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Microbe Assisted Saccharification of Agriculture Waste Using *Bacillus* Sp. Enzyme Cocktail

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Abstract
A lignocellulosic degrading enzyme cocktail producing *Bacillus* sp. was isolated and assessed for its efficient saccharification efficiency. Maximal production of cellulases (100 U/mL), xylanases (108 U/mL) and laccases (56 U/mL), were produced using moistened 0.3% MD5 medium maintained at 37 °C, pH 9, 200 µM $CuSO4$ with 4% inoculum volume after 48h of incubation. An ecofriendly and economical mixed agriculture waste comprising of wheat straw, sugarcane bagasse and rice straw, served as good source for bioethanol production due to its carbohydrate contents. Statistical optimization using response surface methodology was done to carry out the enzyme cocktail treated saccharification of alkali-treated mixed agriculture biomass. Enzyme cocktail was used to treat 100 g of alkali-treated mixed agriculture waste maintained at pH 8.5 and 65ºC for 18h and gave a net yield of 10.2 and 29 mg/mL of reducing sugars and glucose, respectively. This study highlights the potential of *Bacillus* to produce a cocktail of lignocellulolytic enzymes that could be used to produce bioethanol, pulp and fruit industry.

Keywords: *Bacillus,* scarification, agriculture waste, lignocellulosic enzyme, Cocktail,

1. Introduction

Fossil fuels are the major source of global energy demand but their overexploitation contributed to the drastic increase in the level of greenhouse gases *viz.* CO_2 and other toxic gases such as methane, carbon monoxide, and chlorofluorocarbons (Li et al. 2017). At the United Nations Climate Change Conference (COP-26) held in Glasgow in 2021, all members are committed to achieve the aim of net- zero emissions by 2070 and minimize the carbon intensity below 45% (Chadha and Sivamani 2021). Different countries pledge to cut 30% of current methane emissions to net zero by 2070 (Wise 2021). Due to heavy dependency of transportation sector on liquid fuel, it is estimated that by 2070-2080, the world will fall short of petroleum based fuels (Sharma et al. 2020). Although the demand of oil is expected to increase by 57% from 2002 to 2030 so due to environmental concerns globally, there is a need for alternative, sustainable, cheap renewable energy sources.

Biofuel offers many advantages because of their sulfur-free, nontoxic and biodegradable potential over the petroleum-based fuels. Depending upon the raw material, biofuels are categorized into four types: 1stgeneration biofuel which uses cane sugar, wheat, corn, etc., 2ndgeneration biofuel uses lignocellulosic biomass majorly agricultural and woodland residues for production. The 3rd generation biofuels utilize algae and the 4th generation biofuel utilizes engineered cyanobacterial development, which is a new and rapidly evolving area (Ullah et al. 2018).

Among these, 2nd generation (2G) biofuel can act as the best alternative energy source to meet global energy demands. As 500 million metric tons (Mt)/year of agriculture, waste has been generated in India, after the use of residue for domestic or industrial purposes still, there is 92 Mt of residue burned every year. The traditional disposable approaches resulted in enhancing the global warming and reducing the fertility of the soil. The use of lignocellulosic biomass as the feedstock for energy production can resolve these environmental problems or reduce the dependency on other countries for traditional fossil fuels. Cellulosic (40-50%), hemicellulosic (25-30%), and lignin (15-20%) content of lignocellulosic biomass (Mansora et al. 2019) can satisfy the future energy demands of the world.

Conversion of lignocellulosic biomass to bioethanol required pretreatment, hydrolysis, fermentation, distillation, and dehydration processes (Maurya et al. 2015). Various methods have been used for lignocellulosic pretreatment *viz.* physical, chemical, physiochemical and biological. Biological pretreatment using single or multiple lingo-hemicellulolytic enzymes is preferred over others as it is environmentally friendly required less energy, has fewer chemicals requirement and less generated fermentable inhibitors, uninterrupted supply without seasonal variations and high product yield (Maitan-Alfenas et al. 2015). Cellulases, mannanases, xylanases and ligninases are some important enzymes that play a key role. There is a range of organism lingo- cellulolytic enzymes producing organisms (Fang et al. 2021; Sharma et al. 2020; Benatti and Polizeli 2023). Among these, bacterial one are preferred in this study because of their shorter generation time, wider range of pH and temperature adaptability and flexible oxygen tolerance potential. Acinetobacter, Bacillus,

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Cellulomonas, Clostridium, and Pseudomonas have been explored for biomass degradations. *Bacillus* sp. have been extensively explored and used in various industrial applications (Su et al. 2020). The most important characteristic feature of *Bacillus* sp. that makes it an industrial candidate is its easy handling and spore formation under adverse condition of environment, which can be reversed by providing the favorable environment (Zhang et al. 2020). Thus in this study main focus to isolate a cocktail producing *Bacillus* sp. and explored its potential for second generation biofuel generation. The optimal conditions for maximal cocktail production of enzymes and maximal release of sugars during biomass scarification were examined statistically using response surface methodology (RSM).

2. Material and Methods

2.1 Isolation and Screening of lingo cellulolytic enzymes producing bacteria

A soil sample was collected from the Industrial effluent Chandigarh and Nalagarh India, region of India. For isolation, carboxymethyl cellulose (CMC) agar medium (0.5%) was prepared. A 10-fold serial dilution of 1 g of soil sample was plated on CMC agar plates followed by incubated at 37ºC for 72 h of incubation. The growing colonies were sub-cultured on fresh agar plates to purify. The purified isolate was stored at -20 °C for further screening.
Further screening of lignocellulolytic enzymes producing microbe was done using plate assays for cellulase (Kasana et al., 2008), laccase (Bains et al. 2003), mannanase (Harnentis et al. 2013), xylanase (Teather and Wood 1982) and other peroxidases (Tien and Kirk 1984).

2.2 Characterization

The morphological, and biochemical characterization of lignocellulosic degrading enzymes were carried out according to Bergey's Manual of Systematic Bacteriology (Volume 5). Light microscopy was used for cell shape and arrangement determination. Gram staining was done using Himedia Gram-staining kit. Endospore staining, indole reaction, methyl red, voges- proskauer tests, and oxidase test, catalase test, nitrate reduction and citrate utilization tests were performed (Cappuccino 2018; Kannan 2002).

2.3 Enzyme production

Bacterium inoculum was prepared by growing a colony of bacterium in 100 ml nutrient broth (NB) and incubated overnight at 37°C at 150 rpm. 10% (v/w) of inoculum was added to 5 g of wheat bran (containing 5 ml of 1X M162 medium (pH 7.0- 8.0) and incubated at 37°C for 48-96 h. 0.02% Tween 80 was added and agitated at 150 rpm for 30 minute to extract the enzymes. Subsequently, centrifuged at 10,000 rpm for 30 minutes. The obtained supernatant was used as crude enzyme cocktail.

2.4 Enzyme assay

2.4.1 Cellulase assay

Cellulase activity was determined by the amount of reducing sugars released from a reaction mixture of 900 µl of carboxymethyl cellulose (CMC, 0.5%) prepared in 100 mM (pH 7.0) phosphate buffer and crude enzyme of 100 µl, incubated at 55°C for 20 minutes using DNSA protocol (Miller 1959). This reaction mixture was boiled for 15- 20 minutes. Optical density was observed at 470 nm after cooling the mixture. One unit of cellulase activity was expressed as U/ml and defined as the units of enzyme that catalyzes the 1 µmol of glucose released per minute.

2.4.2 Xylanase assay

Xylanase activity was measured by the release of reducing sugars from a reaction mixture of 900 µl of birchwood xylan (0.5%) made in 100 mM of pH 7.0 phosphate buffer with crude enzyme of 100 µl, incubated at 55°C for 15 minutes using DNSA method (Miller, 1959). This reaction mixture was boiled for 15- 20 minutes. Optical density was observed at 540 nm (Bailey et al. 1992).

2.4.3 Laccase assay

A reaction mixture containing 500 µl of 3.0 mM ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) prepared in phosphate buffer of pH 7.0 (100 mM) and crude enzyme preparation of 500 µl allowed to incubate at 55°C for 10 min. The oxidation of ABTS depicted by the change in absorbance at 420 nm (More et al. 2011).

2.5 Optimization of enzyme cocktail production (cellulase, xylanase and laccase) by *Bacillus* sp. using One Variable at a Time (OVAT) analysis by solid state fermentation.

Different types of media mentioned in Table 1 were optimized by OVAT analysis. Further, a range of pH (6.5-10), inoculum size (1-10%), incubation period (24-72 h), and $CuSO_4$ concentration (100-500 µM) also varied to produce the enzymes.

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Table 1 Different media for enzyme cocktail production (g/L)

MD1: modified M162 medium (Degryse et al. 1978)	1) M162 medium (MM): ($CuSO_4 \cdot 2H_2O$, 0.4; $MgCl_2 \cdot 6H_2O$, 2.0; nitrilotriacetic acid,1.0; 0.01M ferric citrate solution, 5 ml) + (micronutrient solution (MS), 10 ml) + Micronutrient solution (MS): H_2SO_4,5.0 ml; $MnSO_4H_2O$, 2.28; $ZnSO_47 \cdot H_2O$,5.0; H_3BO_3,5.0; $CuSO_4 \cdot 5H_2O$,0.250; $Na_2MO_4 \cdot 2H_2O$, 0.250; $COCl_2 \cdot 6H_2O$, 0.450 2) Yeast extract (YE), 2; tryptone (TY), 2
MD2	MM+ soybean meal (SM), 5; YE, 2; TY, 3
MD3	MM+ wheat bran (WB), 5; YE, 2; TY, 3
MD4	MM+ SM, 3; WB, 3; YE, 2; TY, 2
MD5	MM + SM, 3; WB, 3; xylan 1.5
MD6	MM+ SM, 3; WB, 3; YE, 2; TY, 2; xylan 1.5

2.6 Alkali pretreatment of agriculture waste
Agricultural wastes mixture containing wheat straw (40-45%), sugarcane bagasse (40-50%), and rice straw (8-10%) were collected from locals of Hamirpur, Himachal Pradesh. The waste mixture collected in plastic bags, washed, dried and shredded to small pieces and stored for further experiments. 100g of agriculture waste mixture was dispersed in 1000 ml of 5% NaOH in a ratio of 1:10. The mixture was then boiled for 3.5 h on a hot plate, cooled down at room temperature and washed under tap water until no color was rinsed by filtrate.

2.7 Statistical optimization of saccharification of alkali treated agro biomass by *Bacillus* sp. cocktail using RSM
Different parameters with significant factors in a range such as cocktail dosage (2-10 mL), pH (7.0- 10), temperature (40-80ºC), treatment time (3-10 h), were analyzed statistically using RSM using100 g of alkali treated agriculture mixed waste to achieve the maximal digestion of agro biomass. Treated filtrate was checked for maximal release of glucose and reducing sugars. The interactions among the chosen parameters were identified for each enzyme optimization using Central Composite Design (CCD), which had three positive significant factors at five levels for 30 runs. The coded levels investigated were low, high, -alpha and + alpha depicted table 2. Polynomials, contour plots, and 3D graphs were produced using the statistical program Stat-Ease 360 (Llimos et al. 2022). Every experiment was carried out three times.

Table 2: Different ranges and variables selected for optimal saccharification of mixed agro waste

Variables	Range and levels			
	Low	High	-alpha	+alpha
Enzyme cocktail dosage (mL)	5	8	3.5	9.5
pH	7	10	5.5	11.5
Temperature (ºC)	55	75	45	85
Treatment time (H)	5	10	2.5	12.5

2.8 Analysis of Variance (ANOVA)
ANOVA analysis was done to determine the second-order polynomial equation, standard deviation, PRESS, CV%, mean adj R-squared, and pre R-squared, R-squared, adeq Precision, AICC and BIC values using the statistical program Stat-Ease 360 optimization toolbox.

2.9 Glucose and reducing sugars estimation
The estimation of reducing sugars and glucose in filtrate of untreated agro waste and treated agro waste was determined according to protocol of Miller 1959 and Morin and Prox 1974.

3. Result and Discussion
3.1 Isolation, selection and identification of the isolate
Soil samples were collected from Industrial waste area of Chandigarh and Nalagarh region of North India. Serial dilution of soil samples was carried out on CMC agar plates and incubated at 37ºC for 72h. A total of 45 isolates of different morphology were isolated and further screened for production of different lingo cellulolytic enzymes using the plate assays. Among these, only 4 isolates NI-4, -15, -18 and -23 were observed to produce a cocktail of cellulase, laccases and xylanase enzymes observed by appearance of clear zone formation around the colony. All these four isolates NI-4, -15, -18 and -23 were grown on 5% wheat bran medium , pH 7 for 72h at 37°C using solid state fermentation and respective

enzyme activities were determined. NS-18 exhibited extracellular release of enzymes higher cellulase (4.2 U/mL), laccase (4.8 U/mL) and xylanase 2.3 (U/mL) activities and was selected for further studies.

3.2 Morphological and biochemical characteristics

Isolate NI-18 was grown at NB agar at 37ºC for 24h and observed colonies were whitish, irregular and smooth in appearance. Biochemical analysis depicted its Gram positive, rods structure, spore forming, catalase positive and oxidase negative (Table 3) indicated that it belonging to the genus "Bacillus" and have capability of producing cocktail of enzymes. Bacillus have been widely preferred strain due to its highly adaptable metabolism, easier cultivations, utilization of cheaper substrates and higher yields (Park et al. 2021). Bacillus is well recognized for releasing the enzymes xylanase (Bakry et al. 2024; Phukon et al. 2024) and cellulases (Ugras et al. 2024; Su et al. 2020) individually. A limited studies are available in literature, in which Bacillus sp. has been reported to produce the mixture of lignocellulosic enzymes from a single source (Thite et al. 2020; Agarwal et al. 2016 and Amadi et al. 2022).

Table 3: Biochemical characterization of isolate NI- 18

Sr. No.	Test	Result
Culture Characteristics		
1	Colony color	White
2	Colony appearance	Irregular
3	Pigmentation	No
4	Motility	Motile
5	Optimum pH	7.0
6	Optimum temp	37- 40ºC
Morphological Characteristics		
7	Gram staining	+
8	Appearance	Rods
9	Arrangement	Singly placed (sometimes in chain and clumps)
	Surface	
Biochemical Characteristics		
10	Catalase	+
11	Oxidase	-
12	VP (Voges–Proskauer)	+
13	Methyl red	-
14	Indole	-
15	Sucrose hydrolysis	+
16	Xylose hydrolysis	+
17	Lactose hydrolysis	-
18	NaCl (10%)	-
19	Sucrose hydrolysis	+
20	Citrate utilization	+
21	Nitrate reduction	+
22	Endospore	+
23	Urease	-
24	Ornithine	+
25	Arginine	-
26	Mannanase	-
27	Lipase	-

3.3 Optimization of enzyme production (cellulase, xylanase and laccase) by *Bacillus* sp. using OVAT analysis by solid state fermentation

OVAT analysis performed to optimize various media and production conditions. Primarily for enzyme cocktail production from NI-18, wheat bran (5%), pH 7, and temperature 37ºC, incubation time 72, inoculum size 1%, with moisture content (1:1), CuSO4 100 µM, parameters were chosen which resulting in enzymes production as mentioned in section 3.1. Further, different parameters were optimized using the OVAT. Type and different ranges of parameters chosen mentioned in section 2.7. Maximal production of these three enzymes was achieved using MD5 medium maintained at pH 8.0, inoculum volume 5% after 48 h of incubation with addition of 100 µM CuSO4 at 37ºC. 23.8, 24.34 and 22.5 fold increase in cellulase, laccase and xylanase activities which have enzyme activities 100 ± 5.3, 56 ± 6.5, and 108 ± 5.9 U/mL respectively, were achieved. No doubt, *Bacillus* has been explores for cocktail production of pectinase, cellulase and xylanase (Yadav et al. 2020), cellulase and xylanase (Sreena et al. 2016), protease and xylanase (Limkar et al. 2019).

Nevertheless, there have been few findings of *B. cereus's* ability to co-produce the ligno- cellulolytic enzymes. Nkohla et al (2017) have reported co- production of xylanase (390.5 U/mL) and cellulase (102.7 U/mL) at pH 5-6, 25-30°C after 72 and 84 h, respectively of incubation. 60.57 and 28.2 U/mL of xylanase and cellulase co- production were reported from *B. cereus* at 15°C (Patent CN113151123A). This study reported the extracellular co- production of cellulase, xylanase and laccase.

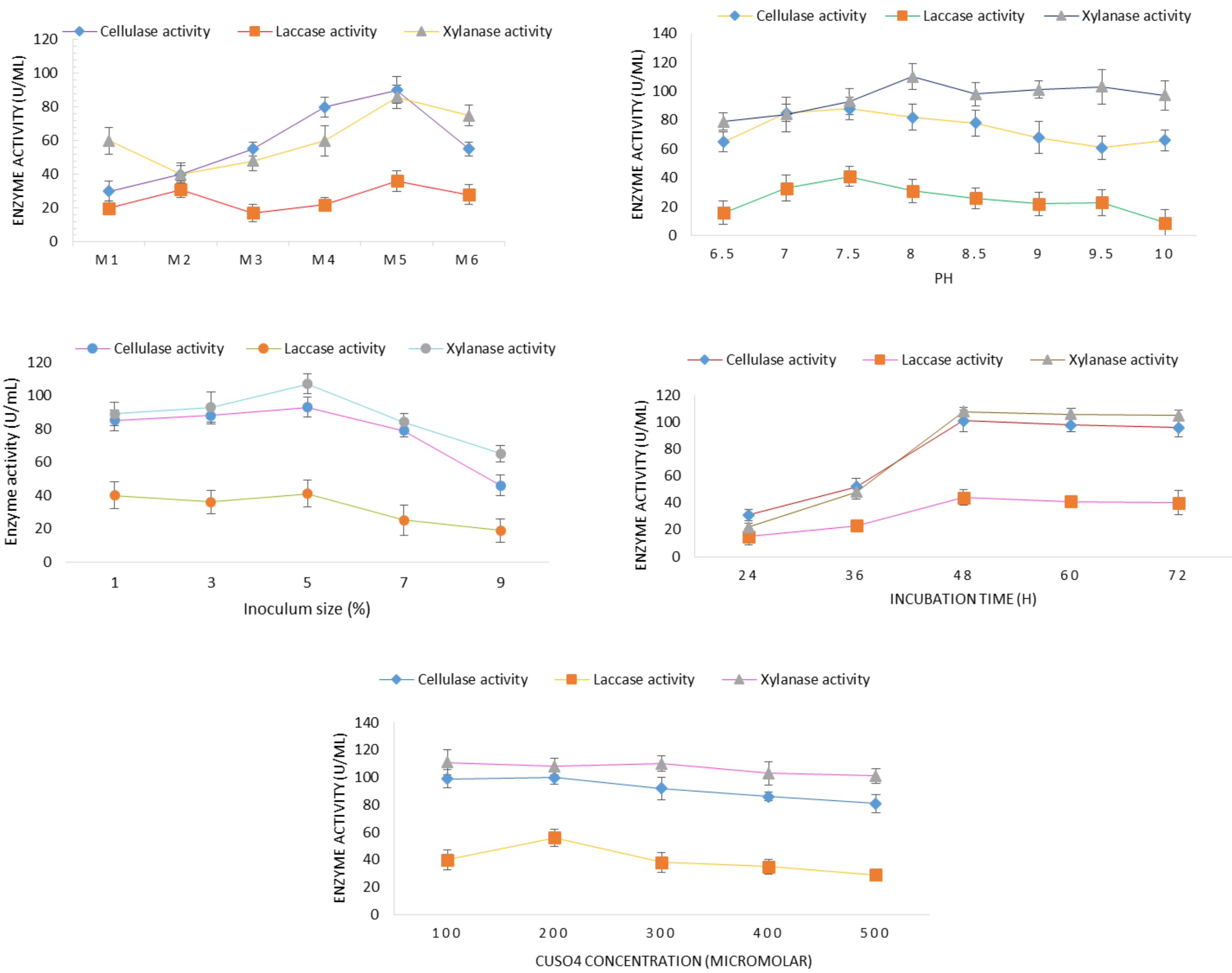

Fig 1. OVAT analysis of maximal enzyme cocktail production from *Bacillus* sp. using solid state fermentation

3.4 Statistical optimization of saccharification of alkali treated agriculture waste by *Bacillus* sp. cocktail using using RSM

The predominant component of lignocellulosic biomass is cellulose (30- 55%), while hemicellulose, the second most prevalent component, accounts for 20%–35% of the biomass and thirdly, 10 - 25 % lignin (Srivastava et al. 2019). In case of rice straw, an additional component accounts for 17% of the ash, which impedes the delignification process (Rosadoo et al. 2021). Therefore, a smaller percentage of rice straw was used in this study compared to sugarcane bagasse and wheat straw. These constituents makes a complex organized structure of biomass. Lignin strengthens the structure by creating crosslinks between the hemicellulosic and cellulosic components. The lignin crosslinks are broken by ligninolytic enzymes, which facilitate the easy access of cellulosic and hemi-cellulosic enzymes. Different hemicellulases and cellulases break down their substrates into simpler sugars (Buckeridge et al. 2017). Different factors such as enzyme cocktail dosage, pH, treatment time and temperature were taken for maximal release of reducing sugars and glucose from agriculture waste. A total of 30 experimental runs were carried out to find out the optimized conditions and validated further comparing the observed values to predicted values. The contour plots, and 3D graphs were showed in Fig 2 and 3 designed using program Stat-Ease 360.

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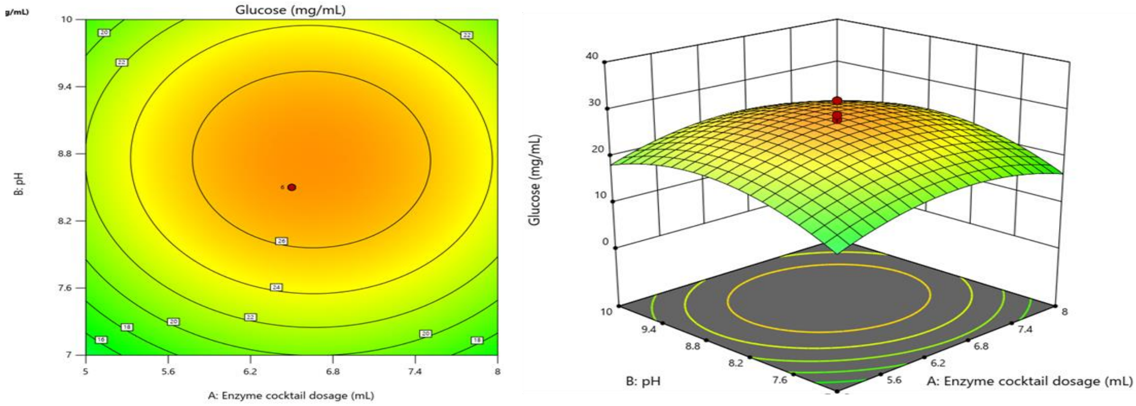

Fig 2a Contour plot (a) and 3D graph (b) showing interaction effect of enzyme cocktail dosage and pH for release of glucose

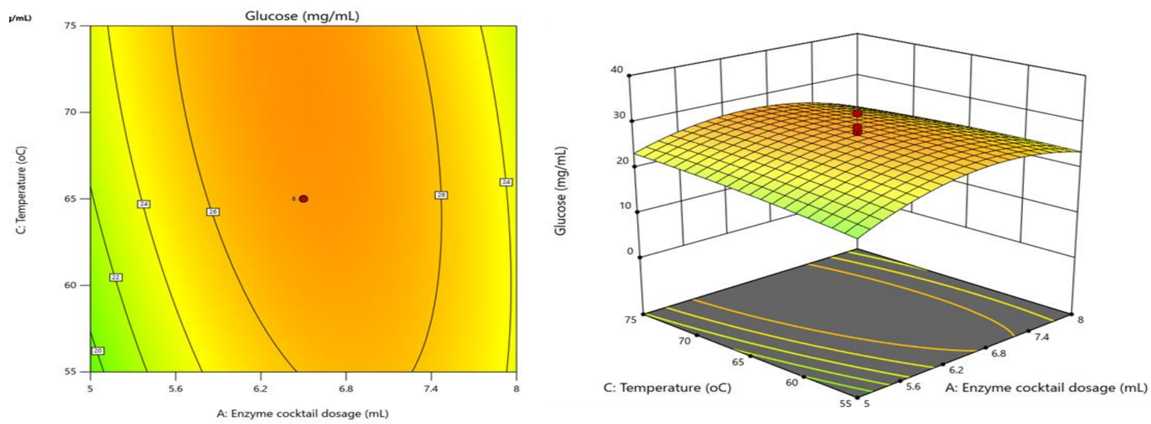

Fig 2b Contour plot (a) and 3D graph (b) showing interaction effect of enzyme cocktail dosage and temperature for release of glucose

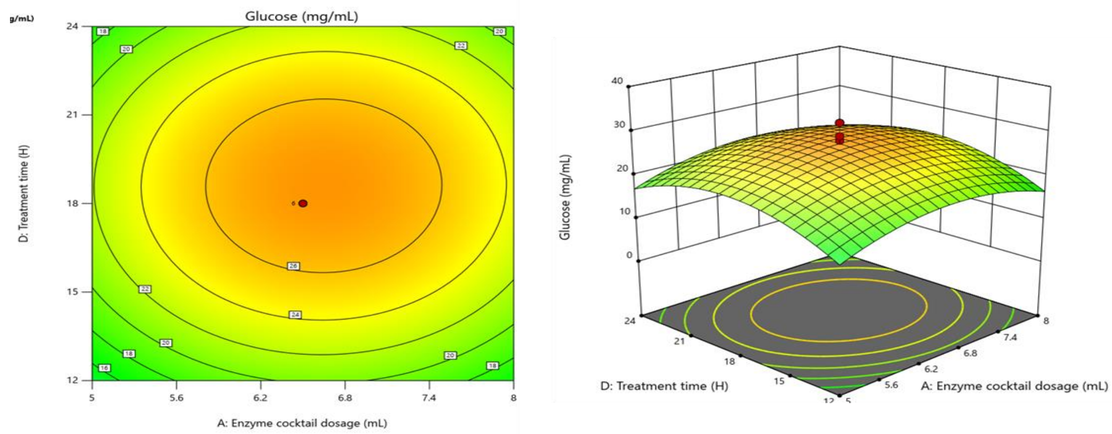

Fig 2c Contour plot (a) and 3D graph (b) showing interaction effect of enzyme cocktail dosage and treatment time for release of glucose

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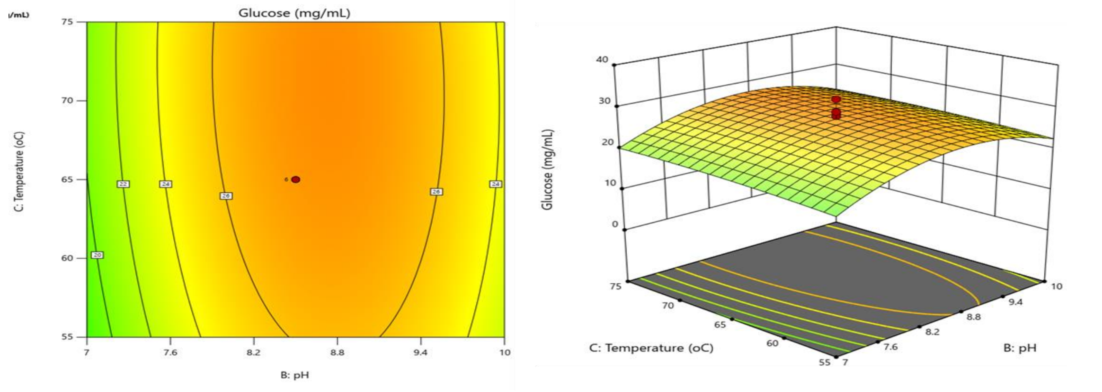

Fig 2d Contour plot (a) and 3D graph (b) showing interaction effect of temperature and pH for release of glucose

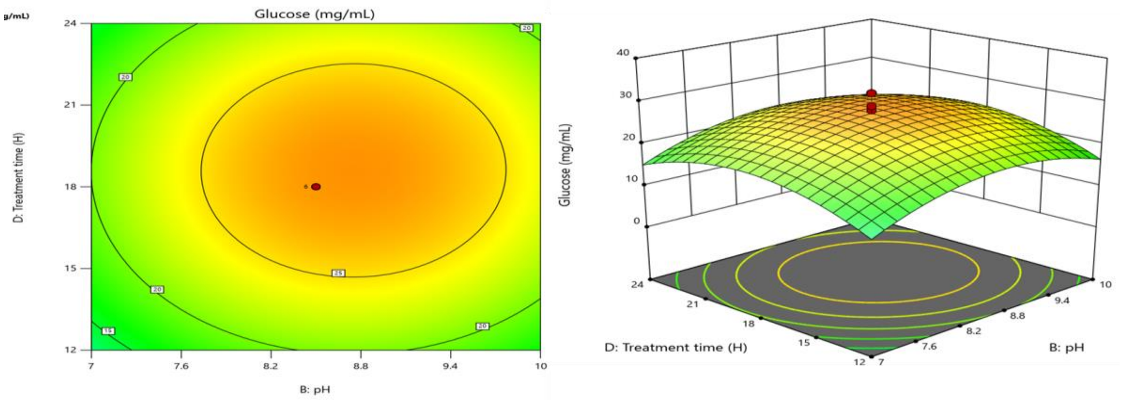

Fig 2e Contour plot (a) and 3D graph (b) showing interaction effect of treatment time and pH for release of glucose

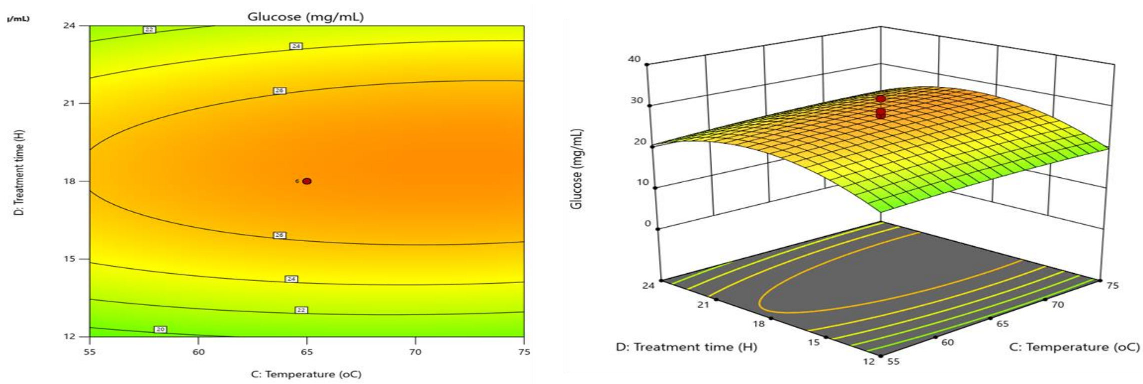

Fig 2f Contour plot (a) and 3D graph (b) showing interaction effect of temperature and treatment time for release of glucose

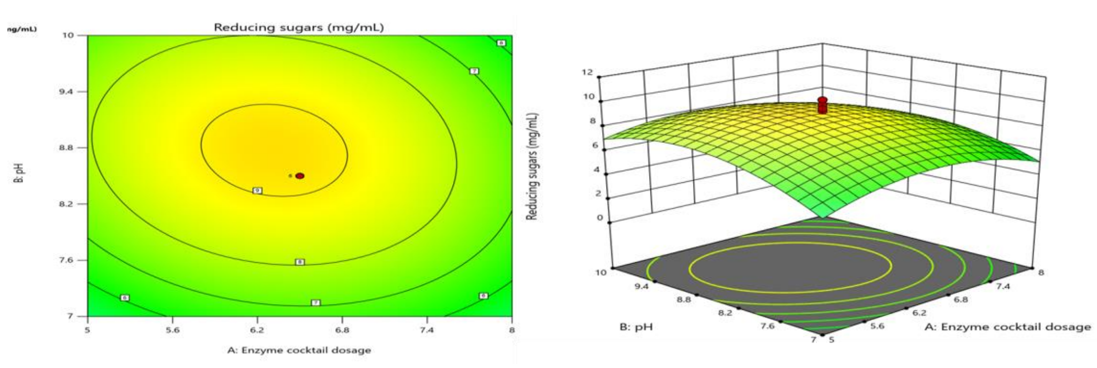

Fig 3a Contour plot (a) and 3D graph (b) showing interaction effect of enzyme cocktail dosage and pH for release of reducing sugars

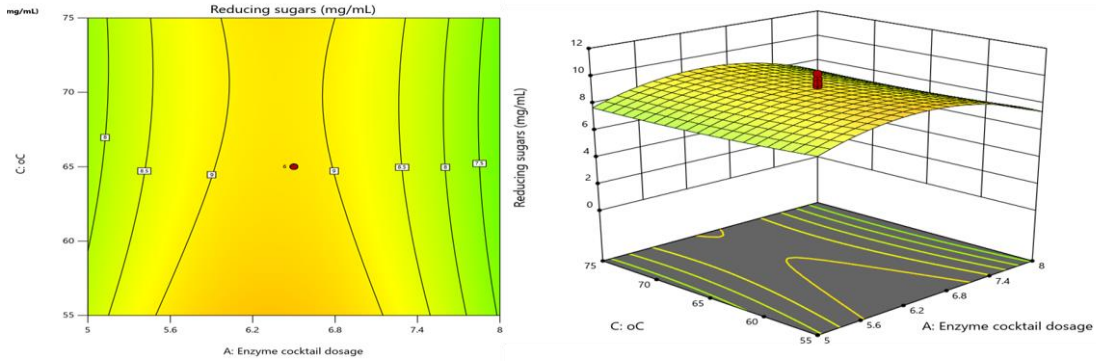

Fig 3b Contour plot (a) and 3D graph (b) showing interaction effect of enzyme cocktail dosage and pH for release of reducing sugars

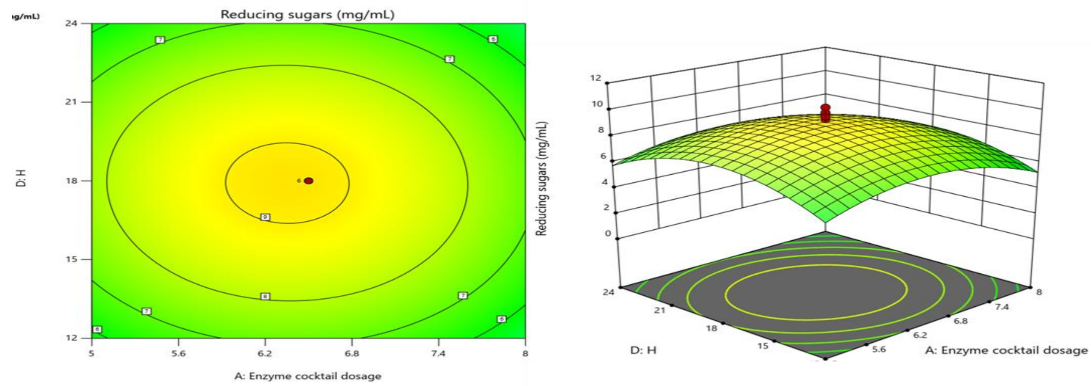

Fig 3c Contour plot (a) and 3D graph (b) showing interaction effect of enzyme cocktail dosage and treatment time for release of reducing sugars

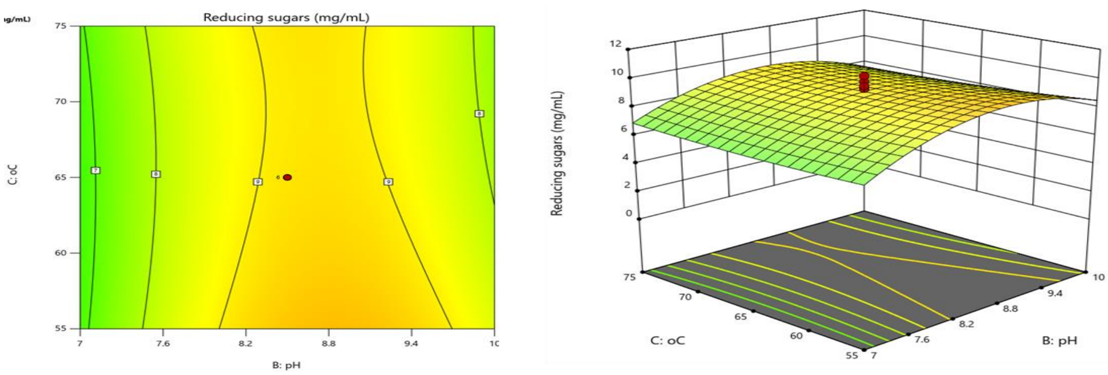

Fig 3d Contour plot (a) and 3D graph (b) showing interaction effect of temperature and pH for release of reducing sugars

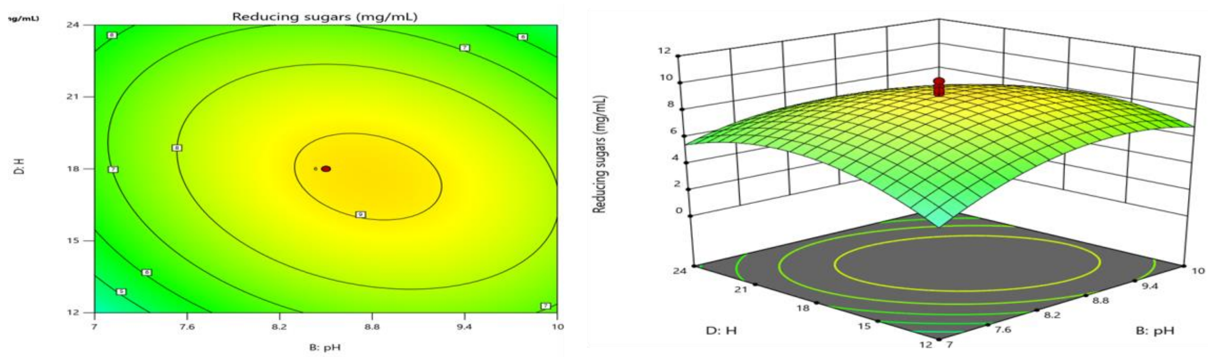

Fig 3e Contour plot (a) and 3D graph (b) showing interaction effect of treatment time and pH for release of reducing sugars

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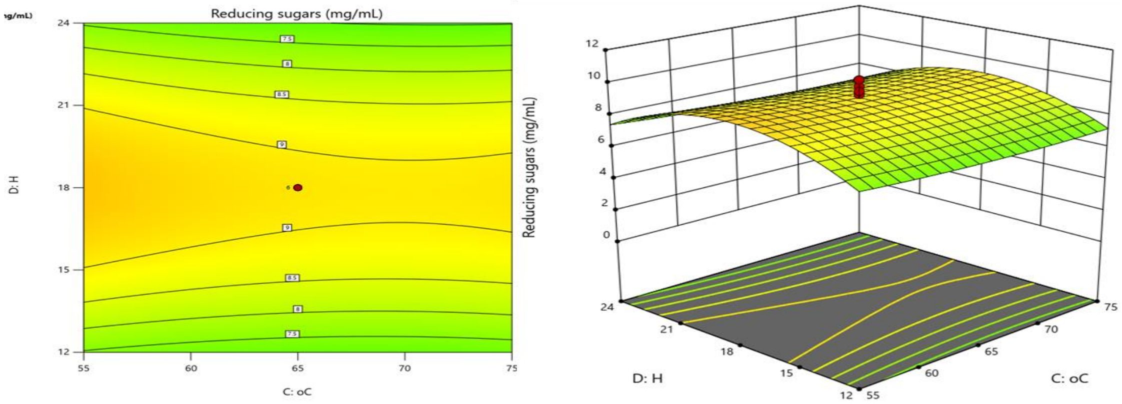

Fig 3f Contour plot (a) and 3D graph (b) showing interaction effect of treatment time and temperature for release of reducing sugars

ANOVA analysis of reducing sugars and glucose released showed in Table 4 and 5. The Model F-value of 7.55 and 9.19 implies that the models are significant for reducing sugars and glucose, respectively. There is only a 0.02 and 0.01% %, respectively chance that an F-value this large could occur due to noise. However, the Lack of Fit F-value of 3.52 and 2.31 Lack of Fit is not significant relative to the pure error, there is a 8.85 and 18.37% chance that a Lack of Fit F-value this large could occur due to noise. 6.5mL enzyme cocktail was used to treat 100 g of alkali-treated agriculture waste maintained at pH 8.5 and 65ºC for 18h. 10.2 and 29 mg/mL of reducing sugars and glucose, respectively were achieved. *B. subtilis* produced a cocktail of cellulase, xylanase, amylase, pectinase and protease was employed for rice straw saccharification and reported to yield of 51.06 g/L of sugars at 5.5 pH, 50°C and 200 rpm after 24 h (Tunio et al. 2024). Vu et al (2022), examined the lignocellulosic breakdown of wheat straw using a consortium of *Bacillus* sp. *viz. B. cereus* and *B. coagulans*, *B. subtilis* and *B. licheniformis*. Pretreatment using *B. cereus* and *B. coagulans* produced 28 mg/gds of reducing sugars. Whereas, *B. subtilis* and *B. licheniformis* reported 18 mg/gds yield of reducing sugars after 24 h, of treatment. No doubt, in our situation, the release of reducing sugars and glucose content is somewhat comparable to these, though the operating conditions and 18 h of optimized sugar release time meet the industry's cost-friendly requirements. The efficiency of the lignification process depends on a number of factors, including the metabolism of the microbe, the type of substrate used and enzyme utilized, the concentration of sugar, and the production of inhibitors during processing.

Table 4: ANOVA analysis maximal release of reducing sugars

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	260.35	14	18.60	7.55	0.0002	significant
A-Enzyme cocktail dosage	2.47	1	2.47	1.00	0.3323	
B-pH	9.00	1	9.00	3.66	0.0751	
C-ºC	0.7704	1	0.7704	0.3130	0.5841	
D-H	0.0938	1	0.0938	0.0381	0.8479	
AB	2.64	1	2.64	1.07	0.3168	
AC	0.0756	1	0.0756	0.0307	0.8632	
AD	0.0506	1	0.0506	0.0206	0.8879	
BC	0.8556	1	0.8556	0.3476	0.5643	
BD	11.73	1	11.73	4.77	0.0453	
CD	0.0506	1	0.0506	0.0206	0.8879	
A^2	73.27	1	73.27	29.76	< 0.0001	
B^2	89.80	1	89.80	36.48	< 0.0001	
C^2	0.8703	1	0.8703	0.3535	0.5610	
D^2	112.13	1	112.13	45.55	< 0.0001	
Residual	36.93	15	2.46			
Lack of Fit	32.34	10	3.23	3.52	0.0885	not significant
Pure Error	4.59	5	0.9177			
Cor Total	297.27	29				
PRESS :192.87; -2 Log Likelihood: 91.37; BIC: 142.39; AICc: 155.65; Std. Dev.: 1.57; Mean: 4.89; C.V. %: 32.11; R^2: 0.8758; Adjusted R^2: 0.7599; Predicted R^2: -0.3512; Adeq Precision: 8.5929						

Table 5: ANOVA analysis maximal release of glucose

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2090.36	14	149.31	9.19	< 0.0001	significant
A-Enzyme cocktail dosage	19.26	1	19.26	1.19	0.2935	
B-pH	81.77	1	81.77	5.03	0.0404	
C-Temperature	12.18	1	12.18	0.7497	0.4002	
D-Treatment time	33.84	1	33.84	2.08	0.1696	
AB	0.2256	1	0.2256	0.0139	0.9078	
AC	26.78	1	26.78	1.65	0.2187	
AD	0.1406	1	0.1406	0.0087	0.9271	
BC	0.6006	1	0.6006	0.0370	0.8501	
BD	0.0756	1	0.0756	0.0047	0.9465	
CD	1.89	1	1.89	0.1163	0.7378	
A^2	573.84	1	573.84	35.31	< 0.0001	
B^2	836.96	1	836.96	51.50	< 0.0001	
C^2	8.27	1	8.27	0.5086	0.4867	
D^2	978.88	1	978.88	60.23	< 0.0001	
Residual	243.77	15	16.25			
Lack of Fit	200.44	10	20.04	2.31	0.1837	not significant
Pure Error	43.33	5	8.67			
Cor Total	2334.13	29				
PRESS :1216.93; -2 Log Likelihood: 147.99; BIC: 199.01; AICc: 212.27; Std. Dev.: 4.03; Mean: 14.04; C.V. %: 28.72; R^2:0.8956; Adjusted R^2: 0.7981; Predicted R^2: 0.4786; Adeq Precision: 9.2160						

Table 6 Comparison of predicted and observed values for sugar release after saccharification

Solution 1 of 100 Response	Predicted Mean	Observed	Std Dev	N
Reducing sugars	8.32667	11.2	1.56899	3
Glucose	27.3333	26.24	4.03131	3

4. Conclusion

Concerning the depletion of renewable sources of fuels and generated pollution from them have demands the alternative ecofriendly alternatives. *Bacillus* strain was isolated and evaluated for the extracellular release of lignocellulosic degrading enzymes as part of an attempt in this area for second generation bioethanol production. A mixture of cellulases, xylanase and laccase was produced and employed for sachharifiication of agriculture waste residues. This allows for the economical and environmentally sustainable use of ample agricultural waste, which has caused problems with burning and dumping that lead to pollution. Further efforts should be required to boost the utilization of these enzymes for its industrial scale utilization.

Statements and Declarations

Conflict of interest

The authors declare no conflict of interests.

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Author contribution
All authors contributed to the study conception and design. Material preparation, data collection and analysis, draft writing, editing were performed by Sunny Banyal. Data analysis and editing was done by Dr. Shikha Kumari and Dr. Vijay Kumar critically revised the work. The final manuscript was read and approved by all the authors.

Conflict of interest
The authors declare no conflict of interests.

Data Availability
The manuscript contains the majority of the data generated in the current work. Any additional information that may be required can be requested from the corresponding author on a reasonable basis.