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Optimization of process variables for enhanced production of urease by indigenous *Aspergillus niger* strains through response surface methodology

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ABSTRACT

Urease is one of the important enzymes secreted by microorganisms in the soil, but can also be produced by microbial fermentation. The present study aimed to explore optimum conditions for maximum urease enzyme production from wheat straw by applying a statistical approach, response surface methodology (RSM), under the solid-state fermentation process. Eight different local strains of *Aspergillus niger* were screened for the production of urease enzyme. Fermentation profile revealed that all the strains showed activity but two strains BMB-02 NSJ and BMB-05 G displayed the higher activity as compared to the other strains. Therefore, two strains explicitly BMB-02 NSJ and BMB-05 G were shortlisted for further optimization studies. Different process parameters including incubation time, temperature, and pH were optimized for urease production. The result showed that both strains of *A. niger* (BMB0-02 NSJ and BMB-05 G) displayed maximum enzyme activity at 144 h (6 days), 35 °C, and pH 9.0, respectively. Moreover, RSM was employed to optimize the different parameters such as biomass, inoculum size, nitrogen content and moisture for both strains. Optimal enzyme conditions for BMB-02 NSJ were biomass, inoculum size, nitrogen, and moisture of 10 g, 5 mL, 0.5 g and 250% ratio to biomass, respectively, whereas biomass, inoculum size, nitrogen and moisture content of 6 g, 3 mL, 150% ratio to biomass without the addition of any nitrogen were found for BMB-05 G. The findings revealed that optimization of different factors have a significant effect on urease production, and RSM-based optimization is a promising and time-saving technique.

1. Introduction

Enzymes are biodegradable, biocompatible, and highly efficient catalysts that can catalyze reactions with great specificity and stereoselectivity under the mild environmental conditions (low temperature and pressure, aqueous medium, etc.) (Asgher et al., 2017; Bilal et al., 2019a,b; Ren et al., 2019; Rehman et al., 2019). All organism including plants, animals, and microorganism are capable of producing enzymes, however, microbial enzymes have gained particular interest for their widespread industrial applications as compared to the other counterparts (Adeel et al., 2018; Khan et al., 2018; Bilal et al., 2019a,b). Microorganisms decompose organic waste and play a vital role in recycling nutrients present in the surrounding environment. In agriculture, enzymes have shown a crucial role in maintaining soil fertility (Mvila et al., 2016; Xi et al., 2017). Enzymes can be produced from different agricultural wastes in the course of hydrolyzing polysaccharides into simple monomeric sugars, which have a variety of uses (Olsson et al. 2003; Imran et al., 2017).

There is an increasing demand to produce new products (food and non-food, chemicals) from food and agro-industrial residues in a more sustainable way than are currently realized or possible. Solid-state fermentation (SSF) and related technologies offer alternative production routes for such biotechnology-based products (Abdul Manan and Webb, 2017). SSF has been used for a long time for traditional manufacture of fermented foods, and mold-ripened cheese. It has received enormous attention as an economically viable and practically acceptable technology for large-scale microbial bioconversion and biodegradation processes for the production of food, enzymes, animal feed, chemicals, bioethanol and pharmaceuticals (Munir et al., 2015; Asgher et al., 2016a,b; Abdul Manan and Webb, 2017; Amin et al., 2017).

Urease is one of the important enzymes secreted by microorganisms in the soil, but can also be produced by microbial fermentation. Belonging to the superfamily of amidohydrolases and phosphotriesterases, this nickel-containing enzyme catalysis the hydrolysis of urea into carbon dioxide and ammonia. Firstly, it converts urea into carbon dioxide and carbamate. The resulting compound carbamate then transformed into ammonia and carbon dioxide. All living organism such as invertebrate, algae, plants and filamentous fungi produce

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Table 1
Fungal strains used in this study.

Sr. No.	Strain name
1	BMB-01 Yellow
2	BMB-02 NSJ
3	BMB-03 White
4	BMB-05 G
5	BMB-09
6	BMB Amp
7	BMB 10 Black
8	BMB 11 Niger

urease. Mostly, fungal and plant urease is homohexamers α6, and bacterial urease is heterotrimers (αβγ) 3. Bacterial ureases have three subunits, one large and two small subunits typically form trimers. Whereas plants and fungi composed of identical subunits and commonly formed as trimers and hexamers (Krajewska, 2009). Ureases are made up of different subunits belonging to the different origin of living organism fungi to plant and bacteria, but they contain similarity in amino acid sequences with Jack bean urease (Saxena et al., 2017; Kappaun et al., 2018).

Ureases has very wide application in the beverage industry to reduce the urea in the alcohol. It is also used in the diagnostic kits for the determination of urea concentration in the blood, and biosensor of hemodialysis for urea detection in the blood. It also finds applications in the manufacturing of plastics, hair conditioners, glues, and fertilizer. It has also important use in the preparation of animal feed. Urease can be used in dermatological products as well as for nonsurgical debridements of nails. It can also be used as a diuretic (Omoregie et al., 2016; Zeinali and Lenjannezhadian, 2018). Due to these facts, the urease requirement in the industry is increasing day by day, that can only be fulfilled by large scale fermentation (Nandy, 2016). Filamentous fungi produce almost 40% of all the enzymes. For this reason, filamentous fungi have a wide application in the fermentation industry. Urease can also be produced by bacteria but their production ratio is lower as compared to fungi (Akhtar et al., 2014). Among the filamentous fungi, *Aspergillus niger* is the best strain for the production of the commercial enzyme such as urease, glucose oxidase, pectinase, glucoamylase and catalase (Bakhtiari et al., 2006).

Response surface methodology (RSM) is an experimental method that is a useful tool to optimize the parameters of fermentation processes by applying mathematics and statistics (Kavitha et al., 2016). RSM has been widely used to optimize microbial fermentation processes, and it can determine the impact of multiple factors (Nawaz et al., 2016). Furthermore, RSM can be used to optimize fermentation conditions such that they meet the nutritional needs of a specific microorganism, thus avoiding the unnecessary addition of excess components in the culture medium (Feng et al., 2017). Compared with other optimization methods, RSM requires fewer trials to calculate the numerous variables and their interactions. Therefore, RSM strategies to maximize the yields of bioactive metabolites are necessary (Ushakiranmayi et al., 2017; Peng et al., 2018; Yun et al., 2018).

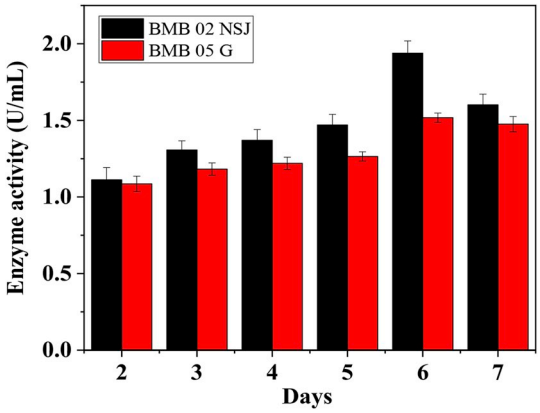

Fig. 2. Time optimization for BMB-02 NSJ and BMB-05 G.

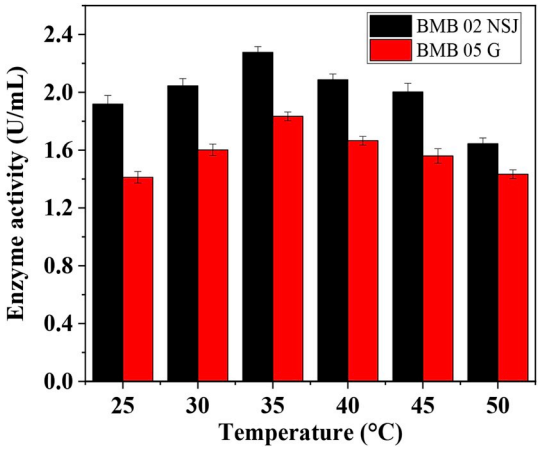

Fig. 3. Optimization of temperature for BMB-02 NSJ and BMB-05 G.

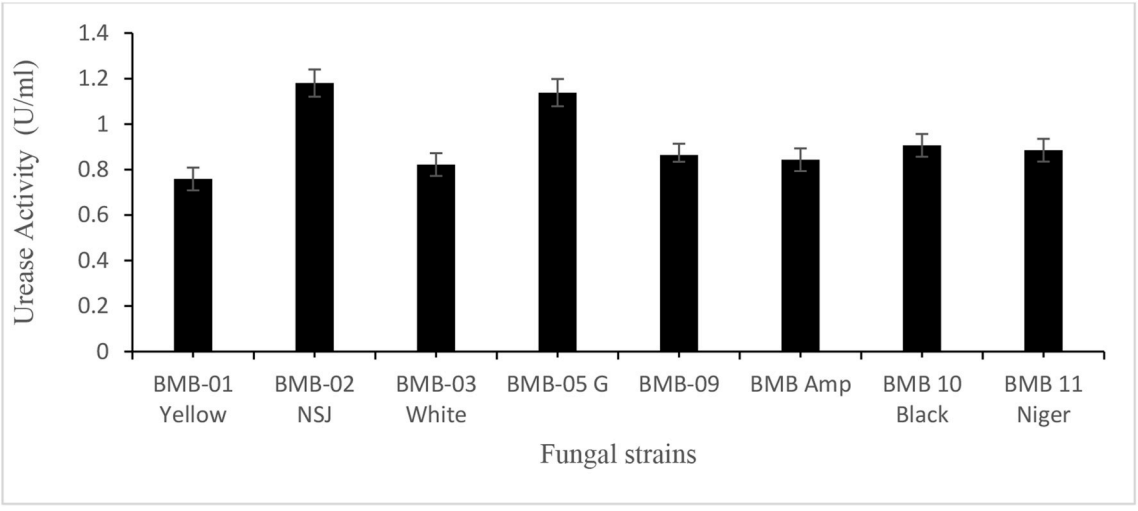

Fig. 1. Screening of local fungal strains for urease enzyme production.

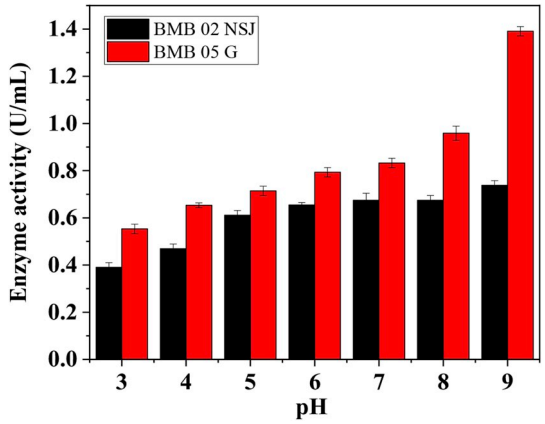

Fig. 4. Optimization of pH for BMB-02 NSJ and BMB-05 G.

Table 2
Optimization and results of response surface methodology-based parameters of BMB-02 NSJ.

Run	Biomass (g)	Moisture content (%)	Inoculum size (mL)	Nitrogen Level (g)	Enzyme activity (U/mL)
1	6	150	3	1.5	2.02
2	2	250	1	2.5	2.02
3	2	250	5	0.5	2.63
4	2	250	1	0.5	1.26
5	2	50	5	0.5	1.36
6	2	50	5	2.5	1.30
7	2	50	1	2.5	1.26
8	6	150	3	1.5	2.02
9	10	250	5	0.5	3.14
10	2	250	5	2.5	1.13
11	10	250	5	2.5	1.45
12	6	150	3	1.5	2.02
13	10	50	5	0.5	1.79
14	2	50	1	0.5	0.88
15	10	250	1	0.5	2.59
16	10	50	5	2.5	1.09
17	6	150	3	1.5	2.02
18	10	250	1	2.5	2.84
19	10	50	1	2.5	1.32
20	10	50	1	0.5	1.26
21	6	350	3	1.5	1.55
22	1	150	3	1.5	1.13
23	6	150	0.5	1.5	2.40
24	6	150	7	1.5	1.45
25	6	150	3	00	2.06
26	6	150	3	1.5	2.02
27	6	150	3	3.5	1.01
28	14	150	3	1.5	1.26
29	6	150	3	1.5	2.02
30	6	50	3	1.5	1.51

Lignocellulose biomass has a very crucial role in fermentation, as it provides all necessary nutrients to the organism used in the process of fermentation. Being an agricultural country, agro-industrial wastes are easily available at low cost in Pakistan. These agro-industrial wastes are a good source of all necessary nutrients such as carbohydrates, protein, and minerals that can be used as a substrate for the synthesis of enzymes by the microorganism. A range of substrates including wheat straw, rice straw, wheat bran, sugar cane bagasse, and husk can be exploited as substrates for the production of enzymes by *A. niger* (Mukhtar and Haq, 2013; Pensupa et al., 2013). In the present research, different culture conditions were optimized for enhanced production of urease from indigenous *A. niger* by using wheat straw as a growth substrate.

2. Materials and methods

2.1. Fungal strains, medium and lignocellulosic substrate

In this research work, eight different types of *Aspergillus niger* strains were used for urease production due to its capability of producing numerous enzymes. All the *Aspergillus niger* strains were obtained from the Department of Biochemistry and Biotechnology, University of Gujrat, Gujrat Pakistan. The detail of strains is listed in Table 1. Potato Dextrose Agar (PDA) medium was prepared for the growth of the spores as it contains all the nutrients required for their growth. Wheat straw (*Triticum*) collected from the surroundings of Dinga (Gujrat Pakistan) was utilized as a lignocellulosic substrate for enzyme production.

2.2. Inoculum preparation

The spores of the *Aspergillus niger* of different strains were grown on PDA agar slants at 30 °C for three days. Later on, the pure colony of each strain was transferred to PDA broth, and flasks were incubated in a shaker (Thermostable IS-20R, Daihan) at 150 rpm and 30 °C for 72 h to get a homogenous inoculum suspension (Rashid et al., 2018).

2.3. Production and harvesting of urease

Triplicate shake flasks containing an appropriate amount of biomass were inoculated with 3 mL of each strain with the help of a micropipette. The inoculated flasks were cotton plugged and kept in an incubator at 30 °C for 5 days. After the speculated incubation time, 50 mL of distilled water was added to each flask and shaken at 150 rpm for 1 h. The mixtures containing crude enzyme extract were filtered and the filtrate containing the enzyme was used for enzyme activity assay (Rashid et al., 2018).

2.4. Enzyme activity assay

For the enzyme activity assay, Weatherburn (1967) method was adopted with slight modification, i.e. Na_2HPO_4 was used in place of NaOH and the time for color development increased from 20 to 30 min. To 100 µL of the sample, 500 µL of urea and potassium phosphate buffer (pH 8) were added. The reaction mixture was kept in a shaking bath at 37 °C for 30 min. After the addition of 500 µL of phenol-sodium nitroprusside solution to 50 µL of the reaction mixture, it was again kept for 30 min at 37 °C. The optical density of the color complex was measured by a spectrophotometer (T80 plus UV/VIS spectrophotometer, PG instruments) at 630 nm.

2.5. Optimization studies

2.5.1. Screening of fungal strain

Eight flasks containing 5 gm biomass in each were inoculated with 3 mL inoculum of used eight different strains. After inoculation, the flasks were incubated at 30 °C for five days, and enzyme assay was performed to shortlist strain for the maximum enzyme activity (Ali et al., 2016).

2.5.2. Time period optimization

For the optimization of time, flasks were filled with 5 g of biomass, autoclaved (Digital Fuzzy Control System, Daihan), and each flask was inoculated with strains. The strains giving the highest yield of enzymes were selected for further use. The inoculated flasks were placed in an incubator for different times ranging from 2 to 7 days followed by the enzyme activity assay.

2.5.3. Temperature and pH optimization

For temperature optimization, flasks containing biomass and inoculum were incubated at different temperatures of 25 °C, 30 °C, 35 °C,

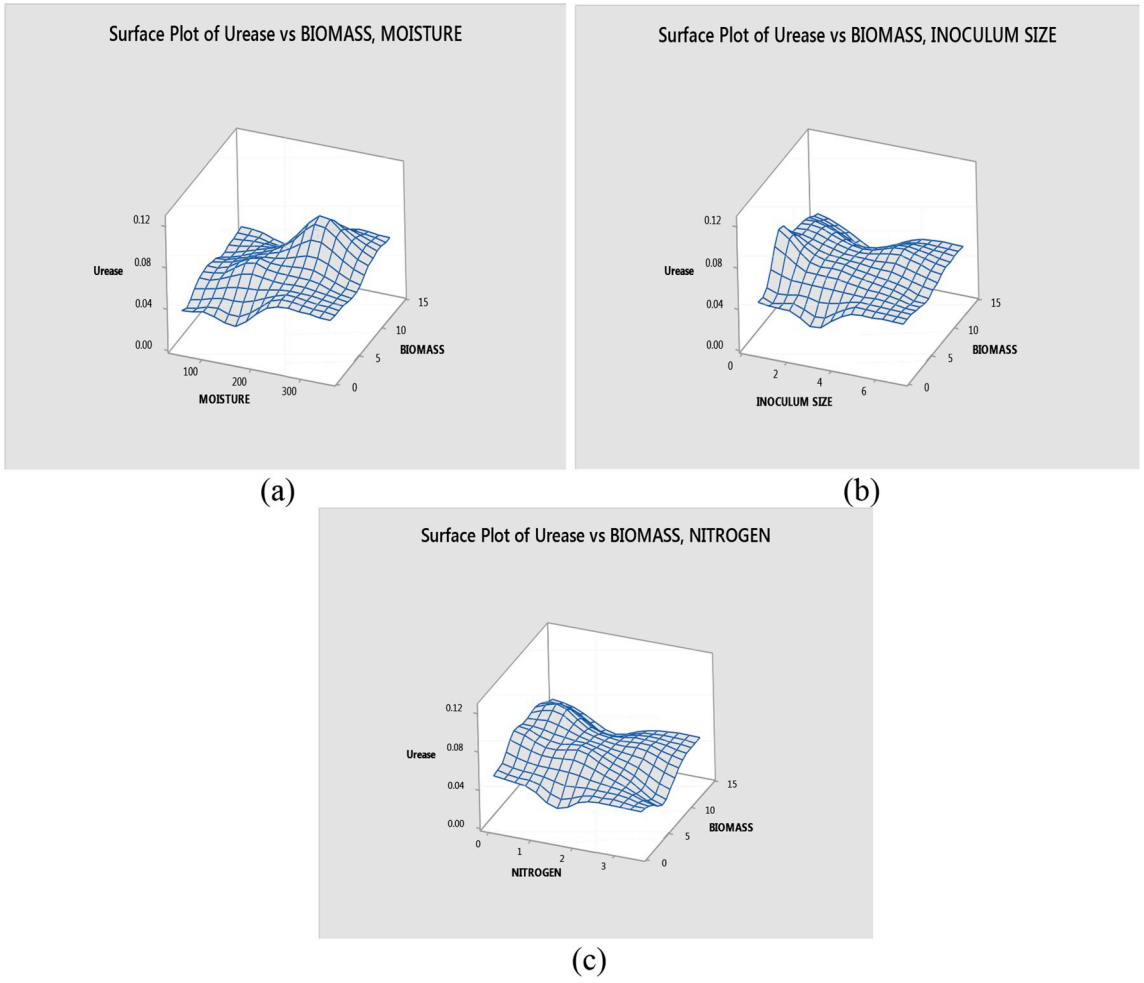

Fig. 5. Response surface plots showing the interactive effects (a) biomass v/s moisture (b) biomass pH v/s inoculum size and (c) biomass v/s nitrogen for urease production by BMB-02 NSJ.

40 °C, 45 °C, 50 °C, 55 °C and 60 °C, followed by same steps of harvesting and enzyme assay to reveal the temperature inducing the maximum enzyme production. In pH optimization, the range selected was ranging from pH 3 to 9. The enzyme assay was performed to check that which pH gives a maximum yield of urease.

3. Optimization through RSM

The time duration of fermentation, temperature, and pH was optimized by "one factor at a time" optimization strategy (Rashid et al., 2018). The parameters included were biomass size (ranging from 1 to 10 g), moisture content (50–250%), nitrogen level (0.5–3 g) and inoculum size (1–5 mL) were optimized by applying RSM in factorial statistical design. Flasks with biomass were autoclaved and inoculated with measured values according to the design. The flasks were placed in an incubator for 6 day at 35 °C. Later on, the harvested enzyme was stored, and the hyperactivity of urease was checked by an enzyme activity assay.

4. Results and discussion

4.1. Screening of fungal strains

Eight fungal strains were inoculated on wheat straw biomass for the selection of fungal strains, which gives maximum enzyme yield after fermentation (Fig. 1). Fermentation profile revealed that all the strains showed activity but two strains BMB-02 NSJ and BMB-05 G displayed high activity as compared to the other strains. In a previous study, thirteen different strains of *A. niger* were studied for the production of urease enzyme and efficient urease producing strains were selected (Faezi et al., 2004). Aggarwal et al. (2015) compared the characteristics of urease secreting two fungal and thirteen bacterial strains for urease production by quantitative and qualitative analysis screening analysis. In another study, different fungal and bacterial strains screening was carried out for the production of urease by identifying various fungal and bacterial strains (Alizadeh et al., 2014).

4.2. Time period optimization for BMB-02 NSJ and BMB-05 G

The time of urease production for both strains was measured. Results showed that both the strains produce maximum urease at day six (Fig. 2). The findings were correlated to Akhtar et al. (2014), who reported the optimum production of urease enzyme by *A. niger* strains after the 6th day of fermentation.

4.3. Temperature optimization for BMB-02 NSJ and BMB-05 G

Temperature is a very crucial parameter and has a great effect on the production of various microbial enzymes. Results in Fig. 3 portrayed that both strains exhibited the highest production of urease at 35 °C, and showed an increase in urease activity up to 35 °C. After this temperature, urease activity starts decreasing and showed the least activity at 50 °C. The optimum temperature of 40 °C has been reported previously by Danial et al. (2015). In another study, Fathima and

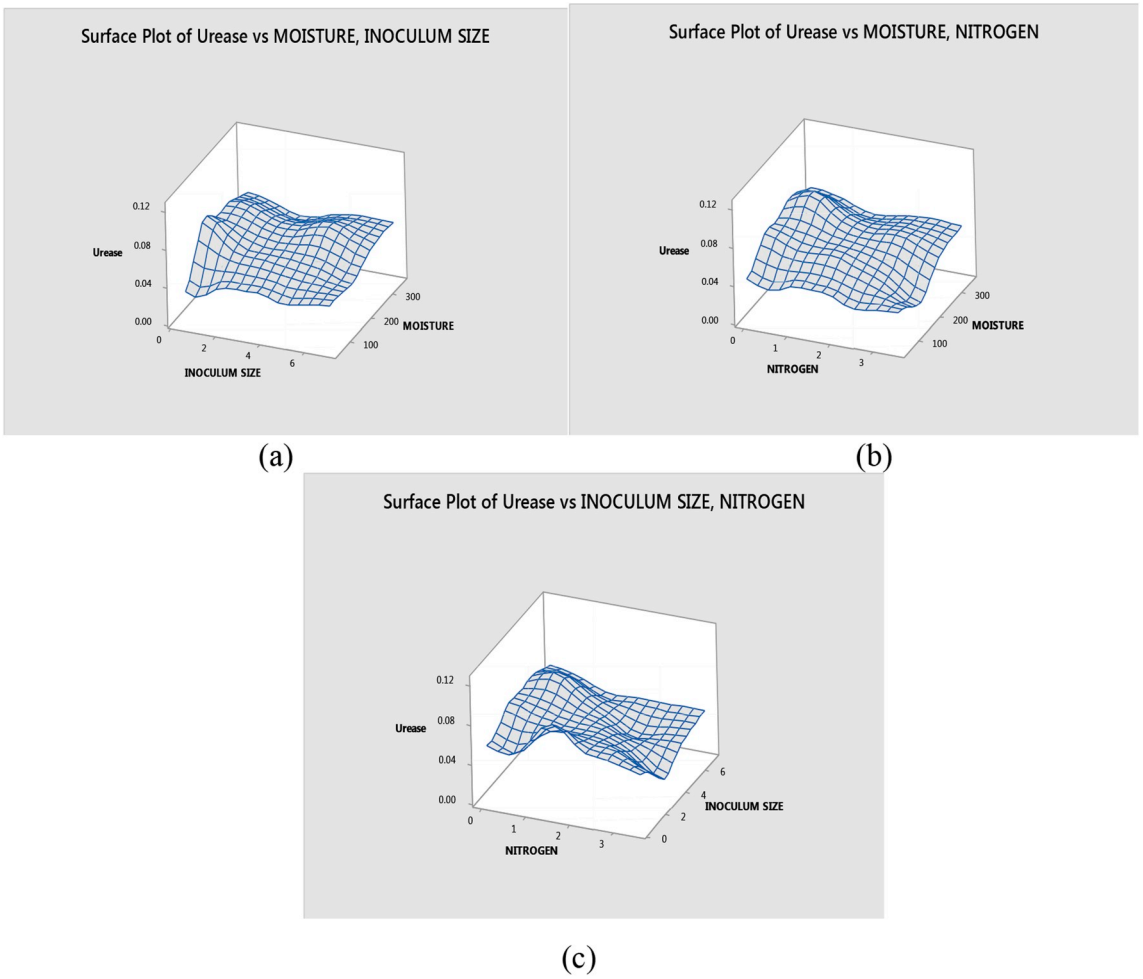

Fig. 6. Response surface plots showing the interactive effects (a) moisture v/s inoculum size (b) moisture v/s nitrogen, and (c) inoculum size v/s nitrogen for urease production by BMB-02 NSJ.

Table 3
Analysis of variance for optimization of urease production by BMB-02 NSJ.

Source	DF	Adj SS	Adj MS	F-value	P-value
Model	14	0.0	0.0	4.10	0.005
Linear	4	0.0	0.0	4.00	0.021
Biomass	1	0.0	0.0	1.37	0.260
Inoculum Size	1	0.0	0.0	0.70	0.417
Nitrogen Level	1	0.0	0.0	0.01	0.942
Moisture Content	1	0.0	0.0	13.81	0.002
Square	4	0.0	0.0	2.68	0.072
Biomass*Biomass	1	0.0	0.0	5.52	0.033
Inoculum Size*Inoculum Size	1	0.0	0.0	3.88	0.068
Nitrogen Level*Nitrogen Level	1	0.0	0.0	0.10	0.753
Moisture Content*Moisture Content	1	0.0	0.0	0.48	0.499
2-Way Interaction	6	0.0	0.0	3.40	0.025
Biomass*Inoculum Size	1	0.0	0.0	2.47	0.137
Biomass*Nitrogen Level	1	0.0	0.0	1.12	0.307
Biomass*Moisture Content	1	0.0	0.0	1.24	0.283
Inoculum Size*Nitrogen Level	1	0.0	0.0	0.64	0.436
Inoculum Size*Moisture Content	1	0.0	0.0	1.58	0.228
Nitrogen Level*Moisture Content	1	0.0	0.0	13.37	0.002
Error	15	0.0	0.0		
Total	29	0.0			

R^2 = 91.21%.

Jayalakshmi (2012) also observed that urease gives maximum activity at 35 °C, while optimizing different physiochemical parameters for urease biosynthesis.

4.4. pH optimization for BMB-02 NSJ and BMB-05 G

A range of pH i.e. 3–9 was tested to optimize the pH for the maximum yield of urease. Fermentation profile revealed that the enzyme shows more activity as the pH increases, and the maximum urease production was achieved at pH 9 for both strains (Fig. 4). Urease activity gradually increases with the increase of pH from 3.0 to 9.0. Urease showed the least activity at pH 3.0 and highest at pH 9.0 as shown in Fig. 4. Since ammonia is produced as a result of urease activity, as ammonia is basic in nature it increases the pH of the fermentation environment (Sujoy and Aparna, 2013). Recently, Phang et al. (2018) optimized the conditions for maximum activity of urease by using different bacterial strains. They evaluated that urease showed maximum activity at an alkaline pH of 9.0, which was in consonance with the Smith et al. (1993). Another study showed that maximum urease activity occurred at pH 8 (Mirbod et al., 2002; Danial et al., 2015). Urease produced by *A. niger and A. nidulans* had an optimum pH of 8.0 and pH 8.5, respectively (Kappaun et al., 2018). They worked on two different strains and found that the urease showed hyperactivity in the basic medium. It can be concluded that fungal urease has maximum activity

Table 4
Optimization and results of response surface methodology-based parameters of BMB-05 G.

Run	Biomass (g)	Moisture content (%)	Inoculum size (mL)	Nitrogen Level (g)	Enzyme activity (U/mL)
1	6	150	3	1.5	2.02
2	2	250	1	2.5	2.02
3	2	250	5	0.5	2.63
4	2	250	1	0.5	1.26
5	2	50	5	0.5	1.36
6	2	50	5	2.5	1.30
7	2	50	1	2.5	1.26
8	6	150	3	1.5	2.02
9	10	250	5	0.5	3.14
10	2	250	5	2.5	1.13
11	10	250	5	2.5	1.45
12	6	150	3	1.5	2.02
13	10	50	5	0.5	1.79
14	2	50	1	0.5	0.88
15	10	250	1	0.5	2.59
16	10	50	5	2.5	1.09
17	6	150	3	1.5	2.02
18	10	250	1	2.5	2.84
19	10	50	1	2.5	1.32
20	10	50	1	0.5	1.26
21	6	350	3	1.5	1.55
22	1	150	3	1.5	1.13
23	6	150	0.5	1.5	2.40
24	6	150	7	1.5	1.45
25	**6**	**150**	**3**	**00**	**2.06**
26	6	150	3	1.5	2.02
27	6	150	3	3.5	1.01
28	14	150	3	1.5	1.26
29	6	150	3	1.5	2.02
30	6	50	3	1.5	1.51

in the basic medium.

4.5. Optimization by RSM

RSM was used for the optimization of biomass content, inoculum size, nitrogen content, and moisture level. The 3-D surface plots were obtained by using the MINITAB software (Version 17) to interpret the results.

4.5.1. RSM for urease optimization by BMB-02 NSJ

The response surface parameters such as biomass, nitrogen content, moisture content and inoculum size were measured for the strain BMB-02 NSJ. From the result, it was found that urease maximum production of urease takes place under the optimal conditions of biomass 10 g, moisture 250%, inoculum size 5.0 mL and nitrogen 0.5 g (Table 2).

4.5.2. Statistical synergistic effect study among different variables for BMB-02 NSJ

The 3-D surface plots represent the fact that in linear relation, the moisture content is a significant contributing factor in urease production, whereas biomass, inoculum size and nitrogen content were not important factors with respect to urease production (Figs. 5 and 6). Moisture content shows *p*-value 0.002, which is highly significant, while biomass, nitrogen level, and inoculum size has 0.260, 0.942 and 0.417 *p*-values, which are not significant. The square relation shows the importance of biomass as production factors because of its p-value, while inoculum size, moisture content and nitrogen level do not have a contribution in the production process, so these are less important factors. Two-way interaction of factors is significant for moisture level versus nitrogen content (Table 3).

Statistically, the produced amount of urease can be determined by applying the following formula;

Urease U/mL = −0.0640 - 0.000129 Sr + 0.01277 Biomass +0.000493 Moisture +0.01660 Inoculum Size.

+ 0.0389 Nitrogen - 0.000721 Biomass*Biomass - 0.000001 Moisture*Moisture.

+ 0.000065 Inoculum Size*Inoculum Size - 0.00187 Nitrogen*Nitrogen.

- 0.000409 Sr*Nitrogen +0.000016 Biomass*Moisture - 0.000739 Biomass*Inoculum Size.

- 0.00068 Biomass*Nitrogen - 0.000015 Moisture*Inoculum Size.

- 0.000056 Moisture*Nitrogen - 0.00787 Inoculum Size*Nitrogen.

When biomass and moisture content studied together, they indicate the significance of moisture content, and non-significant of biomass. Both of these together factors have the least effect. Biomass versus inoculum size showed that biomass has some role in the urease production, while inoculum size has no effect on the enzyme production. When biomass increased from 4 to 8 g, it has many productions and when the amount raised from 8 g, its production decreases. Biomass and nitrogen in combination are reverses of each other. Biomass has some significance in urease production, but nitrogen content has the least impact. Biomass has maximum production in a range from 5 to 7 g and 9–11 g. The moisture versus inoculum size interactions showed that moisture has some importance in urease production, while inoculum size has the least role in urease production. Moisture show increase in urease production when it crosses 120% of moisture and reaches its peak at 300% ratio. Moisture versus nitrogen level also indicates the importance of moisture in urease production. Moisture content also displayed the maximum production in the range from 130 to 320%, while nitrogen has a negligible role. These relations show moisture content in relation to inoculum size and nitrogen level play a significant role in urease production. Inoculum size versus nitrogen relationship displays that both of these have no significant role in urease production. Inoculum size has some effect on a narrow range. Results evidenced that RSM was highly significant because the p-value of the model was 0.005. When the model was analyzed by using Minitab 17 software, its calculated value of R-square was 91.21%. This shows that the experimental values were accurate and have the least error.

4.5.3. RSM for urease optimization by BMB-05 G

Different other parameters for enzyme production was measured by applying RSM. From enzyme assay, it was known that best condition for the enzyme production from fungal strain BMB-05 G was biomass size 6 g, moisture content 150% ratio to biomass, inoculum size 3 mL without urea (Table 4).

4.5.4. Statistical synergistic effect study among different variables by BMB-05 G

The 3D surface plots represent that in linear relation (Figs. 7 and 8), biomass, moisture content and inoculum size were not important factors as these show p-value 0.216, 0.630 and 0.872 respectively, whereas nitrogen content has some importance because its *p*-value is 0.85. The square relation shows the importance of biomass and moisture content as important production factors because of its *p*-value 0.014 and 0.036, respectively, while inoculum size and nitrogen content do not have many contributions in the production process, so these are less important factors. Two-way interaction of factors was found to be significant for biomass versus nitrogen content (Table 5).

Statistically, the produced amount of urease can be determined by applying the following formula;

UreaseU/mL = 0.0103 + 0.000107 Sr+ 0.00603 Biomas-0.000050 Moisture +0.00847 Inoculum Size.

- 0.01477 Nitrogen - 0.000012 Sr*Sr −0.000486 Biomass*Biomass.

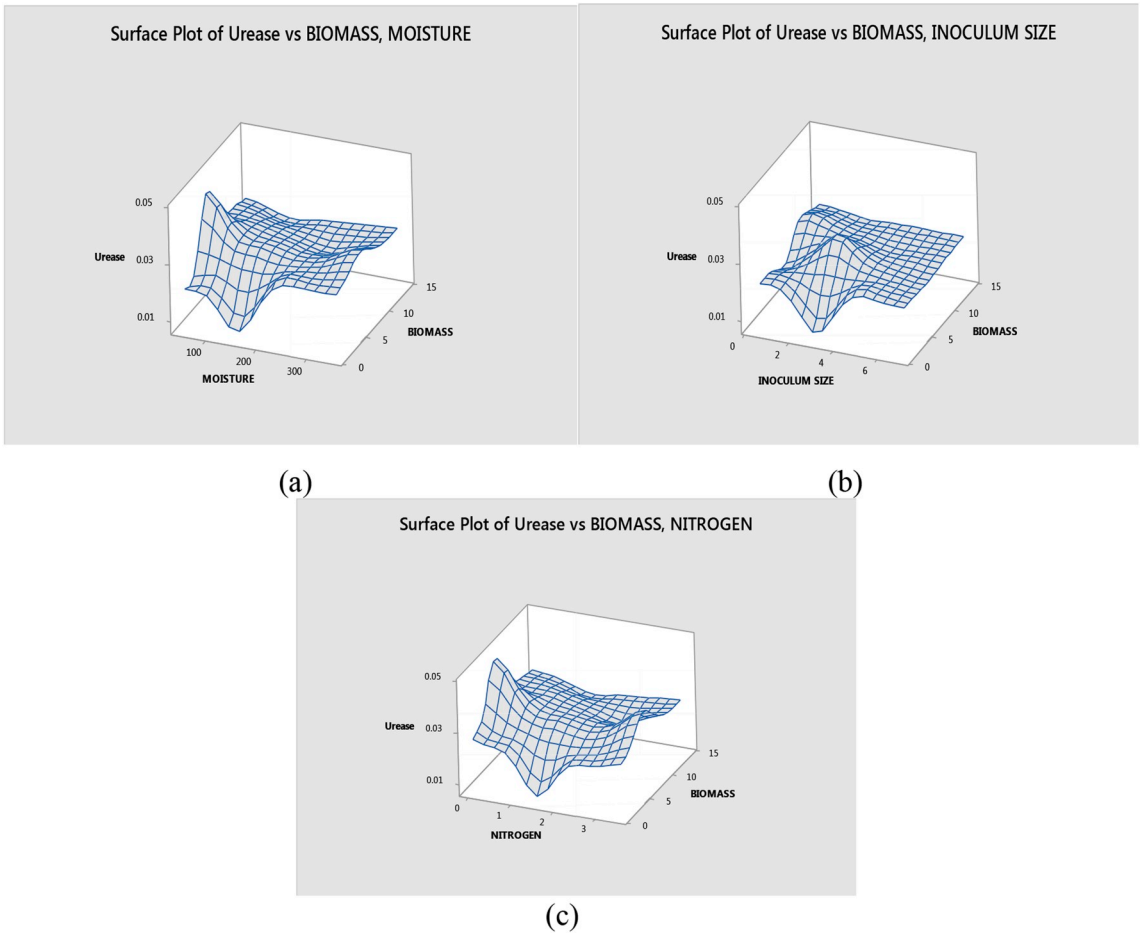

Fig. 7. Response surface plots showing the interactive effects (a) biomass v/s moisture (b) biomass v/s inoculum size and (c) biomass v/s nitrogen for urease production by BMB-05 G.

+ 0.000000 Moisture*Moisture - 0.001254 Inoculum Size*Inoculum Size.

+ 0.00418 Nitrogen*Nitrogen +0.000156 Sr*Biomass - 0.000005 Sr*Moisture.

+ 0.000197 Sr*Inoculum Size- 0.000169 Sr*Nitrogen - 0.000000 Biomass*Moisture.

- 0.000439 Biomass*Inoculum Size - 0.000223 Biomass*Nitrogen.

+ 0.000001 Moisture*Inoculum Size +0.000024 Moisture*Nitrogen.

- 0.000239 Inoculum Size*Nitrogen.

Biomass and moisture content together indicate that significance of biomass content, while non-significance of moisture content. Biomass content showed the effect to a certain range on the production of urease. As the biomass content increase production increase, maximum production takes place from 5 to 7 g. Both of these together had the least effect. Biomass versus inoculum size showed that both of these together have no contribution in enzyme production. Biomass and nitrogen interactions showed that biomass has an effect on urease production, as it increases the production also increase. Maximum production takes place from 4 to 8 g. Whereas the nitrogen level has no effect in urease production and no production takes place even from 1.5 to 2 g. In the relation of moisture versus inoculum size, moisture shows no effect, while inoculum size has importance to a specific range at 2.5–3.5 mL, maximum productions occurred. Moisture versus nitrogen level shows both of these together has no significance in urease production. Inoculum size versus nitrogen together shows a significant role in the production of urease. The enzyme production also increases as the inoculum size and nitrogen level increase. In both of these factors, inoculum size has more effect as compared to the nitrogen level. Maximum production takes place in a range from 2 to 4 mL. Results show RSM is highly significant. When the model was analyzed by using Minitab 17 software, its calculated R-square value of 91.09% shows that the experimental values were accurate and has the least error. The p-value of the model was significant indicating the accuracy of the experimental work of using BMB 2 G for the production of urease.

5. Conclusions

In this research, an industrially important enzyme urease was produced from wheat straw by using eight different fungal strains of *Aspergillus niger*. The enzyme production was optimized by one-factor at-a-time as well as RSM approaches. Optimal enzyme conditions for BMB-02 NSJ were biomass, inoculum size, nitrogen, and moisture of 10 g, 5 mL, 0.5 g and 250% ratio to biomass, respectively, whereas biomass, inoculum size, nitrogen and moisture content of 6 g, 3 mL, 150% ratio to biomass without the addition of any nitrogen were found for BMB-05 G. Most tremendous operating conditions and optimized levels for enhanced urease titer were achieved by unique statistical optimization. The findings revealed that the optimization of different factors have a significant effect on urease production, and RSM-based optimization is a promising and time-saving technique.

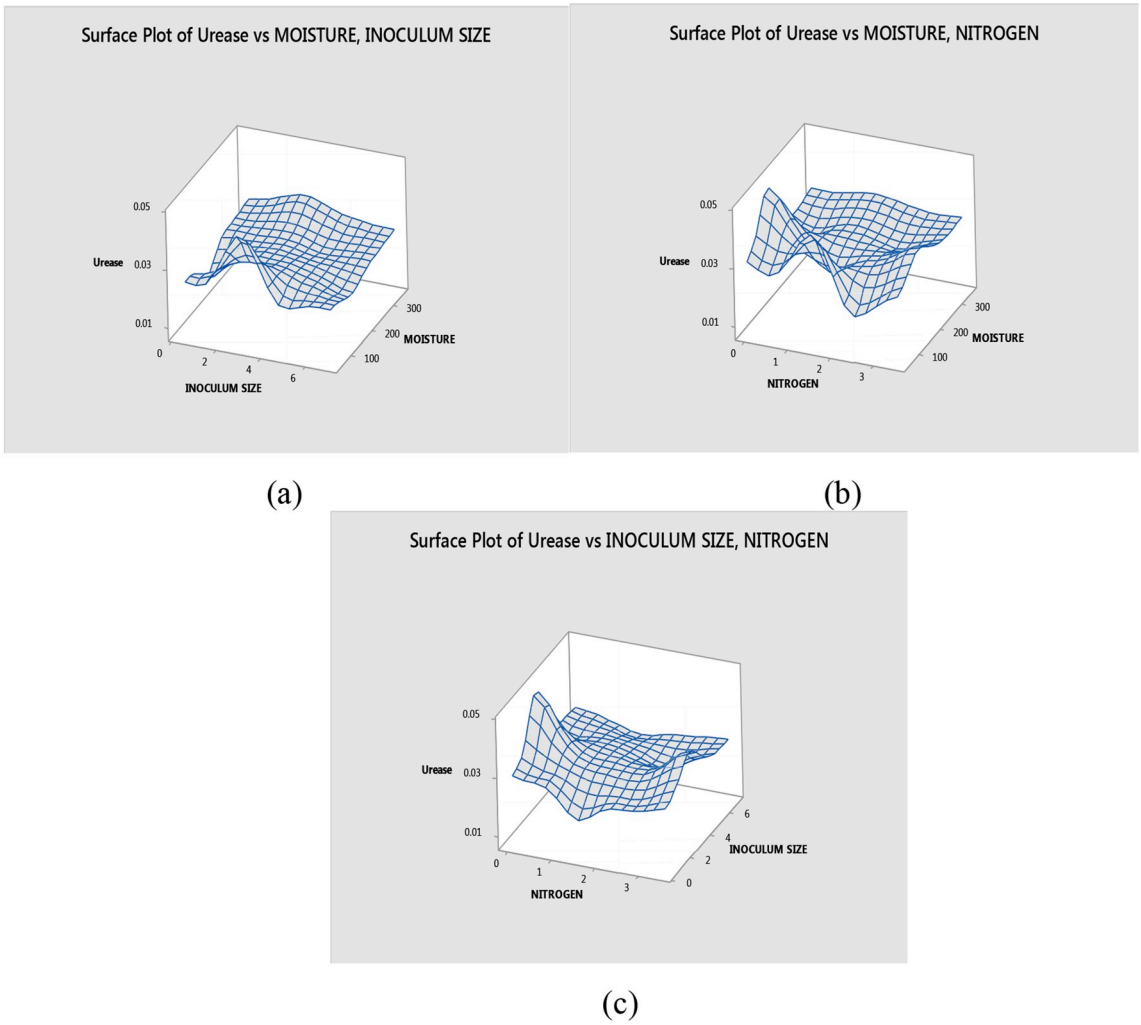

Fig. 8. Response surface plots showing the interactive effects (a) moisture v/s inoculum size (b) moisture v/s nitrogen, and (c) inoculum size v/s nitrogen for urease production by BMB-05 G.

Table 5
Analysis of variance for optimization of urease production by BMB-05 G.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	14	0.0	0.0	2.10	0.083
Linear	4	0.0	0.0	1.37	0.291
Biomass	1	0.0	0.0	1.67	0.216
Inoculum Size	1	0.0	0.0	0.03	0.872
Nitrogen Level	1	0.0	0.0	3.40	0.085
Moisture Content	1	0.0	0.0	0.24	0.630
Square	4	0.0	0.0	4.24	0.017
Biomass*Biomass	1	0.0	0.0	7.79	0.014
Inoculum Size*Inoculum Size	1	0.0	0.0	0.47	0.502
Nitrogen Level*Nitrogen Level	1	0.0	0.0	3.42	0.084
Moisture Content*Moisture Content	1	0.0	0.0	5.29	0.036
2-Way Interaction	6	0.0	0.0	1.52	0.238
Biomass*Inoculum Size	1	0.0	0.0	4.96	0.042
Biomass*Nitrogen Level	1	0.0	0.0	1.67	0.216
Biomass*Moisture Content	1	0.0	0.0	0.62	0.442
Inoculum Size*Nitrogen Level	1	0.0	0.0	0.00	1.000
Inoculum Size*Moisture Content	1	0.0	0.0	1.86	0.193
Nitrogen Level*Moisture Content	1	0.0	0.0	0.00	1.000
Error	15	0.0	0.0		
Total	29	0.0			

R^2 = 91.09%.

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