**Production and optimization of fungal silicase using rice straw under solid state fermentation**

**ABSTRACT**

**Aim**: The present study aimed to convert agricultural residue, specifically rice straw, into a valuable enzymatic product through solid-state fermentation (SSF), using three fungal strains—Penicillium limosum (WF1), Bipolaris sorokiniana (BF1), and Pleurotus ostreatus (P1). The objective was to optimize the production of the silica-degrading enzyme **silicase**, which catalyzes the transformation of silica into plant-available silicic acid.

**Study Area:** Rice straw used in this study was sourced from agricultural fields in Haryana, India. The experimental work was carried out at the Department of Biotechnology, Kurukshetra University, Kurukshetra.

**Study Design and Methodology:** A one-variable-at-a-time (OVAT) strategy was applied to optimize process variables such as substrate form (crude vs. powdered), incubation period, temperature, pH, rice straw-to-water ratio, and the use of nutrient additives. Silicase production was compared between submerged fermentation (SmF) and solid-state fermentation (SSF). Under SSF, the optimized medium consisted of crude rice straw with a 1:5 straw-to-water ratio, incubated at 30 °C and pH 7 for 8 days. Silicase activity was measured spectrophotometrically using a silicate assay kit.

**Results**: Solid-state fermentation significantly outperformed submerged fermentation for enzyme production. Under optimal SSF conditions, silicase activity reached 0.98 U/mL/min for WF1, 0.96 U/mL/min for BF1, 1.00 U/mL/min for P1, and 0.99 U/mL/min for the fungal consortium (WF1+BF1+P1). Supplementation with jaggery and soy protein further enhanced enzyme yield. Crude rice straw supported higher enzyme activity than powdered forms, and incubation beyond 8 days or deviations in pH and temperature resulted in decreased activity.

**Conclusion**: The study demonstrates that rice straw can be efficiently bioconverted into a high-value enzymatic product using solid-state fermentation with silicate-solubilizing fungi. The optimized SSF system, supplemented with cost-effective nutrient additives, provides an eco-friendly and scalable strategy for silicase production. These findings have potential applications in sustainable agriculture, silica bioremediation, and industrial processing of silica-rich biomass.

*Keywords:**Fungal bioconversion; Rice straw; Silicase; Silicon solubilization; Solid-state fermentation; Sustainable agriculture*

1. **INTRODUCTION**

India produces approximately 62 million tons of bio-waste annually, a quantity that has not only increased significantly but has also undergone notable changes in its composition over time. Nearly half of the world's population relies on rice for nutrition and calories, leading to the production of approximately 595.92 million tonnes of rice straw as residual biomass left in fields after harvesting (Alqattaf et al., 2020). This substantial amount poses challenges for waste management strategies. Exploring efficient and sustainable methods to utilize or manage agricultural residues such as rice straw remains critical in addressing both environmental concerns and agricultural productivity (Kaur et al., 2013; Pandey et al., 2000). Silicon, a predominant constituent of soil and rocks, becomes accessible to plants following microbial conversion of insoluble silicates into soluble forms (Handerson and Duff, 1963; Nazir et al., 2010). Silicon is the second most abundant element on Earth after oxygen and functions as an essential growth regulator in certain plants, animals, and microbial systems (Ameen et al., 2019; Schroder et al., 2003). The simplest soluble form of silica is the monomer orthosilicic acid, which is silicon tetrahedrally coordinated to four hydroxyl groups and has the formula Si(OH)₄. Once it is absorbed by plant roots, it moves safely to actively growing plants, where it combines with organic compounds, thereby improving cell wall strength and contributing to stronger plants (Zargar et al., 2019). Silica can be formed from silicon alkoxides, where the central silicon atom is connected to organic moieties like methyl, ethyl, phenyl, or other functional groups, sometimes with silicon directly bonded to carbon atoms. When placed in an aqueous environment, the alkoxy groups generally undergo hydrolysis, especially under acidic or basic conditions. However, organic groups directly bonded to silicon atoms are not hydrolyzed. Silica, present in rice straw, serves as a physical defense mechanism; studies have shown that insects and fungi tend to target plant parts with lower silica content (Zargar et al., 2019).

Microbes play a pivotal role in the ecosystem by actively solubilizing various minerals found in soil, such as silicates and phosphates (Lee et al., 2019). This interaction is crucial for enhancing nutrient availability to plants, particularly in challenging environmental conditions (Jin and Kirk, 2018; Joshi et al., 2006). Silicate-solubilizing microbes play a crucial role in converting non-bioavailable silicon in soil into forms that plants can absorb (Chandrakala et al., 2019). This transformation involves the enzymatic action of "silicase," which consists of a peptide chain structure and catalyzes the conversion of silica (SiO₂) into silicic acid. Silicases hydrolyze both crystalline and amorphous forms of silica, producing free silicic acid as the final product (Schroder et al., 2007). Interestingly, silicases share similarities in their activity and reaction mechanisms with carbonic anhydrases (CAs) (Tomazett et al., 2016). Studies on mRNA transcripts from sea sponges, such as Suberites domuncula, have highlighted these enzymatic similarities, leading to the classification of silicases as a subclass of CAs (Schroder et al., 2007). However, not all subclasses of CAs are reported to solubilize silica by generating silicic acid (Asther et al., 1987; Tomazett et al., 2016).

Pleurotus ostreatus, which can grow on different types of lignocellulosic wastes, can efficiently degrade lignocellulosic biomass under optimized conditions such as pH, temperature, and metal ion concentration. Kaur et al. (2013) reported that P. ostreatus is a silica degrader and degrades more than 45% of the lignin-silica complex. This presents the requirement of economic production of silicase to make it more feasible and cost-effective for industrial use. Microorganisms have widely been exploited as a major source for the production of a diverse range of industrial enzymes (Pandey et al., 2000; Singh et al., 1991). Microbial enzymes are also preferred on an industrial scale due to their easy production process, plentiful supply, and better controllability at all phases of enzyme production (Hernandez et al., 2018; Shaku et al., 1980). Nowadays, fungi are becoming a preferred choice for the isolation and production of different enzymes due to their higher growth rate, multiple enzyme complexes, and ability to tolerate a wide variety of environmental stresses (Zhang et al., 2017). In this study, rice straw was used for the production of silicase enzymes from silica-solubilizing fungi P. ostreatus, Penicillium limosum, and Bipolaris sorokiniana to enhance paddy straw digestibility in a solid-state fungal fermentation-based strategy.

To fill this research gap, the current study focuses on optimizing silicase production under controlled conditions using a silicate-solubilizing fungus (SSF) isolated and identified in a previous study conducted by Mor and Dalal (2024). This study aims to optimize the production of silicase from microbial sources by systematically varying and analyzing key factors. These factors include the type of rice straw (crude vs. powdered), incubation time, temperature, pH levels, the ratio of rice straw to tap water, and the inclusion of various additives. Each parameter will be carefully adjusted to determine its optimal contribution to enhancing silicase yield and activity (Hernandez et al., 2018; Jin and Kirk, 2018; Shaku et al., 1980). This multivariate approach ensures a thorough understanding of how each factor influences enzyme production, laying a foundation for more efficient biotechnological applications. By optimizing their production under controlled conditions and exploring their applications, this research aims to unlock the full potential of silicases in various industrial and biotechnological applications (Rogers and Bennett, 2004; Zhalnina et al., 2015).

**2. MATERIALS AND METHODS**

**2.1 Raw Materials, Chemicals, Plasticware, and Glassware**

Rice straw, an abundant agricultural residue, was chosen as the primary substrate for enzyme production. The straw was collected, air-dried to reduce moisture content, and then precisely cut into uniform pieces of approximately 2-3 cm in length using a mechanical cutter. This preparation step was crucial to ensure a consistent surface area for microbial activity and efficient enzymatic breakdown. All chemicals used in media preparation, biochemical testing, and pretreatment analyses were of analytical grade, ensuring high purity and consistency in experimental results. Reagents were sourced from reputable suppliers, including Hi Media Laboratories Ltd. (India), Sigma Chemicals Ltd. (USA), Rankem (Gurugram, India), and Merck & Co., Inc. (USA). Plasticware and glassware used in the experiments were also selected for their compatibility with the chemicals and conditions used, ensuring the integrity of the experimental procedures.

**2.2 Fungal strains**

The isolation of silica-solubilizing fungi was a key component of our previous research efforts (Mor et al., 2024). During these studies, fungal isolates were subjected to a screening process to assess their ability to solubilize silicates using silica peptone broth (SPB) as the medium. The effectiveness of silicate solubilization was determined by observing colour changes in the SPB, which signaled successful silica solubilization. Through this screening, the most effective silicate-solubilizing fungal isolates were identified and further characterized. The fungal strain was identified as *Penicillium limosum* through 18S rRNA sequencing is labeled as WF1. Another strain exhibited a high degree of similarity to *Bipolaris sorokiniana* isdesignated as BF1. Additionally, a third strain, *Pleurotus ostreatus* (P1), was obtained from the Microbial Type Culture Collection (MTCC 142) at IMTECH, Chandigarh. Following their identification, these fungal isolates—WF1, BF1, and P1—were further investigated for their enzyme production capabilities in subsequent studies, as they were selected for their strong potential to produce silicase, laying the foundation for optimizing enzyme production in future experiments.

**2.3 Inoculum Preparation**

Inoculum preparation began by cultivating fungal strains repeatedly on potato dextrose agar (PDA) to ensure the isolation of pure, uncontaminated cultures. This process was critical to eliminate potential microbial contaminants and guarantee the stability of the fungal strains, leading to consistent and reliable results. Once pure cultures were obtained, uniform discs approximately 5 mm in diameter were aseptically cut from actively growing fungal colonies. These discs were used as standardized innocula, ensuring a consistent starting point for each experiment. The use of fungal discs allowed for equal and reproducible fungal biomass to be introduced into the production medium, which was vital for maintaining uniform growth conditions across all trials. This approach promoted consistency in enzyme production and optimized conditions for silicase and other target enzymes. By using a precise and standardized inoculum, experimental variability was minimized, allowing for reliable comparisons and accurate assessment of the production media’s performance.

**2.4 Production of Silicase: SmF (Submerged Fermentation) vs. SSF (Solid-State Fermentation)**

For solid-state fermentation (SSF), 3 grams of dry crude rice straw were weighed and placed into 250 mL Erlenmeyer flasks, with 15 mL of tap water added to each flask. For submerged fermentation (SmF), a modified Horikoshi medium was prepared, consisting of 0.5% peptone, 0.3% yeast extract, 0.5% potassium nitrate, 0.1% potassium dihydrogen phosphate, 0.1% magnesium sulfate, and 1% substrate in 100 mL of distilled water. Both mixtures were sterilized by autoclaving at 121°C for 15 minutes to eliminate contaminants. After sterilization, a 5 mm fungal disc was introduced into the production media, and the flasks were incubated at 30°C for 8 days under static conditions to provide optimal conditions for fungal growth.

**2.5 Enzyme Extraction and activity**

Silicase enzyme in its crude form was extracted from the production media by centrifugation at 10,000 rpm at 4°C for 20 minutes. The silicase assay was performed using a silicate test kit (Product code 1.14794.0001) from Merck MQuant® Supelco, based on the APHA 4500-SiO2 D+E, ASTM D859-16, and DIN 38405-21 methods. This method involves the solubilization of silica into silicic acid, which is then quantified using a spectrophotometer. For the assay, 5.0 ml of the pretreated sample was pipetted into a test tube. Three drops of Reagent 1 were added and mixed, followed by a 10-minute standing period. Then, three drops of Reagent 2 were added and mixed, followed by the addition of 0.50 ml of Reagent 3 using a pipette. The mixture was then left to stand for 10 minutes before transferring the sample into a cuvette for measurement. The formation of silicic acid was indicated by the development of a blue color, which was measured at 810 nm using a UV-Vis spectrophotometer, with a blank sample (without enzyme) serving as the control. All experiments were performed in triplicate to ensure accuracy. The amount of silicic acid produced was quantified by constructing a calibration curve using known silicic acid standards, and the mean values were reported in the study.

**2.6 Optimization of SSF Variables Using One-Variable-at-a-Time Approach**

To maximize silicase production, various process parameters were considered, including incubation period, temperature, pH, the ratio of rice straw to tap water, and the inclusion of additives. Each parameter was optimized individually using a one-variable-at-a-time approach, with the range of these parameters presented in Table 1. This methodical optimization process ensured the identification of the most favorable conditions for maximizing silicase production.

**Table 1. Optimization of various parameters at different range.**

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| --- | --- |
| **Parameter**  | **Range** |
| Straw | Crude and Powder |
| Ratio of Rice straw and Tap water | 1:5, 1:10, 1:15 |
| Incubation time | 4d, 8d, 16d, 24d |
| Temperature  | 25°C, 30°C, 35°C |
| pH  | 5.0, 7.0 and 9. |
| Additives | Jaggery, Chickpea, Soyabean powder, Khal |

**2.6.1 Optimization of Crude and Powdered Forms**

The production medium, composed of crude rice straw and tap water, was inoculated with a 5 mm disc from 4–7 days-old fungal cultures and incubated at 30ºC under static conditions. On the 8th day, silicase activity was measured in both the crude and powdered forms of the extracted enzyme to evaluate the effectiveness of each form in enzyme production.

**2.6.2 Optimization of Incubation Period**

The optimal incubation period was determined by inoculating the production medium with a 5 mm fungal disc (4–7 days old) and incubating it at 30ºC under static conditions. Silicase activity was measured on the 4th, 8th, 16th, and 24th days to monitor the progression of enzyme production over time.

**2.6.3 Optimization of Incubation Temperature**

The effect of temperature on silicase production was tested by incubating the production medium, inoculated with a 5 mm fungal disc (4–7 days old), at 25ºC, 30ºC, and 35ºC under static conditions for 8 days. After incubation, crude enzymes were harvested and silicase activity was measured.

**2.6.4 Optimization of pH**

To determine the optimal pH for silicase production, the production media were adjusted to pH levels of 5.0, 7.0, and 9.0, inoculated with a 5 mm fungal disc (4–7 days old), and incubated at 30ºC under static conditions for 8 days. Enzyme activity was measured after the incubation period.

**2.6.5 Optimization of Rice Straw to Tap Water Ratio**

To optimize the liquid-to-solid ratio, rice straw was mixed with tap water at ratios of 3:1, 5:1, 10:1, and 15:1. Each mixture was inoculated with a 5 mm fungal disc (4–7 days old), incubated at 30ºC under static conditions, and silicase activity was measured after an 8-day incubation period.

**2.6.6 Optimization of Additives**

Various additives, including jaggery, chickpea husk, soybean powder, and khal, were tested to enhance silicase production. The production medium, supplemented with these additives, was inoculated with a 5 mm fungal disc (4–7 days old) and incubated at 30ºC under static conditions for 8 days. Silicase activity was then measured to determine the most effective additive.

**3. RESULTS AND DISCUSSIONS**

**3.1 Effect of Submerged vs. Solid-State Fermentation on Silicase Production**

The comparison between submerged fermentation and solid-state fermentation (SSF), as depicted in the graph, clearly shows that SSF consistently yields higher enzymatic activity for silicase production across all fungal strains. In this study, SSF resulted in silicase activities of approximately 0.23 U/mL/min for *Penicillium limosum* (W), 0.24 U/mL/min for *Bipolaris sorokiniana* (B), 0.25 U/mL/min for *Pleurotus ostreatus* (P), and the highest activity, 0.26 U/mL/min, for the combination of all three fungi (W+B+P).

In contrast, submerged fermentation exhibited significantly lower enzymatic activities, with *Penicillium limosum* (W) producing around 0.18 U/mL/min, *Bipolaris sorokiniana* (B) yielding 0.16 U/mL/min, and *Pleurotus ostreatus* (P) showing 0.22 U/mL/min. The combination of all three fungi resulted in a maximum activity of approximately 0.22 U/mL/min (Fig. 1).

These results highlight the superiority of SSF over submerged fermentation for maximizing silicase production, particularly when using a combination of the three fungal strains. The higher efficiency of SSF can be attributed to factors such as better aeration, enhanced substrate availability, and conditions that better simulate natural microbial environments, which aligns with previous research emphasizing the advantages of SSF for enzyme production (Pandey et al., 2000).



**Fig. 1. Effect of Submerged or Solid state fermentation for silicase production.**

**3.2 Effect of Crude and Powdered Forms of Rice Straw on Silicase Production**

Rice straw was tested in both crude and powdered forms to evaluate its effect on silicase activity, and the results show that the crude form consistently produced higher silicase activity compared to the powdered form. Specifically, the crude form recorded silicase activities of approximately 0.22 U/mL/min for *Penicillium limosum* (W), 0.25 U/mL/min for *Bipolaris sorokiniana* (B), 0.24 U/mL/min for *Pleurotus ostreatus* (P), and 0.26 U/mL/min for the combination of all three fungi (All). In contrast, the powdered form demonstrated lower silicase activities, with around 0.13 U/mL/min for W, 0.12 U/mL/min for B, 0.11 U/mL/min for P, and 0.16 U/mL/min for the combination of all three fungi (Fig. 2).

These findings suggest that the crude form of rice straw is more favorable for silicase production, likely due to its larger particle size, which may provide a more suitable environment for fungal colonization and enzyme secretion. The coarser texture and greater surface area of the crude straw likely enhance aeration and moisture retention, both of which are important factors for fungal growth and enzymatic activity. This supports previous research that highlights the critical role of substrate particle size and structure in solid-state fermentation processes (Pandey et al., 2000).



**Fig. 2. Effect of crude and powdered form for silicase production.**

**3.3 Effect of Incubation Time on Silicase Production**

This study provides valuable insights into the effect of different incubation periods on silicase activity for fungal strains such as *Penicillium limosum* (W), *Bipolaris sorokiniana* (B), *Pleurotus ostreatus* (P), and their combination (All). The data demonstrate that silicase activity consistently peaked on Day 8 across all fungal strains, highlighting this as the optimal incubation period for maximizing enzyme production.

For *P. limosum* (W), the peak silicase activity was recorded at approximately 0.22 U/mL/min on Day 8, with slightly lower activity (~0.20 U/mL/min) observed on Day 4. The activity decreased further on Day 16 (0.19 U/mL/min) and dropped to 0.16 U/mL/min by Day 25. Similarly, *B. sorokiniana* (B) exhibited maximum silicase activity of about 0.27 U/mL/min on Day 8. On Day 4, the activity was around 0.23 U/mL/min, followed by a decline to 0.21 U/mL/min on Day 16 and a further decrease to 0.19 U/mL/min by Day 25.

In the case of *P. ostreatus* (P), the silicase activity peaked at approximately 0.23 U/mL/min on Day 8, while Day 4 showed slightly lower activity at around 0.20 U/mL/min. By Days 16 and 25, the activity dropped to 0.20 U/mL/min and 0.19 U/mL/min, respectively. The combination of all three fungi (All) showed the highest overall silicase activity, reaching nearly 0.27 U/mL/min on Day 8. On Day 4, the activity was approximately 0.21 U/mL/min, which then increased slightly to 0.23 U/mL/min on Day 16, before reducing again to 0.21 U/mL/min by Day 25 (Fig. 3).

These findings confirm Day 8 as the optimal incubation period for maximizing silicase production across all strains. A consistent decline in enzymatic activity was observed beyond this point, likely due to factors such as nutrient depletion, accumulation of metabolic by-products, or enzyme degradation during prolonged incubation. This trend aligns with earlier studies reporting reduced enzyme activity following extended incubation due to substrate exhaustion and the presence of inhibitory metabolites (Hernandez et al., 2018; Zhang et al., 2017). Therefore, optimizing the incubation period to Day 8 is critical for achieving maximum silicase production efficiency in biotechnological applications.



**Fig. 3. Effect of incubation period on silicase production.**

**3.4 Effect of Incubation Temperature on Silicase Production**

The impact of different incubation temperatures (25 °C, 30 °C, and 35 °C) on silicase activity was assessed for various fungal strains, including *Penicillium limosum* (W), *Bipolaris sorokiniana* (B), *Pleurotus ostreatus* (P), and their combination (All). The results indicate that 30 °C is the optimal temperature for maximizing silicase production across all conditions.

For *P. limosum* (W), the highest activity was observed at 30 °C, with silicase activity reaching approximately 0.20 U/mL/min. At 25 °C and 35 °C, the activity was slightly lower, at 0.19 and 0.18 U/mL/min, respectively. *B. sorokiniana* (B) also exhibited its peak activity at 30 °C (0.26 U/mL/min), while activity at 25 °C and 35 °C was marginally reduced, around 0.23 U/mL/min and 0.22 U/mL/min, respectively.

For *P. ostreatus* (P), silicase production peaked at 30 °C, reaching approximately 0.26 U/mL/min. Comparatively lower activities were observed at 25 °C (0.25 U/mL/min) and 35 °C (0.24 U/mL/min). The combination of all three fungi (All) showed a similar trend, with peak silicase activity of approximately 0.28 U/mL/min at 30 °C, while slightly lower values were recorded at 25 °C (0.27 U/mL/min) and 35 °C (0.26 U/mL/min) (Fig. 4).

These findings suggest that 30 °C is generally the most favorable temperature for silicase production across all fungal strains. Although 25 °C and 35 °C still support moderate enzyme activity, 30 °C consistently results in the highest yield. This observation is consistent with established knowledge that many mesophilic fungi exhibit peak enzyme activity in this temperature range due to optimal metabolic function and enzyme stability (Tomazett et al., 2016). The slight decline in activity at 35 °C may be attributed to the onset of thermal denaturation or reduced enzymatic efficiency at elevated temperatures, although the enzymes retain partial stability. Tomazett et al. (2016) also reported that carbonic anhydrases, such as CA1 (β-class) and CA4 (α-class), produced by *Paracoccidioides* fungi, demonstrated stability between 30 °C and 35 °C. These findings underscore the importance of maintaining an incubation temperature around 30 °C for optimal silicase production and functional enzyme stability.



**Fig. 4. Effect of incubation temperature on silicase production.**

**3.5 Effect of pH of the Medium on Silicase Production**

pH is a crucial regulatory factor in microbiological and biotechnological processes, as it significantly affects enzyme production and activity. This study examined the impact of different pH levels (5, 7, and 9) on silicase activity for various fungal strains, including *Penicillium limosum* (W), *Bipolaris sorokiniana* (B), *Pleurotus ostreatus* (P), and their combination (All). The results indicate that pH 7 was optimal for silicase production across all tested conditions.

For *P. limosum* (W), the highest activity was observed at pH 7, with silicase activity reaching approximately 0.22 U/mL/min. Lower activities were recorded at pH 5 (0.20 U/mL/min), with a further decline to 0.19 U/mL/min at pH 9. Similarly, *B. sorokiniana* (B) exhibited peak activity at pH 7 (0.24 U/mL/min), while activity dropped to 0.20 U/mL/min at pH 5 and to approximately 0.19 U/mL/min at pH 9.

*P. ostreatus* (P) also showed maximal silicase activity at pH 7 (0.25 U/mL/min), with slightly lower values observed at pH 5 (0.22 U/mL/min) and pH 9 (0.21 U/mL/min). The combination of all three fungi (All) demonstrated the highest overall silicase activity, peaking at 0.26 U/mL/min at pH 7. In comparison, activity dropped slightly to 0.23 U/mL/min at pH 5 and to 0.21 U/mL/min at pH 9 (Fig. 5).

These results suggest that pH 7 is the most favorable condition for silicase production, while both more acidic (pH 5) and more alkaline (pH 9) environments led to a noticeable decline in enzyme activity. The decrease in activity under non-neutral pH conditions may be attributed to suboptimal nutrient availability, altered ionization of amino acid residues at the enzyme's active site, or partial enzyme denaturation.

Similar findings were reported by Nazir et al. (2010), who observed that rice straw as a substrate resulted in maximum CMCase activity during solid-state fermentation at pH 6.0. Furthermore, Joshi et al. (2006) emphasized that enzyme production at varying pH levels is closely tied to nutrient solubility and microbial metabolism. pH is recognized as a primary control parameter in microbial processes due to its broad influence on enzyme activity, nutrient uptake, and overall physiological performance (Jin and Kirk, 2018; Joshi et al., 2006).



**Fig. 5. Effect of pH on silicase production.**

**3.6 Effect of Rice Straw to Tap Water Ratio on Silicase Production**

The graph illustrates the optimization of silicase production by adjusting the ratio of rice straw to tap water for various fungal strains, including *Penicillium limosum* (W), *Bipolaris sorokiniana* (B), *Pleurotus ostreatus* (P), and their combination (All). The results show that while a 1:5 ratio consistently yielded the highest silicase activity for *P. limosum* and *B. sorokiniana*, other strains such as *P. ostreatus* and the combined fungal consortium performed better at a 1:10 ratio.

For *P. limosum* (W), the 1:5 ratio resulted in silicase activity of approximately 0.28 U/mL/min, outperforming the lower activities observed at the 1:3 (0.24 U/mL/min) and 1:10 (0.23 U/mL/min) ratios. Similarly, *B. sorokiniana* (B) exhibited maximum activity at the 1:5 ratio, achieving approximately 0.27 U/mL/min, compared to 0.25 U/mL/min at 1:3 and 0.24 U/mL/min at 1:10.

In contrast, *P. ostreatus* (P) showed its highest activity at the 1:10 ratio (0.26 U/mL/min), surpassing the activities at 1:5 (0.22 U/mL/min) and 1:3 (0.23 U/mL/min). Similarly, the combination of all three fungi demonstrated better performance at the 1:10 ratio, with silicase activity reaching approximately 0.30 U/mL/min, compared to 0.29 U/mL/min at 1:5 and 0.27 U/mL/min at 1:3 (Fig. 6).

These findings align with previous research emphasizing that the optimal substrate-to-water ratio in solid-state fermentation can vary depending on the fungal species and their moisture requirements. As noted by Pandey et al. (2000), while adequate moisture is essential for microbial metabolism and enzymatic activity, excessive water may limit oxygen diffusion, dilute nutrients, and reduce enzyme yield. This study suggests that the 1:5 ratio is more effective for strains like *P. limosum* and *B. sorokiniana*, whereas for *P. ostreatus* and the combined fungal culture, a 1:10 ratio offers more favorable conditions. Thus, tailoring the rice straw-to-water ratio to suit specific fungal strains is critical for maximizing silicase production, as the nutrient-moisture balance directly influences enzyme synthesis.



**Fig. 6. Effect of ratio of rice straw and tap water for silicase production.**

**3.7 Effect of Different Additives on Silicase Production**

The study evaluated the effect of different additives on silicase production across several fungal strains, including *Penicillium limosum* (W), *Bipolaris sorokiniana* (B), *Pleurotus ostreatus* (P), and their combination (All). The results, as shown in the graph, indicate that jaggery and soya powder were the most effective additives for enhancing silicase activity across all tested conditions.

For *P. limosum* (W), jaggery resulted in the highest silicase activity, reaching approximately 0.34 U/mL/min, followed by soya powder at around 0.32 U/mL/min. Khal and chickpea husk yielded slightly lower activities of approximately 0.26 U/mL/min and 0.24 U/mL/min, respectively. In the case of *B. sorokiniana* (B), jaggery again led to the highest activity (0.28 U/mL/min), with soya powder close behind at 0.29 U/mL/min. Khal and chickpea husk showed lower activities at around 0.23 U/mL/min and 0.24 U/mL/min, respectively.

For *P. ostreatus* (P), both jaggery and soya powder resulted in relatively high silicase activity—approximately 0.29 U/mL/min and 0.28 U/mL/min, respectively—while chickpea husk and khal produced lower values around 0.25 U/mL/min and 0.24 U/mL/min. The combination of all three fungi (All) demonstrated the highest activity with jaggery (0.30 U/mL/min) and soya powder (0.31 U/mL/min), whereas chickpea husk and khal showed slightly lower enzyme activity levels of approximately 0.24 U/mL/min each (Fig. 7).

These findings suggest that jaggery and soya powder are the most effective additives for maximizing silicase production. The superior performance of jaggery may be attributed to its high sugar content, which supports energy metabolism and stimulates microbial activity. Meanwhile, soya powder likely promotes enzyme production due to its rich protein content and essential amino acids, which are important for fungal growth and enzyme synthesis. In contrast, chickpea husk and khal, which resulted in lower enzyme activity, may lack the optimal nutrient composition or introduce inhibitory compounds that suppress enzyme secretion.

These results highlight the critical role of additive selection in enhancing enzyme yields in biotechnological processes. Similar studies have shown that carbon-rich and nitrogen-rich additives can significantly impact enzyme production by promoting microbial growth and metabolic activity (Shaku et al., 1980). Furthermore, Asther et al. (1987) reported that certain additives, such as fatty acids and surfactants, enhance enzyme activity by improving cell membrane permeability, thus facilitating faster enzyme secretion. Collectively, these findings suggest that further optimizing the use of effective additives like jaggery and soya powder could lead to even greater improvements in silicase production.



**Fig. 7. Effect of additives on silicase production.**

The study optimized several key parameters to maximize silicase production through solid-state fermentation (SSF), which proved more effective than submerged fermentation. SSF allows for better aeration and simulates the fungi’s natural habitat, thus promoting higher enzymatic activity. The use of crude rice straw as a substrate, instead of powdered straw, enhanced enzyme production due to its larger particle size, which improves aeration and moisture retention, allowing fungi to grow and secrete enzymes efficiently. The optimal rice straw to water ratio of 1:5 provided sufficient moisture for fungal growth while preventing nutrient dilution, which is essential in solid-state fermentation processes. A lower ratio could lead to inadequate moisture, while higher ratios may dilute the nutrients, reducing enzymatic activity. The incubation time of 8 days was identified as the optimal period for maximizing enzyme production. Beyond this time, enzyme activity decreased due to nutrient depletion and metabolic by-product accumulation.

**Table 2. Optimized Parameters for Silicase Production**

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| **Parameter** | **Optimized Condition** |
| Fermentation Method | Solid-State Fermentation (SSF) |
| Substrate Form | Crude Rice Straw |
| Rice Straw to Water Ratio | 1:5 (for W, B) 1:10 (for P, All) |
| Incubation Time | 8 Days |
| Incubation Temperature | 30°C |
| pH | 7 |
| Additives | Jaggery and Soya Powder |

Temperature control was critical, with 30°C being the optimal condition for enzyme stability and activity. This temperature supports the growth of mesophilic fungi, which are known to thrive and produce enzymes effectively in this range. Furthermore, maintaining a pH of 7 ensured the optimal environment for enzymatic activity, as both acidic and alkaline conditions tend to reduce enzyme efficiency.

The use of jaggery and soya powder as additives significantly enhanced silicase production. Jaggery, rich in carbohydrates, serves as an excellent energy source, while soya powder provides essential proteins and amino acids needed for fungal growth and enzyme synthesis. These additives contributed to achieving the highest enzyme activity in the study.

**4. CONCLUSION**

This research successfully optimized key parameters for enhancing silicase production through solid-state fermentation. By optimizing factors such as substrate type, incubation time, temperature, pH, and nutrient supplementation, the study increased silicase activity from an unoptimized value of 0.11 U/mL/min to an optimized 0.34 U/mL/min. The most effective conditions were found to be the use of crude rice straw as the substrate, a 1:5 rice straw-to-water ratio, and an 8-day incubation period at 30°C and pH 7. The addition of jaggery and soya powder further enhanced enzyme production, highlighting the importance of nutrient-rich supplements. The findings of this study emphasize the importance of tailoring each aspect of the fermentation process to ensure efficient silicase production. The integration of bacterial strains in the fungal consortium not only enhanced enzyme yields but also offers a promising strategy for industrial-scale applications in fields such as biotechnology, agriculture, and bioremediation, where silica solubilization processes are essential. Future research can explore scaling up this approach or investigating additional bacterial strains to further optimize the process, potentially opening new avenues for more efficient and cost-effective enzyme production systems. The optimized conditions and consortium approach used in this study lay the groundwork for improved enzyme production processes with significant potential for real-world applications, offering an efficient, scalable solution for industries reliant on enzymatic silica solubilization.

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