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Optimisation of Solid-State Fermentation for Enhanced Cellulase Production by Trichoderma Species

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ABSTRACT

Background: Cellulases produced by Trichoderma species under solid-state fermentation (SSF) are central to cost-effective saccharification of lignocellulosic biomass, yet reported yields vary widely with substrate and process design. Objective: This review synthesises published SSF optimisation studies for Trichoderma reesei, T. viride and T. harzianum cellulase production. Methods: A narrative review of peer-reviewed reports using wheat bran, sugarcane bagasse and related agro-residues, examining moisture, pH, temperature, incubation time, nitrogen supplementation and statistical optimisation. Findings: Plackett-Burman screening followed by response-surface or central-composite designs repeatedly delivers several-fold gains in filter-paper and endoglucanase activity over unoptimised baselines. Conclusions: Substrate choice combined with statistically guided process optimisation, rather than any single variable, determines industrially relevant cellulase titres from Trichoderma SSF.

Keywords: *solid-state fermentation; Trichoderma reesei; cellulase; response surface methodology; wheat bran*

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Abstract

Background: Cellulases produced by *Trichoderma* species under solid-state fermentation (SSF) are central to cost-effective saccharification of lignocellulosic biomass, yet reported yields vary widely with substrate and process design. **Objective:** This review synthesises published SSF optimisation studies for *Trichoderma reesei*, *T. viride* and *T. harzianum* cellulase production. **Methods:** A narrative review of peer-reviewed reports using wheat bran, sugarcane bagasse and related agro-residues, examining moisture, pH, temperature, incubation time, nitrogen supplementation and statistical optimisation. **Findings:** Plackett-Burman screening followed by response-surface or central-composite designs repeatedly delivers several-fold gains in filter-paper and endoglucanase activity over unoptimised baselines. **Conclusions:** Substrate choice combined with statistically guided process optimisation, rather than any single variable, determines industrially relevant cellulase titres from *Trichoderma* SSF.

Keywords: solid-state fermentation; Trichoderma reesei; cellulase; response surface methodology; wheat bran

Introduction

Cellulases (EC 3.2.1.4 and related glycoside hydrolases) hydrolyse the β-1,4-glycosidic bonds of cellulose and underpin biofuel, textile, animal-feed, food and paper-pulp industries that together consume a large share of global industrial enzyme output [1]. Because pretreated agricultural lignocellulose is abundant and cheap, converting it into fermentable sugars for bioethanol has driven decades of interest in efficient, low-cost cellulase production [2]. Among filamentous fungi, *Trichoderma reesei* (anamorph of *Hypocrea jecorina*) remains the most extensively exploited cellulase producer, owing to its secretion capacity and the availability of hyperproducing mutants such as RUT-C30, derived through successive rounds of mutagenesis from the wild-type QM6a isolate [3,4]. Related species, including *T. viride* and *T. harzianum*, are also widely studied cellulase sources, particularly on agro-industrial residues [5,6].

Solid-state fermentation, in which fungi grow on moist solid substrate in the near-absence of free water, is generally reported to give higher volumetric cellulase titres, lower catabolite repression and simpler downstream recovery than submerged fermentation for these organisms, though it poses distinct heat- and mass-transfer challenges at scale [7,8]. Because the solid matrix mimics the natural niche in which many filamentous fungi decompose plant litter, SSF is also frequently reported to require less energy input and generate less wastewater than

stirred-tank submerged culture, both of which matter for the economics of an enzyme that must ultimately be produced cheaply enough to compete with fossil-derived chemical processing routes [2,1].

This review does not describe a new experiment; instead, it synthesises published literature on how substrate selection, moisture content, pH, temperature, incubation time, nitrogen supplementation, and statistical experimental design (Plackett-Burman screening and response surface methodology) have been used to optimise SSF cellulase production by *Trichoderma* species, drawing on real reported enzyme activities and fold-improvements from the cited primary studies. The aim throughout is to identify recurring patterns across independent laboratories and substrates rather than to endorse any single "best" protocol, since strain background, substrate composition and reactor geometry all constrain which optimum is achievable in a given system.

Substrate Selection for Solid-State Cellulase Production

Wheat bran is the substrate most consistently associated with high cellulase titres in *Trichoderma* SSF, likely reflecting its favourable particle structure, porosity and residual starch content that support fungal growth alongside cellulose induction [9,10]. Using wheat bran, *T. reesei* RUT C30 produced 3.8 U/gds filter-paper activity after optimisation, a 6.2-fold increase over an unoptimised 0.605 U/gds baseline [9], while *T. reesei* CBS 836.91 achieved 8-10 IU/gds filter-paper activity and 15.6-17.8 IU/gds endoglucanase activity on the same substrate at laboratory scale, values that were largely retained (8.2 and 13.5 IU/gds) after scale-up to a rotating-drum reactor [10]. *Trichoderma* sp. RCK65 grown on wheat bran reached 145 U/gds CMCase, 38 U/gds FPase and 105 U/gds β-glucosidase, with the crude extract achieving roughly 83% saccharification of pretreated *Prosopis juliflora* [11]. Other lignocellulosic residues, including sweet sorghum bagasse for *T. harzianum* HZN11 [6] and porous inert carriers combined with *T. reesei* [12], have also supported strong cellulase output, and agricultural by-products more broadly remain the preferred low-cost carbon source across this literature [13,14]. Figure 1, presented under this section, compares maximum reported cellulase activities from four representative SSF studies.

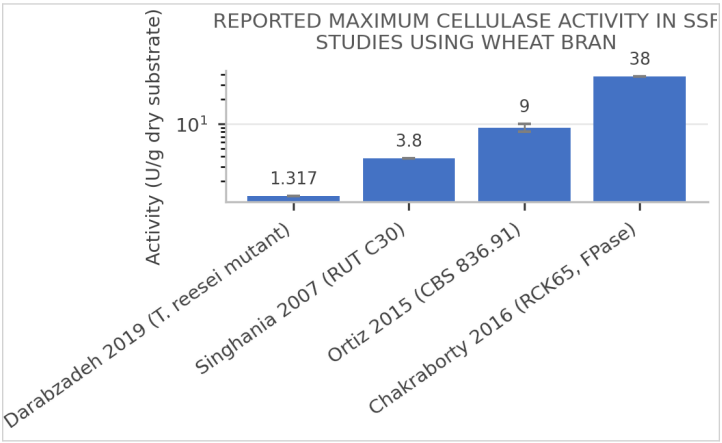
Physicochemical Process Variables: Moisture, pH, Temperature and Incubation Time

Moisture content is repeatedly identified as the single most influential physical variable in *Trichoderma* SSF, since it governs substrate swelling, oxygen diffusion and nutrient accessibility. A mutant *T. reesei* strain achieved maximal FPase activity of 1.317 U/gds at 74% initial moisture, with a Plackett-Burman screen ranking moisture above phosphate supplementation, temperature and incubation time as the dominant factor [15]. *T. reesei* CBS 836.91 performed best at 74-94% moisture and pH 6.4-6.6 [10], while earlier work on RUT C30 similarly converged on near-neutral pH and moderate mesophilic temperatures around 28-30°C [9,16]. Incubation time interacts with these variables: cellulase titres typically rise over the first several days of cultivation before plateauing or declining as substrate nutrients are depleted and proteolysis increases, a pattern reported consistently for *T. reesei* mutants harvested around four days post-inoculation [15] and for *T. viride* on wheat straw, where peak filter-paper and carboxymethylcellulase activities were recorded around 144 hours [17]. Temperature effects are comparatively narrow across the genus: most published optima cluster between 28°C and 30°C, consistent with the mesophilic physiology of *Trichoderma* species even though the fermenting substrate bed can self-heat several degrees above ambient once fungal metabolic activity peaks, a phenomenon that becomes more pronounced, and more difficult to control, as bed depth and scale increase [10,7]. Reviews of SSF more broadly emphasise that these variables cannot be optimised independently, since moisture strongly modulates the effective temperature and aeration regime experienced by the fungal biomass [7,8].

Table 1: Representative substrates used for solid-state cellulase production by Trichoderma species and reported outcomes.

Substrate	Species/strain	Reported outcome	Reference
Wheat bran	Trichoderma reesei RUT C30	3.8 U/gds FPase after optimization (6.2-fold over baseline)	[9]
Wheat bran	Trichoderma reesei CBS 836.91	8-10 IU/gds FPase, 15.6-17.8 IU/gds endoglucanase at lab scale	[10]
Wheat bran	Trichoderma sp. RCK65	145 U/gds CMCase, 38 U/gds FPase, ~83% saccharification yield	[11]
Sweet sorghum bagasse	Trichoderma harzianum HZN11	30.32 U/gds endoglucanase (2.31-fold increase) via CCD	[6]
Wheat straw	Trichoderma viride	Peak FPase and CMCase activity at approximately 144 h	[17]

Figure 1: Reported maximum cellulase activities from representative solid-state fermentation studies on wheat bran using Trichoderma species [15,9,10,11].



Nitrogen Supplementation and Carbon-to-Nitrogen Ratio

Nitrogen availability shapes both fungal growth and the transcriptional induction of cellulase genes. In several *Trichoderma* SSF systems, supplementing wheat bran with inorganic nitrogen or phosphate sources measurably alters enzyme output, though the direction of the effect is strain- and system-dependent: one Plackett-Burman screen found K2HPO4 supplementation raised activity while added nitrogen was

excluded as unhelpful for that particular mutant [15], whereas nitrogen regulatory mechanisms acting through glutamine-sensing pathways are documented more generally as governing cellulase gene expression across filamentous fungi [18]. Comparative work on *Trichoderma* SG2 found that submerged cultures needed carefully balanced peptone, yeast extract and ammonium sulfate to reach appreciable activity, whereas SSF produced substantially higher biomass-hydrolysing enzyme activities under comparable nutritional inputs, underscoring that the solid matrix itself buffers nitrogen effects differently than liquid culture [19]. These findings collectively indicate that nitrogen source and concentration must be screened empirically for each *Trichoderma* strain-substrate combination rather than assumed from submerged-fermentation precedent. Because wheat bran and most other agro-residues already carry appreciable residual protein and free amino nitrogen, the marginal benefit of supplementary nitrogen salts in SSF is often smaller than in defined submerged media, which likely explains why some Plackett-Burman screens rank nitrogen sources as neutral or even mildly inhibitory factors once the natural substrate composition is accounted for [15,11].

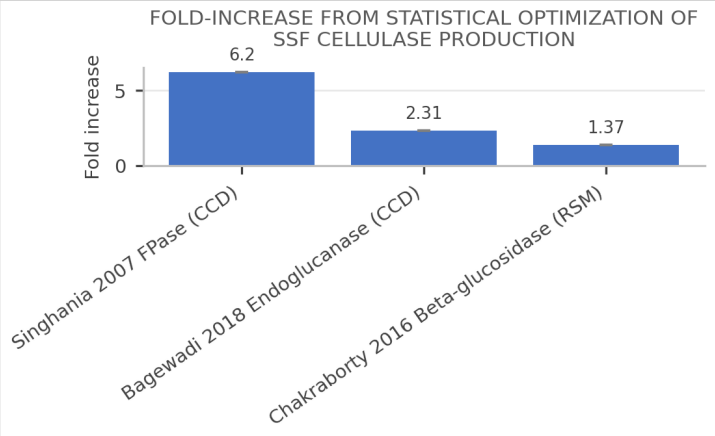
Statistical Optimization Approaches: Plackett-Burman and Response Surface Methodology

Because SSF cellulase production depends on numerous interacting variables, one-factor-at-a-time approaches are inefficient, and the literature has increasingly adopted two-stage statistical designs. Plackett-Burman screening first ranks candidate variables by effect size using a small number of runs, after which the most influential factors are refined using response surface methodology (RSM), typically a central composite or Box-Behnken design [6,20]. Applying this pipeline, *T. reesei* RUT C30 cellulase activity rose 6.2-fold on wheat bran [9], and *T. harzianum* HZN11 endoglucanase production rose 2.31-fold to 30.32 U/gds on sweet sorghum bagasse after optimising protease peptone, lactose, MgSO4 and K2HPO4 levels via a 31-run central composite design [6]. RSM has also been applied to co-production of cellulase alongside xylanase, and to *T. gamsii* M501 for combined enzyme optimisation and downstream saccharification performance [20], while *T. viride* cellulase yields have likewise been improved through RSM-guided design [5]. Table 2, under this section, summarises the design type, optimised variables and reported improvement for four representative studies. Figure 2, also under this section, plots the reported fold-increases achieved by statistical optimisation relative to unoptimised or screening-stage baselines.

Table 2: Statistical optimization designs applied to Trichoderma solid-state cellulase production.

Study/strain	Design used	Key variables optimized	Reported improvement
Trichoderma reesei RUT C30 [9]	Plackett-Burman + central composite design	Moisture content, temperature	6.2-fold increase in FPase activity
Trichoderma harzianum HZN11 [6]	Plackett-Burman + central composite design (31 runs)	Protease peptone, lactose, MgSO4, K2HPO4	2.31-fold increase in endoglucanase activity
Trichoderma reesei mutant [15]	Plackett-Burman screening	Moisture, K2HPO4, temperature, incubation time	Moisture identified as dominant factor (effect +1.16)
Trichoderma reesei RUT C30 [26]	Statistical process optimization	Moisture, inoculum, incubation time	Systematic improvement over unoptimized process

Figure 2: Fold-increase in cellulase or endoglucanase activity achieved through Plackett-Burman screening followed by response surface optimization in three representative studies [9,6,11].



Strain Improvement and Enzyme Complex Considerations

Process optimisation interacts with, rather than substitutes for, strain properties. RUT-C30 itself arose from three rounds of mutagenesis of QM6a that deregulated carbon catabolite repression and increased secretion capacity, a genetic background that partly explains why so many SSF optimisation studies use this strain as a benchmark [3]. Whole-genome sequencing of *T. reesei* revealed a comparatively modest complement of cellulolytic genes relative to some other fungi, implying that high extracellular titres depend heavily on strong secretion and regulatory efficiency rather than gene number alone [4], while broader genomic surveys of the genus continue to identify additional biotechnologically relevant *Trichoderma* strains and gene clusters [21]. Achieved cellulase activity also reflects the composition of the secreted enzyme complex: endoglucanases, cellobiohydrolases and β-glucosidases act synergistically on cellulose of intermediate crystallinity, and imbalances among these components, particularly limiting β-glucosidase activity, can cap overall saccharification performance even when filter-paper activity appears high [22,23]. Recognising this synergy has motivated both substrate- and host-level engineering, including alternative solid carriers that improve fungal-substrate contact and oxygen transfer during SSF [12,14], and recombinant strain work such as *T. reesei* ZU-02, engineered for enhanced cellobiohydrolase output on top of its already high native titres on corn cob residue in submerged culture [24,25] — a reminder that liquid-phase strain benchmarks remain a useful comparator even where SSF is the ultimate production format. Mekala et al. likewise applied a structured statistical optimisation pipeline to RUT C30 in SSF, reinforcing that the same two-stage screening-then-refinement logic recurs across independent laboratories and substrates [26].

Conclusion

Published evidence across *Trichoderma reesei*, *T. viride* and *T. harzianum* consistently shows that no single SSF variable determines cellulase output: substrate choice (wheat bran

performing particularly well), moisture content, pH, temperature, incubation time and nitrogen supplementation interact, and disentangling their effects requires Plackett-Burman screening followed by response-surface refinement. Where this two-stage statistical pipeline has been applied, reported fold-improvements of two- to six-fold over unoptimised baselines are common, and some optimised systems have been carried successfully from flask to rotating-drum reactor scale. Strain background and the balance of endoglucanase, cellobiohydrolase and β-glucosidase activities within the secreted complex remain equally important, so future gains are likely to come from combining statistically optimised SSF conditions with continued strain and substrate engineering rather than from process optimisation alone.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Bizjak R conceived the review, conducted the literature search, and drafted the manuscript. Marchand N contributed the microbiological and strain-background context and critically revised the manuscript. Hofer L contributed the bioprocess-engineering and scale-up framing and approved the final version for submission.

Ethics Statement

Not applicable. This is a narrative review of previously published solid-state fermentation literature and did not involve new experimental data, human participants, or animal subjects.

Data Availability

No new data were generated or analyzed for this review. All sources synthesized are cited in the reference list and are available through their original publishers.

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