Tunable Solvent Systems for Bioprocessing

In any chemical or biochemical process there must be a reaction and a separation - either purification of a product or perhaps recycling of a catalyst. While in many cases both reaction and separation are performed conveniently with the same solvent system, the solvent often is a compromise between the needs for reaction and separation. We have a great deal of experience working with a variety of tunable solvents: these are solvent systems where we can change the solvent characteristics at will by changing reversibly the physicochemical characteristics of the solvent. Examples of such systems include:

- Supercritical fluids (SCFs)
- Nearcritical water (NCW)
- Partially miscible systems
- Gas-expanded liquids (GELs), such as organic solvents and fluorous biphasic systems

We have done extensive work in supercritical fluid (SCF) solvents, and they offer many advantages, but the low solubility of biomolecules precludes their use for this application. Likewise, nearcritical water (NCW) is another tunable solvent, which too has many applications, but the high temperatures rule its use here.

For bioprocess in we shall use the third class of tunable solvents - partially miscible systems where temperature or composition changes can cause changes in phase behavior. Also we use gas-expanded liquids (GELs). The gas is almost always CO2, which is infinitely miscible with most organics (but not water) and can be used to tune solvent power with small pressure variations. In these cases we have used the CO2 as an antisolvent to make phases homogeneous for reaction and heterogeneous for separations.

We have used dimethyl ether (DME)/water mixtures. DME and water have 12-14% mutual solubility at ambient temperature. DME is an organic with polarity and basicity similar to that of acetone, but with a normal boiling point of -25 °C, it is far easier to remove. As a result, we propose the use of DME/water mixtures for homogeneous biocatalysis involving a relatively water-insoluble substrate. DME/water may be superior to traditional organic solvents such as toluene or alcohols in terms of a compromise of hydrophilicity and hydrophobicity to optimize biocatalytic processes. After reaction, we are able to carry out a biphasic downstream separation; the DME-rich phase, containing the

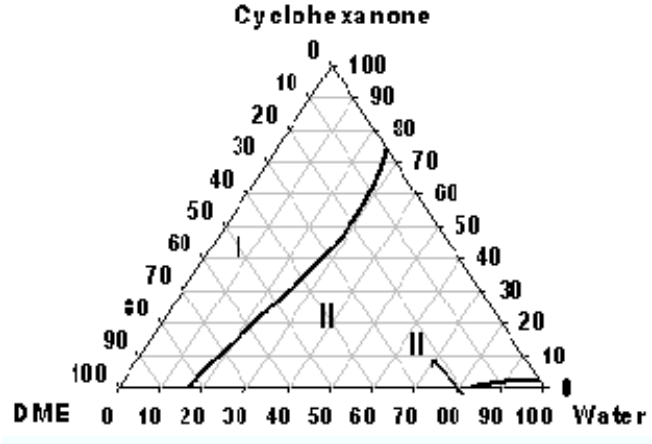

Figure 1: Phase equilibria in the DME-Water-Cyclohexane System at 25°C

product, is separated from the aqueous phase, retaining the catalyst for reuse. Product recovery is facilitated by the simple depressurization and vaporization of the DME, which can be subsequently recondensed for reuse.

Biocatalytic reduction system

We have investigated the DME/water solvent system for reduction of ketones with horse-liver alcohol dehydrogenase (ADH), depicted in Figure 2. NADH-dependent reduction of ketones is catalyzed by horse liver alcohol dehydrogenase and coupled with a regeneration of cofactor NADH catalyzed by formate dehydrogenase (FDH). NADH/NAD+ are cofactors functioning as hydrogen transfer agents. Ammonia formate is used as buffer; formate is also the substrate in NADH regeneration.

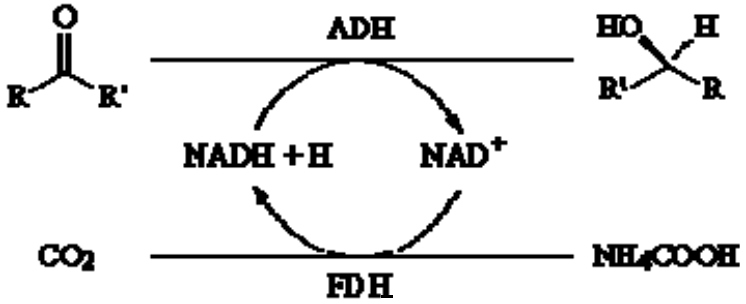

Figure 2: ADH-catalyzed reduction of ketone with cofactor regeneration

Stabilization of NADH

To evaluate the feasibility of a biocatalysis process, we determined the solvent effect of DME on NADH stability. The half-life (t1/2) of NADH was calculated based on the decomposition rate constant in water and in DME/water mixtures of different compositions, as shown in Figure 3. For example, in the presence of 12 mol% of DME, a two-fold increase in the half-life was observed compared to that in pure water. This suggests that the stability of NADH increases as the mole fraction of DME in aqueous phase increases DME stabilizes the valuable cofactor.

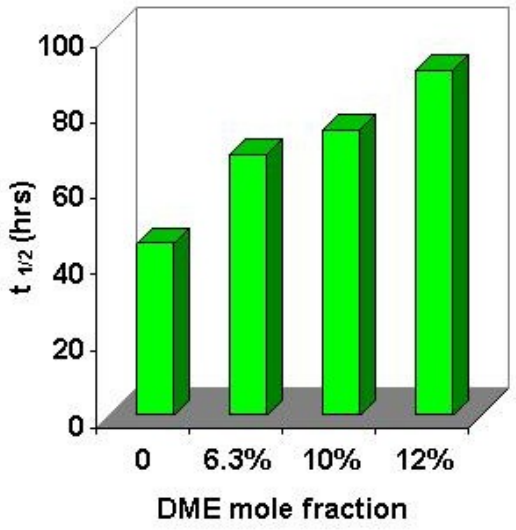

Figure 3: Half-life of NADH in DME/water mixtures at 30 °C (20 mM NH4COOH, pH 6.5)

Activity of HL-ADH and FDH

The activity of HLADH in a monophasic DME/water system was investigated based on the reduction kinetics of ketones (acetone, 2-hexanone, and cyclohexanone). The calculated rate constants (kcat) are expressed relative to the rate constant in pure water for

cyclohexanone, the best of the three substrates for HL-ADH (Table 1). Although organic solvents almost totally deactivate most enzymes, in this case in there is about 10-fold reduction in rate constant when the reaction is conducted in DME/water mixtures for all three substrates.

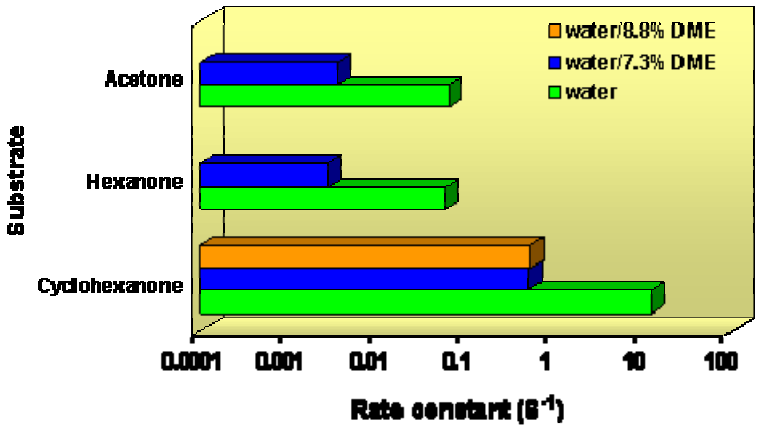

Figure 4: Conservation of enzyme activity: At 30°C, 4~9 × 10^{-4} mmol/L horse-liver ADH (≥ 0.5 U/mg), 0.16 mmol/L NADH, 100 mmol/L NH_4COOH, pH ~ 6.5

Monophasic reaction coupled with biphasic separation

The addition of DME greatly improves the solubility of less water-soluble substrates in a homogeneous DME/water reaction medium. The solubility of hydrophopic substrates is increased by a factor of 3.8 and 5.6 in the presence of 7 and 12 mol% DME in water, respectively. Further, in addition to being a monophasic reaction medium, composition changes can access the biphasic are, which offers a new avenue for facile downstream separation. The organic phase can be used to extract organic products from the aqueous phase, while the biocatalyst will partition predominantly in the aqueous phase. Figure 5 shows the favorable partitioning of reaction products.

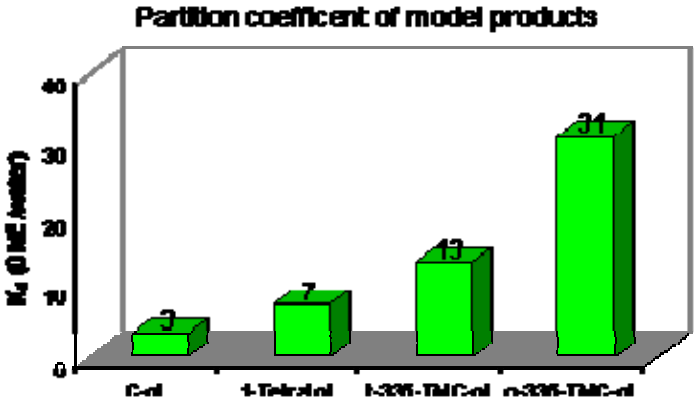

Figure 5: Partition of Hydrophobic Products

Thus the total reaction-separation process envisioned is shown below in Figure 6.

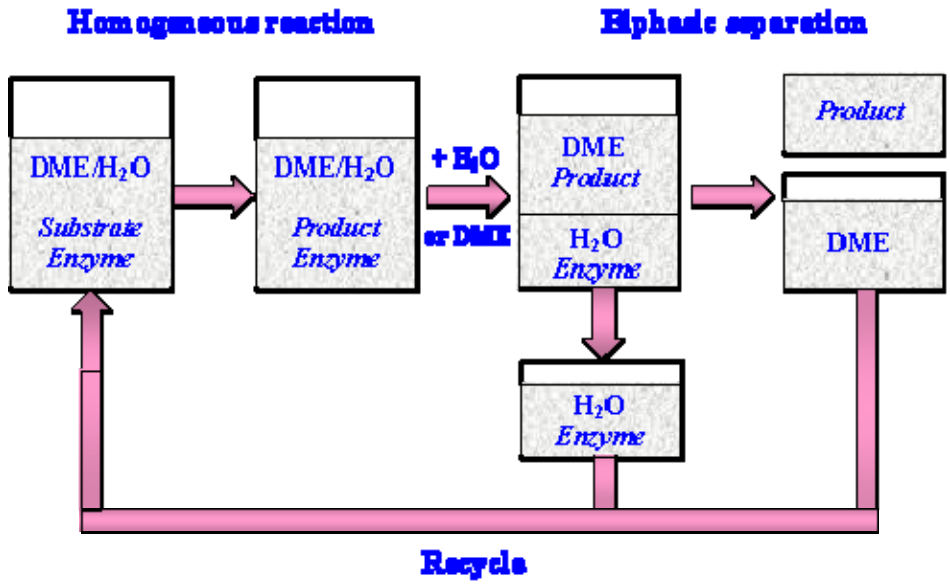

Figure 6: Process for homogeneous enzyme catalysis followed by heterogeneous separatation in the DME-Water system

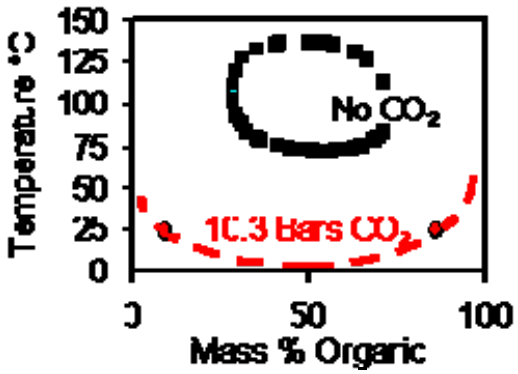

Figure 7: Effect of CO2 on the liquid-liquid phase separation of tetrahydrofuran-water.

Another very promising system is tetrahydrofuran (THF)-water. This system exhibits a closed region of partial miscibility between 70-140°C with the lower critical solution point at 70°C. However, we have found experimentally that using CO2 as an antisolvent, we can lower the LCST by more than 50°C even at very modest pressures of only 10 bar, and at higher, but still modest pressures virtually complete separation occurs. The CO2 is completely miscible with THF and only very slightly soluble in water. The THF is a relatively strong, polar solvent, and the CO2 is a very weak solvent. The mixture of THF-CO2 is relatively immiscible with water - leading to an enormous effect on the phase behavior. Best of all, the phase change is easily reversible with pressure.

Therefore, we plan now to run reactions in miscible THF-water systems at ambient pressure, and after reaction to use modest pressures of CO2 to separate catalysts from products. As an example of such a separation, see Figure 8 below.

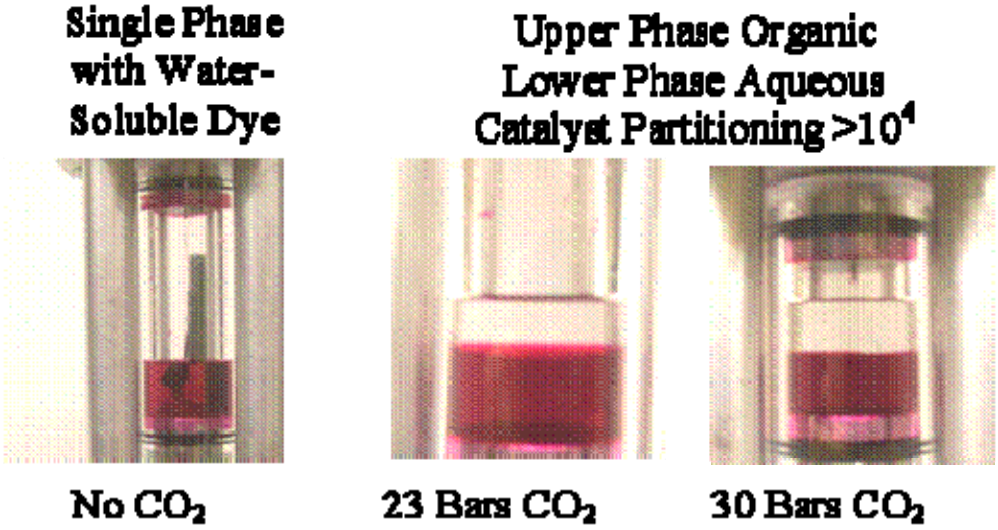

Figure 8: Effect of CO2 on the partitioning of a hydrophilic model catalyst in the THF-water system at 25°C.

As a model reaction, we plan first to test the hydroformalation of a alkene - see Figure 9. Current industrial practice permits these reactions for very short-chain olefins, such as propylene, due to a very slight solubility in water. However, the reaction cannot be carried out in this manner. for longer alkenes. These are typically run biphasic with intense agitation to overcome the mass-transfer limitations. In this system, these reactions could easily be run homogeneously.

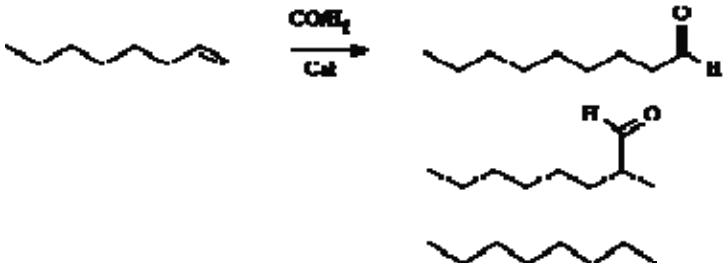

Figure 9: Hydroformylation reaction: Solvent THF-Water-CO2

A further application, only now in the initial stages, involves a reactive separation using enzymes of a racemic mixture of hydrophobic pharmaceutical compounds.