Aerated Extraction Column for Reactive Extraction from Fermentation Broth

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The separation of a product from a fermentation broth can be achieved by reactive extraction. A systematic way to find suitable process conditions based on cascaded option trees has been demonstrated for the example of diamine recovery. Suitable choice of reactive extractant and diluent lead to a high degree of extraction and feasible coalescence characteristics at *p*H values suitable for in-situ extraction. The competing boundary conditions for an efficient process are discussed showing that in general a complex optimization task has to be solved to find the most feasible process conditions. Simulation of extraction-column performance based on single-drop experiments in dedicated lab-scale equipment shows good agreement with pilot-plant experiments up to and including the flooding limit. Finally, to facilitate in-situ removal of a product to avoid product inhibition, an aerated column has been studied, where a dedicated three-phase separator for the top of the column is presented. The results show that even for this challenging task feasible operation conditions can be found.

1. Introduction

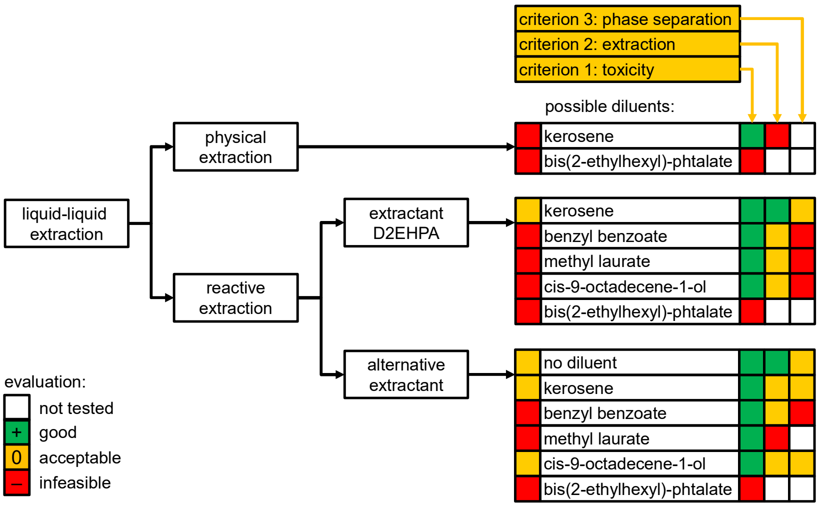
The foreseeable feedstock change in chemical industry towards renewable resources will lead to an increased application of bio-reactions for obtaining the product [1,2]. The challenge in an overall process with a biotechnological step is the primary recovery of the product, because typically the product is obtained at low concentration in an aqueous environment. This means that very selective process steps have to be used to achieve directly a significant enrichment of the desired product component(s) and to minimize correspondingly the flowrates in the first separation step. Here, solvent or reactive extraction are feasible options for first downstream steps. The extractant and the reactive agent can be chosen to maximize selectivity and capacity. Another challenge results, if the first separation shall be realized as in-situ extraction. This requires that the boundary conditions for the survival of the microorganisms are taken into account also during the extraction step. Because some microorganisms require continuous supply with oxygen, the extraction process has to be aerated in that case. This leads to the demand to realize a three-phase separation, where already liquid-liquid phase separation alone in many cases results in significant challenges. As an example case the production of diamine by fermentation has been chosen, because it is a component of potential industrial relevance as monomer for polyamide production. Since diamines can be components for high-performance polymers, such a high-added-value component has the chance to be economically competitive as bio-based product already today.

2. Design of Extraction Process

In the downstream development for diamine removal after fermentation, a variety of options exists to try to solve the challenges mentioned. In order to be able to systematically structure the options, the method of cascaded option trees has been applied [3] as shown in Figure 1. In these option trees all options are collected and the result of evaluation with respect to the relevant criteria is recorded with a color code indicated at the bottom left of Figure 1. Also, unit operations other than extraction had been evaluated but turned out to be obviously less promising than extraction. In further detailing the options physical as well as reactive extraction have been regarded, where different reactive extractants have been considered in various diluents, which were chosen to be characteristic for a variety of solvent classes.

The first criterion considered was toxicity, where the phthalate as diluent has been discarded because of its hormone-like effects on humans. It is obvious that once an option completely fails with respect to one of the criteria, this option does not need to be considered further. Thus, the phthalates do not need to be considered in evaluating the following criteria.

The next challenge to be mastered is the extraction equilibrium, which has to allow efficient and selective removal of the desired product. Reactive selectivity may be especially promising. Figure 2 shows the results obtained for the example extraction of a diamine with D2EHPA (di-(2-ethylhexyl) phosphoric acid) [4]. Equilibration was realized with a mixing device, which allows slow over-head rotation of the sample flasks, which was performed for at least 30 min in a temperature controlled water bath. The phase ratio applied was around 1:1. Plotted is the degree of extraction, which is the fraction of the extracted component, here the diamine, which is transferred into the organic extractant phase at the indicated conditions. Two main parameters are apparently influencing the extraction equilibrium, namely the extractant concentration and *p*H. The *p*H is also relevant when considering in-situ extraction and the extraction concentration directly relates to the capacity of the organic phase.



		possible diluents	criterion 1: toxicity	criterion 2: extraction	criterion 3: phase separation
physical extraction	🟥	kerosene	🟩	🟥	☐
	🟥	bis(2-ethylhexyl)-phtalate	🟥	☐	☐
extractant D2EHPA	🟨	kerosene	🟩	🟩	🟨
	🟥	benzyl benzoate	🟩	🟨	🟥
	🟥	methyl laurate	🟩	🟨	🟥
	🟥	cis-9-octadecene-1-ol	🟩	🟨	🟥
	🟥	bis(2-ethylhexyl)-phtalate	🟥	☐	☐
alternative extractant	🟨	no diluent	🟩	🟩	🟨
	🟨	kerosene	🟩	🟩	🟨
	🟥	benzyl benzoate	🟩	🟨	🟥
	🟥	methyl laurate	🟩	🟥	☐
	🟨	cis-9-octadecene-1-ol	🟩	🟨	🟨
	🟥	bis(2-ethylhexyl)-phtalate	🟥	☐	☐

Figure 1. Degree of extraction as basis for extraction-process design

Unfortunately both parameters also simultaneously influence the third criterion evaluated, namely phase separation after extraction [5]. The ease of phase separation is characterized with a standardized settling cell, which was originally proposed by Henschke together with the quantitative evaluation of the coalescence process [6]. The time to settle in that lab-scale equipment is a quantitative measure, which allows direct judgement on feasibility of phase separation. The parameters obtained from the detailed evaluation of videos taken from this experiment with roughly one liter of two-phase system after 30 s of stirring at 800 min^{-1} allow quantitative design of the technical gravity settler. As shown in Figure 3, settling time for this system depends strongly on *p*H as well as on the phase ratio. It is apparent that for biological systems a rather large scatter of the data occurs. As a rule of thumb, settling times below 300 s allow relatively unproblematic phase separation on technical scale, which can be achieved for all phase ratios at suitable values of *p*H [7]. This still holds even if in-situ extraction is performed, i.e. in the presence of cells. Unfortunately, for *p*H-values close to 7, which would be desirable in this case for in-situ extraction, phase separation becomes slightly worse. It should also be mentioned that in a variety of cases the formation of significant crud layer has been observed, e.g. in the case of low *p*H with an aqueous dispersed phase [8].

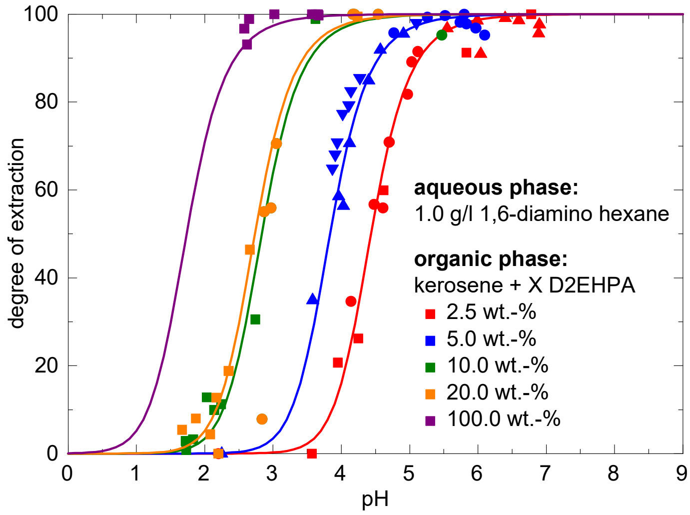


Figure 2. Degree of extraction as basis for extraction-process design at 30°C

Considering these criteria, in overall process optimization a feasible parameter window has to be found. For the example case the following boundary conditions result.

- Good extraction selectivity is characterized via the extraction equilibrium, which is on the one hand side determined by the choice of the reactive extractant, here D2EHPA. Some influence can also be attributed to the diluent, here kerosene. The diluent influence is usually not very pronounced with respect to extraction equilibrium. Finally, in this example *p*H is a major influencing parameter, which strongly affects this ion-exchange equilibrium. Here a sufficiently high *p*H is required to achieve a high degree of extraction, i.e. a sufficient degree of enrichment [4].
- Re-extraction is achieved by adding a base and shifting the *p*H to sufficiently low values so that the diamine in equilibrium can then be found in the aqueous phase. To shift the *p*H, base has to be added leading to salt formation. This back extraction should be possible with as little as possible salt

production. Since the *p*H scale is logarithmic and only small amounts of acid or base are required to shift *p*H around the value of 7, an extractant is desired where the point of inflection in the typical S-shaped curves in Figure 1 is located near this optimal *p*H. In this example, a low extractant concentration is thus desirable.

- A high capacity for the product is on the other hand reached with high extractant concentration.
- In contrast to that, it turns out that good coalescence behavior which allows phase separation in continuous gravity settlers is achieved for low extractant concentrations.
- Crud-formation tendency induced by biomass strongly depends on extractant concentration and *p*H. In the evaluated *p*H-window of 7 and slightly below feasible phase separation has been observed for a variety of phase ratios [5].
- For in-situ extraction, further limitations e.g. on feasible *p*H-range may additionally apply. In the case considered here, a *p*H slightly below neutral is desirable.

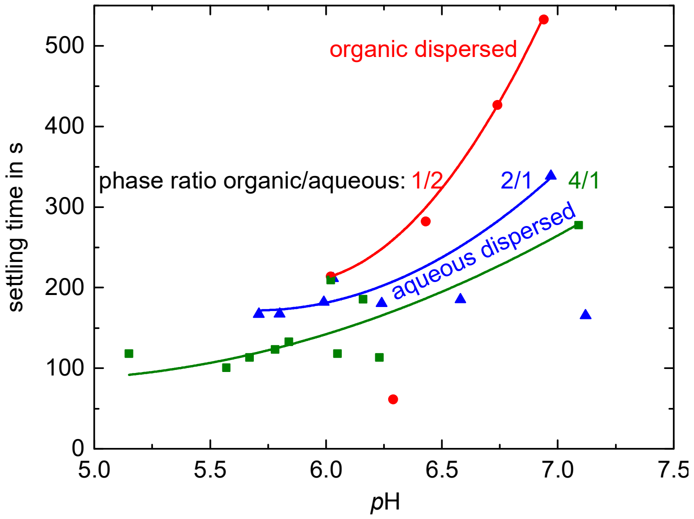


Figure 3. Settling time for the system kerosene with 5 wt-% D2EHPA at 30°C with 0.1g/l diamine, determined with 1 liter of dispersion.

3. Design of Extraction Column

After the extractant has been selected and some basic process parameters defined like *p*H and the choice of the dispersed phase, the extraction equipment has to be designed. An option with high volume-specific separation performance is to use an extraction column. For extraction-column design especially for such complex processes, where during process design only limited amounts of the product are available, drop-based simulation of column performance may be an especially promising approach. In previous work, this method has been developed and it has been shown that even for technical systems, prediction accuracy better than 10% can be expected up to and including flooding limit [9]. This method relies on experiments on lab scale with dedicated equipment to quantify behavior of single drops, e.g. on sedimentation as well as on mass transfer [10,11,12]. The parameters of suitable models are then fitted to these data, which are in turn used in the drop-based column simulation. In the developed tool, ReDrop (REpresentative DROPs), a sufficient number of individual drops can be followed in their behavior, taking sedimentation, mass transfer, breakage and splitting into account, also considering the swarm effect as well as the interactions with the internals. The tool has been extended to account for chemical reactions in either phase as well as at the interface.

The corresponding single-drop experiments on sedimentation velocity and mass transfer have been performed. Then the ReDrop simulations were compared to experimental results obtained in a pilot-plant scale sieve-tray extraction column of 50 mm diameter. As shown in Figure 4, ReDrop allows describing the transient behavior of the extraction-column performance with good accuracy.

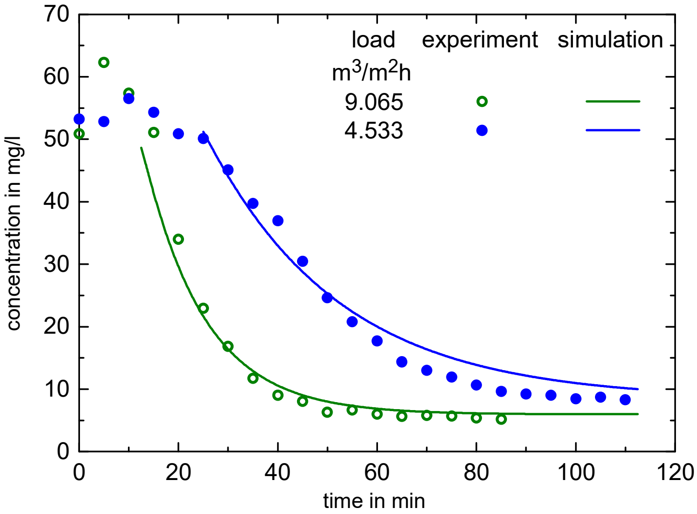


Figure 4. Concentration of diamine at the continuous-phase outlet, organic phase: oleyl alcohol + 5wt-% D2EHPA, aqueous phase: ammonium-phosphate buffer + hexane-1,6-diamine with c_0 =100 mg/l, flow-rate ratio 1/1, pulsation intensity 10 mm/s, 25°C

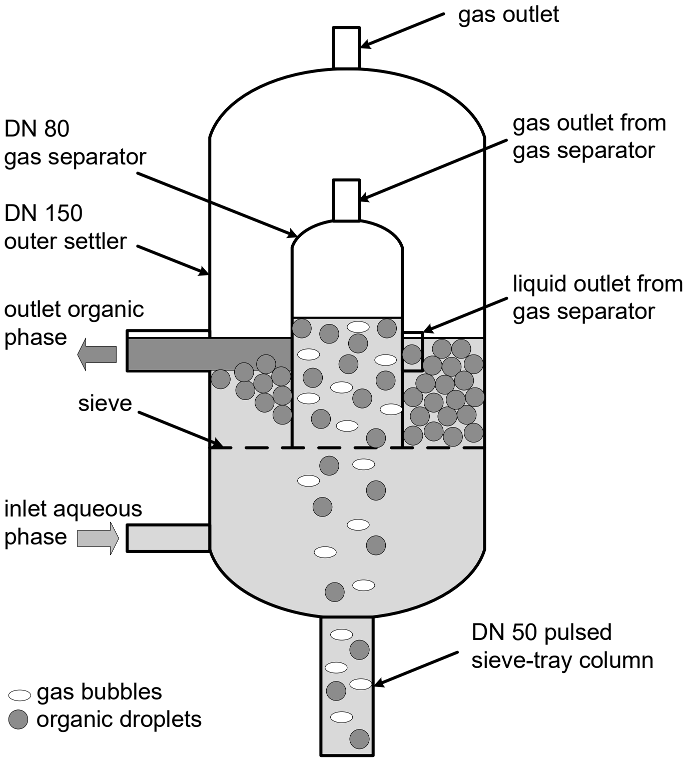


Figure 5. Three-phase separator at extraction-column top for aerated extraction

4. Aerated Column Performance

As mentioned, for in-situ recovery from biotechnological processes e.g. to overcome product inhibition, it is required that the fermentation broth is aerated also during separation. To allow this, the head of the column has been modified with a three-phase separator as shown in Figure 5. All three phases coming from the extraction column – the dispersed phase, gas bubbles and some continuous phase carried along with the dispersed phases – enter the internal gas separator, where the gas is leaving towards the top and the liquids to one side. The liquid phases are then separated in the annular space of the outer separator, where the formerly dispersed phase is exiting to the left.

The results with aeration show that the pulsed sieve-tray column can be operated without problem. The hold-up is significantly increased as compared to the non-aerated case, and the drop size of the organic dispersed phase is reduced, which may even be beneficial for separation performance. The increased hold-up leads to an earlier onset of flooding so that only reduced flowrates are feasible. Since as seen above the degree of extraction of the amine can be approaching essentially unity at reasonable values of pH, meaning essentially complete removal in a single theoretical stage, and because of the usually slow reaction kinetics of fermentation, a low flowrate of organic phase may be fully sufficient to ensure sufficient product removal to avoid product inhibition. For any specific case, this of course requires setting up the basic balances, where in this work it has been shown that even aerated extraction columns are a suitable option to realize this primary separation step technically.

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