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# Surfactant assisted ultrasonication for enzyme extraction from lignocellulosic biomass: Box–Behnken design optimization

Sangeetha Velusamy[a,*], Kannan Kandasamy[a], Sudha Ayyasamy[b], Dhivya Bharathi Nagarajan[b], Harini Sekar[a], Kiruthick Durairaj[a], Abi Prakash Maniraj[a]

[a]*Department of Chemical Engineering, Kongu Engineering College, Perundurai 638060, Tamil Nadu, India, Tel.: 04294 226601; Fax: 04294 220087; emails: vsangeetha@kongu.ac.in (S. Velusamy), kannank@kongu.ac.in (K. Kandasamy), harini44001@gmail.com (S. Harini), kiruthickd.18chem@kongu.edu (D. Kiruthick), mabiprakash99761@gmail.com (M. Abi Prakash)*
[b]*Department of Food Technology, Kongu Engineering College, Perundurai 638060, Tamil Nadu, India, emails: sudhaseshu@gmail.com (A. Sudha), dhivss96@gmail.com (N. Dhivya Bharathi)*

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**ABSTRACT**

Increased population and rapid industrialization have created large quantity of solid waste, different solid waste processing methods used to recover and reusing of its yield's valuable products. The project is aimed to the extraction of enzyme from the mixture of textile sludge and domestic sludge. The solid separation method is adopted to separate the enzyme from textile sludge using ultrasound combined with surfactant (sodium dodecyl). The phases are been separated by disintegration method using ultrasonication of 25 kHz at different time intervals (10, 15, and 20 min) combining with surfactant of 1, 3 and, 5 g, respectively. The response surface methodology based on Box–Behnken design (BBD) was applied to evaluate and optimize the effect of parameters such as sludge ratio (25:75, 50:50, 75:25), ultrasonication time and surfactant dosages. The factors –1, 0, 1 with different combinations in BBD was done in experimental method for separation of phases and they are centrifuged, then the enzyme activity was proved. From the experimental results, for high absorbance values for increased surfactant dosage the enzyme activity also increases (5 g) by optimising ultrasonication time and sludge ratio of 15 min and 50:50. Scanning electron microscopy analysis of sludge samples were done to figure out the effect of disintegration using ultrasonication.

*Keywords:* Textile sludge; Domestic sludge; Surfactant; Ultrasonication; Response surface methodology

## 1. Introduction

Rapid industrialization and increased population generate more solid waste in our environment and different techniques have been adopted to achieve sustainable environment by developing a value-added product from the waste. Solid wastes are highly produced in different industries such as textile industry, food industry, paper, and pulp etc... Developing countries has adopted 3R's (reduce, reuse, recycle) to enhance a valuable product such as enzyme, protein, starch. The valuable products that can be produced from the solid waste were used in different industries as catalyst under many reactions and additives. The usage of enzyme has been increased in animal feed additives, agriculture, and agro-based industries. Enzyme can be extracted from the solid waste by different methods such as adsorption, membrane separation, solid separation, and gel filtration [1]. Usually, commercial enzymes are extracted from plant species, animal cells and microbial species. In which this method increases the enzymatic pre-treatment process that cost high, therefore, to achieve at lower cost the enzymes are extracted from the waste activated sludge

* Corresponding author.

that are produced from different industries [2]. Gel filtration is the process in which the separation is based on their size and molecular shape. Larger the particles are been separated easily than the smaller particles. But this method cannot be applied for very minute particles [3]. In the membrane separation techniques, it uses different type of filters which is more expensive [4]. So, in this experiment solid separation method is adopted to separate the solid and liquid phases where the supernatant is the enzyme and it is further used in agro-based industries or as an additive. The solid phase could also be suspended further using sodium chloride and an amount of enzyme can be extracted from it [5]. The solid separation method involves ultrasonication using high frequency of 20 kHz helps in disintegration of solid and liquid phase to achieve supernatant as an enzyme [6–8]. It is adopted to reduce the sludge volume to achieve better dewaterability and to increase the solubilisation of organic matter during the waste sludge treatment [9]. Ultrasonication combined with surfactant has yield more amount of enzyme [10]. Surfactant usually breaks the extra bounded polymeric substances of solid waste and yields higher enzyme activity [11]. The structured polymeric substances are known as gel and they are combined in a biofilm gel [12] and the incomplete extraction or deactivation of enzyme is due to the biofilm of the bacteria in surface which is prevented from cohesion of lethal ecological factors [13]. Different types of surfactants can also be used such as anionic, cationic, non-ionic surfactants. Ionic surfactants readily mix with water so anionic surfactant sodium dodecyl sulphate surfactant is used [14] which is more feasible and tends to give more foam when it is dissolved in waste water and separates solid and liquid phases readily [15]. Textile sludge and domestic sludge have been chosen for extracting enzyme [16]. The waste activated sludge from the aerated tank is collected and is mixed with different proportion of domestic sludge, which is also collected from the local areas. chemical oxygen demand (COD), biological oxygen demand (BOD) values are characterized to use the wastewater for enzyme extraction [17–21]. Textile sludge and domestic sludge are initially mixed with surfactant, so that extra polymeric substances can be easily broken [22] the ultrasonication at different time interval and surfactant with different dosages were studied [23]. Response surface methodology is the statistical technique which is more helpful in optimizing better experimental design for different experimental runs of dependent factors, using design expert software the number of experimental runs has been reduced to seventeen experimental runs in the laboratory [24–28]. Box–Behnken design is used for different experimental runs of independent factors where it is the three factorial experimental runs [29]. The solid phase and liquid phase are separated using ultrasonication and is refrigerated at 4°C preventing from mixing of these phases for an hour [30]. The refrigerated sample is centrifuged at 3,500 rpm for 30 min [31] and the supernatant is extracted. The aim of this study was to decide the effect of time and surfactant dosages for different combinations using response surface methodology (RSM) model and to optimise enzyme activity by the effect of dependent variables such as ultrasonication time (10–20 min) surfactant dosages (1–5 g) and sludge ratios (25:75, 50;50, 75:25).

## 2. Materials and methods

### 2.1. Textile and domestic sludge

The textile sludge was collected from Bannari Amman Spinning Mills, Perundurai. The sludge was taken from the aerated tank [15] and it is characterized because textile sludge usually has different compositions of heavy metals depends on the type of industrial process and the chemicals involved [32,33]. The characteristics of textile sludge such as pH: 5.86, COD: 1,720 mg/L, BOD: 560 mg/L, TDS: 8,500 mg/L, TSS: 530 mg/L were studied, respectively. Similarly, the domestic sludge was collected from the local area and its characteristic such as pH: 7.5, COD: 680 mg/L, BOD: 880 mg/L were studied, respectively.

### 2.2. Different proportions of sludge

Different ratios of sample were mixed to produce the enzyme. The domestic sludge was taken as 25 mL [25], 50 mL and 75 mL. The surfactant was mixed with different proportions of 1, 3 and 5 g, respectively. The samples were mixed in the ratio of 25:75, 50:50 and 75:25 [34] with the different proportions of surfactants. The different ratio was mixed and it is kept in orbital shaker of 150 rpm for 15 min [33].

### 2.3. Surfactant

Surfactant are the surface-active agent when it was mixed with sludge it tends to create foam and breaks the extra polymeric substances [11]. When the extra polymeric substance matrix is broken the yield of enzyme will be higher, ionic surfactant mixes with water and that forms other compound so, non-ionic surfactant or anionic surfactant was used in the experiment to break the EPS matrix [35–39]. The most feasible anionic surfactant sodium dodecyl was used in the mixture of textile and domestic sludge to enhance the yield of enzyme during disintegration process [40] which is having the molecular formula of $NaSO_4C_{12}H_{25}$ and molecular weight of 288.38. In this experiment 1, 3, and 5 g of surfactant was mixed with the textile and domestic sludge as per the combinations of experimental runs from Box–Behnken design shown in Table 1 [32].

### 2.4. Experimental design

In 1951, Box and Wilson started the work of response surface methodology at first. RSM emphasized practical applications in the chemical and processing fields [41]. RSM was one of the most well-known methods in various variables enhancement. RSM detects the insert and out-turn of

Table 1
Experimental ranges and factors for response surface methodology

| Variables | –1 | 0 | 1 |
|---|---|---|---|
| Domestic waste: textile sludge (mL) - *A* | 25:75 | 50:50 | 75:25 |
| Ultrasonication time (min) - *B* | 10 | 15 | 20 |
| Surfactant (g) - *C* | 1 | 3 | 5 |

the model, but not the inward factors and explicit its abilities inferred by the computer. The main purpose of RSM was to decrease the predicted value of a single out-turn, with ongoing inputs and without any limitations [42]. RSM enables assessment of the impacts of process variables and their interchanges on response variables that has been effectively used for developing, and optimizing biological processes related to food structures, as well as extraction. The motive of this work was to decreasing the substantial number of runs into optimum level [43]. This study involves optimization aided by ultrasonication process using RSM. Box–Behnken design (BBD) is an effective way for fixing response surfaces using three equally dispersed levels. The ultrasonic time (10–20 min), sludge ratio and surfactant dosage were picked as variables. A Box–Behnken design existing of 17 experimental runs were applied to process the variables [44]. In this situation, the maximum enzyme extraction was acquired. The main purpose of this process design is to optimize the response surface which is determined by various process variables. This methodology also estimates the connection between the manageable input process variables and the existed response surfaces. In this study, the Box–Behnken design was pick out for detect the connection between the functions and variables (sludge ratio, ultrasonication time and surfactant dosage) [45]. The optimum working conditions were resulted by using more difficult experimental process like Doehlert matrix (DM), central composite designs (CCD) and Box–Behnken design (BBD). Comparing BBD to other response processes, determined that BBD is slightly more effective than CCD and much more effective than full factorial designs [46]. The Box–Behnken design and the RSM RSM, the effectiveness of the process variables (sludge ratio, ultrasonication time and surfactant dosage) was determined. Experiments were proven under BBD with three variables and each of these were coded as −1, 0 and +1 [47]. The independent variable of RSM in BBD will lie at different point and the centre point is replicated as multidimensional cube. The model which is given by the Eq. (1) [48].

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} x_i x_j + \varepsilon \quad (1)$$

where $Y$ – predicted response; $\beta_0$ – constant co-efficient; $\beta_i$ – linear co-efficient; $\beta_{ii}$ – quadratic co-efficient; $\beta_{ij}$ – cross product co-efficient; $x_i$ – input variables; ε – error.

### 2.5. Ultrasonication

Ultrasonication is the solid phase separation method. It gives sound energy to agitate particles in a sample, for various purposes such as the extraction of multiple compounds from plants, microalgae, and seaweeds [46]. It has low consumed energy, less extraction time, more extraction of yields. During this process, more micro bubbles was formed and then will be kept after few microseconds. Ultrasonication is main purpose for cleaning and removing rusts. Mechanisms of cavitation and bombardment which carry the ultrasonic waves, to be used for cleaning solid deposits particles. Cavitation releases heat and energy of the liquid, which increases temperature that eases for separate the solid phase and liquid phase. In this experiment the frequency of 20 kHz was used to separate solid and liquid phases combining with the anionic surfactant (sodium lauryl sulphate) [49] at different time intervals as 10, 15, 20 min [34]. In 100 mL of sludge sample, the ultrasonicator probe is dipped in the beaker containing the mixed sludge with surfactant at different conditions [50]. The specific energy of the sludge disintegrates was determined using the formula as follows:

$$\text{Specific energy} = \frac{PV}{VTs} \quad (2)$$

where $P$ – power (KW/min); $T$ – sonication time (s); $V$ – volume of sludge sample (L); $T_s$ – total solid concentration (mg/L); specific energy-kJ/kg.

### 2.6. Centrifugation

Centrifugation is a method to separate molecules which has different densities it rotates the particles of solid kept at button and liquid is presented at top when it's operated at high speed. The centrifuge works using the sedimentation of particle where the centripetal acceleration due to denser substances. In the radial direction, particles to move towards outside [51]. It is used to collect cells to precipitate DNA particle, distinguish the differences on phases of molecules. It is a mechanical process applied centrifugal force to separate the components of mixture according to density or particle size. The denser phase will be settled at the bottom and less the dense will present at the top. The supernatant was collected after centrifugation for further test, the remaining solid phases be further suspended with NaCl for further process (or) it can disposed [35]. The sample is centrifuged in disc type centrifuge for about 45 min at 1,500–3,000 rpm. So that the supernatant is easily separated [31]. The supernatant which is liquid phase has high amount of enzyme and is extracted and the solid phase which is settled at the bottom is further suspended to get complete yield of enzyme present in that sludge sample. The removed liquid phase is analysed for enzyme present in the supernatant.

### 2.7. Enzyme activity

The activity that is measured in units which indicate the rate of reaction catalysed by enzyme expressed as a micromoles of transformed substrate per minute. Different methods were adopted to find the activity of amylase, protease, lipase in enzyme [52]. In this experiment the amylase activity is seen using different combination of mixed sludge ratios, surfactant and the ultrasonication time. The amylase activity is measured by reducing sugars that has been released from the starch by using DNSA method using dinitrosalicylic acid (DNS) reagent. The glucosidase activity is measured by p-nitrophenyl and d-glucopyranoside (PNPG) which is used as a substrate. 3,5-dinitrosalicylic acid is used to find the reducing sugars in a sample solution [53]. The carbonyl group (C=O) which is free can be estimated using this method. When the surfactant sodium dodecyl sulphate in water, they form the form of crystallization where the meta bisulphate component of sodium to oxidize first with DNS and keep its properties. Using water and DNS reagent the standard graph is drawn for finding the molasses present in the supernatant using

DNSA reagent. The reducing sugars are estimated by adding the DNS reagent to the sample solution [54]. The monosaccharides and disaccharides are also estimated using the DNS reagent. The procedure for making DNS reagent is dinitrosalicylic acid (DNS)-1 g of DNS is dissolved in 50 mL of distilled water and 5 g of sodium potassium tartrate tetrahydrate is added in small lot after that the solution turns milky yellow in colour [55]. With this reagent the DNSA method was followed, at first 2 mL of starch solution is taken in the test tubes and the starch solution and $\alpha$-amylase was preincubated for 10 min at 37°C, then 2 mL of $\alpha$-amylase was added to the test tube containing 2 mL of starch solutions and it is incubated at 37°C for 10 min. After incubation, 2 mL of DNS Reagent is added to the test tube, and it is mixed well for the absorbance of test solutions is read at 540 nm.

### 2.8. Scanning electron microscopy analysis

The samples of sludge with 1% (w/v) of glutaraldehyde solution at room temperature is fixed for 120 min. After that the sludge sample were washed using distilled water and dehydrated for 5 min using high concentrations of ethanol (25%, 50%, 75%, and 90% v/v) and in absolute ethanol the samples were kept for 20 min. The sample of thickness 110–140 Å are dried and coated with gold in argon atmosphere. The process was inspected using scanning microscope equipment containing probe diameter of 40–60 Å.

## 3. Result and discussion

### 3.1. Statistical analysis and fitting of second-order polynomial equation

According to Table 2 varied factors influences the enzyme activity are surfactant dosages, sludge ratio and ultrasonication time. The specific energy and the enzyme activity for the independent variable was calculated. Actual equations of enzyme activity such as amylase and glucosidase activity were obtained:

$$\text{Amylase Activity} = +1.72 - 0.3052A - 0.1294B + 0.2129C - 0.0945AB - 0.3920AC + 0.0428BC - 0.5531A^2 - 0.3679B^2 - 0.1829C^2 \quad (3)$$

$$\text{Glucosidase Activity} = +1.60 - 0.2936A - 0.1109B + 0.1800C - 0.0552AB - 0.3595AC + 0.0320BC - 0.5310A^2 - 0.4355B^2 - 0.1643C^2 \quad (4)$$

Fisher $F$-test was used for goodness of fit, where multiple correlation for coefficient of $R^2$ were calculated. Quadratic model is suggested where $p$-value Prob. > $F$ is 0.002 and 0.010 for amylase and glucosidase activity and cubic model is aliased. Two different methods were used to find the significant response of enzyme activities. From Table 3 sequential sum of squares $p$-value is less than 0.005 Prob. > $F$ only in the quadratic model so it is significant where the cubic model is aliased for Prob. > $F$ is greater than 0.05. For quadratic model, the $R^2$ values were less than 1, the adjusted $R^2$ value is also less than 1 and the predicted $R^2$ value is 0.5. From the Table 4 model summary statistics, the $R^2$ value of 0.915 and 0.807, adjusted $R^2$ value of 0.807 and 0.671, predicted $R^2$ value of 0.475 and 0.409 for both enzyme activity, quadratic model was suggested and for cubic model was aliased. From the ANOVA Table 5, it was observed that highest $R^2$ for the model terms of value Prob. > $F$ $A$, $C$, $AC$, $A^2$, $B^2$ are significant when $p$-value is less than 0.05. The value which is nearer to 0.05 is significant and having high deviation are not significant for both responses.

Table 2
Experimental design matrix for ultrasonication

| Run | Sludge ratio (mL) $A$ | Ultrasonication (min) $B$ | Surfactant (g) $C$ | Specific energy (kJ/kg TS) | Amylase activity (unit/g VSS) | Glucosidase activity (unit/g VSS) |
|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 6,509.5 | 1.209 | 1.029 |
| 2 | 0 | 0 | 0 | 6,509.5 | 1.209 | 1.029 |
| 3 | –1 | 0 | 1 | 6,509.5 | 0.525 | 0.537 |
| 4 | 0 | 1 | 1 | 8,679.2 | 0.914 | 0.879 |
| 5 | –1 | 1 | 0 | 8,679.2 | 1.975 | 1.872 |
| 6 | 1 | –1 | 0 | 4,339.62 | 1.832 | 1.643 |
| 7 | 0 | 0 | 0 | 6,509.5 | 1.409 | 1.029 |
| 8 | 0 | 1 | –1 | 8,679.2 | 1.512 | 1.426 |
| 9 | 0 | 0 | 0 | 6,509.5 | 1.209 | 1.029 |
| 10 | 1 | 0 | 1 | 6,509.5 | 1.751 | 1.796 |
| 11 | 0 | –1 | –1 | 4,339.62 | 0.795 | 0.632 |
| 12 | 0 | –1 | 1 | 4,339.62 | 0.632 | 0.547 |
| 13 | –1 | 0 | –1 | 6,509.5 | 1.452 | 1.298 |
| 14 | 1 | 0 | –1 | 6,509.5 | 0.194 | 0.121 |
| 15 | 0 | 0 | 0 | 6,509.5 | 1.209 | 1.029 |
| 16 | –1 | –1 | 0 | 4,339.62 | 1.938 | 1.861 |
| 17 | 1 | 1 | 0 | 8,679.2 | 0.572 | 0.342 |

Table 3
Sequential model sum of squares

| Source | Sum of squares | Df | Mean square | *F*-value | *p*-value Prob. > *F* | Remark |
|---|---|---|---|---|---|---|
| | | | Amylase activity | | | |
| Mean vs. total | 24.379 | 1 | 24.379 | | | |
| Linear vs. mean | 1.241 | 3 | 0.413 | 1.672 | 0.221 | |
| 2FI vs. linear | 0.657 | 3 | 0.219 | 0.856 | 0.494 | |
| Quadratic vs. 2FI | 2.185 | 3 | 0.728 | 13.600 | 0.002 | Suggested |
| Cubic vs. Quadratic | 0.121 | 3 | 0.040 | 0.639 | 0.628 | Aliased |
| Residual | 0.253 | 4 | 0.063 | | | |
| Total | 28.839 | 17 | 1.696 | | | |
| | | | Glucosidase activity | | | |
| Mean vs. total | 19.27 | 1 | 19.27 | | | |
| Linear vs. mean | 1.05 | 3 | 0.349 | 1.30 | 0.315 | |
| 2FI vs. linear | 0.533 | 3 | 0.177 | 0.603 | 0.627 | |
| Quadratic vs. 2FI | 2.30 | 3 | 0.765 | 8.22 | 0.010 | Suggested |
| Cubic vs. quadratic | 0.114 | 3 | 0.038 | 0.284 | 0.835 | Aliased |
| Residual | 0.536 | 4 | 0.134 | | | |
| Total | 23.80 | 17 | 1.40 | | | |

Table 4
Model summary statistics

| Source | Std. dev. | *R*-squared | Adjusted *R*-squared | Predicted *R*-squared | Remark |
|---|---|---|---|---|---|
| | | | Amylase activity | | |
| Linear | 0.497 | 0.278 | 0.111 | –0.163 | |
| 2FI | 0.506 | 0.425 | 0.081 | –0.509 | |
| Quadratic | 0.231 | 0.915 | 0.807 | 0.475 | Suggested |
| Cubic | 0.251 | 0.943 | 0.772 | | Aliased |
| | | | Glucosidase activity | | |
| Linear | 0.517 | 0.111 | 0.053 | –0.193 | |
| 2FI | 0.542 | 0.081 | –0.041 | –0.615 | |
| Quadratic | 0.305 | 0.807 | 0.671 | 0.409 | Suggested |
| Cubic | 0.366 | 0.772 | 0.525 | | Aliased |

### *3.2. Effect of independent variables on enzyme activity*

The effect of surfactant dosages, ultrasonication time and the sludge ratio has many interventions in the activity of enzyme. The effect of surfactant dosages has shown increased variation on the activity of amylase and glucosidase. The effect of different sludge ratios has slight variation comparing with the ultrasonication time, this broke the long polymeric chain of carbon present in the textile sludge and those microbial cells are broken down to produce high amount of enzyme. The equivalent results were observed by the study of Anbazhagan and Palani [34] for high yield of enzyme using surfactant. The ranges of ultrasonication time that has been studied are 10, 15, 20 min and the surfactant dosages of 1, 3 and 5 g with the sludge ratios of the 25:75, 50:50, 75:25. Fig. 1 depicts the effect of independent variables on the activity of amylase and glucosidase by varying the surfactant dosages the activity has shown larger variations.

### *3.3. Optimization of independent variables on enzyme activity*

The effect of surfactant dosages and ultrasonication time on enzyme activity was analysed from Table 2 the experiment run for which the enzyme activity is showed high are optimised with the data. From Fig. 2, the optimized value for the effect of surfactant dosages and the ultrasonication time has obtained. The activity of enzyme has been increased on increasing the surfactant dosages using 5 g of surfactant and it yields more amount of enzyme from the sludges. The ratio of textile and domestic sludges were optimized as 50:50 and the ultrasonication time as 15 min, upon increasing or decreasing it does not showed differences on activity of enzyme. The activity of

Table 5
ANOVA of quadratic response surface model for enzyme activity

| Source | df | Amylase activity | | Glucosidase activity | |
|---|---|---|---|---|---|
| | | Coefficient estimate | *p*-value Prob. > *F* | Coefficient estimate | *p*-value Prob. > *F* |
| Model | 9 | 1.72 | 0.0051 | 1.60 | 0.027 |
| *A* | 1 | –0.3052 | 0.0074 | –0.2936 | 0.029 |
| *B* | 1 | –0.1294 | 0.1579 | –0.1109 | 0.338 |
| *C* | 1 | 0.2129 | 0.0353 | 0.1800 | 0.139 |
| *AB* | 1 | –0.0945 | 0.4411 | –0.0552 | 0.727 |
| *AC* | 1 | –0.3920 | 0.0116 | –0.3595 | 0.050 |
| *BC* | 1 | 0.0428 | 0.7227 | 0.0320 | 0.839 |
| $A^2$ | 1 | –0.5531 | 0.0017 | –0.5310 | 0.009 |
| $B^2$ | 1 | –0.3679 | 0.0138 | –0.4355 | 0.022 |
| $C^2$ | 1 | –0.1829 | 0.1490 | –0.1643 | 0.305 |
| Residual | 7 | | | | |
| Lack of fit | 3 | | 0.6284 | | 0.835 |
| Pure error | 4 | | | | |
| Cor. total | 16 | | | | |

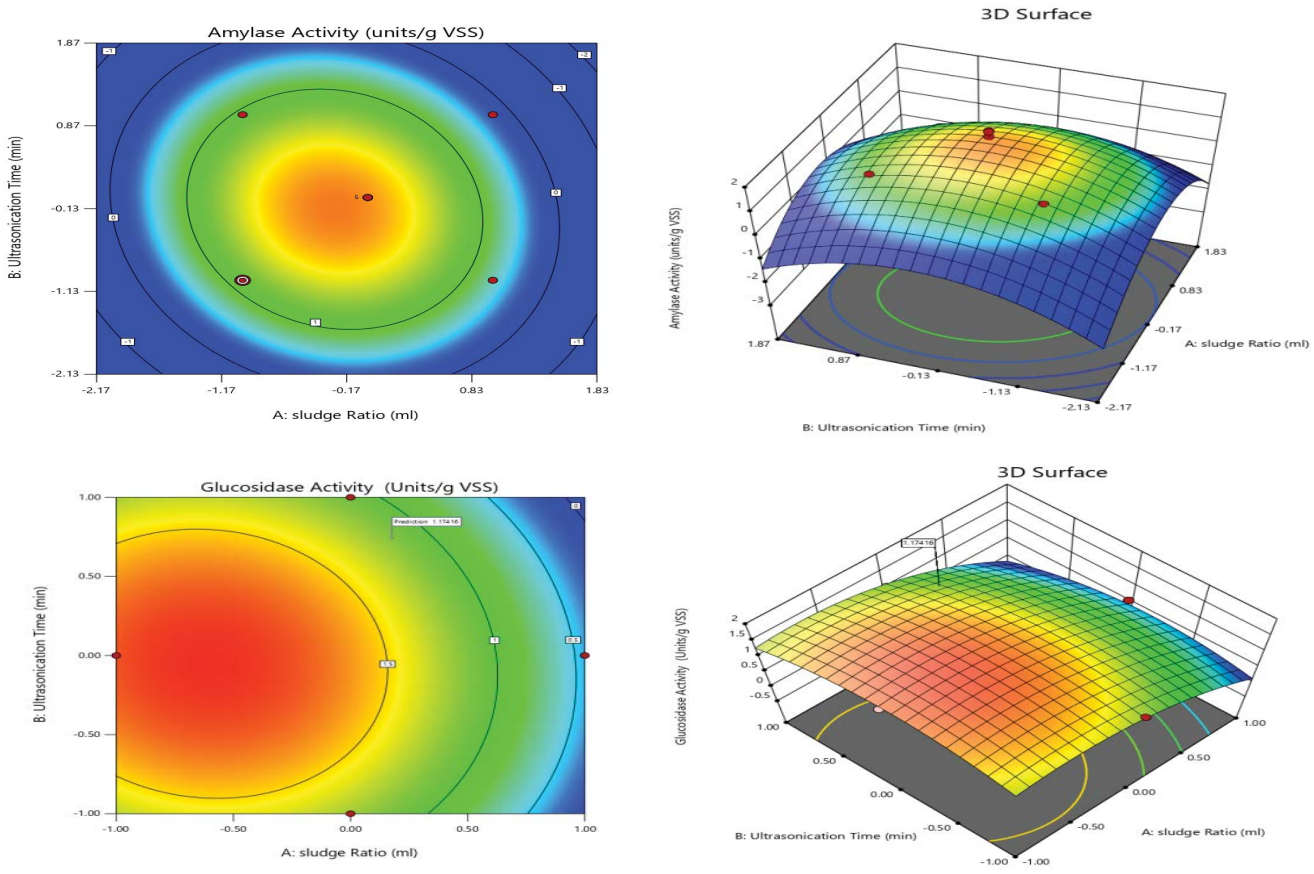


Fig. 1. Effect of independent variable on enzyme activity.

enzyme has increased on increasing the surfactant dosages using 5 g of surfactant and it yields more amount of enzyme from the sludges. The ratio of textile and domestic sludges were optimized as 50:50 and the ultrasonication time is obtained as 15 min, upon increasing or decreasing it does not showed differences on activity of enzyme.

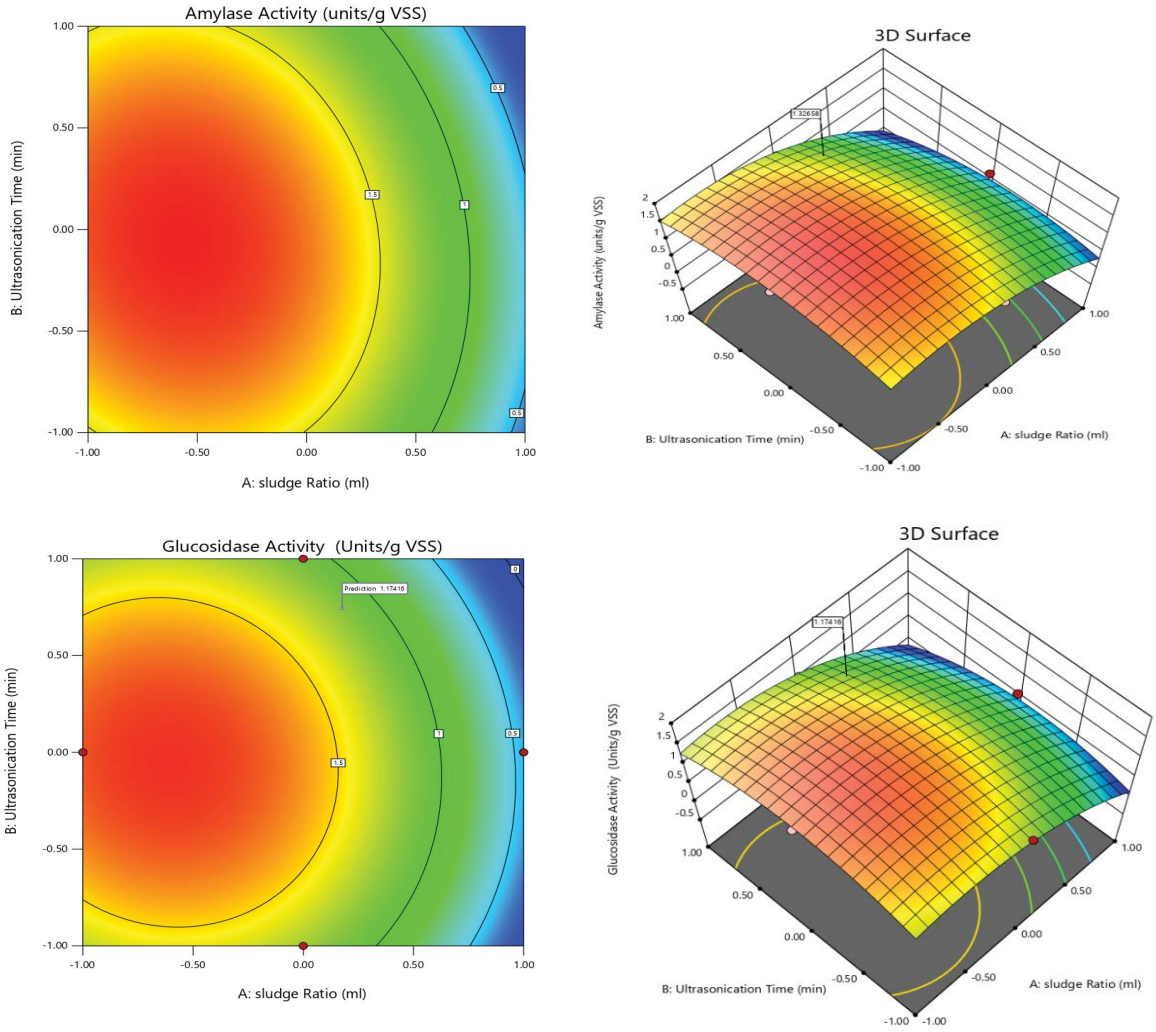


Fig. 2. Effect of independent variable on enzyme activity in optimized experimental conditions.

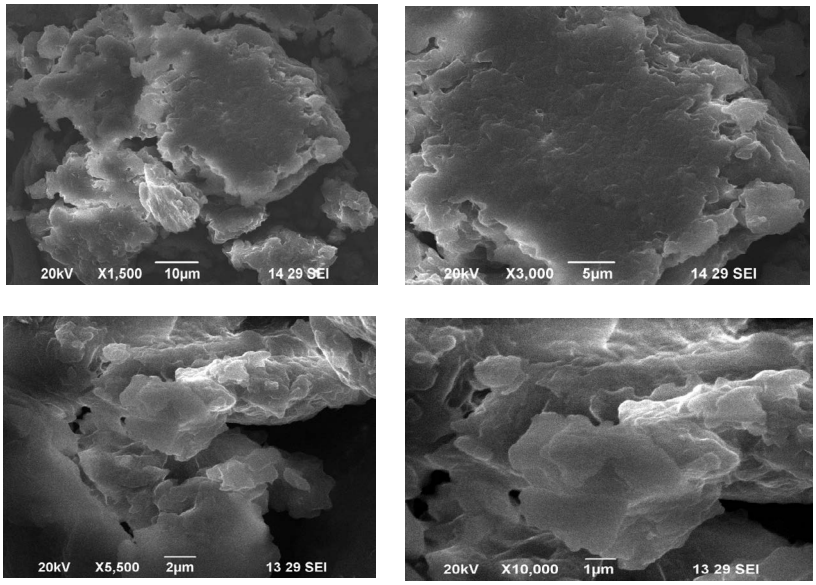


Fig. 3. Microscopic image of sludge flocs after disintegration.

### 3.4. Scanning electron microscopy analysis

Fig. 3 depicts the microscopic image for the disintegration method using an optimized mixed sample in which the sludge is treated with the ultrasonication time of 15 min and surfactant of 5 g. The sludge before disintegration [34] and after disintegration Fig. 3 shows that sludge flocs are observed at different magnification of (x1,500, x3,000, x5,500, x10,000) only before disintegration but after disintegration only at the magnification of x10,000 the sludge flocs are disintegrated into small particles which the surfactant and ultrasonication time has not influenced the mixed sludge because there is no difference in pattern where the size of sludge flocs varies.

## 4. Conclusion

In this study, ultrasonication method is used to extract enzyme from lignocellulosic biomass, The Box–Behnken design in RSM was useful in investigating the independent variables such as ultrasonication time, surfactant dosages, sludge ratios on different experimental runs and it is observed that in RSM method, quadratic model has showed different correlation for coefficient of $R^2$, the ANOVA table in which the coefficients of $R^2$ has showed more variations on comparing experimental and predicted values 0.9 and 0.4 and it is observed that the effect of different ultrasonication time, the effect of varying surfactant dosages has showed changes in enzyme activity, it has showed optimized result on increasing the surfactant dosages of 5 g and the ultrasonication time as 15 min and sludge ratio of 50:50. The sludge flocs has no influence of disintegration method when it is combine with surfactant of 5 g and ultrasonication of 15 min.

## Data availability

No data available

## Declaration

All the authors have no conflict of interest.

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