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this enzyme, characterized by current densities of 30 A/m^2 at a cell potential of 600 mV *vs.* Ag/AgCl electrode. This means that an active layer of *ca.* 9000 U of enzyme activity/m^2 is obtained. As a result, glucose and lactose are converted very efficiently at the electrode.

Conclusions

An effective method for the preparation of enzyme electrodes with high current densities is presented. Based on these current densities and on the characteristics of a carbon electrode with a high specific surface area (35000 m^2/m^3), volumetric productivities of 100–940 g product/l·h are calculated for the production of acetaldehyde and gluconate, respectively. On basis of the results, scaling-up of these electrodes is now under study.

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André Jaquet[a]), **Ian W. Marison**[a]), **Hans-Peter Meyer**[b]), and **Urs von Stockar**[a])*

Introduction

Biotechnological formation of industrially interesting compounds using microbial systems is often limited due to substrate and/or product inhibition [1–4]. The former may be overcome by suitable substrate-feeding regimes, however, the latter is technically more difficult to avoid or overcome. Ideally, it would be necessary to continuously remove the product at a rate similar to the formation rate using an efficient, non-toxic, regenerable, and cheap process which has a high specificity for the targeted substance, the latter usually present in a complex culture solution. This is the concept of *in situ* Product Recovery (ISPR) [2][4–6], which offers a number of important features: *1*) increased productivity due to low accumulation of the inhibiting compound, *2*) product losses by subsequent reactions or unwanted separations are reduced, and *3*) reduction of downstream processing steps is possible [5]. The choice of the most appropriate ISPR technique is essential to obtain these features, however, this choice will depend upon the nature of the product as well as the physico-chemical environment of the production conditions, including the lability of the biological system employed.

Results and Discussion

3-Pyridylacetic acid (PyAA) formed by biotransformation of 3-ethylpyridine with *Pseudomonas oleovorans* has been chosen as the model system for this work, since PyAA is a toxic metabolite which accumulates to a maximum of 15 g l^{-1}. Moreover, 3-pyridylacetic acid is representative of a large family of industrially interesting compounds: the biologically formed carboxylic acids.

Many separation processes exist, which can be essentially classified in four categories (see also [5]) based on:
1) evaporation of the product,
2) extraction of the product,
3) size-selective permeation of the product,
4) immobilization of the product.

The choice of the best method to apply to the desired product requires a detailed knowledge of the respective advantages and disadvantages of each. This can be combined with physico-chemical properties of the inhibiting compound (molecular weight, hydrophobicity/-philicity, volatility, charge), culture conditions (temperature, pH, presence of living cells, substrates, *etc.*) (see *Table*).

From the *Table*, ion exchange, immobilization by biorecognition, electrodialysis, reactive extraction, and its corollary perstraction are the separation processes that are, *a priori*, the best adapted to the *in situ* recovery of 3-pyridylacetic acid. From these, reactive extraction was chosen for extensive study.

Considering the relatively high hydrophilicity of 3-pyridylacetic acid and the fact that pure organic solvents such as hexane, octane, octan-1-ol, chloroform, and methyl isobutyl ketone are not able to extract it (not shown here), it is clear that carboxylic acids can generally not be separated from water by conventional liquid-liquid extraction (LLE) [7–9]. Thus, it is necessary to add a so-called extractant, to increase the distribution coefficient between organic and aqueous phases of the acid. A complexation reaction is then used (liquid-liquid reactive extraction or LLRE), which adds a certain selectivity to the separation [8].

Two different extractants were tested for PyAA separation. The first, a tertiary amine (*Alamine 336* or trioctyl/decylamine, *Henkel KGaA*, Düsseldorf, Germany), is able to react with the protonated

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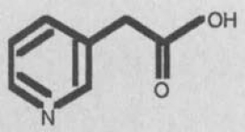
Table. *Evaluation of Separation Processes as a Function of Physico-chemical Properties of 3-Pyridylacetic Acid and Culture Conditions.* Ability of each method is classified from – – (not suitable) to +++ (*a priori* suitable).

Separation Process		
Evaporation	Vacuum fermentation	– –
	Flash fermentation	– –
	Distillation	– –
	Stripping	– –
+ membrane	Membrane distillation	– –
+ membrane	Pervaporation	– –
Extraction	By contact with an organic solvent	–
	Aqueous two-phase system	+
	Reactive	+++
	With supercritical fluid	–
+ membrane (solid or liquid)	**Perstraction**	++
Size-selective permeation	Dialysis	+
	Electrodialysis	++
	Reverse osmosis	– –
Immobilization	Hydrophobic adsorption	+
	Ion exchange	+++
	Biorecognition	++

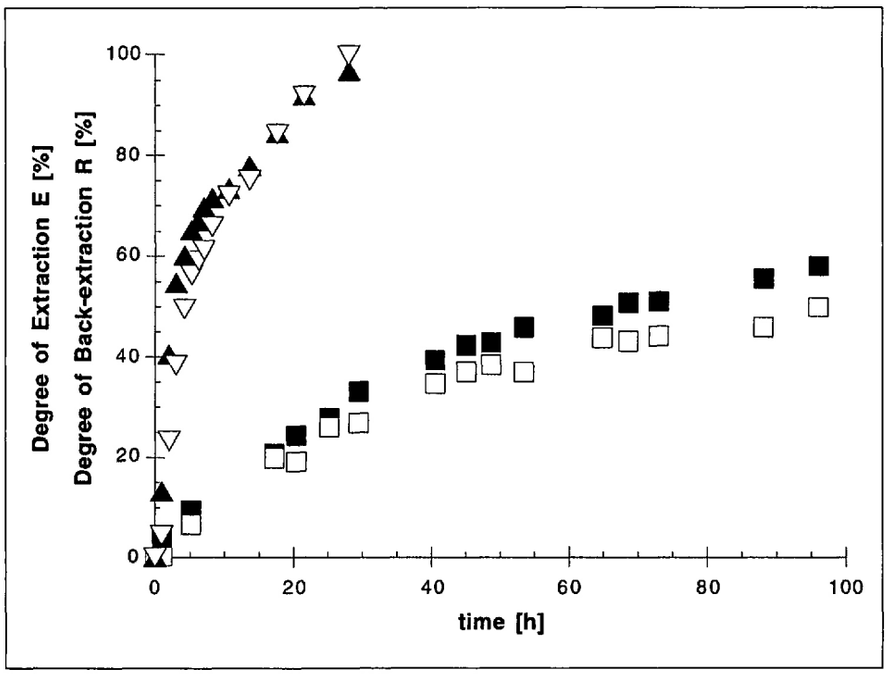


Figure. *Extraction and back-extraction of 3-pyridylacetic acid.* Extractions are represented by full symbols while back-extractions are depicted by empty ones. ■ □: organic phase, a mixture of 0.6M *Alamine 336* diluted in octan-1-ol. Aqueous phase on the extraction side initially contained 0.1M PyAA and 1M NaCl. Aqueous phase for back-extraction, 1M NaOH. ▲▽: organic phase, a mixture of 0.3M *Aliquat 336* diluted in octan-1-ol. Aqueous phase on the extraction side initially contained 0.1M PyAA and 0.5M Na_2SO_4. Aqueous phase for back-extraction, 0.5M H_2SO_4.

form of the carboxylic acid [6a][7–9], while the second, a quaternary amine (*Aliquat 336* or trioctyl/decylmethylammonium chloride, *Henkel KGaA*) can complex the 3-pyridylacetate (PyAAc) and functions as a liquid ion-exchanger [7][8].

A special configuration allowing simultaneous extraction *and* back-extraction of 3-pyridylacetic acid (or 3-pyridylacetate, depending on the extractant that was tested) was used. The results are shown in the *Fig.*

In comparison with *Alamine 336*, *Aliquat 336* is more efficient at separating aqueous solutions of PyAA. In view of pK_{a1} of 3-pyridylacetic acid (3.02 at 20°), this result is not surprising since PyAA at the pH of the culture (7.0) is largely negatively charged (acetate) and, therefore, complexes strongly with the cation trioctyl/decylmethylammonium. Nevertheless, both extractions need several hours for completion, showing clearly that kinetic problems are involved. A larger contact area between the phases (only 72.4 cm^2 here) and better hydrodynamics would result in important improvements to the extraction/back-extraction system, and form the basis of future work.

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