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ENZYME ACTIVITY IN SUPERCRITICAL FLUIDS

Alan J. Russell[‡*] and Eric J. Beckman[‡]

[‡]Department of Chemical Engineering, University of Pittsburgh, Pittsburgh, Pennsylvania, 15261.

*Center for Biotechnology and Bioengineering, University of Pittsburgh, Pittsburgh, Pennsylvania, 15261.

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Address correspondence to: Alan J. Russell
1235 Benedum Hall
Department of Chemical Engineering
University of Pittsburgh
Pittsburgh, PA 15261

INTRODUCTION

Materials, at pressures and temperatures above their critical values, exhibit thermophysical properties which bridge the gap between liquids and gases (see Table 1). A relatively high density imparts a supercritical fluid with the solvating power of a liquid, while a low viscosity simultaneously produces gas-like mass transfer characteristics. In contrast to the behavior of gases and liquids, the physical properties of a supercritical fluid can be tailored over a wide range by modest variations in pressure and/or temperature. Because the solvent power of a fluid, is, to a first approximation, proportional to its density, the solubility of a particular substance in a supercritical fluid is not only a function of the nature of the solvent-solute interaction, but also depends heavily on the system pressure. Careful regulation of the pressure can consequently be used to selectively remove a single compound of interest from a multi-component mixture. The selectivity and high mass transfer rates achievable using supercritical fluids as solvents has spurred successful commercial development in several novel directions, including coffee decaffeination by General Foods, and the development of supercritical fluid chromatography by Lee Scientific and Suprex Corporation.

Table 1: Comparison of Thermophysical Properties of Liquids, Gases, & Supercritical Fluids[a].

Property	Liquids	Gases	SCF's
Density (g/cc)	~1.0	~0.001	0.05-1.0
Viscosity (cp)	~1.0	~0.01	0.05-0.15

[a] CRC Handbook of Chemistry and Physics. D.R. Lide, Ed., CRC Press, Boca Raton, 1990.

Supercritical fluids are particularly attractive solvents where natural products are concerned, in that the critical temperatures of many fluids are below 100 °C (see Table 2), enabling processing without the danger of thermal degradation. For example, carbon dioxide is not only non-toxic and non-flammable, but, because depressurization to atmospheric conditions results in the near-total precipitation of solutes, is also readily recyclable. This latter characteristic both facilitates down-stream recovery of valuable components and reduces the volume of liquid and vapor effluent in a process. It should be noted that nitrous oxide (which is listed in Table 2) can form explosive mixtures with alcohols under supercritical conditions.

Table 2: Physical Properties of Supercritical Fluids[a].

Fluid	T_c (°C)	P_c (atm.)	d_c (g/cc)
Carbon dioxide	31.0	72.8	0.468
Nitrous oxide	36.0	71.5	0.450
Sulfur hexafluoride	45.6	37.1	0.734
Xenon	16.6	57.6	1.110
Ethane	32.3	48.2	0.203
Ethylene	9.2	49.7	0.218
Fluoroform (Freon 23)	25.9	47.7	0.526
Freon 13	28.9	38.7	0.579

[a] CRC Handbook of Chemistry and Physics. D.R. Lide, Ed., CRC Press, Boca Raton, 1990.

Supercritical fluids are attractive non-aqueous solvents for enzyme-catalyzed reactions for several reasons. First, given that non-aqueous enzymatic reactions involves heterogeneous catalysis which is often limited by internal and external diffusion, the high diffusivities inherent to supercritical fluids will facilitate increased reaction rates. This effect has recently been modelled by our group (Kamat et al., 1991; Russell & Beckman, 1991). Secondly, the strong dependence of solubility on pressure in supercritical fluid mixtures permits good substrate solubility and eases downstream product separation and recovery. Finally, the mild temperatures involved allow examination of even thermally sensitive enzymes.

In discussing the activity of enzymes in the presence of a supercritical fluid, the effects of both solvent type and pressure must be considered. In aqueous solution, high pressure has been shown to denature various enzymes, usually but not always irreversibly (Isaacs, 1981). Critically, most of the enzymes studied to date do not display any loss in activity until the pressure exceeds 400 MPa, far above the critical pressures reported in Table 2.

With the exception of some results by Hammond and colleagues using polyphenol oxidase in fluoroform (Hammond et al., 1985), hyperbaric non-aqueous studies of enzyme activity have been conducted solely in carbon dioxide (CO_2). Early work by Weder (1980) showed that exposure to CO_2 at 30 MPa for 1 hour has essentially no effect on the subsequent activity of several different enzymes in aqueous solution, indicating that there is no *irreversible* denaturation of enzymes in supercritical CO_2. Recent research has extended this work to demonstrate that a variety of enzymes retain their activity in the presence of CO_2 at pressures up to 35 MPa over extended

time periods. Blanch and co-workers reported the conversion of disodium p-nitrophenol phosphate to p-nitrophenol using alkaline phosphatase (with small amounts of water) in supercritical CO_2 at 35 °C and 10 MPa (Randolph et al., 1985). Indeed, the enzyme retained full activity after at least 24 hours exposure to the fluid under these conditions.

Subsequent investigations by Blanch considered the activity of cholesterol oxidase in CO_2 at 35 °C and pressures ranging from 7.5 to 12.5 MPa (Randolph et al., 1988). Under these conditions the enzyme retained its activity for at least three days. Predictably, small amounts of co-solvents (primarily alcohols) added to the CO_2 affect the reaction rate significantly, yet the observed changes were contrary to what would be expected based purely on substrate solubility considerations. It was postulated that short-chain alcohols affect to varying degrees the ability of the substrate to aggregate in solution, which in turn influences the reaction rate.

Chi and colleagues conducted hydrolysis and esterification of glycerides using immobilized lipases in CO_2 at 50 °C and 29.4 MPa (Chi et al., 1988). Reaction rates were greater in CO_2 than in hexane, which, according to Chi, is due to the greater solubility of water in the former. Hammond and co-workers demonstrated that polyphenol oxidase is active not only in supercritical CO_2, but also in fluoroform (Tc = 26 °C), at pressures as high as 35 MPa (Hammond et al., 1985). Benzoquinones (the initial product from phenol in the reaction studied) are so unstable that they rapidly oligomerized in the presence of the enzyme. The polymerization was promoted by the low solubility of higher molecular weight species in the supercritical fluid, which limited diffusion away from the aqueous layer surrounding the enzyme.

Taniguchi and co-workers examined the behavior of a variety of enzymes in supercritical CO_2, both with and without alcohol co-solvents, and observed no significant loss in activity (Taniguchi et al., 1987). However, while agreeing that exposure to CO_2 involves no activity loss, Kasche (Kasche et al., 1988) warns that the mechanical stress accompanying a rapid depressurization can denature enzymes. In addition, small amounts of water promote this degradation process. It was observed that as pressure is decreased, the reaction rate increases, ostensibly due to an increasing rate of mass transfer as the pressure decreases. Thus, the increasing mass transfer rates outweigh any drop in substrate solubility as the pressure decreases.

The pioneering work of these research groups and others has clearly demonstrated that enzymes retain their activity under exposure to supercritical CO_2, and that biocatalysis in such environments may have a dramatic impact on the future of industrial chemical processing.

PATENTS

Using the keywords "enzyme" or "protein", and "supercritical fluid", the following relevant patents were recovered. Patent titles and abstracts since 1985 were searched.

Blanch, H. W., Randolph T. and Wilke C. R.

ALKALINE PHOSPHATASE -OR CHOLESTEROL OXIDASE- CATALYSED REACTIONS - IN SUPERCRITICAL FLUID AS SOLVENT TO IMPROVE REACTION TIME AND CONVERSION EFFICIENCY

US 4,925,790, May 5, 1990

Assignee: University of California

Products are obtained from an enzyme-catalyzed reaction as follows: the substrate (I) is contacted with the enzyme (II) in the presence of a fluid under supercritical temp./pressure conditions. (I) is substantially soluble in the fluid under the conditions used. (II) is (a) an alkaline phosphatase; or (b) cholesterol oxidase; and is entrapped with water in reverse micelles. Optionally, the supercritical fluid may also comprise a co-solvent to increase solubility of (I) or products. Preferable fluids include CO_2, or a mixture of CO_2/O_2/co-solvent. (II) is preferably immobilized on a solid support (especially packed or fluidized bed of particles).

Fujimoto, K., Arai, K., and Noguchi, Y.

MANUFACTURE OF EICOSAPENTAENOIC ACID OR DOCOSAHEXAENOIC ACID-ENRICHED FATS AND OILS USING LIPASE IN SUPERCRITICIAL CARBON DIOXIDE.

Japan Kokai Tokkyo Koho, JP 87-01108990, 26 Apr, 1989

Assignee: Nippon Oils and Fats Co. Ltd.

Fats and oils are manufactured by transesterification of glycerides with fatty acids or their lower alcohol esters in supercritical carbon dioxide using lipase with byproducts, i.e. resulting fatty acids or their lower alcohol esters, removed by the gas.

Nakamura, K. and Yano, T.
ENZYMIC PRODUCTION OF INDUSTRIAL CHEMICALS UNDER SUPERCRITICAL CONDITIONS.
Japan Kokai Tokkyo Koho, JP 61021098, 29 Jan, 1986.
Assignee: Ajinomoto Co.

An enzyme and its substrate are reacted in the presence of a supercritical gas. The product is extracted with the supercritical gas. Compounds produced by this method contain the least amounts of impurities. Thus a component containing triolein, Rhizopus delemer lipase, celite, and MTES buffer was placed in a high-pressure reactor. Liquid CO_2 was added to the reactor, and the mixture was stirred at 10 atm. and 32 degree for 5 hours. The reactant was recovered with dry ice which was formed from CO_2 in the supercritical condition by cooling the reactor with a dry-ice-acetone mixture. The dry ice was removed by sublimation at room temperature. The residue was extracted with hexane. The hexane was worked up by column chromatography to yield Me oleate and Me stearate.

LITERATURE

Using the same keywords as the patent search the following (and selected other) articles were highlighted in our search.

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