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PROCESS INTENSIFICATION AND RSM OPTIMIZATION FOR HIGH-YIELD FRUCTOSE CAPRYLATE SYNTHESIS: THE SYNERGISTIC ROLE OF ULTRASOUND AND IONIC LIQUIDS

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ABSTRACT
The production of sugar esters using enzymes is becoming more important as industries move towards using non-toxic and biodegradable surfactants in food, pharmaceuticals, and cosmetics. This study helps to advance green chemistry by finding the best way to synthesize fructose caprylate using ultrasound in ionic liquid media. A special method called Box-Behnken Response Surface Methodology was used to create a highly accurate model that shows how ultrasonic energy can overcome traditional limitations and increase conversion rates by 57% compared to traditional mechanical stirring methods. This indicates a considerable enhancement, and it's because the ultrasonic energy helps mass transfer more effectively. Our model is very good at predicting the results, with an R^2 value of 99.77% and an adjusted R^2 value of 99.35%. This research has important effects on the field of sustainable chemical engineering. It shows that using ultrasonic process intensification with ionic liquid and biocatalysis can make green synthesis faster and more efficient. This study provides a reliable way to improve reaction kinetics in green synthesis. The study provides essential knowledge for the scaling up of bio-based surfactant production and presents validated optimized production parameters for sustainable production processes.
Keywords: Ultrasound-Assisted Synthesis, Ionic Liquids, Sugar Esters, Box–Behnken Design, Response Surface Methodology (RSM), Biocatalyst

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INTRODUCTION

Aligning with the United Nations Sustainable Development Goal SDG-12:Responsible Consumption and Production—has become a global imperative for advancing sustainable chemistry. The optimisation of chemical synthesis methods in terms of efficiency of production constitutes one of the challenges to be faced in this field. In recent years, green chemistry and process intensification have been proposed as non-classical solutions to the above challenge, utilising non-traditional energy inputs such as ultrasound, microwave technology, mechanical activation and microfluidic systems.[1-5] In this respect, the development of ionic liquids (ILs) as green solvents has become increasingly relevant as they offer several key advantages. Notably, they possess very low vapour pressure at ambient conditions, which renders evaporation loss negligible. Additionally, ILs are thermally stable and possess great chemical tunability. Their application to various types of biocatalytic transformations is noteworthy, since in reactions involving sensitive bioactive molecules such as sugars and other carbohydrates, the use of ILs results in the stabilisation of enzymes.[6,7] Importantly, the combination of the properties of ILs with ultrasound cavitation results in an extremely environmentally friendly system, where the ILs act as highly efficient solvents and ultrasounds intensify the mass transfer processes.[8-10] Enzymatic synthesis of sugar esters under ultrasound in ionic liquid media is presented in this paper. The approach to synthesis involves a gentle condition of specific biocatalysis and increased reaction rates under green chemistry principles.[11,12] The use of lipase enzymes, specifically immobilized forms, enhances process selectivity and enables reusability, which results in cost-effective and scalable operations.[13-15] Optimised conditions employing immobilised lipase catalysis permitted the process to be economically and scalably practicable. Sugar esters are biodegradable, non-toxic substances that possess superior emulsifying and antimicrobial characteristics. The compounds show increasing demand in food production and pharmaceutical and

cosmetic industries[16-18]. The commercial production of sugar esters is challenging and time-consuming because sugars are insoluble in organic solvents, and a high temperature is required for their synthesis. A new approach for the synthesis of sugar esters using ultrasound and ionic liquid overcomes these limitations.[19,20] The research on sugar esters for yield improvement through ultrasound and ILs has received moderate studies, yet the kinetic aspects and statistical modelling of these systems remain understudied. The optimisation and kinetic modelling of ultrasound-assisted enzymatic systems require further investigation because these areas remain poorly understood.[8,21–23] The research requires essential systematic studies of these parameters through robust modelling techniques because of their importance. Response Surface Methodology (RSM) serves as an extensive tool that combines experimental design with regression modelling and statistical analysis to optimise complex multivariate systems.[13,17,24] The Box–Behnken Design (BBD) implementation of RSM enables researchers to study variable interactions through efficient experimental runs.[14,17,25] RSM has gained widespread adoption in chemical and biochemical engineering for developing predictive models and optimising processes.[21,24] This research fills this knowledge gap by exploring the possibility of optimizing and modeling ultrasonic-assisted enzymatic synthesis of fructose caprylate using Response Surface Methodology (RSM) with Box–Behnken Design. From the results, optimal reaction conditions could be obtained, and a reliable second-order polynomial kinetic model was developed by analyzing interaction effects of reaction time, temperature and ultrasonic power at constant substrate concentration and amount of enzyme. The study explored the possibility of sugar esters production using a combination of ultrasound technology, green solvents and biocatalysis to develop environmentally friendly production methods.

EXPERIMENTAL

Experimental Design

A Box-Behnken experimental design with three factors and three levels was used to conduct fifteen different experiments to find the optimal fructose caprylate synthesis. The experiment evaluated reaction time from 2 to 20 hours, temperature between 30 and 50 °C and ultrasonic power between 20 and 100 W. These parameters are presented in Tables-1 and 2.

Table-1: Process Variables, their Ranges, and Optimisation Constraints

Variables	Symbol	level		
		-1	0	1
Independent Variables				
Time, hr	X_1	2	11	20
Temperature, 0C	X_2	30	40	50
Power, W	X_3	20	60	100
Dependant variable				
% Conversion	Y			

Materials

The experiments used AR-grade chemicals as-is since no purification was needed before usage. SRL supplied fatty acids that exceeded 99% purity. SRL supplied fructose (with a purity greater than 99%) for the experiments. The METISARK PTE. LTD, Singapore, supplied an immobilised lipase with more than 5000 U/g activity on acrylic resin. 1-butyl-3-methylimidazolium hexafluorophosphate from Avra Synthesis Pvt Ltd.

Table-2: Box–Behnken Design Matrix and Experimental Results for Three-Level, Three-Factor Response Surface Analysis

Run. No	Time, hr X_1,	Temp ^{0}C X_2	Power W X_3	Conversion %, Y
1	2	30	60	0.9
2	2	50	60	20.7
3	20	30	60	24.7
4	20	50	60	49.8
5	2	40	20	7.3

6	2	40	100	14.9
7	20	40	20	17.5
8	20	40	100	64.9
9	11	30	20	5.5
10	11	30	100	12.1
11	11	50	20	10.8
12	11	50	100	53.8
13	11	40	60	23.3
14	11	40	60	23.1
15	11	40	60	22.4

Preparation of Supersaturated Sugar Solution

For each reaction,0.25 mmol of fructose was dissolved in 0.2 mL of distilled water. 0.5 mL of 1-butyl-3-methylimidazolium hexafluorophosphate ([Bmim][PF_6]) was added. The mixture received continuous stirring at room temperature until it formed a clear, uniform solution. Excess water is removed through vacuum evaporation. The supersaturated solution underwent moisture content analysis through weighing before and after vacuum evaporation. Figure-1 shows the experimental setup.

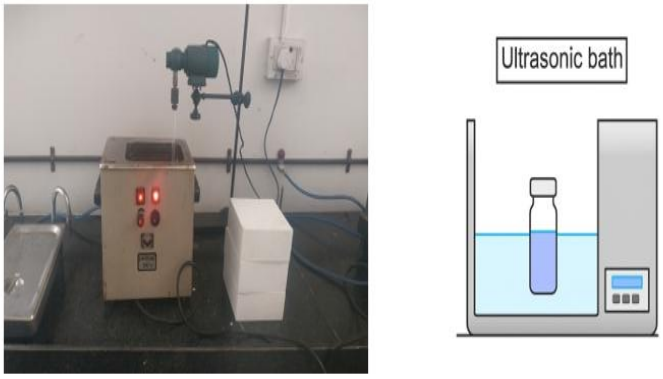

Fig.-1: Experimental Setup (Ultrasonic bath)

Reaction

The sugar solution received 35 milligrams of immobilised enzyme (30 % of the total mass of substrate), 0.072 g (0.5 mmol) of caprylic acid, and 10% by weight of molecular sieves. The vial containing the reaction mixture was submerged in an ultrasonic bath filled with water. The reaction temperature needed to be maintained through a temperature controller provided with an ultrasonic bath. The reactions were performed under set power levels during specified time periods. After completion of the reaction, one millilitre of distilled water was added to the vial mixture. The mixture was centrifuged. Supernatant liquid containing unreacted sugar was analysed for sugar content. The experiments were conducted at different reaction times (2 to 20 hours), temperatures (30–50 °C), and ultrasound powers, as shown in the results. A series of control experiments was performed to evaluate sonication effects by maintaining the same temperature without sonication.

Characterization and Analysis

The DNS method was used to determine the concentration of unreacted sugar in the supernatant liquid. The DNS method is a fast, simple, and cost-effective analytical technique widely used for quantifying reducing sugars in various samples, including biomass, cereals, honey, beverages, and other food products.[26-30] The low solubility of sugar esters (< 0.1 g/L at 25 °C) in both water and the IL solution had minimal impact on the DNS measurements. Standard calibration curves were prepared by plotting absorbance against known sugar concentrations. These curves were subsequently used to estimate the residual sugar concentration in the reaction supernatant (fructose). Sugar conversion (%) was determined from the difference between initial and residual sugar concentrations in the reaction mixture.

Calculation of Conversion

The amount of unreacted sugar (fructose) was determined using the following relation:

Unreacted sugar (mg)=Concentration of sugar in the supernatant (mg/mL) ×Volume of supernatant (mL)

The conversion (%) of sugar to ester was calculated using:

$$\text{Conversion (\%)} = ((\text{Initial amount of sugar} - \text{Unreacted sugar})/\text{Initial amount of sugar})) \times 100$$

Model Fitting

The experimental data from the Box–Behnken Design (BBD) were fitted to a second-order polynomial model relating conversion (%) to reaction time (X_1), temperature (X_2), and ultrasonic power (X_3). The model was fitted using Python with the help of the scikit-learn and NumPy libraries.

RESULTS AND DISCUSSIONS

Preliminary Experiment

Fructose caprylate was synthesised using lipase as a biocatalyst under two experimental conditions: ultrasonic-assisted synthesis at 40 °C (100 W) and mechanically-assisted synthesis at 40 °C. The quantity of unreacted sugar using the DNS method was recorded during the reaction to monitor the conversion degree. The results obtained (Fig.-2) show that a 63% molar conversion was reached after 20 h by ultrasonic-assisted synthesis, while a 40% molar conversion was reached in the mechanically-assisted process, using the same amount of enzyme and experimental conditions. A 57% increase in yield was achieved using process intensification. Therefore, a fundamental requirement for sustainable manufacturing, which is enhancing process yield while decreasing the consumption of resources and materials, was completely fulfilled. Also, SDG 12 (Responsible Consumption and Production), related to the sustainable management of resources and their efficient use, was fulfilled through the reduction of energy and time for synthesis. The results are in line with the existing literature in terms of mass transfer enhancement and effects of decreased viscosity.[31,32] The high viscosity of the ionic liquids studied herein was found furthermore to be decreased positively by ultrasonic treatment. In addition, the results support previous observations regarding the modification activation energy so that the reaction moves towards the mass transfer–controlled regime.

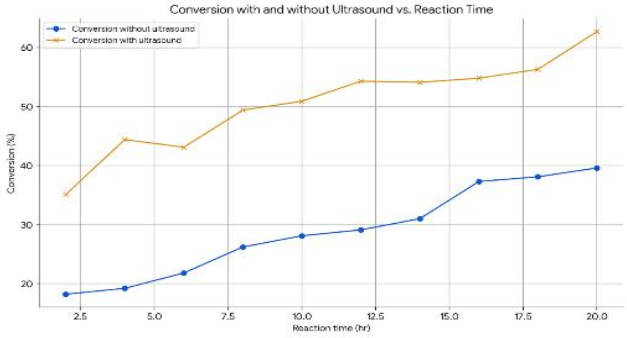

Fig.-2: Preliminary Experiment, Temp 40 ^{0}C, Ultrasound Power 100W, Enzyme Concentration 30 % w/w

Polynomial Model

Based on the fitted second-degree polynomial model using the Box–Behnken Design data, the regression equation for % Conversion (Y) as a function of Time (X_1), Temperature (X_2), and Power (X_3) is:

$$Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{11}X_1^2+\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{22}X_2^2+\beta_{23}X_2X_3 + \beta_{33}X_3^2$$

Using the fitted coefficients, the equation becomes:

$$Y=-239.593+6.415X_1+8.484X_2+2.370X_3-0.180X_1^2-0.094X_2^2-0.013X_3^2-0.082X_1X_2-0.026X_1X_3-0.004X_2X_3$$

The ANOVA results (Table-3) clearly indicate that the second-order polynomial model provides an excellent fit to the experimental data, with a high coefficient of determination (R^2 = 99.77%) and adjusted R^2 of 99.35%. All three components—linear, quadratic, and 2-way interaction terms—were statistically significant with extremely low p-values (< 0.001), and very high F-values, highlighting their substantial contribution to the model. This implies strong synergistic effects among time, temperature, and ultrasonic power in determining conversion efficiency. The overall model is robust and suitable for predictive and optimisation purposes.

Table-3: ANOVA Summary

Source	RÂ² (%)	df	F-value	P-value
Regression	99.77			
Adj. RÂ²	99.35			
Linear	271.24	3	1932.67	0
Quadratic	66.27	3	472.22	0
2-way interaction	511.86	3	3647.24	0
Total model	99.77	9	236.96	0

Mutual Effect of Parameters

Further insight into how the process variables affected the conversion efficiency was gained from the response surface plots based on the Box–Behnken design matrix (Table-2). Figure-3 examines the impact of time (2–20 hours) and temperature (30–50 °C) on conversion when the ultrasonic power is held constant at 60 W. At the lowest combination of time (2 h) and temperature (30 °C), negligible conversion (~0%) was observed, while the highest yield (~51%) was achieved at 20 h and 50 °C. The low conversions at low temperatures are explained by the fact that the lipase has a slightly higher optimal temperature and requires an alkaline pH, as stated in previous reports.[33] The effect of ultrasonic power and reaction time on conversion is shown in Fig.-4 when the temperature is set at 40 °C. The combination of longer reaction times and higher ultrasonic power resulted in a substantial increase in yield, starting from the lowest conversion of ~8.5% at 5 h and 20 W. The data showed that temperature, reaction time and ultrasonic power were important parameters affecting the enzymatic esterification of fructose.

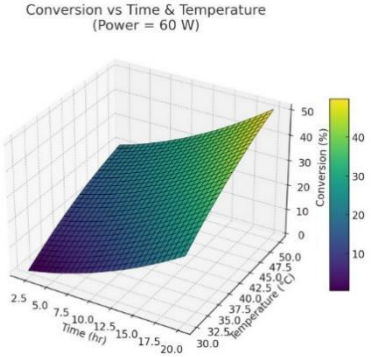

Fig.-3: Response Surface Plot of Fructose Conversion During Fructose Caprylate Synthesis as A Function of Reaction Time and Temperature

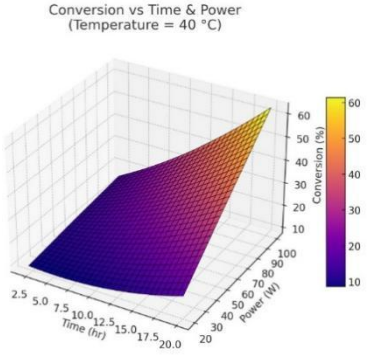

Fig.-4: Response Surface Plot of Fructose Conversion During Fructose Caprylate Synthesis as a Function of Reaction Time and US Power

Previous research has established that moderate increases in ultrasonic power increase the rate of enzymatic reactions by improving mass transfer conditions. Under ultrasonic irradiation, the apparent activation energy increases markedly, indicating a shift toward kinetic control.[34] In addition to the previous study, the current research also found that higher ultrasound intensity combined with longer reaction times resulted in higher conversion rates. At lower temperatures, even when using 60 W ultrasound, no measurable enhancement in enzyme activity was observed. This is likely due to insufficient thermal energy at lower temperatures, which limits the catalytic activity of the enzyme despite ultrasonic agitation.[35] Both plots clearly show that increasing time, temperature, and power

improves conversion. Low temperatures or low powers result in even extended reaction times, failing to activate the enzyme properly

Optimal reaction conditions

The main purpose of this research was to establish the best experimental conditions (reaction time (X_1), temperature (X_2) and ultrasonic power (X_3)) to achieve the highest conversion (%) in the ultrasound-assisted enzymatic synthesis of fructose caprylate in an ionic liquid medium. The optimisation was performed using the L-BFGS-B algorithm, a quasi-Newton method designed. The model predicts that the maximum conversion of ≈ 83% is achieved under the following optimal conditions: Reaction Time (X_1): 20 hours, Temperature (X_2): 50 °C, Ultrasound Power (X_3): 100 W By defining these precise optimal parameters, a platform for technology scale-up for production of green surfactants was established. The technology has low environmental impact per unit of product (SDG-12). Design space upper limits indicated that higher levels of time, temperature and power were required for higher conversion within the investigated experimental domain.

A quantitative mathematical model was developed based on experimental data to predict optimal operating conditions for a biocatalytic reaction. Calculations indicated that optimal operating conditions for maximum conversion within the experimental domain are at upper limits of the investigated design space, i.e., time, temperature and ultrasonic power. Similar results were obtained for different biocatalytic systems. For example, the Box–Behnken model for ultrasound and vacuum-assisted enzymatic synthesis of octyl cinnamate[36] located optimum at the edge of the investigated domain, at approximately 12 h, 75 °C and 120 W for maximum conversion efficiency. Another study[37] was conducted to investigate the effects of reaction time (12–36 hours), enzyme loading and ultrasonication power (90–150 W) on the catalytic activity. The highest conversion of 95.3% was achieved at the upper limit of the design range at 12 hours reaction time, 50% enzyme loading and 120 W ultrasound power, further affirming that elevated parameter settings within the studied range tend to favour enhanced enzymatic activity and product formation.

CONCLUSION

A feasible and efficient method for ultrasound-assisted enzymatic synthesis of fructose caprylate in ionic liquid media was developed and optimized. A robust quadratic model was developed using Box–Behnken Design and Response Surface Methodology. Predicted optimal synthesis conditions were 20 hours at 50 °C and 100 W ultrasonic power. The maximum experimental conversion rate (83%) was 57% higher than that obtained under conventional mechanical agitation. To achieve the sustainable synthesis of sugar esters while overcoming mass transfer limitations and solubility issues, it is necessary to integrate process intensification technologies with green chemistry strategies.

In this work, a green and efficient method for the production of sugar esters, scalable to industrial levels, was developed. And the achievement of this result contributes directly to the United Nations Sustainable Development Goal (SDG) 12: Responsible Consumption and Production. The method proposed for the synthesis of sugar esters constitutes a suitable alternative to conventional industrial processes and may enable the establishment of a new platform for sustainable manufacturing.

Future studies could explore the scaling up of this process to a continuous flow reactor, along with optimisation of the enzyme recycling steps to further increase commercialisation potential. This work provides a robust platform for future technological development to create high-performance, 'green' chemical technologies that will be capable of meeting the challenging production requirements of modern industrial processes.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-12: Responsible Consumption and Production*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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