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Recycling of commercial enzymes in the production of second generation ethanol

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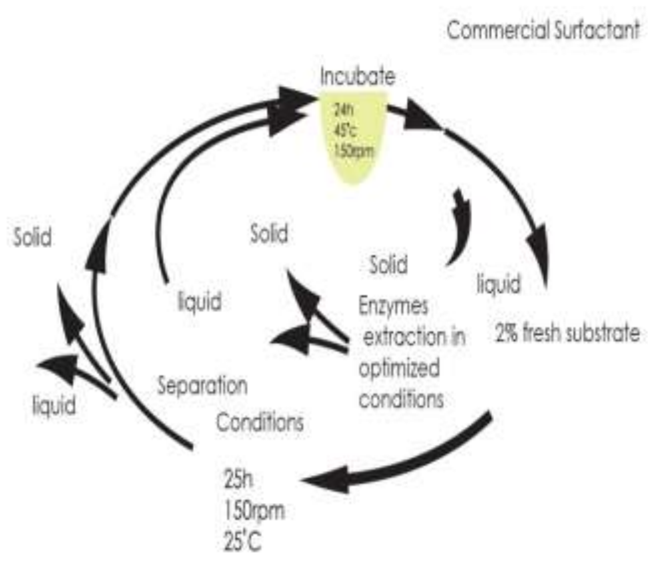
Graphical Abstract



Abstract.
The present work proposes a procedure to evaluate the economic impact of recycling cellulolytic enzymes in the process of ethanol production from sugarcane bagasse. The proposed procedure stems from the experimental results reported about the yield changes obtained at laboratory level when the cellulose from *Aspergillus niger*, Sigma is used when it is recycled in one or two times. 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 recirculating 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. 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. The economic benefits of recycling enzymes increase as installed production capacity increases.

Key words: enzymes; economic impact; ethanol; recycling

Introduction

Most of the bagasse produced in the sugar industry is used as a fuel to generate the required steam, the remaining is used as raw material for other purposes, within which its use for obtaining ethanol has become a possibility [1] 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 [2]. For every 100 t of sugar produced using a conventional model of cooked masses, 75.1 t of bagasse can be obtained [3], 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 [4].

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 [5]. 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 [6].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, [7]. 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 and the recovery of the recycled enzymes are 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) [6]. 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.

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 case where 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 [8].

This first analysis was carried out under the conditions described in Mesa (2010) [8] 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) [6] 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 enzymatic hydrolysis. 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. Figure 1 shows the process described above.

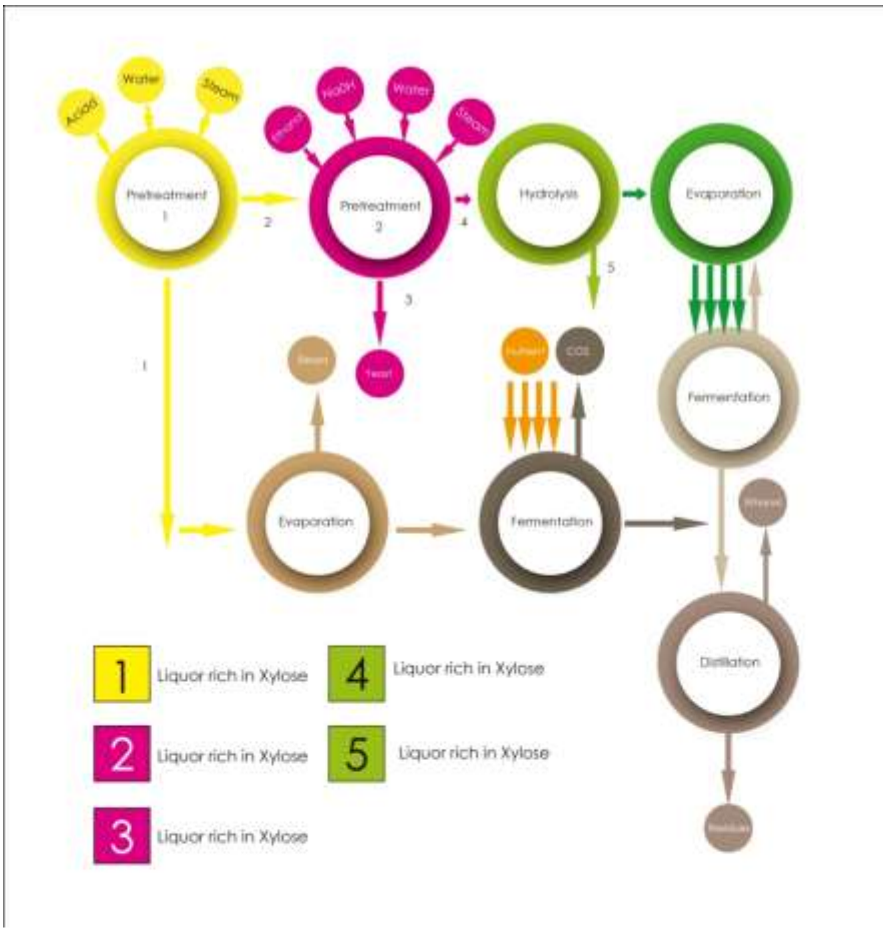


Figure 1. Technological diagram of the ethanol production process from sugarcane bagasse without recirculation of cellulolytic enzymes

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 [9]; recycling of cellulase adsorbed to the hydrolysis residue through absorption on fresh substrates present in the suspension through ultrafiltration [10]. But the one used for this case was that of Barriga in his 2011 thesis [11].

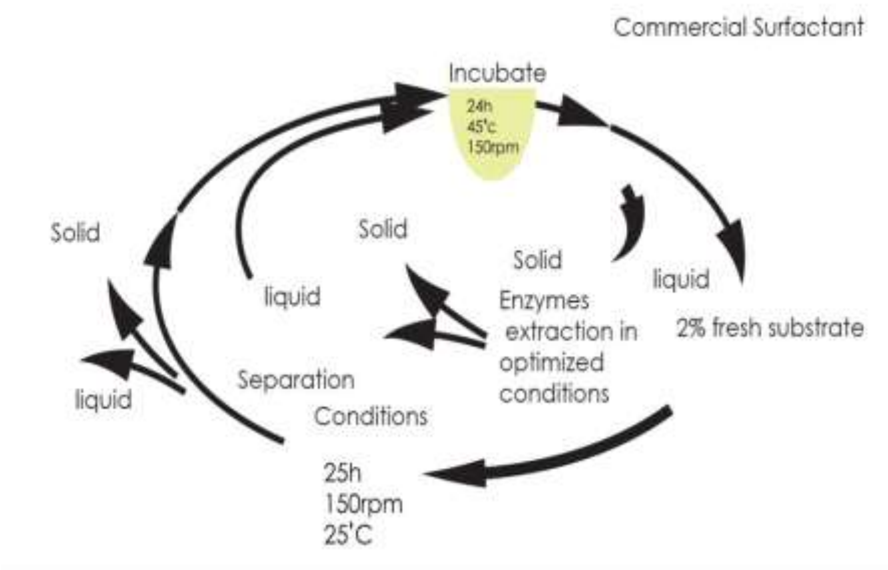


Figure 2. Recirculation strategy in the enzymatic hydrolysis stage.

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) [6]. Figure 3 shows the process diagram considering enzymatic recirculation.

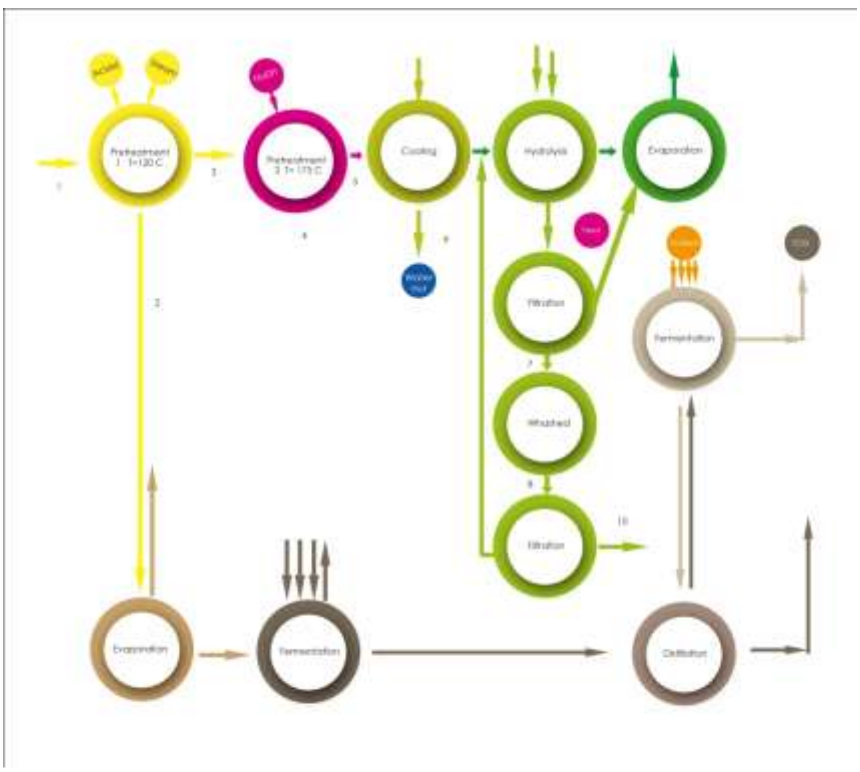


Figure 3. Technological diagram of the ethanol production process from bagasse with recirculation of cellulolytic enzymes

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.

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 recirculating 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 4 represents the proposed procedure.

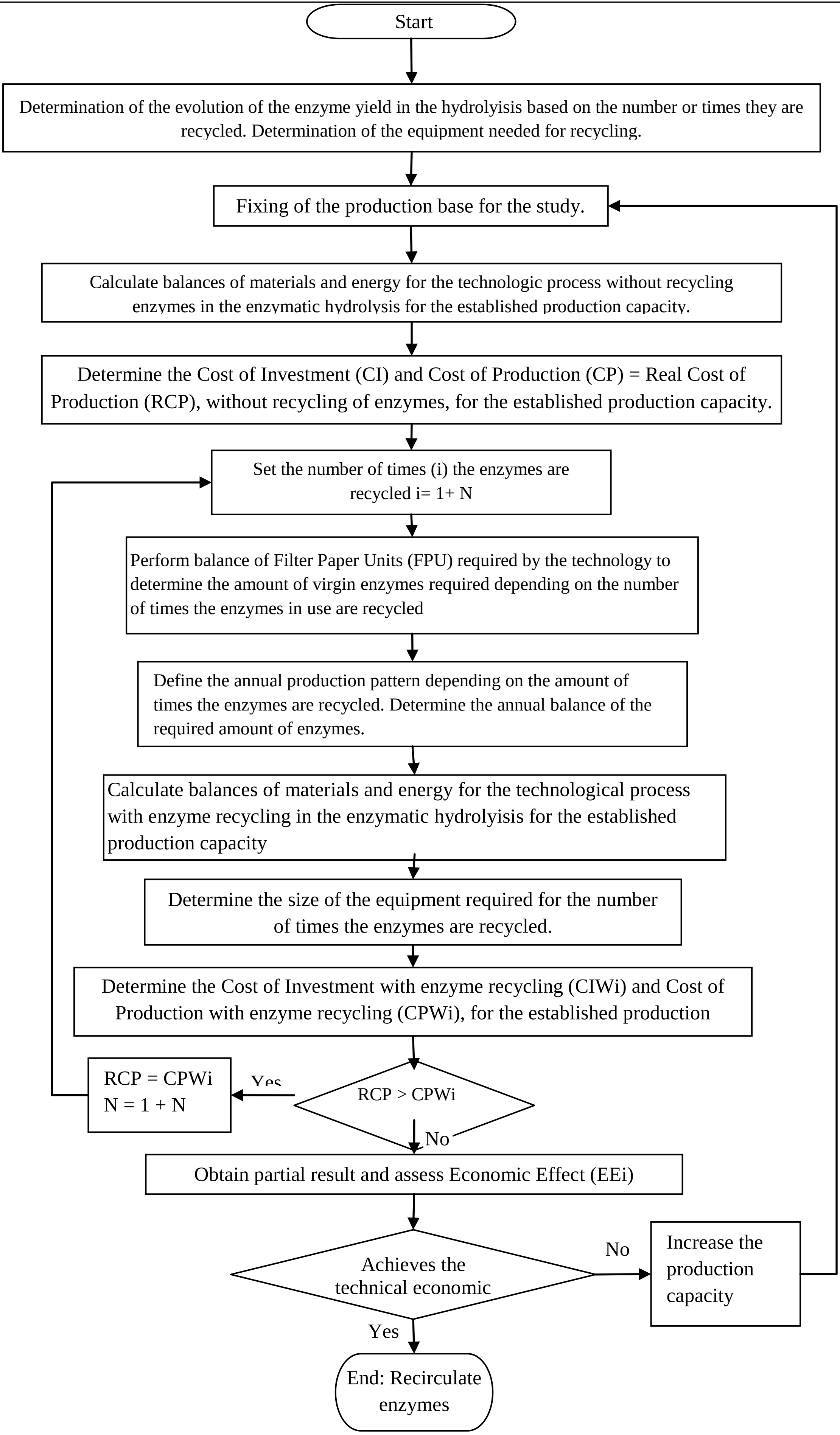


Figure 4. Heuristic diagram to evaluate the economic effect of enzyme recycling

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.

Results and discussion

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 [12,13]. In addition, they were estimated with the help of the Rule of Point 6 [13] and their adjustment to the year 2018 has been extrapolated, using the idea proposed by Aden et al. (2002) [14], to predict the annual cost index for that year by adjusting the annual data since 1957 [15], as shown in Figure 5.

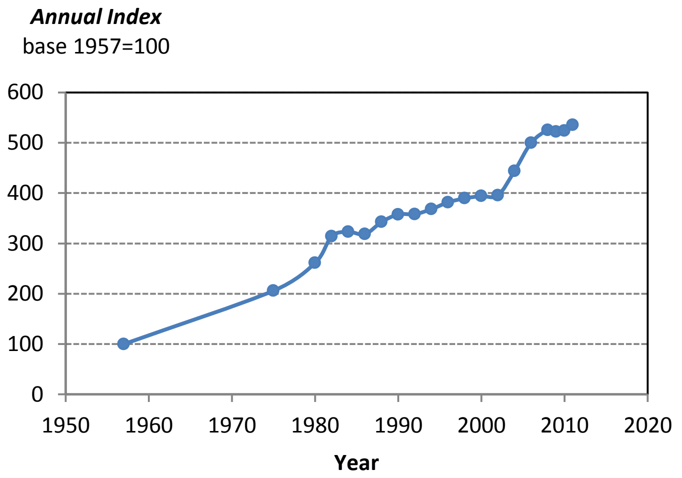


Figure 5. Chart of evolution of the Chemical Engineering Cost Annual Index 1975-2011 and forecast [15]

Table 1 shows a summary of investment components for each ethanol production capacity. This data was calculated taking [8] 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	1,355,227.00	3,068,233.00	1,077,580.00	4,145,813.00
1,000	2,033,468.00	4,603,772.00	1,616,869.00	6,220,641.00
1,500	2,317,457.00	5,246,723.00	1,842,677.00	7,089,400.00

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 COST OF PRODUCTION	PRICE $ / UM	AMOUNT	UM	COST USD x 10^3 / YEAR
I Manufacturing Expenses (A + B + C)				19539.475
A: DIRECT COSTS				17704.534
1. Raw materials				4888.3
• Bagasse	0.007	78 3282 85.71	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/a	2 970.93
• Ammonium Sulfate and yeasts	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 %TPC			784.575
3. Direct supervision 10%	10 % de 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			207.29
6. Supplies % of 5	10			2.07
7. Lab charges% of 2	10			78.45
8. Patents % TPC	1			78.45
B: FIXED CHARGES				472.62
1. Depreciation 10% FCI	10			414.58
2. Local Taxes 1-4% FCI	1			41.458
3. Taxes 0.4-1 % FCI	0.4			16.58
C: INDIRECT COSTS 5-15% TPC	5			392.2875
II. General Expenses (A + B + C)				706.12
A .. Distribution and sales % TPC	2			156.92
B. Management % TPC	2			156.92
C. Research and Development%	5			392.29
D. Financial interests	1			41.45
III. Total Production Cost (TPC) (I + II)				7845.75

Total Production Cost (TPC) = 0.27TPC + 10919.77 = TPC = 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	1,000	1,500
Total Production Cost (USD x 10^3/ year)	7,845.75	14,958.58	20,535.00
Unitary Cost (USD/l)	0.5567	0.53	0.47

Variation of production costs with enzymes recycling.

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

Determination of FPU balances for enzyme recycling.

To analyze the economic impact of the possibilities of obtaining ethanol with enzyme recycling, the results obtained by Mesa et al. (2016) [6] 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 .106 FPU/d. Therefore, for a production strategy operating first with fresh enzymes and then recycling, the following situations will occur:

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 2,970,930.00/y, that is, USD 9,903.1/d.

Operation with enzymes recycled one time.

In the second operation we will also need: 4,428.94.106 FPU/d that will be contributed from the recycled enzyme with a lower yield equivalent to 0.48 [6] and also fresh enzymes, while the yield of the fresh enzyme was 0.72, 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:

Equation 1:

$$4\,428.94.106\,\frac{FPU}{d} = A\left(\frac{0.48}{0.72}\right).30\,\frac{FPU}{g}$$

Where A: 147 631 368 g/d

We can clear B:

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

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

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

Therefore, the costs of recycling enzymes one time decrease in the recycling operation to: USD 3,301.03/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 1,980,619.65/y, a reduction of USD 990,310.35/y in the expense in enzymes without recycling.

This directly translates into the TPC, allowing it to be estimated when the enzymes are recycled once in:

$\mathrm{TPC} = \mathrm{USD}\ 7{,}845{,}750.00 - 990310 = \mathrm{USD}\ 6{,}855{,}440$, for a Cost of USD 0.4864 / l

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 [6], and a first recycling of enzymes in an amount B, being then the balance of FPU as follows:

$$4\,428.94.106\ \frac{\mathrm{FPU}}{\mathrm{d}} = \mathrm{A}\left(\frac{0.271}{0.72}\right).30\ \frac{\mathrm{FPU}}{g} + \mathrm{B.}\left(\frac{0.48}{0.72}\right)30\frac{\mathrm{FPU}}{\mathrm{g}} + \mathrm{C.}\,30\frac{\mathrm{FPU}}{\mathrm{g}}$$

For **A:** $A = 147\,631\,368\ \frac{\mathrm{g}}{\mathrm{d}}.$

And B:

$$\mathrm{B} = 49\,210\,421.33\frac{\mathrm{g}}{\mathrm{d}}$$

We can clear C:

$$C = \frac{4\,428.94.106 - 147\,631\,368\,(0.3764)30 - 49\,210\,421.33\,(0.667)30}{30}$$

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

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

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 17,177.91, 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 1,717,791.00, a reduction of USD 1,253,139/y from 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:

$$\begin{aligned} TPC &= USD\ 7{,}845{,}750 - USD\ 1{,}253{,}140 \\ &= USD\ 6{,}592{,}61 \end{aligned}$$

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.

Estimated production cost with enzyme recycling for a plant of 1000 Hl / d (27,714,300 l/y)

Cost reduction through recycling enzymes once: **USD 1,947,502**

$$TPC = USD\ 14{,}958{,}580 - USD\ 1{,}947{,}500 = USD\ 13{,}011{,}080$$

$$C/l = \frac{TPC}{annual\ production} = \frac{USD\ 13{,}011{,}080}{27{,}714{,}300} = USD\ 0.47/l$$

Cost reduction through recycling enzymes twice: **USD 2,464,370**

$$TPC = USD\ 14{,}958{,}580 - USD\ 2{,}464{,}370 = USD\ 12{,}494{,}210$$

$$C/l = \frac{TPC}{annual\ production} = \frac{USD\ 12{,}494{,}210}{27{,}714{,}300} = USD\ 0.45/l$$

Estimated production cost with enzyme recycling for a plant of 1500 Hl / d (4,500,000 l/y)

Cost reduction through recycling enzymes once: **USD 3,056,773.69**

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

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

Cost reduction through recycling enzymes twice: **USD 3,868,042**

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

$$C/l = \frac{TPC}{annual\ production} = \frac{USD\ 16{,}666{,}958}{43{,}500{,}000} = 0.383\ USD/l$$

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. It is summarized as follows:

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 990,310.35
1,000	USD 2,464,370.00
1,500	USD 3,868,042.00

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 have been calculated according to the established references and the results are summarized in Table 5 as follows:

Table 5. Cost of equipment acquisition

Capacity (Hl/d)	500	1000	1500
Total (USD)	689,074.177	1,308,443.79	1,926,855.009

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

	Projected amount (USD)		
Concept	***500 Hl/d***	***1000 Hl/d***	***1500 Hl/d***
Fixed Capital Investment (FCI)	2,466,885.55	4,684,228.76	6,898,140.93
Working Capital (WC)	274,098.39	520,469.86	766,460.10
Total Working Capital (TWC)	2,740,983.95	5,204,698.62	7,664,601.04

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

	FCI (USD)		
Capacity (Hl/d)	**Without recycling**	**Recycling one time**	**Recycling two times**
500	0.00	809,704.28	2,466,885.55
1000	0.00	1,618,853.088	4,684,228.76
1500	0.00	2,424,727.914	6,898,140.93

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	5,857.40
1000	10,496.00
1500	15,490.40

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)	2,148,797.75	6,646,880.46	11,147,785.25
Internal Rate of Return - IRR (%)	30	60	89
Payback Period (years)	6	3	2

Conclusions

1. It is feasible to apply a procedure to determine the economic impact of recycling enzymes in the production of ethanol from sugarcane bagasse.
2. Recycling enzymes in the technological process of ethanol production from sugarcane bagasse has economic benefits by reducing costs of enzyme purchase.

3. The economic benefits of enzyme recycling increase as the installed production capacity increases.
4. The 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 investment needed 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 1500 Hl respectively.

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