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ENZYME FACILITATED ENANTIOSELECTIVE TRANSPORT OF (L)-LACTIC ACID THROUGH MEMBRANES

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Production of ethyl lactate in esterification reaction by candida antarctica lipase enzyme was studied The optimal conditions of the synthesis of ethyl lactate was obtained to be: 20 µl/ml lactic acid, 160 µl/ml ethanol, 15 mg/ml Novozym 435 lipase enzyme. The yield obtained is over 90% and the enantiomeric exess is about 20% at temperature of 40 °C in case of 24 hours reaction time under 150 rpm revolution rate . In the present study, the enantioselective transport of the (L)-enantiomer from racemic lactic-acid has been presented through a lipase-facilitated supported liquid membrane (SLM) containing ionic liquids

Keywords: D,L-lactic acid, enantioseparation, esterification, supported liquid membrane.

Introduction

A growing interest has been developed for the synthesis and production of environmentally benign solvents and green chemicals.

The significance of lactic acid arises from its utility as a monomer for biodegradable solvents and polymers in which the hydroxyl and the carboxyl functional groups permit it to participate in a wide variety of chemical reactions [1].

The reactivity of an alcohol as lactic acid (or its esters or amides) may undergo xanthation reaction with carbon bisulphide, esterification with organic acids and dehdrogenation or oxygenation to form pyruvic acid or its derivatives. The acid reactions of lactic acid are those that form salts. It can also undergo esterification reactions with various alcohols [2].

Lactic acid, occurrence of which is widespread in nature, can be produced in large quantities by fermentation at low cost and its esterification is an important step for both lactic acid purification and for the production of the environmentally benign solvents. Lactic acid esters are nontoxic and biodegradable materials having excellent solvent properties and could potentially replace toxic and halogenated solvents for a wide range of industrial and consumer uses [3, 4].

Lactic acid has many pharmaceutical and cosmetic applications as formulations in topical ointments, lotions, anti acne solutions, humectants, parenteral solutions and dialysis applications, for anti carries agent. Calcium lactate can be used for calcium deficiency therapy and as anti caries agent. Its biodegradable polymer has medical applications as sutures, orthopaedic implants, controlled drug release etc. Polymers of lactic acids are biodegradable thermoplastics. These polymers are transparent and their degradation can be controlled by adjusting the composition, and the molecular weight. Their properties approach those of petroleum derived plastics. Lactic acid esters like ethyl/butyl lactate can be used as green solvents. They are high boiling, non-toxic and degradable components [5].

An enzyme often shows a high selectivity for a target substrate; therefore, we can utilize the enzyme as a biocatalyst for the resolution of optically active materials. In an aqueous solution, lipase catalyzes the hydrolysis reaction of ester compounds however, the use of lipase in nonaqueous media enables its reverse reaction namely esterfication [6]. This property can be utilized for the resolution of racemic alcohols or carboxylic acids through the esterification by lipase.

Only a few reports have been published concerning the enzymatically catalyzed esterification reaction of lactic acid and alcohols [7]. In these reactions there is a potential risk that lactic acid will act both as acyl and nucleophile donor, thus yielding dimers and oligomers of lactic acid. To avoid this, one could either protect the hydroxyl group of the lactic acid or use an enzyme with which lactic acid is not effective as a nucleophile agent. Here, the second alternative was utilised using a commercially available lipase from Candida antarctica as catalyst [8].

Moreover, lipases have been employed in preparing many flavour and fragrance esters under conditions that are milder than those used in the industry. In particular, the lipase from Candida antarctica fraction B (CAL-B) is a robust lipase in organic syntheses, showing high catalytic efficiency in the resolution of chiral esters and

amines via esterification, transesterification [9], aminolysis, and ammonolysis reactions, including regio-chemo-, and geometric selectivity [10].

Nowadays, enantioseparation of l(+)-lactic acid from the racemic mixture is important and challenging task for food and pharmaceutical industries. Among various alternatives for lactic acid recovery, the supported liquid membrane (SLM) appears to be a promising method because it potentially offers high flux and very selective separation. In addition, only small amounts of expensive carriers are required [11].

Membrane separation methods, involving liquid membranes, are very promising new processes for enantioseparation [12–15]. However, only a little work has been conducted in the liquid membrane area engaging with the separation of lactic acid enantiomers: Hadik et al. [16, 17] studied the enantioselective transport of d,l-lactic acid facilitated by their synthesized chiral selector N-3,5-dinitrobenzoyl-l-alanine octylester through supported liquid membrane or solid chiral membrane.

Tailoring of ionic liquids (IL) to achieve good partitioning of target solutes and acceptable viscosity of IL may lead to interesting results. The reactive mechanism of the solute extraction promotes partitioning and the separation selectivity. Water extraction highly influences solvent extraction [18, 19].

A dual mechanism with the formation of reverse micelles and the formation of a hydrated complex has been proposed. Kinetics of these processes may greatly influence the transport rate. Influence of an anion structure of ILs on the extraction by them is discussed in papers of Martak et al. [20].

Employing ionic liquids as a liquid membrane phase resulted in the stabilization of the SLM, because ionic liquids have negligible vapor pressure and are water-immiscible [21].

In the present study, we have investigated in the enantioselective transport of the (L)-enantiomer from racemic lactic acid through a lipase-facilitated SLM based on ionic liquids.

Materials and methods

Materials

Enzyme: triacilglicerol hydrolase, E.C. 3.1.1.3. Novozym 435, Novo Nordisk (Basvaerd, Danmark), 1U = 7000 μmol PLU/g, 15 min, 1 atm (PLU: propyl-laurate, water content: 1–2%,

Lipase from porcine pancreas Type VI-S, lyophilized powder from Sigma ,

(D,L)-lactic acid 90% (Reanal, Hungary), Ethanol (Spektrum 3D, Hungary).

Ionic liquid:
Trihexyltetradecylphosphonium bromide, >95%,

1-Ethyl-3-methylimidazolium bis(trifluoromethyl-sulfonyl)imide 99%,

Triisobutylmethylphosphonium Tosylate,>95% from Iolitec.

Gas chromatograph: ACME 6100, Column: LIPODEX E 0,2 μm 25 m x 0,25 m, FID detector,

Shaking incubator: GFL 3031.

Ethyl lactate synthesis

Reactin mixtures contained 20 μl/ml lactic acid and 160 μl/ml ethanol. Enzyme preparations were added to 15 mg/ml reaction solution. Reactions were performed in 10 ml screw-capped glass bottles, at temperature of 40 °C for 24 hours. Solution was shaked at 150 rpm. Samples were taken from the reaction solutions at intervals of time and analyzed.

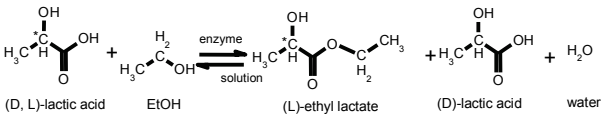

Figure 1: Reaction scheme for the enantioselective esterification of lactic acid with ethanol

Membrane

Hydrophobic poly(propylene) membrane filter (47 mm diameter, 0.2 μm pore size, 170 μm thickness) supplied by Sterlitech. (Kent, USA) were used as supporting membranes.

Membrane module using a pair of plastic cells (each cell had a volume of 25 ml).

Immobilization of the liquid membrane: the porous supporting membrane was submerged in 5 ml of IL and a vacuum from a rotary pump was applied for 1 h to remove all of the air from the pores of the membrane. After procedures, the membrane was left to drip overnight to remove excess ionic liquid from the membrane surface. To determine the amount of ionic liquid immobilized in the supporting membrane, all the membranes were weighted before and after impregnation.

Analysis

The enantiomers of ethyl lactate were analyzed by gas chromatograph 6100 model from ACME (the Republic of Korea) with FID detector and chiral column (LIPODEX E 0,2 μm 25 m x 0,25 m).

The selectivity was calculated in terms of the percentage enantiomeric excess (ee., %) and the separation factor (a) where

$$e.e.(L)\% = \frac{L-D}{L+D} * 100,$$

L is L-lactic acid concentration,
D is D-lactic acid concentration.

Results and discussion

Production of ethyl lactate in esterification reaction by candida antarctica lipase was studied. Novozyme 435 (immobilized *Candida antarctica* lipase B) was used for the present study as a biocatalyst, because this enzyme has been confirmed, in the previous observation, to be the most effective to esterify lactic acid.

The influence of ethanol, lactic acid and enzyme concentration, initial water content and reaction time have been studied on both the ester yield and enantiomeric excess.

Effect of molar ratio of lactic acid to alcohol

Ratio between the lactic acid and ethanol was varied while the other parameters were held constant. An enzyme concentration of 15 mg/ml was used for all experiments.

The lactic acid concentration was then held constant while the ethanol concentration was increased to obtain a ratio of 10:1, and vice versa, to obtain a ratio of 1:10 (*Fig. 2*).

Results, shown in *Fig. 1*, indicate that the most favorable ethanol to lactic acid ratio was equal to 7, where the conversion of the ester formation reached the value of 85%.

The highest value of the enantimeric excess was obtained at alcohol to lactic acid ratio of 5. This can be regarded as optimal value.

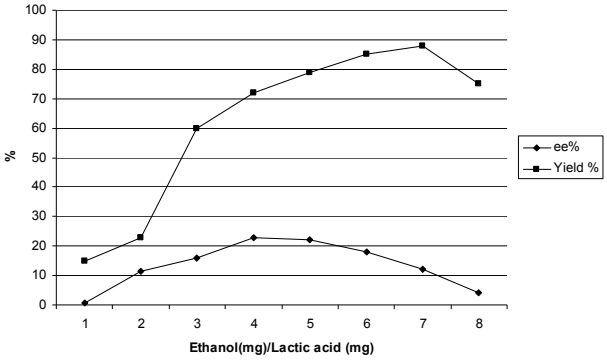

Figure 2: Effect of the molar ratio of alcohol to lactic acid on enantioselectivity of esterification of racemic lactic acid with ethanol, catalyzed by candida antartica

Effect of the temperature on activity of lipase

The heat energy from the reaction temperature may affect enzymatic rate and functional group of substrate involved in the reaction, and therefore, reactions must be experimented to find the optimum temperature in order to obtain the best yield (*Fig. 3*).

The effect of reaction temperature using Novozyme 435 on the esterification activity is shown in Fig. 2. The results show that the highest value of activity of lipase was obtained at 40 °C, which can be regarded as the optimal reaction temperature.

The relative activity was found to be increased with the temperature between 30 °C to 40 °C.

In temperatur range above 50 °C, the relative yield was drastically decreased and it further decreased with an increase of temperature up to 70 °C, probably due to inactivation of the catalyst.

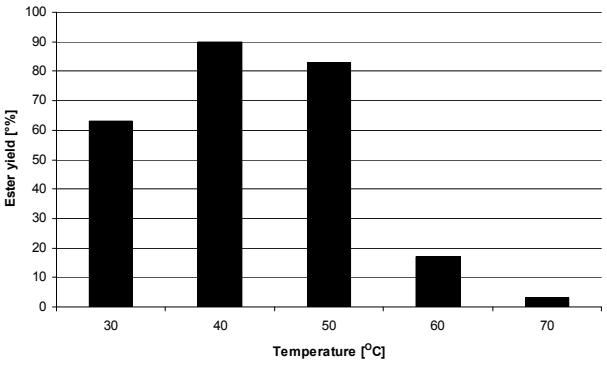

Figure 3: Effect of temperature on the relative value of esterification activity of immobilized lipase

Effect of the enzyme concentration

Reactions were carried out at different enzyme levels (5–20 mg/ml) using constant concentrations of alcohol and acid substrates.

When enzyme concentration was increased the ester yield increased but the enantiomeric excess was constant (*Fig. 4*).

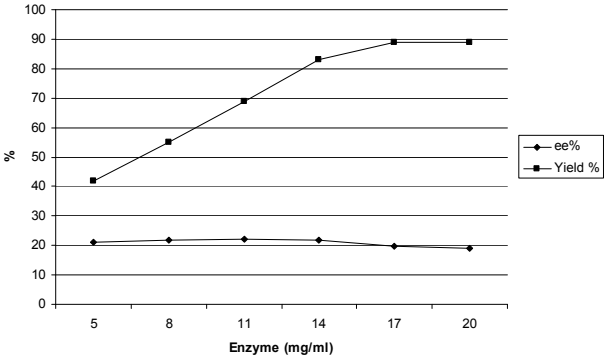

Figure 4 : Effect of enzyme concentration on the enzymatic esterification of lactic acid by ethanol

Influence of the initial water activity

It is well known that while water is essential for the catalytic activity of enzymes in organic media, excess of water molecules reverses the direction of the esterification reaction. As a consequence, there is an optimal water level that maximizes the enzyme activity in organic media; control of the water content is thus important for optimizing esterification reactions in organic solvents.

The highest enantioselectivity was observed at 4 w/w % water concentration. This is the consequence of different effects of water activity on the reaction rates with the two isomers (*Fig. 5*).

According to our results, it appears that the lower the water activity is, the higher is the synthetic activity of *C. antarctica* lipase. Best conversion was achieved for water concentration less than 2 w/w %.

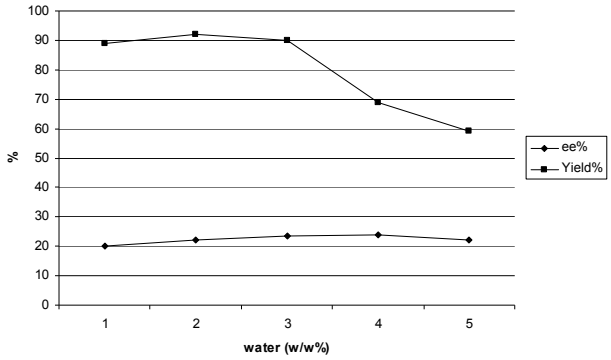

Figure 5: Effect of water activity on the enantioselectivity and ester yield in the esterification of lactic acid

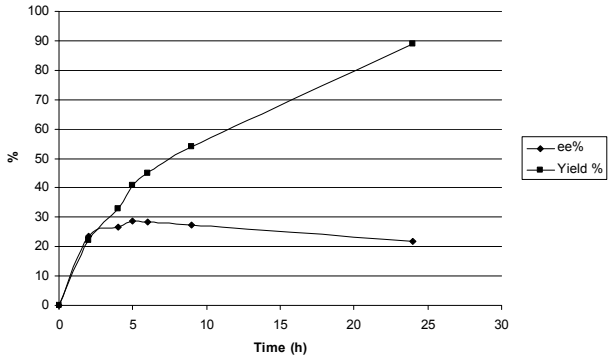

Figure 6: A time course of the esterification between lactic acid and ethanol by Novozymes 435

The optimal conditions of the synthesis of ethyl lactate were 20 µl/ml lactic acid, 160 µl/ml ethanol, 15 mg/ml Novozym 435 lipase enzyme. After shaking the reaction mixture with 150 rpm at 40 °C. The measured yield is over 90%. A high enantiomeric excess 20% is seen up to 4–6 h of reaction. Results are shown in *Fig. 6*.

Membrane separation

Lipase from *Candida antarctica* (CRL) is applicable for catalyzing esterification in the feed phase and another lipase from porcine pancreas (PPL) is an ester hydrolysis catalyst in the receiving phase. Lipases are usually known as ester-hydrolysis catalysts, however, some of them, such as CRL are able to catalyze ester synthesis. (L)-lactic acid is selectively esterified by CRL in the feed phase, and the resulting ester dissolves into the ionic liquid phase of the SLM and diffuses across the SLM. In the receiving phase, PPL catalyzes the ester hydrolysis to produce the initial lactic acid and ethanol, which are water soluble. Finally, the (L)-lactic acid is selectively transported through the SLM, based on the enantioselectivity of lipases [21].

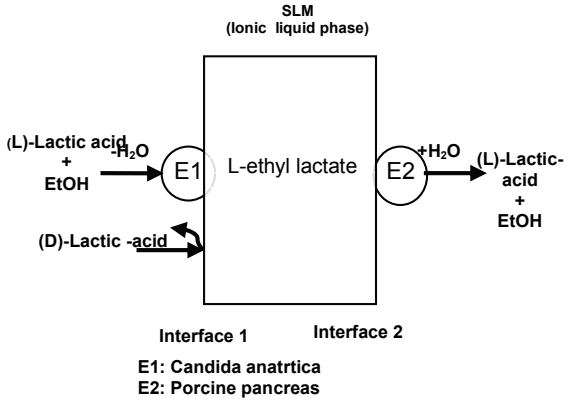

Figure 7: Schematic diagram of enantioselective transport of (L)-lactic acid through a lipase-facilitated SLM based on an ionic liquid

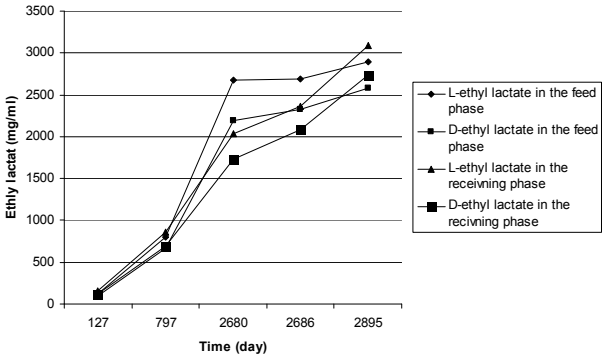

Figure 8: Lipase-facilitated transport of lactic acid through the SLM based on trihexyltetradecylphosphonium bromide

The results of experiments in *Fig. 8* show the transport of both LA enantiomers. When only the *Candida antartica* lipase were present in the feed and porcine pancreas weren't in the receiving phase, the concentration of (*L*)-ethyl lactate and (*D*)-ethyl lactate increases with time in the feed and receiving phases. The increasing rates of *L*-ethyl lactate in the phases were higher than those of *D*-ethyl lactate meaning that the lipase catalyzed reactions drove the selective transport of *L* ethyl lactate through the SLM. The highest enantiomeric excess was 20%

In all the used ionic liquid were almost the same effects of lactic-acid enantioselectivity.

Conclusion

The resolution of lactic-acid by esterification with ethanol catalysed by a commercial immobilized Candida antarctica lipase B was succesfully carried out in a membrane bioreactor containing an SLM based on ionic liquids. This system integrated the enantioselective

catalytic action of the enzyme and the selective permeability of the isomers through the SLM.

Various process variables such as the nature of the liquid membrane phase, the enzyme concentration in the feed compartment, temperature and the nature of ethanol used as acyl-donor were investigated in order to optimize the integrated reaction/separation process.

In conclusion, our investigations demonstrate that the coupling of lipases with an SLM based on IL provides a promisingbasis for the development of environmentally friendly methodologies for practical production of enantiomerically pure or enriched compounds.

ACKNOWLEDGEMENT

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