Article

Optimization of Technological Variables to Produce a Probiotic for Shrimp

Optimización de las variables tecnológicas en la producción de un probiótico para camarones

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ABSTRACT

Aim: To optimize the technological variables engaged in the manufacture of a probiotic for shrimp.

Methods: This research was based on experimental designs oriented to phenomenological mathematical modeling of the process to identify the main design variables. The product cost sheet was used to create the economic model that fits such variables. The objective-function was to minimize the unit cost of the product. Restrictions of design variables were established, and their optimal values were estimated through the use of multi-criteria computer optimizing tools.

Results: The initial sucrose concentration produced in the culture medium was 132 g/L, at a shaking speed of 1.67 s^{-1}, air flow equal to 0.025 L/L.h, and fermentation time 8.5 h, which minimized the unit cost of the product to 5.31 $/L of product. This value was quite below $ 31.50/L, which is the price of imported probiotic *Epicin*, used to breed shrimp larvae.

Conclusions: The sale of this product produces a revenue of $ 478 483.20/year to the Center of Genetic Engineering and Biotechnology of Camagüey, leading to savings of approximately $ 40 000 US yearly in savings for Yaguacam Basic Production Company, through import substitution. Additionally, its use has led to a reduction in antibiotic use, increased quality and quantity of post larvae, and an overall positive impact for the company.

Key words: cost sheet; mathematical-economic models; optimization; probiotics; import substitution.

RESUMEN

Objetivo: Optimizar las variables tecnológicas que inciden en el proceso de producción de un probiótico para camarones.

Métodos: Se emplearon diseños experimentales orientados a la modelación matemática fenomenológica del proceso para identificar las variables de diseño fundamentales. Se utilizó la ficha de costo del producto para confeccionar el modelo económico en función de estas variables. Se definió como función objetivo minimizar el costo unitario del producto. Se establecieron las restricciones de las variables de diseño y se determinaron sus valores óptimos mediante el empleo de herramientas computacionales de optimización multicriterio.

Resultados: Se obtuvo que una concentración inicial de sacarosa en el medio de cultivo igual a 132 g/L, una velocidad de agitación de 1,67 s^{-1}, un flujo de aire igual a 0,025 L/L.h y un tiempo de fermentación de 8,5 h minimizan el costo unitario del producto hasta 5,31 $/L de producto, valor muy inferior a los 31,50 $/L que es el precio del probiótico de importación *Epicin* que se emplea en la cría de larvas de camarones.

Conclusiones: La venta del producto genera una ganancia neta de 478 483,20 $/año para el Centro de Ingeniería Genética y Biotecnología de Camagüey, un ahorro de cerca de 40 000 USD anuales a la Unidad Empresarial de Base Yaguacam por concepto de sustitución de importaciones y su uso ha permitido la disminución del empleo de antibióticos, el incremento de la calidad y cantidad de las postlarvas y un impacto económico positivo en la entidad.

Palabras clave: ficha de costo; modelos económico-matemáticos; optimización; probióticos; sustitución de importaciones

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INTRODUCTION

Worldwide, aquaculture has undergone a significant increase in production, due to the growing interest in fresh water foods, and the increase in world population. As part of this industry, intensive shrimp culture is one of the sectors with the fastest evolution in many tropical countries. However, this development has faced animal disease issues, which appear as a consequence of indiscriminate use of chemicals and antibiotics to prevent mortality in larvae and juveniles, as well as the deterioration of the environmental conditions inside culture ponds (United Nations Organization for Food and Agriculture [FAO], 2018).

The above can be observed in the ever-increasing trend to ban and/or regulate the utilization of these substances, and replace them by more human-friendly practices, that protect the environment. Some of them include the use of probiotic microorganisms (FAO, 2018).

In Cuba, enormous efforts are being made to substitute the use of antibiotics in shrimp culture, with imports of several kinds of products, such as probiotics that improve survival in the ponds (Kumar *et al.*, 2015; Toledo, Castillo, Carrillo & Arenal, 2018). These products have a high cost in the international market; hence, creating a national alternative at a lower cost, with similar uses would bring economic benefits.

To achieve that, the Center of Genetic Engineering and Biotechnology, in Camagüey (CIGB), Cuba, isolated and identified bacterium *Bacillus licheniformis*, C-232, with antagonistic activity against chitinolitic strains which are pathogenic to shrimp *Litopenaeus vannamei*, the most widely known intensive cultured species. Accordingly, a fermentative technology was developed to produce a probiotic at a unit cost of $ 27.33 per L of the product.

This value is lower than the value of the imported probiotic (31.50 $/L), though the profit margin is very little, thus making the productive process unprofitable. Besides, it hinders massive introduction of the product in shrimp production at the competitive costs of the national market.

Consequently, the aim of this paper is to optimize the main technological variables that have an effect on the production process of a probiotic for shrimp. This is based on multi criteria economic-mathematical modeling, and computational techniques, in order to reduce the unit cost of the product, increase income, meet the national demands, and cut imports of a similar product at a higher cost.

DEVELOPMENT

The probiotic for shrimp consumption has been conceived to culture post-larvae of the species, as a microbial food supplement, and as a safe and natural alternative solution to the utilization of chemicals and antibiotics in the culturing ponds. In Cuba, there are two entities engaged in this, in Cienfuegos and Granma provinces. They are the potential clients which, by 2009, required 7 682 L of the probiotic (Arenal, 2009).

The technological development of the product was based on those demands, and the product would be manufactured at the productive development plant of CIGB-Camagüey, which has a 50 L fermenter.

However, unpublished preliminary studies done at the CIGB-Camagüey, as part of the project development, have demonstrated that its use is advantageous in other stages of intensive cultured shrimp, thus raising the national demands of the product.

The introduction of the process in an all-set plant would only require a study of production costs of the probiotic, following these steps:

1. Preliminary definition of the annual productive capacity.
2. Technological proposal of the process.
3. Definition of design or independent variables.
4. Development of the model for estimation of annual production costs, and unit cost.
5. Selection of objective function, and the restrictions of the design variables.
6. Implementation of optimum design.
   6.1. Implementation of optimization.
   6.2. Validation of the optimum design variables.
   6.3. Analysis of sensitivity toward optimization, and physical interpretation of optimization results.
   6.4. Analysis of the structure of production cost.

Such optimization will allow for the generation of values of the technological variables that cut donw the unit cost of the probiotic, to values way below the price of the similar imported product, which is $ 31.50/L.

1. Preliminary definition of the annual productive capacity

The pilot plant installed has a fermenting capacity of 50 L effective volume, which meets the probiotic demands identified in Cuba (7 682 L) (Arenal, 2009). Producing a daily batch for 300 days a year, including the necessary maintenance stops, possible breakdown, and batch validation, 15 000 L of the product can be produced. Considering 88% of success (percent of successful fermentations based on the historical occurring frequency of contaminations defined in the product cost sheet), the real productive capacity of the plant is 45 L, which guarantees 13 200 L of the product, a value higher than the predicted demand.

2. Technological proposal of the process.

The strategy suggested is discontinuous culture, which poses lower risk of contamination. Since the probiotic action of the product depends on cell viability, the cells should be in their stationary growth stage.

Basically, the technological proposal would be made of four stages: preparation of raw materials, fermentation, formulation, and filling.

3. Definition of design or independent variables.

The design variables were selected according to the results achieved in kinetic studies done to formulate the phenomenological model of the fermentation process under limiting conditions, without oxygen restriction.

These studies allowed for selection of the initial concentration of sucrose (S_0), as an important variable to consider in terms of technology optimization, which remarkably influences on the production cost, as well as fermentation time (t_f). It was also demonstrated that the speed of shaking (N), and the volumetric air flow (q_a) directly affect the production cost, since they have a remarkable influence on oxygen transference, and therefore, cellular growth.

4. Model design to estimate the annual cost of production and unit cost

The cost sheet of the product was based on a production technology to design an economical model. It was made considering the design variables selected, and it relied on the definition of unit cost of production (CU_P), as the annual cost of production (CP) divided by the annual liters of the product (P_A).

$$CU_P = \frac{CP}{P_A} \qquad (1)$$

The annual cost of production (CP was determined as the production cost of a batch (CP_l, multiplied by the amount of batches to be produced in a year (Nla). (ec. 2), and it was regarded as current time (t_{da}) divided by the sum of time of fermentation (t_f), and an auxiliary time between batches (t_a) to prepare the fermenter for a new process (ec. 3)

$$CP = CP_l \cdot Nla \qquad (2)$$

$$Nla = \frac{t_{da}}{t_a + t_f} \qquad (3)$$

According to the cost sheet of the probiotic (document with restricted use at CIGB-Camagüey), the items of the production cost analyzed were:

- Cost of raw materials and other materials (C_{RMM})

- Cost of auxiliary facilities (C_{AF})
- Cost of direct labor (C_{DL})
- Other direct expenses (C_{ODE})
- Cost of laboratory (C_L)
- Depreciation of direct tangible fix assets $(C_{DTFA}\ )$
- Cost of equipment maintenance or repair (C_{EMTR})
- Cost of productive services received (C_{PS})
- Indirect costs of production (I_{PC})
- General and administrative costs (A_C)

The production cost of a batch was defined as:

$$I_{CP} = D_{CP} + I_{CP} + C_A \qquad (4)$$

The direct costs of production were made of:

$$D_{CP} = C_{RMM} + C_{AF} + C_{DL} + C_{ODE} \qquad (5)$$

The cost of raw materials included the components of the culture medium, and those to prepare the inoculum, based on the optimum carbon-nitrogen ratio (7.5-4.0 g/L), determined experimentally (Rizo, González, Ramos, and Pérez, 2015).

The cost of the other raw materials was estimated from the consumption index set in the cost sheet and the purchase price of each, for a given production volume, and the quantity of batches produced in a year.

The cost of materials was integrated by the cost based on miscellaneous materials, such as filters, electrodes, hoses, etc., and the costs of the filling material for the final product, which included 1 L bottles and caps, as well as labels.

The cost of auxiliary facilities included the cost of water used to clean the main equipment, steam generation for sterilization of the culture medium, cold water to control the temperature of the fermenter and cooling after sterilization, compressed air for aeration, electric power for mechanical shaking, and general operation of the main equipment.

Three operators are required for proper plant functioning in 24-h shifts, to cope with fermenting stages, formulation, and filling. Besides, an employee is required for document management, as well as two laboratory assistants, and a head of the production plant. The mean salary of an employee at CIGB is $ 493.00. According to the new payroll suggested, considering the tax on social security (14%) and the tax on labor use (25%), the annual fix labor cost was $100 150.43/year.

The estimation of costs related to other direct expenditures was based on the sum of the laboratory expenses, depreciation of tangible fix assets, costs of maintenance and repair of equipment, and the cost of productive services, according to the equation:

The laboratory costs include the economic expenditures for supplies, materials, salaries of staff that conducts laboratory analyses needed for quality control of production, and process control. The analytical techniques used to release batches are concentration of viable cells, concentration of phytopathogenic pollutants, concentration of nonpathogenic pollutants, organoleptic characteristics, control of the volume in each container, and process control techniques to check culture media, and the purity of inocula.

The cost sheet of the product showed that TFAs are depreciated at a rate of $ 4.78/batch. In order to determine maintenance expenses and the cost of productive services received, consumption indexes previously approved at the CIGB-Camagüey for the production of the probiotic were used according to the cost sheet.

The estimation of indirect costs was performed according to the sum of indirect tangible fixed assets; the consumption of indirect auxiliary material; expenditures in maintenance and exploitation of indirect equipment; indirect expenditures in terms of salary and vacation; social security; and the tax on labor use. In this kind of facility, indirect expenditures are fixed expenses per batch produced, according to the index shown in the cost sheet.

For their part, the general and administrative expenditures were estimated according to a fixed index per batch that includes equipment maintenance and exploitation expenses; depreciation of tangible fixed assets; consumption of fuel, oils, and electric power; food; water expenses; salary and vacations; social security; and the tax on labor use.

The economic model developed according to cost entries analyzed, and the design variables chosen, are shown in Table 1.

Table 1. Economic model of the process

Production cost entry	Equation
Cost of raw materials and other materials	$C_{RMM} = \frac{6624 \cdot S_0 + 1051560}{4 + t_f}$
Cost of auxiliary facilities	$C_{AF} = \frac{330.96q_a \cdot t_f + 4036824 \cdot N^3 \cdot t_f + 144.72}{4 + t_f}$
Cost of direct labor use	$C_{DL} = 100150.43$
Other direct expenses	$C_{ODE} = \frac{440856}{4 + t_f}$
Indirect production costs	$I_{CP} = \frac{570240}{4 + t_f}$
General and administrative costs	$C_A = \frac{181440}{4 + t_f}$

Source: self-made

5. Selection of objective function, and restrictions of design variables.

The objective function related to obtaining a minimum unit cost to produce the probiotic subjected to structural restrictions, and no negativity in the design or independent variables: initial concentration of sucrose (S_0), fermentation time (t_f), shaking speed (N), and the volumetric flow of air (q_a).

Objective function:

$OF = \min(UC_P)$

Structural restrictions:

$0 < S_0 \leq 150\ g/L$

$1 \leq q_a \leq 1.67\ vvm$

No negativity restrictions:

$t_f > 0$

$N > 0$

The initial concentration of sucrose should be less than 150 g/L, since higher values inhibit growth and affect cellular viability (Larroche, Sanromán, Du, and Pandey, 2017; Voget and Todaro, 2014), and the volumetric air flow must be between 1 and 1.67 vvm (culture air volume per minute), because cavitation occurs in the shaker, along with the appearance of residence in the aqueous phase for a short time in the equipment, when the values are higher, which also affects cell growth (Larroche Sanromán, Du, and Pandey, 2017; Voget and Todaro, 2014).

Implementation of optimum design task

To perform optimization based on minimum production cost, the physical model of the process was used (Table 3), which describes its dynamic phenomenologically, according to different levels of design variables and the economic model (see Table 1), which identifies the main entries that affect the production cost of the probiotic, in terms of the initial sucrose concentration (S_0), fermentation time (t_f), shaking speed (N), and air volumetric flow (q_a).

Table 2. Physical model of the process

Definition	Equation
Maximum growth speed	$\mu_{max} = \mu_{opt} \cdot \frac{(T - T_{max})(T - T_{min})^2}{(T_{opt} - T_{min})[(T_{opt} - T_{min})(T - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T)]} - pH_{min})\{1 - exp(c_3(pH - pH_{max}))\}]^2$
Saturation constant	$K_S = -1.1355 - 0.1779.pH + 0.1006.T + 0.0364.pH^2 - 0.0076.pH.T - 7.4333.10^{-4}.T^2$
Constant of death	$K_d = -8.7814 + 0.9905.pH + 0.3150T - 0.0449pH^2 - 0.0102pH.T - 0.0033T^2$
Biomass-substrate yield	$Y_{X/S} = 8.0433 - 2.5067.pH + 0.0355T + 0.2048pH^2 - 0.0034pH.T + 1.170210^{-4}.T^2$
Constant of cell maintenance	$m_S = 11.414 - 3.8736.pH + 0.0788T + 0.4536pH^2 - 0.0615pH.T + 0.0046T^2$
Speed of live cell mass increase	$rX_v = \frac{dX_v}{dt_f} = \mu_{max} \frac{S}{K_S + S} \cdot \mu_{maxO} \frac{O}{K_O + O} \cdot X_v - K_d.X_v$
Speed of dead cell mass increase	$\frac{dX_d}{dt_f} = K_d.X_v$

Speed of substrate consumption	$\frac{dS}{dt_f} = -\left(\frac{\mu_{max}\frac{S}{K_S+S}.\mu_{maxO}\frac{O}{K_O+O}.X_v - K_d.X_v}{Y_{X/S}} + e_S.X_v + kp.P.S\right)$
Speed of product degradation	$\frac{dP}{dt_f} = -(\alpha.\mu_{max}\frac{S}{K_S+S}.\mu_{maxO}\frac{O}{K_O+O}.X_v - K_d.X_v + \beta.X_v)$
Speed of oxygen consumption	$\frac{dO}{dt_f} = k_La(O^* - O) - \left(\frac{\mu_{max}\frac{S}{K_S+S}.\mu_{maxO}\frac{O}{K_O+O}.X_v - K_d.X_v}{Y_{X/O}} + e_O.X\right)$
Volumetric coefficient of oxygen transference	$k_La = 63\,241.6\left(\frac{P_g}{V_L}\right)^{2.72}.\vartheta_s^{3.18}.\mu_a^{3.75}$
Apparent viscosity	$\mu_a = k_{c_0}0.4133.e^{0.0046\ S_0} + k_{C_1}.X$

Source: self-made

Previously set optimum temperature and pH values equal to 45 ºC and 6.5, respectively, were used.

The determination of kinetic values of the physical model was done by adjusting the experimental data to the model proposed. To achieve that, a model for direct search was applied, using optimization function *patternsearch*, under an optimum-accelerated approach through the generalized direct search algorithm based on the initial conditions of variables to be optimized, and the restrictions set (Koo Yeo, 2018; Singh, 2017). The starting point of parameter optimization were chosen according to the programmer's experience. The method of multiple startups was used, since there is no mathematic procedure to validate that the optimal condition achieved by multifactorial optimization is not a local end (Villaverde, Frohlich, Weindl, Hasenauer, and Banga, 2018). This method allowed for exploration of function convergence from multiple initial values of the independent variables, and validation of the optimum values achieved. The objective function was minimization of the sum of the square of normalized errors divided by zero and one, for each variable. Every calculation was performed through Matlab v.9.3 (The MathWorks Inc., 2017).

6.1. Implementation of optimization

The optimization function *patternsearch*, and the method of multiple startups were used for multifactorial optimization. The heuristic diagram that describes the procedure implemented for process optimization using *Matlab* v.9.3 (The MathWorks Inc., 2017) is shown in Fig. 1.

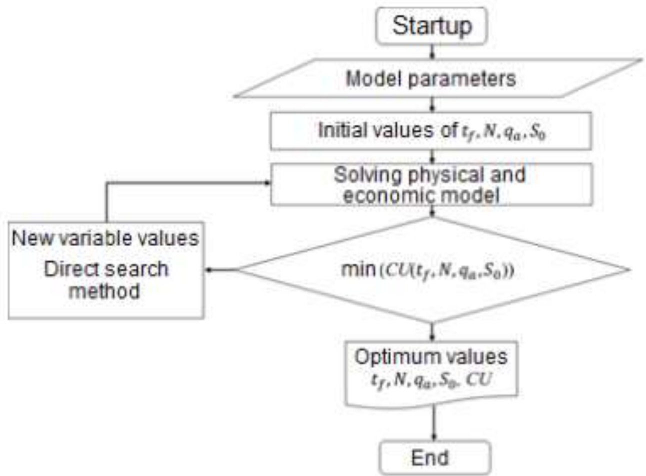

Fig.1. Heuristic diagram for the optimization process used

Source: self-made

In the optimum conditions, the unit cost is set in $ 5.31/L, which represents a net annual profit close to $ 478 483.20/year, and a 4.48-fold reduction of the current unit cost. This value is inferior to the purchasing price of imported product Epicin ($ 31.50/L), which is presently used in the post-larval culture of shrimp.

In these conditions of minimum unit cost, the minimum values of the design variables chosen are initial concentration of sucrose (132 g/L), shaking speed (1.67 s^{-1}), air flow (0.025 L/L.h), and fermentation time (8.5 h).

With these values, the concentration of live cells increases to 25 g/L in relation to the cellular densities achieved using the above mentioned-technology.

Although due to process optimization the initial concentration of the substrate increased from 75 g/L used in the current production process, to 132 g/L, the unit cost was 4.48-fold lower than its value. Hence, the positive economic effect for the producing company (CIGB-Camagüey) is evident.

6.2. Validation of optimum design variables

Three fermentations were performed under optimum conditions to validate the optimum parameter: temperature (45 ºC), pH (6.5), shaking speed (1.67 s^{-1}) (100 rpm), air flow (0.025 L/L.h) (1.5 vvm), fermentation time (8.5 h), and pressure (0.1 MPa) (1 bar). The culture medium contained 132 g sucrose and 70.4 yeast extract. The initial concentration of the biomass was 0.3 g/L.

Fig. 2 shows the fit of average dynamics to the process model of three fermentations, under optimum conditions in a 50 L bioreactor. As shown, there is a high level of fit between the experimental finding and the model, which provides satisfactory validation of the optimum parameters found in conditions of minimum unit cost of production.

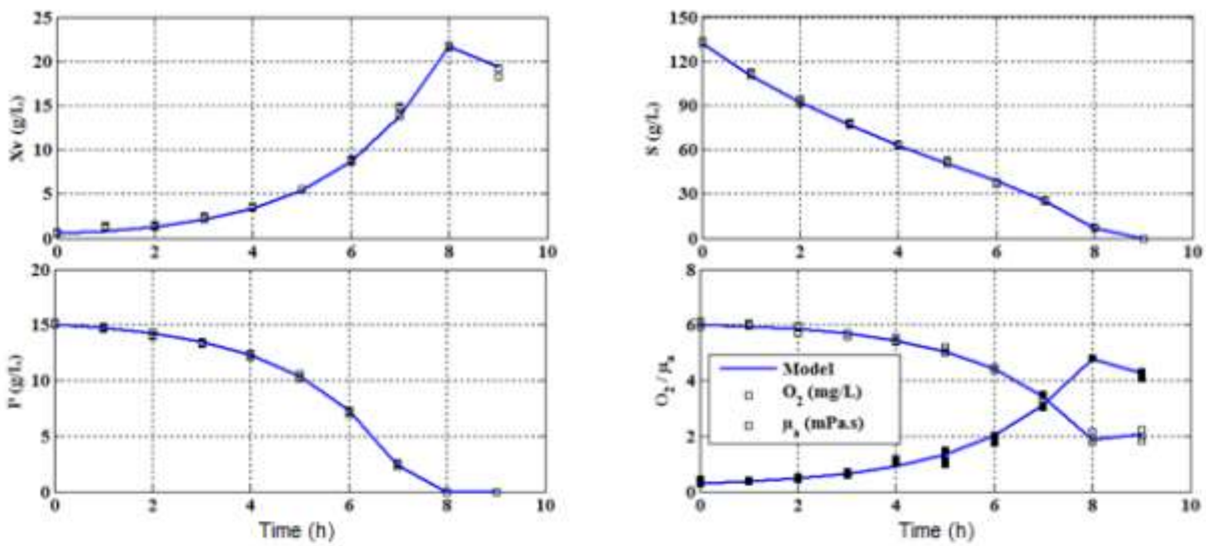

Fig.2. Validation of optimum operation parameters in a 50 L scale

Source: self-made

6.3. Sensitivity analysis within optimum conditions and physical interpretation of optimization results.

The analysis of sensitivity was performed with the evaluation of the objective function, modifying each variable in the –50%-+50% range, and keeping the other one fixed to the optimum level. The corresponding optimum level was assigned a zero percent deviation. Fig. 3 shows the sensitivity graph obtained.

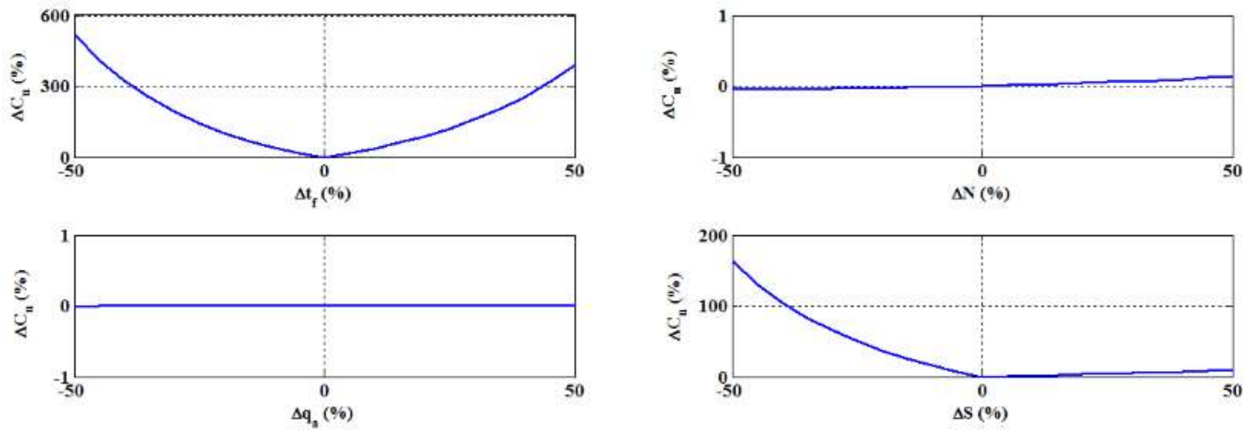

Fig.3. Sensitivity of the objective function around the optimum for each variable

Source: self-made

This figure shows that the unit cost is very sensitive in relation to fermentation time, at sucrose concentrations below the optimum levels. Increases of this variable of up to 50% do not have a significant effect on cost; however, it is not advisable, since growth inhibition may take place due to high substrate concentration. Unit cost is practically insensitive to shaking speed and air flow, which can be explained by the low contribution of these design variables to the total cost of production.

6.4. Analysis of the structure of production cost

In fermentation processes, the cost of raw materials generally accounts for 40-50% of the cost of production (Petrides, 2015); however, a detailed analysis of the structure of production costs (Fig. 4) shows that the cost of raw materials in this process only represents about 28% of the production cost, which is given by the simplicity of the medium composition. The high cost of direct labor use associated to production is significant in the analysis of cost structure. This may be caused by the fact that CIGB-Camagüey is part of the business system of BioCubaFarma, which, as a Higher Organization of Business System (OSDE), implements a payment system dependent of production. The entities that make up this Company manufacture product with high added value that generate large sums in terms of salaries, and profit distribution.

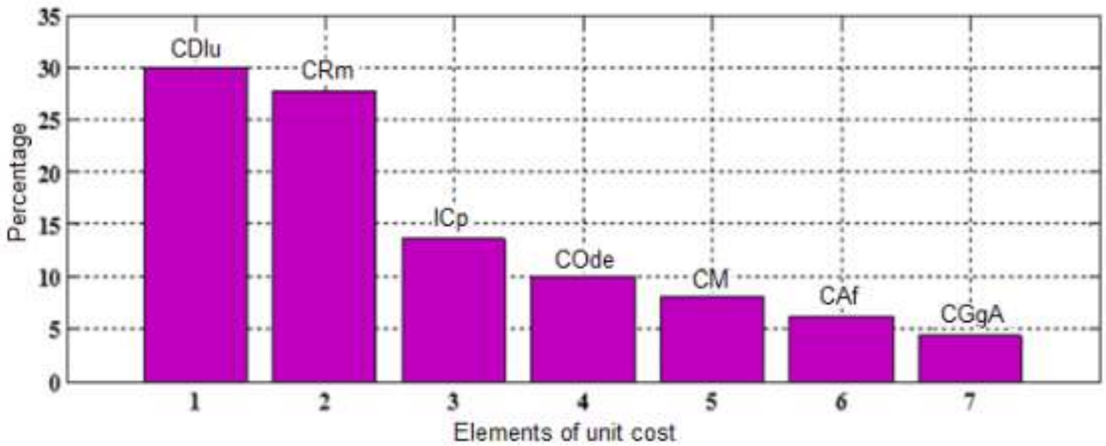

Fig.4. Influence of cost components on total production cost

Source: self-made

The Pareto diagram in Fig. 5 shows that with the costs of raw materials, yeast extract takes up almost 80% of the total cost. This suggests that any action taken toward reducing the purchasing cost of this product, would have a favorable effect on the unit cost of production of the probiotic.

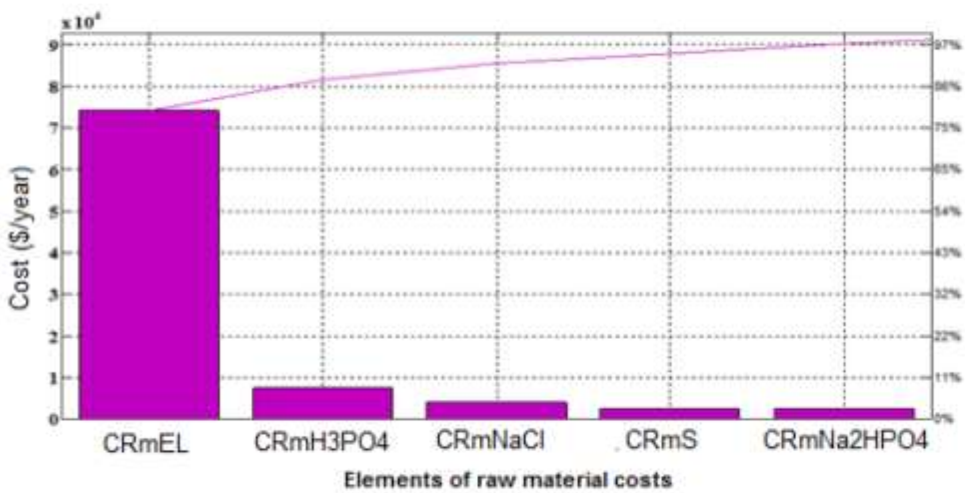

Fig.5. Pareto diagram to define the influence of cost components of raw materials

Source: self-made

Fig. 6 shows a unit cost sensitivity analysis in relation to variations around the current price of yeast extract. As shown, an increase of the price over 15% equals, and even surmounts, the value of the current unit cost.

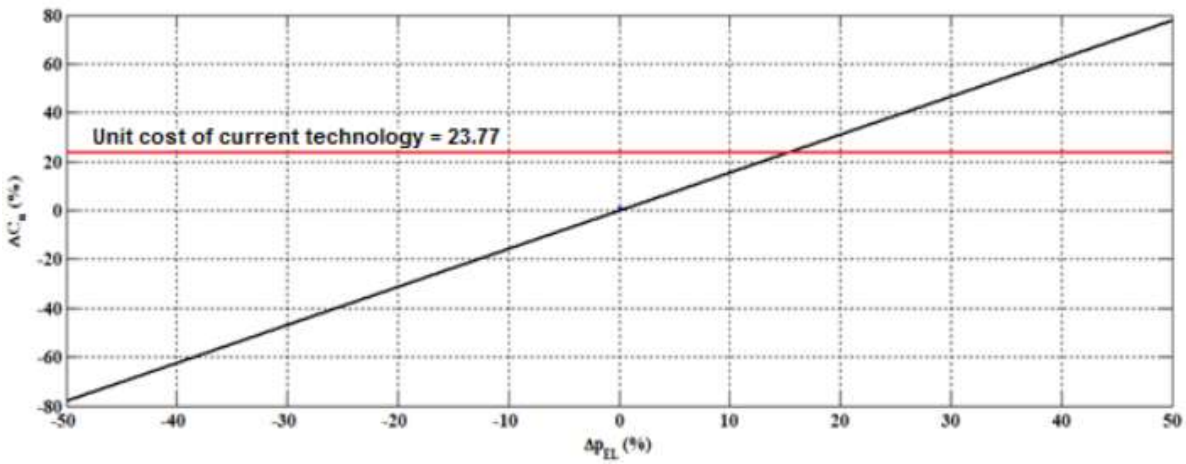

Fig.6. Sensitivity analysis of unit cost in relation to variations around the current price of yeast extract

Source: self-made

Social-economic impact on Cuban shrimp culture

Until 2009, the Company for shrimp culture (UEB Yaguacam), in the province of Cienfuegos, Cuba, applied an imported product named Epicin to handle the bacterial burden of pathogens on larvae, the water of ponds, and to raise larval quality.

As a result of isolation and further studies done in relation to probiotic strain C-232 (*Bacillus licheniformis*), it was demonstrated that it keeps the bacterial burden of pathogens (*Vibrios spp*. and *Pseudomonas spp*.) lower than in the animals and water treated with Epicin, which increases larval survival, and contributes to import substitution. In turn, the animals treated with this strain showed higher larval quality than the one rehabilitated with the imported product, seen in the larger number of gill ramifications, weight, and size.

By 2009, these results allowed the CIGB-Camagüey to sell that company this probiotic for shrimp consumption, which has caused a positive impact to the shrimp rearing company, with more than $ 40 000 USD annual income, thanks to the substitution of Epicin.

Its use has enabled the reduction of antibiotic use, along with quality increases, and the number of post-larvae obtained, with an ensued increase in the company's income.

CONCLUSIONS

An initial concentration of sucrose equal to 132 g/L at a shaking speed of 1.67 s^{-1}, air flow of 0.025 L/L.h, and fermentation time of 8.5 h, are the main conditions for the production of a probiotic for shrimp, at a unit cost of $ 5.31/L of the product.

The optimum values of the design variables allow for production cost reductions, in relation to the previous process, in approximately $ 4.5.

As a result from technology optimization, and the introduction of probiotic production at the pilot plant installed, the profit of CIGB-Camagüey accounts for $ 478 483.20/year.

The production of this probiotic at CIGB-Camagüey, at a cost below the purchasing cost of a similar product abroad, offers UEB Yaguacam the possibility of saving import expenses currently reaching 40 000 USD annually, and reducing the cost of shrimp post-larvae production in Cuba.

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Conflicts of interest

All the authors of this paper declare that this manuscript is original, and therefore it has not been submitted to another journal. The authors are responsible for all the contents included, and certify the absence of plagiarism, interest or ethical conflicts among them, which exempts the journal from any ethical and/or legal commitment in that sense.

Author contribution statement

Mariela Rizo Porro: Main author, redaction of the original manuscript, correction suggested by reviewers, in charge of the theoretical reference frame of the study, development of mathematical models, experimental design and development, implementation and analysis of computing optimization, article design, and drafting of conclusions.

Nemecio González Fernández: Review of the theoretical reference frame, advisory in experimental design, and laboratory work, advisory in the analysis of data, information gathering, and review and analysis of the results and conclusions.

Luis Beltrán Ramos Sánchez: Review and translation into English of the abstract, review of the theoretical reference frame, advisory in the analysis of data, development of mathematical models, computing programming of optimization, and analysis review of results and conclusions.