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Performance comparison of precipitation strategies for recovering succinic acid from carob pod-based fermentation broths

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ABSTRACT

Experiments were performed for comparing and selecting the most appropriate precipitation strategy for succinic acid (SA) recovery from carob pod extract fermentation broths. The performances of three downstream options – employing calcium hydroxide, magnesium hydroxide or ammonia – were investigated and compared from operational, techno-economical and sustainability viewpoints. The highest SA recovery (84.3%) was obtained with ammonia, whereas the calcium-based treatment proved inadequate. Sustainability indicators favoured magnesium-based treatment, closely followed by the ammonia-based one. A preliminary economic analysis favours magnesium-based treatment, which appears to be the most feasible option. However, materials recycling and possible by-products commercialization could well position also ammonia-based treatment.

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Introduction

Succinic acid (SA), a saturated four-carbon dicarboxylic acid, can be produced via two routes: petrochemical and biological. While the petrochemical route is based on the chemical transformation of maleic anhydride, the biological route makes use of a variety of microorganisms, including bacteria and yeasts, to ferment natural carbohydrates, thus offering several advantages such as the possibility of obtaining diverse platform chemicals from renewable resources^[1–3] with a lower net fossil energy consumption.^[4] Because of this, SA has been recognized as one of the most promising building block molecules that can be derived from natural sources.^[5–7]

To provide conditions for large-scale SA bioproduction, current research has been focused on three main areas: 1) screening and improvement of SA-producing microorganisms, including metabolically engineered strains;^[8–10] 2) optimization of the fermentation process operation;^[11] and 3) use of different types of prospective feedstocks.^[12–14] Referring to this last category, SA production by *A. succinogenes* 130Z using carob pod water extracts as a feedstock was recently reported.^[15] The carob tree (*Ceratonia siliqua* L.) is a Mediterranean perennial tree that produces pods containing seeds (10% w/w) and a highly rich sugar pulp (90% w/w), which may constitute as

much as 50% of the carob pod mass.^[16] Although the worldwide carob pod production accounts for almost 400,000 tons/year, a large portion of this is discarded since it is considered a by-product of the carob locust bean gum industry. Thus, carob pod represents a promising feedstock for the bio-industry. Indeed, the volumetric productivity of 1.67 g SA/L h achieved by Carvalho *et al.* when using a carob pod extract as substrate^[17] is one of the highest available in the literature regarding bacteria-based fermentations,^[18] which justifies the interest in further researching this SA production method.

Equally important as achieving a high SA productivity during the fermentation step is to develop an efficient downstream process, which allows one to recover the SA obtained in a useful form. Owing to the large variety of SA biosynthetic conditions that can be applied, the type and sequence of recovery steps need to be adapted to address the removal of particular impurities; therefore, no universal solution exists. Because of this, downstream processing has been found to be the most economically demanding part of the SA production process, accounting for 50–80% of the associated costs.^[7,19]

As for other carboxylic acids, the first SA downstream processing method that was developed involves precipitation of its carboxylate anion with calcium. According to the patent by Datta *et al.*,^[20] calcium hydroxide is added to the fermentation broth in order

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to neutralize it and, at the same time, precipitate the produced SA as calcium succinate. This intermediate product is filtered off from the fermentation broth and then treated with concentrated sulphuric acid, thus generating calcium sulphate (gypsum) as a by-product in an equimolar amount. Free SA is obtained by ion-exchange chromatography, after which the product is further concentrated and crystallized by evaporation. Although relatively high SA yields can be obtained through this method, the consumption of reactants that cannot be regenerated or recycled is high, which increases the process costs. In addition, some authors suggest that the by-product, calcium sulphate, is not likely to be commercialized because of smell and colour impurities.^[21] Thus, the process has raised concerns regarding its economic and environmental impact-related aspects.^[22]

Several other possible SA recovery techniques have been proposed and studied. These include membrane filtration,^[19,23,24] vacuum distillation,^[22] liquid-liquid extraction,^[25] salting-out extraction,^[26] direct crystallization^[27,28] and electrodialysis^[11,29] among others. Yet, most of them have only been tested on a laboratory scale. However, since some companies and consortia have now begun to implement large-scale bio-based SA productions, it becomes important to evaluate which SA recovery technique(s) is(are) the most feasible one(s).

Currently, there are four known commercial bio-SA producers: BioAmber, Reverdia, Myriant and Succinity.^[2,30] Among them, BioAmber and Reverdia have each developed and commercialized yeast processes to produce SA, which use a minimal amount of base for pH control, thus leaving most of the carboxylic acids present in their acidic forms. As a consequence, the steps needed to recover the SA in a purified form are minimized compared to bacteria-based fermentations.^[30] The other two companies, Succinity and Myriant, have indeed patented bacteria-based fermentations. And, quite interestingly, it has been suggested that the downstream processes that they most likely will implement are those based on precipitation-involving schemes.^[2,30] Presumably, Succinity is (will be) employing a magnesium hydroxide-based precipitation process, whereas Myriant will be making use of ammonia, to precipitate the succinate during fermentation.^[2,31]

Both precipitation processes are relatively similar, besides the difference in employing either magnesium or ammonia as a cation to precipitate the succinate. The magnesium-based precipitation, patented by de Haan *et al.*,^[32] employs magnesium hydroxide (or another magnesium-containing species, *e.g.*, magnesium carbonate) for pH control during fermentation, resulting in the accumulation of magnesium succinate

as the main fermentation product. After biomass separation, hydrochloric acid is added to convert the obtained magnesium succinate into a free SA, producing magnesium chloride as a by-product.

Similarly, an SA precipitation method with ammonia has been patented.^[33,34] In the scheme proposed by Yedur *et al.*, di-ammonium succinate needs to be obtained, either by using an ammonium cation-based material to maintain neutral pH in the fermenter or by substituting the ammonium cation for the cation of the succinate salt formed in the fermenter. After biomass separation, di-ammonium succinate is converted into a free SA and di-ammonium sulphate by the addition of sulphuric acid. For both schemes, the resulting SA is separated from the by-product by precipitation (after a concentration step) and subsequently further purified if required.

The use of two different precipitation techniques in newly developed industrial processes is worthy of attention. Therefore, this study was conducted with the objective of experimentally investigating and comparing the performance of three precipitation-based downstream options for the specific case of carob pod-based fermentation broth. The comparison includes not only the downstream operational aspects, but also preliminary techno-economical and sustainability considerations.

Experimental

Materials

Fresh SA-containing broth was obtained from batch fermentations using carob pod water extracts as a carbon source and *Actinobacillus succinogenes* 130Z as an SA-producing strain, as described by Carvalho *et al.*^[15] The fermentations were carried on a 1 L benchtop-bioreactor with a working volume of 900 mL, sparged with CO₂ at 0.05 vvm, at 37°C, and stirred at 250 rpm. The carob pod aqueous extracts were obtained through the procedure described in.^[15] The culture medium contained per litre 10.0 g yeast extract, 8.5 g of NaH₂PO₄ · H₂O, 15.5 g of K₂HPO₄ and 12.6 g of NaHCO₃, and during the fermentation process, 5 M NaOH solution was added to keep the pH at 6.8. The fermentation broths of two different batches were centrifuged for 15 minutes at 15,300 g to remove the cells, recovering 1.8 L of the uniform supernatant solution. The concentrations of SA, methanoic acid (formic, FA) and acetic acid (AA) in the broth were equal to approximately 20.0, 14.3 and 13.3 g/L, respectively, corresponding to mass fractions of 0.42 for SA, 0.30 for FA and 0.28 for AA.^[15]

The model solution employed in some experiments included all the components of the culture medium

(8.5 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 15.5 g of K_2HPO_4 and 12.6 g of NaHCO_3), along with the three carboxylic acids in the following concentration: SA 20.0 g/L, FA 14.3 g/L and AA 13.3 g/L.

All inorganic salts (analytical grade), hydrochloric acid (37% purity) and sulphuric acid (98% purity) were purchased from Panreac. For the ammonia-based precipitation, ammonia solution 25% w/w (reagent grade) was employed. Roasted carob flour was purchased from a local store. All reagents were employed without further purification.

Precipitation with calcium hydroxide

The precipitation procedure, schematically presented in Fig. 1a, applied was adapted from Li *et al.*^[27] and performed as follows. First, $\text{Ca}(\text{OH})_2$ slurry (30%, w/v) was slowly added to 100 mL of acidified supernatant of the fermentation broth. The mixture obtained was vigorously stirred at room temperature until the solution pH increased to 12.5–13.0. The obtained slurry was immediately filtered through a 0.2 μm Whatman filter using a vacuum pump. The filtrate was recovered, and the precipitate was further washed with 50 mL of distilled water, which was also recovered. Next, the filter cake was re-dissolved in 100 mL of distilled water, and H_2SO_4 concentrated solution (48.15%, w/v) was added while stirring the mixture at room temperature until the solution pH decreased to 1.5–2.0. Again, the obtained slurry was filtrated through a 0.2 μm Whatman filter using a vacuum pump, the SA-containing filtrate was recovered and the precipitate was washed with distilled water (50 mL), which was also recovered. The filter cake was then dried at 70°C to a constant weight and the weight was registered. The filtrates and the washing water (WW) compositions were analysed by HPLC as described in Analyses section

Precipitation with magnesium hydroxide

For each of the two experiments performed (see Fig. 1b), 100 ml of the previously homogenized and centrifuged fermentation broth was used as in the $\text{Ca}(\text{OH})_2$ precipitation tests (previous section). In one of the experiments (P21), the solution was titrated with 5M NaOH to obtain a pH equal to 6.8, whereas in the other, the pH was left unaltered (P22). Then, a defined amount of $\text{Mg}(\text{OH})_2$ was added to the solution, which was stirred vigorously. The minimal required amount of $\text{Mg}(\text{OH})_2$ necessary to achieve a ratio of $\text{Mg}(\text{OH})_2$ to SA of 1 to 2 (w/w) was set, taking into consideration the information provided in De Haan *et al.*^[32] However, since the fermentation broth

also contained other acids that could react with Mg^{2+} , higher proportions of this hydroxide were used in the second experiment (1.0 and 2.5 (w/w) for P21 and P22, respectively). To solubilize completely the $\text{Mg}(\text{OH})_2$ present, the solutions were heated up to 72°C while stirring at 300 rpm. They were then left to cool down to 50°C, and concentrated hydrochloric acid (37%) was added until a pH of around 1.5 was reached. Since the procedure requires the use of a concentrated solution of magnesium chloride (close to 30% (w/w)), the solutions were left to evaporate in an airflow chamber at room temperature for a time period of 5 days, during which the volumes were monitored. When the solution volumes dropped to below 20 mL each, crystals had already formed, and the liquid phases were separated from the solids by decantation and stored for later HPLC analyses. The solids were left for one more day in the airflow chamber in order to dry them completely; their solid weights were registered and then re-dissolved in 50 mL of distilled water at room temperature. Samples of these final solutions were stored for HPLC analyses.

Precipitation with ammonia

As in the previous cases (see Fig. 1c), 100 ml of the homogenized and centrifuged fermentation broth was employed in two experiments. In one of the experiments (P31), the solution was titrated with 5M NaOH to a pH equal to 6.8, whereas the other was left unaltered (P32). Then, 10 mL of 25% (w/w) ammonia solutions was added to each of the solutions. The amount was determined by titrating the P31 solution until a pH of 11 was reached, and for P32 the same volume was employed. Both solutions were agitated vigorously for 10 minutes and then left to evaporate at room temperature in an airflow chamber for two days to obtain a di-ammonium succinate concentration of 25–30% (w/v). When the volumes of the solutions had reduced to two-third of the original ones, they were titrated with a H_2SO_4 concentrated solution (48.15%, (w/v)) until a pH of 1.5 was reached. Then, the solutions were filtered through a 0.2 μm Whatman filter using a vacuum pump and left again (for approximately three more days) in an airflow chamber until the volumes dropped below 20 mL and crystals had formed. The liquid phase was separated from the solids by decantation and stored for later HPLC analyses. The solids were left for one more day in the airflow chamber in order to dry them completely; their weights were registered, and then the solids were re-dissolved in 50 mL of distilled water at room temperature. Samples of these final solutions were also analysed by HPLC.

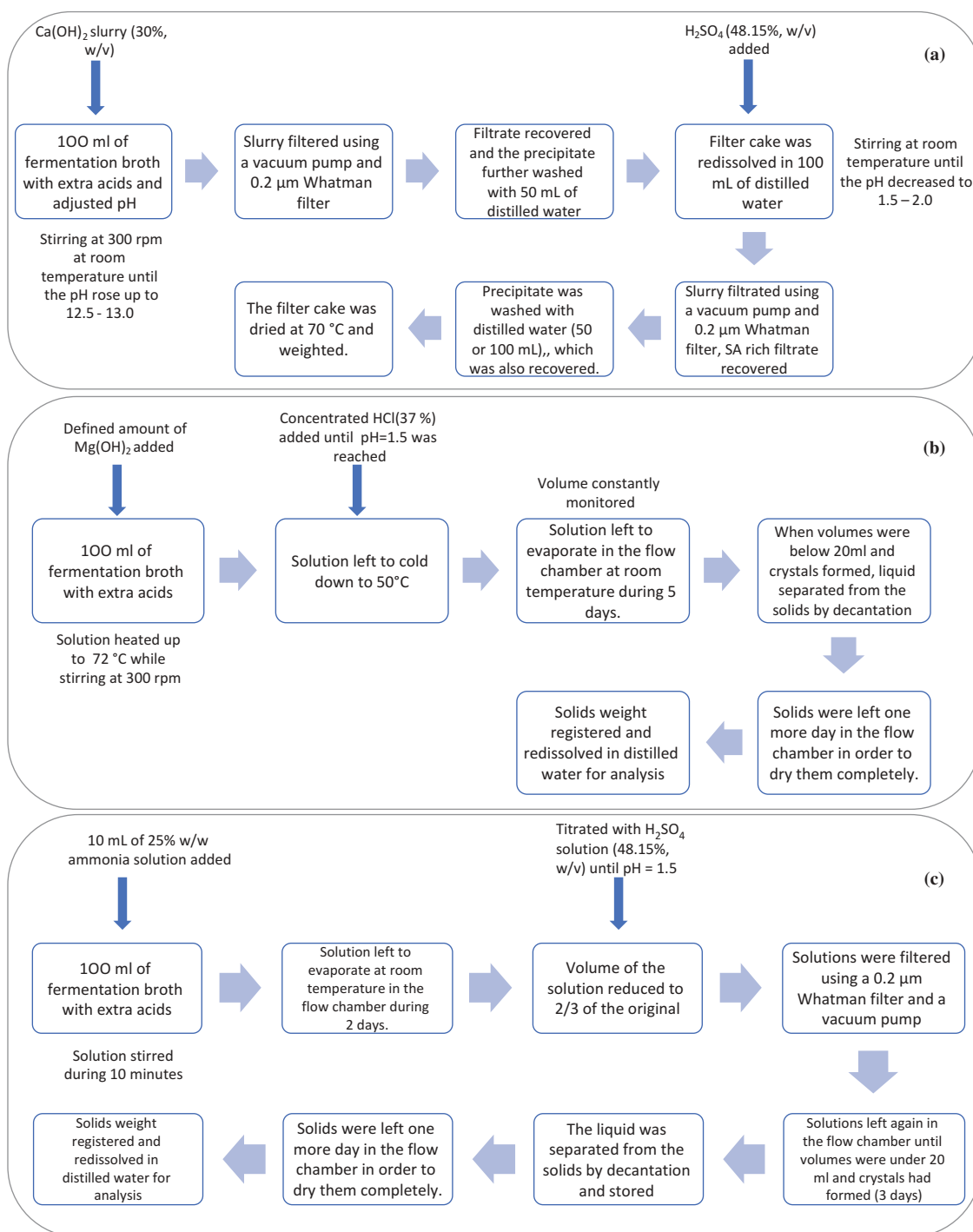


Figure 1. Methodologies of the performed precipitation experiments: **a)** with calcium hydroxide, **b)** with magnesium hydroxide and **c)** with ammonia solution.

Analyses

The concentrations of carboxylates (succinate, acetate and carboxylate) in all samples were determined by high-performance liquid chromatography (HPLC) using a Metacarb 87H column (Varian) and a refractive index detector (RI-71, Merck). The column was eluted at 30°C with 0.01 N H₂SO₄ aqueous solution at a flow

rate of 0.5 mL/min. The R² values of the corresponding calibration curves in all cases were above 0.999 (data not shown). Sugars (glucose, fructose and sucrose) were also analysed by HPLC, at the same operating conditions, but using a Hi-Plex H column (Agilent).^[15] The pH was measured with a commercial pH meter (Basic 20 from Crison).

Evaluation of precipitation performance

The precipitation schemes employed were evaluated and compared in terms of SA recovery and material intensity and waste-related indicators. For SA recovery (R), the following equation was used:

$$R = \frac{\text{Recovered mass of SA}}{\text{Total mass of SA originally present}} \quad (1)$$

The material intensity and waste-related parameters of choice were mass intensity (MI) and E-factor (EF) and calculated as the quotient between the waste produced or the total mass used in a process or a process step divided by the mass of the product obtained,^[35] defined by Eqs. (2) and (3), respectively.

$$EF = \frac{\sum^w \text{aste mass}}{\sum^p \text{roduct mass}} \quad (2)$$

$$MI = \frac{\text{Total mass used in a process or process step}}{\text{Mass of final product}} \quad (3)$$

It should be noted that for the EF factor, the waste mass was defined as everything but the desired product and was estimated as the total amount of waste produced in the process excluding water. A higher EF means more waste and, hence, a greater negative environmental impact.^[36]

For the economic analysis, the cost in USD per kg of recovered SA was calculated using the following equation:

$$\text{Cost} = \frac{\sum(\text{Amount of reagent used} \times \text{unit cost})}{\text{SA recovery}} \quad (4)$$

where the amount of reagent used is given in kg or L (depending on the reagent) per litre of fermentation broth, the unit cost is given in USD per kg or L and the SA recovery is given in kg per litre of fermentation broth.

Results and discussion

Precipitation with calcium hydroxide

The main characteristics of the experiments performed are provided in Table 1. Four experiments (P1–P4) were initially carried out using the methodology described in the previous section in order to test the influence of the solution pH on the process performance in terms of SA, AA and FA recoveries.

According to the procedure, SA should be recovered in the second filtrate, after reaction of calcium succinate with the added sulphuric acid. The recovered masses of the compounds in each step (first filtrate, first WW, second filtrate and second WW) were calculated, and the data obtained was compared. As can be observed in Fig. 2, in all cases, a significant amount of the SA was “lost” already in the first filtrate together with the other two acids. A considerable amount of acids was also present in the first WW. As a consequence, in the second filtrate and second WW, the SA recovery was minimal. Notably, the highest SA recovery in the set of experiments was obtained in experiment P4, indicating that when sodium and calcium are both present in the solution, succinate has a lower tendency to precipitate with calcium.

Table 1. Summary of operating conditions and SA recovery for the performed experiments.

Method	Experiment	Description	SA recovery (%)
Calcium hydroxide	P1	Conditions as described in Section 2.3.	1.76
	P2	Same as P1, but targeted pH equal to 12, when adding Ca(OH) ₂	1.74
	P3	Same as P1, but targeted pH equal to 10, when adding Ca(OH) ₂	0.59
	P4	Same as P1, but pH was not adjusted	12.52
	P5	Used fermentation broth, extra acids were added, no NaOH was used. The same amount of Ca(OH) ₂ (as in P4) was added. Overnight cooling was performed. The first filtrate was not washed	26.35
	P6	Same as P5, but used five times the amount of Ca(OH) ₂	21.01
	P7	Same as P5, but used 10 times the amount of Ca(OH) ₂	26.67
	P8	Same as P7, but pH was adjusted to 6.8 with 5M NaOH	16.50
	P9	Used a model solution. No use of NaOH. The same amount of Ca(OH) ₂ (as in P4) was added. Filtrated immediately without cooling. No washing of the first filtrate was performed	33.67
	P10	Same as P9, but left for cooling overnight	24.08
	P11	Fermentation broth with extra acids. Left for shaking 24 hours at 37°C. The same amount of Ca(OH) ₂ (as in P5) was added	28.68
Magnesium hydroxide	P12	Same as P11, but used five times the amount of Ca(OH) ₂	24.52
	P13	Same as P11, but performed with a model solution	23.32
	P21	pH was adjusted to 6.8 with 5M NaOH. The amount of Mg(OH) ₂ added was equal to that of the SA present in the broth	53.96
Ammonia	P22	The solution pH was not modified after adding the extra acids, the amount of Mg(OH) ₂ was 2.5 times the one of SA	75.10
	P31	pH was adjusted to 6.8 with 5M NaOH, and titrated with 25% (w/w) ammonia-containing solution to pH 11	83.58
	P32	The pH of the solution was not modified after adding the extra acids and titrated with the same amount of ammonia as in P31	58.61

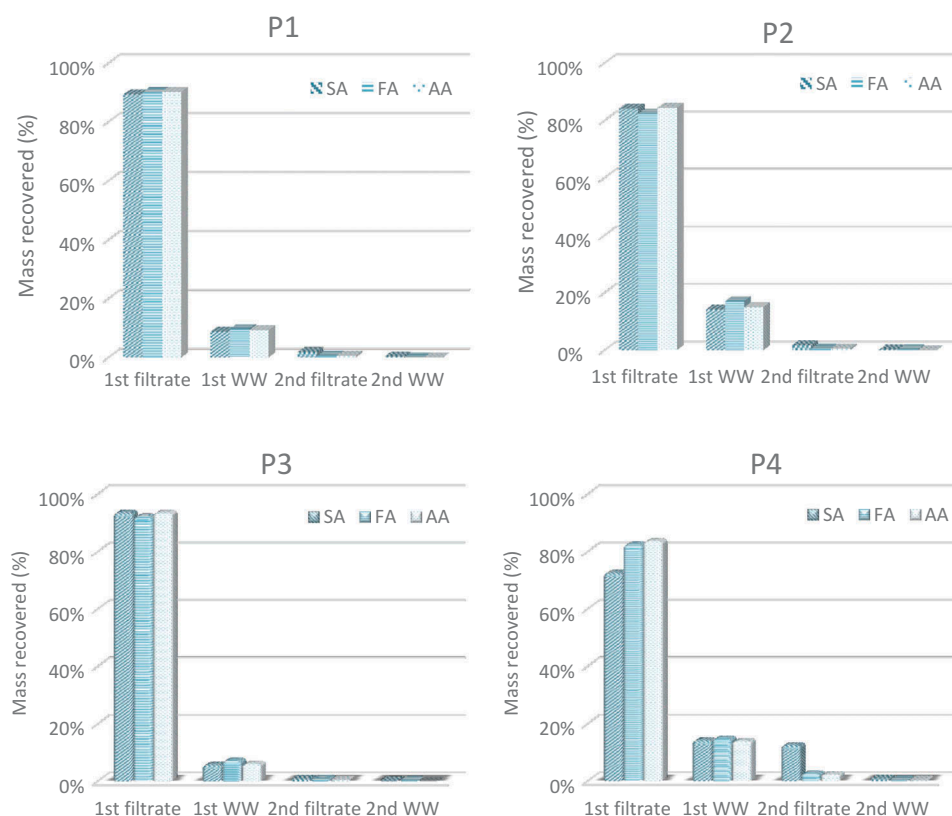


Figure 2. Percentages of the recovered acid masses during the $\text{Ca}(\text{OH})_2$ precipitation process for the first set of experiments (P1–P4).

The HPLC analyses showed that the individual concentrations of glucose, sucrose and fructose were all below 1.0 g/L (total sugar concentration of 0.5 g/L), which was anticipated since the SA fermentation stops due to sugar depletion. When sulphuric acid was added to the dissolved filter cake, the remaining sugars were dehydrated in a reaction that produces carbon and water,^[37] which practically eliminated them in the second filtrate (the total sugar concentration was below 0.1 g/L).

A second set of experiments was designed taking as a base experiment P4. In this case, the effect of temperature was evaluated, performing the first filtration after cooling the fermentation broth supernatant down to 4°C.^[27] It was also decided to omit the first washing of the precipitate formed and to increase the time between the addition of the $\text{Ca}(\text{OH})_2$ slurry and the subsequent filtration so the flasks were cooling during 16 hours before performing the first filtration.

Another variable that was investigated was the effect of the added amount of $\text{Ca}(\text{OH})_2$. Dedicated experiments were performed adding 5 and 10 times the amount of $\text{Ca}(\text{OH})_2$ supplied in experiment P4. Finally, to evaluate the possible precipitation interference impact of carob pod ingredients present, two experiments (P9 and P10) were performed using the

model solution described in the Materials section. The summary of the conditions applied for this second set of experiments (P5–P10) is presented in Table 1.

By employing the concentrations measured by HPLC, the recovery of each acid was calculated. As can be observed in Fig. 3, the biggest SA recovery occurred in the first filtrate, although there was still some recovery associated with the second filtrate. The increase in the SA recovered from the second filtrate ranged from 16% in experiment P8 to 33% in experiment P9. The lowest recovery was obtained in experiment P8, which was the only experiment where NaOH was added, suggesting that the presence of additional sodium ions interferes with the reaction of calcium ions with the succinate, thus diminishing its recovery. Indeed, the difference in recovery between experiments P7 and P8, which differ in the addition of sodium hydroxide, is approximately 10%. This is the same difference found between experiments P1 and P4, which difference is also due to the presence of sodium, indicating a consistent decrease in SA recovery when sodium is present in the solution.

Another interesting finding emerged comparing experiments P9 and P10, for which the only difference was that in P10 the solution was left for cooling at 4°C

overnight before performing the first filtration. Since the “lost” SA in P10’s first filtrate was proportionally larger than the SA amount lost in the same step for P9, it can be settled that the cooling-down step was not helpful to increase the degree of SA recovery.

Among the experiments performed with a real fermentation broth (P5–P8), the highest second filtrate recovery of SA was obtained in P7 (26.7%), which used 10 times the amount of $\text{Ca}(\text{OH})_2$ supplied to experiment P4. However, this percentage was very close to the 26.3% of SA recovery obtained for the same stage in experiment P5, which indicates that a large excess of calcium is not a determining factor to increase the degree of SA recovery. This 0.4% difference

in SA recovery suggests a high reproducibility of the results obtained, since experiments P5 and P7 differ only in the amount of calcium hydroxide added.

Despite the slight improvement in this new set of experiments, higher SA recoveries are desirable. Therefore, additional experiments (P11–P13; see Table 1) included an intermediate agitation step before performing the first filtration.^[22] This was performed by placing the flasks on a shaker at 37°C, where they were kept under agitation at 200 rpm during 20 hours. However, the results for this set of experiments (Fig. 4a) showed SA recoveries in the second filtrate of 28.7% for P11, 24.5% for P12 and 23.3% for P13, which were very similar to the results obtained in the previous set of experiments (P5–P10).

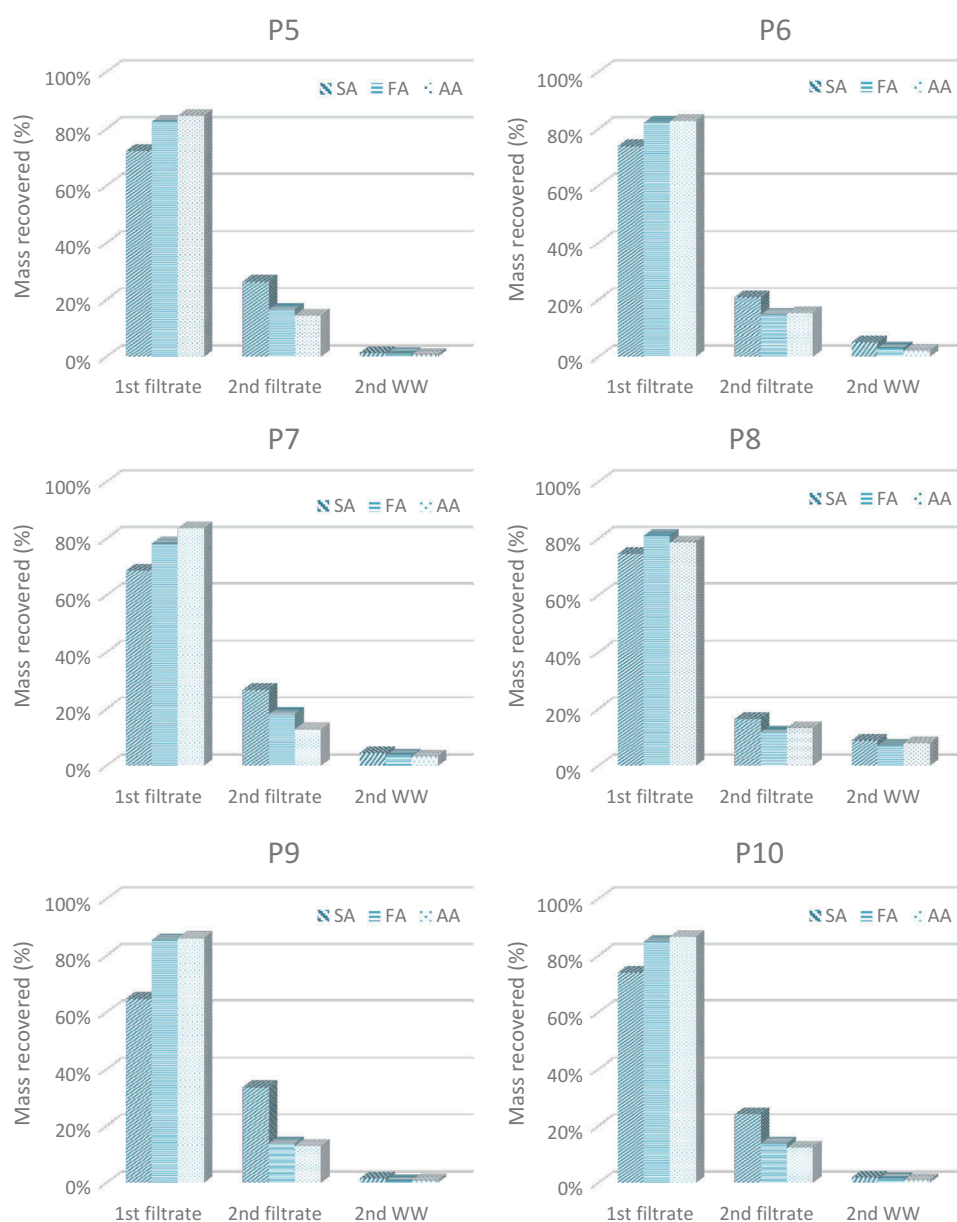


Figure 3. Percentages of the recovered acid masses during the $\text{Ca}(\text{OH})_2$ precipitation process for the second set of experiments (P5–P10).

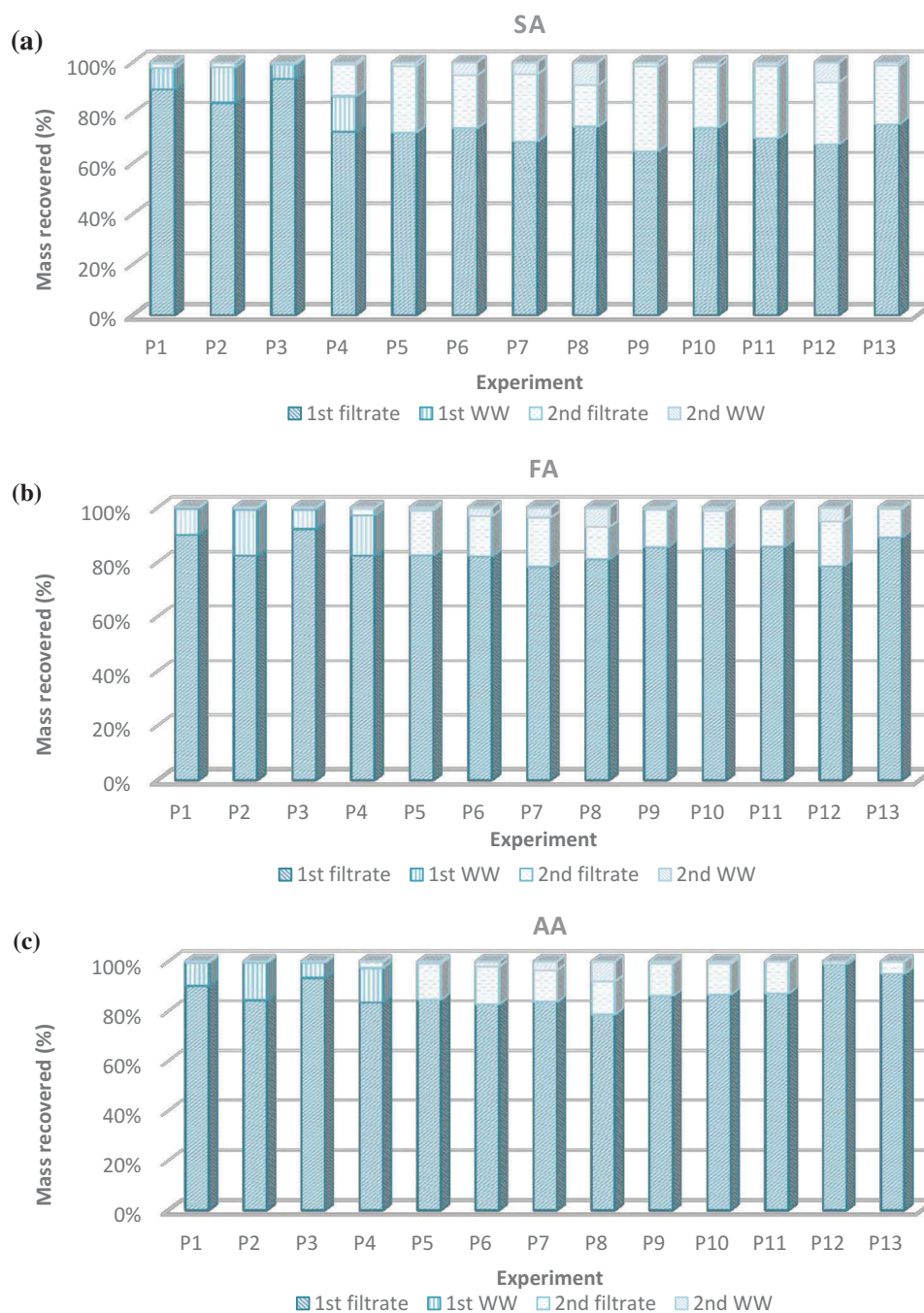


Figure 4. Succinic (a), formic (b) and acetic (c) acids recoveries after each stage of precipitation with $\text{Ca}(\text{OH})_2$ for the experiments performed.

The results for all $\text{Ca}(\text{OH})_2$ precipitation experiments are shown in Fig. 4. As can be observed in Fig. 4a, the highest succinate recovery (33.7%) corresponded to experiment P9, which was performed with a model solution and without any pH adjustment (initial pH equal to 3.68). With an approximately 5.0% less recovery, the second best result corresponded to experiment P11. These recoveries are in agreement with the 36% recovery obtained by Luque *et al.* when employing a modified calcium precipitation method.^[22] The

results obtained indicate that employing $\text{Ca}(\text{OH})_2$ as an SA precipitant in carob pod-based fermentation broths is not an efficient method. To be improved, the first filtrate could be eventually recycled to the fermentation vessel, as suggested by Datta *et al.*^[20]

Regarding the recoveries of the other two carboxylic acids, Figs. 4b and 4c show that practically in all experiments, around 80% of FA and AA was removed in the first filtrate, a higher percentage than for SA, which means that their preferential removal takes place. When

the first filter cake was washed (P1–P4), the remaining FA and AA were eliminated in the first WW, but in the rest of the experiments, they reached the product stream (second filtrate), so the calculated purity of SA in the referred stream was around 60% (w/w) on a dry basis.

Precipitation with magnesium hydroxide

Contrary to the $\text{Ca}(\text{OH})_2$ precipitation technique, where multiple experiments were performed aiming at eventually achieving the recoveries mentioned in the patent by Datta *et al.*,^[20] in the case of the technique with $\text{Mg}(\text{OH})_2$ only two experiments were performed since by following a procedure similar to the one reported by De Haan *et al.*,^[32] close SA recoveries were obtained.

Composition analysis of the redissolved crystals only resulted positive for SA. Sugar absence can be explained, as in the previous section, by their dehydration reaction with acid (in this case hydrochloric acid) and the subsequent formation of carbon.^[37] The absence of FA and AA could be explained by their high volatilities, which have caused them to evaporate together with water during the concentration process.

The SA recovery was calculated for the remaining liquid and the redissolved precipitate, and the results obtained are presented. As can be observed in Table 2, for both experiments, the SA recovered in the precipitate was more than 50% of the amount originally present, being 54% and 75% for P21 and P22, respectively. Additionally, the calculated SA purities in the precipitate were 21.9% for P21 and 13.3% for P22, and the precipitate did not contain either sugars or other carboxylic acids. Mass balances were performed for both experiments, resulting in 8.1% (P21) and 6.2% (P22) differences between the original amount of SA in the fermentation broth and the sum of the SA recovered from both crystals and the remaining solution.

These results are notable, especially when compared to those obtained in the experiments with calcium hydroxide. According to these recoveries, it proved highly beneficial to perform all the neutralization with $\text{Mg}(\text{OH})_2$ (and therefore employ a higher amount of it), instead of previously titrating the fermentation broth with another base, such as NaOH. De Haan *et al.*^[32] reported SA recoveries of 86% in the case of using $\text{Mg}(\text{OH})_2$ as a precipitant agent. Although these values are

higher than the ones obtained in the present study, it must be considered that the values reported by^[32] were for model solutions that only contained the substances of interest. It seems reasonable to anticipate lower SA recoveries and lower purities of the precipitate when a real fermentation broth, containing a variety of substances, is treated. Therefore, the results obtained in the present study clearly show that $\text{Mg}(\text{OH})_2$ is a potentially good candidate for the recovery of SA from carob pod-based fermentations. However, the magnesium-based precipitation requires heating of the solution to achieve complete salt dissolution, which should be taken into consideration for the cost analysis.

Precipitation with ammonia

The potential of using ammonia for SA recovery has been evaluated performing experiments P31 and P32 (Table 1). As previously performed, the SA recoveries were calculated for the remaining liquid and for the redissolved precipitate, and are presented in Table 2. The SA recovery was similar to the one obtained in the magnesium-based process (83.6 and 58.6% for P31 and P32, respectively). The composition analysis showed that, as in the magnesium-based process, the redissolved precipitate only contained SA among the analysed compounds (carboxylic acids and sugars).

It appears that titration of the fermentation broth with NaOH before adding the ammonia solution did not affect the process performance, since the highest SA recovery was obtained in experiment P31. However, the precipitation process yielded better results for experiment P32 since the precipitate purity was 48.7%, compared to 14.1% for P31, the former being the highest value obtained in all the precipitation tests performed. The mass balances for experiments P31 and P32 resulted, respectively, in 5.9 and 10.4% differences between the original SA mass present in the solutions compared to the SA mass recovered at the end of the experiments.

Yedur *et al.*^[34] employed ammonia as an SA precipitant using a real fermentation broth, although they did not clearly indicate the source of carbohydrates that had served as a feed, obtaining an SA recovery of 78%. In the present study, the obtained SA recovery of 83.6% (experiment P31) was noticeably higher, thus indicating that ammonia is a promising precipitant for SA recovery from carob pod-based fermentation broths. The diammonium sulphate fraction, which still contains a considerable amount of SA, can be directly used as fertilizer or thermally cracked into ammonia and ammonium bisulphate. Other potential advantages of this route, compared to the calcium-based SA precipitation option, are that ammonia is a relatively cheap

Table 2. Percentage of succinic acid recovered in the magnesium hydroxide and ammonia precipitation experiments.

Experiment	Magnesium hydroxide		Ammonia	
	P21	P22	P31	P32
Crystals	54.0%	75.1%	83.6%	58.6%
Liquid	46.0%	24.9%	16.4%	41.4%

industrial titrant and that a more valuable salt is co-produced. However, some reservations still remain since ammonia could become inhibitory for the SA-producing microorganisms and the salt co-product needs to be marketed. Additionally, significant amounts of SA still present in the di-ammonium sulphate-containing stream should be recovered to improve the overall process yield and economy.^[2]

Comparison of the precipitation techniques

Taking into account the promising results obtained with $Mg(OH)_2$ and ammonia, it was decided to compare the process performance not only on the basis of SA recovery, but also in terms of process sustainability (green) indicators such as EF and MI. The SA recoveries for the best cases obtained in each of the three precipitation techniques studied are presented in Fig. 5. As can be seen, the highest SA recovery was achieved through precipitation with ammonia, followed by the precipitation with magnesium hydroxide. The lowest SA recoveries were achieved with calcium hydroxide.

To determine and compare the process sustainability indicators, the following assumptions were made.

- Since all experiments were performed with identical initial volumetric amounts of fermentation broth, all calculations were performed without considering it as an input to the downstream process. As is common practice,^[36] water was not considered in the calculations.
- For the ammonia and magnesium precipitation experiments, only the dry mass corresponding to SA in the precipitates formed was considered as product and the mass of the rest of the precipitate was considered as waste. Additionally, the liquid

phase, from which the precipitates were recovered at the end of the experiments, was considered as waste.

For the calcium precipitation experiments, since the final product consisted of a diluted solution of succinic and other carboxylic acids, only the actual mass of SA was considered. As can be observed (Fig. 6), the EF value varied widely from 300 for experiment P1 to 9 for experiment P11. These EF numbers are within the range presented by Pinazo *et al.*^[31] for the production of SA from natural carbohydrates.

As mentioned, the higher the EF value, the higher is the associated negative environmental impact; therefore, the best results in terms of this indicator were obtained for experiments P11, P5 and P13.

Regarding the precipitation processes MI, the best result was obtained for experiment P11 (MI = 12), followed by P5 and P22 (MI equal to 14 and 15, respectively). According to this parameter, it appears that magnesium hydroxide precipitation is the best technique among the three techniques that were evaluated.

A preliminary economic analysis was also performed for the experiments with the highest SA recovery of each technique. For each technique, the costs of the chemicals involved obtained from commercial suppliers for industrial-grade purities^[38] were taken into account, and the results obtained (see Table 3) showed that the precipitation with magnesium hydroxide would have the lowest cost of the three techniques, less than half the cost for recovering 1 kg of SA via precipitation with calcium hydroxide. The most expensive method will be precipitation with ammonia, at around five times the cost estimated for the magnesium hydroxide-based technique.

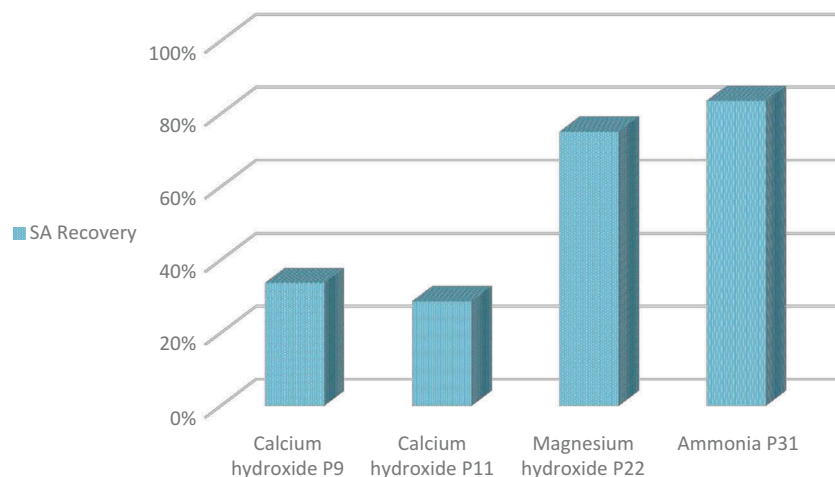


Figure 5. Comparison of succinic acid recoveries for representative cases of the three precipitation techniques.

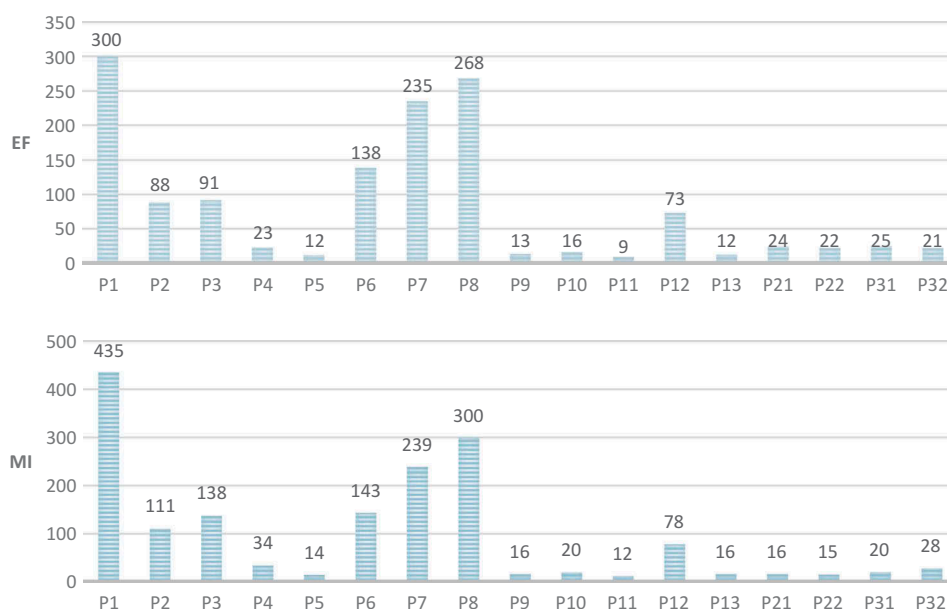


Figure 6. E-Factor and mass intensity (MI) parameters obtained in the experiments.

Table 3. Preliminary operating cost estimation for alternative SA downstream options.

Method	Cost (€/kg SA recovered)
Precipitation with calcium hydroxide	6.86
Precipitation with magnesium hydroxide	2.72
Precipitation with ammonia	14.30

Conclusion

- The highest SA recovery (83%) and purity (48.7%) were achieved employing ammonia-based downstream.
- Inclusion of an evaporation step eliminated FA and AA present in the broth.
- The lowest EF and MI values were obtained for the magnesium-based downstream strategy, followed by the ammonia-based strategy.
- A preliminary economic analysis suggests that the cost per kg of SA recovered would be significantly lower for the magnesium-based treatment compared to the ammonia-based treatment. Since a reasonably high SA recovery (75%) can be achieved by this treatment option, it appears as the most feasible option for carob pod fermentation broths, containing SA as the main product.

It can be anticipated that materials recycle and/or commercialization of by-products will also play an important role when selecting the most appropriate downstream strategy for a defined case. Further SA enrichment/purification can be achieved, depending on the requirements of the specific application, through

more selective separation techniques, including chromatography and/or membrane-assisted treatment.

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