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Large Scale Simulation Of Cyanovirin Production From Conventional And Biotechnological Techniques

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Abstract. This paper's primary purpose is to determine the best cyanovirin-N n large-scale technique for production based on its pre-feasibility reached using the traditional method (applying recombinant soybean) and the biotechnological method (employing Nostoc ellipsosporum cultures in bioreactors). Cyanovirin-N is a lectin capable of inhibiting HIV type 1 and 2 infections. Hence the importance of optimizing production and improving cost-effectiveness for its use as immediate treatment in HIV patients is crucial for further researches. In various studies, it is confirmed that the problem lies in the low production of cyanovirin at the level of Nostoc ellipsosporum. However, this method is essential to find a solution since the operating conditions can be controlled and not dependent on environmental factors as in traditional processes. The results obtained using SuperPro Designer® software indicate that the soybean produces a greater quantity of crystallized cyanovirin-N (744.48kg/h), but it is not profitable due to the annual operating cost (USD) $ 458,892,000. The latter is due to its comparison to the results using Nostoc ellipsosporum. This latter reveals lower cyanovirin-N crystals (0.36kg/h). However, the annual operating cost significantly decreases to (USD) $ 24,236,000, almost 85% less than the cost compared to the traditional method. It should be noted that the culture medium used for Nostoc ellipsosporum offers better conditions for protein synthesis and opens the way to future studies that standardize profitable methods in the production of this lectin.

Keywords. Cyanovirin; Simulation; Large scale; Bioreactor.

Introduction

Cyanovirin-N is a protein substance produced by Nostoc ellipsosporum and can irreversibly inhibit membrane fusion in HIV-1,2 infection. Its recombinant production is being carried out, and a topical formulation for its use in humans is under development (Wilson et al., 2020). In addition, scientists in Brazil modified soybeans with the Nostoc ellipsosporum gene to obtain higher protein production. Also, a study (Deepika et al., 2015) was performed to optimize the CV-N (cyanovirin-N) production extracted from the cyanobacterium Nostoc ellipsosporum using components such as PHA extract, glucose, Fe-EDTA, and micronutrients. Results showed that the optimization improves the production of the protein, as

demonstrated by the authors. Meaning above that the PHA extract is a significant factor in improving protein synthesis (Deepika et al., 2015).

In other studies, biologically active pure rCV-N was isolated from lyophilized soybean powder with a yield of 350 μg/g dry seed weight by a combination of aqueous extraction, ethanolic precipitation, and C-18 reverse phase chromatography. First, pure rCV-N was obtained from the ethanolic precipitate. However, after ethanolic precipitation (67% EtOH), the soluble fraction also contained rCV-N, and purification was more difficult as other contaminating proteins were co-purified with rCV-N in almost all fractions (O'Keefe et al., 2015). This research suggests that soybean is more profitable for producing protein, and its purification can be improved to reduce economic costs.

On the other hand, the cyanovirin production is not enough to guarantee the development of medicines, although the antiviral activity was known, it had not been possible to use it on a large scale in pharmaceutical products since the production of this protein in algae is minimal (Citler et al., 2008; Araque et al., 2020). In addition, various microorganisms were modified to achieve higher production but did not give good results. Therefore, cheap and efficient production systems are sought (Rybalko et al., 2008).

Likewise, it is confirmed that the problem lies in the cyanovirin production at the Nostoc ellipsosporum level due to the lack of the process of optimization at an industrial level, since low performance increases its costs, which is not feasible for use as a natural treatment in HIV patients (Qi et al., 2000). Other research highlights that patients spend about 300 thousand dollars annually on traditional treatments since the global market for biotechnological products drugs registers its trademark for a value of 149 billion dollars for the year 2010 (Shattock et al., 2000) being inaccessible in patients with low economic resources.

In order to solve the production problems mentioned above, the professional SuperPro Designer® simulator will be used in this research, which facilitates the modeling, design, and optimization of integrated processes in a wide range of industries such as pharmaceuticals, biotechnology, agrochemicals, food, waste treatment, and water purification, among others (Benitez et al., 2018).

Finally, the present work aims to perform simulations to obtain cyanovirin-n, taking into account parameters that affect the performance of obtaining the protein substance so that it is sufficient and, at the same time, profitable by reducing the process costs. Based on the latter, this paper's primary goal is to determine the best cyanovirin-N n large-scale technique for production based on its pre-feasibility reached using the traditional method (applying recombinant soybean) and the biotechnological method (employing Nostoc ellipsosporum cultures in bioreactors). The first simulation will be carried out with Nostoc ellipsosporum and the second with modified soybean (Glycine max) based on the advances obtained to optimize the simulations with Nostoc ellipsosporum better thus be able to identify which process can be more profitable (Caicedo et al., 2020). Unit operations will be used to extract the protein from the seed, and with the help of a specifically designed bioreactor, the development of the cyanobacteria will be optimized.

Materials and methods

The simulation was carried out using Nostoc ellipsosporum, a cyanobacterium capable of producing cyanovirin-N under optimal conditions.

The substrates used for the simulation of the optimization of the process with N. ellipsosporum for 2 liters consists of 2 g of glucose, 20 mL micronutrient solution with the following components: H_3BO_3, $MnCl_2$ *

$4H_2O$, $ZnSO_4$ * $7H_2O$, Na_2MoO_4 * $2H_2O$, and $CuSO_4$ * $5H_2O$, 100 ml of Fe-EDTA solution that was prepared by dissolving Na_2EDTA and $FeSO_4$ * $7H_2O$ and 20 ml of PHA extract that was extracted from P. vulgaris (red beans). Based on previous researches (Deepika et al., 2015;), [11], biomass to substrate yield (Yxs) is set up with a value of 0.74.

An inoculation train process was used to reach 20.000 L of production level. Then, a maceration was carried out and mixed with surfactant (distilled water) to obtain an aqueous solution of the red bean seed. Later the extract passed through a disk centrifuge, and finally, the PHA was obtained in the supernatant, which feeds the final bioreactor (Srivatsava et al., 2013).

Super Pro Designer® software was used for the simulations of obtaining CV-N with Nostoc ellipsosporum, using a 2-liter tank as inoculum. Subsequently, a stepped process was carried out until reaching a 20.000-liter tank, fed with PHA extract since the highest biomass production is expected.

Once the biomass of the 20,000-liter tank is obtained, the product is pumped to a centrifuge. After storing the product in a storage tank, a rotary mill is connected to break the cells. Finally, a centrifugation and storage process is repeated. First, the out-stream is subjected to ultrafiltration in order to separate the CV-N from the cellular debris. Then, a flash evaporator is used to remove the water. Also, a crystallizer is used to obtain cyanovirin-N crystals for its production into tablets. The simulations for obtaining cyanovirin-N using the seed were made by the unit operations already established, which is the same procedure to obtain soybean oil: milling, sieving, and solid-liquid extraction with ethanol were carried out after storing, cleaning, and dehulling the soybean to obtain crude oil (Caicedo et al., 2020; Srivatsava et al., 2013).

For the purification of rCV-N, a flash evaporator was used to separate the protein from the ethanol used in the previous operation. Then, crystals of pure rCV-N for its production into tablets are produced using a crystallizer.

Results and Discussions

This work's primary goal is to analyze the feasibility of obtaining Cyanovirin-N from the traditional method using recombinant soybean and the biotechnological method utilizing Nostoc ellipsosporum cultures in bioreactors. In addition, mass flow analysis on each unit operating is considered at starting point for the profitability analysis.

Operating conditions and culture media and biomass yields were set up according to bibliographic references. Figure 1 shows the specific process diagram set up in SuperPro Designer Software for large Cyanovirin-N production using a biotechnological technique applying Nostoc ellipsosporum cultures in bioreactors

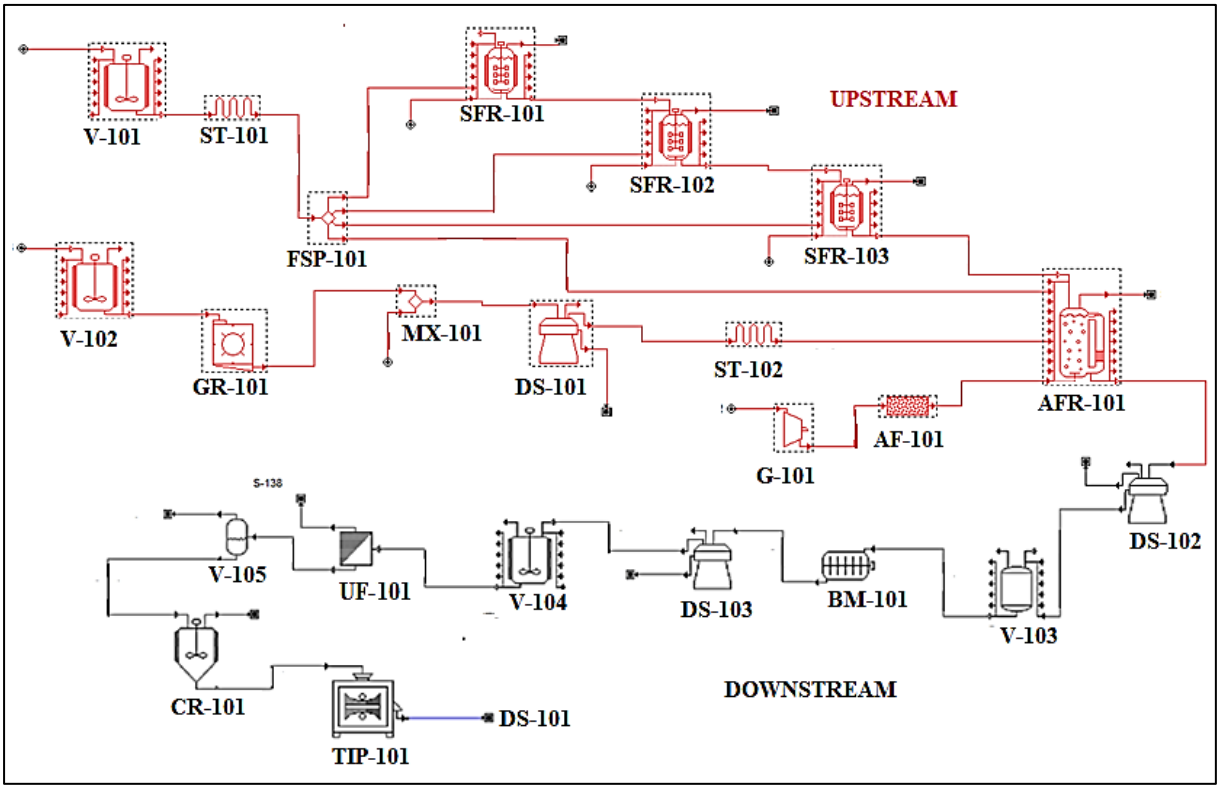

Figure 1. Industrial plant Scheme proposed for Cyanovirin-N production from Nostoc ellipsosporum cultures.

As shown in Figure 1, different equipment is set up for large-scale Cyanovirin-N production: V-101, V-102, V-103.V-104, and V-105 are mixing/storage tanks for different purposes. ST-101 is an installed heat sterilization system for removing contaminants before Nostoc ellipsosporum cultures. SFR-101, SFR-102, and SFR-103 account for seed fermenters. AFR-101 is the airlift bioreactor. DS-101, DS-102 and DS-103 are centrifuges. BM-101 is a bead milling for cell disruption, and MF1 and MF2 are microfiltration modules. Table 2 defines the general input and output balance of all the components used in operation, which is estimated to be 2351.12 kg/h, which in the year is 18620 t.

Table 1. Overall mass balance for Cyanovirin-N production from Nostoc ellipsosporum

COMPONENT	IN (kg/h)	OUT (kg/h)	OUT (kg/yr)
Biomass	0.00	0.21	1,628.93
Carb. Dioxide	0.00	11.42	90,417.51
cristales CVN	0.00	0.36	2,872.00
CuSO4	0.12	0.07	543.46
Ditritos	0.00	6.96	55,114.79
EDTA Disodium	6.00	3.49	27,648.00
Glucose	16.00	5.97	47,247.84
H_3BO_3	1.76	1.26	9,964.80
MnCl2	1.14	1.11	8,814.24
Na2MoO4	0.25	0.20	1,544.54
Sucrose	24.00	12.21	96,705.36
Water	1,960.80	1,965.81	15,569,240.40
Zinc Sulfate	0.14	0.01	99.36
TOTAL	2,351.12	2,351.12	18,620,849.83

According to Table 2, a summary of the fixed capital costs necessary to carry out the project can be observed, which projects a total value of USD 31,541,000. The latter is the required budget for the operation of the entire plant. It should be noted that the value of the direct physical costs is around USD 17,142,000.

Table 2. Fixed Capital Estimate Summary

Total Plant Direct Costs (TPDC)	
1. Equipment	5,201,000
2. Instalation	2,162,000
3. Pipe-line	1,820,000
4. Instrumentation	2,081,000
5. Insulation	156,000
6. Electrical	520,000
7. Building	2,341,000
8. Yard Improvements	780,000
9. Auxiliary Facilityes	2,081,000
TPDC	17,142,000
Total Indirect Plant Costs (TPIC)	
10. Engineering	4,286,000
11. Construction	6,000,000
TPIC	10,285,000
Total Plant Costs (TPC = TPDC + TPIC)	

TPC	27,427,000	
Contrators Fee and Contingency (CFC)		
12. Contrators Fee	1,371,000	
13. Contingency	2,743,000	
CFC = 12 + 13	4,114,000	
Direct fixed Capital (DFC = TPC + CFC)		31,541,000

Table 3 shows the main specifications and costs of each piece of equipment used in the simulation to obtain cyanovirin-N. It is estimated that a value of USD 5,201,000 should be required for the equipment.

Table 3. Equipment Costs for Cyanovirin-N production from Nostoc ellipsosporum

Name	Description	Cost (USD)
V-101	Blending Tank; Vessel Volume = 2218,70 L	207,000
SFR-101	Seed Fermentor; Vessel Volume = 10,69 L	477,000
SFR-102	Seed Fermentor; Vessel Volume = 106,87 L	477,000
SFR-103	Seed Fermentor; Vessel Volume = 1068,66 L	629,000
AFR-101	Air-Lift Fermentor; Vessel Volume = 10741,42 L	115,000
AF-101	Air Filter; Rated Throughput = 46077,22 L/h	7,000
ST-101	Heat Sterilizer; Rated Throughput = 1996,83 L/h	288,000
G-101	Centrifugal Compressor; Compressor Power = 24,15 kW	70,000
DS-101	Disk-Stack Centrifuge; Throughput = 10,25 L/h	106,000
ST-102	Heat Sterilizer; Rated Throughput = 10,54 L/h	114,000
GR-101	Grinder; Rated Throughput = 0,20 kg/h	71,000
V-102	Blending Tank; Vessel Volume = 0,22 L	141,000
DS-102	Disk-Stack Centrifuge; Throughput = 2009,82 L/h	467,000
V-103	Receiver Tank; Vessel Volume = 56,07 L	49,000
BM-10	Bead Mill; Bead Volume = 11,21 L	59,000
DS-103	Disk-Stack Centrifuge; Throughput = 50,44 L/h	106,000
V-104	Blending Tank; Vessel Volume = 48,60 L	141,000
UF-101	Ultrafilter; Membrane Area = 1,75 m2	27,000
V-105	Flash Drum; Vessel Volume = 1,45 L	1,000
CR-101	Crystallizer; Vessel Volume = 0,83 L	394,000
TP-101	Tablet Press (Pharma); Discrete Throughput = 3626,61 entities/h	216,000
	Unlisted Equipment	1,040,000
	TOTAL	5,201,000

The total operating costs in an industrial scale process are discriminated according to raw materials, labor, process monitoring for quality control, waste disposal, and costs of services required or utilities (energy,

heating steam, and cooling water). The results are shown in Figure 2. Based on Figure 4, labor-dependent costs presents high operating costs due to their influence on 50 % regarding all operating costs for producing cyanovirin at a large scale using the biotechnical way. The latter considering also the increase in equipment maintenance, insurance policies, local taxes, and depreciation costs.

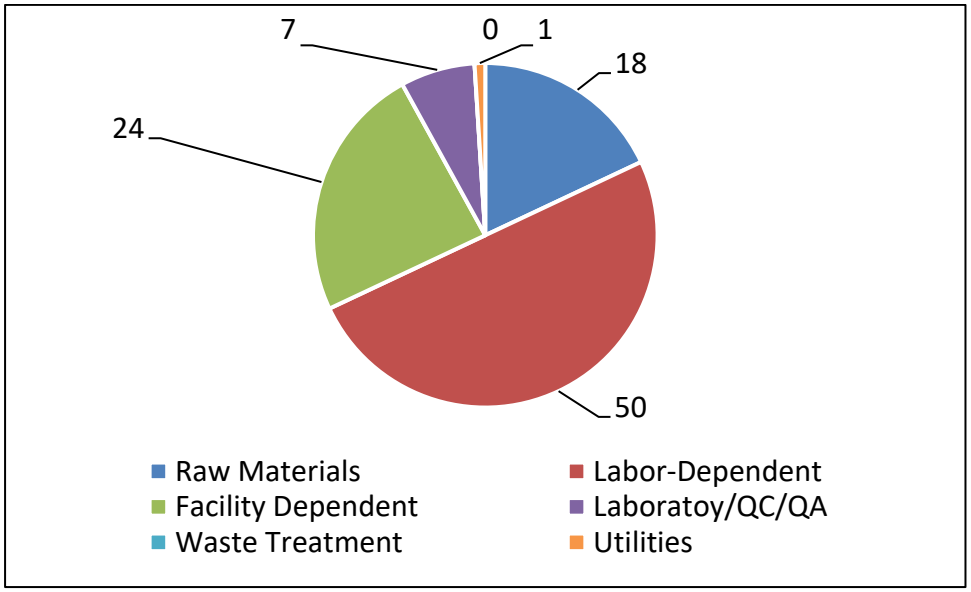

Figure 2. Annual Operating cost estimations for Cyanovirin-N large scale production using Nostoc ellipsosporum [%].

As shown in the previous Tables and Figure 2, total capital investment for this project of (USD) $ 34,617,000 and an operating cost of USD 24,236,000 per year is estimated. Based on the previous results, plant cell cultures in bioreactors offer great potential for producing secondary metabolites, with essential applications in the chemical, pharmaceutical, or food industries (Hernandez et al., 2020). For example, using the biotechnological method is an effective process for obtaining cyanovirin-N, a product of great importance to be applied as a medicine against HIV.

Motivated to compare the pre-feasibility of obtaining cyanovirin using recombinant soybean and the biotechnological method using Nostoc ellipsosporum cultures, Figure 3 shows the Cyanovirin production based on the conventional way explained before in the material section from soybean oil.

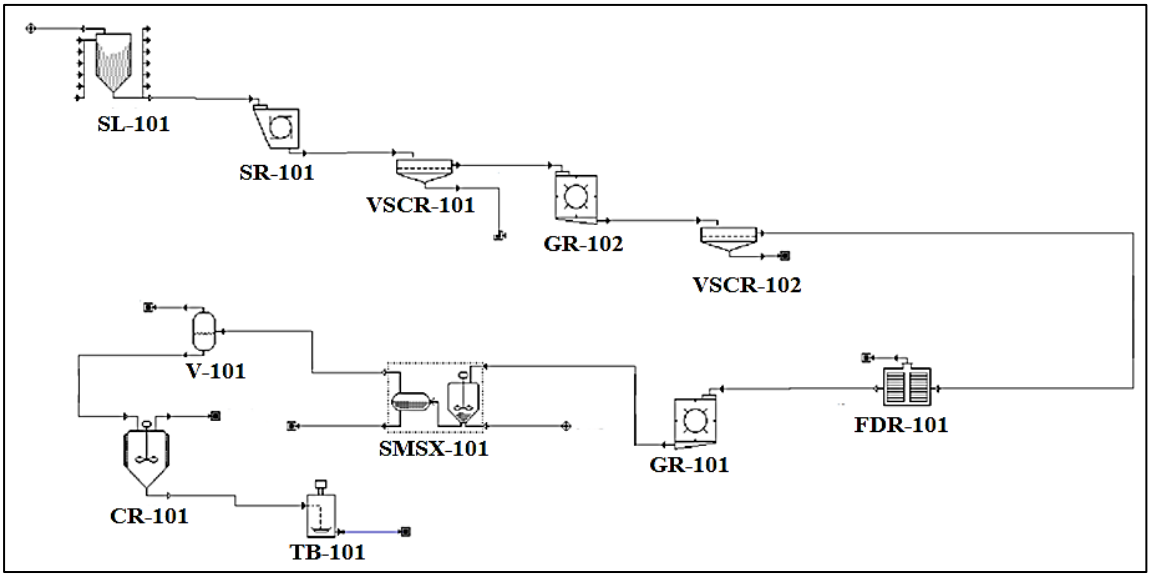

Figure 3. Industrial plant Scheme proposed for Cyanovirin-N production from soybean oil.

The process mainly focused on milling (FDR-101), sieving (GR-101) and solid-liquid extraction (SMSX-101) after storing (SL-101), cleaning (VSR-101), and dehulling (SR-101 and SR-102) the soybean to obtain crude oil (Caicedo et al., 2020).

The purification of rCV-N, a flash evaporator (V-101), is proposed. Finally, a crystallizer (CR-101) is installed to produce crystals of pure rCV-N.

The results obtained from the simulation in SuperPro Designer® using unit operations for the extraction of cyanovirin-N from the modified soybean are those presented in Figure 4.

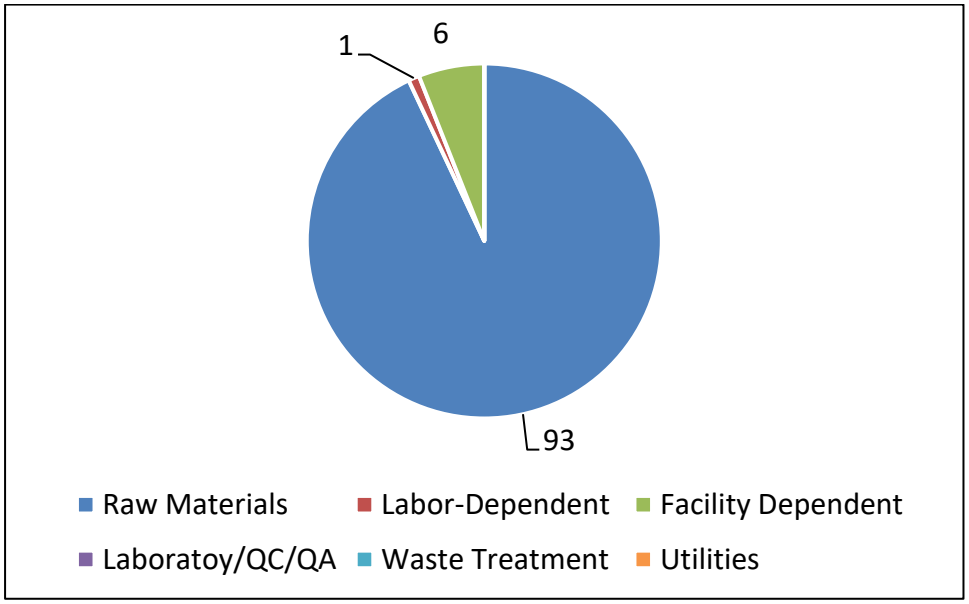

Figure 4. Annual Operating cost estimations for cyanovirin-N large-scale production using the conventional technique [%].

According to the data, it is estimated total capital investment of USD 184,491,000. Likewise, according to Figure 4, an operating cost of USD 458,892,000 per year is stipulated. Therefore, the results obtained using SuperPro Designer® software indicate that the soybean produces a greater quantity of crystallized cyanovirin-N (744.48kg/h), but it is not profitable due to the annual operating cost mentioned before.
The simulations were carried out to compare two different processes that serve as viable alternatives for the production of cyanovirin on an industrial scale. It is shown that the process with cell cultures allows better control of operating conditions and, in turn, the quality of production, unlike the traditional process with recombinant soybean, which requires more labor, this increases the probability of contamination and costs in lectin purification.
Based on revenue on biotechnological industry received last 2020 ($ 10 billion per year), it is evidenced that this type of process should be implemented. Due to the commercial value of 0.1 mg of recombinant cyanovirin equivalent to about $ 610 to $ 1,230.00 (USD), novel bioprocessing technologies are required to be implemented to reduce its value and thus be able to use it as an effective and viable treatment to prevent HIV, reaching the pharmaceutical market at a lower cost.
It has been observed that pretreatment of HIV virions with CV-N reduces their infectivity without producing toxicity in host cells, a quality that makes it an important candidate as a topical vaginal microbicide (Pacheco et al., 2020; Ouafae et al., 2015).
Based on the above, it is essential to obtain more excellent CV-N production worldwide and depend on the countries that produce recombinant soybean seed and be able to produce on a larger scale with novelty methods for improving process conditions.

Conclusions

It was possible to compare the methods of obtaining cyanovirin at an industrial level, taking into account the traditional method and the biotechnological way. It was evidenced that the production of cyanovirin from Nostoc ellipsosporum is 2872 kg/year and has an estimated value per unit (100 mg) of 0.84 USD, unlike the production with modified soybean seed in which 589,628,200 kg/year is obtained and a value around 0.16 USD (subject to environmental conditions), observing a decrease in the prices compared to the current cost ranging from $ 610 to $ 1,230.00 (USD) for 0.1 mg of recombinant cyanovirin. Finally, pre-feasibility studies showed feasibility in both methods, but the biotechnological method has the advantage that it can be developed anywhere regardless of environmental conditions.

Conflicts of interest: All authors declare that there is no conflict of interest that could jeopardize the validity of the results presented.

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