

Article

Evaluation of Ionic Liquids as In Situ Extraction Agents during the Alcoholic Fermentation of Carob Pod Extracts

Sergio Sanchez-Segado ^{1,*}, María José Salar-García ^{2,*}, Víctor Manuel Ortiz-Martínez ^{2,*},
Antonia Pérez de los Ríos ^{2,*}, Francisco José Hernández-Fernández ^{2,*} and
Luis Javier Lozano-Blanco ^{1,*}

¹ Department of Chemical and Environmental Engineering, Technical University of Cartagena, Campus Muralla del Mar C/Doctor Fleming s/n, Cartagena, 30202 Murcia, Spain

² Department of Chemical Engineering, Faculty of Chemistry, University of Murcia, 30071 Murcia, Spain

* Correspondence: sergio.segado@upct.es (S.S.-S.); msg53090@um.es (M.J.S.-G.); victormanuel.ortiz@um.es (V.M.O.-M.); aprios@um.es (A.P.d.I.R.); fjhernan@um.es (F.J.H.-F.); luisja.lozano@upct.es (L.J.L.-B.)

Received: 18 July 2019; Accepted: 15 October 2019; Published: 18 October 2019



Abstract: Anhydrous ethanol is a promising alternative to gasoline in fuel engines. However, since ethanol forms an azeotrope with water, high-energy-consumption separation techniques such as azeotropic distillation, extractive distillation, and molecular sieves are needed to produce anhydrous ethanol. This work discusses the potential development of an integrated process for bioethanol production using ionic liquids and *Ceratonia siliqua* as a carbohydrate source for further fermentation of the aqueous extracts. A four-stage counter-current system was designed to improve the sugar extraction yield to values close to 99%. The alcoholic fermentation of the extracts showed ethanol concentrations of 95 g/L using the microorganism *Saccharomyces cerevisiae*. The production of anhydrous ethanol through extractive distillation with ethylene glycol was simulated using CHEMCAD software, with an energy consumption of 13.23 MJ/Kg of anhydrous ethanol. Finally, several ionic liquids were analyzed and are proposed as potential solvents for the recovery of bioethanol for the design of an integrated extraction–fermentation–separation process, according to their ability to extract ethanol from aqueous solutions and their biocompatibility with the microorganism used in this study.

Keywords: carob pod; bioethanol; extractive distillation; ionic liquids; biocompatibility; liquid–liquid extraction

1. Introduction

The global demand of energy is expected to rise sharply in the next decades as a consequence of the increase in worldwide population. The energy demand is projected to increase by 50% or more by 2030 [1]. The depletion of conventional fossil fuel reserves together with the growing concerns for environmental protection have aroused considerable interest in promoting alternative and renewable sources of energy. The shift towards more sustainable energy sources is thus unavoidable, and in this context, biofuels are seen as promising candidates to replace fossil fuels in the short term [2]. Biofuels containing energy from geologically recent carbon fixation offer environmental benefits as their employment reduces the atmospheric dioxide carbon concentration, the emission of hydrocarbons and particulate matter, and the discharge of sulfur compounds [3].

Bioethanol is especially attractive as an alternative to fossil fuels, reason why its global production has significantly increased in the last years. Bioethanol is an environment-friendly oxygenated fuel containing over 34% of oxygen, with enhanced combustion efficiency (15% higher) in comparison to

gasoline. The renewable sources for bioethanol generation largely include sugars, starch, lignocellulosic biomass, and algae [4,5]. While third-generation bioethanol obtained from algal biomass is still under development and has been demonstrated at laboratory scale, sugars, starch, and lignocellulosic biomass have already shown their potential as commercial feedstock for bioethanol. Nevertheless, their treatment requires different approaches, since raw materials such as cane need a single extraction process to obtain fermentable sugars, while starchy crops such as wheat and corn require a previous hydrolysis stage to transform starch into sugar [6–8].

One of the agricultural crops with high carbohydrate content is *Ceratonia siliqua*, also known as carob [9,10]. The carob tree is native of the Mediterranean region, and its cultivation has been promoted for the revitalization of coastal agriculture in dryland areas [11]. The average worldwide production of carob pods for the period 2010–2013 was 165,990 tons, and the main producers were Spain (26.31%), Italy (16.62%), Portugal (13.77%), Morocco (12.27%), Greece (12.05%), and Turkey (8.53%) [10].

Carob is a drought-resistance evergreen tree requiring low maintenance and producing several products like seeds and pods. The carob pod can be employed for animal feeding or to produce carob powder that in turn can be exploited for human consumption. However, the high content of tannin in carob pods greatly limits this application. Carob pods can be also used for bioethanol production through fermentation with *Saccharomyces cerevisiae*, as has been shown in previous works. Sugars need to be extracted from carob pods, and this can be achieved with water extraction before the subsequent fermentation process [12].

On the other hand, the energy requirements in the process of bioethanol production offer a future challenge. Bioalcohol needs to be separated from the aqueous medium in which fermentation is performed [13,14]. To achieve the desired purity of bioethanol, distillation is usually employed, but this stage is highly energy-consuming. Liquid–liquid extraction of bioethanol has been proposed as an alternative method, but the solvent used has to display at the same time water immiscibility and high polarity for the extraction of alcohol molecules. Both features are rarely found in conventional organics. Unique compounds such as ionic liquids (ILs), however, have shown a promising capacity to separate polar substances such as ethanol from aqueous media [15].

Room-temperature ionic liquids (RTILs) are organic salts usually formed by an organic cation and an inorganic anion that remain in the liquid state at room temperature. RTILs offer advantageous properties versus organic solvents, such as negligible vapor pressure, physical-chemical tunability, and non-flammability [16,17]. Because of these features, they are regarded as a real option to substitute organic solvents in liquid–liquid extraction processes both in bulk form and in membrane-based technologies [18,19].

This work discusses the potential development of an integrated process for bioethanol production using ionic liquids and *C. siliqua* as bioethanol feedstock. Firstly, the sugar extraction process from carob pods is analyzed. Sugar extraction was performed at different liquid/solid ratios (L/S) for 30 min at 25 °C. A four-stage counter-current system was designed to improve the sugar extraction efficiency from carob pods. After the fermentation of carob pod extracts with *S. cerevisiae*, bioethanol recovery was approached through simulation tests. Finally, several ionic liquids are proposed and analyzed as potential solvents to recover bioethanol for the design of an integrated extraction–fermentation–separation process.

2. Materials and Methods

2.1. Characterization of the Carob Pod

Carob pods purchased from a local supplier were physically and chemically characterized. The physical characterization consisted in the determination of their particle size distribution by sieve analysis. For that, 100 g of carob pod was sieved using for 10 min a set of sieves with 2.0, 1.0, 0.5, 0.2, and 0.1 mm mesh opening and a vibrating sieve machine. Sugar, ash, protein, and fibre analyses were carried out according to the methodology proposed by Mahtout et al. [20].

2.2. Sugar Extraction

The sugar extraction was carried out using 250 g of carob pod and 750 mL of water at a fix stirring speed of 1000 r.p.m in a stirred tank reactor at room temperature. The as-received carob pod and the average size fraction of the sieved carob pod (D50) were tested until the extraction yield was constant, with the aim to study the influence of the particle size over the extraction time and to ascertain a suitable particle size for the as-received material. Samples were withdrawn at periodic time intervals to analyze the total sugars content.

Extraction System with Alternate Washing Steps

A four-stage counter-current system was designed to improve the sugar extraction efficiency from carob pod. Different L/S ratios were tested according to the operation conditions described in Table 1, using the D50 fraction. Liquid–solid separation was carried out using a 0.05 mm sieve under vibration for 5 min.

Table 1. Operation conditions for the four-stage counter-current system. L/S: liquid/solid ratios.

Experiment	Carob Pod (g) Each Stage	Water (mL) Each Stage	L/S Ratio	Temperature (°C)	Time (mins)	Stirring Speed (r.p.m.)
1	250	500	2.00	25	30	1000
2	100	375	3.75	25	30	1000

2.3. Fermentation Test

The anaerobic fermentation was carried out in a 3 L fermenter with several sampling devices and temperature and stirring control. The aqueous extract from the sugar extraction tests with a concentration of 200 g/L was used as fermentation medium supplemented with ammonium phosphate (3.2 g/L), potassium sulphate (1 g/L), and magnesium sulphate (1.8 g/L). The pH was adjusted to 3.5–4, using diluted sulphuric acid. The resulting solution was sterilized by heating up to its boiling point and then cooled at 35 °C. This solution was fed into the fermenter at a fixed temperature of 35 °C and at a fix stirring speed of 125 r.p.m. Free cells of *S. cerevisiae* at concentration of 10, 15, and 25 g/L were used for ethanol production. The evolution of the fermentation process was determined by density measurement of the hydro-alcoholic solutions obtained and by gas chromatography using an HP-INNOWAX column (30 m × 0.53 mm × 0.25 µm, Agilent). A medium consisting of 200 g/L of pure saccharose prepared in the same conditions as described above and 15 g/L of *S. cerevisiae* cells was used as a control test.

2.4. Ethanol Recovery Simulation

Ethanol recovery from the fermentation broth through extractive distillation with ethylene glycol (EG) was simulated using CHEMCAD software and the NRTL thermodynamic model, according to the flowsheet shown in Figure 1. The ethanol/water mixture with 10% mol of ethanol was distilled in the column C1 to produce an ethanol/water mixture with azeotropic composition.

Ethanol was dehydrated in column C2 with the aid of EG and recovered as a head product. The bottom product of column C2 was expanded from 100 to 26.34 kPa and fed into column C3 for EG recovery as a bottom product. The decision variables used in the simulation after sensitivity analysis of the simulation flowsheet in Figure 1 are shown in Table 2.

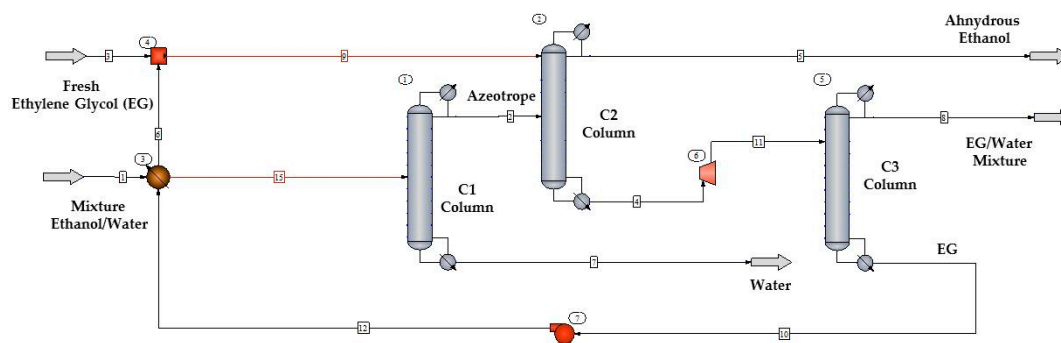


Figure 1. Extractive ethanol recovery process flowsheet.

Table 2. Decision variables used in the simulation.

Stream	Ethanol (kmol/h)	Water (kmol/h)	EG (kmol/h)	Temperature (°C)	Pressure (kPa)
Ethanol/Water mixture	10.00	90.00	0.00	25	100
Fresh EG	0.00	0.00	3.70	80	100
Column	N° Stages	Feed Stage	Bottom Water (kmol/h)	Bottom EG (kmol/h)	Reflux ratio
C1	30	15	88.54	0.00	8.00
C2	30	Azeotrope (15) EG (3)	1.56	73.31	0.5
C2	12	6	0.00	69.65	1.00

2.5. Evaluation of Ionic Liquids as Potential Solvents for the Development of In Situ Alcoholic Fermentation Processes

In order to address the use of ILs as in situ extraction agents to recover ethanol from fermentation broths, it is required to study their water solubility, microorganism biocompatibility, and ethanol extraction power. The ionic liquids used in this study were: 1-octyl-3-methylimidazolium{Bis(trifluoromethyl)sulfonyl}imide[OMIM⁺][NTf₂⁻], Methyltrioctylammonium{Bis(trifluoromethyl)sulfonyl}imide[MTOA⁺][NTf₂⁻], 1-butyl-3-methylimidazolium{Bis(trifluoromethyl)sulfonyl}imide[BMIM⁺][NTf₂⁻], 1-butyl-3-methylimidazolium hexafluorophosphate[BMIM⁺][PF₆⁻], 1-octyl-3-methylimidazolium tetrafluoroborate[OMIM⁺][BF₄⁻], tetradecyl(trihexyl)phosphonium dicyanamide [Hex₃TDP⁺][dca⁻], tetradecyl(trihexyl)phosphonium bromide [Hex₃TDP⁺][Br⁻], tetradecyl(trihexyl)phosphonium chloride [Hex₃TDP⁺][Cl⁻], ethylpyridinium {bis(trifluoromethyl)sulfonyl}imide [EPy⁺][NTf₂⁻]. These liquids were used as received without further purification.

2.5.1. Water Solubility Tests

Water solubility tests were carried out using the cloud-point method. For that, 2 μL of IL was added to 1 mL of water until complete dissolution after vigorous stirring. The additions stopped when saturation was not reached after adding more than 100 μL of IL.

2.5.2. Ionic Liquids Biocompatibility with *S. cerevisiae*

Ionic liquids biocompatibility was measured through growth inhibition in liquid medium and the agar diffusion test. For the growth inhibition tests, a liquid culture medium composed of D-glucose (20 g/L), peptone (20 g/L), yeast extract (10 g/L), and 3% (v/v) of ionic liquid was used. The ionic liquid content of the liquid medium was selected on the basis of previous research works, which report that a significant toxicity is observed for concentrations of ILs ranged between 2% and 5% (v/v) [21,22]. The liquid medium with 1 g/L of *S. cerevisiae* was incubated at 30 °C with continuous shaking. Samples were withdrawn at regular time intervals to measure the OD₆₆₀ using a 1650 PC Shimadzu

UV–Vis spectrophotometer and calculate the specific growth rate by selecting two time points in the exponential growth phase.

For the agar diffusion tests, a solid medium with the same composition mentioned above was placed in a Petri dish. Wells of 6 mm diameter were impregnated with 50 µL of each IL in sterile conditions and placed over dish. The radius of the inhibition zone around the wells was recorded.

2.5.3. Ethanol Extraction Tests

For the extraction tests, a 10% v/v ethanol aqueous solution was put in contact with the same volume of water-insoluble IL at 30 °C. The mixture was shaken for 2 min to facilitate ethanol transfer into the IL phase and left at a constant temperature to complete the phase separation. Samples from the aqueous phase were taken at 24, 48 h, and 21 days and analyzed through gas chromatography as described in Section 2.3. The ethanol extraction percentage was calculated according to Equation (1).

$$E(\%) = C_{IL} (C_{IL} + C_W)^{-1} \tag{1}$$

where C_{IL} and C_W are the ethanol equilibrium concentrations in the ionic liquid and the aqueous phase, respectively. Measurements were carried out in triplicate, and the ethanol content in the aqueous phase was determined by gas chromatography following the same procedure described above. The ethanol concentration in the ionic liquid was determined by taking the difference between the initial and the final ethanol concentrations in the aqueous phase.

3. Results and Discussion

3.1. Physical and Chemical Characterization of Carob Pod

The sieve analysis results are shown in Figure 2. As can be seen, the average calculated particle size was 0.57mm. The chemical analysis of the as-received carob pod is presented in Table 3.

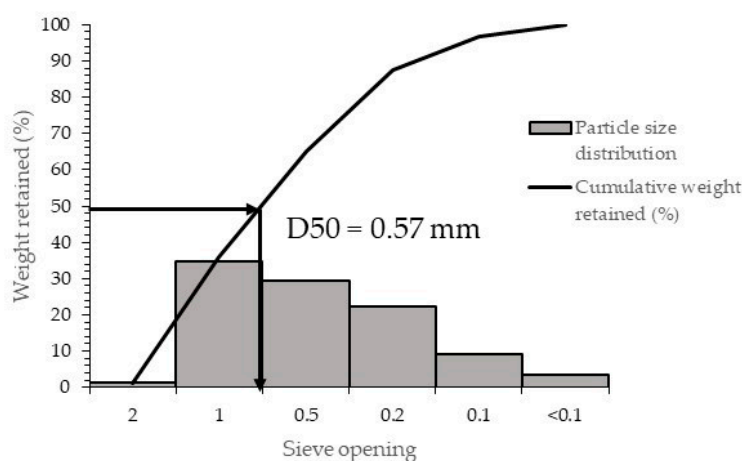


Figure 2. Particle size distribution of the as-received carob pod.

Table 3. Chemical analysis of the carob pod. The results are expressed as %w/w.

Humidity	Ash	Fibre	Protein	Total Sugars	Reducing Sugars
14.41 ± 1.90	3.30 ± 0.46	12.24 ± 2.45	4.15 ± 0.93	49.50 ± 7.60	28.35 ± 2.93

The results presented in Table 3 show that the carob pod is a carbohydrate-rich material and thus a valuable feedstock for fermentation processes after sugar extraction. The feasibility of the extraction of sugars from carob pod is discussed in the following section.

3.2. Sugar Extraction

The extraction results are presented in Figure 3. The time required to achieve total sugars extraction from the as-received material was approximately 360 min, in contrast with the 30 min required for extraction from the D50 fraction. This result points out the suitability of the D50 particle size to carry out the sugar extraction process.

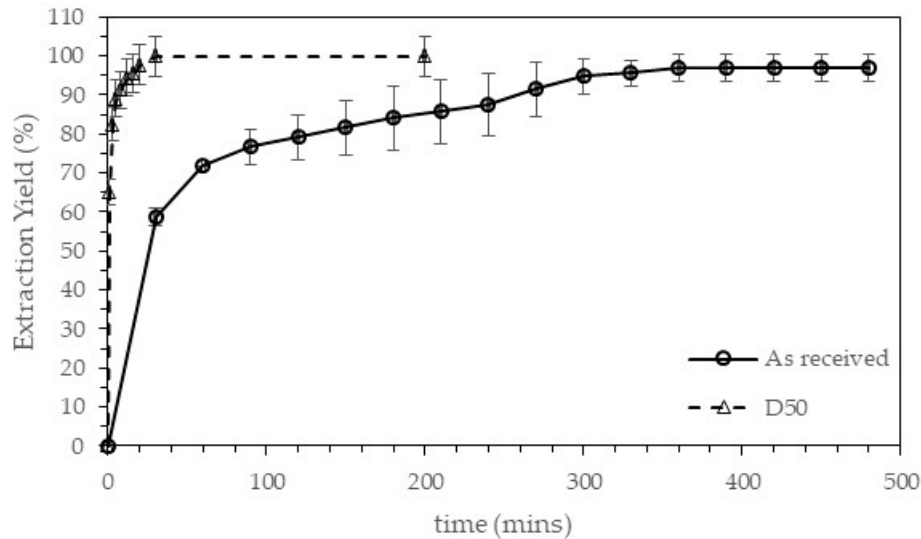


Figure 3. Sugar extraction from carob pod.

These differences are attributed to the better solid–liquid contact and the lower sugar diffusion resistance compared to the bulk solution when the particle size is small [10–12]. However, solid swelling was observed during the extraction process; therefore, the solution retention by the solid waste after solid–liquid separation was high, leading to significant sugar losses. To solve this problem, an extraction system with alternate washing steps was designed, as shown in Figure 4.

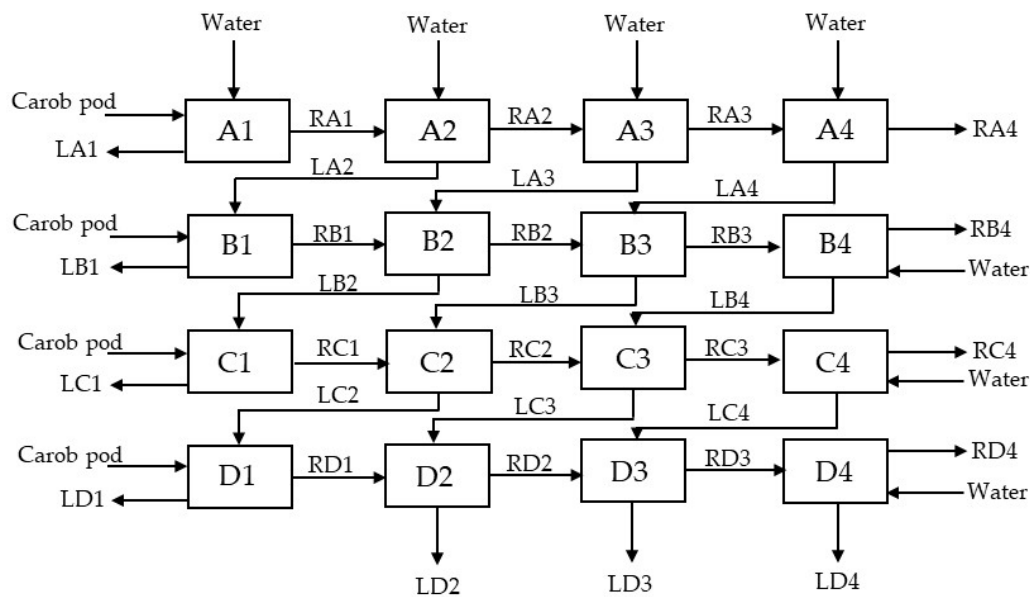


Figure 4. Sugar extraction system with alternate washing steps. L lines represent the flow of the liquid solution. R lines represent the flow of the solid waste.

Two full sets of experiments in the conditions described in Table 1 were carried out with the aim of achieving sugar losses lower than 10%. The results of each set are presented in Table 4.

Table 4. Counter-current experiments results.

Experiment	Total Sugars Feed (g)	Total Sugars in L1 Streams (g)	Total Sugars in LD2-LD4 Streams (g)	Total Sugars in Solid Residues RA4-RD4 (g)	Extraction Yield (%)
1	495.0± 37.6	171.7± 7.7	240.1± 9.6	82.2± 7.2	83.2
2	198± 15.1	142.4± 6.5	52.9± 2.1	3.1± 0.3	98.6

For experiment 1 using an L/S ratio of 2.00, the concentrations of the combined liquid streams LA1 plus LD1 and LD2 plus LD4 were 301.24 g/L and 159.51 g/L, respectively. Both combined streams were suitable to be fed to the fermenter for bioethanol production. However, even after the washing steps, sugar losses in the solid residue accounted for more than 16% of the total sugars fed into the system. On the other hand, in experiment 2, the concentrations of the combined streams LA1 plus LD1 and LD2 plus LD4 were 168 g/L and 48 g/L, respectively. From an industrial point of view, the 168 g/L stream can be directly fed to the fermenter, while the 48 g/L stream can be used as a pre-culture medium for the bioethanol-producing microorganisms. In addition, the use of an L/S ratio of 3.75 seems a more sensible approach because of the higher extraction yield. However, sugar concentrations close to 200 g/L are preferred for fermentation, that is why an L/S ratio of 3.00 was used for the fermentation tests.

3.3. Fermentation of Carob Pod Extracts

The ethanol concentration increased during fermentation until a maximum of 95 g/L was reached after 20 h, independently of the yeast concentration, as shown in Figure 5. Higher ethanol productivities were achieved at higher yeast concentrations. However, after 15 h of fermentation, the productivity value remained constant at 4 g/L*h in all cases. This behaviour can be attributed to the ethanol inhibitory effects on the free cells.

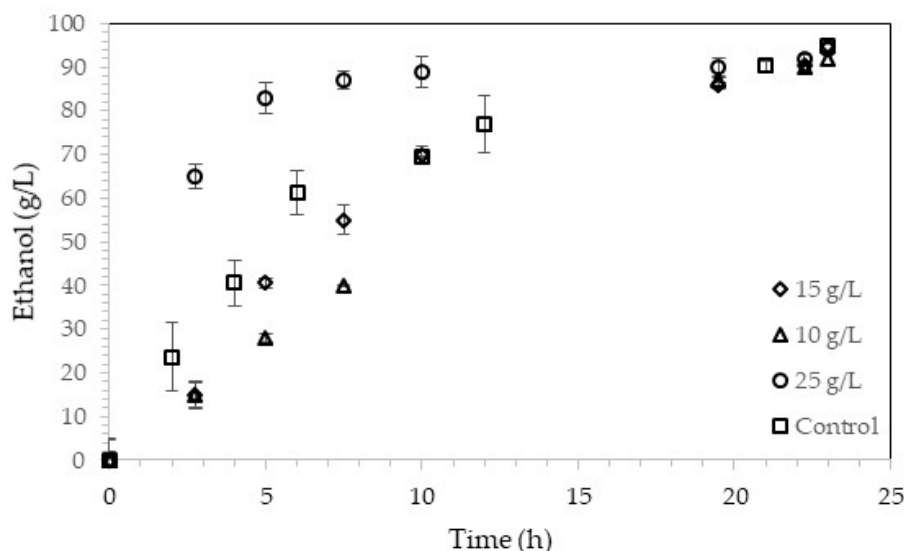


Figure 5. Fermentation kinetics of the aqueous extracts with free cells of *Saccharomyces cerevisiae*.

3.4. Ethanol Recovery

Extractive distillation with ethylene glycol is one of the most common industrial methods for the dehydration of ethanol [23]. The results obtained according to the process flowsheet and process parameters mentioned in Section 2.4 are presented in Tables 5 and 6.

Table 5. Stream composition.

Stream	Total Flow (Kmol/h)	Stream Composition (Mole Fraction)		
		Ethanol	Water	EG
Fresh EG	1.00	0.000	0.000	1.000
Mixture Ethanol/Water	100.00	0.100	0.900	0.000
Azeotrope	11.41	0.868	0.132	0.000
Water	88.63	0.001	0.999	0.000
Anhydrous Ethanol	9.63	0.996	0.002	0.002
EG/Water Mixture	5.47	0.060	0.272	0.669
EG	69.72	0.000	0.010	0.999

Table 6. Energy requirements.

Column	Energy Requirements (MJ/h)		
	Reboiler Duty	Condenser Duty	Specific Energy Requirements (Reboiler Duty/Kg Anhydrous Ethanol)
C1	3638.54	4012.04	8.23
C2	1936.73	561.00	4.38
C3	262.77	669.34	0.59

In Table 7, a summary of the energy consumed per Kg of anhydrous ethanol is presented for the traditional distillation and dehydration processes used in the ethanol industry.

Table 7. Energy requirements for traditional industrial ethanol distillation and dehydration processes [23].

Process	Energy Requirements (MJ/Kg)
Low pressure distillation	11.72
Azeotropic distillation	
Pentane	10.05
Benzene	15.49
Diethyl ether	12.56
Extractive distillation	
Gasoline	9.21
Ethylene Glycol	18.84

A typical extractive distillation with gasoline or ethylene glycol has an energy requirement ranging from 9.21 to 18.84 MJ/Kg of anhydrous ethanol, on the basis of the energy required in the columns reboiler [23,24]. In our process, the energy consumption was 13.23 MJ/Kg of anhydrous ethanol, which within the normal range. Moreover, as can be seen in Table 6, the fractional distillation carried out in column C1, accounted for the 62% of the energy required to produce 1 Kg of anhydrous ethanol as a consequence of the formation of the water–ethanol azeotrope. This fact highlights the need to develop in situ extraction processes to recover ethanol using suitable solvents for further energy-efficient ethanol distillation.

3.5. Evaluation of Ionic Liquids as Potential Solvents for the Development of In Situ Alcoholic Fermentation Processes

3.5.1. Water Solubility Tests

The water solubility of a wide variety of ILs with different anion and cation structures was measured. The results showed that the anion structure strongly affects the water solubility of an ionic liquid, since this parameter changes significantly for ILs containing the same cation but a different anion. In this case, the water solubility showed the following trend: $[dca^-]$, $[Cl^-] > [BF_4^-] > [PF_6^-] > [NTf_2^-]$. Regarding the cation, the longer the alkyl chain, the lower the water solubility of the IL.

For instance, in the case of imidazolium-based ionic liquids, the water solubility according to the length of the alkyl chain of the cation shows the following trend: $[\text{bmim}^+][\text{NTf}_2^-] > [\text{omim}^+][\text{NTf}_2^-]$, $[\text{bmim}^+][\text{PF}_6^-] > [\text{omim}^+][\text{PF}_6^-]$, and $[\text{bmim}^+][\text{BF}_4^-] > [\text{omim}^+][\text{BF}_4^-]$. Table 8 summarizes the ILs considered to be non-water-soluble, whose water solubility is lower than 10 % ($V_{\text{IL}}/V_{\text{water}}$).

Table 8. Selection of ionic liquids and their water solubility.

Ionic Liquid	Abbreviation	Solubility (% $V_{\text{IL}}/V_{\text{Water}}$)
1-octyl-3-methylimidazolium {Bis(trifluoromethyl)sulfonyl}imide	$[\text{OMIM}^+][\text{NTf}_2^-]$	<0.04
Methyltrioctylammonium {Bis(trifluoromethyl)sulfonyl}imide	$[\text{MTOA}^+][\text{NTf}_2^-]$	<0.02
1-butyl-3-methylimidazolium {Bis(trifluoromethyl)sulfonyl}imide	$[\text{BMIM}^+][\text{NTf}_2^-]$	<0.28
1-butyl-3-methylimidazolium hexafluorophosphate	$[\text{BMIM}^+][\text{PF}_6^-]$	<0.50
1-octyl-3-methylimidazolium tetrafluoroborate	$[\text{OMIM}^+][\text{BF}_4^-]$	<1.4
Tetradecyl(trihexyl)phosphonium dicyanamide	$[\text{Hex}_3\text{TDP}^+][\text{dca}^-]$	<2
Tetradecyl(trihexyl)phosphonium bromide	$[\text{Hex}_3\text{TDP}^+][\text{Br}^-]$	<2
Tetradecyl(trihexyl)phosphonium chloride	$[\text{Hex}_3\text{TDP}^+][\text{Cl}^-]$	<2
Ethylpyridinium{Bis(trifluoromethyl)sulfonyl}imide	$[\text{EPy}^+][\text{NTf}_2^-]$	<2

3.5.2. Ionic Liquids Biocompatibility with *S. cerevisiae*

As can be seen in Figure 6, the growth rate of the yeast in the presence of $[\text{MTOA}^+][\text{NTf}_2^-]$ and $[\text{BMIM}^+][\text{NTf}_2^-]$ (0.47 h^{-1} and 0.30 h^{-1} , respectively) was very similar to that observed in the absence of the IL (0.43 h^{-1}). By contrast, the growth rate of *S. cerevisiae* in the presence of $[\text{Hex}_3\text{TDP}^+][\text{Cl}^-]$, $[\text{Hex}_3\text{TDP}^+][\text{Br}^-]$, $[\text{EPy}^+][\text{NTf}_2^-]$, $[\text{OMIM}^+][\text{BF}_4^-]$, and $[\text{Hex}_3\text{TDP}^+][\text{dca}^-]$ was zero or near zero. The results show that ionic liquids containing imidazolium cations along with hydrophobic anions, such as $[\text{PF}_6^-]$ and $[\text{NTf}_2^-]$, allow the yeast to grow normally. On the contrary, ionic liquids combined with more hydrophilic anions, such as $[\text{BF}_4^-]$, inhibit the growth of *S. cerevisiae*, showing toxicity toward this microorganism. Regarding pyridinium- and phosphonium-based ILs such as $[\text{EPy}^+][\text{NTf}_2^-]$, $[\text{Hex}_3\text{TDP}^+][\text{Cl}^-]$, $[\text{Hex}_3\text{TDP}^+][\text{Br}^-]$, and $[\text{Hex}_3\text{TDP}^+][\text{dca}^-]$, they exhibited low biocompatibility with *S. cerevisiae*, hindering its growth. The results show that the higher the water solubility of the IL, the higher its interaction with the microorganism, and therefore, the lower the biocompatibility. Besides water solubility, ILs structure has also significant influence on their toxicity to microorganism. For this reason, the selection of the optimum IL to perform ethanol extraction is a compromise between low water solubility and an appropriate structure of the IL. According to the different anions analyzed, $[\text{NTf}_2^-]$ showed higher biocompatibility than $[\text{PF}_6^-]$ and $[\text{BF}_4^-]$.

In line with the toxicity results shown in this work, Pfruender et al. reported the biocompatibility of water-immiscible ILs such as $[\text{MTOA}^+][\text{NTf}_2^-]$, $[\text{BMIM}^+][\text{NTf}_2^-]$, and $[\text{BMIM}^+][\text{PF}_6^-]$ with *S. cerevisiae* by using viability assays performed after incubation of 20 g L^{-1} of the yeast strain FasB His6 at $27 \text{ }^\circ\text{C}$ at 300 rpm during 20 h [25]. Other non-water-soluble ILs have also been employed to increase the 2-phenylethanol concentration in biphasic systems by using *S. cerevisiae* as an in situ product removal catalyst. After analyzing the effect of the IL structure on the yeast growth, it was concluded that ILs containing $[\text{NTf}_2^-]$ anions are more biocompatible than those containing $[\text{PF}_6^-]$ or $[\text{BF}_4^-]$. On the other hand, it was also observed that the biocompatibility of the IL with the yeast decreases as the length of the alkyl side chain on the imidazolium ring increases [26]. All these results are in good agreement with those reported in this work.

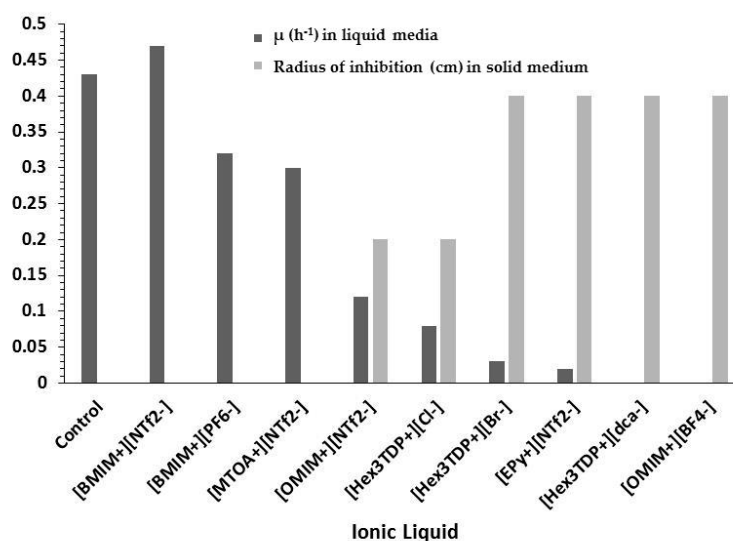


Figure 6. Growth rate of *S. cerevisiae* in liquid media with 3% of ionic liquid (v/v) (■) and radius of inhibition in solid media (■).

3.5.3. Ethanol Extraction Tests

The percentage of ethanol extracted from an aqueous solution with 10% (v/v) of ethanol by using the non-water-soluble ionic liquids selected is shown in Table 7. Figure 7 depicts the evolution of the extraction percentage obtained with each IL over time, measured after 1, 2, and 21 days.

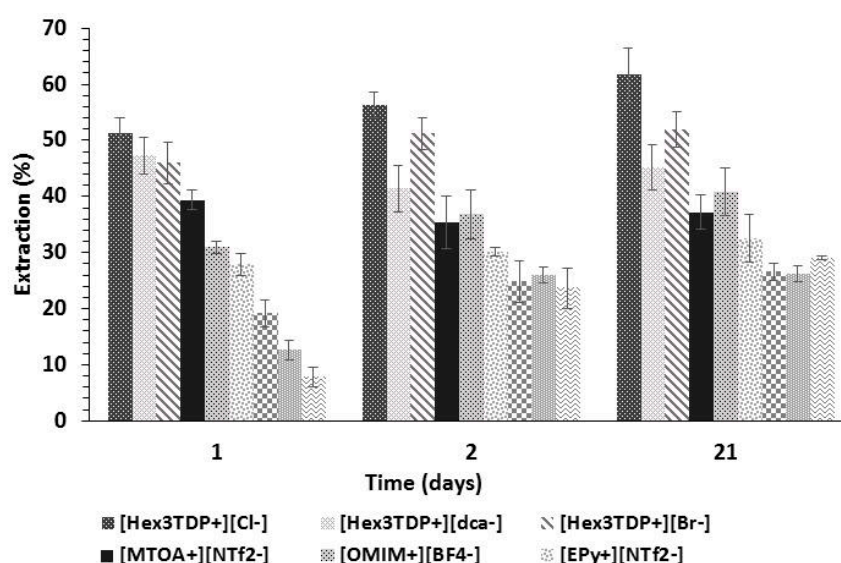


Figure 7. Percentage of ethanol extracted over time by using the water-immiscible ionic liquids selected.

The results of the extraction process showed that the ionic liquids containing phosphonium- or ammonium-based cations were capable of extracting higher amounts of ethanol than the rest of ionic liquids after 1 day of operating time (51.3% and 39.4%, respectively). Similar results were obtained by Neves et al., who also reported high values of ethanol extraction by tetradecyl(trihexyl)phosphonium-based ILs [15]. Other alcohols, such as butanol, have also been extracted from aqueous solutions with ammonium-based ionic liquids, reporting promising results (extraction percentage up to 90%) [27]. The high extraction capacity of these ionic liquids might be related to the localized charges and small-volume ions. Unlike the imidazolium cation in which the positive charge is delocalized, in the case of phosphonium- and ammonium-based ILs, the positive

charge is located in the phosphorus and ammonia atoms, respectively. In the same way, small anions such as chloride, bromide, or dicyanamide show a negative charge localized in one atom, whereas in the case of NTf_2^- , the charge is localized in five atoms. Imidazolium-based ILs yielded a lower extraction percentage of ethanol than those containing phosphonium- or ammonium-based cations. during the whole experiment. Regarding the anion structure, it was observed that the extraction percentage of ethanol decreased as both the anion size and the delocalization of the negative charge increased.

4. Conclusions

The present study revealed the feasibility of sugar extraction from carob pod using a four-stage counter-current system and an L/S ratio of 3.75, with sugar losses below 2%. The alcoholic fermentation of the aqueous extracts with a sugar concentration of 168 g/L using *S. cerevisiae* showed that a maximum ethanol concentration of 95 g/L was achieved after 20 h, independently of the initial concentration of the microorganism.

According to the biocompatibility and ethanol extraction tests, the ILs $[\text{Hex}_3\text{TDP}^+][\text{Cl}^-]$ and $[\text{MTOA}^+][\text{NTf}_2^-]$ are potential candidates for the development of in situ extraction–fermentation processes.

The extractive ethanol recovery simulation using ethylene glycol showed that 62% of the energy consumed in the process can be attributed to the production of the azeotropic ethanol–water mixture. Current research works have used ILs as entrainers to replace ethylene glycol and other solvents commonly employed in ethanol extractive distillation to produce anhydrous ethanol [28,29]. However, there are not many data and experimental studies about ethanol–ionic liquids binary mixtures. These data are of crucial importance to develop single distillation processes for the production of anhydrous ethanol.

Author Contributions: L.J.L.-B. conceived, designed, and supervised the experiments. S.S.-S., M.J.S.-G., and V.M.O.-M. performed the experiments and contributed to the preparation of the paper and data analysis; F.J.H.-F. and A.P.d.l.R. supervised the experiments and contributed to the preparation of the paper and data analysis.

Funding: This research was funded by the Fundación Séneca, grant number 20957/PI/18 and The Ministry of Economy and Competitiveness (MINECO) grant number RTI2018-099011-B-I00.

Acknowledgments: The authors would like to thank the University of Murcia and the Technical University of Cartagena for their administrative and technical support.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Shuba, E.S.; Kifle, D. Microalgae to biofuels: ‘Promising’ alternative and renewable energy, review. *Renew. Sustain. Energy Rev.* **2018**, *81*, 743–755. [[CrossRef](#)]
2. Zabed, H.; Sahu, J.N.; Suely, A.; Boyce, A.; Faruq, G. Bioethanol production from renewable sources: Current perspectives and technological progress. *Renew. Sustain. Energy Rev.* **2017**, *71*, 475–501. [[CrossRef](#)]
3. Demirbas, A. Biofuels securing the planet’s future energy needs. *Energy Convers. Manag.* **2009**, *50*, 2239–2249. [[CrossRef](#)]
4. Kar, Y.; Devenci, H. Importance of P-Series Fuels for Flexible-Fuel Vehicles (FFVs) and Alternative Fuels. *Energy Sources Part A Recover. Util. Environ. Eff.* **2006**, *28*, 909–921. [[CrossRef](#)]
5. Alrefai, R.; Benyounis, K.; Stokes, J. Integration Approach of Anaerobic Digestion and Fermentation Process Towards Producing Biogas and Bioethanol with Zero Waste: Technical. *J. Fundam. Renew. Energy Appl.* **2017**, *7*, 1–6. [[CrossRef](#)]
6. Devarapalli, M.; Atiyeh, H.K. A review of conversion processes for bioethanol production with a focus on syngas fermentation. *Biofuel Res. J.* **2015**, *2*, 268–280. [[CrossRef](#)]
7. Gupta, A.; Verma, J.P. Sustainable bio-ethanol production from agro-residues: A review. *Renew. Sustain. Energy Rev.* **2015**, *41*, 550–567. [[CrossRef](#)]
8. Sarris, D.; Papanikolaou, S. Biotechnological production of ethanol: Biochemistry, processes and technologies. *Eng. Life Sci.* **2016**, *16*, 307–329. [[CrossRef](#)]

9. Saharkhiz, S.; Mazaheri, D.; Shojaosadati, S.A. Evaluation of bioethanol production from carob pods by *Zymomonas mobilis* AND *Saccharomyces cerevisiae* in solid submerged fermentation. *Prep. Biochem. Biotechnol.* **2013**, *43*, 415–430. [CrossRef]
10. Yatmaz, E.; Turhan, I. Carob as a carbon source for fermentation technology. *Biocatal. Agric. Biotechnol.* **2018**, *16*, 200–208. [CrossRef]
11. Sanchez, S.; Lozano, L.; Godínez, C.; Juan, D.; Perez, A.; Hernández, F. Carob pod as a feedstock for the production of bioethanol in Mediterranean areas. *Appl. Energy* **2010**, *87*, 3417–3424. [CrossRef]
12. Turhan, I.; Bialka, K.L.; Demirci, A.; Karhan, M. Ethanol production from carob extract by using *Saccharomyces cerevisiae*. *Bioresour. Technol.* **2010**, *101*, 5290–5296. [CrossRef]
13. Aditiya, H.; Mahlia, T.; Chong, W.; Nur, H.; Sebayang, A. Second generation bioethanol production: A critical review. *Renew. Sustain. Energy Rev.* **2016**, *66*, 631–653. [CrossRef]
14. Robak, K.; Balcerek, M. Review of Second Generation Bioethanol Production from Residual Biomass. *Food Technol. Biotechnol.* **2018**, *56*, 174–187. [CrossRef] [PubMed]
15. Neves, C.S.; Granjo, J.F.O.; Freire, M.G.; Robertson, A.; Oliveira, N.M.C.; Coutinho, J.A.P. Separation of ethanol–water mixtures by liquid–liquid extraction using phosphonium-based ionic liquids. *Green Chem.* **2011**, *13*, 1517. [CrossRef]
16. Zhang, Q.; Zhang, S.; Deng, Y. Recent advances in ionic liquid catalysis. *Green Chem.* **2011**, *13*, 2619. [CrossRef]
17. Zhao, H.; Xia, S.; Ma, P. Use of ionic liquids as ‘green’ solvents for extractions. *J. Chem. Technol. Biotechnol.* **2005**, *80*, 1089–1096. [CrossRef]
18. Chiappe, C.; Pieraccini, D. Review Commentary Ionic liquids: Solvent properties and organic reactivity. *J. Phys. Org. Chem. J. Phys. Org. Chem.* **2005**, *18*, 275–297. [CrossRef]
19. De Los Ríos, A.P.; Hernández-Fernández, F.J. *Ionic Liquids in Separation Technology*, 1st ed.; Elsevier: Amsterdam, The Netherlands, 2014.
20. Mahtout, R.; Ortiz-Martínez, V.M.; Salar-García, M.J.; Gràcia, I.; Hernández-Fernández, F.J.; Ríos, A.P.D.L.; Zaidia, F.; Sanchez-Segado, S.; Lozano-Blanco, L.J. Algerian Carob Tree Products: A Comprehensive Valorization Analysis and Future Prospects. *Sustainability* **2018**, *10*, 90. [CrossRef]
21. Wood, N.; Ferguson, J.L.; Gunaratne, H.Q.N.; Seddon, K.R.; Goodacre, R.; Stephens, G.M. Screening ionic liquids for use in biotransformations with whole microbial cells. *Green Chem.* **2011**, *13*, 1843. [CrossRef]
22. Zhu, S.; Yu, P.; Lei, M.; Tong, Y.; Zheng, L.; Zhang, R.; Ji, J.; Chen, Q.; Wu, Y. Investigation of the toxicity of the ionic liquid 1-butyl-3-methylimidazolium chloride to *Saccharomyces cerevisiae* AY93161 for lignocellulosic ethanol production. *Pol. J. Chem. Technol.* **2013**, *15*, 94–98. [CrossRef]
23. Kumar, S.; Singh, N.; Prasad, R. Anhydrous ethanol: A renewable source of energy. *Renew. Sustain. Energy Rev.* **2010**, *14*, 1830–1844. [CrossRef]
24. Ravagnani, M.; Reis, M.; Filho, R.M.; Wolf-Maciel, M. Anhydrous ethanol production by extractive distillation: A solvent case study. *Process. Saf. Environ. Prot.* **2010**, *88*, 67–73. [CrossRef]
25. Pfruender, H.; Jones, R.; Weuster-Botz, D. Water immiscible ionic liquids as solvents for whole cell biocatalysis. *J. Biotechnol.* **2006**, *124*, 182–190. [CrossRef]
26. Sendovski, M.; Nir, N.; Fishman, A. Bioproduction of 2-Phenylethanol in a Biphasic Ionic Liquid Aqueous System. *J. Agric. Food Chem.* **2010**, *58*, 2260–2265. [CrossRef]
27. Garcia-Chavez, L.Y.; Garsia, C.M.; Schuur, B.; De Haan, A.B. Biobutanol Recovery Using Nonfluorinated Task-Specific Ionic Liquids. *Ind. Eng. Chem. Res.* **2012**, *51*, 8293–8301. [CrossRef]
28. Pereiro, A.B.; Araújo, J.M.M.; Esperança, J.M.S.S.; Marrucho, I.M.; Rebelo, L.P.N. Ionic liquids in separations of azeotropic systems—A review. *J. Chem. Thermodyn.* **2012**, *46*, 2–28. [CrossRef]
29. Figueroa, J.; Lunelli, B.H.; Filho, R.M.; Maciel, M.W. Improvements on Anhydrous Ethanol Production by Extractive Distillation using Ionic Liquid as Solvent. *Procedia Eng.* **2012**, *42*, 1016–1026. [CrossRef]

