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RECYCLING OF COMMERCIAL ENZYMES IN THE PRODUCTION OF SECOND GENERATION ETHANOL

RECICLADO DE ENZIMAS COMERCIALES EN LA PRODUCCIÓN DE ETANOL DE SEGUNDA GENERACIÓN

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ABSTRACT

The present work proposes a procedure to evaluate the economic impact of recycling cellulolytic enzymes in ethanolproduction from sugarcane bagasse process. Three scenarios of ethanol-producing plants of different capacities are set to make the theoretical calculations. The proposed procedure starts from the experimental results reported on the yield changes obtained at the laboratory level when the original enzyme is used and when it is recycled on one or two occasions. From the technological demands of enzymatic quality, some necessary mixtures are established so the levels of addition of original enzyme are evaluated for different levels of recycling of enzymes. The procedure then includes possible scenarios for recycling the enzymes one or several times and it establishes the economic impacts regarding reduction of raw materials. Since the process of recycling enzymes is planned for an industrial installation, economic estimates of the investment are made for a given capacity with and without recycling of enzymes. For the three capacities, the investment and production costs are estimated, as well as the investments required to be able to recycle the enzymes in the enzymatic hydrolysis stage. Recovery of investments is also considered and projected. The economic benefits of recycling enzymes increase as installed production capacity increases.

Key words: enzymes; economic impact; ethanol; recycling.

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RESUMEN

El presente trabajo propone un procedimiento para evaluar el impacto económico de reciclar enzimas celulolíticas en el proceso de producción de etanol a partir del bagazo de caña de azúcar. El procedimiento propuesto parte de los resultados experimentales reportados sobre los cambios de rendimientos obtenidos a nivel de laboratorio cuando se utiliza la enzima original y cundo se recicla en una o en dos oportunidades. Partiendo de las demandas tecnológicas de calidad enzimática se establecen mezclas necesarias para los requerimientos tecnológicos y con ello se evalúan los niveles de adición de enzima original para diferentes niveles de reciclado de las enzimas. El procedimiento incluye entonces escenarios posibles de recirculación de una o varias veces las enzimas y establece los impactos económicos en lo referente a ahorro de materias primas. Dado que el proceso de reciclado de enzimas se planea para una instalación industrial, se realizan estimados económicos de la inversión para una capacidad dada sin reciclado de enzimas y con reciclado de enzimas. Para las tres capacidades, se estiman los costos de inversión y producción, así como las inversiones necesarias para reciclar las enzimas en la etapa de hidrólisis enzimática. La recuperación de la inversión también se considera y proyecta. Los beneficios económicos del reciclado de las enzimas aumentan a medida que aumenta la capacidad de producción instalada.

Palabras clave: enzimas; impacto económico; etanol; reciclado.

1. INTRODUCTION

Most of the bagasse produced in the sugar industry is mostly used as a fuel to generate the required steam. The remaining is used as raw material for other purposes, among which its use for obtaining ethanol has become a possibility (Baudel et al., 2005) due to the need to find new sources of fermentable sugars to increase the use of installed capacities and even create new capacities for ethanol production (García et al., 2015). For every 100 t of sugar produced using a conventional model of cooked masses, 75.1 t of bagasse can be obtained (Gálvez, 2000), which shows the need to optimize its use.

With regard to the treatment, for the cellulose and hemicellulose to be hydrolyzed to soluble monomeric sugars, enzymatic hydrolysis is the best way to achieve an effective cost in the production of ethanol (Bhatia et al., 2012).

Enzymatic hydrolysis is clearly preferred from an environmental point of view. However, economic viability requires the development of active cellulases at high temperatures, low pH, with highly specific activity and resistant to glucose inhibition (Mesa et al., 2010). In addition, the structural differences between different cellulosic substrates influence the development of the enzymatic degradation process. The limiting step in the hydrolysis speed is the degradation of lignin, since it is a material very resistant to biodegradation; therefore, it affects the biodegradability of the material. The main products of cellulose hydrolysis are cellobiose and glucose, while hemicellulose produces pentoses, hexoses and uronic acids. Some of these byproducts present a great challenge for the chemical industry because they can be the raw material not only of ethanol but of several biodegradable compounds.

Recirculation is a potential alternative to reduce the cost of enzymes, using their relatively high stability and high affinity for cellulose (Mesa et al., 2016). The main difficulties to enzymatically hydrolyze lignocellulosic materials are related, on one hand, with the low specific activity of the enzymes currently available, and therefore with the need for a high consumption of them during the process, (Lynd et al., 2002).

Among the advantages, it is known that enzymes are not consumed in the reactions that they catalyze; therefore, they are potentially recyclable. Recycling can reduce costs significantly associated with the enzymatic process.

Currently there are strategies that allow the enzymes to be reused to reduce the cost of the raw material. In some articles it is found that, in order to reduce the cost of enzymes, the production efficacy of the enzyme, the activity and recirculation of cellulose enzymes to be used in subsequent hydrolysis (González, 2006) and the recovery of the recycled enzymesare assessed. However, there is limited efficiency in the recovery of enzymes after hydrolysis.

The proposed procedure is based on the need for the systematic evaluation of the impact strategy of the recirculation of enzymes in the process of obtaining ethanol from bagasse reported by Mesa et al., (2016). Based on the criterion that all technology has to be economically feasible, it is necessary to complement the technological analysis with the economic ones. With this goal, it was considered to carry out a technical-economic analysis on the impact of the enzymes recirculation.

2. MATERIALS AND METHODS

The proposed procedure for the technical - economic analysis with the goal of evaluating the impact of recycling enzymes in the production of ethanol using sugar cane bagasse as raw material for the production of ethanol, started from a casewhere considerations are as follows:

The bagasse is generated in a sugar factory that has an distillery of ethanol obtained from sugar syrup. The cost of transportation of the bagasse is assumed to be covered by the sale of sugar. The bagasse storage area is in wet piles (approximately 60% humidity), before being transported to the pre-treatment area (Mesa, 2010).

This first analysis was carried out under the conditions described in Mesa (2010) for the case of the application of two pretreatment stages to sugarcane bagasse and the configuration of enzymatic hydrolysis and fermentation separately. Enzymatic hydrolysis was carried out, as reported by Mesa et al., (2016) with an enzymatic load of cellulase of 10 FPU / g of pretreated substrate in dry base, 2.5% of surfactant based on dry fiber and 10% solids in the enzymatichydrolysis. The enzymatic hydrolysis was carried out for 24 hours as well as the alcoholic fermentation.

The glucose concentration values obtained for the pretreated substrate at 24 hours of enzymatic hydrolysis was 52.45 ± 0.25 g / L. Ethanol concentration obtained from the fermentation was 21.22 g / L corresponding to a yield of 79.35%. Under these conditions, 5.55 kg of bagasse would be needed to obtain 1 liter of ethanol, only considering the glucan fraction. For each ton of bagasse, 180.12 liters would be obtained, corresponding to 62.02% of the theoretical potential for this raw material from the glucan fraction. Albernas-Carvajal et al., (2015) present a chart based in math calculation for this process.

For the recirculation of enzymes, some considerations from the scientific literature stand out: recycling cellulase adsorbed to the hydrolysis residue present in the suspension by adsorption and recycling of cellulase desorbed from the hydrolysis residue present in the suspension (Benkun et al., 2011); recycling of cellulase adsorbed to the hydrolysis residue through absorption on fresh substrates present in the suspension through ultrafiltration (Maobing et al., 2009). But the one used for this case was that of Barriga, 2001 in histhesis, and illustrated below in Figure 1.

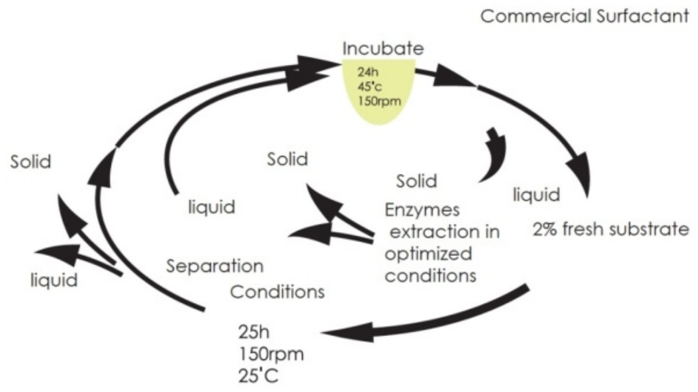

Figure 1. Recirculation strategy in the enzymatic hydrolysis stage

The impact analysis of the enzymes recycling was made based on the previous case, considering the enzymatic recirculation according to the results referred to by Mesa et al (2016). Figure 2shows the process diagram considering enzymatic recirculation.

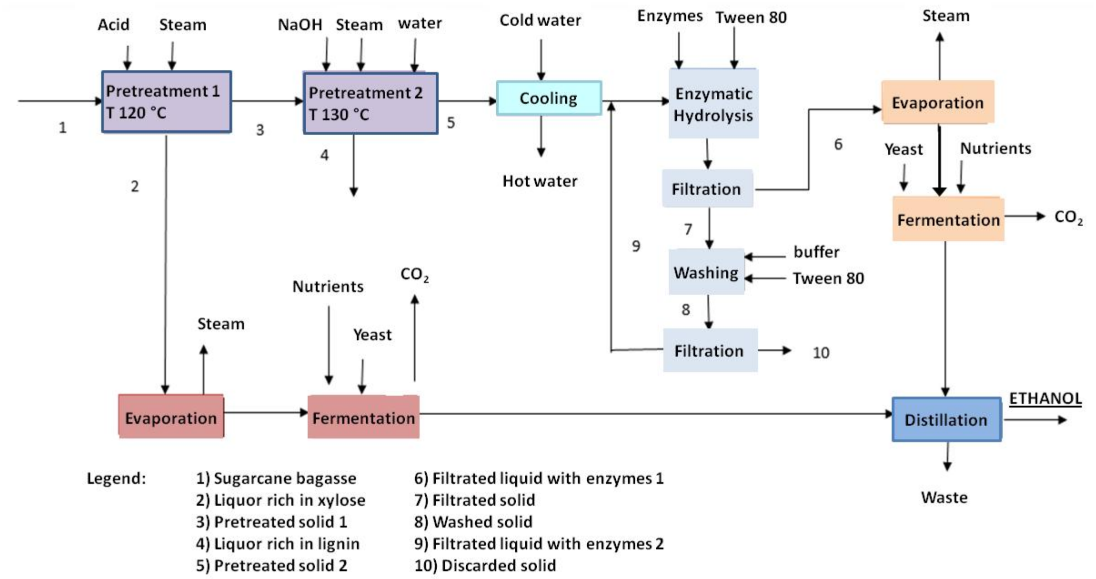

Figure 2. Technological diagram of the ethanol production process from bagasse with recirculation of cellulolytic enzymes

The proposed procedure stems from the experimental results reported on the yield changes obtained at the laboratory level when the original enzyme is used and when it is recycled once or twice.

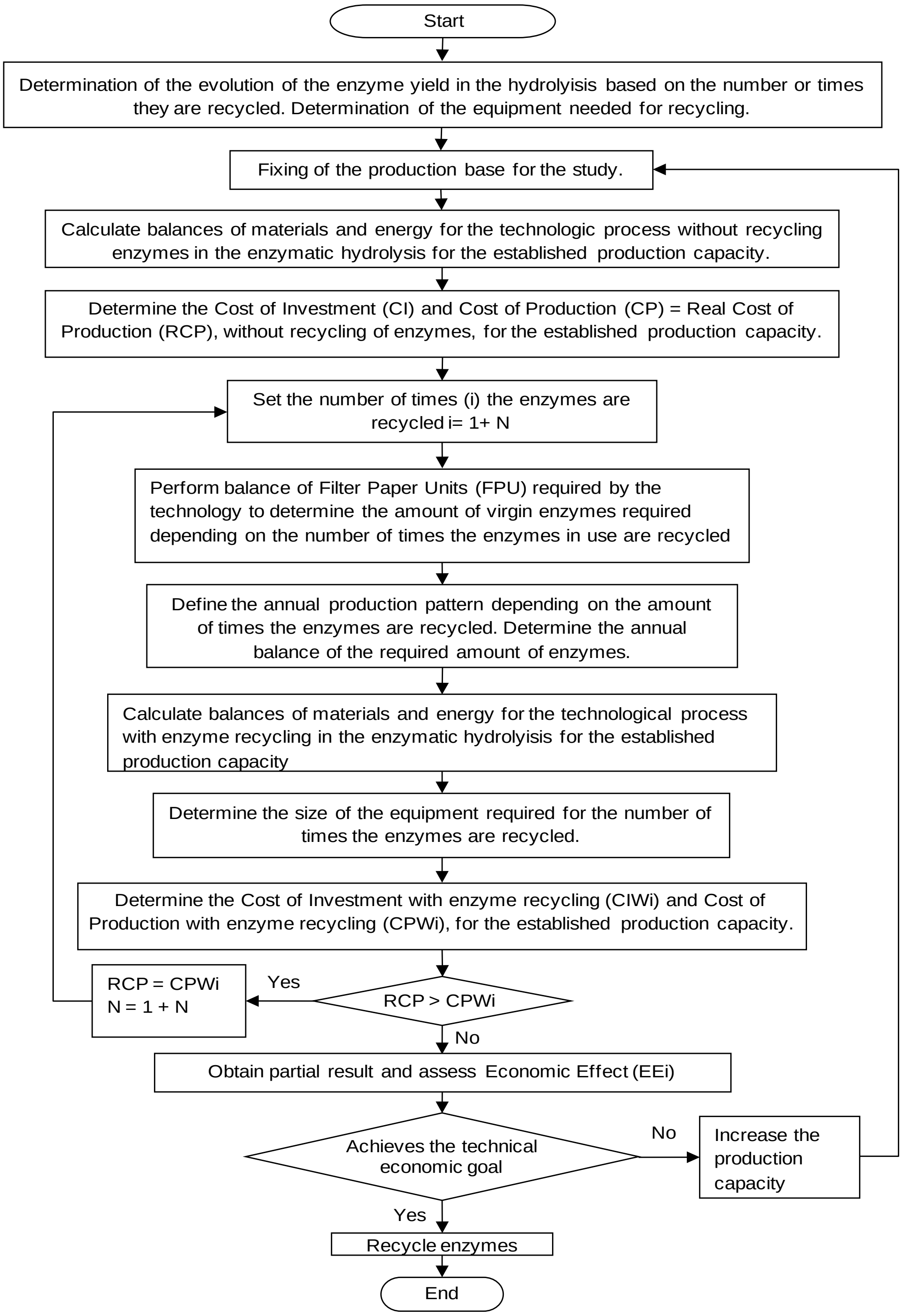

Figure 3. Heuristic diagram to evaluate the economic effect of enzyme recycling

From the technological demands of enzymatic quality, some necessary mixtures are established for the technological requirements and with that the levels of addition of original enzyme are evaluated for different levels of recycling of enzymes. The procedure then includes possible scenarios for recycling the enzymes one or several times and it establishes the economic impacts regarding reduction of raw materials. Since the process of recycling enzymes is planned for an industrial installation, economic estimates of the investment are made for a given capacity with and without recycling of enzymes. For this, starting from the material and energy balances, the investment and production costs are estimated, as well as the investments required to be able to recycle the enzymes in the enzymatic hydrolysis stage. The heuristic diagram of figure 3represents the proposed procedure.

Finally, economic technical analysis are carried out to evaluate the effectiveness of the enzyme recycling by measuring the recovery of investments required for this activity in industrial conditions.

3. RESULTS AND DISCUSSION

3.1. Determination of investment values

To calculate the costs of the equipment, real values of industrially installed equipment have been used indistinctly, updating the values through the annual cost indices and estimates of equipment of the scientific literature, which are also updated (Peters and Timmerhauss, 1991; Perry et al., 1958). In addition, they were estimated with the help of the Rule of Point 6 (Peters and Timmerhaus, 1991) and their adjustment to the year 2018 has been extrapolated, using the idea proposed by Aden et al., (2002), to predict the annual cost index for that year by adjusting the annual data since 1957 (González and Castro, 2012), as shown in Figure 4.

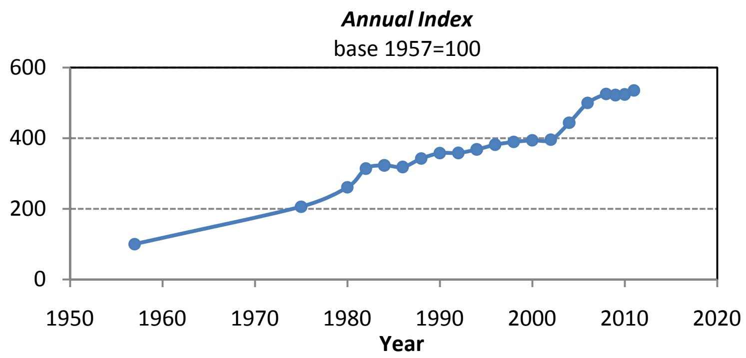

Figure 4. Chart of evolution of the Chemical Engineering Cost Annual Index 1975-2011 and forecast (González and Castro, 2012)

Table 1 shows a summary of investment components for each ethanol production capacity. This data was calculated taking Mesa's (2010) data as source.

Table 1. Summary of investment components for each ethanol production capacity without recycling enzymes

Installed Capacity for Ethanol Production (Hl/d)	*Cost of equipment acquisition, (USD)*	*Direct cost, (USD)*	*Indirect cost, (USD)*	*Invested Fixed Capital, (USD)*
500	1 355 227	3 068 233	1 077 580	4 145 813
1000	2 033 468	4 603 772	1 616 869	6 220 641
1500	2 317 457	5 246 723	1 842 677	7 089 400

3.2. Determination of production cost without enzyme recycling.

Using as a base for calculation the facilities when enzymes are not recycled, the obtained amounts are shown in Table 2 below.

Table 2. Production cost estimation without enzyme recycling for a plant of 500 Hl/d and an availability of 94% per year. Production: 7 046 400 l/y

Total Production Cost	*Price $/UM*	*Amount*	*Unit of Measurement (UM)*	*Cost USD/Year*
I. Manufacturing Expenses (A + B + C)	-	-	-	19 539 475
A:DIRECT COSTS	-	-	-	17 704 534
1. Raw materials	-	-	-	4 888 3
• Bagasse	0.007	78 3282	Kg/y	548 29
• Acid	0.9	783 282 85	Kg/y	704 95
• Ethanol	0.3	253 780 00	Kg/y	76 14
• Cellulose enzyme	2.236	1 328 682.11	106 FPU/y	2 970 93
• Ammonium Sulfate and	127 5	22 44	t/y	2 86
• Ammonium Phosphate	290 0	22 44	t/y	6 51
• Na0H	0 38	1 522 689 29	Kg/y	578 62
2. Operation work	10	-	-	784 575
3. Direct supervision 10% operation work	10 % of 2	-	-	784575
4.Utilities and services	-	-	-	115.67
• Steam	0 00673	327 260 43	Kg/y	22.02
• Water	0 0001	936 359 47	Kg/y	93.65
5. Maintenance and repairs % of FCI	5	-	-	207 290
6. Supplies % of 5	10	-	-	20729
7. Lab charges% of 2	10	-	-	78 450
8. Patents % TPC	1	-	-	78 450
B: FIXED CHARGES				472 620
1. Depreciation 10% FCI	10	-	-	414 580
2. Local Taxes 1-4% FCI	1	-	-	41 4580
3. Taxes 0.4-1 % FCI	0 4	-	-	16 580
C: INDIRECT COSTS 5-15% TPC	5	-	-	392 287.5
II. General Expenses (A + B + C)	-	-	-	706 120
A. Distribution and sales %	2	-	-	156 920
B. Management % TPC	2	-	-	156 920
C. Research and Development% TPC 5	5	-	900 815 34	392 290
D. Financial interests	1	-	-	41 450
III. Total Production Cost (TPC) (I + II)	-	-	-	7 845 750

Total Production Cost (TPC) = 0.27TPC + 10919.77 =USD 14 958 58

Cost per Liter (C/l)=14 967 25/(300 x 923.81)= USD 0.53/l

The same calculations in Table 2 were applied for two other projected plant capacities (1 000 Hl/d and 1 500 Hl/d) and the corresponding results obtained are shown in Table 3 below.

Table 3. Total production cost estimations without enzyme recycling for different plant capacities

Capacity (Hl/d)	500	1000	1500
Total Production Cost (USDx10^3/ year)	7 845 75	14 958 58	20 535 00
Unitary Cost (USD/l)	0.5567	0.53	0.47

3.3. Variation of production costs with enzymes recycling.

For this analysis, the enzyme balance proposed in the procedure was necessary.

3.3.1. Determination of FPU balances for enzymerecycling.

To analyze the economic impact of the possibilities of obtaining ethanol with enzyme recycling, the results obtained by Mesa et al., (2016) were considered.The raw materials are the same, except the amount of enzyme to be added that decreases as enzymes are recycled in the process.

According to the established parameters, 1 328 682 11 FPU/y are demanded, which for 300 days of annual production represent 4 428 94 106FPU/d. Therefore, for a production strategy operating first with fresh enzymes and then recycling, the following situations will occur:

3.3.2. Operation with fresh enzymes.

For the first operation, 4 428 94 106 FPU/d are needed, which for enzymes with 30 FPU/g causes A: 147 631 368 g/d or 147 631 39 kg/d to be required. The annual cost for the use of enzymes is then: USD 2 970 930/y, that is, USD 9 9031/d.

3.3.3. Operation with enzymes recycled one time.

In the second operation we will also need: 4428 94FPU/d that will be contributed from the recycled enzyme with a lower yield equivalent to 048 (Mesa et al., 2016) and also fresh enzymes, while the yield of the fresh enzyme was 0 72, (Mesaet al., 2016) so the contribution for the FPU required would be then those contributed by the recycled enzymes and a necessary amount of fresh enzymes.Then, the balance for the required FPU/d will be:

$$4\,428\,94.106\,\frac{FPU}{d} = A\,\left(\frac{0.48}{0.72}\right).30\,\frac{FPU}{g} \qquad (1)$$

Where A: 147 631 368 g/d

We can clear B:

$$b = \frac{\left(4\,428\,94.106\,\frac{\mathrm{FPU}}{\mathrm{d}} - \mathrm{A}\left(\frac{0.48}{0.72}\right).30\,\frac{\mathrm{FPU}}{\mathrm{g}}\right)}{30\ \mathrm{FPU}\ /\ \mathrm{g}} \qquad (2)$$

$$\mathrm{b} = \frac{4\,428\,940\,000\,\frac{\mathrm{FPU}}{\mathrm{d}} - \ 147\,631\,368\,\frac{\mathrm{g}}{\mathrm{d}}\left(\frac{0.48}{0.72}\right).30\,\frac{\mathrm{FPU}}{\mathrm{g}}}{30}\,\mathrm{FPU/g}$$

$$b = (4\,428\,940\,000\ -\ 2\,952\,627\,360)/30$$

$$b = (1\,476\,313\,333.33)/30\ \mathrm{g/d}\ =\ 49\,210\,421.33\ \mathrm{g/d}$$

Therefore, the costs of recycling enzymes one timedecrease in the recycling operation to: USD 3301.03/d, and in the two days of operation to 13,204.13 USD, which means that for 300 d/y (where the two operation conditions take place 150 times) the total expense is of USD 1980619.65/y, a reduction of USD 990 310.35/y in the expense in enzymes without recycling.This directly translatesinto the TPC, allowing it to be estimated when the enzymes are recycled once in:

$$\text{TPC} = \text{USD } 7\ 845\ 750.00 - 990\ 310 = \text{USD } 6\ 855\ 440,$$

for a Cost of USD 0.4864 / l

3.3.4. Operation with enzymes recycled up to two times:

In a third operation, a second recycling of enzymes will take place in an amount A with a yield of 0.271 (Mesa et al., 2016), and a first recycling of enzymes in an amount B, being then the balance of FPU as follows:

$$4\ 428\ 94.106\,\frac{\text{FPU}}{\text{d}} = \text{A}\left(\frac{0.271}{0.72}\right).30\ \frac{\text{FPU}}{g} + \text{B}.\left(\frac{0.48}{0.72}\right)30\,\frac{\text{FPU}}{\text{g}} + \text{C}.30\,\frac{\text{FPU}}{\text{g}} \qquad (3)$$

ForA: $A = 147\ 631\ 368\ \frac{\text{g}}{\text{d}}$

And B: $\text{B} = 49\ 210\ 421.33\,\frac{\text{g}}{\text{d}}$

We can clear C:

$$C = \frac{4\ 428\ 94.106\,\frac{\text{FPU}}{\text{d}} - \text{A}\left(\frac{0.271}{0.72}\right).30\frac{\text{FPU}}{\text{g}} - \text{B}.\left(\frac{0.48}{0.72}\right)30\frac{\text{FPU}}{\text{g}}}{30\,\frac{\text{FPU}}{\text{g}}} \qquad (4)$$

$$C = \frac{4\ 428\ 94.106 - 147\ 631\ 368\ (0.3764)30 - 49\ 210\ 421.33\ (0.667)30}{30}$$

$$C = \frac{4\ 428\ 940\ 000 - 1\ 667\ 053\ 407.46 - 984\ 700\ 530.81}{30}$$

$$C = 59\ 239\ 535.39\ g/d$$

Which implies that the costs of enzymes, when recycled one more time, again decrease in the operation of the second recycling to: USD 3,973.78 and in the three days of operation to USD 17177.91, where the work conditions alternate between: a) without recycling;b) with a first recycling; and c) with a second recycling, which means that, for 300 d/y(where these combinations take place in a total of 100 times)the total expense is of USD 1717791.00, a reduction of USD 1253139/yfrom the expense in enzymes when there is no recycling. This, directly translated to the TPC, allows estimating it when the enzymes are recycled twice in:

$$TPC = USD\ 7\ 845\ 750 - USD\ 1\ 253\ 140 = USD\ 6\ 592\ 61$$

and for a Cost of USD 0.4678/l.

The balance is similar whether the recycling is applied once or twice. It makes possible to estimate the cost reduction for these two instances for facilities of greater capacities.

3.3.5. Estimated production cost with enzyme recycling for a plant of 1000 Hl / d (27714300 l/y)

Cost reduction through recyclingenzymesonce: USD 1947502

$$TPC = USD14\ 958\ 580 - USD1\ 947\ 500 = USD13\ 011\ 080$$

$$C/_l = \frac{TPC}{annualproduction} = \frac{USD13\ 011\ 080}{27\ 714\ 300} = USD\ 0.47/l(5)$$

Cost reduction through recycling enzymes twice: USD 2464370

$$TPC = USD14\ 958\ 580 - USD2\ 464\ 370 = USD12\ 494\ 210$$

$$C/_l = \frac{TPC}{annualproduction} = \frac{USD12\ 494\ 210}{27\ 714\ 300} = USD\ 0.45/l$$

3.3.6. Estimated production cost with enzyme recycling for a plant of 1500 Hl / d (4500000 l/y)

Cost reduction through recycling enzymes once: USD 3056773.69

$$TPC = USD\ 20\ 535\ 000 - USD\ 3\ 056\ 770 = USD\ 17\ 478\ 230$$

$$C/_l = \frac{TPC}{annual\ production} = \frac{USD\ 17\ 478\ 230}{43\ 500\ 000} = 0.4017\ USD/l(6)$$

Cost reduction through recycling enzymes twice: USD 3868042

$$TPC = USD\ 20\ 535\ 000 - USD\ 3\ 868\ 042 = USD\ 16\ 666\ 958$$

$$C/_l = \frac{TPC}{annual\ production} = \frac{USD\ 16\ 666\ 958}{43\ 500\ 000} = 0.383USD/l \qquad (7)$$

3.4. Necessary investments for enzyme recovery.

In contrast to the above, as recycling is increased, greater filtering capacities must be created for the recovery of enzymes.

For each of the production capacities, a balance of recycled enzymes is established, necessary to design the enzyme recycling facility. Table 4 summarizes it below:

Table 4. Cost reductions through recycling enzymes for each of the installed capacities

Production Capacity (Hl/d)	***Annual cost reduction due to enzyme recycling***
500	USD 990310.35
1000	USD 2464370.00
1500	USD 3868042.00

3.5. Design of the facilities for enzymatic recycling.

The equipment to be used in each facility according to the production capacity is selected from the commercial literature to estimate the investment costs. The different spaces, machines and supplies havebeen calculated according to the established references and the results are summarized in Table 5 as follows:

Table 5. Cost of equipmentacquisition

Capacity (Hl/d)	500	1000	1500
Total (USD)	689074.177	1308443.79	1926855.009

Investment necessary to implement the improvements in the ethanol-producing plant needs to be carefully calculated and justified. The production of ethanol with demonstrated reduction of costs merits the corresponding analysis of amounts invested, recovery times and projected benefits. Table 6 below shows the summarized amounts projected for investment. Said amounts are the result of calculations that included construction expenses, workforce salaries, maintenance, purchase and installation of equipment, etc. for the three different scenarios of production capacity.

Table 6. Calculation of the investments amounts for recycling of traditional bagasse enzymes

Concept	***Projected amount (USD)***		
	500 Hl/d	***1000 Hl/d***	***1500 Hl/d***
Fixed Capital Investment (FCI)	2466885.55	4684228.76	6898140.93
Working Capital (WC)	274098.39	520469.86	766460.10
Total Working Capital (TWC)	2740983.95	5204698.62	7664601.04

Calculations were made for the different amount of investments needed for the different capacities and the considered processes regarding not recycling, recycling once and recycling twice. Table 7 shows the summarized results.

Table 7. Investment amounts for enzyme recycling

Capacity (Hl/d)	***FCI (USD)***		
	Without recycling	***Recycling one time***	***Recycling two times***
500	0.00	809704.28	2466885.55
1000	0.00	1618853.088	4684228.76
1500	0.00	2424727.914	6898140.93

3.5 Design of the facility for enzymatic recycling

The equipment to be used in enzymatic recycling also needs to incorporate specific equipment, improvements and adaptations in the plant, as well as continuous expenses due to their operation. Table 8 below shows a summary of the mentioned calculated production costs in each installation according to the production capacity:

Table 8. Production costs when recycling enzymes for each of the installed capacities

Capacity (Hl/d)	***Year Costs (USD)***
500	5857.40
1000	10496.00
1500	15490.40

Table 9 below has a summary of the main economic indicators for investment recovery: Net Present Value o, which shows a positive net value; the Internal Rate of Return, which shows a high and attractive percentage; and the Payback Period, where the expected moment when the investment will be amortized comes at relatively near times in the future.

Table 9. Economic indicators for investment recovery

Indicator/Production	***500 Hl/d***	***1000 Hl/d***	***1500 Hl/d***
Net Present Value - NPV (USD)	2148797.75	6646880.46	11147785.25
Internal Rate of Return - IRR (%)	30	60	89
Payback Period (years)	6	3	2

4. CONCLUSIONS

1. After calculating the possible economic impact of recycling enzymes in the production of ethanol from sugarcane bagasse, based on the laboratory results of products obtained and enzyme cost reduction, it is feasible to apply this procedures in a real factory to reduce costs.
2. Recycling enzymes in the technological process of ethanol production from sugarcane bagasse has economic benefits by reducing costs of enzyme purchase, in different amounts depending on the installed capacity of the ethanol-producing plant; USD 990 310.35 for a 500 Hl/y plant, USD 2 464 370.00 for a 1 000 Hl/y plant, and USD 3 868 042.00 for a 1 500 Hl/y plant.
3. Given the calculated amounts stated above, it is clear that, the greater the installed capacity, the greater and more significant the cost reduction is. Considering the Total Production Costs for each case, and without taking into account the necessary amount to invest in the recycling infrastructure, the reduction percentage is 12.62% for a 500 Hl/y plant, 16.47% for a 1 000 Hl/y plant and 18.84% for a 1 500 Hl/y plant.
4. Said costs of investments to achieve recycling of enzymes are subject to projection by traditional methods, but it is necessary to refine the considered purchase of some equipment due to shortage of supply catalogs.
5. Recovery of the investmentneeded for the recycling of commercial enzymes in the production of ethanol is estimated at 6, 3 and 2 years for an installed capacity of 500, 1000 and 1500Hl respectively.

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