0273-2289/99/77–79/0473/$13.00

Separation Optimization for the Recovery of Phenyl Ethyl Alcohol

Sarah A. Priddy,*[,1,2] Thomas R. Hanley,[1] and W. Thomas Effler[2]

[1]University of Louisville, Department of Chemical Engineering, Louisville, KY 40292, E-mail: sapast01@starbase.spd.louisville.edu; and [2]Brown-Forman Corporation, Louisville, KY

Abstract

Phenyl ethyl alcohol is a compound that occurs naturally in flower petals and in many common beverages, such as beer. Desire for the floral, rose-like notes imparted by phenyl ethyl alcohol has created a unique niche for this chemical in flavor and fragrance industries. Phenyl ethyl alcohol can be produced by *Saccharomyces cerevisiae* via bioconversion. Often this method of production results in extremely low yields, thus placing a great deal of importance on recovery and purification of the valuable metabolite.

To determine the best method for recovering the chemical, a primary recovery step and a secondary recovery step were developed. The primary recovery step consisted of comparing dead-end filtration with crossflow ultrafiltration. Crossflow ultrafiltration was ultimately selected to filter the fermentation broth because of its high flow rates and low affinity for the product. The secondary recovery step consisted of a comparison of liquid–liquid extraction and hydrophobic resin recovery. The hydrophobic resin was selected because of its higher rate of recovery and a higher purity than the liquid–liquid extraction, the current practice of Brown-Forman.

Index Entries: Phenyl ethyl alcohol; *Saccharomyces cerevisiae*; crossflow ultrafiltration; hydrophobic resin; bioconversion.

Introduction

Phenyl ethyl alcohol is a compound that occurs naturally in flower petals and in many common beverages, such as beer *(1)*. Despite this unpretentious origin, this compound serves a dual purpose to the flavor and fragrance industry; it is an extremely valuable aroma and flavor constituent. Until recently, flavor and fragrance manufacturers have relied heavily on chemical synthesis of this product *(2)*. For phenyl ethyl alcohol and

*Author to whom all correspondence and reprint requests should be addressed.

many other flavor compounds, it is simply easier and more cost effective to proceed in this manner. However, current socioeconomic trends in consumerism show that "natural" products are highly sought, partly because of the demands of a health-conscious society. This push has caused flavor and fragrance suppliers to search for alternatives to the more traditional chemical synthesis in order to give consumers the "natural" product desired.

In earlier research into natural flavor production, it was discovered that the yeast strain *Saccharomyces cerevisiae* produces phenyl ethyl alcohol via a biosynthetic pathway *(3,4)*. Several fermentations using this strain of yeast were attempted using a rich medium. These experiments produced relatively low levels of this desired chemical, rendering this method of production unprofitable *(5)*. Clearly, optimization of this fermentation would be the easiest way to maximize flavor production and increase profitability.

After optimization of fermentation, problems associated with product recovery and concentration were encountered. Most consumers of phenyl ethyl alcohol demand a high-purity product, necessitating a high rate of recovery and subsequent product concentration. Traditional methods of recovery, such as dead-end filtration, distillation, and extraction, have been effective but often resulted in the death of the yeast culture *(6)*. Fortunately, ultrafiltration and resin-bed applications have proved to be suitable options.

The purpose of this article is to produce phenyl ethyl alcohol with a greater yield and a higher purity, allowing subsequent sale to more consumers at a higher price. This effort is attempted in a two-stage process, testing the current method against a more innovative method, including a primary recovery and a secondary recovery/purification step. The primary recovery tests two methods of filtration; the conventional dead-end filtration is tested against a crossflow membrane ultrafiltration. Here, such issues as efficiency, time, and throughput are investigated. In the secondary recovery/purification step, two methods of product purification are tested. The first, liquid–liquid extraction, is currently used. The second method utilizes a hydrophobic resin. Material balances determine the efficiency of each step. The most effective process for the primary recovery step is used to produce the feed for the secondary recovery/purification step.

Materials and Methods

Organism and Fermentation

The yeast strain used throughout these experiments was *S. cerevisiae*, obtained from Red Star (Milwaukee, WI), under the commercial name Côtes des Blancs®. Dry stock cultures were stored at approx 4°C refrigeration.

A rich medium was utilized for the fermentation broth, including dextrose, l-phenylalanine, and water. The pathway is shown in Fig. 1. The vessel used was a 3785-L stainless-steel fermenter equipped with agitation and sparge capabilities.

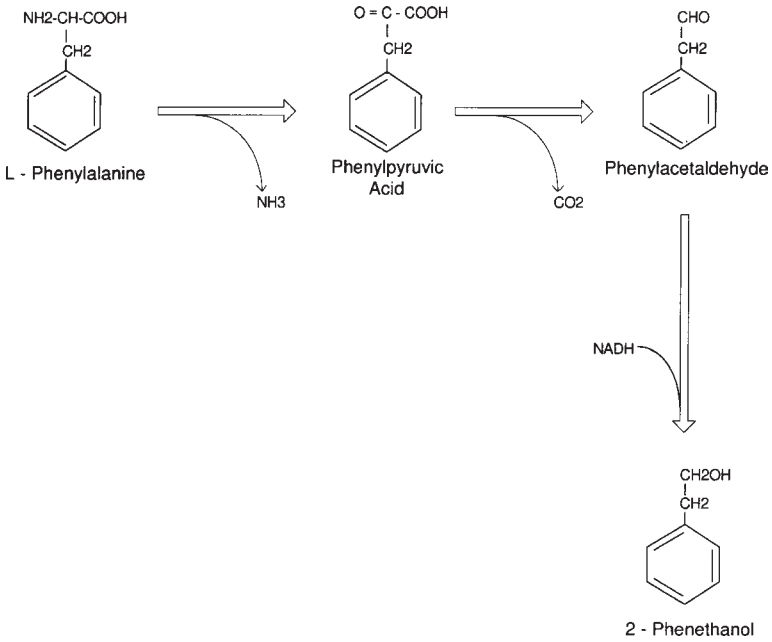

Fig. 1. Phenyl ethyl alcohol pathway.

The fermentations in these experiments were performed in a fed-batch mode. They were fed at two points with dextrose and yeast when the dextrose concentration of the broth ranged between 0 and 5 g/L. The points of feeding were determined using a YSI Inc. (Model 2700; Yellow Springs, OH) metabolite analyzer.

Plate and Frame Filter

The dead-end filtrations were carried out in an Ertel® plate and frame filtration unit. This unit consisted of six frames 30 cm in diameter. The filter pad used were Cellulo® 0.5- and 0.2-µm exclusion filter pads.

Crossflow Ultrafilter

The crossflow ultrafiltrations were carried out in a Romicon®-WF4 ultrafiltration apparatus (Koch Membrane Systems; Wilmington, MA). The cartridges used in these filtrations were the 0.2-µm and the 10,000 mol-wt cutoff Romicon hollow fiber cartridges from Koch Membrane Systems, Inc. (Wilmington, MA).

Liquid–Liquid Extraction

Liquid–liquid extractions were carried out in a 3785-L stainless-steel vessel equipped with agitation capabilities. Each liquid–liquid extraction was carried out in a batch mode.

Hydrophobic Resin

These experiments used a proprietary hydrophobic resin. This resin is a macroporous, weak-base functionalized adsorbent, and is composed of a dimethylamine functionalized chloromethylated copolymer of styrene and divinylbenzene.

The apparatus consisted of a 2.5-cm diameter glass column with a Teflon® stopcock (Aldrich, Milwaukee, WI). The filtrate was gravity-fed into the column at varying flow rates. The column was filled with 20 mL of resin, which was supported with a small ball of glass wool. The entire column was held by a ring stand over a collection vessel.

To prepare the resin, 3% (by weight) solution of sodium hydroxide was prepared with 60°C distilled and deionized water. Approximately 10 bed volumes of this solution were back-washed through the column. To remove all traces of the sodium hydroxide, copious amounts of 82°C water were flushed through the system. The water rinse proceeded until the pH of the water leaving the column equaled that of the water entering the column. To ensure that the column was completely clean, several bed volumes of a solution of ethyl alcohol and water were fed to the column and then drained by gravity. This resin was regenerated in the same manner as it was prepared.

Analysis

All phenyl ethyl alcohol detections and quantitations were carried out by high-performance liquid chromatography (HPLC) by Dr. John Keck of the Analytical Division of Research and Development of the Brown-Forman Corporation (Louisville, KY).

Results and Discussion

Fermentation

Predetermined amounts of dextrose, yeast, l-phenylalanine, and water were added to the 3785-L stainless-steel reaction vessel. The initial pH of the medium was adjusted to 8.0 and the temperature to approx 30°C. This broth was agitated at a rate of 40–50 rpm for the first 8 h. A slow sterile-air sparge was introduced into the fermentation broth, although the dissolved oxygen concentration was not tested. The fermentation was allowed to proceed for 4 d, after which the dextrose concentration was 0 g/L, thus indicating the time for fermentation feeding. In this fermentation, dextrose is utilized by the yeast for both cell maintenance and fermentation. One of the byproducts of fermentation, nicotinamide-adenine dinucleotide (NADH), is consumed in the phenyl ethyl alcohol pathway. The acceptable concentration range for dextrose before feeding was 0–5 g/L. The fermentation was fed according to a determined feeding schedule and allowed to react for an additional 4 d, at which time the fermentation was fed for the final time. Upon completion of the fermentation, the following phenyl ethyl alcohol and ethanol concentrations were obtained, as shown in Table 1.

Table 1
Initial, Final, and Intermediate Phenyl Ethyl Alcohol Concentrations for 3785-L Fermentation

Time	Concentration of phenyl ethyl alcohol, % by wt
Initial	Not detected
After first feeding	0.15
After second feeding	0.19
Final	0.23
Final ethyl alcohol concentration	6.57

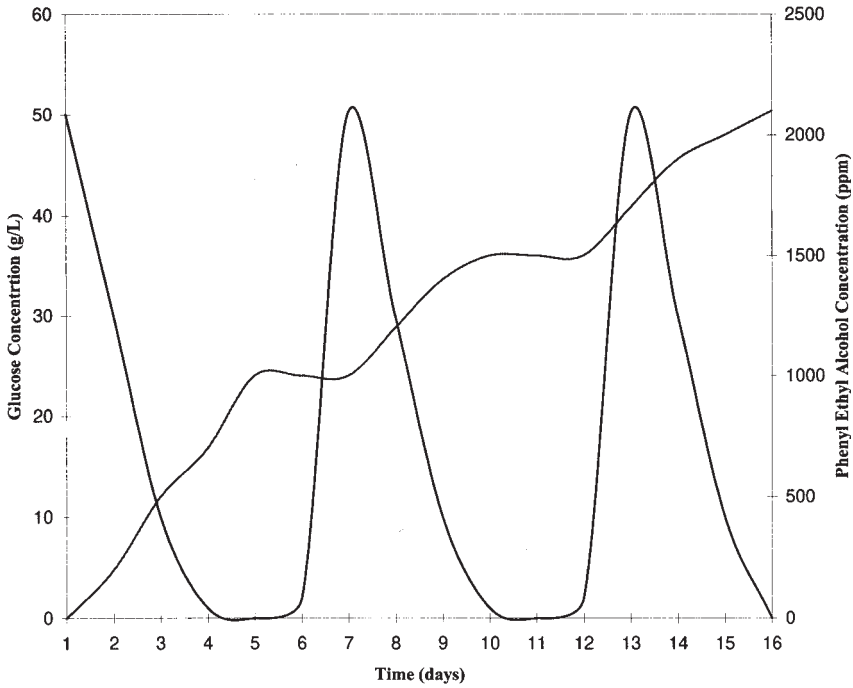

Fig. 2. Phenyl ethyl alcohol and glucose concentration versus time.

The fermentation was allowed to proceed for 4 d longer than would have been predicted. Previous research suggested that the fermentation should only take 9–12 d *(7)*, but the fermentation in this experiment took 16 d. The previous research done at Brown-Forman by Darren Priddy stated that the fermentation should be fed as soon as the glucose concentration of the broth neared zero *(8)*. This fermentation was allowed to rest for 2 d after the broth glucose level reached zero, which allowed all dead or dying yeast to settle to the bottom of the reactor, making subsequent filtering easier. Unfortunately, this may have caused the living yeast to begin to lose viability and cause yields to be lower than was expected. The time course is shown in Fig. 2.

Table 2
Phenyl Ethyl Alcohol Concentrations
Based on Initial Filtration Results from Plate and Frame Filtration

Sample	Phenyl ethyl alcohol concentration, % by wt
Prefiltration	0.139
Postfiltration	0.117

Primary Recovery

Dead-End Filtration

When the first filtration trial was attempted with the single-pass filtration planned, the filter fouled in a matter of seconds. The filter cake that remained behind in this filtration was extremely thick and wet, whereas dry cake was desired.

In the initial study done for the dead-end filtration, the filter press was assembled and operated in the usual manner. Initial filtration results indicated that the filter pads and diatomaceous earth used to filter the broth were actually absorbing some of the desired product. These results are shown in Table 2. These concentrations suggest that this value could be as high as 16% lost. Although it was understood that the porosity of both the diatomaceous earth and the filter pads had the potential for trapping phenyl ethyl alcohol, this phenomenon had not been observed in similar systems. The filter pads and filter aid do not have the same effect on ethyl alcohol and water systems. If the loss of phenyl ethyl alcohol was significant, the economics of using plate and frame filtration would be adversely affected.

The best filtration rate that was achieved with the plate and frame-filter press was approx 4 L/min. In addition to the slow rate of filtration, the massive amount of solids in the fermentation broth caused the filter pads to occlude quickly. Although this problem was alleviated to a great extent by the initiation of a three-stage filtration using filter pads of decreasing exclusion, the solid content was still overwhelming and the filter pads had to be replaced frequently.

Crossflow Ultrafiltration

The membranes used in this ultrafiltration were checked for affinity for phenyl ethyl alcohol. Several filtrations were attempted without a loss of volume to the membrane.

The filtration rates achieved in this crossflow ultrafiltration were larger than those with the plate and frame filtration. The filtration rates achieved in the ultrafiltration portion of experimentation ranged from 12.5 to 15 L/min. Even using the smallest exclusion of 10,000 mol wt, the flow rate was quadruple that achieved with the plate and frame filtration unit. In addition to the faster flow rate, this filter needed fewer cleanings during the course of

Table 3
Phenyl Ethyl Alcohol Concentrations
and Recovered Volumes for Liquid–Liquid Extractions
with Oleyl Alcohol, Propylene Glycol, and Ethyl Acetate

Solvent	Layer	Initial volume, mL	Volume recovered, mL	Concentration of phenyl ethyl alcohol, ppm	K[a]
Oleyl alcohol	Top	50	50	10,150	8.83
	Bottom	500	500	1150	
Propylene glycol	Top	500	510	1250	10.80
	Bottom	50	40	13,500	
Ethyl acetate	Top	50	45	22,760	151.73
	Bottom	500	505	150	

[a]K, organic phase concentration/aqueous phase concentration.

the large-scale filtration. During this filtration, the ultrafiltration unit needed to be cleaned once during the filtration. In these cases the thick, yeast-rich retentate was discarded, which accounts for the void volume losses recorded.

Based on the first two filtration criteria, affinity for phenyl ethyl alcohol and filtration flow rate, ultrafiltration seemed like the best opportunity. The ultrafiltration method also had a decreased down-time between filtrations and did not need extra filter preparation steps, such as the addition of a filter cake. However, void volume losses from the ultrafiltration unit were approx 133 out of 3730 L. These losses amount to almost 3.6% of the total recoverable volume. In the 133 L of filtrate discarded because of ultrafilter cleanings, 1/3 kg of phenyl ethyl alcohol could no longer be recov ered. The current selling price of $935/kg results in almost $300 lost and unrecoverable *(9)*.

Secondary Recovery/Purification

Liquid–Liquid Extraction

The solvents that were selected for the liquid–liquid extraction (oleyl alcohol, propylene glycol, and ethyl acetate) were often used in other food and fragrance applications *(10)*. The solvents were evaluated according to two major criteria: separation and recovery. Both the ethyl acetate and the oleyl alcohol partitioned very well; neither exhibited any emulsion layer or difficult separation. This was not the case with the propylene glycol. Partitioning was not successful with this extraction. The propylene glycol formed small bubbles in the broth layer. These bubbles eventually fell back into the propylene glycol layer, but the time requirement was much greater than with the other solvents. Results from these extractions are shown in Table 3.

Based on the major criteria used for selection of solvent, ethyl acetate seemed to be the best choice. With both the oleyl alcohol and the propylene glycol solvents, nearly half of the phenyl ethyl alcohol present in the broth initially was not extracted. Oleyl alcohol recovered 46.9% and propylene glycol recovered 47.9%. In this respect, the ethyl acetate extraction surpassed the others in terms of efficiency, with a recovery of 94.5%.

To test the use of the ethyl acetate solvent on a larger scale, the extraction was carried out on ultrafiltered fermentation broth. For the small-scale extraction with ethyl acetate, a precipitate formed at the two-phase interface. This precipitate was likely to be a salt that resulted from organic acids remaining in the ultrafiltered fermentation broth, and could be eliminated through pH adjustments *(11)*. The pH adjustment eliminated the particulate at the broth–ethyl acetate interface.

The broth was extracted three times with ethyl acetate to ensure complete recovery of the phenyl ethyl alcohol. Although some of the phenyl ethyl alcohol remained in the aqueous layer, further extractions with ethyl acetate were deemed economically undesirable. It can also be seen that the concentrations in the ethyl acetate layers were about three times as high as those in the broth. The results from this larger scale extraction is shown in Table 4.

From these results, it was calculated that approx 8.5 kg of phenyl ethyl alcohol were recovered. The total amount of phenyl ethyl alcohol produced, based on final broth concentration and final broth volume, was 9.1 kg. This value did not neglect losses caused by ultrafiltration. Efficiency of recovery was then about 93.5%, an acceptable recovery. This value represents the amount recovered from the three-stage extraction procedure and does not take into account the amount that may be recovered with increased stages or a countercurrent column. This also represented a concentration of the original 3400 L of broth to 2566 L of ethyl acetate, a reduction of 25%.

Hydrophobic Resin

It was already known that phenyl ethyl alcohol was extremely hydrophobic *(12)*. However, it was not known how well this new hydrophobic resin would work at adsorbing phenyl ethyl alcohol. Batch resin studies were tested first. In these studies, a baseline resin capacity was discovered to be at a ratio of between 1:20 and 1:40 of resin to filtered fermentation broth. One important discovery from these batch experiments was that the recovery of phenyl ethyl alcohol from the fermentation broth was nearly 100%.

Having completed some preliminary batch studies, hydrophobic resin column studies were started. Since ultimate scale-up and production implementation would necessitate the use of a column, these studies were crucial. A variety of tests were performed in this mode. These first involved varying the flow rate of liquid through the column. It was discovered that for maximum binding, a flow rate of 0.1 mL/min/mL of bed volume was

Table 4
Phenyl Ethyl Alcohol Concentrations and Recovered Volumes
for the Liquid–Liquid Extraction of the 3785-L Ultrafiltered Fermentation Broth

Tank #	Extraction #	Volume of ethyl acetate added, L	Volume of ethyl acetate recovered, L	Phenyl ethyl alcohol in ethyl acetate, % by wt	Phenyl ethyl alcohol in broth, % by wt	K[a]
1	1	624.5	416	0.71	0.02	35.5
	2	416.0	469	0.20	0.01	20.0
	3	416.0	397	0.06	0.0054	11.1
2	1	624.5	394	0.79	0.03	26.3
	2	416.0	469	0.22	0.02	11.1
	3	416.0	420	0.05	0.0077	6.5

[a]K, organic phase concentration/aqueous phase concentration.

Table 5
Phenyl Ethyl Alcohol Concentrations Resulting
from the Column Elution with Ethyl Acetate Post Sodium Hydroxide

Washing	Volume of ethyl acetate recovered, mL	Volume of water recovered, mL	Concentration of phenyl ethyl alcohol, ppm
1	45	5	51,100
2	75	2	15,400

required. At this flow rate the capacity of the resin was determined to be approx 30 bed volumes.

Because of the resin's extreme affinity for the phenyl ethyl alcohol, elution of the resin to recover the bound product was difficult. A variety of techniques were attempted, including elution with ethanol, warm ethanol, warm sodium hydroxide solutions, and ethyl acetate.

The elutions with ethanol and warm ethanol met with moderate success. However, the sodium hydroxide elutions were not successful. The sodium hydroxide solution did not remove an appreciable amount of phenyl ethyl alcohol and only seemed to wash the resin of any other bound particles that may have been in the broth. Although the high pH of this elution did not meet with success, elutions of this type with varying pH need to be investigated further. Conversely, the subsequent elution of the bed with ethyl acetate was successful. These results from this experiment, as seen in Table 5, show that the ethyl acetate elution increased recovery and purity of the resulting phenyl ethyl alcohol product. Whereas only 62.7% of the phenyl ethyl alcohol in the original 3 L of broth was recovered, 68.7% of the bound phenyl ethyl alcohol was recovered. The concentration of one phenyl ethyl alcohol product was 5.11%. This was the highest concentration seen in the resin studies and was a concentration five times greater than the liquid–liquid extractions achieved.

Conclusions

Fermentation

1. The fed batch technique produces the highest observed yield, with a yield increase of 47% over normal batch yields.
2. The 2-d waiting period instituted after the fermentation broth glucose concentration reaches zero decreased phenyl ethyl alcohol yield by 8% over control fermentations.

Primary Recovery

Ultrafiltration provides a better method of recovery than dead end filtration.

1. Ultrafilter membranes show no affinity for phenyl ethyl alcohol, whereas the plate and frame-filter media absorbed up to 16% of the product.
2. The ultrafilter apparatus is easier to operate than the plate and frame-filter press.
3. The flow rate achieved with the ultrafilter is approximately four times greater than that of the plate and frame-filter press.
4. The hollow-fiber filtration cartridges have exclusion capabilities 50 times greater than the plate and frame filter pads.

Secondary Recovery/Purification

1. Ethyl acetate performed better than the other solvents tested.
   a. Ethyl acetate did not form an emulsion layer with the fermentation broth.
   b. Ethyl acetate removed nearly twice as much phenyl ethyl alcohol from the broth as did the two other solvents tested, oleyl alcohol and propylene glycol.
2. Hydrophobic resin utilization provides a better purification than liquid–liquid extraction.
   a. The hydrophobic resin removed 98% of the broth phenyl ethyl alcohol prior to breakthrough, a 5% increase over liquid–liquid extraction.
   b. The concentration of the phenyl ethyl alcohol product from the hydrophobic resin purification is five times greater than that realized in the liquid–liquid extractions.
   c. Use of the hydrophobic resin caused an overall reduction of volume from broth to extract of 95%, whereas the liquid–liquid extraction only reduced the volume 25%.

Acknowledgments

This research was supported by the Brown-Forman Corporation of Louisville, KY.

References

1. Gabelman, A. (ed.) (1984), *Bioprocess Production of Flavors, Fragrance and Color Ingredients*, Wiley, New York, pp. 1–17.
2. Häuser, A. and Münch, T. (1997), *ASM News* **63,** 551.
3. Fukuda, K., Watanabe, M., Asano, K., Ueda, H., and Ohta, S. (1989), *Agricult. Biol. Chem.* **54,** 269–271.
4. Patterson, R. L. S., Charlwood, B. V., MacLeod, G., and Williams, A. A. (1992), *Bioformation of Flavours*, The Royal Society of Chemistry Press, Cambridge, UK.
5. Priddy, D. L. (1993), Interoffice correspondence provided by Brown-Forman Corporation, Louisville, KY.
6. Atkinson, B. and Mauituna, F. (1991), *Biochemical Engineering and Biotechnology Handbook*, Stockton, New York, pp. 907–1021.
7. Priddy, D. L. (1995), Interoffice correspondence provided by Brown-Forman Corporation, Louisville, KY.

8. Priddy, D. L. (1996), Interoffice correspondence provided by Brown-Forman Corporation, Louisville, KY.
9. Catalog Handbook of Fine Chemicals, (1996–1997), Aldrich, Milwaukee, WI.
10. Gabelman, A. (ed.) (1984), *Bioprocess Production of Flavors, Fragrance and Color Ingredients*, Wiley, New York, pp. 34–58.
11. Priddy, D. L. (1998), Brown-Forman Corporation, personal communication.
12. Priddy, D. L. (1998), Brown-Forman Corporation, personal communication.