SUGAR ESTER SYNTHESIS IN HIGH PRESSURE ACETONE-CO_2 SYSTEM

Huan Phan Tai*, Gerd Brunner.
Institute of Thermal and Separation Processes,TUHH, Germany
huan.phantai@tu-harburg.de

Abstract
Esterification of palmitic acid and glucose by Novozyme 435 was perfomed in CO_2 expanded acetone. The study included investigating key process parameters such as pressure, temperature, substrate and enzyme ratios. An amount of acetone up to 3% (v/v) is required to ensure that the reaction takes place in an expanded liquid phase. A good esterification performance could be found with 30% wt. enzyme related to the amount of dissolved fatty acid at an optimum temperature of 50°C and a pressure of 65 bar. Additionally, a new mechanism for removal of water as a by-product of the reaction is discussed, which is due to the multi-phase distribution of acetone-CO_2-water-glucose system.

Keywords: *Sugar fatty acid ester, esterification, lipase, supercritical CO_2, expanded liquid*

INTRODUCTION

Sugar fatty acid esters (SFAE) are non-ionic surfactants consisting of a sugar as hydrophilic group and a fatty acid as lipophilic group. They can be different in the degree of substitution (DS), defined as the number of hydroxyl groups esterified with fatty acids. Sugar esters with DS 1 to 3 are hydrophilic, absorbable, and digestible. Besides, SFAE have a wide range of hydrophilic-lipophilic balance (HLB) depending on DS and type of sugar and fatty acids, and could be used as both water in oil (W/O) or oil in water (O/W) emulsifiers.
SFAE are commonly used as food emulsifier because they are tasteless, odorless and nontoxic. On the other hand, with the property of being non irritant to the eyes and skin, they could be used in pharmaceuticals and cosmetics. Moreover, they could be biodegradable. With their wide application, sugar esters are produced in a huge amount. For example, sucrose esters are produced at 4000 Tons/year [1]. There are two processes for synthesis of sugar esters using chemical processes and enzymatic methods. Chemical processes are mainly performed at high temperatures in the presence of alkaline catalysts [2]. Therefore the process needs a high energy input, has poor selectivity for the products which are colored. When the bio-surfactants are produced by an enzymatic reaction, they are preferred for food, cosmetic or pharmaceutical applications. Because the HLB value of sugar esters can be adjusted by the number of hydroxyl groups from the free sugar substituted with fatty acid chains, enzyme synthesis shows a great potential, because the selectivity is better. Energy requirement is also reduced because the enzymes react at a modest temperature (ranging from 40 to 60°C). Recently, supercritical carbon dioxide ($SCCO_2$) has been investigated as a medium for enzymatic reaction. This brings benefit by accelerating the reaction rate reducing mass transfer resistance. Besides, products and

reactants are easily separated. However, solubility of sugar in pure $SCCO_2$ is too small [3]. Therefore, organic solvents expanded by $SCCO_2$ could become alternative reaction media. The purpose of this research is using high pressure CO_2 to support the enzymatic synthesis of glucose palmitate in acetone, simultaneously removing water. The key process parameters will be investigated and a reaction mechanism will be suggested.

MATERIALS AND METHODS

Materials: Palmitic acid (>99%) and glucose (99.8%) were supplied by Merck, Darmstadt, Germany. The immobilized enzymes Novozyme 435 was obtained from Novo Nordisk A/S , Bagsvaerd, Denmark, and acetone (>99.8%) was purchased from Riedel-de Häen, Seelze, Germany.

Experimental Method: The experimental set-up as shown in Figure 1 consists basically of a CO_2 cylinder, a cooler, a high pressure pump, an autoclave of 500ml equipped with a mechanical shaking system and a heating mantle. The reactor was loaded with reactants, solvents and enzyme, then desired temperature was set. The reactor was pressurised to the desired pressure with pre-cooled carbon dioxide. A pressure gauge and a temperature indicator were installed to ensure that the system operated at constant pressure and temperature throughout the reaction. Duplicate runs were carried out for each experimental condition with a reproducibility of ± 5%.

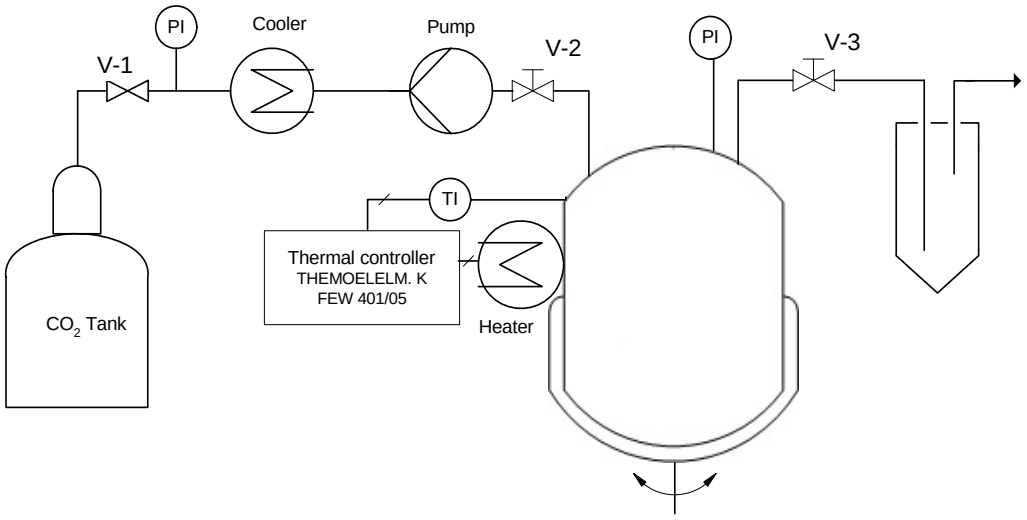

Figure 1: Flow-sheet of the reactor unit

Analytical method: The degree of esterification (conversion) was determined by calculating the residual palmitic acid amount in the mixture after the reaction. The acid content in the samples was quantified by gas chromatography (Hewlett Packard HP 5890A capillary gas chromatograph) with intergrator (HP3396 Series II). The stationary phase was a J & W Scientific fused silica (DB-5ht) column (30m×0.25mm i.d. with 0.1 µm coating). Carrier gas was Nitrogen (2 l/min). The oven temperature was maintained at 150°C for 2 min and raised to 320 °C at a gradient of 10 °C/min. Squalane was used as internal standard. Injection volume was 1µl.

RESULTS

Effect of acetone concentration

Pure $SCCO_2$ can dissolve a very limited amount of glucose [3]. Therefore adding a polar organic solvent to improve glucose solubility is required. Acetone is selected because it is accepted by EEC directives_88-344-CEE as an extraction solvent in the manufacturing of food products and additives [4]. The phase distribution in the reactor is dependent on the amount of acetone introduced into the reactor. With a small amount, acetone is totally dissolved in $SCCO_2$. An exceeding amount of acetone leads to an expanded saturated liquid mixture, here CO_2 saturated acetone [5] [6]. Figure 2 shows the initial reaction rate vs the acetone concentration. At 3% (v/v) acetone there is a maximum, where the reaction takes place in the expanded liquid phase. An increased amount of acetone at a constant substrate quantity dilutes the substrate concentration and slows down the reaction. The reaction is not favored at an acetone level of 2% or less because acetone and CO_2 form a gaseous phase, which cannot dissolve much glucose. A good contacting of substrates and the active site of the enzyme is required. The best condition is that the reaction takes place in the expanded liquid phase. Pfohl et al [7] reported that CO_2 does not dissolve more than 3% of common cosolvents at pressures below 10 MPa. Therefore, no significant glucose concentration can be expected in the vapor phase.

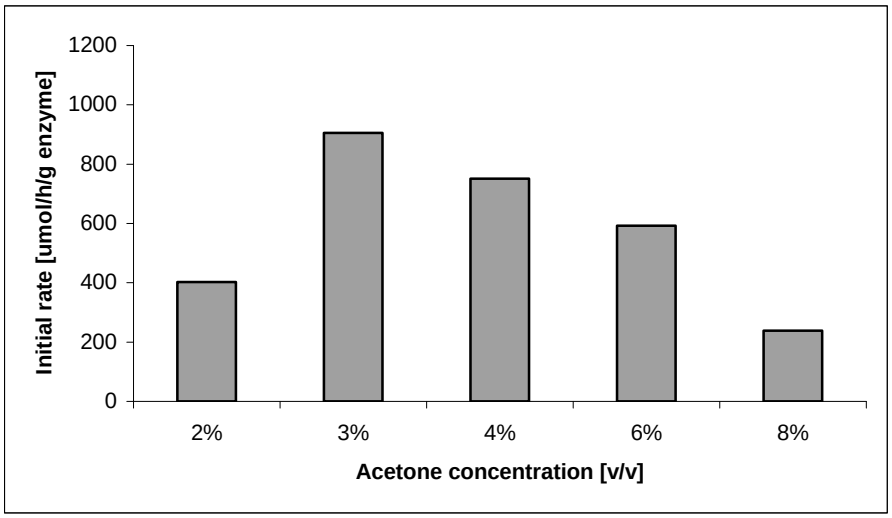

Figure 2: Effect of acetone concentration on the initial reaction rate. Reaction conditions: 50mg palmitic acid, 35mg glucose, 15mg Novozyme 435, at 65 bar and 50°C.

Enzyme concentration

In general, sugar is only slightly soluble into high pressure CO_2-acetone [7]. As a result, despite an exceeding amount of glucose is initially being loaded into the reaction vessel, the concentration of glucose in the reaction medium is kept constant at certain temperature and pressure. Therefore, esterification depends on the amount of fatty acid dissolved in the mixture. Figure 3 shows the conversion depending on the amount of enzyme, based on the amount of dissolved palmitic acid. It is observed that first the amount of enzyme increases the total conversion, since more active sites are available for the reaction. However, at higher loadings of the enzyme, the reaction slows down because of agglomeration of the enzyme particles, which

lowers their activity and decreases reaction rate and conversion. The optimum amount of enzyme was recorded at 30% Novozyme 435 related to the amount of dissolved palmitic acid.

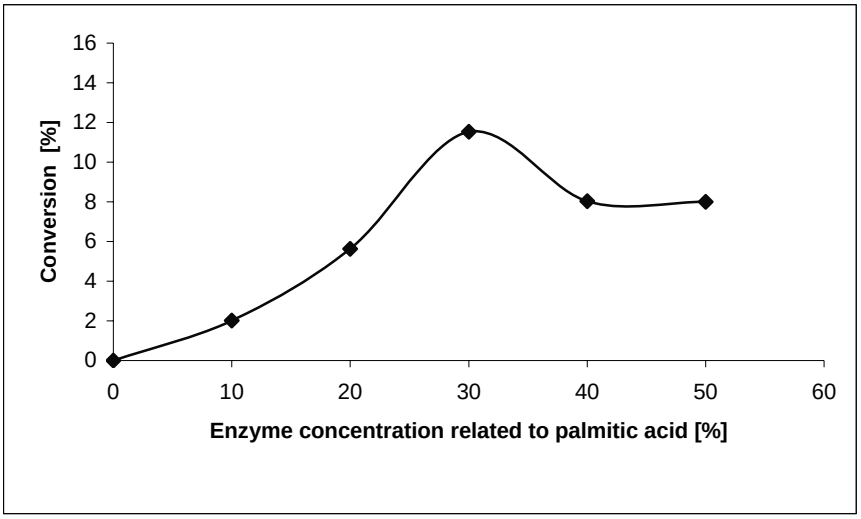

Figure 3: Effect of Novozyme 435 concentration on conversion in 2 hours with 50mg palmitic acid, 35mg glucose, 20ml acetone at 65bar and 50°C.

Temperature effect

It is observed that the conversion increases with temperature (Figure 4). Conversion at 50 or 60°C is better than the one at 40°C. However, there is no significant difference between the conversion obtained at 50°C and 60°C.

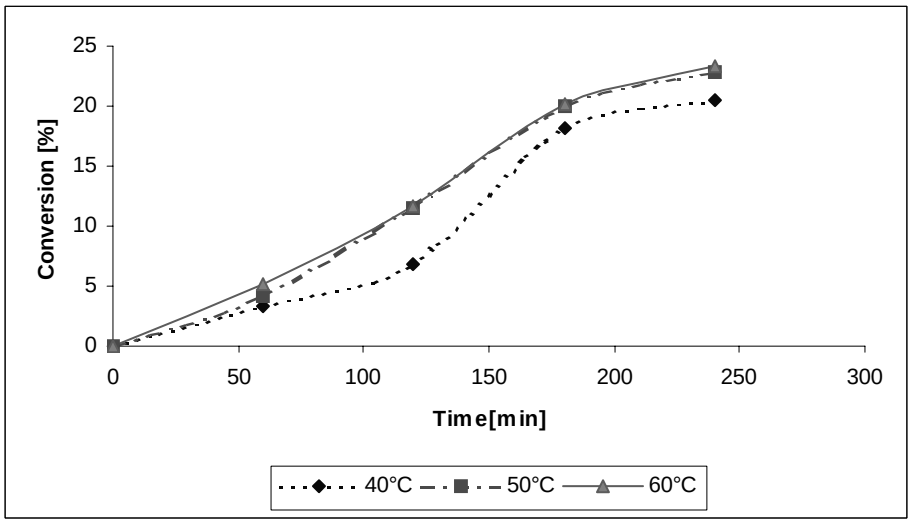

Figure 4: Temperature effect on the conversion. Reaction conditions: 50mg palmitic acid, 35mg glucose, 15mg Novozyme 435 and 20ml acetone at 65bar.

At higher temperature, the internal energy and the average distance of the substrate molecules are increased, leading to reduction of the inter-hooking of the substrate molecules. On the other hand, the effect of pressure and temperature are related [8]. Changing temperature will also change the

solubility of glucose and palmitic acid. Beside that, temperature has an effect on the configuration of the active site of the enzyme. With this type of high pressure CO_2-acetone system, Novozyme 435 has an optimum stability temperature in the range of 50 to 60°C.

Pressure effect

The effect of pressure on the esterification reaction is shown in Figure 5. It is observed that the reaction could not take place without applying $SCCO_2$. At atmospheric pressure, there is no reaction found at 40°C and a very low conversion at 60°C. When CO_2 is introduced into the system, from up to 65 bar, the reaction is accelerated. This can be explained by effect of high pressure CO_2 on the viscosity and the phase distribution of the system. Viscosity will be strongly reduced when the organic solvent is saturated by $SCCO_2$ [6]. Mass transfer is therefore improved. In addition, more substrate is transported to the active sites of the enzymes, speeding up the conversion rate. Phase distribution is well defined under CO_2 pressure. The amount of glucose and water can be distributed between light and heavy phases of a high CO_2 pressurized acetone system [7]. As a result, the level of water bound around the active site of the enzyme can be controlled by the pressure applied. However, pressure has also a strong effect on the volume expansion [9] [10]. An increase in pressure increases the volume of the reaction mixture and reduces the concentration of the substrate. In this study, a pressure of 65 bar is suggested because there is no significant difference between the reaction rate at 65 and 105 bar.

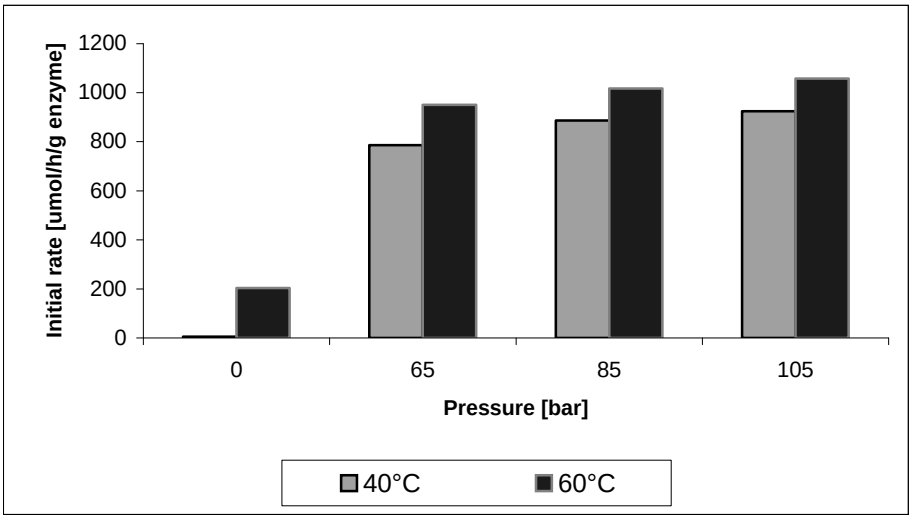

Figure 5: Effect of pressure on the initial reaction rate. Reaction conditions: 50mg palmitic acid, 35mg glucose, 15mg Novozyme 435 and 20ml acetone.

Reaction mechanism

Based on phase diagram reported by Pfohl et al. [7], a reaction mechanism is suggested as shown in Figure 6. The reaction is performed in a heterogeneous system, where most of the glucose is not dissolved and is suspended in the reaction mixture. A pseudo dynamic equilibrium between glucose, palmitic acid, glucose palmitate, acetone, CO_2 , and H_2O is proposed. Solid glucose will be continuously dissolved in the light phase (enriched acetone and CO_2), converted into glucose palmitate through enzymatic reaction. Glucose palmitate will be precipitated as a solid, if its concentration increases beyond the solvent capacity of the expanded liquid mixture. Water as a

by-product is transferred to the water phase. By this way, excess water could be removed without using any water removal tools such as adding a molecular sieve or using distillation.

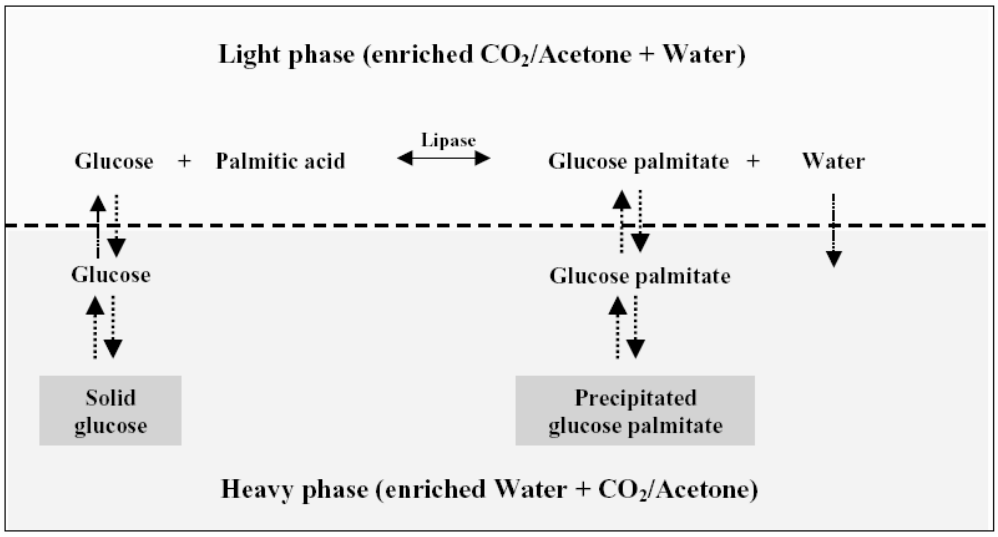

Figure 6: Reaction mechanism for esterification of palmitic acid and glucose in a high pressure acetone-CO_2 system.

CONCLUSION

Glucose palmitate could be enzymatically synthesised in a high pressure acetone-CO_2 system. At a pressure of 65 bar and with an amount of 3% (v/v) acetone, the reaction takes place in the expanded liquid mixture. By this way, water as a by product is separated and the esterification can continously take place. Novozyme 435 appears as a good catalyst for this type of reaction with an optimum temperature of 50°C. Acetone saturated with CO_2 could become a medium for esterification of fatty acids and polar compounds.

REFERENCES :

1. Hill, K. and O. Rhode, Fett/Lipid, **1999**. 101: p. 25.
2. Tomasik, *Chemical and Functional Properties of Food Saccharides*. **2003**: CRC. 440.
3. Yau, J.-S. and F.-N. Tsai, The Journal of Supercritical Fluids, **1994**. 7(2): p. 129.
4. Otero, C., et al., Journal of Molecular Catalysis B: Enzymatic, **2001**. 11(4-6): p. 883.
5. Wendland, M., et al., The Journal of Supercritical Fluids, **1994**. 7(4): p. 245.
6. Sih, R., et al., The Journal of Supercritical Fluids, **2008**. 43(3): p. 460.
7. Pfohl, O., et al., Fluid Phase Equilibria, **1996**. 124(1-2): p. 22.
8. Brunner, G., *Gas Extraction*. **1994**: Steinkopff, Darmstadt, Springer, New York.
9. Kordikowski, A., et al., The Journal of Supercritical Fluids, **1995.** 8(3): p. 205.
10. De la Fuente Badilla, J.C., et al., The Journal of Supercritical Fluids, **2000.** 17(1): p. 13.