Insights into the economic viability of cellulases recycling on bioethanol production from recycled paper sludge
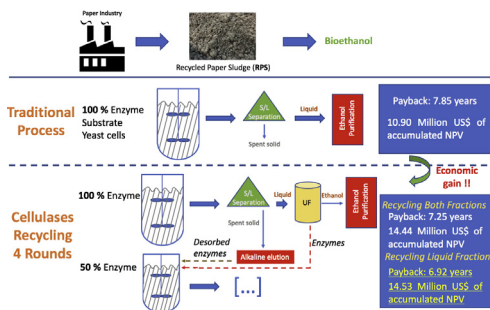
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GRAPHICAL ABSTRACT

ARTICLE INFO

ABSTRACT

The economics of Recycled Paper Sludge conversion into ethanol was here assessed with emphasis on integrating a cellulase recycling system.

Without cellulases recycling this process presented positive economic outputs (payback period of 7.85 years; 10.90 Million US$ of accumulated NPV) despite the modest ethanol titers. Recycling both free and solid-bound enzymes allowed considerable savings of enzyme but also an increase on annual costs (0.88%), resulting on a superior economic output: payback period decreased to 7.25 years; accumulated NPV increased to 14.44 Million US$. Recycling exclusively the liquid fraction enabled a clear costs reduction, however, also total ethanol decreased, attenuating the abovementioned benefits. Targeting higher ethanol concentrations, superior solids consistencies were also evaluated. Despite a costs reduction, total ethanol decreased due to a higher ethanol retention on the solid. A sensitivity analysis further revealed that the cost of enzymes and ultrafiltration membrane may be critical on enzyme recycling economic feasibility.

1. Introduction

Over the last years, cellulosic ethanol is increasingly establishing itself as a feasible alternative to fossil fuels. This can have an important boost on small and local economies, while at the same time eliminating the potential competition with food crops. The cost of both feedstock and the enzymes is critical regarding the economic performance of this process (Johnson, 2016; Liu et al., 2016; Pirzadah et al., 2017). Thus, more attention has been given to residues (from forest, agriculture, industry, etc.) rather than energy crops, since they may have a negative

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cost that arises from the processing required to avoid environmental impact associated to their disposal. Recycled Paper Sludge (RPS) is a substrate generated in great amounts worldwide by paper-manufacturing industry (Monte et al., 2009). More specifically, it results from the treatment of the effluents generated on paper recycling processes (Marques et al., 2008a). It has a limited number of handling options, being usually disposed in landfills. Nevertheless, this material also contains a considerable amount of sugars that can be valorized through multiple products, namely bioethanol. In fact, several studies have already demonstrated the practical feasibility of RPS for the production of bioethanol (Marques et al., 2008a; Gomes et al., 2016, 2018) or lactic acid (Marques et al., 2008b). Chen et al. (2014) have recently demonstrated that from an economic point of view, the conversion of different paper manufacturing residues is in fact viable to implement on an industrial scale.

Despite all the progresses achieved by enzyme manufactures, cellulases still harbor a significant fraction of ethanol final cost (Gomes et al., 2015). For this reason, numerous strategies have been studied towards a reduction on enzyme cost. Still, the number of studies demonstrating the practical feasibility of enzyme recycling strategies are rather small. Furthermore, to the better of our knowledge, no work currently exists making this assessment for recycled paper sludge material and specifically applying an ultrafiltration process, gaining even more relevance as these processes are usually associated with a very high cost.

Here, an overall analysis on the economic viability of cellulase recycling in the specific context of bioethanol production from RPS was performed. The economics of RPS conversion was first analyzed considering no enzyme recycling, which represents the first study of this kind for this particular material. Afterwards, based on experimental data gathered in previous research (Gomes et al., 2016, 2018), the impact of cellulase recycling was assessed. Finally, a sensitivity analysis demonstrated the effect of different variables on the process economics and the validity of the main findings of this work.

2. Materials and methods

2.1. General assumptions

The scenarios described on this work consider some major assumptions (Table 1). The feedstock is *n*RPS, the RPS residue after carbonates neutralization, with the following composition (dry mass basis): 34.1% cellulose; 7.9% xylan; 4.8% protein; 3.5% fat; 29.3% ash; 20.4% lignin (Marques et al., 2008a). The process of *n*RPS conversion

will be integrated on an existing paper mill facility that generates this residue, hence, no transportation costs are considered. For economic evaluation purposes, a null cost is associated to *n*RPS. This way, the economic gain from avoiding disposal costs were not considered here. For all scenarios, it was assumed an average uptake of 200 dry tons (*refers to metric tons*)/day of *n*RPS and a total annual operating period of 8520 h.

2.2. Process description

Different scenarios were considered. The base case scenario – BaseSc - refers to the process where enzymes are not recycled. Based on results from a previous study (Gomes et al., 2018), a recycling scenario was elaborated which considers the recovery of both liquid and solid fraction of enzymes (Rec2Frac). A simpler process, with only liquid fraction recycling, was also elaborated (Rec1Frac). The later was not tested experimentally and thus is based on some assumptions. Considering the low final ethanol titers (approx. 3.4% (w/w)) achieved using a solid content of 22% (w/v), a scenario homologous to Rec2Frac was also considered for an initial solid loading of 28% (w/v) (Rec2Frac28%). Finally, a scenario reporting to the valorization of the final solid through a combustion-gasification process was also analyzed (BaseScComb).

2.2.1. BaseSc – RPS conversion into ethanol without cellulase recycling

On the base case approach *n*RPS (approx. 42% carbohydrates) is mixed with water in a liquid to solid ratio of 4.55 (22% (w/v) consistency) for BaseSc, or 3.57 (28% (w/v) consistency) for Rec2Frac28%. A cellulase cocktail is then added at an enzyme loading of 20 FPU/g$_{cellulose}$. This mixture is incubated at 40 °C until a cellulose conversion of 86% is achieved. Afterwards, sugars syrup is inoculated with yeast cells and the temperature set to 35 °C, enabling glucose conversion into ethanol, carbon dioxide and biomass. After hydrolysis and fermentation, the liquid (containing ethanol, residual glucose and soluble enzymes) and the final solid residue are separated. The liquid stream then proceeds to an ethanol purification stage using distillation, enabling a final purified stream with 99.5% (w/w) ethanol (Fig. 1A). An extended description of this process has been reported by Gomes et al. (2016, 2018).

2.2.2. Rec2Frac and Rec1Frac – RPS conversion into ethanol with enzyme recycling

For scenarios with enzyme recycling, some modifications are introduced after hydrolysis (Fig. 1B). Following the main solid-liquid

Table 1
Main techno-economic assumptions of the study.

| Main economic assumptions		Overall process specifications	
Total operating hours	8520	RPS upgrade capacity	200 tones/day
Project lifetime	15 years	Solids loading	22 or 28% (w/v)
Income Taxes	21%	Hydrolysis temperature	40 °C
Annual Interest Rate	8%	Fermentation temperature	35 °C
Ethanol selling price	0.986 US$/kg	Enzymes load	20 FPU/g$_{cellulose}$
Cost of materials		Cellulose conversion	86%
RPS	0 US$/ton	Glucose-ethanol Yield	0.493 g/g$_{glucose}$
Cellulases	4 US$/kg$_{cellulase}$	Glucose-CO2 Yield	0.430 g/g$_{glucose}$
NaOH	400 US$/ton	Glucose-cells Yield	0.065 g/g$_{glucose}$
H$_2$SO$_4$	85 US$/ton	**Solid-liquid separation**	
Cost of utilities		Final solid humidity	50% (w/w)
LP steam	7.56 x 10^{-3} US$/kg	**Ultrafiltration**	
Electricity	0.14 US$/kWh	Feed/Retentate	20
Cooling water	Ulrich and Vasudevan (2006)	Filtrate flux	200 L(m^{2}h)
UF membrane	400 US$/m^2	UF membrane lifetime	1000 h
Labor		**Enzyme Recycling**	
Operator pay rate	5 US$/hour	Number recycling rounds	4
Number of Shifts	3	Fresh enzyme added	50%
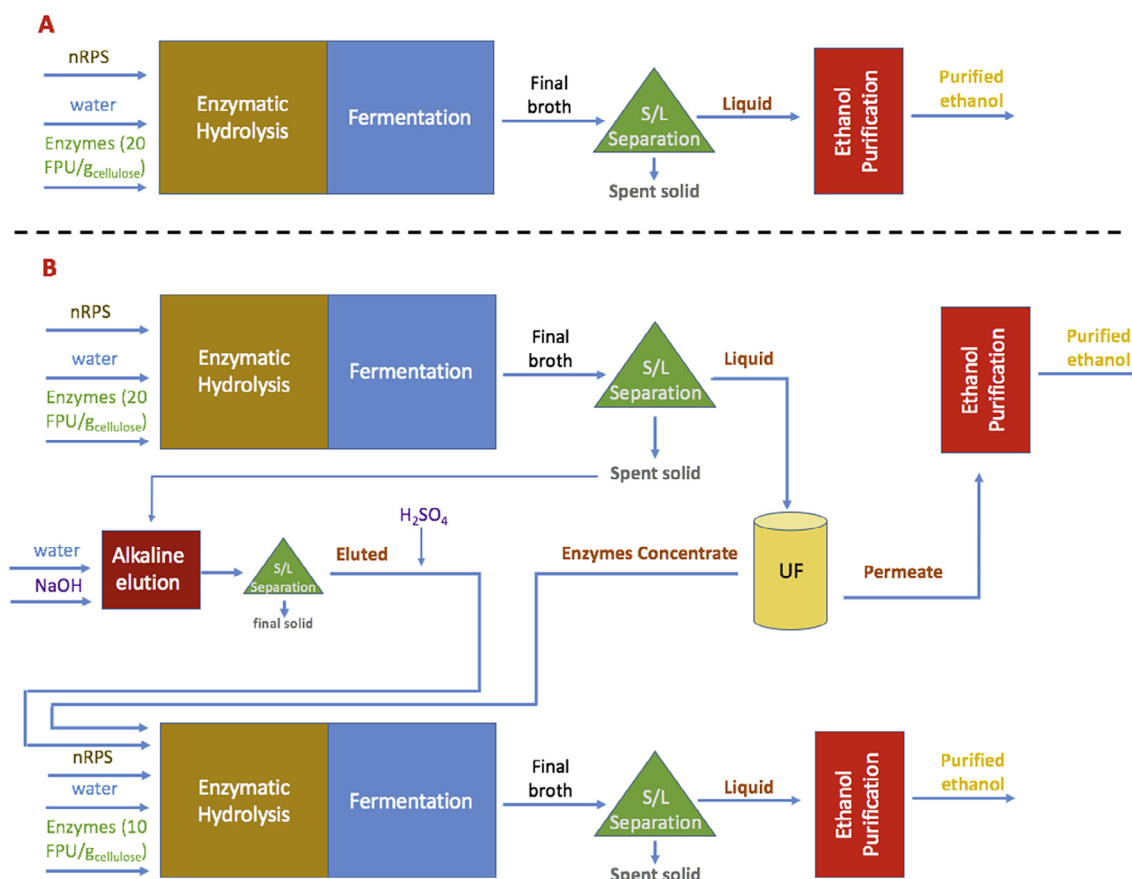
Fig. 1. Overall scheme of *n*RPS conversion into ethanol (A) without and (B) with enzymes recycling.

separation process, the liquid fraction is concentrated by ultrafiltration (UF) enabling the separation of cellulases (on the concentrate) from water and ethanol (and other low-molecular weight solutes). The solid is resuspended in water and the pH increased to 9 (with NaOH) to allow enzymes desorption from the solid. This mixture is then centrifuged and the pH of the liquid fraction adjusted to 5 (with H_2SO_4). This stream with desorbed enzymes is mixed with the enzyme concentrate obtained from the ultrafiltration and used to process a new batch of fresh substrate. For the new hydrolysis run, 50% of the initial enzyme loading is added to compensate activity loss occurring during the overall process. As experimentally validated (Gomes et al., 2018), this strategy is applied during 4 consecutive rounds, after which 100% of enzyme is once again employed. Enzyme recycling concerning exclusively the liquid fraction (Rec1Frac) is conducted in a similar way, omitting the enzyme recovery from the final solid residue; only the enzyme ultrafiltrated from the liquid fraction is thus reutilized in this scenario on the subsequent round.

2.2.3. BaseScComb – valorization of final solid residue by a combustion-gasification process

The final solid obtained after hydrolysis and fermentation still contains some components that can be further converted into energy through a thermo-chemical process. Specifically, the final dry biomass undergoes initially a devolatilization (pyrolysis) where it is decomposed into carbon, hydrogen, oxygen and ash, according to its elemental composition. All these components go into a combustion chamber where they react with oxygen to produce CO_2, CO, H_2O and heat. The char obtained on pyrolysis and combustion passes to a reduction zone where gasification occurs producing CO_2, CO, H_2 and CH_4. Ash and remaining char are separated from the syngas using a cyclone. The generated synthesis gas from gasification has a high energy content

which can be used directly as fuel in a gas engine to produce electricity. An internal combustion engine burns the gaseous fuel to produce electricity by means of a generator. A more detailed description can be found on García et al. (2017a, 2017b).

2.3. Process modeling and simulation

A simulation model was constructed for each scenario using Aspen Plus v8.6 (Aspen Technology, Inc., USA). These models were used to generate the correspondent mass and energy balances, providing the data for the economic analysis. Process simulation assumed a continuous approach based on a fixed value of substrate uptake, in this case 200 tons/day of *n*RPS, and conversion rates experimentally obtained. As to simulate the abovementioned enzyme recycling system, the models assumed four production lines, which simulates four sequential hydrolysis rounds, each with a RPS uptake capacity of 50 tons/day. Each stream of recovered enzymes is subsequently re-introduced in another production line, properly simulating also the recovery of other products, such as ethanol. To ensure that this particular type of simulation design does not introduce any effects in the process costs, e.g. resulting from different equipment sizes, all scenarios (including those without enzyme recycling) followed this approach.

2.3.1. Enzymatic hydrolysis

The stream of water and RPS is heated to a temperature of 40 °C and mixed with a proper amount of enzyme. Inside a stoichiometric reactor, Reaction1 occurs considering a cellulose conversion of 86% (Gomes et al., 2018). Excepting for water, which is also used for cellulose hydrolysis, all other components are inert.

1 Cellulose + 1 H_2O —> *n* Glucose
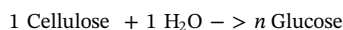
2.3.2. Fermentation

Stream from enzymatic hydrolysis is temperature-changed to 35 °C entering then a yield reactor where glucose is converted into multiple products according to a specific yield ($g/g_{glucose}$): ethanol, 0.493; CO_2, 0.430; yeast cells, 0.065; unconverted glucose, 0.012.

2.3.3. Solid-liquid separation

Final broth from hydrolysis and fermentation is fed into a filtration system (rotary drum filter) enabling a total separation of solid components (hemicellulose, remaining cellulose, lignin, ashes, yeast cells) from the liquid fraction. Considering the experimental values of solid humidity after solid-liquid separation, a value of 0.5 was set for the mass fraction of liquid in the final solid.

2.3.4. Ethanol purification stage

Liquid stream obtained from solid-liquid separation is processed by an ethanol purification stage composed by three steps. Distillation initially allows to increase ethanol concentration from 3 to 5 % to 70% (w/w). Posteriorly, a rectification column enables an increase to nearly 88% (w/w). A final step of dehydration removes a considerable amount of the remaining water, using in this case a molecular sieve, resulting on a final stream of purified ethanol with a concentration of 99.5% (w/w).

2.3.5. Alkaline washing

On a mixing vessel, the solid from the primary solid–liquid separation is resuspended in water, at the same consistency as initially adopted for hydrolysis. This is then mixed with a given amount of NaOH, which was calculated considering a pH shift of 4.8 to 9 for a total volume equal to the solid suspension. Afterwards, this suspension passes through another process of solid–liquid separation (similar to the initial one) being the liquid fraction pH reduced to 4.8 by the addition of H_2SO_4 in a mixing vessel, where it is stored until further use.

2.3.6. Enzyme concentration

Liquid fraction from the primary solid–liquid separation is fed into a UF system (10 kDa cut-off Polyethersulfone (PES) membrane) which produces two main streams: a permeate, containing mostly water and ethanol; the retentate, referring to a cellulases concentrate, containing a large majority of the enzymes, but also some residual levels of ethanol, water and other components. Permeate and retentate volumes are set assuming a concentration factor of 20, as considered in some case studies available within SuperPro Designer (Intelligen Inc.) platform.

2.4. Economic assessment

The capital, operating costs and revenues were estimated. The ethanol selling price was set as 0.986 US$/kg. Cost of equipment was calculated with Aspen Economic Analyzer v8.6 considering the specifications obtained from process simulation. Fix capital investment was estimated based on the purchasing cost of the equipment and other additional factors accounting for delivery (10% of purchasing costs), direct costs (% of purchasing costs: installation 47%, instrumentation and control 36%, piping 68%, electrical systems 11%, buildings 18%, yard improvements 10%, service facilities 70%) and indirect costs (% of purchasing costs: engineering and supervision 33%, construction expenses 41%, legal expenses 4%, contractor's fee 22%, contingency 44%) (Peters et al., 2003). The costs were estimated on an annual basis, taking in account the following categories: raw materials, utilities, maintenance, labor, fixed & general, overhead and capital depreciation. Costs of the raw materials were estimated according to the mass balances, and the correspondent market prices. Utilities were estimated based on the energy balances and after energy integration internally conducted by Aspen. Maintenance costs refers to maintenance (6% of fix capital investment) and operating supplies (15% of maintenance). Fixed & general refer to taxes (2% of fix capital investment), insurance (1% of fix capital investment), and general cost (20% of labor,

supervision and maintenance). Plant overhead was estimated as 60% of labor, supervision and maintenance (Peters et al., 2003).

2.4.1. Cost of raw-material, chemicals and enzymes

As RPS is commonly associated with a negative (disposal) cost, and assuming the integration of this process on an existing facility that generates this residue, a null price was considered for this material. Also, the neutralized RPS (after carbonates removal) was considered as the initial raw-material.

A cost of 4 US$/$kg_{enzyme}$ was assumed on this study considering different sources providing values on this range (Chen et al., 2014, NREL, 2013, 2015). It is worth of note however, that this represents an indicative value. For H_2SO_4 and NaOH an indicative price of 0.085 and 0.4 US$/kg was assumed, respectively, considering multiple estimations from different suppliers (e.g. MS SANSA Co., Ltd; Wuhan Kangzheng Science and Technology Co., Ltd).

2.4.2. Cost of utilities, labor and consumables

The cost of the main utilities was considered as follows: LP (low-pressure) steam, 7.56×10^{-3} US$/kg; electricity, 0.14 US$/kWh; cooling water price was defined according to Ulrich and Vasudevan (2006); UF PES membranes, which require regular replacement, were given a cost of 400 US$/$m^2$. This was based on several case studies available within SuperPro Designer software. Total labor costs were calculated as the sum of operating labor, operation supervision (15% of operating labor) and laboratory charges (15% of operating labor), assuming 3 shifts of 8 h per day and an operator pay rate of 5.00 US$/hour. The number of operators per shift was calculated according to the method reported by Peters et al. (2003) which considers mainly the plant capacity and the complexity of their processes.

2.4.3. Estimation of UF costs

SuperPro Designer was used to estimate the cost of the equipment and the consumption of electricity and membrane used on the UF process. For that purpose, it was considered a Feed/Retentate ratio of 20, a filtrate flux of 200 L/(m^2h) and a membrane replacement periodicity of 1000 operating hours. Electricity consumption was calculated considering an average level of 0.00065 kWh/L/hour, as employed by Alriols et al. (2014).

2.4.4. Main economic parameters

To assess the overall economic performance of the different scenarios, the Net Present Value (NPV) accumulated after the project lifetime (15 years) was estimated. For this, it was considered a total project life-time of 15 years, an annual interest rate of 8% and an income tax rate of 21%. Capital depreciation was computed following the straight line method. Total project life-time was established based on previous studies referring to similar processes (Chen et al., 2014; NREL, 2013, 2015). Annual interest rate was defined in accordance to the average values reported by the European Central Bank for Europe in 2018.

3. Results and discussion

3.1. Overall analysis of production indicators

Table 2 presents the global mass balance for the base case scenario (BaseSc) regarding the consumption and production of the main elements of the process. The main products are CO_2, yeast biomass and ethanol, in addition to the final spent solid obtained. CO_2 is released to the atmosphere, while yeast cells are discarded with the final residue. Ethanol thus represents the only source of revenue. Within the context of lignocellulosics conversion, however, a broader range of final products should be considered to enable a full solid valorization and an improvement of the process economics. In addition to the hemicellulose that can also be converted to sugars, large amounts of lignin (31% (w/w)$_{dry\ basis}$) are present on the final residue. This fraction can thus still be

Table 2

Mass flows for the main components of ethanol production from RPS (base case scenario).

Type	Designation	Flow (kg/h)
Consumption	*nRPS*	8333
	Cellulose	*2842*
	Xylan	*658*
	Lignin	*1700*
	Ash	*2442*
	Protein	*400*
	Fat	*292*
	Water	37,660
	Enzymes	100
	Cooling water	516,142
	LP Steam	9205
	Electricity	*n.a.*
Production	Purified ethanol	1163
	Ethanol	*1156*
	Water	*6*
	CO_2	1168
Disposal	Water	1869
	Ethanol	187
	Glucose	27
	Protein	400
	Fat	292
	Cellulase	99
	Ash	2442
	Cellulose	398
	Hemicellulose	658
	Lignin	1700
	Cells	177

n.a. – not applicable.

used for the production of added-value chemicals, such as aromatics (van Haveren et al., 2007). Due to the high ash content of this solid (45% (w/w)$_{dry\ basis}$), it can also be incorporated into cements and used for the manufacturing of construction materials – i.e. bricks – which already represents one of the few valorization routes for RPS (Monte et al., 2009). The capture of CO_2 for food and beverage applications could also enable important gains, while adding to a significant reduction of carbon footprint.

Regarding ethanol, a significant loss during production is observed. Considering the ethanol present on the purified stream – 1156 kg/hour – and the initial cellulose uptake, an overall conversion rate of 0.407 kg$_{ethanol}$/kg$_{cellulose}$ can be estimated, which it is not in agreement with the production specifications previously detailed on section 2.3 (86% cellulose conversion; 0.493 glucose-to-ethanol conversion yield; this would lead to 0.471 kg$_{ethanol}$kg$_{cellulose}^{-1}$). Indeed, a significant fraction remains retained on the solid during the processes of liquid-solid separation (Fig. 2), hence the lower overall conversion rate. This is due to the high water-retention capacity of the final solid. As it can be seen on Table 2, due to both solid retention and the distillation process, a total of 186.58 kg/hour of ethanol is redirected to disposal, representing nearly 13.9% of total production.

Also, the concentration of ethanol entering the purification stage is rather low (Fig. 2). This can be mainly attributed to 1) a moderate level of initial solids concentration and 2) low level of available sugars in the raw-material. After solid–liquid separation, ethanol concentration in the liquid stream was 3.4% (w/w), below the critical level of 4% commonly reported for an economically viable fermentation process (Hahn-Hägerdal et al., 2006). As it will be detailed next, this will have a considerable impact on the economics of the overall process, both in terms of capital investment and operational cost.

3.2. Economic viability of RPS conversion into ethanol

To assess whether the described process is economically viable, both production costs and incomes needs to be analyzed. Raw-Materials represented one of the main contributors for total costs (due mostly to enzymes), accounting for nearly 35% of total costs, only being exceeded by Capital Depreciation (Table 3). This can be partially explained by the fact that *n*RPS have a null cost associated and no other major chemicals are required, since pre-treatments are unnecessary. Capital depreciation, however, is more complex since it is mostly determined by the initial capital investment but also by the economic parameters of the study, namely the Annual Interest Rate, Income Taxes and the Period of the project. The total cost of equipment was approximately 2.97 Million US\$ (*cf.* Fig. 2), not considering the ethanol purification stage, which can be considered quite acceptable taking into account the total capacity of the facility (200 tons RPS/day). For an approximate capacity of 60 dry tons of paper residues processed per day, Chen et al. (2014) reported equipment costs of 4.76 Million US\$. On the other hand, the ethanol purification was remarkably costly, representing nearly 79.5% of total cost. This may be explained by the low ethanol concentrations in the beginning of the purification stage (3.4%), resulting on a critical increase on equipment dimensions and heat transfer utilities consumption (Katzen et al., 1999).

Considering the annual ethanol production – 9.906×10^6 kg – and a base selling price of 0.986 US\$/kg, the estimated annual ethanol income is approximately 9.768 Million US\$. If we further also consider the total annual operational cost – 10.28 Million US\$ - an approximate ethanol production cost of 1.04 US\$/kg can be estimated. Taking into account the expected annual incomes and total costs, the main economic indicators were estimated. The payback period of the process is 7.85 years and after project life-time (15 years) there is a positive accumulated NPV of 10.90 Million US\$, suggesting that the present scenario has a positive economic output. A null cost for *n*RPS seems to be highly advantageous; considering instead an *n*RPS price of 10 or 20 US \$/ton the accumulated NPV would be 3.39 and -4.85 Million US\$, respectively (*data not shown*). Still, the payback period for the investment can be considered slightly high when compared to similar industrial processes, due to the significant costs of ethanol purification derived from the modest ethanol titers achieved.

3.3. Impact of the solids gasification process

Taking into account the considerable amounts of solid residue and its potential for energy generation, the impact of a combined combustion-gasification system on the process economics was assessed. Unexpectedly, there was an increase in utilities cost, resulting from a remarkably higher requirement of electricity (Table 3). Even though this process allowed the generation of approximately 1302 kWh, it also caused a critical increase on total electricity requirements from 100.7 kWh (in the base case) to 1650 kWh. Consequently, the net energy balance for electricity on this case was -347.8 kWh, hampering any economic gain from this strategy. Furthermore, due to the additional equipment required, there was also a critical increase on equipment cost in the range of 6.7 Million US\$. This unexpected result can be partially explained by the high humidity of the residue (50% (w/w)), thus requiring considerable amounts of energy (Pellegrini and de Oliveira, 2007). According to different authors, solid humidity should be below 20% (Zainal et al., 2001; Lv et al., 2004). Another possible explanation may be the rather high ash content of the final residue – approx. 45% (w/w)$_{dry\ basis}$ – which is not converted into energy (García et al., 2017b; Munalula and Meincken, 2009).

3.4. Economic impact of integrating cellulases recycling

Considering the results and specifications described by Gomes et al. (2016, 2018), a cellulases recycling scenario was envisioned assuming the recovery of both liquid and solid fractions, with only 50% of fresh enzyme being utilized on each recycling stage (Rec2Frac). As expected, there was a clear reduction in the total cost of enzyme – 38% – and consequently the corresponding contribution to the total costs
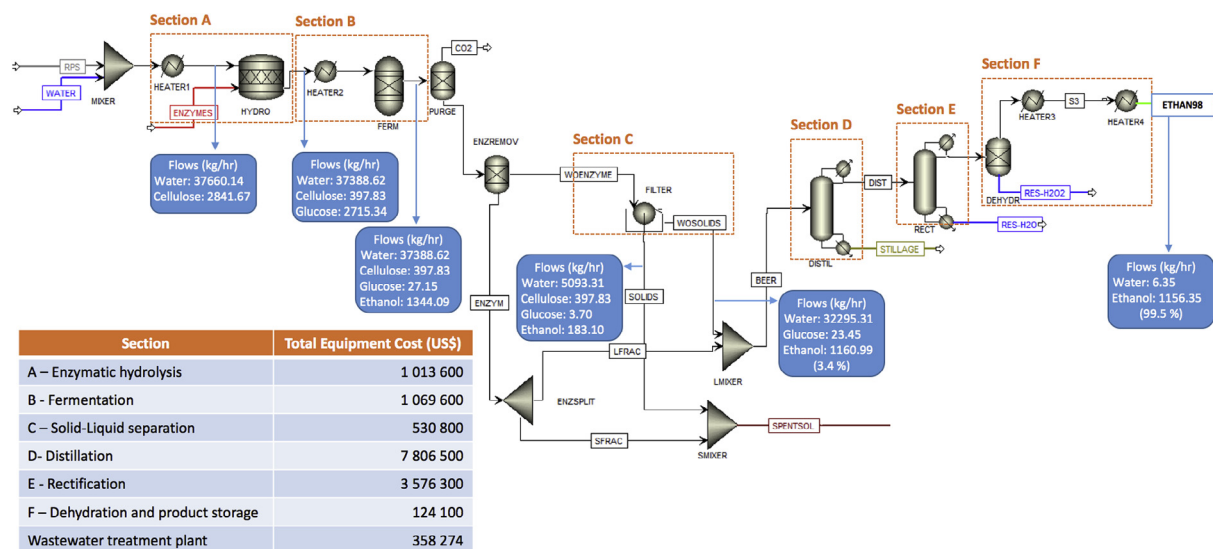
Fig. 2. Overall scheme of the simulation model for the base case scenario. The mass flows for the main components and total equipment cost of each section are presented.

Table 3
Effects of the integration of solids combustion (BaseScComb) and enzyme recycling (Rec2Frac) over annual operational costs and materials and utilities consumption of ethanol production from RPS (BaseSc).

Item	Base case (BaseSc)	Solids combustion (BaseScComb)	Enzyme Recycling (Rec2Frac)	Variation Rec2Frac to BaseSc (%)
Raw Materials[1]	3.55	3.55	2.26	− 36.3
RPS (10^3 tones)	71.00	71.00	71.00	
Enzymes (tones)	847.4	847.4	529.6	
Water (10^3 tones)	320.9	320.9	286.6	
NaOH (tones)	–	–	0.085	
H_2SO_4 (tones)	–	–	0.085	
Utilities[1]	1.31	1.59	2.03	+ 55.0
Electricity (10^3 kWh)	857	2964	1949	
Cooling water (10^6 tones)	4.40	3.95	8.20	
LP steam (10^3 tones)	78.4	78.4	78.4	
UF membrane (m^2)	–	–	1030	
Maintenance[1]	1.00	1.50	1.13	+ 13.0
Labor[1]	0.17	0.17	0.17	–
Fixed and General[1]	0.64	0.94	0.72	+ 12.5
Plant Overhead[1]	0.61	0.87	0.68	+ 11.5
Capital Depreciation[1]	3.00	4.49	3.38	+ 12.7
Total[1]	10.28	13.12	10.37	+ 0.88

[1] Values presented in Million US$.

Table 4
Overall economic indicators of ethanol production from RPS for the different scenarios.

Indicator	BaseSc	BaseScComb	Rec2Frac	Rec1Frac	Rec2Frac28
Ethanol Production (10^6 kg/year)	9.91	9.91	10.15	9.70	9.83
Gross Income (Million US$/year)	9.77	9.77	10.01	9.57	9.69
Allocated Cost (Million US$/year)	10.28	13.12	10.37	9.76	9.94
Ethanol Production Cost (US$/kg)	1.04	1.32	1.02	1.01	1.01
Payback period (years)	7.85	–	7.25	6.92	7.01
Accumulated NPV (Million US$)	10.90	–	14.44	14.53	14.91

decreased from 33% on the base case to 21% (Table 3). Even though some chemicals are required for enzymes recovery from the solid (NaOH and H_2SO_4), the cost of other materials other than enzymes actually decreased from 0.16 to 0.14 Million US$. This probably occurred due to an almost insignificant cost of these chemicals, but also to an inferior water requirement on this case, possibly resulting from a small improvement on process water re-utilization. Oppositely, the cost of utilities increased 55% with UF membrane, used to separate enzymes from ethanol, representing an additional 0.41 Million US$. Also

electricity consumption increased, resulting from additional operations such as solid–liquid separation, alkaline washing and the UF procedure. Interestingly, there was an increase on heat transfer utilities, possibly coming from some changes on ethanol purification requirements and from enzyme recovery steps. Consequently, there was an increase on the equipment's total cost, in the range of 1.46 Million US$: 1.29 Million US$ associated to enzyme recovery from the solid and 0.166 Million US$ to UF procedure. The annual cost from Capital Depreciation also increased in a similar rate – 12.7% – but also from Maintenance,
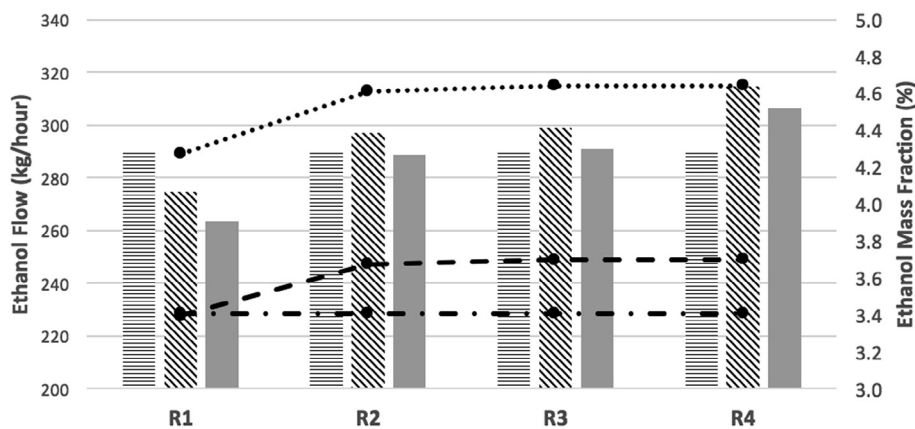
Fig. 3. Variation of ethanol flow (≡BaseSc ▨ Rec2Frac ▪Rec2Frac28) and its mass fraction (▪ · ▪ BaseSc ▪ ▪Rec2Frac ᴵᴵᴵᴵᴵRec2Frac28) for different scenarios along the different rounds.

Fixed and General and Plant Overhead, all strictly related to the complexity of the facility (Table 3).

Although total annual costs increased from 10.28 (base case) to 10.37 Million US$ (approx. 0.88%), the annual income also raised from 9.77 to 10.01 Million US$ (Table 4), resulting from a superior ethanol production, which increased from 9.91 to 10.15 x 10^6 kg. It seems that this system enabled the recovery of an important fraction of ethanol that otherwise would stay on final solid. Opposing to the base case scenario with a constant ethanol production flow of 290.2 kg/hour, in the present scenario it gradually increased from 274.6 to 297, 298.8 and 314.7 kg/hour on the 2nd, 3rd and 4th round, respectively (Fig. 3).

Considering the annual incomes and operational costs, there was a notorious economic gain in the process comparatively to the base case: the payback period decreased from 7.85 to 7.25 years; accumulated NPV after project lifetime increased from 10.90 to 14.44 Million US$. Overall, this suggests that cellulases recycling under similar conditions may be advantageous from an economic perspective.

Taking into account the considerable costs of adsorbed enzymes recovery, it was then hypothesized a scenario with exclusive recovery of soluble (non-adsorbed) enzymes. For sake of simplicity, we assumed that the initial enzyme activity in each round would be the same as for the previous scenario, where solid bound enzymes were also recovered, without the need to increase the fresh enzymes added. As expected, there was a small reduction on electricity consumption due to a less complex enzyme recycling process (Table 5). On the other hand, the reduction on equipment requirements had a greater impact totalizing

an annual reduction of 0.47 Million US$ on costs due to Maintenance, Fixed and General, Plan Overhead and Capital Depreciation. Overall, total costs decreased nearly 5.9%, from 10.37 to 9.76 Million US$/year (Table 5). As the solid fraction is no longer used, the ethanol being recovered alongside alkaline washing step is no longer recovered, resulting on a decrease in annual ethanol production to 9.7 x 10^6 kg. Unexpectedly, this is slightly inferior (−2.1%) to the yield registered for base case, probably because the ethanol-containing stream has to go through an additional purification stage (UF). As this fraction is re-circulated, more ethanol is lost. Accordingly, simplifying the cellulase recycling system yielded a superior economic performance as payback period decreased to 6.92 years and the accumulated NPV increased to 14.53 Million US$.

It is worth mentioning that although these results may provide some economic insights into general strategies of enzyme recycling, they are highly specific for RPS material. As recently shown by Gomes et al. (2018), recycling viability is strongly dependent on the enzymes fractionation and recovery at the end of the process, which itself may strongly vary according to multiple factors, one of the most important being the employed substrate and its composition (Rodrigues et al., 2014, 2015).

3.5. Cellulase recycling using increased solids loading

It was initially demonstrated that ethanol purification represents a major part of total equipment costs due to low final ethanol titers.

Table 5
Effects of the elimination of solid fraction recycling (Rec1Frac) and the increase of solids loading (Rec2Frac28%) over annual operational costs and materials and utilities consumption of ethanol production from RPS with enzyme recycling (Rec2Frac).

| Item	Enzyme Recycling (Rec2Frac) A	Enzyme Recycling (Rec1Frac) B	B-A (%)	28% Solids (Rec2Frac28%) C	C-A (%)
Raw materials[1]	2.26	2.27	+ 0.44	2.24	− 0.88
RPS (10^3 tones)	71.00	71.00		71.00	
Enzymes (tones)	529.6	529.6		529.6	
Water (10^3 tones)	286.6	311.0		212.8	
NaOH (tones)	0.085	–		0.085	
H_2SO_4 (tones)	0.085	–		0.085	
Utilities[1]	2.03	1.87	− 7.88	1.81	− 10.8
Electricity (10^3 kWh)	1949	1628		1789	
Cooling water (10^6 tones)	8.20	5.31		7.37	
LP steam (10^3 tones)	78.4	78.4		69.4	
UF membrane (m^2)	1030	1030		793	
Maintenance[1]	1.13	1.04	− 7.96	1.09	− 3.54
Labor[1]	0.17	0.17	–	0.17	–
Fixed and General[1]	0.72	0.66	− 8.33	0.70	− 2.78
Plant Overhead[1]	0.68	0.63	− 7.35	0.66	− 2.94
Capital Depreciation[1]	3.38	3.11	− 7.99	3.27	− 3.25
Total[1]	10.37	9.76	− 5.88	9.94	− 4.15

[1] Values presented in Million US$.
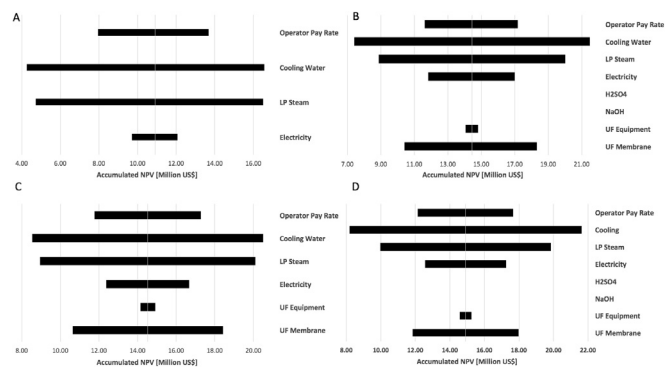
Fig. 4. Accumulated NPV after project lifetime under ranging levels (+100% to -100%) of different variables cost for (A) BaseSc, (B) Rec2Frac, (C) Rec1Frac and (D) Rec2Frac28%.

Considering that, a hypothetic scenario was envisioned with a solids concentration of 28%, instead of 22%, assuming the recycling of both fractions of enzymes. Due to a reduction on total working volume, there was a reduction on equipment related costs on an average level of 3%. Specifically referring to the ethanol purification stage, no differences were detected on rectification and dehydration steps but clear improvements were observed on distillation, with the cost of equipment decreasing from 8.2 to 7.7 Million US$ (-6.1%). As expected there was a notable increase of the final ethanol titers, from around 3.5% (w/w) (BaseSc and Rec2Frac), to 4.6% (Rec2Frac28%) (Fig. 3). Ultimately, this also led to a decrease on utilities of around 10.8% (Table 5), although part of this reduction may also be assigned to a small decrease on UF membrane usage, as the total volume for filtration is also reduced.

Even though final ethanol titers slightly increased, the actual total mass flow of ethanol recovered after purification has decreased from 1185.15 to 1149.03 kg/hour. This occurs because the solid obtained after separation contains a higher amount of ethanol. Consequently, also the gross annual income decreased from 10.01 to 9.69 Million US$. Despite this reduction, this strategy still enabled a slight improvement of the economic output: the payback-period decreased from 7.25 to

7.01 years, while the accumulated NPV increased from 14.44 to 14.91 Million US$ (Table 4).

It should be referred that, as for the scenario with only liquid fraction recycling, the increased solid loading scenario is also hypothetic, hence, their results should be seen as projections of promising recycling scenarios that might be interesting to validate.

3.6. Sensitivity analysis

The abovementioned results are based on assumptions that were set for nuclear variables, defined according to current values employed on similar processes and/or based on literature reports. While for some cases relevant variations are not foreseen, some are susceptible to fluctuations. To understand the validity of the results referred above, it is necessary to assess the impact of such fluctuations. Aiming that, the impact of the variation of some parameters on the accumulated NPV was evaluated (Fig. 4). The accumulated NPV showed to be highly sensitive to both ethanol selling price and enzyme cost, presenting a relative total amplitude above 1800 and 600%, respectively (*due to considerable scale differences, these are not plotted on the graphs*). Additionally, also the cost of UF membrane and heat transfer utilities (cooling water and LP steam) showed a considerable influence on NPV (Fig. 4). UF membrane base price is considerably high, 400 US$/m^2, which justifies in part its impact. For the case of heat transfer utilities this can be explained by the low ethanol titres and consequent higher consumption of this item.

It was further aimed to investigate the range of relevant variables cellulase recycling is economically viable, i.e. has a superior economic output comparatively to the base case. Fig. 5 shows a comparison of the accumulated NPV variation for different scenarios as a function of ranging values of Ethanol Selling Price, Enzyme Cost and UF membrane cost.

Ethanol selling price has a clearly significant effect in the economics of the process for all scenarios, however, it seems to show no apparent influence on the decision of whether cellulase recycling is viable or not. Differently, as expected, enzyme cost evidenced an important role on this decision. As observed on Fig. 5A, the accumulated NPV is superior for the scenarios of cellulase recycling (comparatively to the base case) for a wide range of enzyme cost. Furthermore, it can be seen that
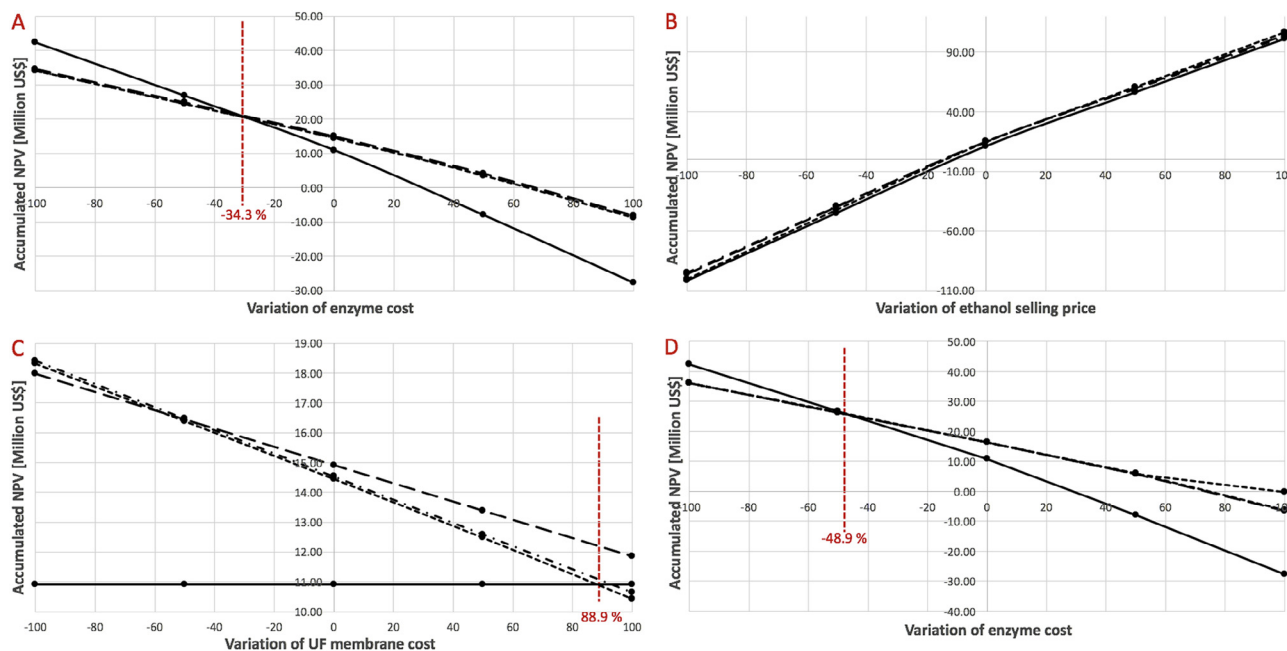

Fig. 5. Variation of the accumulated NPV for varying levels of (A) enzyme cost, (B) ethanol selling price, (C) UF membrane cost and (D) enzyme cost (assuming 200 US$/m^2 of UF membrane) under different scenarios (——BaseSc ·····Rec2Frac · - ·Rec1Frac - — Rec2Frac28).

enzymes price must decrease at least 34.3% (to a value of 2.63 US$/kg) for enzyme recycling being no longer of interest; beyond this barrier, accumulated NPV for the base case becomes superior to the recycling scenarios. Considering the recent achievements on enzyme cost reached by industry, one may expect that this scenario would be achieved in the near future. On the other hand, this is also dependent on other assumptions. For instance, if the base price for UF membrane is halved to 200 US$/m² (Fig. 5D), enzyme recycling consequently gains more relevance and the range for its economic viability becomes wider. Thus, the cost of UF membrane seems to importantly affect the possible benefit of enzyme recycling. Accumulated NPV for the recycling scenarios is superior to the base case in a wide range of membrane cost values. Specifically considering the scenario with recycling of both fractions employing 22% solids (Rec2Frac), cellulases recycling only becomes unviable when membrane cost increases above 88.9% of its base cost. Opposing to enzyme cost, such variations, however, are not expected to occur in the near future.

4. Conclusions

This work provides relevant insights into the economics of enzyme recycling on bioethanol production from RPS. It demonstrates that bioethanol production from RPS is viable despite its modest final titres. Under conditions already experimentally validated, the recycling of both enzyme fractions was economically superior to the base case. Eliminating the recovery of solid fraction importantly affected total annual costs enabling a superior economic output despite a slightly inferior ethanol production. Finally, a sensitive analysis suggested that recycling viability is particularly susceptible to the cost of enzyme and UF membranes, hence, significant changes on these may dictate its unviability.

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