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Batch and semicontinuous production of L-ascorbyl oleate catalyzed by CALB immobilized onto Purolite® MN102

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ABSTRACT

L-Ascorbyl oleate is recognized as efficient liposoluble antioxidant and biosurfactant which can be synthesized by using immobilized lipases as catalysts in organic media. However, development of economical production process is still a challenge, particularly in (semi)continuous systems. Hereby, we present application of novel immobilized lipase preparation, lipase B from *Candida antarctica* hydrophobically adsorbed onto styrene divinylbenzene based support, for the synthesis of L-ascorbyl oleate in batch bioreactor (BB) and fluidized bed bioreactor (FBB) with recirculation of reaction medium. Reaction kinetics was described by Ping Pong bi bi mechanism with low acyl donor inhibition. Considerably higher reaction rates (~40%) were accomplished in FBB. Hydrodynamic study revealed that reaction is kinetically controlled and unhindered external and internal mass transfer was enabled in both systems. However, much higher shear stress occurred in BB (10.46 Pa) comparing to FBB (5×10^{-2} Pa). After 12 consecutive reaction cycles lasting 24 h, 23.13 g of product per gram of biocatalyst was synthesized in a FBB. Within this study, usage of new immobilized enzyme in semicontinuous system enabled high reaction rates and satisfying operational stability in the synthesis of L-ascorbyl oleate, offering good prospect for further process development.

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1. Introduction

Vitamin C (L-ascorbic acid) is among the most abundant natural hydrosoluble antioxidants. Due to its polar nature, it is insoluble in lipids and products with high lipid content, which renders it unsuitable for application therein (Liu et al., 1996). Besides that, vitamin C molecule is unstable and easily oxidized to dehydroascorbic acid (Halliwell and Gutteridge, 2007). With the goal of its stabilization and lipophilization, it could be acylated to form derivatives—fatty acid ascorbyl esters (FAAE). FAAE's application as additives in lipophilic food, cosmetics and pharmaceutical products is preferable to synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG) and tertiary butylhydroquinone (TBHQ), considering results of some toxicological studies

(Kaitaranta, 1992; Watson, 2002). Depending on side acyl chain characteristics, they could be applied as liposoluble antioxidants, biosurfactants, sources of vitamin C in food and cosmetics (Stojanović et al., 2013b). Recent studies indicate that area of application could be even wider due to their numerous beneficial biological effects, such as protective effect towards high density lipoproteins (HDL) (Lloyd and Lynch, 2011), human erythrocytes and lymphocytes (Ross et al., 1998), participation in the recycling of endogenous α -tocopherol (May et al., 1996) and synergistic effect with endogenous antioxidants, in general (Liu et al., 1998), chemopreventive (Rao et al., 1995) and antimicrobial activity (Kharat et al., 2014).

Enzymatic synthesis of FAAE, being green process under mild reaction conditions, has been widely investigated and optimized throughout last two decades (Bezbradica et al., 2017; Karmee, 2009;

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Nomenclature

A_b	Biocatalyst surface area (m^2)
A_r	Archimedes number
C	Substrate concentration in solution (mol m^{-3})
D_a	Damköhler number
D	Diffusivity of substrate in the solvent ($\text{m}^2 \text{s}^{-1}$)
D_{ef}	Effective diffusivity ($\text{m}^2 \text{s}^{-1}$)
D_h	Hydraulic diameter of interstitial gaps in the layers of particles (m)
d	Largest inner diameter of bioreactor (m)
d_p	Particle diameter (m)
h	Height of the bioreactor (m)
k	Solid–liquid mass transfer coefficient (m s^{-1})
l	Dimension of micro whirls (m)
M	Correction factor which determines the influence of the walls of the column to the D_h value
M_s	Molar mass of solvent (g mol^{-1})
n	Stirring rate (s^{-1})
Ne'	Modified power factor
OA	Oleic acid
P	Power consumption (W)
Re	Reynolds number
Sc	Schmidt number
Sh	Sherwood number
T	Temperature of reaction (K)
t	Tortuosity
T_f	Tortuosity factor
U	Fluid velocity (m s^{-1})
Up	Superficial speed (m s^{-1})
V	Liquid volume in the tank (m^3)
$\dot{V}$	Flow rate ($\text{m}^3 \text{s}^{-1}$)
VC	Vitamin C
V_m	Maximum initial reaction rate ($\text{mol m}^{-3} \text{s}^{-1}$)
V_m''	The maximum reaction rate per a unit of biocatalysts surface area ($\text{mol m}^{-2} \text{s}^{-1}$)
$V_{m,C}$	Molar volume of carbon on temperature of boiling ($14.8 \times 10^{-3} \text{ dm}^3 \text{ mol}^{-1}$)
$V_{m,H}$	Molar volume of hydrogen on temperature of boiling ($3.7 \times 10^{-3} \text{ dm}^3 \text{ mol}^{-1}$)
$V_{m,O}$	Molar volume of oxygen on temperature of boiling ($7.4 \times 10^{-3} \text{ dm}^3 \text{ mol}^{-1}$)
$V_{m,s}$	Molar volume of substrate on temperature of boiling ($\text{dm}^3 \text{ mol}^{-1}$)
V_{rm}	Reaction mixture volume (m^3)
v	Initial reaction rate ($\text{mol m}^{-3} \text{s}^{-1}$)
x_i	Mass percent

Greek symbols

ε	Fluidized bed porosity
ε_p	Particle porosity
$\langle \varepsilon \rangle$	Energy dissipation rate per unit mass ($\text{J kg}^{-1} \text{s}^{-1}$)
μ	Dynamic viscosity of solvent (Pa s)
ν	Kinematic viscosity of the medium ($\text{mol m}^{-3} \text{s}$)
ρ	Density of the liquid phase (kg m^{-3})
ρ_i	Density of the component (kg m^{-3})
ρ_p	Density of the particles (kg m^{-3})
τ	Shear stress (Pa)
φ	Association factor of solvent

Stojanović et al., 2013b). Several microbial lipases, such as lipase B from *Candida antarctica*, *Rhizomucor miehei* and *Thermomyces lanuginosus* lipase, were proven as efficient biocatalysts for the reaction in the majority of studies, wherein the application of commercial

immobilized preparations was predominant (Karmee, 2009). As a reaction media, instead of hydrophobic solvents commonly applied for lipase-catalyzed esterifications, in which vitamin C is not soluble, more polar solvents such as *tert*-alcohols and acetone (Stojanović et al., 2015) or unconventional reaction mediums, such as ionic liquids (Chen et al., 2008; Park et al., 2003; Yang et al., 2012) were favorable. Different types of acyl donors (carboxylic acids, methyl, ethyl or vinyl esters and triglycerides) served as efficient substrates for achieving high yields of variety of ascorbyl esters (Karmee, 2009). Ascorbyl esters derived from unsaturated fatty acids (UFA) are particularly valuable, due to their widespread application in food and cosmetics. Despite significant achievements in optimization of key experimental factors resulting with high yields of ascorbyl esters, enzymatic syntheses of FAAE in packed bed or fluidized bed reactors were rarely examined, although they are the best solution for cost-efficient large-scale production. Series of ascorbyl esters were synthesized by using saturated fatty acids as acyl donors in a mechanically stirred continuous system in acetone with Chirazyme® L-2 C2 as a catalyst and productivities of approximately 60 g of esters per liter per day and products concentrations of 17–21 mmol l⁻¹ were accomplished (Watanabe et al., 2003). Kuwabara et al. (2003) used packed bed bioreactor system composed of two columns set in a row—one with vitamin C and one with biocatalyst, Chirazyme® L-2 C2, and products concentrations of 14–17 mmol l⁻¹ were achieved, with both, saturated and unsaturated acyl donors. In another research, Novozym® 435 was applied in a packed bed reactor configuration (one column with a biocatalyst and one with molecular sieves) for the synthesis of lard based ascorbyl esters in *tert*-amyl alcohol and highest yield of 50.5% was reported (Zhao et al., 2014).

Hereby, we present application of *C. antarctica* lipase B hydrophobically immobilized onto styrene divinylbenzene based support, Purolite® MN102, as a catalyst for the synthesis of L-ascorbyl oleate from oleic acid and vitamin C in *tert*-butanol as a reaction medium. Although covalent immobilization is regarded as method which provides high operational stability of biocatalysts and offers possibility of enzyme properties adjustment, adsorption of lipases onto hydrophobic supports enables high lipase loading in a simple procedure, preservation of catalytic activity and satisfying stability (Abaházi et al., 2016; Adlercreutz, 2013; Idris and Bukhari, 2012). Oleic acid was selected as suitable acyl donor since ascorbyl oleate had been regarded as a very strong antioxidant in emulsions and bulk oil systems (Lonostro et al., 2000; Stojanović et al., 2015; Viklund et al., 2003), efficient emulsifier (Adamczak and Bornscheuer, 2009; Kharrat et al., 2014; Reyes-Duarte et al., 2011), and potential antileishmaniasis agent (Kharrat et al., 2014). Novel enzyme preparation exhibited favorable properties within our previous study, since it provided high activity and stability with low estimated costs of its industrial-scale production (Ćorović et al., 2017), which along with excellent chemical and thermal stability of support and high particle size uniformity recommend immobilized preparation for application in fluidized bed enzymatic reactors.

Hence, initial experiments were focused on kinetic study to define adequate kinetic model, which will give insight into reaction mechanism and substrate inhibitions and provide data for evaluation of diffusion properties of immobilized enzyme in subsequent hydrodynamic calculations. After assessing mass transfer and shear stress effects, more detailed optimization of process conditions was undertaken and efficiency of fluidized bed bioreactor and batch reactor was compared with respect to achieved product concentrations and reaction rates. To the best of our knowledge, only few studies dealing with (semi)continuous lipase-catalyzed production of FAAE were published so far. Nevertheless, reaction was not performed in fluidized bed bioreactor system, despite of its inevitable advantages over packed bed configuration such as lower pressure drops and higher temperature

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