Biotechnol. Bioprocess Eng. **1999**, 4, 1-11

Extractive Recovery of Products from Fermentation Broths

Joong Kyun Kim[1]*, Eugene L. Iannotti[2], and Rakesh Bajpai[2,3]

[1]Major of Biotechnology, Division of Food Science and Biotechnology, Pukyong National University, Pusan 608-737, Korea
[2]Department of Agricultural Engineering, University of Missouri, Columbia, MO 65211, U.S.A.
[3]Department of Chemical Engineering, University of Missouri, Columbia, MO 65211, U.S.A.

Considerations of partition coefficients, selectivity, biocompatibility, and waste generation are important in selection of appropriate solvents to be used for extractive recovery of products from fermentation broths. Several selection criteria can be used based upon the nature of different species present in the broth. These criteria, along with examples of specific case studies, were presented. These serve not only in screening of useful solvents, but also in pointing to the specific modes of operation of recovery-coupled bioprocesses.

Key words: extractive recovery, fermentation, partition coefficient, selectivity, biocompatibility

INTRODUCTION

Biological production of chemicals from non-fossilized resources is very attractive due to the relatively moderate conditions utilized in such processes, specificity of transformations, and regenerative nature of raw materials. Bioprocesses are generally considered environmentally friendly also. On the other hand, these processes form product streams that are dilute and contain a number of contaminating chemicals. Hence, recovery of products from these product streams is tedious and costly. Efficient, economic, fast, and robust methods of product recovery are necessary for the successful production of chemicals by bioprocessing. Extraction, driven by preferential partitioning of desired compound(s) between two immiscible liquid phases, is a commonly used unit operation in chemical processing industry and is also extensively used in pharmaceutical industry (Table 1). It lends to continuous operation, and principles of scale-up of extractions are well known. As a result, extraction has been extensively investigated for recovery of chemicals produced by bioprocessing.

Since end-product inhibition limits the concentration of the desired fermentation products in a number of bioprocesses [13,14], many attempts have been made to integrate the production process with recovery operations [15-19]. Extractive fermentation is a technique where extraction is carried out as the product is formed in the bioreactor and the inhibitory product(s) are selectively taken out of the aqueous culture medium thus relieving inhibition. *In-situ* extraction involves introducing the extractants directly in the bioreactor. Extractive fermentation has been used successfully for kinetic enhancement as well for concentration of the desired product. Extraction is attractive not only for heat-labile and high boiling-point metabolites, but also for low boiling point products that form azeotropes with water at low concentrations. Higher reactor productivities are also obtained due to higher cell concentrations in the bioreactor [20].

Table 1. Extractions in pharmaceutical industry

Antibiotics	[1]
Vitamin B-12	[2]
Prednisolone	[3]
Macrolides	[4]
Rifamycin	[4]
Chloramphenicol	[4]
Polyenes	[5]
Penicillin	[6]
Cephalosporin	[7]
Erythromycin	[8]
Oxytetracycline	[9]
Tetracycline	[10]
Bacitracin	[11]
Dactinomycin	[12]

Key considerations in extractive fermentations include solvent selection and deciding a strategy of operation of such recovery-coupled systems. Different aspects of these considerations have been discussed in this paper. Several examples have been provided and the directions of new developments have been identified.

SOLVENT SELECTION

Systematic solvent screening is necessary for successful development of an extractive fermentation process. The key criteria for solvent screening are the distribution coefficient (ratio of product concentrations in solvent and in aqueous phases), selectivity (ratio of distribution coefficients of the product and of other impurities between the two phases), and biocompatibility of the solvent system. The important solvent characteristics are density, viscosity, interfacial tension, polarity, volatility, and flammability. An ideal solvent should posses the following characteristics: a low cost, environmental safety, non-toxicity, low viscosity, biocompatibility, complete immiscibility with aqueous phase, high distribution coefficient and a high selecti-

* Corresponding author
Tel: +82-51-620-6186 Fax: +82-51-620-6186
e-mail: jkkim@nuri.net

vity for the desired product, and easy recovery of the desired product from the solvent phase.

Miscibility of solvent and aqueous phases is economically important. Cost of solvent required to make-up for losses is a major concern in solvent extractions [21]. High solvent-solubility in aqueous phase is likely to be associated with higher toxicity for the microorganisms (loss of biocompatibility); it increases the cost of waste treatment as well. Partitioning of water in solvent phase generally increases the cost of product recovery from the solvent phase.

Viscosity of solvent influences the efficiency of extraction through its effect on mass transfer coefficients. As an example, Alamine 336 in oleyl alcohol turned out to be an excellent extractant for lactic acid from fermentation broth [22]. However, the high viscosity of oleyl alcohol resulted in prediction of low mass-transfer coefficients in membrane extractions, which in turn would cause accumulation of higher concentrations of lactic acid in the fermentation broth and low rates of its production [22]. Highly viscous solvents require more power for pumping and mixing also. Interfacial tension has a direct bearing on phase contacting during extraction and in the solvent recovery operation. Low interfacial tension causes stable emulsions resulting in difficulties during separation of phases, while high interfacial tension causes high energy requirements to maintain sufficient contact area for mass transfer [17].

Screening of solvents for their suitability for extraction of a product from fermentation broths can be conducted systematically from a computer database. Programs such as UNIFAC and UNIQUAC can be used to calculate liquid-liquid equilibria (LLE) data for different solvent-solute-aqueous medium systems. From the LLE data, distribution coefficients, selectivity, and the solubilities of the products and solvents in aqueous phase are calculated. Subsequent screening of the selected solvents for biocompatibility to the cells is often conducted experimentally to further narrow down the choices [18,23,24].

Distribution and Partition Coefficients

Distribution coefficient (k_d) of a compound is defined as the ratio of its concentration in the solvent phase to that in aqueous phase. The greater the value of the distribution coefficient, the less the ratio of solvent to water will be required for effective extraction. For dissociating compounds, the pH of aqueous solution displays a strong effect on k_d. This observation is a result of the fact that only the undissociated form of a compound is transferred into the generally nonpolar organic solvents. Therefore, another term 'partition coefficient' (m) is defined as a ratio of the concentration of undissociated product in the organic phase to that in aqueous phase. For nondissociating products, the values of k_d and m are same. The partition coefficients of organic acids have been shown to be independent of pH of the aqueous broth [25]. It is thus important for effective extraction to maintain pH of aqueous broths near pK value of the product.

Distribution and partition coefficients of several fermentation products in various solvents are listed in Tables 2 and 3. There are some apparent wide variations in k_d data reported in literature due to variations in purity of solvents, pH, temperature, analytical methods, etc. [26]. In general, two key parameters of the solute and the solvent determine the value of distribution coefficients of different solutes between aqueous and organic phases: the numbers of electron donor/acceptor (Lewis basicity/acidity) and the Hildebrand solubility parameters (polar/nonpolar character). These numbers for a number of chemicals are available from handbooks and can be used to make a tentative list of solvents that may be used for further screening using LLE-data.

The distribution coefficients of polar chemicals tend to increase with increasing numbers of polar groups in the solvent-structure [26]. An increasing order of extractability of ethanol in various solvents is hydrocarbon = halocarbon < ether < ketone < amine < ester < alcohol = organic phosphate < carboxylic acids [26,27]. In ethanol-solvent-water system, ethanol and water have both electron donating and electron accepting capabilities, and ethanol has a higher electron donor number than water. Therefore, it is not surprising that Lewis acids (such as carboxylic acids) form better extractants than Lewis bases (such as amines and ketones). Amongst carboxylic acids, those with lower chain length have higher k_d values than those with longer chain length.

As solvents, alcohols, esters, and phenols have higher distribution coefficients for ethanol, butanol, and acetone from aqueous solutions [17,28-30], while saturated and unsaturated hydrocarbons have poor values of k_d due to their nonpolar characteristics. Branched chain alcohols appear to be better for ethanol extraction [26, 31]. This is a result of changes in basicity caused by steric effects due to branched chains. For extractions of acetone and butanol, k_d values decrease as the molecular weight of hydrocarbon solvent increases [17]. Incorporation of halogen and nitrogen-bearing groups in the solvent improves the distribution coefficients slightly. Accordingly, fluorocarbons such as Freon 11 (monofluorotrichloro-methane) and Freon 21 (monofluorodichloromethane) have been proposed as solvents for extraction of C_2-C_5 alcohols from fermentation broths [32].

C_5 and C_6 carboxylic acids have reasonably high distribution coefficients for ethanol. The fact that k_d decreases with increase in chain length of the acid [30] is perhaps related to decreasing solvent polarity, as measured by Hildebrand solubility parameter [30,33, 34]. For the same reason, with kerosene as a solvent, k_d values of long-chain organic acids are larger than those of lower chain length.

Distribution coefficients of chemicals between two phases can be affected by reactions in either phase. Addition of enzyme lipase in a two phase system consisting of an organic acid (valeric, hexanoic, octanoic, or oleic acid) as solvent and aqueous phase, improved the partitioning of ethanol [30] in the nonaqueous phase. The enzyme catalyzed a reaction between ethanol and the organic acids, forming esters which are very efficiently partitioned in the solvent phase. For the same reason, strong Lewis bases such as high molecular weight tertiary amines and trioctyl phosphine oxide (TOPO) can improve the partitioning of organic acids in conventional solvents such as ethyl acetate or diethyl ketone [22]. Tertiary amines offer advantage over TOPO due to their lower cost, greater volatility, and generally higher distribution coefficients [36,37]. Alamine 336 and Adogen 364 are among the most

Table 2. Distribution coefficients (k_d) and partition coefficients (m) of alcohols and acetone

Product	Solvent	k_d	Ref.
Ethanol	Heptane	0.056	[17]
	n-Dodecane	0.045	[17]
	Decane	0.02	[17]
	n-Tridecane	0.04	[17]
	Tetradecane	0.10	[17]
	Hexadecane	0.02	[17]
	Hexane	0.07	[17]
	Hexanes mix	0.05	[17]
	Cyclohexane	0.0066-0.066	[17,26]
	Phenylcyclo-hexane	0.0078	[26]
	Cyclooctane	0.02	[17]
	Isopentane	0.20	[17]
	2,2,4-trimethyl-pentane	0.02	[17]
	1-Heptene	0.04	[17]
Ethanol	Benzene	0.046-0.092	[17,23,31]
	Ethylbenzene	0.029	[26]
	Diethylbenzene	0.022	[26]
	Cumene	0.028-0.18	[17,26]
	Toluene	0.034-0.085	[29,30,31,35]
	o-Xylene	0.02-0.03	[17,26]
	m-Xylene	0.06	[17]
	p-Xylene	0.06	[17]
	1,2,3,4-Tetra hydronaphthalene	0.0029-0.05	[17,26]
Ethanol	1-Pentanol	0.078	[17]
	1-Hexanol	1.0-1.2	[31,35]
	1-Octanol	0.50-0.64	[17,26,31,35]
	2-Octanol	0.60	[28,39]
	1-Heptanol	0.75	[17]
	1-Nonanol	0.71-0.73	[31]
	1-Decanol	0.39-0.57	[29,31]
	1-Tridecanol	0.22	[26]
	4-Decanol	0.32	[26]
	1-Dodecanol	0.21-0.59	[18,29,31]
	3-Methylcyclo-hexanol	0.93	[31]
	3-Methyl-3-pentanol	1.3	[31]
	4-Methyl-2-pentanol	1.1	[31]
	2,2-Dimethyl-3-octanol	0.31	[26]
	2,4-Dimethyl-3-pentanol	0.59	[26]
	2,4-Dimethyl-3-heptanol	0.38	[26]
	2,6-Dimethyl-4-heptanol	0.53	[31]
	3,7-Dimethyl-3-octanol	0.40	[26]
	2,3,4-trimethyl-3-pentanol	0.82	[31]
	2-Ethyl-1-butanol	0.69-1.03	[26,28,31,35]
	2-Ethyl-1-hexanol	0.47	[17,31]
	2-Ethyl-1-heptanol	0.48	[26]
	3-Ethyl-3-pentanol	1.1	[31]
	3-Ethyl-3-heptanol	0.44	[26]
	3-Phenyl-1-propanol	0.64-0.77	[26,28]
	Texanol	0.36	[26]
	Fine Oxacol	0.034-0.16	[29,30]
	Oxacol	0.022-0.20	[29,30]
	Oleyl alcohol	0.22-0.24	[29,30]
	C-20 Guerbet alcohol	0.15-0.17	[29,30]
Ethanol	Phenol	2.15	[17]
	o-Isopropylphenol	1.4	[17]
	0-tert-Butylphenol	1.4	[29]
Ethanol	2-Butanone	0.93	[17]
	4-Methyl-2-pentanone	0.34	[17]
	3-Pentanone	0.34	[17]
	3-Heptanone	0.23	[17]
	2,6-Dimethyl-4-heptanone	0.088	[26]
	Methylisobutylketone	0.5	[31]
	Diisobutylketone	0.18-0.20	[31]
	Isobutylheptyl ketone	0.12-0.14	[31]
	Isophorone	0.79	[31]
Ethanol	Valeric acid	1.13	[19,27]
	Hexanoic acid	0.944-1.1	[19,27,31]
	Octanoic acid	0.525-0.65	[19,27,31]
	Oleic acid	0.047-0.171	[17,19,29,30]
	Isostearic acid	0.06	[29]
	Ricinoleic acid	0.17	[29]
	Nonaoic acid	0.464	[27]
	Neodecanoic acid	0.23	[31]
	2-Ethyl hexanoic acid	0.51-0.55	[31,35]
	2-Ethyl-4-Methyl-n-pentanoic acid	0.49	[31]
Ethanol	Methyl acetate	0.91	[17]
	Ethyl acetate	0.70	[17]
	Vinyl acetate	0.21	[17]
	Butyl acetate	0.26-0.34	[17,26]
	Isobutyl acetate	0.21	[26]
	tert-Butyl acetate	0.24	[26]
	n-Propyl acetate	0.30	[17]
	Ethyl formate	0.23	[17]
	Ethyl butyrate	0.39	[17]
	Ethyl propionate	2.53	[17]
	n-Butyl phthalate	0.10	[17]
	Dibutyl phthalate	0.651	[18]
	n-Hexyl ether	0.037	[26]
Ethanol	Methylene chloride	0.10	[31]
	Carbon tetra-chloride	0.021	[31]
	Dichloromethane	0.28	[17]
	Tettrachloro-methane	0.038	[17]
	1,2-Dichloro-ethane	0.074-0.12	[17,31]
	1,1,1-Trichloro-ethane	0.09-0.12	[17,31]
	Freon 11	0.011-0.21	[17,26]
	Freon E	0.21	[30]
	Freon 113	0.094	[26]
	Freon 214	0.0087	[26]
	n-Decylbromide	0.0099	[26]
	Chloroform	0.12	[26]
Ethanol	Castor oil	0.08-0.22	[29,30]
	Olive oil	0.04	[29]
	Tung oil	0.01	[29]
	Tempura oil	0.02	[29]
Ethanol	PPG P-1200	0.58	[28]
	PPG 1000	0.51	[18]
Ethanol	1,2,4-Trichloro benzene	0.06	[17]
	Nitrobenzene	0.091-0.092	[17,31]
	Octadecafluoro-decalin	0.74	[30]
Ethanol	Tributyl phosphate	0.54-0.886	[18,26,28]
	Triisobutyl phosphate	0.65	[26]
	Tris(2-methylbutyl) phosphate	0.44	[26]
	Tri-2-ethylhexyl-phosphate	0.23	[26]
	Diamyl amyl phosphate	0.56	[26]
Ethanol	Amberlite XLA3	0.0044	[26]
	Adogen 364	0.017	[26]
	Adogen 368	0.04	[26]
	Adogen 464	0.48	[26]
Ethanol	52% Toluene (w/w) + 48% ethylhexanoic acid	0.39	[35]
	85% Hexan-1-ol + 15% toluene (w/w)	0.87	[35]
	75% Hexan-1-ol + 25% toluene (w/w)	0.72	[35]
	85% 2-Ethyl-1butanol (w/w) +15% 2-ethylhexanoic acid	0.88	[35]
	50% Hexan-1-ol + 50% 2-ethyl-1-butanol(w/w)	1.03	[35]
	85% Hexan-1-ol + 15% 2-ethylhexanoic acid (w/w)	0.83	[35]
	85% 2-Ethyl-1-butanol + 15% toluene (w/w)	0.89	[35]
Ethanol	62% (w/w) 2-Ethyl-hexanoic acid in methylisobutyl ketone	0.81	[31]
	42.9% (w/w) 2-Ethyl-hexanoic acid in methylisobutyl ketone	0.76	[31]
	48% (w/w) 2-Ethyl-hexanoic acid in toluene	0.27	[31]
	50% (w/w) 2-Ethyl-hexanoic acid in diidiobutyl ketone	0.41	[31]
	50% (w/w) Neo-decanoic acid in isobutyl heptyl ketone	0.30	[31]
	50% (w/w) Neo-decanoic acid in toluene	0.16	[31]
	50% (w.w) Adogen 368 in diisobutyl ketone	0.13	[31]
	50% (w.w) Adogen 368 in tetrachloroethane	0.30	[31]
	25% (w.w) Adogen 368 in diisobutyl ketone	0.21	[31]

(Continued on the next page)

Table 2.

Product	Solvent	k_d	Ref.
Isopro-panol	Oleyl alcohol	0.90	[30]
	C-20 Guerbe alcohol	0.88	[30]
	Castor oil	0.61	[30]
Butanol	Heptane	0.21	[17]
	n-Dodecane	0.13	[17]
	Decane	0.16	[17]
	n-Tridecane	0.14	[17]
	Tetradecane	0.12	[17]
	Hexadecane	0.11-0.148	[15,17]
	Hexane	0.02	[17]
	Hexanes mix	0.14	[17]
	Cyclohexane	0.27	[17]
	Cyclooctane	0.56	[17]
	Isopentane	0.13	[17]
	2-Methylpentane	0.30	[17]
	2,2,4-Trimethyl-pentane	0.20	[17]
	1-Heptane	0.40	[17]
Butanol	Benzene	0.70	[17]
	Cumene	1.70	[17]
	Toluene	0.93	[17]
	o-Xylene	0.54	[17]
	m-Xylene	0.57	[17]
	p-Xylene	0.74	[17]
	1,2,3,4-Tetra-hydronaphthalene	0.51	[17]
Butanol	1-Octanol	5.6-7.33	[15,17]
	1-Heptanol	6.62	[17]
	1-Pentanol	7.48	[17]
	2-Ethyl-1-hexanol	6.09	[17,40]
	Hexanol	9.91	[15]
	Decanol	6.20	[15]
	Undecanol	5.55	[15]
	Dodecanol	5.14	[15]
	Fine oxocol	3.0	[41]
	Oxocol	4.7	[30]
	Oleyl alcohol	3.21-4.3	[15,30]
	C-16 Guerbet alcohol	4.5	[30]
	C-20 Guerbet alcohol	3.5	[30]
Butanol	Phenol	24.0	[17]
Butanol	2-Butanone	3.50	[17]
	4-methyl-2-pentanone	4.02	[17]
	3-Pentanone	4.50	[17]
	3-Heptanone	3.22	[17]
Butanol	Oleic acid	1.61- 3.0	[17,30]
	Isostearic acid	2.2	[30]
Butanol	Methyl acetate	3.37	[17]
	Ethyl acetate	4.62	[18]
	Vinyl acetate	2.40	[17]
	Butyl acetate	3.58	[17]
	n-Propyl acetate	4.34	[17]
	Ethyl formate	1.75	[17]
	Ethyl butyrate	2.86	[17]
	Ethyl propionate	3.48	[17]
	n-Butyl phthalate	1.36	[17]
Butanol	Castor oil	2.6	[30]
	Corn oil	0.653	[15]
Butanol	1,2,4-Trichlorobenzene	0.46	[17]
	Nitrobenzene	0.93	[17]
Butanol	Dichloromethane	2.14	[17]
	Tetrachloromethane	0.40	[17]
	1,2-Dichloroethane	1.07	[17]
	1,1,1-Trichloro- methane	0.61	[17]
	Monofluorotri-chloromethane	0.23	[17]
	Freon E	0.31	[17]
Butanol	Octadecafluoro-decalin	0.65	[30]
	Kerosene	0.127	[15]
Acetone	Heptane	0.16	[17]
	n-Dodecane	0.10	[17]
	Decane	0.11	[17]
	n-Tridecane	0.31	[17]
	Tetradecane	0.07	[17]
	Hexadecane	0.08	[17]
	Hexane	0.17	[17]
	Hexane mix	0.15	[17]
	Cyclohexane	0.18	[17]
	Cyclooctane	0.15	[17]
	Isopentane	0.14	[17]
	2-Methylpentane	0.21	[17]
Acetone	2,2,4-Trimethyl-Pentane	0.15	[17]
	1-Heptene	0.21	[17]
Acetone	Benzene	0.97	[17]
	Cumene	1.40	[17]
	Toluene	0.63	[17]
	o-Xylene	0.41	[17]
	m-Xylene	0.53	[17]
	p-Xylene	0.54	[17]
	1,2,3,4-Tetrahydro-naphthalene	0.34	[17]
Acetone	1-Octanol	0.52	[17]
	1-Heptanol	0.65	[17]
	1-Pentanol	0.88	[17]
	2-Ethyl-1-hexanol	0.58	[17]
	Fine oxocol	0.14	[30]
	Oxocol	0.089	[30]
	Oleyl alcohol	0.52	[30]
	C-16 Guerbet alcohol	0.44	[30]
	C-20 Guerbet alcohol	0.34	[30]
Acetone	Phenol	8.45	[17]
Acetone	2-Butanone	1.37	[17]
	4-methyl-2-pentanone	1.08	[17]
	3-pentanone	1.32	[17]
	3-Heptanone	0.84	[17]
Acetone	Oleic acid	0.27-0.29	[17,30]
	Isostearic acid	0.15	[30]
Acetone	Methyl acetate	1.35	[17]
	Ethyl acetate	1.44	[17]
	Vinyl acetate	1.36	[17]
	Butyl acetate	0.97	[17]
	n-Propyl acetate	1.16	[17]
	Ethyl formate	1.37	[17]
	Ethyl butyrate	0.83	[17]
	Ethyl propionate	1.12	[17]
Acetone	n-Butyl phthalate	0.57	[17]
Acetone	Dichloromethane	4.91	[17]
	Tetrachloromethane	0.45	[17]
	1,2-Dichloroethane	2.05	[17]
	1,1,1-Trichloroethane	1.04	[17]
	Monofluoroteichloro-methane	0.38	[17]
	Freon E	0.74	[30]
	Castor oil	0.44	[30]
Acetone	1,2,4-Trichloro-benzene	0.54	[17]
	Nitobenzene	1.05	[17]
	Octadecafluoro-decalin	0.12	[30]

Table 3. Distribution coefficients (k_d) and partition coefficients (m) of organic acids

Product	Solvent	Concentration aq. phase(g/L)	k_d	Ref.
Lactic acid	n-Heptadecane	-	0.01	[40]
	Oleyl alcohol	60.5	0.001	[22]
	Hexanoic acid	72.5	0.076	[22]
	Hexanoic acid	73.2	0.067	[22]
	Tributyl phosphate	56.5	0.186	[22]
	Tributyl phosphate	-	0.9	[40]
	Cumene	67.5	0.001	[22]
	Cumene	76.2	0.001	[22]
	Kerosene	52.3	0.0004	[22]
	Methyl crotonate	71.1	0.063	[22]
	Methyl crotonate	66.3	0.066	[22]
	Aliquot 336	-	1.0	[40]
	30% TOPO (w/w) in cumene	-	1.0-1.4	[40]
	Kerosene saturated with TOPO	-	0.6-0.8	[40]
	25% (w/w) Aliquot 336 in cyclooctane	pH=5.5	4.5	[40]
	21% TOPO (w/w) in kerosene	91.0	0.110	[22]
	21% TOPO (w/w) in kerosene	76.7	0.113	[22]
	21% TOPO (w/w) in kerosene	56.5	0.150	[22]
	21% TOPO (w/w) in kerosene	31.9	0.166	[22]
	10% TOPO (w/w) in kerosene	91.0	0.081	[22]
	10% TOPO (w/w) in kerosene	82.6	0.064	[22]
	5% TOPO (w/w) in kerosene	99.1	0.042	[22]
	5% TOPO (w/w) in kerosene	84.6	0.039	[22]
	Alamine 336	-	0.759	[49]
	15% Alamine (w/w) in kerosene	96.2	0.023	[22]
	15% Alamine (w/w) in kerosene	80.7	0.021	[22]
	15% Alamine (w/w) in oleyl alcohol	74.8	0.168	[22]
	15% Alamine (w/w) in oleyl alcohol	86.5	0.147	[22]
	50% Alamine (v/v) in oleyl alcohol	-	2.62	[49]
	50% di-n-Octyl amine in oleyl alcohol	-	11.1	[49]
	tri-n-Hexyl amine	-	1.27	[40]
	50% tri-n-Hexyl amine in oleyl alcohol	-	1.27	[40]
Formic acid	23% (w %) TOPO in n-heptane/ n-hexanol (2:1 vol)	0.394	12.7	[42]
	Tributyl phosphate (TBP)	0.394	2.9-3.0	[42]
	44.1 wt% TBP in Chevron 25	0.394	1.0	[42]
	8.57% Triisooctyl amine in n-heptane/n-hexanol (2:1 vol)	0.394	5.6	[42]
Acetic acid	50 vol% Amberlite LA-1 in Chevron 25	1.16	1.27	[38]
	50 vol% Amberlite LA-1 in Chevron25	3.78	2.26	[38]
	30 vol% Amberlite LA-1 in chloroform	0.0469	4.48	[38]
	Amberlite LA-1	2.52	4.22	[38]
	50 vol% Amberlite LA-2 in Chevron25	0.53	3.82	[38]
	50 vol% Amberlite LA-2 in Chevron25	2.19	4.48	[38]
	30 vol% Amberlite LA-2 in chloroform	0.0218	9.86	[38]
	Amberlite LA-2	1.83	6.49	[38]
	50 vol% Adogen 283-D in Chevron25	0.983	9.65	[38]
	50 vol% Adogen 283-D in Chevron25	2.63	4.55	[38]
	50 vol% Adogen 283-D in Chevron25	3.85	3.46	[38]
	30 vol% Adogen 283-D in chloroform	0.0133	32.11	[38]
	Adogen 283-D	0.383	33.4	[38]
	20 vol% Adogen 381 in 2-Heptanone	3.23	1.72	[38]
	30 vol% Adogen 381 in chloroform	0.0448	7.79	[38]
	20 vol% Adogen 364 in 2-Heptanone	2.9	1.98	[38]
	30 vol% Adogen 364 in chloroform	0.0381	9.69	[38]
	20 vol% Adogen 368 in 2-Heptanone	3.11	1.83	[38]
	30 vol% Adogen 368 in chloroform	0.0447	8.28	[38]
	20 vol% Adogen 363 in 2-Heptanone	3.44	1.58	[38]
	30 vol% Adogen 363 in chloroform	0.0623	7.82	[38]
	20 vol% Alamine 336 in 2-Heptanone	2.99	2.24	[38]
	30 vol% Alamine in chloroform	0.0465	9.68	[38]
	50 vol% Methyl ditridecyl in Methyl isoamyl ketone	2.91	3.54	[38]
	1.0 wt% triosooctyl amine in chloroform	0.5	0.44	[42]
	4.2 wt% triosooctyl amine in chloroform	0.5	2.1	[42]
	8.7 wt% triosooctyl amine in chloroform	0.5	5.1	[42]
	7.03 wt% triosooctyl amine in Chevron	0.5	0.16	[42]
	6.47 wt% triosooctyl amine in n-heptane/chloroform (2:1 vol)	0.5	0.55	[42]
	8.57 wt% triosooctyl amine in n-heptane/chloroform (2:1 vol)	0.5	1.5	[42]
	5.14 wt% triosooctyl amine in nitrobenzene	0.5	1.2	[42]
Acetic acid	8.0 wt% triosooctyl amine in n-heptane/n-hexanol (1:1 vol)	0.5	2.3	[42]
	8.13 wt% triosooctyl amine in n-heptane/n-hexanol (1:2 vol)	0.5	3.4	[42]
	7.57 wt% triosooctyl amine in n-hexanol	0.5	5.3	[42]
	3.84 wt% triosooctyl amine in n-hexanol	0.5	0.34	[42]
	4.2 wt% triosooctyl amine in chloroform	0.5	2.4	[42]
	2.15 wt% triosooctyl amine in chloroform	0.5	0.38	[42]
	18.6 wt% triosooctyl amine in chloroform	0.5	9.9	[42]
	44.7 wt% triosooctyl amine in chloroform	0.5	9.0	[42]
	82.4 wt% triosooctyl amine in chloroform	0.5	2.0	[42]
	Tri-n-octyl amine	0.5	0.94	[42]
	Chloroform	0.5	0.028	[42]
	n-Hexanol	0.5	0.88	[42]
	Nitrobenzene	0.5	0.06	[42]
	n-Heptane/Chloroform (2:1)	0.5	0.02	[42]
	Chevron25	0.5	0.009	[42]
	15.2 wt% TBP in Chevron25	0.5	0.28	[42]
	44.1 wt% TBP in Chevron25	0.5	0.93	[42]
	71.2 wt% TBP in Chevron25	0.5	1.6	[42]
	Tributyl phosphate	0.5	2.2-2.7	[42]
	12.5 wt% Tributyl phosphine oxide in Chevron25	0.5	2.1	[42]
	22.2 wt% Tributyl phosphine oxide in Chevron 25	0.5	3.4	[42]
	13 wt% Dibutyl Phosphate in Chevron25	0.5	0.45	[42]
	37.3 wt % Tributyl phosphine oxide in Chevron 25	0.5	4.5	[42]
	6.4 wt% Triphenyl phosphine oxide in chevron 25	0.5	0.16	[42]
	50 wt% TOPO in 2-ethyl-1-hexanol	1.55	1.12	[38]
	50 wt% TOPO in 2-heptanone	1.55	2.83	[42]
	50 wt% TOPO in Chevron25	1.55	2.01	[38]
	22 wt% TOPO in Chevron25	0.189	3.12	[38]
	22 wt% TOPO in Chevron25	1.27	1.33	[38]
	22 wt% TOPO in Chevron25	3.2	0.766	[38]
	22 wt% TOPO in Chevron25	7.45	0.45	[38]
	3 wt% TOPO in kerosene	0.713	0.324	[43]
	3 wt% TOPO in kerosene	1.33	0.223	[43]
	3 wt% TOPO in kerosene	2.69	0.148	[43]
	3 wt% TOPO in kerosene	2.772	0.149	[43]
	3 wt% TOPO in kerosene	4.918	0.098	[43]
	3 wt% TOPO in kerosene	5.167	0.101	[43]
	3 wt% TOPO in kerosene	5.268	0.095	[43]
	3 wt% TOPO in kerosene	7.766	0.07	[43]
	3 wt% TOPO in kerosene	7.822	0.078	[43]
	3 wt% TOPO in kerosene	7.828	0.072	[43]
	3 wt% TOPO in kerosene	10.11	0.062	[43]
	3 wt% TOPO in kerosene	10.171	0.062	[43]
	3 wt% TOPO in kerosene	12.338	0.055	[43]
	5 wt% TOPO in kerosene	0.351	0.689	[43]
	5 wt% TOPO in kerosene	1.196	0.4	[43]
	5 wt% TOPO in kerosene	2.556	0.241	[43]
	5 wt% TOPO in kerosene	2.556	0.241	[43]
	5 wt% TOPO in kerosene	4.991	0.149	[43]
	5 wt% TOPO in kerosene	9.647	0.1	[43]
	5 wt% TOPO in kerosene	14.24	0.077	[43]
	18 wt% TOPO in kerosene	1.126	1.165	[43]
	18 wt% TOPO in kerosene	2.41	0.814	[43]
	18 wt% TOPO in kerosene	5.385	1.494	[43]
	18 wt% TOPO in kerosene	8.799	0.343	[43]
	10.5 wt% TOPO in n-heptane/ n-hexanol (2:1 vol)	0.5	1.3	[42]
	18.1 wt% TOPO in n-heptane/ n-hexanol (2:1 vol)	0.5	3.1	[42]
	25.9 wt% TOPO in n-heptane/ n-hexanol (2:1 vol)	0.5	4.7	[42]
	44.2 wt% TOPO in n-heptane/ n-hexanol (2:1 vol)	0.5	4.4	[42]
	58.6 wt% TOPO in n-heptane/ n-hexanol (2:1 vol)	0.5	3.5	[42]
	21.8 wt% TOPO in Chevron25	0.5	3.8-4.8	[42]
	5.5 wt% TOPO in Chevron25	0.5	0.8	[42]
	n-Heptane/n-Hexanol(2:1 vol)	0.5	0.30	[42]
	Kerosene	0.01		[32]

(Continued on the next page)

Table 3.

Product	Solvent	m	Ref.
Propionic acid	n-Hexane	0.005	[25]
	Cyclohexane	0.006	[25]
	Benzene	0.043	[25]
	Toluene	0.034	[25]
	Xylene	0.030	[25]
	n-Butanol	3.2	[25]
	n-Pentanol	2.95	[25]
	Carbon tetrachloride	0.015	[25]
	Chloroform	0.11	[25]
	Nitrobenzene	0.16	[25]
	Diethyl ether	1.75	[25]
	Diisopropyl ether	0.80	[25]
	Methyl isobutyl ketone	2.15	[25]
	Cyclohexane	3.30	[25]
	Kerosene	0.03	[32]
Lactic acid	Diethyl ether	0.10	[25]
	Diidopropyl ether	0.04	[25]
	Methyl isobutyl ketone	0.14	[25]
	n-Butanol	0.73	[25]
	Isobutanol	0.66	[25]
	n-Pentanol	0.40	[25]
	n-Hexanol	0.37	[25]
	n-Octanol	0.32	[25]
Butyric acid	Kerosene	0.19	[32]
Valeric acid	Kerosene	0.73	[32]
Caproic acid	Kerosene	3.10	[32]
Pyruvic acid	Diethyl ether	0.16	[25]
Succinic acid	Diethyl ether	0.15	[25]
	Methyl isobutyl ketone	0.19	[25]
	n-Butanol	1.20	[25]
	Isobutanol	0.96	[25]
	n-Pentanol	0.66	[25]
	n-Octanol	0.26	[25]
Fumaric acid	Diethyl ether	1.50	[25]
	Methyl isobutyl ketone	1.40	[25]
	n-Butanol	3.30	[25]
	Isobutanol	4.60	[25]
Maleic acid	Diethyl ether	0.15	[25]
	Methyl isobutyl ketone	0.21	[25]
	Isobutanol	0.92	[25]
	Diethyl ether	0.02	[25]
	Methyl isobutyl ketone	0.04	[25]
	Isobutanol	0.36	[25]
Itaconic acid	Diethyl ether	0.35	[25]
	Methyl isobutyl ketone	0.55	[25]
	Isobutanol	1.8	[25]
Tartaric acid	Diethyl ether	0.003	[25]
	Methyl isobutyl ketone	0.02	[25]
	Isobutanol	0.16	[25]
Citric acid	Diethyl ether	0.009	[25]
	Methyl isobutyl ketone	0.09	[25]
	n-Butanol	0.29	[25]
	Isobutanol	0.30	[25]

effective extractants for acetic acid, where there is a strong effect of amine molecular weight due to their hydrocarbon-like character [38]. Another method to improve the distribution coefficients is to use a mixture of solvents [31,35]. A mixture of diisobutyl ketone and C_8-C_{10} amines provides a good compromise between low reactivity and high distribution coefficient for extraction of acetic acid.

Diluents are commonly used in the solvent phase to introduce desirable modifications in the physical properties of the solvents and/or to improve distribution coefficients. Amines used in a diluents must be polar in nature in order to have high k_d [36]. Ketones as diluents for tri C_8-C_{10} amine extractants offer the best compromise between low reactivity and high k_d for organic acids [36]. A C_9 ketone such as diisobutyl ketone gave the most attractive combination of high k_d and adequate relative volatility for regeneration by distillation for extraction of acetic acid.

For carboxylic acids, the partition coefficients are generally very low (~0.003) in aliphatic hydrocarbons, 2~3 in aliphatic acids and ketones, and about 10 or more in organophosphates (such as TOPO and di-2-ethylhexylphosphoric acid) [25]. With kerosene as extractant, long chain organic acids are preferably extracted [33]; partition coefficients are 3.10 for caproic acid and 0.01~0.03 for short chain organic acids.

Selectivity

The ability of a solvent to remove a product selectively from water is described by separation factor, defined as the ratio of distribution coefficient of the product to that of water. If the separation factor is greater than unity, the solvent preferentially extracts product rather than water. Another important parameter is selectivity with respect to specific contaminants, as defined earlier. Bajpai *et al.* [22] investigated the selectivity of lactic acid with respect to glucose present in the fermentation broth. Since the partition coefficient of sugar in TOPO/kerosene solutions was ~0.002, it was possible to get very high selectivities of lactic acid in such system.

For ethanol, the separation factor is dependent upon the value of Lewis acidity/basicity (electron donor/acceptor capacity), isomeric configuration, and molecular weight of the solvent [31]. Lewis acids have higher separation factors than Lewis bases. Similarly, branched chain solvents of high molecular weight are preferred for extraction of alcohols and carboxylic acids. The separation factor in mixed solvent systems is dependent upon the functional groups in solvent molecules. Since the interactions between solvent and product molecules are complex, the separation factors for mixed solvents can not be predicted simply on the basis of pure solvent data.

High separation factors (>50) for ethanol are found mainly among alcohols and esters [28]. Branched-chain chemicals have higher values of separation factors than their linear-chain counterparts. 2-Ethyl-1-butanol has a separation factor of 103.8 for ethanol. For extraction of butanol, alcohols have only a moderate separation factor even though the distribution coefficients are high. 2-Ethyl-1-butanol is an exception (separation factor of 280 for butanol). Organic acids and esters show a high selectivity for butanol; for the same distribution factors, acids provide higher selectivity [26,30,31].

Selectivities can be significantly improved by introducing halogen groups in hydrocarbons. Fluorinated hydrocarbons (such as Freon 11) show a separation factor of 200 for ethanol [19]. Chlorinated hydrocarbons (1,1,1-Trichloroethane) and aromatics (1,2,4-Trichlorobenzene) also have high separation factors for acetone (200 and 110, respectively) and butanol

(120 and 92, respectively) [17]. Unfortunately, these solvents do not possess high enough distribution coefficients for these compounds. There is a natural tradeoff between selectivity and distribution coefficient. Mixed solvent systems offer a potential for achieving reasonably high values of both and should be explored. As an example, mixing 2-ethylhexanoic acid and methyl isobutylketone provides a large improvement in distribution coefficient of ethanol with only a slight decrease in selectivity [24].

Biocompatibility

In extractive fermentations, microorganisms are exposed to solvent systems in one form or the other. Hence, the biocompatibility of the solvents is very important. Generally, the solvents with high distribution coefficients are also toxic to the cells. The toxicity manifests itself particularly in abnormal functions of the cell membrane in the microbial cells resulting in the hindrance of the nutrient-transport system or in the leakage of metabolites [37,44]. Various measurements of solvent toxicity have been used in literature [29,33,34,37,40,41,44-50]. These include changes in cell density [37,45], maximum specific growth rate [40, 49], sugar consumption [44], conversion [46], product concentration [29], gas production [41,47], and cellular activity [34,50]. Another set of commonly available toxicity data deals with LD_{50} solubility (ratio of the dose to kill 50% of population of rats to the solubility of solvent in water) of chemicals. These data are readily available [48] and can be used to identify a set of solvents for further screening of biocompatibility. For photosynthetic microorganisms, the rate of increase of oxygen concentration in culture broth in presence of light is a measure of photosynthetic activity and can be used to quantify the biocompatibility of solvents [33].

The exposure of cells to a separate solvent phase (water immiscible portion of the solvent) tends to increase solvent toxicity compared to when the solvents are present only in soluble form [47,48]; reduction of concentrations to 10% of the saturation value reduced solvent toxicity substantially. Hence, solvent toxicity is divided into two types: molecular toxicity when the solvent concentration in the aqueous phase is less than or equal to saturation value, and interfacial toxicity caused by exposure of cells to dispersed solvent phase. Molecular toxicity is a result of modulation of enzymatic activity and membrane permeability mediated by solvent molecules, and is essentially unavoidable if the cells are exposed to the extracted liquid phase. Interfacial toxicity, on the other hand, is a result of coating of cells by the solvent, disruption of cell wall, and/or extraction of key nutrients/nucleotides into the separate solvent phase [44,45]. Interfacial toxicity can be avoided by preventing the cells from being exposed to a separate solvent phase.

Toxicity is strain and solvent specific. For example, 2-ethyl-1-hexanol is less toxic than 1-octanol to *Clostridium acetobutylicum* even though the two solvents have the same molecular weight [45]. Freon E (fluorinated ether) and octadecafluorodecalin are nontoxic to *C. acetobutylicum* cells; however the partition coefficients of butanol in these solvents are low [30]. Mixtures of toxic solvents with high distribution coefficient for a solute with non-toxic solvents having low distribution coefficients have been used to mitigate the effects of solvent toxicity [15].

Table 4. Maximum specific growth and product formation rates by *Lactobacillus delbrueckii* [51]

Solvent	μ_{max} (h^{-1})	ν_{max} (h^{-1})
Control	0.45	1.8
Kerosene (Chevron) Saturated	0.35	1.7
Kerosene (Chevron) Excess	0.22	1.3
Kerosene (Chevron) + TOPO (5-20%, w/v)	0.35	1.7
Kerosene (Fisher) Saturated	0.13	1.8
Hexadecane Saturated	0.45	1.8
Hexadecane Saturated + TOPO (2.5, 5, 10%, w/v)	0.39	1.8
Isooctane Saturated	0.45	1.8
Isooctane Excess	0.12	1.8
Dodecane Saturated	0.20	1.8
Dodecane Excess	0.20	1.8
Tributyl phosphate Saturated	0.04	0.7
Tributyl phosphate Excess	0.01	0.9

For *Lactobacillus delbrueckii*, an increasing solvent toxicity is seen for alkane = cumene < ketone < tertiary amine < secondary amine < quaternary amine [40]. This is almost the order of increasing partition coefficients of lactic acid, which suggests a strong correlation between solvent toxicity and distribution coefficient. Clearly, a compromise between these two important factors must be achieved in practice. In general, solvents with low polarity and high molecular weight are desirable as solvents because these have low toxicities for microbial cells.

In fermentations where cell growth and product formation both occur, it is necessary to distinguish between the effect of solvent on growth and product formation processes. Bajpai *et al.* [51] conducted lactic acid fermentations in presence of different solvents and then elucidated the effect of solvents on growth and product formation with the help of a mathematical model. The results are presented in Table 4. Many of the solvents showed an effect on growth only. This information points to a necessity to investigate immobilized-cell extractive-fermentations for lactic acid production and recovery. This study clearly underscores a need to differentiate between the effects on the two processes (growth and production formation). Unfortunately, such information is rarely available.

Solvent Regeneration

Successful regeneration of the solvent and recovery of product from the solvent phase are important for any viable extractive fermentation. Distillation under reduced pressure is a common method of solvent regeneration. However, care must be taken to avoid any undesirable reactions during this operation. As an example, alcohols are excellent solvents for extraction of acetic acid from fermentation broths. However, there is a potential for irreversible formation of acetals during distillation [36]. Similarly, with the use of secondary amines for extraction of acetic acid, formation of amides may result in losses of the solvent [52]. If distillation is to be used as a method of regeneration, the solvent should have sufficient relative volatility with respect to the extracted product [53].

Back extraction is another method for solvent regeneration [22,33,40]. This is particularly convenient for lactic acid where back extraction with alkali-solution

results in very efficient solvent regeneration. Undissociated acids partition preferentially in the alkali phase, where these dissociate and can not partition back [22,33,41] into the solvent phase. This feature has been used by Bajpai *et al.* [22] in an innovative fashion to counter the low distribution coefficient of lactic acid in solvent phase, while producing a concentrated stream of almost pure sodium lactate. Solvents have been regenerated by flash vaporization [54] as well as by washing with hot water [55].

IN-SITU EXTRACTION

A number of researchers have investigated in-situ extractions to remove inhibitory products from fermentation broths [18,41,47,56]. Solvent is continuously introduced in a chemostat and overflow containing the dispersed solvent in the broth is separated into solvent and aqueous phases. The solvent is regenerated to recover the product and recirculated. Part of the aqueous stream may also be recirculated through the chemostat. Dougalis *et al.* [54] used such a system to recover ethanol from fermentation broth where >96% conversion of a 300 g/L glucose feed with a productivity of ~11 g/(L/h), based upon the aqueous fermenter volume, was obtained. Ishii *et al.* [41] also used an in-situ system to increase the amount of butanol produced by *C. acetobutylicum* 2.6 fold. In such systems, the suspended cells form closely packed layers at the solvent interface and interfere with the process of mass transfer [57]. Also, since the cells are present in the vicinity of the solvent interface, they are affected by the interfacial toxicity too. This fact reduces the choice of solvents that can be used in direct in-situ extraction.

Use of immobilized cells circumvents these undesirable phenomena. Immobilized cells have been shown to be more viable than free cells in the presence of solvents [20,27,33,44]. The immobilized cells also possess a higher metabolic activity than the free cells at high glucose concentrations [27]. Minier and Goma [55] used such system (without solvent recovery and recirculation) successfully to ferment 409 g/L glucose with yeast. Less than 200 g/L glucose had been fermented under non-immobilized conditions. However, as the extractant (dodecanol) got saturated, the extraction stopped in immobilized condition, too. Clearly, the limited capacity of extraction is a measure limitation and continuous regeneration of solvents is necessary. Our own experience with lactic acid too points in the same direction [16]. On the other hand, instability of the system, leakage of cells from the immobilization matrix, and decrease in the viability of the entrapped cells cause some serious problems [58]. Cell viability in the gel-matrix has been enhanced by entrapment of a small amount of sterol and unsaturated fatty acids [20] and of soybean oil [59] along with the immobilized cells. Entrapment of cells in alginate beads with double gel-layers has been reported to prolong productivity of the immobilized systems without cell leakage [60].

Use of reverse-micelles containing organic phase has been suggested for in-situ extractions [61-65]. However, it has mainly been applied to the recovery of proteins from fermentation broths and very little work has been done involving its use for the removal of industrial chemicals.

EXTRACTIONS OUTSIDE THE BIOREACTOR

A decision to conduct the extraction outside the fermenter opens up many possibilities, such as removing the cells from broth before it enters the extractor, coupling the fermentation/recovery system to back extraction for removal of product and regeneration of the solvent. In a system used by Roffler *et al.* [53], the whole broth including the cells was recycled through a Karr reciprocating-plate extraction column where acetone and butanol were extracted using oleyl alcohol flowing countercurrently. A reduction in energy requirements and 70% increase in productivity were reported, compared to batch fermentation followed by recovery of products, while utilizing a sugar stream of 300 g/L concentration [66]. The solvent-cell contact caused interfacial toxicity for the cells, as expected. This problem can be avoided by using membranes to remove the cells before extracting. Cho and Shuler [67] used a multimembrane bioreactor containing hydrophobic and hydrophilic membranes for ethanol fermentation and recovery using tributyl phosphate as an extractant. The specific membrane configuration allowed an easy removal of carbon dioxide evolved during this fermentation.

In a system designed by Bajpai *et al.* [22], the problem of interfacial toxicity of solvent was solved by an innovative use of microporous-membranes where the back extraction of lactic acid is also carried out simultaneously. A hollow-fiber membrane module was used as a countercurrent extractor where aqueous broth was pumped on the shell side of the membrane unit and the solvent system was pumped through the tube side. Since the two phases were physically separated by the membrane, with membrane pores creating the surface area for mass transfer, it was also possible to use an emulsion of concentrated alkali in the solvent phase (kerosene saturated with trioctyl phosphine oxide). The alkali droplets in the solvent phase back-extracted the lactic acid from organic phase and thus created a sink for the extracted lactic acid. This feature overcame the problem of low partition coefficient of lactic acid in the solvent phase and at the same time resulted in a high selectivity. It was possible to operate this system and achieve a concentrated stream of sodium lactate without any ionic and nonionic impurities. On the other hand, severe fouling of the membrane was observed and upwards of 10% reduction in solute flux was observed within 24 hours. Upon scanning electron microscopic examination of the membrane, fouling appeared to be mainly due to deposition of TOPO crystals on the membrane. It was not clear, however, if there was any fouling on the broth side and whether the deposition of TOPO crystals occurred during the extraction or during the post processing of the membrane for scanning electron microscope (SEM) observation. This feature needs to be investigated further.

AQUEOUS TWO-PHASE EXTRACTION

Aqueous two phase system is an attempt to overcome organic solvent toxicity while, at the same time, ensuring reasonable distribution coefficients for the product in the extracting phase. The underlying principle for this extraction procedure is that addition of polymers such as dextran and polypropylene glycol (PPG) in the

aqueous solution results in formation of two phases in which cells, proteins, and several products preferentially partition. This techniques has been used for recovery of toxins [68], ethanol [69,70], proteins [71,72], 6-amino penicillinic acid [73], acetic acid [74], prednisolone [75], and lactic acid [76,77]. It is also adaptable for continuous operation and lends itself to scale-up [78]. However, the high costs of polymers, recovery of product from polymer solutions, and low selectivities are some of the key problems that need to be addressed [40].

CONCLUSION

Effective and biocompatible extractants have been identified for several fermentation systems. A systemic approach to solvent screening is essential for selection of the most appropriate solvent-system in different fermentations. Dispersion-free (membrane) extractive fermentations offer several distinct advantages over conventional dispersion-based processes and should be explored further. However, the problems related to membrane fouling and cost of membranes must be addressed in order to make systems involving membranes more attractive.

REFERENCES

[1] Karr, A. E. (1970) Isolation of substantially pure courmermycin A(sub 1), US Patent 3, 527, 750.

[2] Fenton, W. A. and L. E. Rosenberg. (1978) Improved techniques for the extraction chromatography of cobalamins. *Anal. Biochem.*, 90: 119-125.

[3] Nobile, A. (1958) Process for production of dienes by corynebacteria. US Patent 2,837,464.

[4] Hoover, J. R. E. and C. H. Nash (1977) p. 852-919 In: Kirk Othmer (ed.) *Encyclopedia of Chemical Technology*. Vol. 2, John Wiley & Sons Inc., New York.

[5] Mallams, A. K. (1970) *Chem. Eng. Prog. Symp. Ser.* 66: 21-47.

[6] Souders, M., G. J. Pierotti, and C. L. Dunn, (1970) Recovery of penicillin by extraction with a pH gradient. *Chem. Eng. Prog. Symp. Ser.* 66(100): 37-42.

[7] Wildfeuer, M. E. (1985) Approaches to cephalosporin C purification from fermentation products. *ACS Symp. Ser.*, 271: 155-174.

[8] Kostareva, M. G., K. K. Eremina, A. N. Fedorova, G. A Bityukskaya., M. I. Vyatkina, and T. L. Vasil'era (1971) Comparative evaluation of different solvents for erythromycin. *Antibiotiki (Moscow)* 16(2): 124-127.

[9] Sobin, B. A., A. C. Finlay, and J. H. Kane (1950) Terramycin and its production. US Patent 2,516,080.

[10] Pal, C., M. Gavrilescu, S. User, D.Urescu, and L. Natalia-Margineau (1971) Preparation of tetracycline base. Romanian Patent 53,908.

[11] Meischer, G. M. (1974) Recovery of bacitracin. US Patent 3, 795, 663.

[12] Waksman, S. A. and H. B. Woodruff (June 9, 1945) Actinomycin. US Patent 2, 378, 876.

[13] Herrero, A. A. (1983) End-product inhibition in anaerobic fermentation. *Trend. Biotechnol.* 1(2): 49-53.

[14] Bajpai R. K. and E. L. Iannotti (1988) Product Inhibition, Chapter 8, p. 207-241 In: L. E. Erickson and D. Y.-C. Fung, (eds.) *Handbook on Anaerobic Fermentations*. Marcel Dekker, Inc., New York, Basel.

[15] Evans, P. J. and h. Y. Wang (1988) Enhancement of butanol formation by *Clostridium acetobutylicum* in the presence of decanol-oleyl alcohol mixed extractants. *Appl. Environ. Microbiol.* 54(7): 1662-1667.

[16] Wang, C. J., R. K. Bajpai, and E. L. Iannotti (1991) Non-dispersive extraction for recovering lactic acid. *Appl. Biochem. Biotechnol.*, 28/29: 589-603.

[17] Dadger, A. M. and G. L. Foutch (1985) Evaluation of solvents for the recovery of *Clostridium* fermentation products by liquid-liquid extraction. *Biotechnol. Bioeng. Symp. Ser.* 15: 611-620.

[18] Kollerup, F. and A. J. Daugulis (1986) Ethanol production by extractive fermentation solvent identification and prototype development. *Can. J. Chem. Eng.* 64: 598-606.

[19] Shere, S. M. and D. J. Gannon (1984) Extractive fermentation using fluorinated hydrocarbons. Proceedings of the 5th Can. Bioenergy R & D Seminar, 548-552.

[20] Nagasimha, M., M. Azuma, S. Noguchi, K. Inuzuka, and H. Samejima (1984) Continuous ethanol fermentation using immobilized yeast cells. *Biotechnol. Bioeng.* 26: 992-997.

[21] Essien, D. E. and D. L. Pyle (1987) Fermentation ethanol recovery by solvent extraction. *Sep. Biotechnol.* Ch. 23: 320-332.

[22] Bajpai, R. K., E. L. Iannotti, C. J. Wang, and B. Su (1990) Simultaneous fermentation and recovery of lactic acid. The AIChE Spring National Meeting, Orlando, Florida.

[23] Kollerup, F. and A. J. Daugulis (1985) Screening and identification of extractive fermentation solvents using a database. *Can. J. Chem. Eng.* 63: 919-927.

[24] Kollerup, F. and A. J. Daugulis (1985) Ethanol Production. *Energy Processing/Canada*, May/June 26-30.

[25] Kertes, A. S. and C. J. King (1986) Extraction chemistry of fermentation product carboxylic acids. *Biotechnol. Bioeng.* 28: 269-282.

[26] Roddy, J. W.(1981) Distribution of ethanol-water mixtures to organic liquids. *I & EC Proc. Des. Dev.* 20: 104-1108.

[27] Barros, M. R. A., J. M. S. Cabral, and J. M. Novais (1987) Production of ethanol by immobilized *Sccharomyces bayanus* in an extractive fermentation system. *Biotechnol. Bioeng.* 29: 1097-1104.

[28] Matsumura, M. and H. Markl (1984) Elimination of ethanol inhibition by perstraction. *Biotechnol. Bioeng.* 28: 534-541.

[29] Honda, H., M. Taya, and T. Kobayashi (1984) Ethanol fermentation associated with solvent extraction using immobilized growing cells of *Sccharomyces cerevisiae* and its lactose-fermentable fusant. *J. Chem. Eng. Japan* 19(4): 268-273.

[30] Barros, M. R. A., A. C. Oliveira, and J. M. S. Cabral (1986) Integration of enzyme catalysis in an extractive fermentation progress. *Stud. Org.*

Chem. (Amsterdam) 29: 185-196.
[31] Munson, C. L. and C. J. King (1984) Factors influencing solvent selection for extraction of ethanol from aqueous solutions. *I & EC Proc. Des. Dev.* 23: 109-115.
[32] Levy, P. F., J, E. Sanderson, and D. L. Wise (1981) Development of a process for production of liquid fuels from biomeass. *Biotechnol. Bioeng. Symp. Ser.* 11: 239-248.
[33] Frenz, J., C. Largeau, and E. Casadevall (1989) Hydrocarbon recovery and biocompatibility of solvents for extraction from cultures of *Botryococcus braaunii*. *Biotechnol. Bioeng.* 34: 755-762.
[34] Brink, L. E. S. and J. Tramper (1985) Optimization of organic solvent in multiphase biocatalysis. *Biotechnol. Bioeng.* 27: 1258-1269.
[35] Mitchell, R. J., A. Arrowsmith, and N. Ashton (1987) Mixed solvent systems for recovery of ethanol from dilute aqueous solution by liquid-liquid extraction. *Biotechnol. Bioeng.* 30: 348-351.
[36] Ricker, N. L., E. F. Pittman, and C. J. King (1980) Solvent extraction with amines for recovery of acetic acid from dilute aqueous industrial streams. *J. Sep. Proc. Technol.* 1(2): 23-30.
[37] Yabannavar, V. M. and D. I. C. Wang (1987) Bioreactor system with solvent extraction for organic acid production. *Ann. NY Acad. Sci.* 506: 523-535.
[38] Ricker, N. L., J. N. Michaeals, and C. J. King (1979) Solvent properties of organic bases for extraction of acetic acid from water. *J. Sep. Proc. Technol.* 1: 36-41.
[39] Matsumura, M. and H. Markl (1986) Elimination of ethanol inhibition by perstraction. *Biotechnol. Bioeng.* 28: 534-541.
[40] Roffler, S. R., H. W. Blanch, and C. R. Wilke (1984) In situ recovery of fermentation products. *Trend. Biotechnol.* 2(5): 129-136.
[41] Ishii, S., M. Taya, and T. Kobayashi (1985) Production of butanol by *Clostridium acetobutylicum* in extractive fermentation system. *J. Chem. Eng. Japan* 18: 125-130.
[42] Wardell, J. M. and C. J. King (1978) Solvent equilibria for extraction of carboxylic acids from water. *J. Chem. Eng. Data*, 23(2): 144-150.
[43] Golob, J., V. Grilc, and B. Zaduik (1981) Extraction of acetic acid from dilute aqueous solutions with trioctylphosphine oxide. *I & EC Proc. Des. Dev.* 20(3): 433-435.
[44] Bar, R. and J. L. Gainer (1987) Acid fermentation in water-organic solvent two-liquid phase systems. *Biotechnol. Prog.* 3(2): 109-114.
[45] Shukla, R., W. Kang, and K. K. Sirkar (1988) Toxicity of organic solvents to *Clostridium acetobutylicum* for extractive ABE fermentation. *Appl. Biochem. Biotechnol.* 18: 315-324.
[46] Datta, R. (1981) Acidogenic fermentation of corn stover. *Biotechnol. Bioeng.* 23: 61-77.
[47] Playne, M. J. and B. R. Smith (1983) Toxicity of organic extraction reagents to anaerobic bacteria. *Biotechnol. Bioeng.* 25: 1251-1265.
[48] Hayward, G. And I. Lau (1989) Toxicity of organic solvents to fatty acid forming bacteria. *Can. J. Chem. Eng.* 67: 157-161.
[49] Honda, H., Y. Toyama, H. Takahashi, T. Nakazeko, and T. Kobayashi (1995) Effective lactic acid production by two-stage extractive fermentation. *J. Ferment. Bioeng.* 79(6): 589-593.
[50] Fan, X., Y. Li, K. Zhou, and Y. Su (1993) Biocompatibilities of organic solvents with *Lactobacillus delbrueckii*. *Chin. J. Biotechnol.* 9(4): 263-271.
[51] Bajpai, R., J. K. Kim, and G. Preckshot (1991) Extractions in recovery of fermentation products: An overview and a case study. The 4th World Congress of Chemical Engineering, Karlsruhe, Germany.
[52] Schugerl, L. (1989) Reactive extraction in biotechnology. *Sep. Biotech.* Ch. 20: 260-269.
[53] Roffler, S. R., H. W. Blanch, and C. R. Wilke (1987) Extractive fermentation of acetone and butanol: Process design and economic evaluation. *Biotechnol. Prog.* 3(3): 131-140.
[54] Daugulis, A. J., D. E. Swaine, F. Kollerup, and C. Groom (1987) Extractive fermentation integrated reation and product recovery. *Biotechnol. Lett.* 9(6): 425-430.
[55] Minier, M. and G. Goma (1981) Production of ethanol by coupling fermentation and solvent extraction. *Biotechnol. Lett.* 3(8): 405-408.
[56] Taya, M., S. Ishii, and T. Kobayashi (1985) Monitoring and control for extractive fermentation of *Clostridium acetobutylicum*. *J. Ferment. Technol.* 63(2): 181-187.
[57] Yabannavar, V. M. and D. I. C. Wang (1991) Strategies for reducing solvent toxicity in extractive fermentations. *Biotechnol. Bioeng.* 37: 716-722.
[58] Crabbe, F. and C. W. Tse, and P. A. Munro (1986) Effect of microorganisms on rate of liquid extraction of ethanol from fermentation broths. *Biotechnol. Bioeng.* 28: 939-943.
[59] Gianetto, A, B. Ruggeri, V. Specchia, G. Sassi, and R. Forna (1988) Continuous extraction loop reactor (CELR): alcoholic fermentation by fluidized entrapped biomass. *Chem. Eng. Sci.* 43(8): 1891-1896.
[60] Tanaka, H., S. Irie, and H. Ochi (1989) A novel immobilization method for prevention of cell leakage from the gel matrix. *J. Ferment. Bioeng.* 68: 216-219.
[61] Martinek, K., A. Levashov, N. Klyachko, Y. L. Khmelnitski, and I. V. Berezin (1986) Micellar enzymology. *Eur. J. Biochem.* 155: 453-468.
[62] Golken, K. E. and T. A. Hatton (1987) Liquid-liquid extraction of low molecular-weight proteins by selective solubilization in reversed micelles. *Sep. Sci. Technol.* 22: 831-841.
[63] Shiomori, K., Y. Kawano, R. Kuboi, and I. Komasawa (1995) Effective purification method of large molecular weight proteins using conventional AOT reverse micelles. *J. Chem. Eng. Japan* 28(6): 803-809.
[64] Pieres, M. J., M. R. Aires Barros, and J. M. S. Cabral (1996) Liquid-liquid extraction of proteins with reverse micelles. *Biotechnol. Prog.* 12(3): 290-301.
[65] Woll, J. L., T. A. Hatton, and M. L. Yarmush (1989) Bioaffinity separations using reversed micellar extraction. *Biotechnol. Prog.* 5(2): 57-62.
[66] Roffler, S. L., H. W. Blanch, and C. R. Wilke (1988) In situ extractive fermentation of acetone and butanol. *Biotechnol. Bioeng.* 31: 135-143.
[67] Cho, T. and M. L. Shuler (1986) Multimembrane bioreactor for extractive fermentation. *Biotechnol. Prog.* 2(1): 53-60.
[68] Puziss, M. and C. G. Heden (1965) Toxic pro-

duction by *Clostridium tetani* in biphasic liquid cultures. *Biotechnol. Bioeng.* 7: 355-366.
[69] Mattiasson, B., B. Hahn-Hagerdal, and P. Albertsson (1981) Extractive bioconversion of cellulose to ethanol. *Biotechnol. Lett.* 3(2): 53-58.
[70] Kuhn, I. (1980) Alcoholic fermentation in an aqueous two-phase system. *Biotechnol. Bioeng.* 22: 2393-2398.
[71] Venancio, A., J. A. Teixeira, and M. Mota (1993) Evaluation of crude hydroxypropyl starch as a bioseparation aqueous-phase-forming polymer. *Biotechnol. Prog.* 9: 635-639.
[72] Harve, R. and R. K. Bajpai (1998) Production and purification of tartrate dehydrogenase Role of aqueous two-phase extraction. *Appl. Biochem. Biotechnol.* 70-2: 677-686.
[73] Hahn-Hagerdal, B., E. Andersson, M. Larsson, and B. Mattiasson (1983) Extractive bioconversions in aqueous two-phase systems. *Ann. N. Y. Acad. Sci.* 413: 542-544.
[74] Mattiasson, B. and B. Hahn-Hagerdal (1983) Utilization of aqueous two-phase systems for regenerating soluble immobilized preparations of biocatalysts. p. 121-134 In: B. Mattiasson (ed.) *Immobilized Cells and Organelles*. Vol. 1. CRC Press, Boca Raton, FL.
[75] Kaul, R. and B. Mattiasson (1986) Extractive bioconversion in aqueous two-phase systems. *Appl. Microbiol. Biotechnol.* 24: 259-265.
[76] Kwon, Y. J., R. Kaul, and B. Mattiasson (1996) Extractive lactic acid fermentation in poly(ethyleneimine)-based aqueous two-phase system. *Biotechnol. Bioeng.* 50: 280-290.
[77] Planas, J., D. Lefebvre, F. Tjerneld, and B. Hahn-Hagerdal (1997) Analysis of phase composition in aqueous two-phase systems using a two-column chromatographic method: Application to lactic acid production by extractive fermentation. *Biotechnol. Bioeng.* 37: 716-722.
[78] Tjerneld, F. (1992) Aqueous two-phase partitioning on an industrial scale. p. 85-102 In: J. M. Harris (ed.) *Poly(ethylene glycol) chemistry: Biotechnical and biomedical applications*. Plenum Press New York, NY.