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Lipase Catalyzed Synthesis of Fatty Acid Xylose Esters and their Surfactant Properties

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Enzymatic synthesis of non-ionic surfactants from renewable sources represents a new challenge in biotechnology. In this paper, lipase catalyzed synthesis of xylose esters with fatty acid in organic medium, was performed. Influence of reaction parameters such as biocatalyst amount, temperature, lipase preparations, organic solvents and the use of different fatty as acyl donor was studied. Critical micellar concentration and surface tension value of these amphiphilic compounds have been determined.

Key Words: Sugars esters, Fatty acids, Surfactant, Enzymatic catalysis.

INTRODUCTION

Carbohydrate fatty acid esters, synthesized from renewable resources, are non-ionic surfactants and have a range of applications in the food, cosmetics, oral-care, detergent and pharmaceutical industries[1]. The main properties of these compounds are their biodegradability and their non-toxicity. Sugar esters can be synthesized using either chemical or biological catalysts. Although the poor selectivity towards the various hydroxyl group in sugars and the formation of coloured side-products[2] have focused attention on the more selective enzymatic process, using lipases[3]. Enzymatic catalysis[4] in organic media allows obtaining pure products due to the lipase specificity. Besides catalysis is conducted under mild reaction conditions and can be performed in non-toxic solvents compared to the chemical synthesis[5]. Here, we describe, the enzymatic preparation of fatty acid esters of D-xylose in anomeric position and their surfactant properties. These compounds are of great importance in cosmetics because of skin and eye tolerance[6]. Lipase catalyzed synthesis of xylose laurate and stearate esters, was performed by esterification reaction, using two acylant agents and three kinds of lipase: the *Candida antartica* lipase (CAL B), *Pancreatic porc* lipase (PPL) free and immobilized on celite at the laboratory. Experimental conditions, such as biocatalyst concentration, temperature, kind of lipase *etc.* were optimized[7]. We have also determined the critical micellar concentration and the surface tension area per molecule values of synthesized fatty acid xylose esters have been determined.

EXPERIMENTAL

Porcine pancreas lipase (Type II) was purchased from Sigma Co. (USA) were used as received, without further purification. Novozym 435 (*Candida antarctica* type B lipase immobilized on acrylic resin) was provided by Novo Nordisk (Bagsvaerd, Denmark). Xylose (Sigma-Aldrich) lauric acid, stearic acid from Merck were tested as starting materials. All other chemicals used in this work were of analytical grade and used without further purification.

Xylose esters synthesis: D-xylose (200 mg) was first dissolved in one volume of ethyl methyl ketone for one night. After that, the acyl donor was added, the mixture equilibrated for 15 min and the biocatalyst finally incorporated. Aliquots were removed at intervals, filtered and analyzed qualitatively by thin layer chromatography and quantitatively by volumetric titration.

Lipase immobilization: Celite (60 mg) was added to 10 mL of 0,1 M phosphate buffer (pH = 8) containing the *Pancreatic porc* lipase (100 U/mL). The reaction was then stirred with a magnetic bar at 4 ºC and 100 rpm for 0.5 h. 20 mL of cold acetone were then added. After 2 h, the suspension was filtered. The immobilized enzyme was washed with acetone, dried in a *vacuum* desiccator and then stored at -18 ºC.

Analysis: The sugar content was quantified by calculating the residual fatty acid amount in the reaction mixture. Samples were analyzed by volumetric method: 0.1 g of sample of reaction mixture was diluted in 20 mL of 0.1 wt % phenolphthalein solution in absolute ethanol and then titrated with standardized sodium hydroxide solution of 0.1 M in water.

RESULTS AND DISCUSSION

Synthesis of xylose monolaurate with different lipases: Lipase catalyzed esterification was performed in ethyl methyl ketone, at 40 ºC and 60 ºC with xylose/lauric acid ratio: 1:1.

Reactions were catalyzed with three kinds of lipase, *Candida antartica* B lipase (CAL B), *Porcine pancreatic* lipase (PPL), free and immobilized[8]. Lipase from *Candida antartica* B is the most frequently used enzyme for sugar ester synthesis in organic solvent[9]. Highest yields (65 %) of xylose laurate were obtained with immobilized lipase CAL B. Fig. 1 shows the reaction kinetics for xylose laurate production.

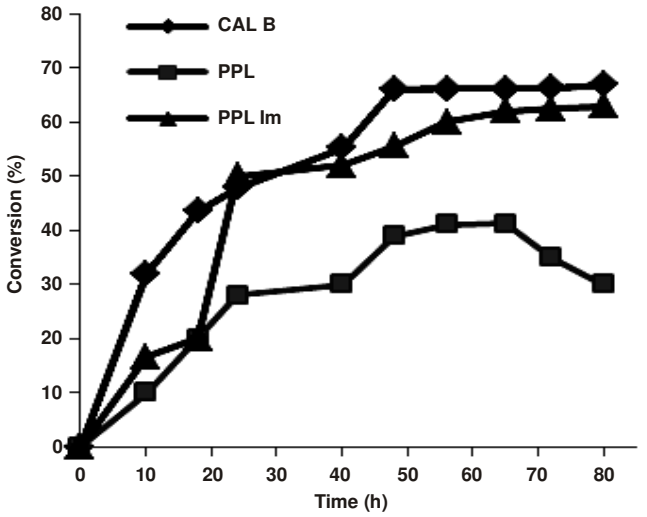

Fig. 1. Reaction kinetics for xylose laurate production. Reaction conditions: 1.33 mmol D-xylose, 1.33 mmol lauric arid, 5 mL ethyl methyl ketone, 30 mg (CAL B, PPL Im) at 60 ºC, 50 mg (PPL) at 40 ºC, molecular sieves (4Aº) in equimolar quantity to the biocatalyst, 250 rpm.

Results (Fig. 1) show that the highest conversion is obtained with CAL B, 67 % in ethyl methyl ketone. We have shown that PPL Im adsorbed to celite can catalyze efficiently xylose laurate synthesis and the obtained conversion reached 63 %. The main advantage of enzyme immobilization is her reduced cost compared with CAL B which is a very expensive lipase. Results obtained with both lipases are similar.

Influence of organic solvent: Effect of different organic solvents on lipase-catalyzed synthesis of xylose laurate with PPL Im at 60 ºC was studied. It is important, that selected organic solvents dissolves enough substrate to allow the lipase catalyzed esterification and that the product solubility is at the same time low enough to facilitate crystallization necessary to achieve a favoured equilibrium for ester formation[10]. Furthermore, it should not affect lipase activity and selectivity. Esterification was performed in *t*-butanol, ethyl methyl ketone (EMK) and tetrahydrofuran (THF). Only ethyl methyl ketone is biocompatible for the production of food additives, while *t*-butanol is not permitted for the manufacture of food additives. The difference in the reaction performance is presented in Fig. 2.

The highest conversion was obtained in ethyl methyl ketone, 63 % after 72 h, at 60 ºC. Final conversion after 72 h of reaction performance at 60 ºC was 45 % in THF and 34 % in *t*-butanol. In all solvents, liquids samples could be easily taken from the liquid phase at all-time intervals and titrated[11].

Influence of biocatalysts concentration: Influence of biocatalyst concentration was also studied, varying enzyme loading from 10 to 100 mg (5 to 50 % w/w of substrate).

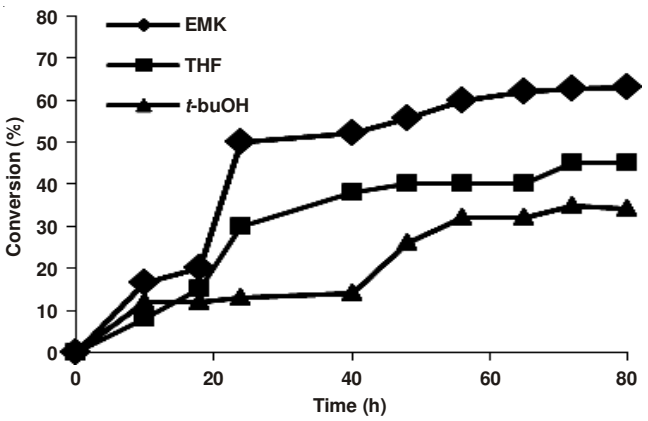

Fig. 2. Influence of organic solvents on lauric acid conversion, Reaction conditions: 1.33 mmol D-xylose, 1.33 mmol lauric acid, 5 mL ethyl methyl ketone, 30 mg PPL Im, 30 mg molecular sieves (4Å) at 60 ºC and 250 rpm

Experiments were conducted to determine the minimum amount of lipase that maximizes the amount of xylose laurate synthetized during the reaction performance. The influence of biocatalyst (CAL B and PPL Im) concentration on the conversion is shown in Fig. 3. Lauric acid conversion increased with increasing the lipase amount till 30 mg (15 % w/w of substrate) and with further increase of lipase amount, a decrease in conversion was observed. This may be due to the water produced[12]. Water is the by-product of the reaction and excessive production of water can cause the reversible reaction, which results in decreasing of the fatty acid conversion. After 72 h of reaction, the highest conversion of 67 % was obtained with CAL B and 63 % with PPL Im, when the lipase concentration was 15 % w/w of substrate. Similar behaviour has been reported for the esterification of glucose with palmitic acid in hexane[13].

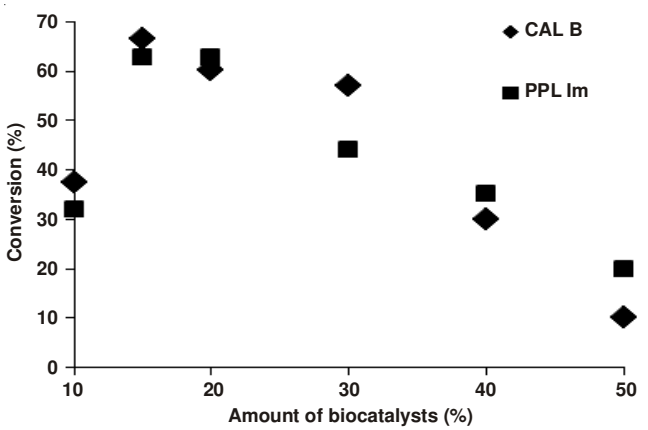

Fig. 3. Influence of biocatalyst concentration on xylose monolaurate conversion. Reaction conditions: 1.33 mmol D-xylose, 1.33 mmol lauric acid, 5 mL ethyl methyl ketone, molecular sieves (4 Å) in equimolar quantity to the biocatalyst (CAL B, PPL Im) and 250 rpm.

Influence of temperature on ester production: To study the influence of temperature on immobilized PPL Im activity, esterification reactions were performed at 40, 60 and 80 ºC, in ethyl methyl ketone. Temperature effect on the conversion is shown in Fig. 4. The conversion after 72 h of reaction increased with increasing temperature to 60 ºC (63 %). Higher temperature

(above 60 ºC) resulted in poor conversion levels because of the obviously relatively low enzyme activity. At a high temperature (80 ºC), the conversion obtained with PPL Im was 32 %. Lipase did not lose its catalytic activity and the immobilization of PPL has increased its thermic stability. Coulon *et al.*[14] and all have observed that an increase in temperature causes a decrease in the conversion of fructose oleate synthesis with the CAL B.

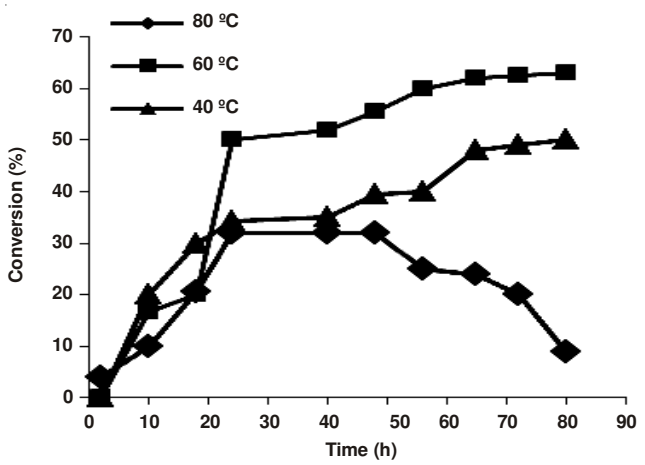

Fig. 4. Influence of temperature on ester conversion, Reaction conditions: 1.33 mmol D-xylose, 1.33 mmol lauric acid, 5 mL ethyl methyl ketone, 30 mg PPL Im, molecular sieves (4Å) in equimolar quantity to the biocatalyst, 250 rpm

Fatty acids with different chain length as acyl donor: Two fatty acids were tested in the esterification reactions, at molar ratio of fatty acid/xylose of 1/1 in ethyl methyl ketone, at 60 ºC.

Influence of fatty acids with different chain length as acyl donors on the conversion is shown Fig. 5.

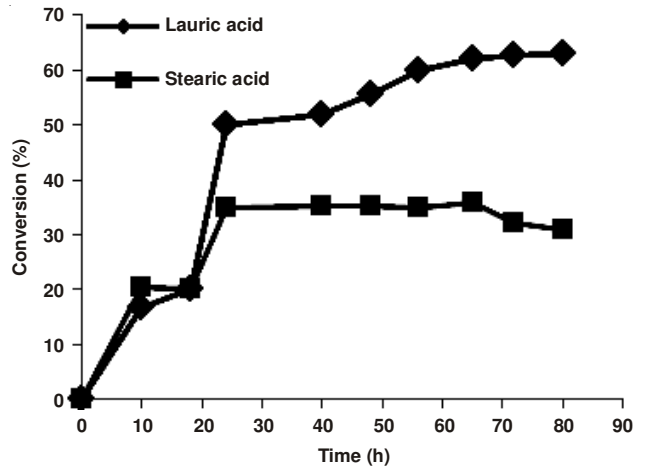

Fig. 5. Influence of fatty acid with different chain length on conversion Reaction conditions: 1.33 mmol D-xylose, 1.33 mmol fatty acid, 5 mL ethyl methyl ketone, 30 mg PPL Im, 30 mg molecular sieves (4Å), 250 rpm

Using lauric acid and stearic acid, conversion of 63 % and 31 % were respectively observed after 72 h of reaction time. The final conversion was higher for lauric acid. High initial rate was observed for both xylose laurate (1-*O*-dodecanoyl-D-xylopyranose) and stearate (1-*O*-Octadecanoyl-D-xylopyranose) formation. Obtained results are in agreement with those reported in the synthesis of glucose fatty acid esters in acetone[15].

Surface-active properties: Surface active properties[16] of compounds synthetized from renewable resources, such as fatty acids and sugars, have increasing interest due to advantages with regard to performance, consumer's health and environmental compatibility17 compared with petrol-derived standard products.

From the surface tension/concentration curves (Fig. 6) at 25 ºC the critical micellar concentration which is associated to the hydrophobicity18 of the molecules (CMC) and the area per molecule whose value indicates the minimum area per surfactant molecule at the air/aqueous solution interface (A) were calculated.

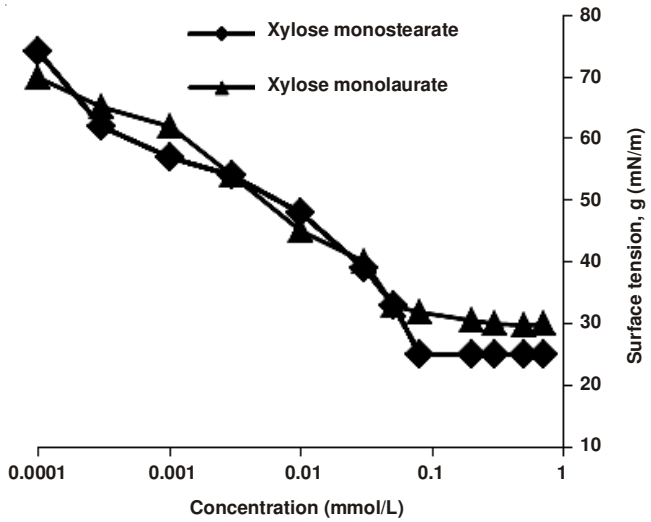

Fig. 6. Surface tension of synthetized fatty acid esters

Table-1 summarizes the surface parameters of synthetized compounds using the Gibbs adsorption[19]. In All cases they have the ability to decrease the surface tension of water until a constant value γ_{CMC} and show a clear CMC in the surface tension/log C curves in the millimolar range.

TABLE-1
SURFACE ACTIVE PROPERTIES OF SYNTHETIZED XYLOSE FATTY ACID ESTERS

Compound	CMC (mmol/L)	γ_{CMC} (mN/m)	A (Å^2)
Xylose monolaurate	0.15	30,0	24,5
Xylose monostearate	0.09	25,0	43,0

CMC: critical micellar concentration, γ_{CMC}: surface tension at CMC, A: area of polar head per molecule

1-*O*-Dodecanoyl-D-xylopyranose exhibit excellent surfactant properties characterized by a surface tension of 30.5 mN/m for a CMC of 0.17 mmol/L. The area per molecule, calculated from the slope of the surface tension curve is 24.5 Å^2. 1-*O*-Octadecanoyl-D-xylopyranose is characterized by a lower CMC and appears more hydrophobic than 1-*O*-dodecanoyl-D-xylopyranose, which is however a more efficient surfactant. The surface tension data obtained are in agreement with those reported for 5-*O*-lauroyl-D-xylofuranose[20].

Conclusion

Enzymatic xylose esters synthesis with fatty acids was performed in a small amount of organic solvent. Influence of different kinds of lipase on reaction rate was studied. The best results were obtained with immobilized CALB. The highest conversion of 67 % was achieved in ethyl methyl ketone, after 72 h of reaction. On another hand and to our best of knowledge, this is the first study showing that the immobilized lipase, PPL Im can catalyze efficiently the synthesis of xylose laurate. This lipase exhibit comparable catalytic activity with CAL B. The obtained conversion is 63 %. Synthetized compounds have significant surfactant properties.

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