



ISSN : 2347 - 2243

Indo - American Journal of Life Sciences and Biotechnology



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Optimisation of Cellulase Production by *Trichoderma reesei* Through Solid-State Fermentation on Rice Straw

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Received: July 18, 2024 Accepted: October 21, 2024 Published: March 12, 2025

DOI: [10.62644/iajlb.2025.v22.i1.pp1-10](https://doi.org/10.62644/iajlb.2025.v22.i1.pp1-10)

ABSTRACT

Rice straw is among the most abundant agricultural residues in the world, and *Trichoderma reesei* is the best-known fungal source of the cellulases that could turn it into fermentable sugar. Growing the fungus directly on moistened straw, a process called solid-state fermentation, is often proposed as a cheap way to make the enzyme, and many reports describe process conditions that were optimised for it. This narrative review asks what the published literature supports about that practice and where it falls short. It draws on work on the biology and regulation of the fungus, the composition and recalcitrance of rice straw, the engineering of solid-state beds, enzyme assay, and the statistical designs used to optimise culture conditions, and it reports no new experimental data. Moisture, temperature, particle size, nitrogen supply and pretreatment all act through a small number of physical routes, notably water availability, heat removal and gas exchange, which is why optima found in one vessel often fail to transfer to another. Activity figures are further limited by assay choice and by the lot-to-lot variability of straw. The review concludes that optimisation of this system is feasible and worthwhile, that reported optima should be read as conditional on substrate, strain, vessel and assay, and that cost and scale-up questions remain largely unanswered.

Keywords: *Trichoderma reesei*; Cellulase; Solid-state fermentation; Rice straw; Process optimisation; Response surface methodology; Narrative review

Citation: Pawlak, Olabarria and Vermeulen [2025]. Optimisation of Cellulase Production by *Trichoderma reesei* Through Solid-State Fermentation on Rice Straw. *Indo-Am. J. Life Sci. Biotechnol.* 22(1):1-10. DOI: [10.62644/iajlb.2025.v22.i1.pp1-10](https://doi.org/10.62644/iajlb.2025.v22.i1.pp1-10)

Article Class: Narrative Review

1. Introduction

Few biological catalysts have been studied as long as the cellulases of *Trichoderma reesei*. The fungus was found deteriorating cotton canvas during the Second World War, and the enzymes it secretes to attack

cellulose have since become the benchmark for industrial hydrolysis of plant biomass (1, 2). Its genome sequence confirmed what its secretion profile had long suggested, namely a compact enzymatic toolkit that is rich in cellulases



and hemicellulases and modest in almost everything else (3). Strain improvement over several decades has produced hypersecreting mutants that are the basis of many commercial preparations (4, 5).

Rice straw is an obvious place to point such an enzyme. It is left in the field after every harvest, it is produced wherever rice is grown, and it is often burned because there is little else to do with it (6, 7). A process that grows the fungus on the straw itself, with only enough water to wet the particles, would turn a disposal problem into an enzyme supply. That is the idea behind solid-state fermentation, and it explains why papers on optimising cellulase production on rice straw keep appearing (8, 9).

The difficulty is that an optimum is only as good as the system it was found in. The title of this article describes a familiar kind of study, one in which several culture variables are adjusted until enzyme activity peaks. We report no such experiment. No new laboratory data are presented here and no local results are claimed; the article instead asks what the published literature on this topic can and cannot tell a reader who wants to reproduce or extend such work. A reader of an optimisation paper wants an answer to a plain question: would this have worked on my straw?

The review proceeds from the organism to the substrate, then to the physics of the bed and the statistics of optimisation. It ends with a frank account of the limits of the evidence. The aim is a fair account of what is established and what is merely repeated.

2. Literature Review

2.1 The Fungus and Its Enzymes

The cellulase system of *T. reesei* is a mixture rather than a single enzyme. Cellobiohydrolases attack the ends of cellulose chains, endoglucanases cut within them, and beta-glucosidases finish

the job by splitting cellobiose into glucose; the first two are secreted in large amounts and the last is comparatively scarce (10, 11). The structure of the main cellobiohydrolase, with its long tunnel that threads a single chain through the active site, explains much about how crystalline cellulose is handled (12, 13).

The balance matters for anyone measuring performance. A preparation rich in the first two enzyme classes can look excellent on a filter paper assay and still release glucose slowly if beta-glucosidase is short, a gap that is usually filled by adding the enzyme from another source or by choosing a different fungus altogether (14, 15). The point is not new, but it is easily lost when a single activity figure is quoted as if it summarised the whole system.

Expression is tightly controlled. Cellulase genes are switched on in the presence of inducing sugars, switched off when glucose is plentiful, and coordinated with those of hemicellulases through a handful of transcription factors (16, 17). One of these factors, ACE1, was described as a repressor of cellulase and xylanase expression (18), and the picture has become more intricate since. Metabolic engineering has tried to exploit the circuitry (19), but for a process on whole straw the practical consequence is simpler: what the fungus is given to eat shapes what it secretes (20).

2.2 Rice Straw as Substrate

Rice straw is a lignocellulose, built from cellulose microfibrils embedded in hemicellulose and lignin. Its composition is unremarkable in that respect, but it carries an unusually large mineral fraction, silica in particular, which makes it abrasive and slow to degrade (7, 21). Standard detergent fibre methods separate the fractions well enough to compare lots, although they were designed for animal nutrition rather than for fermentation (22).



Recalcitrance is the central obstacle. The tight association of the polymers, the crystallinity of cellulose and the physical barrier of lignin all restrict enzyme access (23), and a long literature on pretreatment tries to loosen them (24, 25). Table 1 lists the usual options and their main drawbacks. The choice is not obvious, because harsh pretreatment can release compounds that inhibit the fungus and gentle pretreatment may leave too much of the structure intact. In solid-state work the trade-off is sharper still, since washing away inhibitors also washes away soluble nutrients. An optimum that vanishes when the straw lot changes is not one a plant manager would rely on.

Table 1. Pretreatment options for rice straw before solid-state cultivation.

Option	Main effect	Main concern
Milling only	Smaller particles, more surface (26)	Lignin and silica untouched
Alkali	Removes part of the lignin (25)	Chemical use; washing
Dilute acid	Dissolves hemicellulose (24)	Inhibitors; corrosion
Hydrothermal	Opens the fibre structure (25)	Energy demand
Biological	Gentle delignification (27)	Slow; carbohydrate loss

Note: Qualitative summary; effects depend on conditions and on the straw lot.

2.3 Solid-State Cultivation

Solid-state fermentation grows microorganisms on a moist solid with no free-flowing liquid between the particles (28). Filamentous fungi suit it well, since hyphae can penetrate particles and tolerate low water activity, and several reviews argue that the format gives higher enzyme titres per unit of substrate than submerged culture for some fungi (29). The same reviews are cautious about generalising. The advantage is real in some systems and

absent in others, and the reasons are not always understood (30).

Table 2 sets out the usual contrasts. The attraction is the ability to use raw residues directly and to avoid large volumes of liquid (8, 31). The cost is physical. A fungus growing in a packed bed releases metabolic heat, the bed conducts it poorly, and the temperature at the centre can climb well beyond the set point (32, 33). Oxygen must diffuse through the voids between particles while carbon dioxide must leave, and the voids shrink as the mycelium grows. Such differences look small on paper, yet they often decide whether a process survives scale-up.

Table 2. Solid-state and submerged cultivation compared for cellulase production.

Aspect	Solid-state	Submerged
Free water	Little; held in the substrate (28, 30)	Abundant, continuous liquid phase
Heat removal	Difficult in deep beds (32, 33)	Easy; stirring and jackets
Mixing and sampling	Limited; beds are heterogeneous	Simple and representative
Substrate	Raw residues can be used directly (8)	Usually soluble or finely suspended
Enzyme recovery	Extraction step needed	Filter or centrifuge the broth
Scale-up	Engineering still developing (34)	Well established

Note: Qualitative comparison of general tendencies; individual systems vary.

2.4 Cellulase Assay and What a Number Means

Reported activity depends heavily on how it is measured. The filter paper assay standardised for cellulase work reports total saccharifying activity, while separate assays report endoglucanase or beta-glucosidase activity (35). Most rely on the colour reaction of reducing sugars with dinitrosalicylic acid (36), which



responds to any reducing sugar and so also to the xylose and other sugars released from hemicellulose in a straw extract.

Two papers that use the same unit name may therefore not be comparable. Extraction method, buffer, dilution and the choice of reporting activity per gram of dry substrate or per millilitre of extract all alter the figure. Cost studies that treat enzyme as a commodity make the opposite point, that the metric which matters economically is the sugar released from real biomass per unit cost, not an assay unit (37). This is why the same fungus can look excellent in one report and unremarkable in the next.

2.5 Statistical Optimisation

The language of optimisation in this field comes from experimental design. Varying one factor at a time is simple, yet it cannot detect interactions and can mislead badly when factors depend on each other (38). Screening designs such as that of Plackett and Burman let many factors be tested in few runs (39). The path of steepest ascent and its descendants move the experiment toward better conditions (40), and response surface designs then fit a quadratic model around the promising region (41, 42).

These tools are powerful and easy to misuse. A quadratic model describes the region sampled and nothing beyond it, a good fit statistic is not proof that an optimum is real, and predicted optima need confirmation in fresh runs. Studies on the cellulase production of *T. reesei* RUT C30 on solid substrates have used exactly this sequence (43), and Figure 1 sketches it together with the points at which it commonly fails. Replicated centre points give an honest estimate of pure error, and a design with very few runs can pass its own fit checks while describing noise. Reporting the design matrix, the coded levels and the residuals lets a reader check the model for themselves. Without them an optimum is a number, not a result.

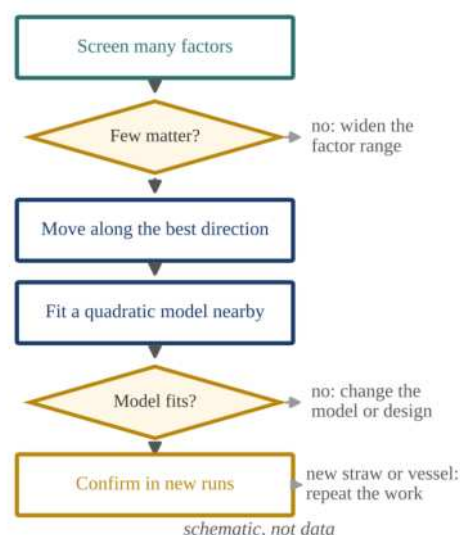


Figure 1. Sequential workflow for statistical optimisation, with the points at which it can fail (schematic, not data).

3. Materials and Methods

3.1 Scope and Question

The review asks one question in two parts. What does the published literature support about the conditions under which *T. reesei* produces cellulase on rice straw by solid-state fermentation, and how far do reported optima transfer between substrates, vessels and laboratories? The question was divided into the biology of the fungus, the nature of the substrate, the physics of the bed, the measurement of enzyme and the statistics of optimisation, and each part is treated in turn.

3.2 Literature Assembly

The review draws on peer-reviewed work in these five areas, together with a small number of classic papers on experimental design that remain the standard references. Sources were chosen by the authors because they are well known, were repeatedly cited in later work or addressed a specific mechanism. Only works published on or before 2025 are cited.

We did not run a formal systematic search and we do not claim one. This is a



narrative synthesis, and the choice of sources reflects our judgement. The practical consequence is that the review is a better guide to the structure of the problem than to the frequency of any finding, and no numerical results from individual studies are pooled or quoted. Reviews of this kind are more useful when they say where the evidence stops than when they smooth it over.

3.3 Appraisal Framework

To judge how far a report of an optimum can be trusted, we asked four questions. Was the substrate described well enough to be obtained again, including its pretreatment and particle size (26)? Was the vessel and its heat and gas exchange reported (33)? Was the assay named and its limits stated (35, 36)? Was the optimum confirmed in runs that were not used to build the model (42)? Table 3 collects the process variables that these questions concern.

Table 3. Process variables in solid-state cellulase production and how they act.

Variable	Main route of action	Usual difficulty
Moisture	Water availability, void space (31)	Too little stalls growth; too much blocks air
Temperature	Growth rate, enzyme stability (32)	Metabolic heat raises the bed
Initial pH	Enzyme production, nutrient uptake (20)	Drifts as the fungus grows
Particle size	Surface area and packing (26)	Fine particles pack, coarse ones resist
Nitrogen source	Biomass and protein synthesis (43)	Excess can suppress enzyme
Inoculum	Lag time, early colonisation	Rarely standardised

Note: The routes shown are the authors' reading of the cited literature, not a measured ranking.

4. Results

4.1 What the Process Looks Like

Across the literature the process follows a recognisable sequence (Figure 2). Straw is dried and reduced in size, sometimes pretreated, then moistened with a mineral salts solution and sterilised. It is inoculated with spores, incubated while heat and gas are managed, and finally extracted with buffer so that the enzyme can be assayed (8, 9). Reports differ mainly in the pretreatment step and in how the bed is contained, whether in flasks, trays or drums.

Each step hides decisions. Moistening fixes the water available to the fungus. Sterilisation can alter the straw. Inoculation fixes how fast the surface is colonised. Extraction decides how much of the enzyme is recovered, since cellulases bind to residual fibre and are only partly released. A paper that reports the optimum of one step without describing the others leaves the reader unable to repeat it, however carefully the optimum itself was found. It is a habit worth keeping even when the answer seems obvious.

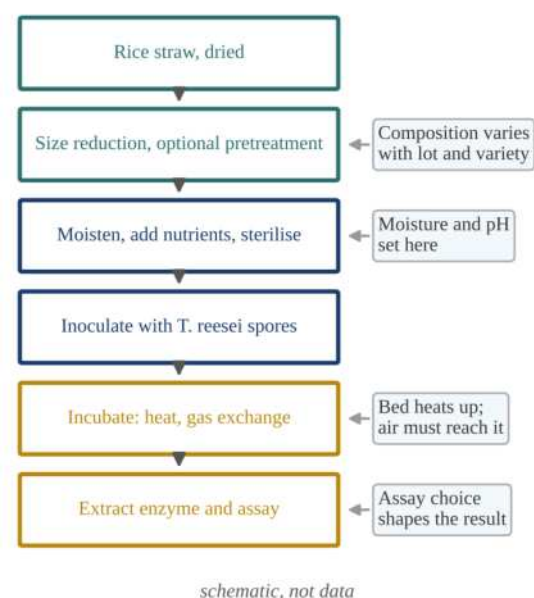


Figure 2. Outline of a solid-state cellulase process on rice straw (schematic, not data).



4.2 The Physical Routes Behind the Variables

Reviewers of the field repeatedly note that many culture variables are not independent. Moisture, temperature, particle size and bed depth all act mainly through water availability, heat removal and gas exchange (31, 32), whereas pH, nitrogen supply and inoculum act more directly on growth and on induction of the enzyme (Figure 3). The grouping is our reading and not a measured ranking, but it helps explain a common experience.

An optimum moisture level found in a small flask reflects a balance between water for the fungus and air in the voids, and the balance moves with particle size and with how tightly the bed is packed (26). An optimum temperature found in a thermostatted flask says little about a deeper bed that heats itself. The numerical values are therefore properties of the whole arrangement, not of the fungus alone. The point is not that a study is poor, only that its optimum travels less far than its abstract suggests.

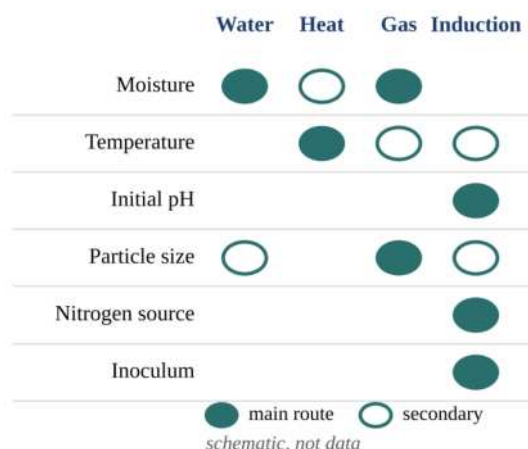


Figure 3. Qualitative matrix linking process variables to the physical routes they act through (schematic, not data).

The same reasoning applies to nutrients. Nitrogen supplied in a form the fungus can use promotes biomass, and the balance between growth and enzyme output

depends on what else is limiting (20, 43). A nitrogen source that looks best in one straw lot may look poorer in another that already carries more of it.

4.3 Reported Optima and Their Conditions

Papers on this topic commonly report a set of best conditions, typically a moisture level, an incubation temperature, an initial pH and an inoculum size, followed by an activity figure. We found no case in which such a set was shown to hold for a different straw lot, a different vessel or a different laboratory, though absence of evidence is not evidence of failure. Work with other fungi on agricultural residues points the same way: the best residue and the best conditions depend on the organism and the preparation (26, 44).

The wording of the conclusions is also telling. Papers tend to state that conditions were optimised, which implies a global maximum, when what was found is the best point within the ranges tested. If the true optimum lies outside the ranges, the design cannot reveal it, and a flat response near the edge of a range is a signal to widen it. Read this way, a flat response in a small design is not evidence that the factor does not matter.

4.4 Cost and Scale

Solid-state production is often presented as cheap because the substrate is cheap. The case is less simple. Enzyme production is one part of a larger chain, and the economics of cellulosic biofuels have been shown to be sensitive to what the enzyme costs and how much of it is needed (37). Savings on substrate can be offset by labour, by handling of bulky solids, and by the difficulty of extracting enzyme from a wet bed and concentrating it (34, 45).

Scale-up is where bench optima are most often tested and most often fail, because heat and gas transfer scale differently from



volume (32, 33). Engineering studies of bioreactor design are the right place to look for guidance, and the optimum conditions from flasks are best viewed as a starting range for them, not a recipe. Such differences look small on paper, yet they often decide whether a process survives scale-up.

5. Discussion

5.1 Interpretation

The main finding of this review is conditional. Solid-state cultivation of *T. reesei* on rice straw is a sound idea and can plausibly give useful cellulase, but a reported optimum belongs to a particular combination of strain, straw lot, pretreatment, vessel and assay. The biology of the fungus and the physics of the bed are well described; the link between them for rice straw specifically is not as well described as the volume of optimisation papers suggests (8, 11). This is why the same fungus can look excellent in one report and unremarkable in the next.

A second point concerns what is being optimised. Maximising activity per gram of substrate is not the same as minimising the cost of a unit of sugar released, and the two can point in different directions (37). Pretreatment that raises enzyme yield may cost more than it returns, and a fungus that makes slightly less enzyme on untreated straw may be the better choice economically.

Three findings would change this reading. A study that transferred an optimum between vessels and substrate lots with little loss would show that the conditions are more robust than argued here. A study that showed reproducible enzyme yields on unsterilised straw would change the cost picture. And a comparison of several fungi on the same straw under the same conditions would put the dominance of *T. reesei* in context (14).

5.2 Limitations

This is a narrative review and shares the limitations of that form. Source selection reflects judgement, no formal search or quality scoring was done, and no individual study is weighed by size or design. Important work may have been missed, and readers who need a count of studies or a pooled estimate should not use this article for that.

The review also relies on evidence from other fungi, other residues and other assays to fill gaps for rice straw, and these are imperfect proxies. The grouping of variables by physical route is our own synthesis. It is plausible and consistent with the sources, but it has not been tested as a model. Nothing here argues against solid-state fermentation; it argues for measuring what actually happens inside the bed.

5.3 Directions for Future Work

A study that could settle the question would describe the straw lot, its pretreatment and its composition, and would report the vessel with its heat and gas exchange. It would state the assay and its limits, use a design that includes interactions, and confirm the optimum in independent runs on a second lot. Reporting such details costs little and would make reports comparable. That is a modest claim, and it is the one the literature currently bears.

Three needs stand out. First, a formal systematic review with a transparent search of solid-state cellulase studies, ideally with an appraisal of design quality. Second, work that tests whether an optimum found at small scale holds in beds of realistic depth. Third, techno-economic accounts that include handling, extraction and concentration of the enzyme, not only the cost of the substrate. These points apply to any lignocellulosic substrate, not only to rice straw.



6. Conclusion

Growing *Trichoderma reesei* on rice straw to make cellulase is biologically plausible and technically feasible, and statistical optimisation is a reasonable way to improve it. The literature does not yet show that reported optima transfer between straw lots, vessels or laboratories, and its enzyme activity figures depend heavily on how they were assayed. A culture given time to adapt behaves differently from one that is not.

For the practitioner the message is practical rather than discouraging. Describe the straw, treat heat and air as variables, name the assay, confirm the optimum in fresh runs, and count the costs beyond the substrate. For the researcher, the opportunity lies in studies designed to test transfer rather than to produce another single best condition.

7. Acknowledgements

The authors thank colleagues who commented on earlier drafts of this review.

8. Funding

This work received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

9. Conflict of Interest

The authors declare no conflict of interest.

10. Author Contributions

L.P. conceived the review and wrote the sections on the fungus and its enzymes. M.O. wrote the sections on the substrate and solid-state cultivation. M.V. wrote the sections on bioprocess engineering, assay and statistical optimisation. All authors reviewed and approved the final text.

11. Ethics Statement

This is a review of published literature. No people or animals were involved, so no ethical approval was needed.

12. Data Availability

No new data were generated. All sources are cited in the reference list.

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