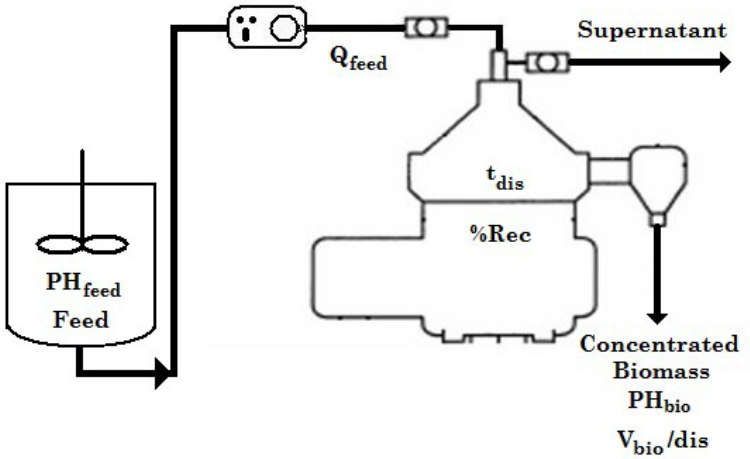


Figure 2. Diagram of the disk-stack centrifuge and the main parameters involved in the study. Source: created by the authors.

2.8 Selection of the discharge duration time

During all the experimental runs carried out, the discharge duration time, which consists of the sum of the rotor opening and closing times, plus the time that the open rotor takes to discharge the concentrated biomass, was set at 15 s (See Table 4) according to values reported in [4]. On the other hand, the rotor rinsing variant was not used, since, according to recommendations of several specialists, it does not significantly influence the performance or the control parameters of the process, thus avoiding a possible increase in the contaminating microbial load and the potential dilution of the concentrated biomass obtained.

Table 4. Duration time of one discharge. Source: created by the authors.

Parameter	Value (s)
Time to completely open the rotor	4
Time that lasts the opened rotor	5
Time to completely close he rotor	6
Total	15

2.9 Determination of the recovery percentage (%Rec)

The recovery percentage was determined according to (3) for each of the experimental runs performed.

$$\%Rec = \frac{PH_{bio} \cdot V_{bio}}{PH_{feed} \cdot V_{feed}} \tag{3}$$

Where PH_{bio} is the final wet weight of concentrated biomass (g/L), V_{bio} is the final total volume of concentrated biomass obtained (g/L), PH_{feed} is the initial wet weight of diluted cell culture before centrifugation (fed) (g/L), and V_{feed} is the initial total volume of diluted cell culture before centrifugation (L). This parameter is a measure of the amount of biomass recovered from an initial value of the same and measures the real efficiency of any recovery operation used in a biotechnological process that involves centrifugation.

2.10 Statistical analysis of the data obtained

In order to statistically analyze and validate the data obtained for each of the response parameters considered in the experimental design, that is: wet weight of the concentrated biomass (PH_{bio}) and % recovery (%Rec), as well as to carry out the optimization of the operational input parameters (feed flowrate, wet weight of the feed and time between discharges), the statistical package Statgraphics Centurion® XV.II was used. Among the statistical options used in this study, which are contained in this program, are:

- Analysis of a variable: Includes the statistical summary of the data, the frequency table, and the statistical validity for a given confidence interval (%).
- Canonical correlations: Identifies associations between two sets of parameters.
- Experiment analysis: Provides estimated effects, variance analysis, and regression coefficients for output parameters, as well as response surface graphs, main effects graphs, surface contour graphs, and standardized Pareto chart.
- Multiple response optimization: This option determines the desirability of the experiment and finds the combination of experimental factors that simultaneously optimize several responses. It also provides estimated response surface graphs, surface contour graphs, and overlay graphs.
- Control charts: Allows determining whether the process is statistically controlled and whether it shows stable behavior.

3. RESULTS AND DISCUSSION

Table 5 shows the results for the wet weight of concentrated biomass (PH_{bio}) and % recovery (%Rec) for each of the experimental runs performed, as well as other parameters of interest such as total volume of diluted culture fed (V_{feed}), volume of concentrated biomass per discharge (V_{bio}/dis) and total centrifugation time ($t_{centrif}$).

Table 5. Experimental runs performed and results obtained. Source: created by the authors.

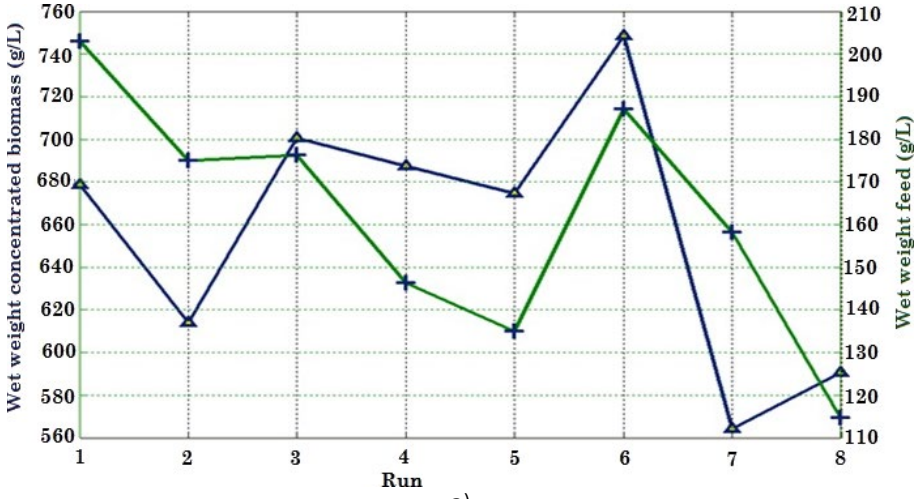
Run	Inlet				Outlet			
	Q_{feed} (L/h)	PH_{feed} (g/L)	t_{dis} (min)	V_{feed} (L)	PH_{bio} (g/L)	%Rec	V_{bio} / dis. (L)	$t_{centrif}$ (h)
1	30.6	203.00	5	58	678.50	97.23	0.90	1.89
2	52.4	175.00	4	60	613.75	93.47	0.96	1.14
3	52.4	176.25	5	56	700.75	98.54	0.85	1.07
4	66.6	146.25	4	62	687.25	99.36	0.88	0.93
5	48.8	135.00	5	55	674.75	98.61	0.92	1.13
6	54.8	187.00	5	58	748.50	97.40	0.78	1.06
7	40.0	158.25	4	59	564.25	96.27	1.10	1.48
8	42.4	114.75	4	61	590.50	98.56	1.00	1.44
Average	48.5	161.94	5	59	657.28	97.43	0.92	1.26

According to the literature [3], [4], [21], the main factors that influence the degree of separation, i.e. the percentage of recovery obtained in a disk-stack centrifuge, as well as the final concentration of the solids in the discharge, are: the difference in density between the components of the liquid to be centrifuged, its viscosity, the shape and size of the particles to be separated, the concentration of solids present in the liquid to be centrifuged, the time between discharges selected, and the feed flowrate. Figure 3 (a-f) shows the interactions that exist between the input and output parameters considered.

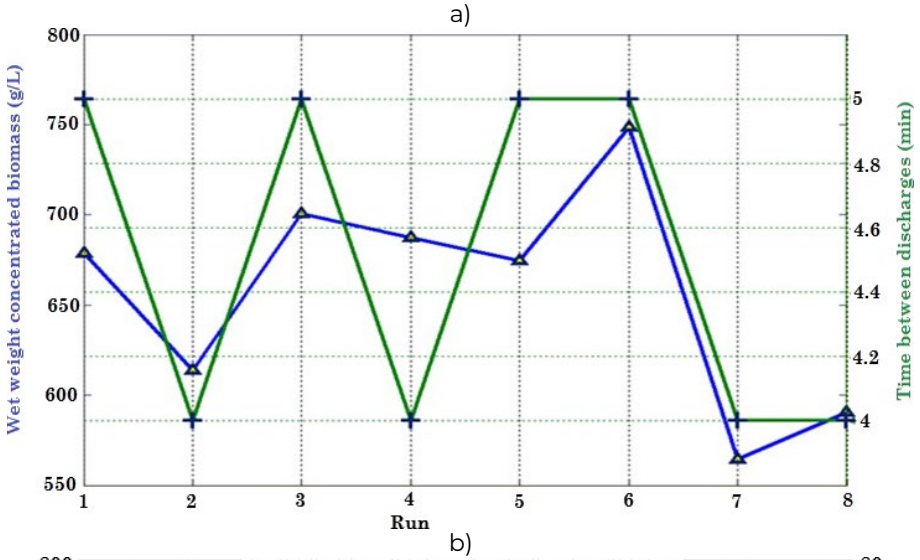
3.1 Analysis of the concentrated biomass wet weight results

According to the results shown in Table 5, the lowest PH_{bio} value obtained (564.25 g/L) corresponded to run No. 7. This run presents the fourth lowest feed wet weight (158.25 g/L), the second lowest feed flow (40 L/h) and the lowest time between discharges (4 min). In this case, since the feed flowrate is small, either an increase in the time between discharges or an increase in the concentration of solids (biomass) in the liquid to be centrifuged is required, in order to accumulate and concentrate the cells until reaching the desired final concentration value (> 600 g/L). It is noteworthy that in run No. 1 the feed flowrate is 23.5 % lower than that used in run No. 7, while the concentration of the culture to be centrifuged used in this run is 22 % higher than that used in run No. 7, also presenting a longer time between discharges (5 min). All this made it possible for the PH_{bio} value obtained in run No. 1 to be about 1.2 times higher than that of run No. 7 (Figure 4).

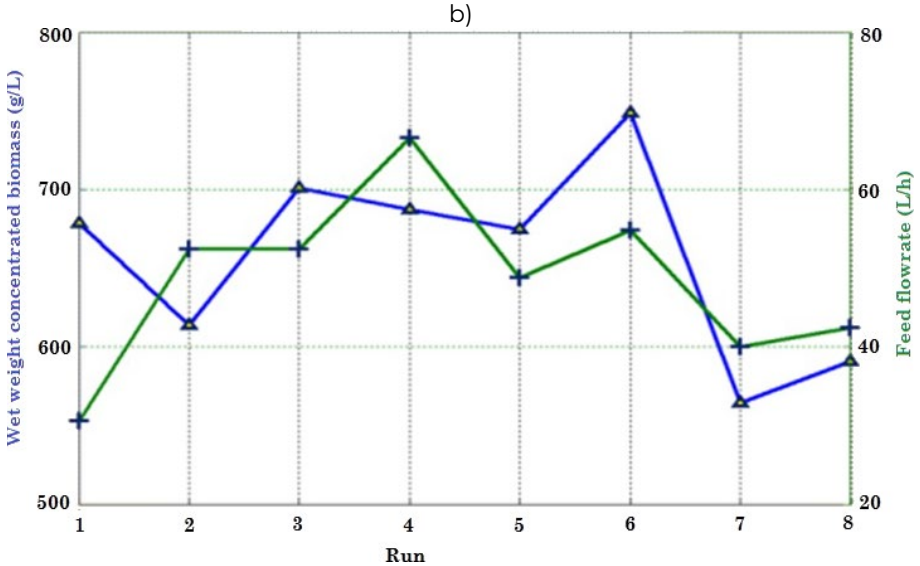
On the other hand, when comparing the values obtained for runs 3 and 6, in which the same value of time between discharges (5 min) was used, and the values of the feed flowrate approximately equal to each other (52.4 L/h and 54.8 L/h for runs 3 and 6, respectively), but with a difference in the wet weight of the feed liquid of approximately 11 g/L (176.25 g/L and 187 g/L for runs 3 and 6, respectively), a difference in the wet weight of the discharge of almost 50 g/L is obtained (700.75 g/L and 748.5 g/L for runs 3 and 6, respectively) (See Figure 5). This indicates that, for approximately equal values of Q_{feed} and t_{dis}, an increase in the concentration of solids in the liquid to be centrifuged (PH_{feed}) will cause an increase in the concentration of biomass in the discharge (PH_{bio}).


Wet weight concentrated biomass (g/L)
Wet weight feed (g/L)
Run
760
740
720
700
680
660
640
620
600
580
560
210
200
190
180
170
160
150
140
130
120
110
1
2
3
4
5
6
7
8

a)


Wet weight concentrated biomass (g/L)
Time between discharges (min)
Run
800
750
700
650
600
550
5
4.8
4.6
4.4
4.2
4
1
2
3
4
5
6
7
8

b)


Wet weight concentrated biomass (g/L)
Feed flowrate (L/h)
Run
800
700
600
500
80
60
40
20
1
2
3
4
5
6
7
8

c)

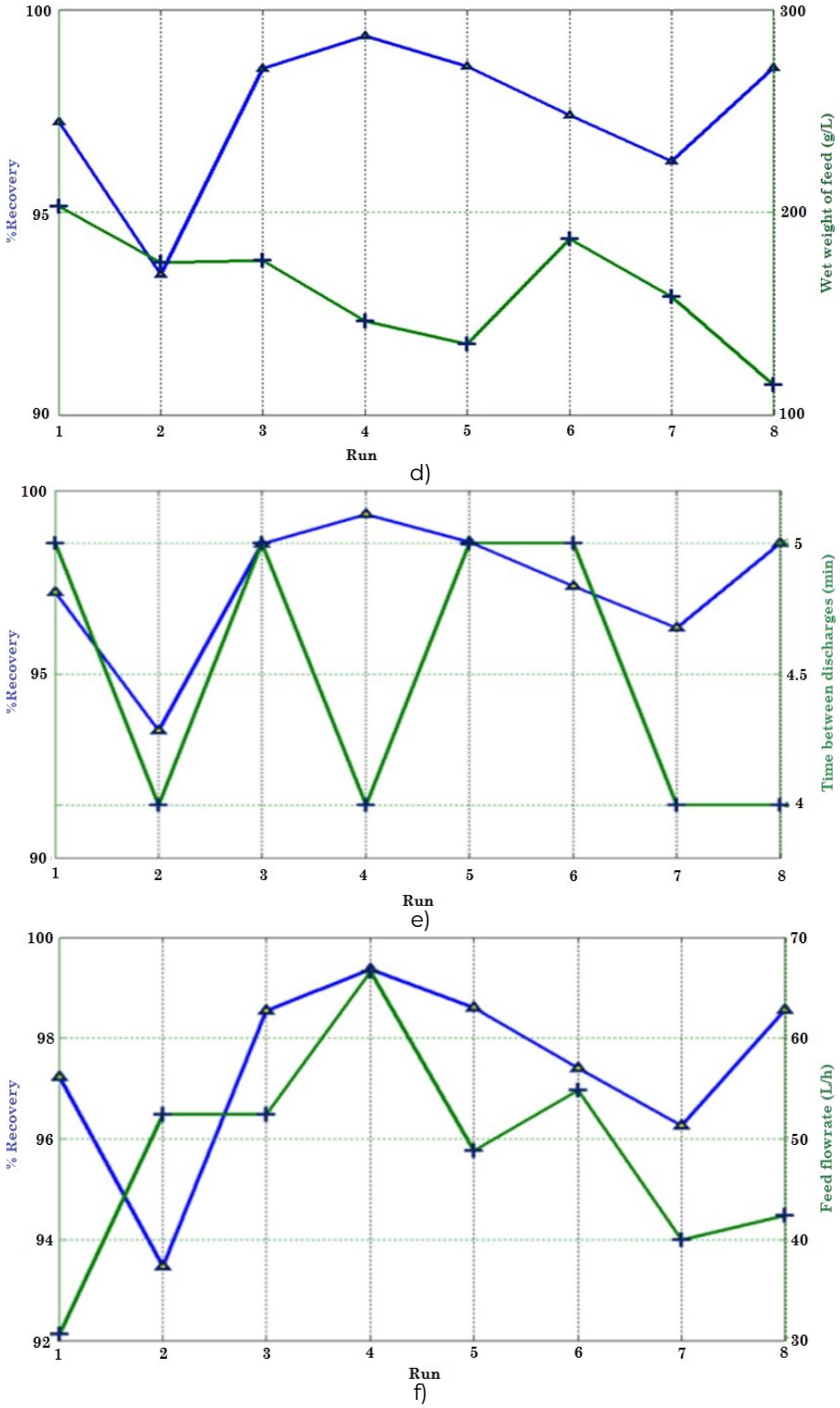


Figure 3. Calibration curve of the feed flowrate to the disk-stack centrifuge evaluated, using a Watson-Marlow® peristaltic pump, model 604 U/R. a) Wet weight of concentrated biomass vs. Wet weight of feed, b) Wet weight of concentrated biomass vs. Time between discharges, c) Wet weight of concentrated biomass vs. Feed flowrate, d) %Recovery vs. Wet weight of feed, e) %Recovery vs. Time between discharges, and f) %Recovery vs. Feed flowrate. Source: created by the authors.

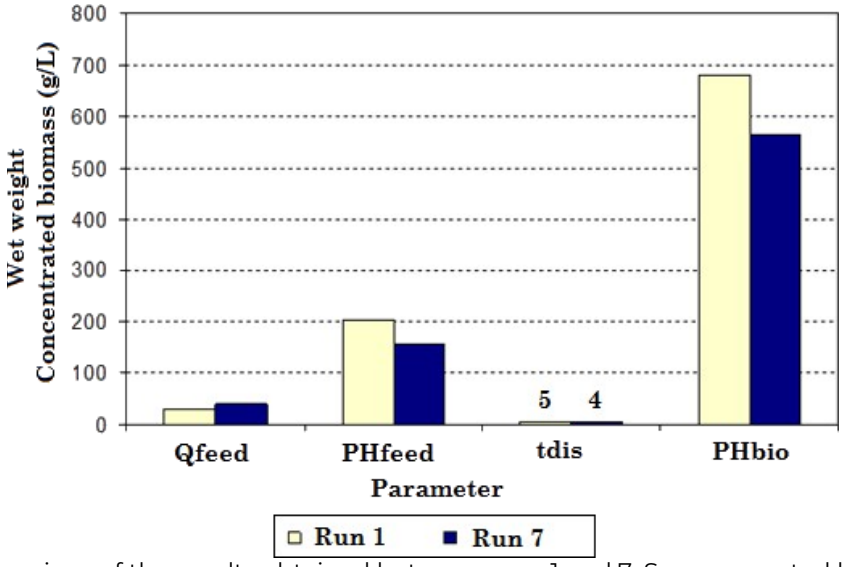


Figure 4. Comparison of the results obtained between runs 1 and 7. Source: created by the authors.

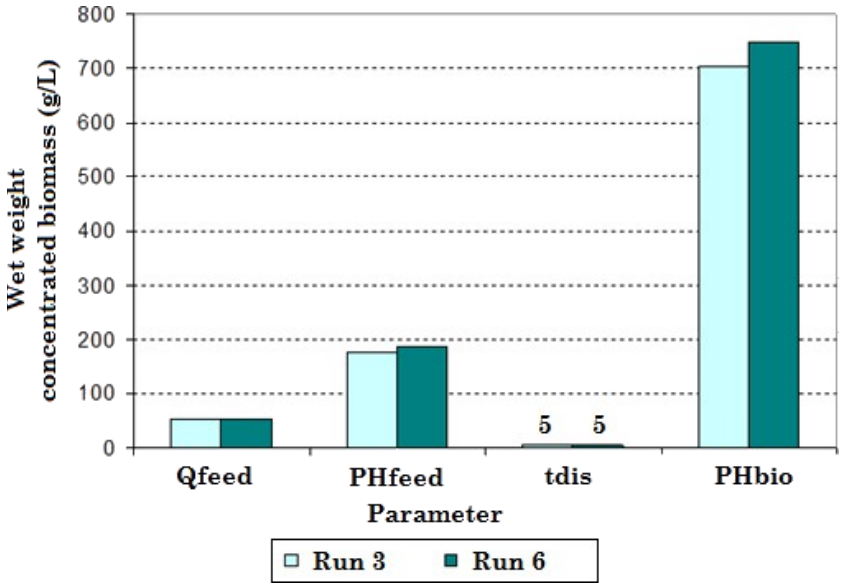


Figure 5. Comparison of the results obtained between runs 3 and 6. Source: created by the authors.

Comparing the results obtained in runs 1 and 5, it can be observed that the Q_{feed} of run No. 1 is 37.3 % lower than that of run No. 5, while the PH_{feed} used in run No. 5 is approximately 33.5 % lower than that of No. 1. In both runs, t_{dis} is kept constant, with a value equal to 5 min. However, for these operating conditions, PH_{bio} values were obtained with a difference of only 3.75 g/L between them (See Figure 6 The above shows that, to obtain the same PH_{bio} results, with an increase in PH_{feed}, Q_{feed} must be reduced, keeping t_{dis} constant.

Regarding the results obtained for runs 2 and 3, it can be observed that the feed flowrate conditions for both runs are identical (52.4 L/h), as well as the input wet weight values (they only have a difference between them of 1.25 g/L), while the time between discharges was modified by 1 min (4 min in run No. 2, and 5 min in run No. 3), which was sufficient to obtain PH_{bio} values between both runs with a difference of 87 g/L (See Figure 7).

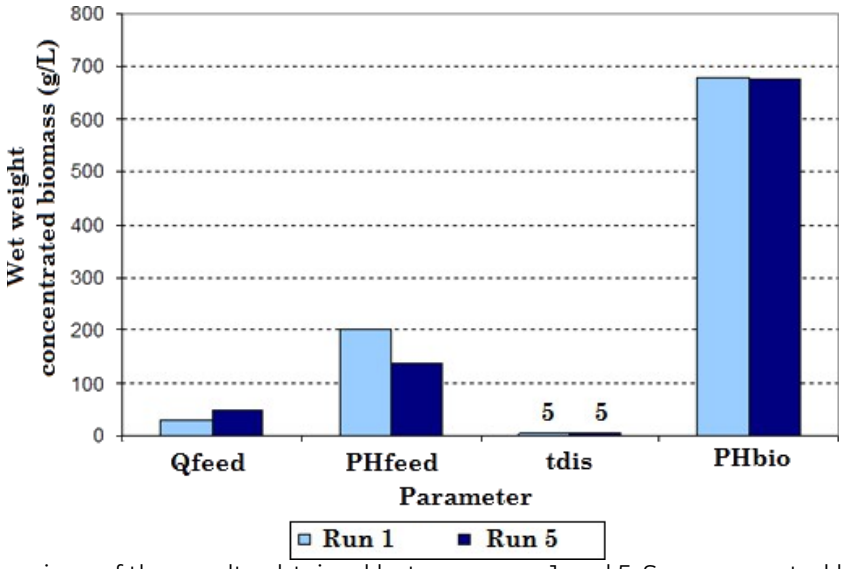


Figure 6. Comparison of the results obtained between runs 1 and 5. Source: created by the authors.

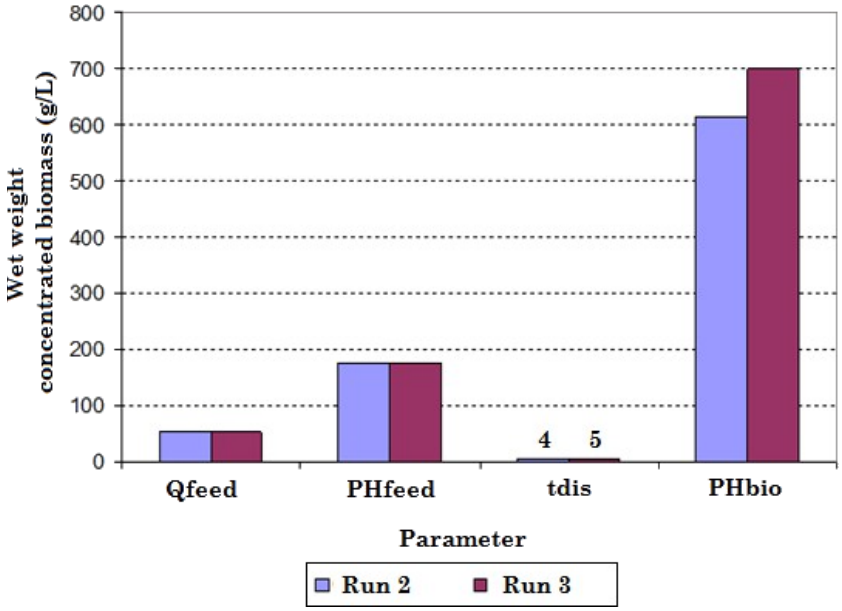


Figure 7. Comparison of the results obtained between runs 2 and 3. Source: created by the authors.

The above is due to the fact that in run 3 the residence time of the biomass within the solids sedimentation zone of the equipment increased, thus enabling a greater quantity of this product to accumulate with the subsequent increase in its concentration. Therefore, it follows from the above that, keeping both the feed flow and the initial concentration of the culture to be centrifuged constant, the increase in the time between discharges will bring about an increase in the concentration of biomass in the discharge. This can be observed in all those runs where the time between discharges applied is the maximum possible (5 min), independently of the values presented by the remaining independent parameters. 66.7 % of the PH_{bio} values obtained above 600 g/L correspond to times between discharges of 5 min, which corroborates the previous conclusion. These results agree with that reported by [9], where it is indicated that the residence time in a semi-continuous disk centrifuge constitutes a key parameter to obtain a desired concentration of biomass in the concentrated biomass stream, for a given density of cells in the feed.

Analyzing the results obtained in runs 2 and 4 where equal times between discharges were applied for both runs (4 min), it can be observed that the concentration of the culture to be fed used in run No. 4 is 16.4 % lower than that used in No. 2. while the feed flowrate of run No. 4 is 21 % higher than that used in No. 2. In this case, the PH_{bio} value obtained in run No. 4 was 73.5 g/L higher than that achieved in No. 2 (See Figure 8). This result shows that Q_{feed} significantly influences the PH_{bio} value obtained in a centrifugation process using clarifying disc centrifuges. It should also be recognized that the feed flowrate used in run No. 4 (66.6 L/h) had the highest value of all those used during the experiment, which is very close to the statistical optimum obtained (63.9 L/h).

Figure 8. Comparison of the results obtained between runs 2 and 4. Source: created by the authors.

The important influence that the feed flowrate has on the biomass concentration to be obtained can also be observed by comparing the results obtained in runs No. 7 and 8, where run No. 8 had a pH_{feed} value almost 1.4 times lower than that used in run No. 7, however, its feed flowrate rate is only 2.4 L/h higher than that applied in run No. 7. The time between discharges remained constant for both runs (4 min). Under these operational conditions, the PH_{bio} result obtained for run No. 8 was 26.25 g/L higher than the value of this parameter obtained for run No. 7. That is, the run that had the highest feed flow rate while keeping the t_{dis} parameter constant obtained the highest biomass concentration at the outlet (Figure 9). This coincides with what was reported by [9] where it is established that the increase in the feed flow in semi-continuous disk centrifuges provides less clarification of the supernatant stream, and therefore a higher concentration of biomass in the concentrate stream, due to a shorter residence time of the solids in the centrifuge.

Finally, analyzing the results obtained for runs No. 4 and 5, it can be observed that for an approximately constant initial concentration (the difference in wet weight of input between both runs is only 11.33 g/L), the decrease in the time between discharges for run No. 4 (4 min) with respect to that used in No. 5 (5 min) was balanced by an increase in its inlet flowrate (66.6 L/h) compared to that of run 5 (48.8 L/h), obtaining PH_{bio} values with a difference between them of only 12.5 g/L. (see Figure 10). This indicates that, to obtain a similar value of PH_{bio}, if the time between discharges is reduced, the feed flow rate must be increased while keeping the wet weight of the feed constant. In all runs, the wet weight value of concentrated biomass was above 560 g/L, obtaining an average value of 657.28 g/L for this parameter.

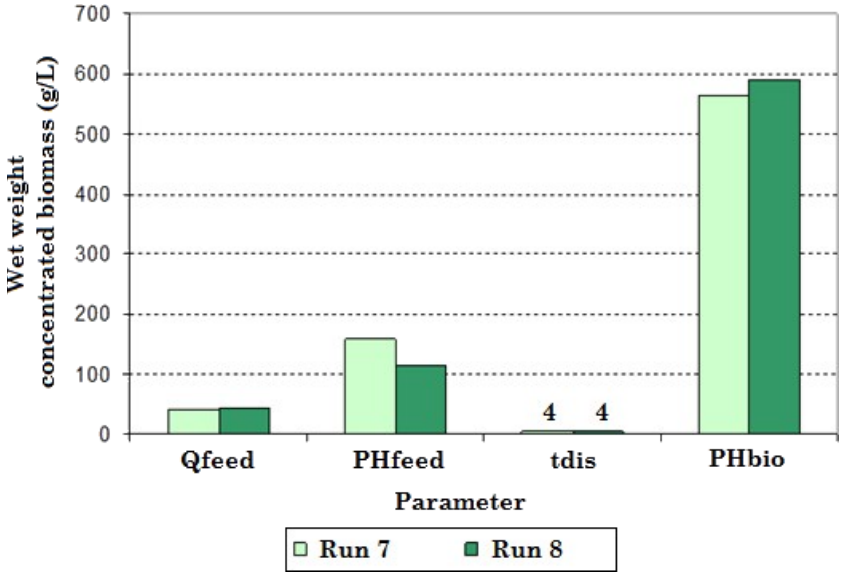


Figure 9. Comparison of the results obtained between runs 7 and 8. Source: created by the authors.

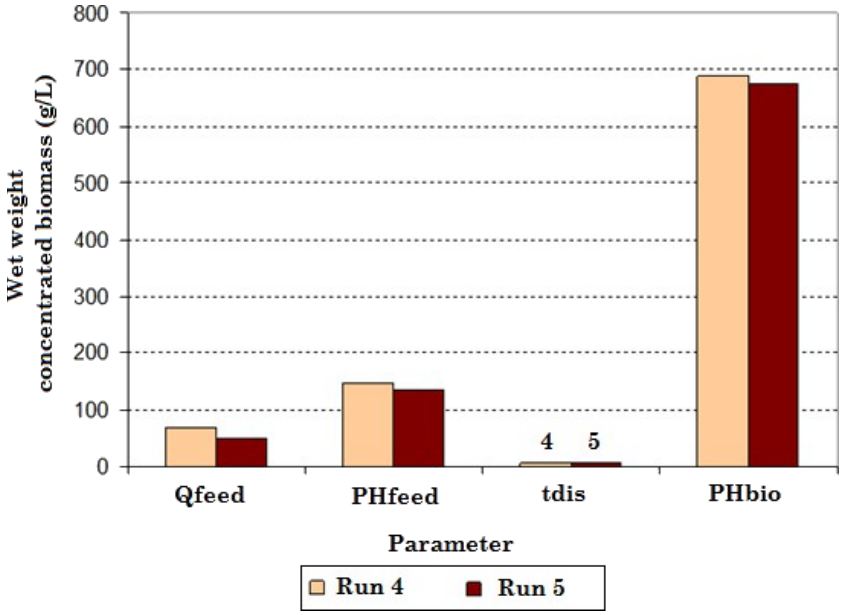


Figure 10. Comparison of the results obtained between runs 4 and 5. Source: created by the authors.

3.2 Analysis of recovery percentage results

According to Table 5, the highest recovery percentage value (99.36 %) was achieved in run 4 for the highest feed flowrate (66.6 L/h), the third lowest wet weight value of the feed (146.25 g/L) and the shortest discharge time (4 min). On the other hand, the lowest % Rec value (93.47 %) was achieved in run 2 for the third highest feed flowrate (52.4 L/h), the fourth highest wet weight value of the feed (175.00 g/L) and the shortest time between discharges (4 min). In all runs the %Rec value was above 92 %, with an average value of 97.43 %.

3.3 Relationship between the wet weight of concentrated biomass (PH_{bio}) and the volume of biomass obtained per discharge (V_{bio}/dis)

Consistent with the results shown in Table 5 and Figure 11 there is an inversely proportional relationship between PH_{bio} and the volume of biomass obtained per discharge (V_{bio}/dis).

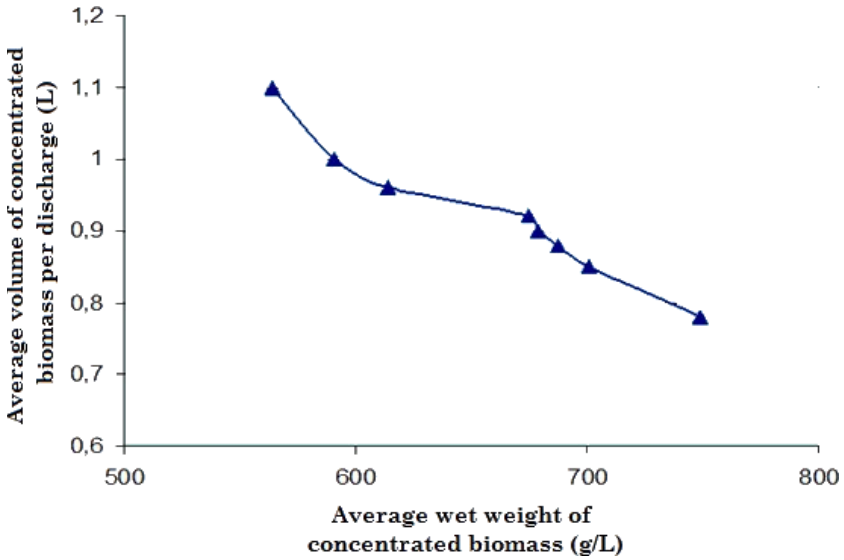


Figure 11. Relationship between the average volume of concentrated biomass per discharge (Vbio/dis) and the average wet weight of the concentrated biomass (PHbio). Source: created by the authors.

In this case, the higher the average wet weight (concentration) of the biomass at the centrifuge outlet, the lower the volume of concentrated biomass that will be obtained for each discharge performed. This has to do with the operational conditions established for each experimental run in question, which in turn influences the degree of compaction and dehydration experienced by the biomass as it accumulates in the solid's accumulation space within the centrifuge rotor, as well as its residence time inside the equipment. The longer the residence time (accumulation) of the solids (cells) inside the equipment (i.e., the longer the time between discharges), the greater the degree of compaction and dehydration they will undergo, since the cells that sediment put pressure on those already accumulated (or sedimented) within the solids accumulation space of the equipment, which causes the water contained within the accumulated biomass to escape to the outside. This is a phenomenon that some authors [2], [4] call compaction-dehydration, which extracts a certain volume of water accumulated around the sedimented cells by centrifugation, due to the accumulation of the same, thus reducing the total accumulated volume of concentrated biomass. This manifestation can also be observed in Figure 12, where the longer the residence time (longer time between discharges) the higher the concentration (PH_{bio}) and the lower the volume (V_{bio}) of the concentrated biomass discharge.

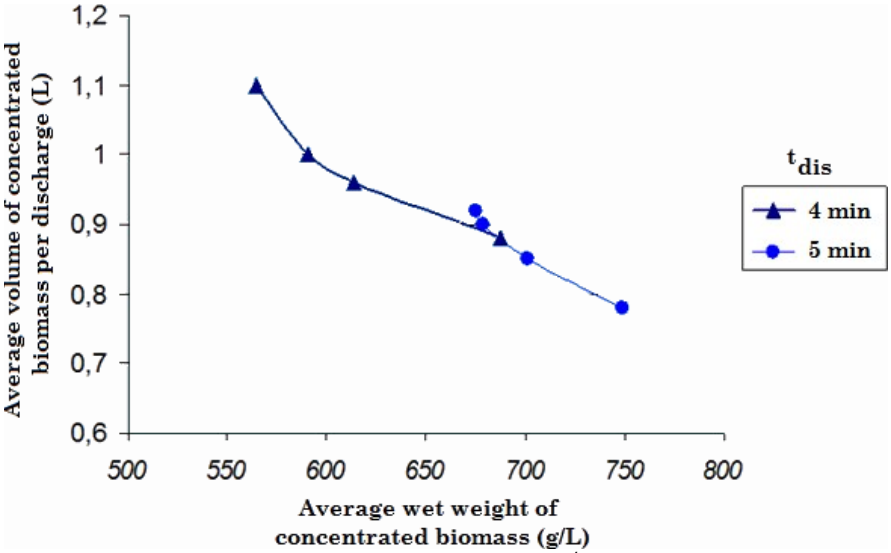


Figure 12. Comparison between the values obtained for V_{bio}/dis with PH_{bio}, with respect to the time between discharges. Source: created by the authors.

3.4 Analysis of the statistical results

Below are the statistical results obtained for the parameters wet weight of concentrated biomass (PH_{bio}) and percentage of recovery (%Rec), using the statistical software Statgraphics Centurion® XVII.

3.4.1 Wet weight of concentrated biomass (PHbio)

Table 6 shows the summary of the statistical results obtained, Table 7 presents the estimated effects between the variables, Table 8 the analysis of variance, Table 9 the regression coefficients and Table 10 the canonical correlations for the variable wet weight of concentrated biomass (PH_{bio}).

Table 6. Summary of the statistical results for PHbio. Source: created by the authors.

Parameter	Value
Count	8
Average	657.281
Standard deviation	61.9602
Coefficient of variation	9.42673 %
Minimum	564.25
Maximum	748.50
Range	184.25
Standardized skewness	- 0.279732
Standardized kurtosis	- 0.514947
F-ratio	5.49
P-value	0.0668

Table 7. Estimated effects between variables. Source: created by the authors.

Effect	Estimate	Standard Error	V.I.F
Average	650.163	10.3115	
A: Qfeed	132.546	33.43	1.42623
B: PHfeed	19.9649	28.2159	2.02965
C: tdis	102.565	20.561	1.68957
AB	14.8034	138.181	16.6239
AC	- 37.8339	107.893	14.366
BC	42.0902	24.9432	1.3089

Table 8. Analysis of variance. Source: created by the authors.

Source	Sum of squares	D.F.	Mean square	F-Ratio	P-Value
A: Q_{feed}	7 866.90	1	7 866.90	15.72	0.1573
B: PH_{feed}	250.54	1	250.548	0.50	0.6080
C: t_{dis}	12 452.30	1	12 452.30	24.88	0.1260
AB	5.743	1	5.743	0.01	0.9321
AC	61.534	1	61.534	0.12	0.7853
BC	1 424.95	1	1 424.95	2.85	0.3406
Total Error	500.42	1	500.429		
Total (corr)	26 873.40	7			

Table 9. Analysis of variance. Source: created by the authors.

Coefficient	Estimate
Constant	541.177
A: Q_{feed}	11.0964
B: PH_{feed}	- 5.86494
C: t_{dis}	- 4.49676
AB	0.012
AC	- 2.102
BC	1.238
R^2	98.1378 %
R^2 adjusted	86.9648 %
Standard error of estimate	22.3703
Mean absolute error	4.95906

Table 10. Canonical correlations. Source: created by the authors.

Interaction	Canonical correlation	Lambda of Wilks	Chi-Squared	P-Value
PH_{feed} vs. PH_{bio}	0.458025	0.790213	1.29499	0.2551
Q_{feed} vs. PH_{bio}	0.411438	0.830719	1.02005	0.3125
t_{dis} vs. PH_{bio}	0.747842	0.440732	4.50625	0.0338

According to the results shown in Table 6, it is observed that the P-Value obtained is greater than 0.05 (0.0668), which indicates that there are no statistically significant differences between the values obtained for the PH_{bio} variable, for a confidence level of 95.0 %. On the other hand, the values obtained for Standardized Skewness and Standardized Kurtosis are within the range of - 2 to + 2 (- 0.279732 and - 0.514947, respectively), indicating that there are no significant deviations from normality, and that the data therefore comes from a normal distribution.

According to the Standardized Pareto Chart shown in Figure 13a, the parameter that influences the most from the statistical point of view in the PH_{bio} values obtained is the time between discharges. This effect can also be observed in Table 8, where the P-Value obtained for this variable is less than 0.15 (0.1260), indicating that it exerts a statistically significant influence on the PH_{bio} variable at a confidence level of 85.0 %. By analyzing the Control Chart obtained for the PH_{bio} variable (Figure 13d), it can be summarized that the data come from a system in a state of statistical control, applying a confidence level of 95.0 %, that is, the data are statistically stable. An equation of the adjusted statistical model was also obtained, which is shown below (4).

$$PH_{bio} = 541.177 + (11.096 \cdot Q_{feed}) - (5.864 \cdot PH_{feed}) - (4.496 \cdot t_{dis}) + (0.0120 \cdot Q_{feed} \cdot PH_{feed}) - (2.101 \cdot Q_{feed} \cdot t_{dis}) - (1.237 \cdot PH_{feed} \cdot t_{dis}) \quad (4)$$

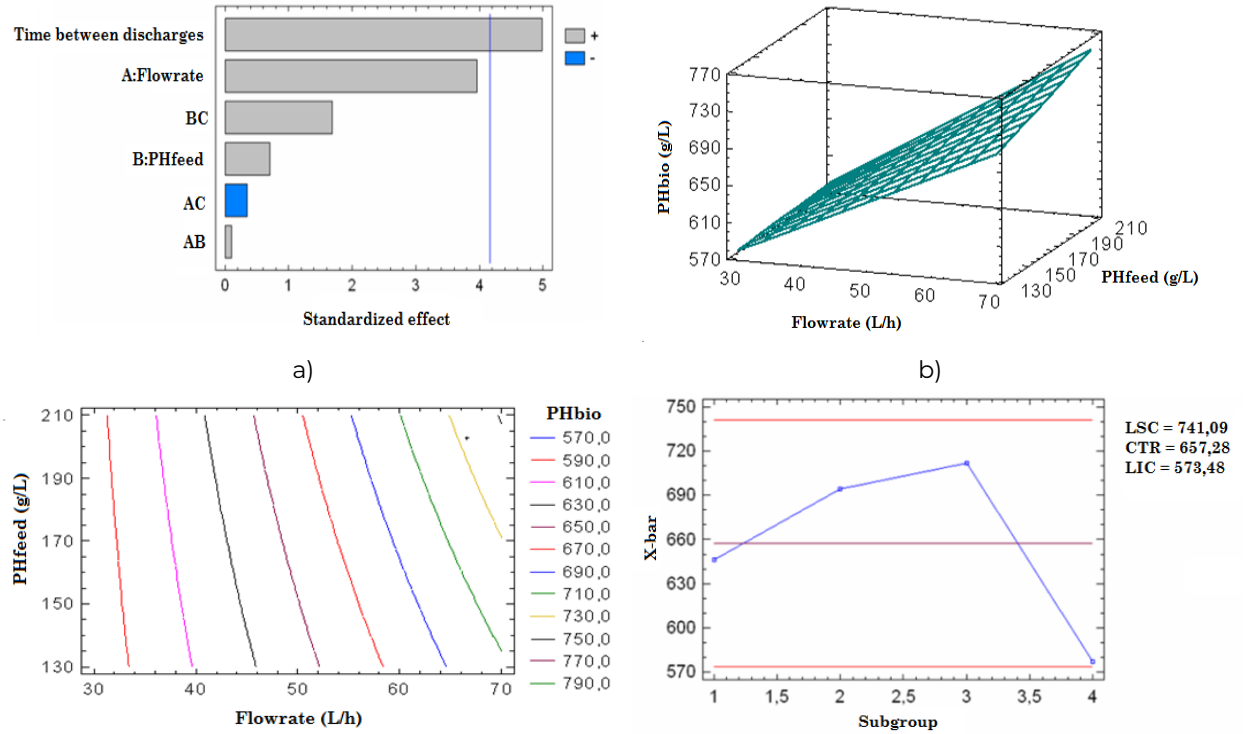


Figure 13. Different graphs obtained for the variable PH_{bio} using the statistical software Statgraphics Centurion® XV.II. a) Standardized Pareto Diagram, b) Response Surface, c) Surface Contours, and d) Control Chart. Source: created by the authors.

The value of the parameter R^2 (Table 9) obtained for this adjusted statistical model explains 98.1378 % of the variability of PH_{bio}.

Equation (4) is represented graphically in Figures 13b and 13c, while the statistical parameters and regression coefficients obtained for this model can be observed in Tables 8 and 9, respectively. Finally, with respect to the results obtained for the Canonical Correlations, it can be observed that the only variable that influences, from a statistical point of view, the value of PH_{bio} obtained is the time between discharges (t_{dis}), since the P-Value for this parameter is below 0.05 (0.0338), taking a representativeness level of 95 %. It is worth noting that 87.5 % of the PH_{bio} values obtained during the experimental runs carried out are above the minimum value required for this parameter (600 g/L) before being formulated.

3.4.2 Recovery percentage (%Rec)

Table 11 shows the summary of the statistical results obtained, Table 12 presents the estimated effects between the variables, Table 13 the analysis of variance, Table 14 the regression coefficients and Table 15 the canonical correlations for the variable %Rec.

Table 11. Summary of the statistical results obtained for %Rec. Source: created by the authors.

Parameter	Value
Count	8
Average	97.43
Standard deviation	1.87824
Coefficient of variation	1.92778 %
Minimum	93.47
Maximum	99.36
Range	5.89
Standardized skewness	- 1.72693
Standardized kurtosis	1.41282
F-ratio	1.95
P-value	0.2863

Table 12. Estimated effects between variables. Source: created by the authors.

Effect	Estimate	Standard Error	V.I.F
Average	96.187	0.225865	
A: Q_{feed}	3.15441	0.73226	1.42623
B: PH_{feed}	- 5.03265	0.618049	2.02965
C: t_{dis}	2.62667	0.450374	1.68957
AB	- 12.6557	3.02675	16.6239
AC	7.88098	2.36332	14.366
BC	2.90613	0.546362	1.3089

Table 13. Analysis of variance. Source: created by the authors.

Fuente	Sum of squares	D.F.	Mean square	F-Ratio	P-Value
A: Q_{feed}	4.45557	1	4.45557	18.56	0.1452
B: PH_{feed}	15.9201	1	15.9201	66.31	0.0778
C: t_{dis}	8.16705	1	8.16705	34.01	0.1081
AB	4.19776	1	4.19776	17.48	0.1494
AC	2.67003	1	2.67003	11.12	0.1855
BC	6.79313	1	6.79313	28.29	0.1183
Total error	0.240104	1	0.240104		
Total (corr.)	24.6944	7			

Table 14. Regression coefficients. Source: created by the authors.

Coefficient	Estimate
Constant	168.45
A: Q_{feed}	- 0.135227
B: PH_{feed}	0.0438607
C: t_{dis}	- 33.0972
AB	- 0.0103396
AC	0.437832
BC	0.0854745
R^2	99.0277 %
R^2 adjusted	93.1939 %
Standard error of estimate	0.490004
Mean absolute error	0.108625

Table 15. Canonical correlations. Source: created by the authors.

Interaction	Canonical correlation	Lambda of Wilks	Chi-Square	P-Value
PH_{feed} vs. %Rec	0.408088	0.833464	1.00191	0.3168
Q_{feed} vs. %Rec	0.213548	0.954397	0.256715	0.6124
t_{dis} vs. %Rec	0.293125	0.914078	0.494118	0.4821

Regarding the statistical results obtained for the variable %Rec, according to the P-Value (Table 11) obtained (0.2863), which is greater than 0.05, it can be inferred that there are no statistically significant differences between the results obtained for this variable, for a confidence level of 95.0 %. The statistical parameters Standardized Skewness and Standardized Kurtosis are within the established range (-2 to +2), with -1.72693 and 1.41282. respectively, thus indicating that the data obtained do not present significant deviations from normality, that is, the values present a normal distribution.

The Standardized Pareto Chart obtained (Figure 14a) indicates that none of the input variables considered has a statistically significant influence on the %Rec parameter. This can also be verified by analyzing Table 13, in which none of the variables involved (A, B or C) has a P-Value less than 0.05, as well as in the Canonical Correlations (Table 15), in which the P-Value of none of the parameters involved is below 0.05, with a confidence level of 95 %.

Along with the Control Chart obtained for this parameter (Figure 14d), the process is in a state of statistical control under a confidence level of 95 %. The equation of the statistical model adjusted for this variable is the following (5):

$$\%Rec = 168.45 - \left(0.135 \cdot Q_{feed}\right) + \left(0.043 \cdot PH_{feed}\right) - \left(33.097 \cdot t_{dis}\right) - \left(0.010 \cdot Q_{feed} \cdot PH_{feed}\right) + \left(0.437 \cdot Q_{feed} \cdot t_{dis}\right) + \left(0.085 \cdot PH_{feed} \cdot t_{dis}\right) \quad (5)$$

The R^2 value obtained (Table 14) indicates that the adjusted model explains 99.027 % of the variability of the data obtained. Equation (5) can be observed graphed in Figures 14b and 14c, while the Statistical Parameters and Regression Coefficients obtained for the adjusted model can be observed in Tables 13 and 14, respectively.

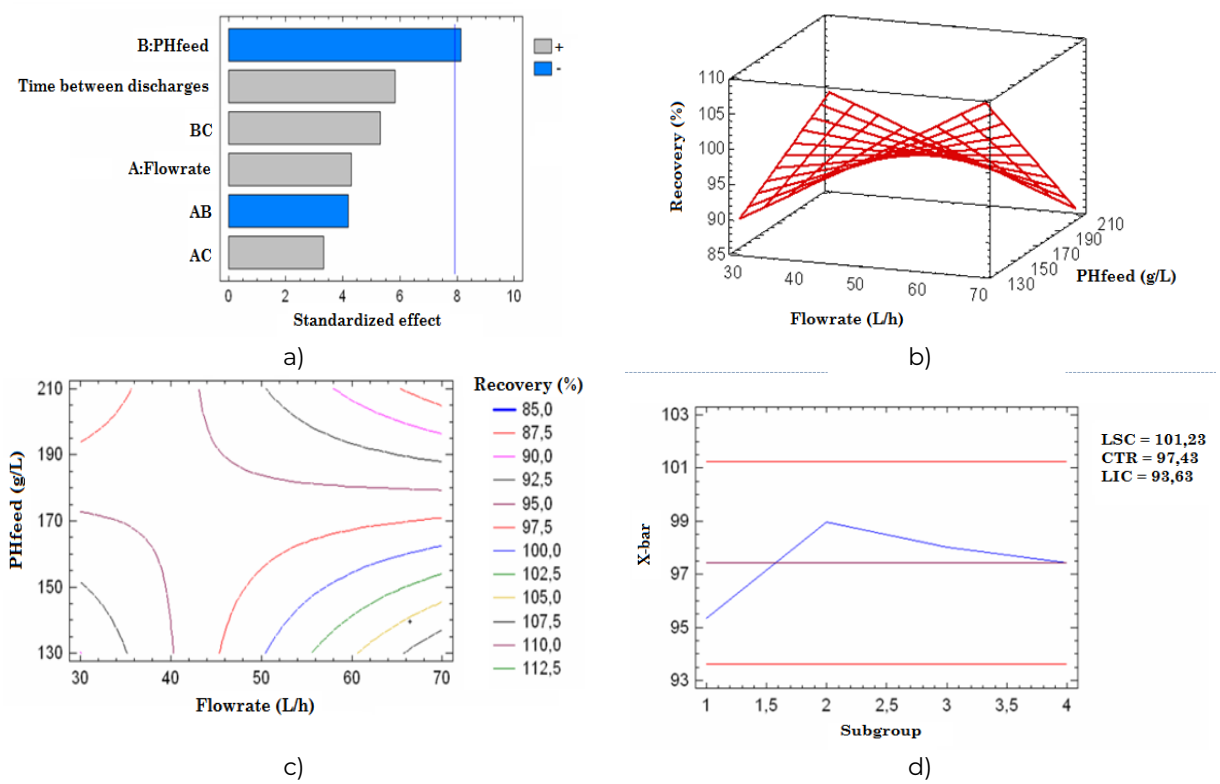


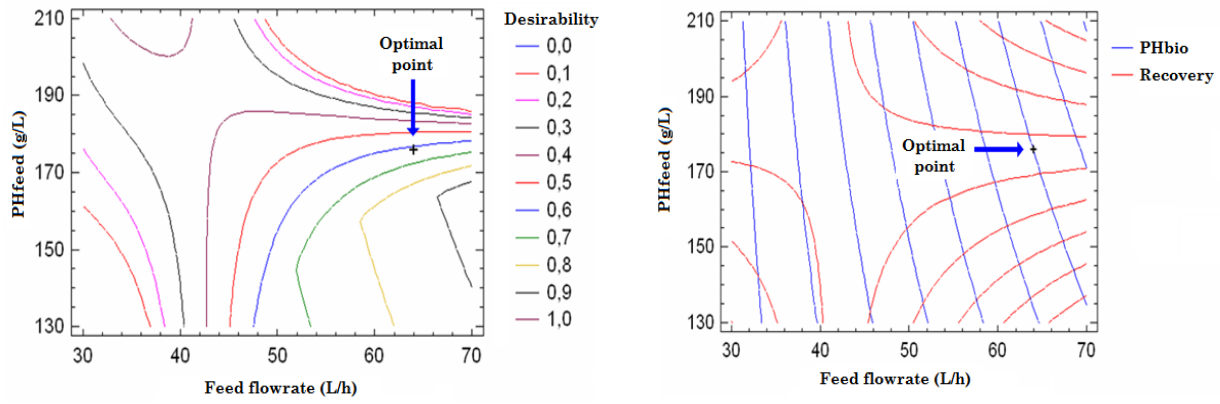
Figure 14. Different Figure s obtained for the variable %Rec using the statistical software Statgraphics Centurion® XV.II. a) Standardized Pareto Diagram, b) Response Surface, c) Surface Contours, and d) Control Chart. Source: created by the authors.

3.5 Optimization of the results

The optimization of the data obtained was carried out in order to find that combination of values of the input parameters that maximize the result to be obtained for the wet weight of the concentrated biomass (PH_{bio}) and the percentage of recovery (%Rec). For this, the "Multiple Response Optimization" option contained in the Statgraphics Centurion® XV.II statistical package was used. In relation to the above, it was found that, to maximize the results of %Rec (up to 95 %) and PH_{bio} (up to 600 g/L), the main input parameters of the process should present the following optimal values (optimal point):

- Wet weight of the feed culture (PH_{feed}): 176.0 g/L.
- Inlet flowrate of the feed culture (Q_{feed}): 63.9 L/h ≈ 64 L/h.
- Time between discharges (t_{dis}): 4.997 min. ≈ 5 min.

Figures 15a and 15b show the Estimated Response Surface Contour Plot and the Overlay Plot, respectively, which show the response surface of the optimized parameters, indicating the optimal point obtained.



a) b)

Figure 15. Contour plots. a) Estimated Response Surface, b) Overlay Plot.
Source: created by the authors

3.6 Results of the application of optimal operating conditions to the new scale of the production plant (1,000 L)

The volume of cell culture that will be obtained at the end of the fermentation stage for the new proposed plant with a higher industrial capacity, which is 1 000 L, must be diluted to obtain a wet weight value (PH_{feed}) of 176 g/L before being processed by the disk centrifuge, according to the optimal results obtained for this variable (see previous section). Taking into account that the final cell culture obtained in a fermentation of *Tsukamurella paurometabola* strain C-924 reaches an average of 350 g/L of biomass concentration (unpublished data), then the total volume of culture after being diluted to 176 g/L prior to centrifugation will be calculated by (6):

$$V_{feed} = \frac{PH_{cult} \cdot V_{cult}}{PH_{feed}} = \frac{350 \cdot 1\,000}{176} = 1\,988\ L \tag{6}$$

Where PH_{cult} is the wet weight of the cell culture at the end of fermentation (g/L), V_{cult} is the final volume of the cell culture at the end of fermentation (L), and V_{feed} is the volume of culture diluted to 176 g/L before centrifugation (L).

If this volume of diluted culture (1 988 L) is fed to the disk-stack centrifuge at a flow rate of 64 L/h, then the harvesting operation will last about 31 h, which is approximately half the time obtained if both tubular centrifuges are used (68 h), which increases the speed of the centrifugation process as well as the integrity, efficiency and quality of the harvested concentrated biomass. However, as previously verified, if the values of the parameters concentration of the culture to be fed to the centrifuge (PH_{feed} = 176 g/L) and the desired biomass concentration at the centrifuge outlet (PH_{bio} = 600 g/L) are kept constant, the feed flow rate (Q_{feed}) can be increased if the time between discharges (t_{dis}) is sufficiently reduced. Thus, if Q_{feed} is increased to 75 L/h, the total duration of the process will be approximately 27 h, while if the flow rate reaches 100 L/h, the process would last 19 h. With regard to these results, it is recommended to carry out additional experimental studies in order to determine a new set of values that the three main operating parameters considered should present, i.e. Q_{feed}, PH_{feed} and t_{dis}, with the objective of increasing the feed flow rate to a value close to 100 L/h, in order to shorten the duration of the centrifugation operation, all without affecting the desired biomass concentration (600 g/L) and the final recovery percentage (> 95 %). This would bring with it a greater benefit in terms of safety and control of the quality parameters of the desired final product (concentrated biomass), as well as with respect to the efficiency and robustness of the centrifugation operation itself.

4. CONCLUSIONS

The following conclusions can be drawn from the studies carried out. The input parameter that statistically has the greatest influence on the wet weight of concentrated biomass (PH_{bio}) value is the time between discharges (t_{dis}), while none of the input parameters considered has a statistically significant influence on the percentage recovery parameter (%Rec). By using the proposed semi-continuous disk centrifuge, PH_{bio} and %Rec values above the minimum required (600 g/L and 95 %, respectively) can be achieved for the harvest-recovery stage of the bionematicide HeberNem® according to the approved procedure, obtaining average PH_{bio} and %Rec values of 657.28 g/L and 97.43 %, respectively. Mathematical correlations were also obtained that describe the influence of the independent parameters PH_{feed}, Q_{feed} and t_{dis} on the response parameters PH_{bio} and %Rec. The optimum values obtained for the parameters PH_{feed}, Q_{feed} and t_{dis} were 176.0 g/L, 64 L/h and 5 min, respectively, which should be applied during the centrifugation operation in order to obtain a concentrated biomass with a PH_{bio} greater than 600 g/L and a recovery percentage greater than 95 %. If a disk-stack centrifuge is used to process 1.988 L of diluted cell culture per batch under a feed flow rate of 64 L/h, the harvesting operation would be reduced by 37 h compared to the use of tubular centrifuges to process a similar volume of cell culture. The disk-stack centrifuge evaluated can be satisfactorily used to harvest *Tsukamurella paurometabola* strain C-924 cells, the active ingredient of the HeberNem-S® product.

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CONFLICT OF INTEREST

The authors of the article proceed to declare that there is no conflict of interest, whether financial, professional, or personal.

AUTHOR CONTRIBUTIONS

Rafael Marcos Pimentel Pérez: Conceptualization, Proposal of the research idea, Review, Correction, and final approval of the manuscript.

Amaury Pérez Sánchez: Experimental runs, Writing the Introduction.

Jesús Zamora Sánchez: Support in the experimental runs, Writing the materials and methods.

Yovisleydis López Sáez: Experimental runs and support in the operation of the equipment.

Rutdali María Segura Silva: Generation of the graphics, Writing the results and discussion.

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