Analysis and Design of Extractive Fermentation Processes Using a Novel Short-Cut Method

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Supporting Information

ABSTRACT: One means of increasing the productivity of microbiological processes is to remove toxic inhibiting products as they are formed through extractive fermentation. The aim of this work was to develop a short-cut tool to allow the design and analysis of extractive fermentation processes. For this purpose, a rigorous model considering both the fermentation kinetics and the application of a thermodynamic phase equilibrium model was developed for batch and continuous regimes. The outcomes and regularities found during the analysis of extractive fermentation using the rigorous model were considered to develop the short-cut tool. The short-cut method was based on the principles of topological thermodynamics and was applied to several case studies in this article. This method enables preliminary information to be acquired and used during the subsequent rigorous simulation, enabling a decrease in the calculation efforts and in the number of experimental runs.

1. INTRODUCTION

Most industrial microbiological processes are carried out in the batch regime. However, the features of microbial metabolism are among the main causes for the low concentration of target metabolites obtained in this regime. This is explained by the inhibition effect caused by the end products on either the cell growth rate or the product biosynthesis rate, as occurs in the production of acetone and butanol, lactic acid, and ethanol.1–3 Similarly, different metabolic intermediate or byproducts can be inhibitory, as in the case of acetic acid and lactate during ethanologenic fermentation using Clostridium thermocellum from cellulosic substrates.4,5 On the other hand, high concentrations of the carbon source, particularly glucose, can inhibit both the growth and product formation rates, as in the case of alcoholic fermentation.6 A rational approach to enhancing the fermentation performance is to remove the toxic inhibiting products as they are formed in the culture broth. To reach this aim, a biocompatible extracting agent (solvent) can be added to the medium in such a way that the product migrates to the solvent phase. This is the principle of extractive fermentation combining liquid−liquid extraction with the fermentation process (reaction−separation integration).

Continuous processes have several advantages compared to batch processes, mainly because of their higher productivities, reduced capital costs, lower maintenance and operation requirements, and better process control. In addition, continuous processes allow the product inhibition effect to be decreased through a cascade of continuous reactors.5 Nevertheless, end-product inhibition cannot be completely neutralized by implementing continuous processes. Extractive fermentation is an attractive alternative to overcome this difficulty.

Extractive fermentation can be considered as an integrated process in which a reaction process (fermentation) is combined with a separation operation (liquid extraction).5 Thus, extractive fermentation could have the key to reduce the costs of fuel ethanol production.7,8 In the early 1980s, Minier and Goma9 reported the operation of a continuous extractive fermentation system for the conversion of a glucose-based medium into ethanol using immobilized yeast cells and n-dodecanol as the solvent. In that work, the solvent improved the fermentation performance by reducing the inhibition effect of ethanol and enabling the use of concentrated solutions of glucose (up to 409 g/L). At this point, it is worth noting that the relationship between the glucose concentration and the water activity imposes an additional upper limit because of the availability of free water needed for biomass growth. In this case, the minimum water activity for yeast growth is about 0.90,10 which corresponds to 485.4 g/L glucose in the broth, so the increase in the glucose concentration due to product removal by extractive fermentation does not limit the yeast growth rate. To enhance ethanol production, a process with further integration through the simultaneous achievement of saccharification and extractive fermentation (in the case of polymeric carbohydrate substrates such as starch or cellulose) was proposed by Moritz and Duff.11 This kind of integrated processes has also been applied for the production of other metabolites. Wayman and Parekh12 demonstrated an increase in the substrate conversion due to the effect of the extracting agent (dibutylphthalate) on the fermentation of culture media for producing a mixture of acetone and butanol.

The task of modeling such integrated processes as extractive fermentation is crucial for the appropriate design of the process.

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itself, as well as its operation and control. However, the mathematical modeling of extractive fermentation processes has not been widely studied. In early works, some models were developed. For example, Weihammer and Blast proposed a simple model based on the mass balance of different components to describe extractive fermentation for ethanol production with *Clostridium thermohydrosulfuricum* using oleyl alcohol as the solvent. Kollerup and Daugulis developed a mathematical model to describe the extractive fermentation process for the continuous production of ethanol from a glucose-containing medium. Fournier developed a more rigorous description that considered the use of the UNIFAC (universal functional activity coefficient) activity model to illustrate the behavior of both liquid phases during continuous extractive fermentation. Nevertheless, these models do not predict where the liquid—liquid equilibrium occurs under the specific conditions of extractive fermentation, so these rigorous models should be refined.

Rigorous modeling enables deep insight into a process to be obtained, to find appropriate operating conditions to maximize its performance. Nevertheless, this type of model requires a significant amount of initial information that is difficult to establish. In general, the initial values to run the models should be delimited to find solutions with physical meaning because of the complexity of the equations describing the underlying phenomena. In the case of processes such as distillation, some short-cut methods have been developed for this purpose (e.g., McCabe—Thiele method for conventional distillation) as an aid to delimit the initial conditions and start a rigorous study [e.g., through the mass, equilibrium, summation, and heat (MESH) equations]. These methods should be simple enough to be used before rigorous modeling or experimental runs, when little information is available, but robust enough to discard those conditions where the performance of the process is poor. This type of short-cut tool has not been proposed for extractive fermentation processes. This work represents an attempt to provide such a tool.

The objective of this work was to propose a novel short-cut method based on the principles of topological thermodynamics to analyze extractive fermentation processes. The aim of this method is to obtain preliminary information to be used during the subsequent rigorous simulation by constraining the initial set of possible operating conditions. In this article, the description and results leading to the short-cut method are presented as follows: (i) The rigorous modeling framework that was developed to study extractive fermentation processes for batch and continuous regimes is described; for this purpose, one case study is presented, and the overall modeling considerations are included in the Supporting Information. (ii) Considering the results and regularities found during the analysis of the process by the rigorous model, the short-cut method is introduced. (iii) Two case studies demonstrating the application of the proposed short-cut method are presented; experimental data published in previous works are used. (iv) The usefulness and suitability of the short-cut method is discussed on the basis of the obtained results. (v) Additional modeling details and results are provided in the Supporting Information.

### 2. Modeling of Extractive Fermentation

To analyze the extractive fermentation process, two regimes were considered: batch simultaneous (in situ) fermentation and extraction and continuous simultaneous fermentation and extraction. A rigorous model was developed by coupling the kinetic relationships and the liquid—liquid equilibrium taking into account that the time scale of the growth kinetics occurs at slower pace that the phase splitting. Thus, during each calculation step, the biological transformations defined by the fermentation kinetics occurring in the aqueous phase provide the concentrations of sugars, biomass, and products. These concentrations were taken as the starting values for solving the liquid—liquid equilibrium using activity models. For a detailed description of the modeling procedure, see Appendix A of the Supporting Information. The results obtained from this model allowed valuable information to be acquired to validate the short-cut method, which is the main issue of the present work. The following assumptions were considered during the development of the overall rigorous model of extractive fermentation: (i) The substrate uptake, biomass formation, and product biosynthesis are carried out only in the aqueous phase; hence, no reactions occurred in the organic (solvent) phase. (ii) The product is the main component migrating to the solvent phase; small amounts of water can migrate to the organic phase depending on the solvent. (iii) No migration to the solvent phase of substrates and biomass occurs. (iv) Solvent is bio compatible with the microorganisms and has no effect on the fermentation process. (v) Stirring of the bioreactor ensures total mixing between liquid phases and produces no damage to the growing cells.

The extractive fermentation process is depicted in Figure 1 for the continuous regime. Two influent streams enter the bioreactor and are mixed throughout the system operation. When the process microorganism is present, the product is formed as soon as these streams are mixed. At this point, the product streams from the decanter located after the bioreactor (extract and raffinate) are in liquid—liquid equilibrium. Here, it is necessary to introduce the concept of pseudoinitial composition, which forms part of the analysis of the statics and that is the basis for the short-cut method developed in this work. The pseudoinitial mixture corresponds to the molar or mass composition obtained by the virtual mixing of all of the inlet flows involved in the process, taking into account the advance of the reaction (characterized by the effluent streams). Thus, the pseudoinitial mixture reacts to a certain degree to immediately separate into two liquid phases. The ratio that the amount of pseudoinitial mixture (represented by

![Figure 1. Simplified diagram of the continuous fermentation process with ethanol removal by liquid–liquid extraction (extractive fermentation): *F*, influent aqueous stream; *F*, influent solvent stream; *Q*, effluent aqueous stream; *Q*, effluent solvent stream.](image-url)
M in eq 1) and amount of the extract is introduced in the balances.

The configuration of continuous extractive fermentation involves the continuous feeding of culture medium and solvent into the reactor. Similarly, both aqueous and solvent phases are continuously removed from the system in a separate way (see Figure 1). Stream Q leaving the bioreactor contains cells, culture medium, and solvent. During the phase splitting in the decanter, the cells and exhausted culture medium remain in the effluent aqueous stream (Q_E), whereas the effluent solvent stream (Q_R) removes the extracted product. Depending on the product properties, the organic phase with the extracted product can be sent to a flash vaporization unit or other separation device, where it is collected and regenerated solvent is obtained. The solvent can be recycled back to the extractive fermentation bioreactor. The aqueous stream leaving the system can be sent to wastewater treatment or to additional downstream operations if a valuable product is present. If needed, the cells can be recovered from this stream. In general, the mass flow rate (in liters per hour) of influent aqueous stream (F_Qa) is greater than the flow rate of stream Q_A (denoted as Q_E) because of the migration of the extractable product to the solvent phase. Likewise, the flow rate of the influent solvent stream (F_Qr) is less than the flow rate of the effluent solvent stream (Q_R). The material balance can be applied for the case when the solvent contains small amounts of the product as a result of incomplete regeneration of the extracting agent. For a detailed representation of the mass balance equations of this process, see Appendix A (Supporting Information). One of the main problems during chemostat operation is the low concentration of the cell biomass. To overcome this drawback, recycling of the cell biomass can be implemented. In this way, this recycling provides some decoupling from hydraulics, leading to the compensation of toxicity effects and improving the volumetric productivity. The proposed rigorous model can be slightly modified to consider such types of recirculation streams.

During batch extractive fermentation, the culture medium loaded into the bioreactor contains the nutrients required by the microbial cells. Once the fermentation starts, solvent is added to extract the end product formed. The bioreactor is stirred to disperse the solvent phase. At the end of cultivation, the medium is discharged, and then the solvent and aqueous phases are separated in a decanter. The solvent can be regenerated to be used for subsequent batches. The volume ratio of the solvent and aqueous phases (R_v) was changed to analyze its effect on the extraction parameters. The features of rigorous modeling for batch extractive fermentation are shown in Appendix A of the Supporting Information.

2.1. Case Study 1: Modeling of Ethanol Production by Extractive Cofermentation. In this case study, ethanol production by an extractive cofermentation process was analyzed using detoxified lignocellulosic hydrolyzate. This hydrolyzate was obtained through several steps: pretreatment of the lignocellulosic biomass, detoxification of the pretreated biomass to remove compounds such as furfural formed during the previous step, and hydrolysis of the exposed cellulose contained in the biomass.

2.1.1. Batch Regime. The kinetic model of batch alcoholic fermentation was taken from Leksawasdi et al.,20 who developed it from experimental data. This model describes the simultaneous consumption by a recombinant Zymomonas mobilis strain of the two main substrates contained in the detoxified lignocellulosic hydrolyzates: glucose and xylose. In addition, it takes into account the biomass growth and ethanol production. The fatty alcohol n-dodecanol was selected as the solvent. Similar analysis was performed using oleyl alcohol as the solvent.

Based on the model of Leksawasdi et al.,20 the simulation of the batch alcoholic fermentation from lignocellulosic hydrolyzate was performed with an initial glucose concentration of 100 g/L and an initial xylose concentration of 50 g/L. The culture behavior can be observed in Figure 2a, where a decline in cell concentration because of the relatively high amounts of ethanol in the broth can be seen after 25 h. The model of these authors captures the inhibition of the growth rate due to the presence of ethanol. According to this model, if the ethanol concentration is greater than the threshold ethanol concentration (28.9 g/L), the end-product inhibition effect can be clearly noticed (see the model in Appendix A of the Supporting Information).

The use of more concentrated culture media leads to the underutilization of expensive feedstocks that cannot be

Figure 2. Comparison of conventional and extractive batch cofermentations for ethanol production using Z. mobilis. The curves were calculated by the rigorous model using the kinetic expressions taken from Leksawasdi et al.20 (a) Batch cofermentation with no solvent addition. (b) Batch extractive cofermentation with addition of n-dodecanol as the extraction agent (R_v = 2.0); the addition of the solvent was performed at 5 h of cultivation. S1, glucose; S2, xylose; X, cells; P, ethanol in the aqueous phase; P*, ethanol in the solvent phase.
transformed into ethanol despite their availability in the broth. According to the model of Leksawasdi et al.\textsuperscript{20} which also captures the limitation in growth rate at high substrate concentrations, when a medium containing up to 400 g/L fermentable sugars is employed, an ethanol concentration of about 71.2 g/L is reached only after 80.5 h of cultivation.

The addition of solvent allows the removal of ethanol from the aqueous phase, as depicted in Figure 2b. If n-dodecanol is added to the medium once at 5 h of cultivation, the ethanol concentration in the aqueous phase is reduced, leading to a lower end-product inhibition effect. In this case, the solvent is not removed from the culture broth until the total depletion of the substrates. At that time, ethanol-enriched solvent is regenerated by flash vaporization. The total ethanol productivity for batch extractive fermentation (evaluated as the mass of ethanol recovered from both phases per liter of working aqueous volume per unit of time) using n-dodecanol depends on the solvent volume/aqueous volume ratio ($R_V$). The rigorous model showed a total ethanol productivity of 1.89 g/(L h) when the substrates were exhausted. Considering only the solvent phase, the ethanol productivity reached 0.82 g/(L h). For comparison, the ethanol productivity when no solvent was used reached 1.49 g/(L h). On the other hand, the concentration profiles of both substrates and cells in the aqueous phase indicated that the extractive effect of the solvent enabled the reduction of the cultivation time for the same initial substrate concentration until both sugars were exhausted: from 44 h without solvent addition to 27 h when n-dodecanol was added (see Figure 2). For oleyl alcohol, the total ethanol productivity and ethanol productivity from the solvent phase were 1.98 and 0.63 g/(L h), respectively.

The behavior of productivity as a function of the solvent volume/aqueous volume ratio ($R_V$) was also assessed. Higher ratios led to lower productivities. For high ratios ($R_V$ above 8.1 for n-dodecanol), the algorithm developed by the authors of this article (based on the rigorous model) predicts the formation of homogeneous mixtures that do not allow the extraction of ethanol. Modeling results for volume ratios lower than 0.54 predict a homogeneous mixture as well. These outcomes were obtained using the UNIFAC equations. At this point, the algorithm enables the activity model used to be shifted to calculate the liquid–liquid equilibrium of the system under evaluation. Thus, the nonrandom two-liquid (NRTL) equation predicts homogeneous mixtures for the system water–ethanol–n-dodecanol where phase splitting exists according to the experimental data provided by Kirbaşlar et al.\textsuperscript{21} as can be seen in the Supporting Information (Figure B1). In the case of the UNIQUAC (universal quasichemical) model, the liquid–liquid envelope is located above the experimental envelope. The UNIFAC model was demonstrated to be appropriate for describing the liquid–liquid equilibrium of the analyzed system by comparison to the data of Kirbaşlar et al. Consider the analysis of one raffinate point with the following composition (in mass fraction): 0.024 n-dodecanol, 0.423 ethanol, and 0.553 water. UNIFAC predicts a point with the composition 0.01 n-dodecanol, 0.43 ethanol, and 0.56 water, whereas UNIQUAC predicts the composition 0.00 n-dodecanol, 0.43 ethanol, and 0.57 water. NRTL does not predict any phase splitting at this point. A similar analysis for oleyl alcohol was also performed, giving a similar behavior.

2.1.2. Continuous Regime. Some simulation results of continuous ethanologenic fermentation without solvent addition using the model of Leksawasdi et al.\textsuperscript{20} and considering a glucose concentration of 100 g/L and a xylose concentration of 50 g/L in the feed stream are provided in the Supporting Information (see Figure A1). The dilution rate ($D = F/V$, where $F$ is the flow rate of the influent or effluent stream, in liters per hour, and $V$ is the volume of culture medium, in liters) corresponding to cell wash-out is about 0.32 h\textsuperscript{-1}. The improvement of the process when the solvent is employed is evident in Figure 3, where the distribution of ethanol between the two immiscible liquid phases occurs. Thus, the addition of n-dodecanol leads to a 3-fold increase in total ethanol productivity compared to maximum ethanol productivity for the process without any solvent [5.17 g/(L h) at $D = 0.15$ h\textsuperscript{-1}]. This comparison was done for the extractive fermentation process with a dilution rate of the feed aqueous stream ($D_A = F_A/V_A$, where $V_A$ is the volume of the aqueous phase, in liters) equal to 0.25 h\textsuperscript{-1}. Considering productivity only from the solvent phase, the increase reaches 78.2%. These same values for the case when oleyl alcohol was used as solvent are 2.29 times and 9.9%, respectively. In addition, the concentrations of both substrates at maximum total ethanol productivity are lower than in the case of conventional continuous culture, where substrates are not completely utilized. These results were obtained for a ratio between the inlet solvent stream and the inlet culture medium (aqueous) stream ($R$) of 2.0. The coupled algorithm was employed for simulation of the process with varying $R$ values for an inlet dilution rate of 0.25 h\textsuperscript{-1}, that is, a dilution rate corresponding to the maximum ethanol productivity in the previous simulations. For n-dodecanol, the best results were obtained for values above 4, corresponding to an increased amount of consumed substrates. Both the total productivity and the productivity for ethanol recovered from the solvent phase approach constant values: 18.2 and 15.5 g/(L h), respectively. Note that the substrate consumption was found to increase with increasing feed flow rate ratio, including the possibility of using more concentrated media in the continuous regime.
Based on the proposed modeling approach, the simulation of the extractive cofermentation process was performed modifying both $R$ and $D_{AI}$. The results for the case of $n$-dodecanol are presented in Figure 4, where the positions of the pseudoinitial compositions of the system and trajectories through which it moves for each set of inlet conditions are represented in the ternary water–ethanol–$n$-dodecanol diagram. In this figure, each point on the curves represents one pseudoinitial mixture for given inlet conditions of $R$ and $D_{AI}$. Then, the mixture separates into two liquid phases in equilibrium (extract and raffinate) that are considered as the steady states of the system. The points with identical $D_{AI}$ values are represented by a single line. The zone of manipulated variables where higher productivities were obtained was found to correspond to dilution rates near wash-out conditions (below 0.32 h$^{-1}$) and to higher values of $R$ (near the solubility limit of the extract, i.e., 7.8). The solubility (binodal) curve obtained using the rigorous model agreed well with the experimental data obtained by Kirbaşlar et al.\textsuperscript{21} (see Appendix B of the Supporting Information).

The results presented in this section were obtained for a composition of the feed aqueous stream (nutritive medium) of 100 g/L glucose and 50 g/L xylose. If these concentrations were changed, the analysis of the system would become more complex.\textsuperscript{17} The simulation of the process utilizing more concentrated culture broths is illustrated in the Supporting Information (see Table C1 in Appendix C). The best values for the total productivity and productivity of ethanol recovered from the solvent phase were found to correspond to an inlet concentration of total sugars of approximately 600 g/L.

### 3. DEVELOPMENT OF A SHORT-CUT METHOD FOR ANALYSIS OF EXTRACTIVE FERMENTATION

In the past 20 years, the analysis of statics that is based on the principles of a thermodynamic-topological approach has been successfully applied to integrated processes such as reactive distillation. This methodology defines whether this type of process can be carried out, as well as the spectrum of feasible initial operating conditions.\textsuperscript{18} However, the analysis of statics has not yet been applied to extractive fermentation processes. In a previous work,\textsuperscript{17} the first steps for developing a short-cut tool were accomplished, but without the validation of the data obtained using actual experimental data for both the batch and continuous regimes.

Starting from the well-known representation of liquid–liquid equilibrium in ternary diagrams showing the state of the system in terms of the fermentation product, water as the main component of the culture medium, and solvent as the extractive agent, it is possible to add another key component involved in the biochemical transformation, namely, the substrate. Thus, a quaternary diagram is obtained (see Figure 5). In this sense, any point in the quaternary diagram can represent the pseudoinitial composition of the system. If this composition falls into the space of heterogeneous mixtures (the goal of an

![Figure 4. Representation in the ternary diagram of the steady states achieved during the rigorous simulation of alcoholic extractive cofermentation using $n$-dodecanol for different operating conditions. Concentration of sugars in feed aqueous stream: glucose, 100 g/L; xylose, 50 g/L. The diagram was amplified in the lower part. The units of the inlet aqueous dilution rate ($D_{AI}$) are h$^{-1}$.](image1)

![Figure 5. General representation of extractive fermentation in the concentration simplex. The dashed lines represent the process trajectory.](image2)
extractive fermentation process), the system, after having undergone some biochemical transformation, will move toward the extract and raffinate compositions following the tie lines of the liquid—liquid equilibrium. This displacement from the starting point toward the product streams can be considered as the trial trajectory of the process analogously to the trial trajectory of a reactive distillation process. Thus, the system moves from the pseudoinitial point to the corresponding steady states (extract and raffinate), namely, the product streams. The basis for the extension of these principles from reactive distillation to extractive fermentation were comprehensively discussed in a previous work.

The procedure for locating the steady states in the concentration simplex using the proposed short-cut method can be conceptually divided into the following steps:

1. Locate the point representing the composition of the feed aqueous stream (nutritive medium). This point is located on the edge representing the binary compositions water—substrate (see point A in Figure 5).
2. Convert the substrate into the fermentation product (microbial transformation). This conversion is represented by the displacement of point A toward point B located in the plane representing the ternary compositions substrate—product—water.
3. Add the extracting agent (feed solvent stream). This is represented by line BC in Figure 5. The addition of the solvent is accomplished until point D is reached within the zone of heterogeneous compositions.
4. Separate the system into two liquid phases. The compositions of the effluent solvent stream, or extract (E), and effluent aqueous stream, or raffinate (W), are obtained through the tie lines.

The initial information needed to apply the proposed short-cut method includes data on the fixed points (pure substances) and the solubility curve of the system (liquid—liquid equilibrium). The final aim of the method is to determine the zone of feasible operating conditions with high productivities. This is illustrated in the following cases studies.

Case Study 2: Application of the Short-Cut Method for Analysis of Ethanologenic Extractive Cofermentation. In ref 17, the basis of the proposed short-cut method was preliminarily applied to the production of ethanol by extractive fermentation using n-dodecanol as the solvent. In this work, some complementary issues are disclosed and applied to other systems in a systematic way. The main components (sugars—water—ethanol—n-dodecanol) are represented in a quaternary diagram to determine the process trajectory, including the biochemical transformation. For the representation of the reaction trajectory, considering that the overall fermentation process is irreversible, a stoichiometric approach was employed. In this case, the cultivation process can be described as

\[
\text{C}_6\text{H}_{12}\text{O}_6 \xrightarrow{Z.\text{ mobilis}} 2\text{C}_2\text{H}_5\text{OH} + 2\text{CO}_2
\]

\[
3\text{C}_5\text{H}_{10}\text{O}_5 \xrightarrow{Z.\text{ mobilis}} 5\text{C}_2\text{H}_5\text{OH} + 5\text{CO}_2
\]

The substrate is represented as the sum of both sugars (glucose and xylose) to simplify the graphical representation of the process trajectory. The location of the pseudoinitial mixture is determined considering a substrate concentration of 150 g/L (point A in Figure 6). The maximum stoichiometric product yield from substrate (0.511 g of ethanol/g of sugar consumed) was considered as well. This implies the total conversion of the sugars, and therefore, the system behavior can be represented in the ternary diagram ethanol—water—n-dodecanol (see Figure 7). Thus, the complete transformation of sugars into ethanol is shown by line AB (Figure 6), where B is the state of the system where the total amount of produced ethanol is represented. The ternary diagram requires the inclusion of the binodal curve. Experimental data or commercial simulators can be used to obtain this curve separating the zone of homogeneous and heterogeneous mixtures. In this work, this curve was obtained by the algorithm developed.

Point B is the starting mixture for the liquid—liquid equilibrium. Line BC represents the addition of the solvent (n-dodecanol) to the aqueous medium containing the product (ethanol), as shown in Figure 7. In this figure, vertical lines correspond to the points representing the operating conditions related to the feed solvent stream/feed aqueous stream ratio (R). This fact was deduced from the modeling results shown in Figure 4. The intersection of these vertical lines with line BC (the pseudoinitial mixture indicated by point D) represents the theoretical conditions corresponding to the mixture before phase separation (equivalent to the feed mixture in a liquid extractor). The location of point D determines the value of
ratio $R$, and vice versa. To graphically represent the conditions linked to $R$ in the ternary diagram, mass balance equations should be employed. The sum of the mass flow rates of the feed aqueous stream ($F_A$) and the feed solvent stream ($F_E$) gives the mass of the overall mixture before phase separation

$$\rho_A F_A + \rho_E F_E = M$$  \hspace{1cm} (1)

where $M$ is the mass flow rate of the pseudoinitial mixture (in grams per hour) and $\rho_A$ and $\rho_E$ are the densities (in grams per liter) of the feed aqueous and solvent streams, respectively. The corresponding equation for the outlet streams (extract and raffinate), assuming that there are no significant changes in the densities of the two phases, is

$$\rho_A Q_A + \rho_E Q_E = M$$  \hspace{1cm} (2)

The product balances for the pseudoinitial mixture are given by

$$\rho_A F_A x_A + \rho_E F_E x_E = M x^*$$  \hspace{1cm} (3)

$$\rho_A Q_A x_A^R + \rho_E Q_E x_E^R = M x^*$$  \hspace{1cm} (4)

where $x_A$ is the product mass fraction corresponding to point $G$ in Figure 7, namely, the product composition when the substrate contained in the feed aqueous stream is converted into the product before the virtual mixing leading to the pseudoinitial mixture, that is, the amount of product appearing in the system due to the fermentation before the liquid–liquid extraction; $x_E$ is the product mass fraction in the feed solvent stream (equal to zero when fresh solvent is used); $x_A^R$ is the product mass fraction in the raffinate (point $W$ in Figure 7), $x_E^R$ is the product mass fraction in the extract (point $E$); and $x^*$ is the composition of the pseudoinitial mixture (point $D$).

To determine $F_E$ from a given $F_A$ having chosen a point $D$ on line BC, the values of $x_A$ and $x^*$ are directly read from the ternary diagram and used in the equation

$$F_E = \frac{F_A (x_A - x^*)}{(x^* - x_E)} \left(\frac{\rho_A}{\rho_E}\right)$$  \hspace{1cm} (5)

In this way, the ratio $R = F_E / F_A$ is readily calculated. The vertical line representing the ratio $R$ links the base of the ternary diagram to point $D$ (line $R_2$ in Figure 7). Alternatively, for given $F_A$ and $R$, the location of point $D$ in the diagram is determined from eq 5 by solving for the product composition

$$x^* = \frac{\rho_A F_A x_A + \rho_E F_E x_E}{\rho_A F_A + \rho_E F_E}$$  \hspace{1cm} (6)

To determine the flow rates of the outlet streams ($Q_A$ and $Q_E$), the points corresponding to the extract (E) and raffinate (W) can be located in the diagram by using the tie line passing through point D. With the compositions of W and E read from the diagram, the flow rates can be calculated from eqs 2–4 as follows

$$Q_A = \frac{(x^* - x_E)(\rho_A F_A + \rho_E F_E)}{\rho_A (x_A^R - x_E^R)}$$  \hspace{1cm} (7)

$$Q_E = F_E + \frac{F_E (F_A - Q_A)}{\rho_A}$$  \hspace{1cm} (8)

Extractive fermentation is aimed at reducing end-product inhibition. To take this effect into consideration, it is necessary to locate the inhibition boundary in the ternary diagram. If the composition of the raffinate is above this boundary, the microorganisms limit their growth because of an excessive concentration of the product in the aqueous phase. Therefore, the ethanol concentration in the outlet aqueous stream (raffinate) should not be greater than the ethanol inhibition boundary whose location is determined by point I in Figure 7. The intersection of the tie line passing through point I with the balance line BC (point J) represents the working conditions related to the ratio $R$ limited by ethanol inhibition (line $R_3$ in Figure 7). The pseudoinitial mixtures located to the left of line $R_3$ are not advantageous for the system, and therefore, the zone of feasible operating conditions can be limited by this line. In Figure 8, this zone is represented by the area delimited by the line $R_3$ and the right branch of the binodal curve (the gray shaded zone). When the concentrations of sugars in the feed aqueous stream increase up to values lower than the maximum solubility of these sugars in water to avoid crystallization (as shown in Figure 6), the total amount of product formed based on the stoichiometry increases as well, and therefore, point B is displaced toward product along the edge of the ternary diagram corresponding to water–product binary mixtures. This new point should be below the line corresponding to the substrate solubility boundary determined by point H (Figure 8). This boundary is shown as a shaded plane in Figure 6, although it should be strictly represented by a surface. In this way, the operating zone for extractive cofermentation can be constrained to a more reduced area delimited by line $R_3$ and the right branch of the binodal curve (the dark gray shaded zone until the bottom edge of the diagram in Figure 8). When the initial substrate concentrations in the feed stream are changed, the vertical lines showing the values of $R$ are displaced in comparison to the original for starting point B in Figure 7.

We now analyze the extreme case when the feed aqueous stream has the maximum allowable concentration of sugars. From the stoichiometry of ethanologenic fermentation, this condition corresponds to an inlet concentration in the feed aqueous stream of approximately 600 g/L of total sugars. Assuming the maximum theoretical yield, the total amount of ethanol that could be produced from this amount of sugars is 0.511 g/g. This yield implies a theoretical starting ethanol concentration of 306.6 g/L, which corresponds to an ethanol mass fraction of about 0.31 (point L). This value determines the position of the substrate solubility boundary for water–ethanol mixtures (point H in Figure 8). Since the concentration of ethanol in the aqueous phase (raffinate) should not be above...
The ethanol inhibition boundary (approximately 10% w/w), the operation conditions represented by the ratio $R_3$ should be such that the ethanol content in the raffinate be equal or less than the ethanol content of the point I to avoid product inhibition. Thus, the zone delimited by the dark gray shaded area (until the bottom edge of the diagram) is the zone of feasible steady states for given operating conditions of the extractive fermentation process with the maximum allowable concentrations of sugars in the aqueous feed stream.

For instance, let us consider a continuous ethanologenic extractive fermentation process using Z. mobilis with a 0.11 L/h feed aqueous stream containing 295 g/L glucose, an inlet solvent stream flow rate/aqueous stream flow rate ratio of 4.0, a working aqueous volume of 4.8 L, and a working solvent volume of 1 L (the conditions reported by Bruce et al.23). The location of the pseudoinitial mixture (point D in Figure 8) can be found by using eq 6. Then, the corresponding compositions of the extract and raffinate can be located using the corresponding tie line (indicated by two rhombuses on the binodal curve in Figure 8). Thus, the ethanol content of point D and the compositions of the extract and raffinate read from the diagram are 3.3%, 2.5%, and 4.5%, respectively (by weight). According to these data, the total ethanol productivity of the system reaches 15.92 g/(L h). The total ethanol productivity calculated by the rigorous model using the developed algorithm is 15.13 g/(L h), which is an acceptable difference for a short-cut method. The experimental data reported by Bruce et al.23 are close to the results of the proposed short-cut method (see Table 1). Hence, the proposed method enabled the determination of the feasibility of operating parameters and an estimation of the productivities. From the rigorous simulation, this zone corresponds to the regimes with higher total ethanol productivities (see Figure 4).

### 3.2. Case Study 3: Application of the Short-Cut Method for Analysis of Lactic Acid Extractive Fermentation

Lactic acid is a biotechnological commodity with many applications in the food, chemical, and pharmaceutical industries. One of the main challenges in lactic acid production by fermentation is the end-product inhibition effect. Extractive fermentation has the potential to reduce this effect, thereby improving the separability and recovery of this acid from culture broths.

The main results obtained from the rigorous modeling of an extractive fermentation process for lactic acid production are shown in Appendix D of the Supporting Information. The short-cut method presented here was applied to the lactic acid extractive fermentation of a glucose-based medium using a mixture of tri-$n$-decylamine (TDA) and oleyl alcohol as the extracting agent. To establish the lactic acid inhibition boundary, an inhibitory product concentration of 100 g/L was assumed. The analysis followed the same procedure as described in the preceding section for ethanol extractive fermentation. Figure 8 can be used to explain the application of this method. From the stoichiometry of lactic acid fermentation, a maximum theoretical yield of 1 g of lactic acid/g of glucose was considered. The equivalent starting lactic acid concentration after microbial conversion was 10% (w/w), which is represented by point B. In this case, the feed aqueous stream flow rate was set to 0.5 L/h. To delimit the operating zone, the ratio $R$ defined by the lactic acid inhibition boundary was calculated using eq 5, reading the $x_A$ values of point G and the $x^*$ values of point J from the ternary diagram. The ratio $R$ obtained was 1.40 (line $R_2$). Then, the zone constrained by the substrate solubility boundary was also defined using the same equation and considering the $x_A$ values of point H and the $x^*$ values of point K. The corresponding ratio $R$ was 6.7 (line $R_3$). In this way, the operating zone with high productivities for the maximum allowable glucose concentration in the feed aqueous stream (600 g/L) was located to the right of line $R_2$ until the right branch of the binodal curve. This zone ensures that no
end-product inhibition occurs during the processing of the concentrated medium. Consider the case of a feed aqueous stream containing 40 g/L glucose being employed for an extractive fermentation process with a ratio R of 2.0. By applying eq 6, the location of the pseudoinitial mixture represented by point D in Figure 8 is defined. From point D and the corresponding tie line, the compositions of the extract and raffinate can be read directly from the ternary diagram. With this information, the productivity of this process was estimated by the short-cut method to be 20.24 g/(L h). The productivity calculated by the rigorous model was 19.38, indicating a very good approximation (see Table 1).

4. DISCUSSION

This article presents a novel short-cut method for analyzing extractive fermentation processes. Before discussing the advantages of such a method, it is necessary to evaluate the proposed rigorous model, which provides an appropriate description of the phenomena involved during extractive fermentation. This information was crucial for developing the short-cut tool.

In this work, a rigorous model was used to obtain a systematic framework to assess extractive fermentation processes under two time regimes. This framework included the two main phenomena involved in the process: fermentation kinetics and liquid—liquid equilibria. The solution procedure implied the application of iterative methods to describe the system behavior, simultaneously considering the complex nonlinear relationships of the two phenomena. From the mathematical point of view, the problem represents several difficulties related to the nonsmooth character of some functions employed, as in the case of the activity models needed to calculate the phase equilibrium and some kinetic expressions that exhibit a highly nonlinear nature. The properties of these functions hinder the convergence of numerical methods implemented within the range of valid physical solutions, especially in the case of continuous extractive fermentation where the user should introduce the starting guess values.

In the case of the batch regime, the proposed modeling framework allows for the generation of the concentration profiles of each of the components involved and the determination of the effect of the solvent on the process performance in terms of productivity. In addition, the model enables the exploration of the influence of the amount of solvent added to the bioreactor, for example, through the evaluation of the effect of the solvent volume/aqueous medium volume ratio (Rv). The results obtained from the modeling of batch ethanologenic extractive fermentation showed a 27% increase in the total ethanol productivity when n-dodecanol was used (Rv = 2.0) compared to the conventional process without solvent. For oleyl alcohol, the increment reached 32%. The best values of total productivity correspond to lower values of Rv. Moreover, the productivity for ethanol recovered from the aqueous phase was higher than the ethanol productivity of the process without any solvent. In this way, the advantage of this integrated reaction—separation process lies in the length of the fermentation process, which can be significantly reduced compared to that of the process without solvent. The reduction of the ethanol concentration in the aqueous phase allowed the biomass to reach a higher final concentration during the stationary phase in comparison to the corresponding concentration when no solvent was added (1.8 g/L without solvent, 4.1 g/L for n-dodecanol, and 3.8 g/L for oleyl alcohol; see Figure 2). However, considering the total amount of ethanol produced relative to that from the conventional batch process, the results are not conclusive for the integrated process. In the latter case, the ethanol should be recovered from the two phases by employing a decanter, unlike in the conventional process, where the ethanol is contained in only one phase (the culture broth) and a decanter is not needed.

The overall amount of ethanol that can be extracted by batch extractive fermentation is practically the same as that obtained from conventional batch fermentation. This indicates that liquid—liquid extraction is not good enough to neutralize the inhibition effects of ethanol and substrates on the batch cultivation process for the case of n-dodecanol. The situation is quite similar for oleyl alcohol. Furthermore, the concentration of ethanol in the solvent phase (16.3 g/L) is too low for a cost-effective separation by flash vaporization or distillation. It is important to obtain high relative concentrations of the product in the solvent phase to reduce energy expenditures during the subsequent separation steps. Thus, the results obtained for batch ethanologenic extractive cofermentation using n-dodecanol do not justify the complexity of this integrated process. These values cannot be considered promising when taking into account the fact that ethanol productivities in conventional batch and fed-batch processes are in the ranges of 1–3 g/(L h) for batch regime and 9–31 g/(L h) for fed-batch alcoholic fermentations. Therefore, the search for an appropriate solvent is of paramount importance. Undoubtedly, the proposed model can significantly contribute to the selection of a suitable solvent (or mixture of solvents) by employing computer-aided techniques such as those described by Wang and Achenie.

To enhance the effectiveness of batch extractive fermentation, a mixture of solvents can be used. As mentioned previously for lactic acid production, one solvent with high extractive properties (TDA) can be mixed with a solvent with good biocompatibility (oleyl alcohol). In this case, the overall process performance was improved, considering that the lactic acid concentration in the resulting organic phase was significantly higher than that in the aqueous phase. This behavior predicted by the rigorous model (see Appendix D of the Supporting Information) can be corroborated in some works reporting experimental data for analogous processes. For example, Gao et al. implemented an extractive fermentation process employing this same solvent system but using S. cerevisiae as the lactic acid-producing microorganism because it can grow well and produce lactic acid efficiently at lower pH than lactic acid bacteria (see Table 1).

Continuous extractive fermentation offers the advantage of reaching significantly higher productivities relative to those of batch processes. The results obtained in this work show the possibility of reaching total ethanol productivities as high as 30.3 g/(L h) compared to the productivities of conventional continuous ethanologenic fermentations, which are in the range of 5–20 g/(L h). The simulations performed indicate that microorganisms can grow at higher rates when the inhibiting product is continuously removed from cultivation broth. Ethanol would become inhibitory to growth from glucose and xylose above a threshold level of approximately 27–29 g/L according to Leksawasdi et al. The ethanol levels in the aqueous phase depend on the changes in the biomass and substrate concentrations according to Monod-type kinetics, but
they are strongly influenced by the migration of ethanol to the solvent phase, as can be observed in Figure 3.

For process economy, it is very important to employ the minimum possible amount of the extracting agent to reduce the solvent costs, pumping expenditures, and energy consumption during solvent recovery. Although high values of the ratio $R$ allow the highest ethanol productivities to be reached, operation of the system at elevated $R$ values is not economically viable (large amounts of solvent employed). Therefore, for the case of n-dodecanol, $R$ values near 4.0 are suggested considering the production costs. For oleyl alcohol, the recommended value is 5.5.

The representation of the steady states of the extractive fermentation process in the ternary diagram using the results generated by the algorithm based on the rigorous model enables interesting information about the system behavior to be obtained. This information was the basis for the structure and configuration of the short-cut method. Thus, the operating conditions related to $R$ can be represented as vertical lines in that diagram, where each vertical line represents the geometric location of pseudoinitial mixtures with identical inlet conditions of the feed flow rates ($F_E$ and $F_A$). The points representing these pseudoinitial mixtures can lead to the steady states of the system represented by the compositions of the extract and raffinate associated with these points. From Figure 4, it can be observed that, at low $R$ values, the pseudoinitial points with different inlet aqueous dilution rates lie on the same vertical line in a separate way. Thus, the variation of $D_A$ for the same value of $R$ leads to different ethanol productivities. Again, from this diagram, it is evident that higher productivities can be achieved for greater values of $R$ and $D_A$, which are limited by wash-out conditions and the zone of homogeneous mixtures, specifically, the curve representing the raffinate compositions. At high values of the ratio $R$ (above 4.0), pseudoinitial points with different $D_A$ values tend to be clustered in a very narrow zone corresponding to higher productivities. This indicates that values of $R$ higher than 4.0 do not provide significant productivity increases, whereas they do elevate the production costs. In this way, the zone of more favorable operating conditions can be preliminarily located. The information derived from Figure 4, which, in turn, was obtained using the proposed rigorous model, allowed a new short-cut method for evaluation of extractive fermentation processes to be deduced and developed. This article has illustrated this method and demonstrated its application to actual experimental systems, as discussed later.

Taking into account that one of the main features of most fermentations (including those used for the production of ethanol and lactic acid) is the impossibility of working with concentrated media. Continuous extractive fermentation offers the possibility of employing elevated substrate concentrations to achieve higher amounts of product. This fact was reflected by the rigorous model and the short-cut method. For the case of ethanologenic extractive cofermentation, high values of the ratio $R$ favor the consumption of both substrates, although marginal increases of productivity are reached for values above 4.0. The results predicted by the proposed model for concentrated media are shown in Table C1 of the Supporting Information. These results can be compared with those obtained by Kollerup and Daugulis, who used a model in which the ethanol distribution coefficient ($K_{E_{DOL}}$) was fixed and the substrate limitation and substrate inhibition effects on both the growth and ethanol production rates caused by high substrate concentrations were not taken into account. They considered an extremely high glucose concentration of 750 g/L as a means for enhancing the total ethanol productivity, which reached 82.6 g/(L h) at a very high value of the influent solvent dilution rate of 5 h$^{-1}$. However, calculations using the algorithm developed in this work indicate that substrate inhibition does not allow such high productivities to be attained. In addition, the model proposed in the present work takes into account a rigorous calculation of liquid–liquid equilibrium through activity models such as NRTL, UNIQUAC, and UNIFAC, such that the values of $K_{E_{DOL}}$ change for different operating conditions according to the proposed rigorous algorithm. A comparison of some key indicators obtained from the model of Kollerup and Daugulis to the data resulting from the rigorous modeling approach described in this article is presented in Table 1.

It is necessary to assess the suitability of the rigorous model developed relative to real reported data for continuous processes. The first reported work on extractive fermentation for ethanol production using microorganisms other than yeasts was published by Bruce et al. They employed a reactor in which Z. mobilis cells were cultivated continuously for more than 225 h using a commercial solvent mostly composed of oleyl alcohol to remove the ethanol produced by the bacteria. They assessed the process performance at high glucose concentrations. Under these conditions, the culture exhibited an oscillatory behavior that implied difficulties during system operation. When solvent was continuously added to the culture broth, these oscillations ceased, and almost all of the glucose was consumed, thus increasing the ethanol productivity and yield. The rigorous model was used to analyze this process under the reported conditions, giving outcomes that were near the experimental data presented by Bruce et al. For this purpose, the kinetic model of Lekawasdi et al. was applied. This model is relevant considering that it allows for the description of growth inhibition by both glucose and ethanol. In addition, the UNIFAC model was utilized. A comparison of the data calculated by the rigorous model and the data obtained in the experimental work is provided in Table 1. The deviations of the data can be explained by the fact that the kinetic model was not specifically developed for this system. Note especially the agreement between the productivity calculated by the model and the obtained experimental value: 15.1 and 15.6 g/(L h), respectively.

The proposed model can be used for predicting some performance indicators of different configurations of extractive fermentation processes. Although the modeling approach was developed for systems with suspended cells, the liquid–liquid extraction phenomenon is the same for several types of extractive fermenters, and therefore, the software can be employed for their preliminary performance analysis. Thus, the model predicted a 2.11-fold increase in ethanol productivity for a process employing a hollow-fiber-based fermenter using immobilized yeasts under the same conditions as reported for such a process by Kang et al., as shown in Table 1.

On the other hand, the selectivity of the extractive agent plays a crucial role in the extractive fermentation process. Comparing the two solvents studied, the selectivity of n-dodecanol (about 28.5) is slightly higher than that of oleyl alcohol (about 25.9). This can be evidenced from the modeling data indicating a 78.2% increase of ethanol productivity from the solvent phase (PrE) for n-dodecanol and only a 9.9%
increase for oleyl alcohol. In the most favorable situation, the addition of a proper solvent should exhibit higher ethanol concentrations in the solvent phase than in the aqueous phase. In this way, the ethanol should be mainly extracted from the solvent phase and not from the aqueous phase, as in the case of the two solvents analyzed. This was the case for TDA/oleyl alcohol extraction of lactic acid as shown in Appendix D of the Supporting Information. In addition, the use of oleyl alcohol allows the biocompatibility of the solvent system to be improved, as indicated elsewhere.28,29

The short-cut method presented in this work was tested for two continuous systems: ethanologenic extractive cofermentation and lactic acid extractive fermentation. This method features a high simplicity, requires a minimum of information about the process, and allows the feasible zone of operating conditions to be delimited and some performance indicators such as volumetric productivity to be estimated. The data obtained by this short-cut method can be used for reducing the space of inlet operating conditions to be analyzed through rigorous simulation. In addition, the short-cut method can provide insight into the impossibility of accomplishing an extractive fermentation process for specific conditions.

The information on the operating conditions corresponding to these delimited zones can be employed as the starting point for developing a preliminary optimization strategy. Because the region of feasible steady states can be determined in the ternary diagram (see Figure 8), the ranges of such manipulated variables as the inlet dilution rate, the ratio R, and the concentration of the sugars in the inlet aqueous stream are known and can be bounded for solving an optimization algorithm. In a previous work,17 GAMS software was employed to find the optimal values of the mentioned variables that maximized the total ethanol productivity. Once a global panorama of the space of operating conditions and their optimal values for the studied process have been obtained, experimental runs should be performed to confirm the validity of the given theoretical approach.17 In this manner, the acquired insight into the process will enable the reduction of expensive experimental work in the search for optimal operations, which is the main goal of both short-cut and rigorous simulation methods. As shown in Table 1, experimental data available in the open literature for extractive fermentation allowed for a comparison with the results obtained in this work using the rigorous model and the short-cut method, despite the fact that some reported operating conditions (immobilized cells, different process microorganisms, and coupled systems using a separate extractor—decanting unit) were different from those proposed in this work. Finally, the main issues analyzed in this work should be considered as a theoretical development prior to the needed experimental runs that will be undertaken in the future for specific extractive fermentation systems.

5. CONCLUSIONS

Continuous extractive fermentation has been demonstrated to be an efficient technology compared to conventional batch and continuous fermentations. The coupling of the fermentation kinetics to thermodynamic equilibrium models enables different ways of improving the productivity and performance of extractive fermentation to be analyzed. The procedures studied in this work exemplify this advantage. The determination of the best values of operating parameters is required to improve the performance indexes of the process. With this aim, rigorous modeling is a powerful tool allowing for analysis of the process before costly experimental runs at the pilot-plant and industrial levels are executed. The modeling approach for extractive fermentation presented in this article differs from most models already published in the rigor with which it describes the thermodynamic and kinetic phenomena.

The proposed short-cut method for extractive fermentation based on the principles of thermodynamic-topological analysis allows preliminary information to be obtained and used during subsequent rigorous simulation. This approach enables the calculation time and number of experimental runs to be decreased. Moreover, it helps to determine which data are required and the space of initial conditions where experimental efforts should be focused.
(22) Gutiérrez, L. F. Estudio y diseño de procesos reacción–extracción simultáneos (Study and simultaneous reaction–extraction processes). Universidad Nacional de Colombia, Manizales, Colombia, 2008 (in Spanish).
(26) Sánchez, O. J.; Cardona, C. A., Producción de Alcohol Carburante: Una Alternativa para el Desarrollo Agroindustrial (Fuel Ethanol Production: An Alternative for the Agro-industrial Development); Universidad Nacional de Colombia: Manizales, Colombia, 2008 (in Spanish).