Continuous operation is possible not only with carrier fixed enzymes but also with native (soluble) enzymes retained in reactors by means of an ultrafiltration membrane. This concept is especially useful for coenzyme depending multi enzyme systems, when the coenzyme also can be retained in the reactor. This is achieved by covalent coupling, e.g. NAD, to water soluble polyethylene glycol (PEG) of MW 10,000 to 40,000. Thus, L-leucine was produced from the corresponding α-keto acid at a productivity of 243 g/L/d for 24 days.

Fig. 1. Enzyme membrane reactor.
In a membrane reactor (1) continuous homogeneous catalysis becomes possible as illustrated in Fig. 1. This is due to the difference in size between the biocatalyst and the product. By means of a recirculating pump the reaction mixture is conveyed along a hollow fiber at high linear velocity. Secondary enzyme membranes, due to concentration polarization, can be avoided in spite of product flux across the membrane. Substrate is added at constant flux across a sterile filter by means of a dosing pump. Due to some catalyst inactivation and incomplete enzyme retention respectively, some enzyme supplementation is necessary in order to operate the system at constant productivity. The enzyme supplementation can be controlled via product analysis for constant conversion. Such a system has a number of advantages: homogeneous catalysis with no activity loss during enzyme fixation, no transport limitations, no contaminating chemicals, low investment per unit activity, high activity per volume, constant productivity, and ultrafiltered product. Enzyme membrane reactors are easy to clean, sterilize, and control. Membrane cost is normally no limitation, but the stability of the enzyme(s) in solution has to be reasonable. Up till now, as with carrier fixed enzymes, it has been mainly hydrolases that have been used in membrane reactors. The racemic resolution of amino acids by means of acylase was commercialized in an enzyme membrane reactor in 1981 (2).

The real potential of membrane reactors becomes evident with coenzyme depending systems. Since coenzymes, such as NAD (transport metabolites), are only effective if they can move between two enzymes, continuous homogeneous catalysis in a membrane reactor is a promising system if the coenzyme can be retained in the reactor together with the enzymes. This is achieved by covalent coupling of the cofactor to a water soluble polymer, like PEG with a MW of 10,000 to 40,000. Details for the preparation of the NAD-derivatives are given by Bueckmann (3).

**Fig. 2.** L-amino acid bioreductive amination from the corresponding α-keto acid.