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Original Research Paper

Response surface methodology and artificial neural network optimized synthesis of enzymatic 2-phenylethyl acetate in a solvent-free system

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ABSTRACT

2-Phenylethyl acetate (2-PEAc) is a colorless to pale yellow liquid with a floral or fruit odor which has been applied widely in food, perfumes, cosmetics, shampoos, soaps and household products. To conform to the "natural" interests of consumers, a solvent-free system using immobilized lipase to catalyze the transesterification of 2-phenethyl alcohol and vinyl acetate was investigated. The solvent-free system offering the advantages of maximization of substrate concentration and greater volumetric production are benefited for industrial production. In this study, an experimental design was used to develop response surface methodology (RSM) and artificial neural network (ANN) models. The effect of synthesis parameters on the molar conversion of 2-PEAc was evaluated. Two models were statistically compared by the coefficient of determination, root mean square error and absolute average deviation, based on the validation data set. The coefficient of determination (R^2) calculated from the validation data for RSM and ANN models were 0.92 and 0.99, respectively. While both models showed good predictions in this study, the ANN model was more precise compared to the RSM model.

1. Introduction

2-Phenylethyl acetate (2-PEAc) is a clear, colorless to pale yellow liquid with a floral or fruit odor caused by a major emission of aroma esters from a fully opened rose flower (Shalit et al., 2003). 2-PEAc is a valuable flavour and fragrance compound which has been approved by the FDA as GRAS (2857) as a flavoring agent for direct addition to food (21 CFR 172.515); The worldwide consumption of 2-PEAc is more than 100 metric tons per year; It is an important supplement fragrance in many foods, perfumes, cosmetics, shampoos, soaps and household products (McGinty et al., 2012). Customarily, 2-PEAc is produced by chemical synthesis (Manabe et al., 2002). Due to the steady growing demand for natural materials, the synthesis of such esters by lipase-catalyzed reactions under mild conditions has received a great deal of attention for producing these valuable products (Chen et al., 2011; Pande et al., 2013; Ravn et al., 2012).

Lipase (triacylglycerol ester hydrolase, EC 3.1.1.3), catalyzing direct esterification or transesterification reactions in organic solvents, has been performed to produce esters, such as sugar ester (Kourist et al., 2010), wax ester (Kuo et al., 2012), structured lipids (Pande et al., 2013), and biodiesel (Padhi et al., 2012). For lipase catalyzed transesterification, vinyl acetate (VAc) is an effective acyl donor because the leaving group is an enol that immediately tautomerizes to acetaldehyde, thereby driving the reaction in a forward direction to achieve a high reaction rate and conversion (Akoh and Yee, 1998; Majumder and Gupta, 2010). Besides, VAc is a liquid ester at reaction temperature with less inhibition to lipase and thus able to use as a reaction solvent (Kuo et al., 2013). The solvent-free system is a simple mixture of substrates, offering the advantages include maximization of substrate concentration, greater volumetric production, and cost savings in reactor design and product separation. Enzymatic processes in solvent-free systems are useful due to their simplicity and decreased environmental hazards (Lee and Akoh, 1998; Shimada et al., 1999).

Recently, response surface methodology (RSM) and an artificial neural network (ANN) approach have been applied for optimization and process modeling. The development of an optimum enzymatic synthesis procedure to improve the yield conversion to reduce production costs will be more attractive for manufacturers and consumers. RSM is a collection of mathematical and statistical techniques for designing experiments, building models, evaluating

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the relative significance of several independent variables and determining optimum conditions for desirable responses (Chen et al., 2002; Ju et al., 2012). ANN is an empirical modeling tool analogous to biological structures, and can identify highly complex relationships from input and output data. ANN is a powerful modeling tool used to learn and generalize the behavior of most complex and non-linear processes. To date, ANN has been applied for the modeling of various bio-chemical processes, for example, biosorption (Bingöl et al., 2012), enzyme production and immobilization (Ebrahimpour et al., 2008; Zhang et al., 2010), fermentation (Zafar et al., 2012), wastewater treatment (Lee et al., 2011) and natural compounds extraction (Sinha et al., 2013).

In this study, a 5-level-4-factor central composite rotatable design (CCRD) with RSM- and ANN-based models was developed to predict the relationship between the experimental variables (substrate concentration, enzyme amount, reaction time and temperature) on the synthesis yields of 2-PEAc. The RSM and ANN models were statistically compared by the coefficient of determination (R^2), root mean square error (RMSE) and absolute average deviation (AAD). The predicted conversion using ANN and RSM models is discussed.

2. Methods

2.1. Materials

Immobilized lipase Novozym® 435 (10,000 U/g; Propyl laurate units) from *Candida antarctica* B (EC 3.1.1.3) supported on a macroporous acrylic resin was purchased from Novo Nordisk Bioindustrials Inc. (Copenhagen, Denmark). 2-Phenethyl alcohol (2-PE), 2-phenylethyl acetate and vinyl acetate were purchased from the Sigma Chemical Co. (St Louis, MO, USA). A molecular sieve 4 Å was purchased from Davison Chemical (Baltimore, MD, USA). All of the chemicals employed were analytical reagent grade.

2.2. Enzymatic synthesis of 2-PEAc

All of the materials were dehydrated by a molecular sieve 4 A° for 24 h before use. The reactions were performed in a capped glass tube (diameter 1.5 cm) consisting of different molar concentrations of 2-PE, and supplemented with VAc to a total volume of 3 mL. Then, different amounts of immobilized enzyme were added to initiate the reaction. The reaction mixture was agitated in an orbital shaking bath (150 rpm) at different temperatures and reaction times. All reactions were carried out in duplicate. Liquid samples were withdrawn from the reaction mixture and analyzed on a gas chromatograph (GC).

2.3. Experimental design

A 5-level-4-factor CCRD was employed in this study; it required 27 experiments. To avoid bias, the 27 runs were performed in a totally random order. The variables and their levels selected for the study of 2-PEAc synthesis were a substrate concentration (300–1900 mM; 2-PE), reaction time (1–5 h), reaction temperature (40–60 °C) and enzyme amount (50–250 U). All of the experiments were performed in a solvent-free system. Table 1 shows the independent factor (X_i), levels and experimental design in terms of being coded and non-coded. Each experimental point was carried out in duplicate.

2.4. Analytical methods

2-PEAc in a reaction mixture was diluted and quantitated by injecting 1 μL of the reaction mixture into a GC (Agilent 7890A) equipped with a flame ionization detector (FID) and MTX-65TG fused-silica capillary column (30 m × 0.25 mm i.d.; film thickness 0.1 μm; RESTEK, USA). Injector and FID temperatures were set at 230 °C and 250 °C, respectively, and the oven temperature was maintained at 80 °C for 2 min followed by a ramp of 20 °C min^{-1} to 100 °C. It was maintained at 100 °C for 3 min, followed by a final ramp of 50 °C min^{-1} to 230 °C, which was maintained for 1.5 min. Nitrogen was used as the carrier at a constant flow rate of 3.5 ml min^{-1}. Calibration curves were prepared from a 2-PE and 2-PEAc standard. The molar conversion was defined as (mmol 2-PEAc production per mmol of initial 2-PE) × 100%.

Table 1
Central composite rotatable design and observed experimental data for 5-level–4-factor response surface analysis.

Treatment no.[a]	Factor				Observed conversion (%)
	Substrate concentration X_1 (mM)	Time X_2 (h)	Temp X_3 (°C)	Enzyme amount X_4 (PLU)	
1	1[b] (1500)	1 (4)	−1 (45)	1 (200)	45.36 ± 8.06
2	0 (1100)	0 (3)	0 (50)	0 (150)	42.10 ± 2.79
3	0 (1100)	0 (3)	0 (50)	0 (150)	36.14 ± 2.03
4	0 (1100)	2 (5)	0 (50)	0 (150)	61.16 ± 0.64
5	−1 (700)	−1 (2)	1 (55)	−1 (100)	39.45 ± 1.34
6	0 (1100)	0 (3)	−2 (40)	0 (150)	48.83 ± 8.57
7	2 (1900)	0 (3)	0 (50)	0 (150)	30.53 ± 6.72
8	1 (1500)	−1 (2)	1 (55)	1 (200)	36.08 ± 0.08
9	−1 (700)	−1 (2)	−1 (45)	−1 (100)	37.13 ± 1.17
10	1 (1500)	1 (4)	1 (55)	1 (200)	64.39 ± 1.98
11	1 (1500)	1 (4)	1 (55)	−1 (100)	40.65 ± 3.21
12	−2 (300)	0 (3)	0 (50)	0 (150)	89.63 ± 2.53
13	1 (1500)	−1 (2)	−1 (45)	−1 (100)	19.26 ± 2.94
14	0 (1100)	−2 (1)	0 (50)	0 (150)	21.80 ± 8.90
15	0 (1100)	0 (3)	2 (60)	0 (150)	37.72 ± 0.24
16	−1 (700)	1 (4)	−1 (45)	−1 (100)	52.93 ± 2.05
17	−1 (700)	1 (4)	−1 (45)	1 (200)	95.96 ± 1.61
18	0 (1100)	0 (3)	0 (50)	0 (150)	40.66 ± 0.17
19	0 (1100)	0 (3)	0 (50)	2 (250)	55.11 ± 4.51
20	1 (1500)	−1 (2)	−1 (45)	1 (200)	35.29 ± 5.45
21	−1 (700)	1 (4)	1 (55)	1 (200)	66.64 ± 5.14
22	1 (1500)	−1 (2)	1 (55)	−1 (100)	30.99 ± 0.00
23	−1 (700)	1 (4)	1 (55)	−1 (100)	53.06 ± 1.81
24	−1 (700)	−1 (2)	−1 (45)	1 (200)	79.19 ± 0.79
25	0 (1100)	0 (3)	0 (50)	−2 (50)	32.09 ± 6.07
26	−1 (700)	−1 (2)	1 (55)	1 (200)	93.66 ± 0.72
27	1 (1500)	1 (4)	−1 (45)	−1 (100)	33.64 ± 4.83

[a] The treatments were run in a random order.
[b] The values −1, 0, and 1 are coded levels.

3. Results and discussion

The aim of this study was to employ RSM and ANN in combination with the experimental design for the evaluation of enzymatic synthesis of 2-PEAc in a solvent-free system. The independent variables selected in the experimental design were: substrate concentration (X_1), reaction time (X_2), reaction temperature (X_3) and enzyme amount (X_4). Their ranges and levels are given in Table 1. Two models were used to evaluate the influence of the experimental factors and their interactions on 2-PEAc synthesis and to predict conversion from different input values. The optimum conversions by two models were compared and the results presented.

3.1. RSM model

The obtained responses, according to the CCRD experiments, are presented in Table 1. Among the various treatments, the highest molar conversion (95.96 ± 1.61%) was treatment no. 17