Research on the Production of Single-Cell Protein from Gibberellin Fermentation Waste Liquid Using Microbial Methods

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ABSTRACT Efficient monitoring and intelligent regulation of industrial bioprocesses are increasingly important for sustainable resource utilization and advanced manufacturing systems. This study investigates the production of single-cell protein (SCP) from gibberellin fermentation waste liquid using the unconventional yeast *Nectaromyces rattus*, aiming to achieve simultaneous pollutant removal and biomass valorization. Bottle culture experiments and tank reactor simulations were conducted to evaluate the effects of wastewater dilution ratio and glucose supplementation on microbial growth, nitrogen assimilation, and SCP production. The results indicate that under a 12-fold wastewater dilution with 10 g/L glucose addition, corresponding to an optimized C/N ratio of approximately 18:1, the dry cell weight reaches 192.336 g/L and the ammonia nitrogen removal efficiency attains 85.54%. Furthermore, systematic analyses of strain adaptation, fermentation optimization, and quality evaluation demonstrate the feasibility of integrating biological treatment with high-value protein production for industrial wastewater recycling. The proposed strategy establishes an effective framework for sustainable biomanufacturing and process intensification. In addition, its reliance on real-time reactor monitoring, multiparameter process control, and intelligent optimization provides valuable engineering references for electromagnetic sensing technologies, wireless monitoring architectures, antennaassisted industrial instrumentation, and advanced signal acquisition systems used in next-generation bioprocess automation.

INDEX TERMS single-cell protein, gibberellin fermentation waste liquid, unconventional yeast, electromagnetic sensing, wireless bioprocess monitoring

I. INTRODUCTION

THE rapid expansion of gibberellin industrial fermentation has generated high-concentration organic waste water that increasingly threatens the water quality standards required for textile dyeing and processing, making its treatment a serious environmental problem urgently needing to be addressed. Effective management of these complex pollutants is essential to ensure that the textile industry maintains the high-purity water resources necessary for sustainable production and environmental compliance. This wastewater is characterized by a total organic carbon content of 12.3% and a free ammonia nitrogen content of 0.78%. While traditional physicochemical treatment methods have some applicability, their development is limited by high operating costs and the potential for secondary pollution. Microbial technology, on the other hand, is economical, efficient, and environmentally friendly, and is gradually becoming one of the important means to achieve the resource utilization of wastewater. Unconventional yeasts possess robust tolerance and protein synthesis capabilities, offering significant advantages in degrading textile processing waste while simultaneously producing single-cell proteins on a large scale. This integration of biological treatment with high-value byproduct recovery has important theoretical and practical significance for promoting sustainable technological innovation and resource efficiency within the textile industry.

II. THE SIGNIFICANCE OF RESEARCH ON THE PRODUCTION OF SINGLE-CELL PROTEIN FROM GIBBERELLIN FERMENTATION WASTE LIQUID BY UNCONVENTIONAL YEAST

The research on the technology of preparing single-cell protein from unconventional yeast using gibberellin fermentation waste liquid has important theoretical and practical significance (as shown in Figure 1).

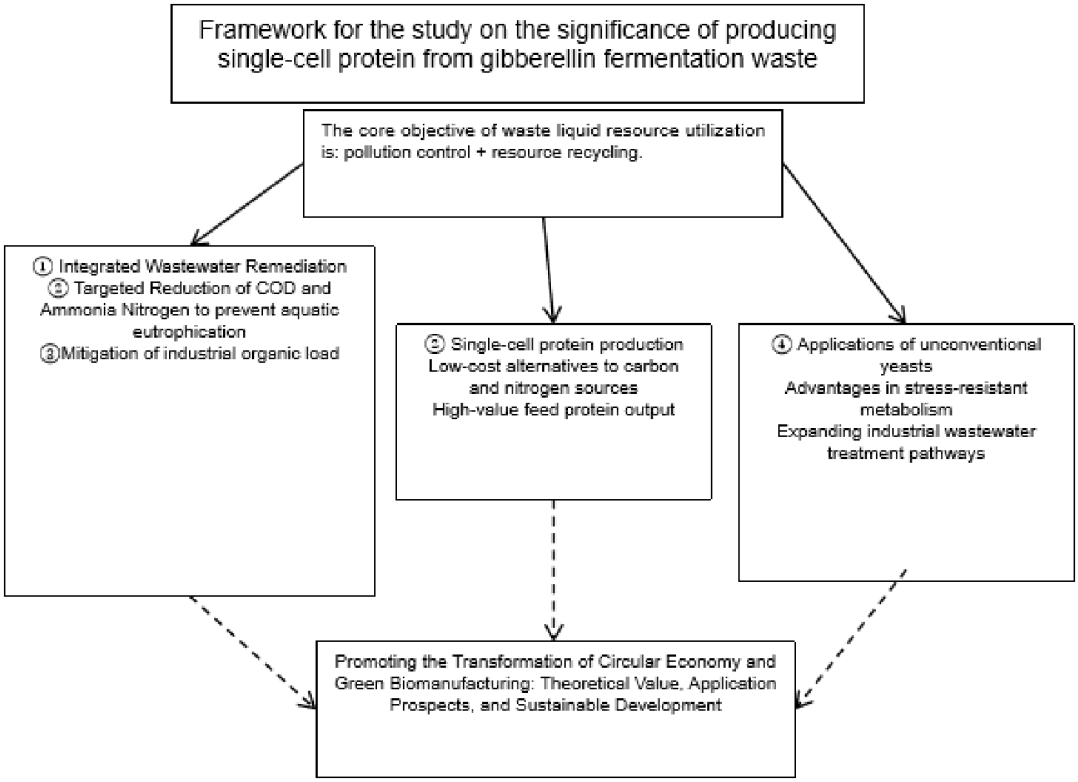

FIGURE 1. Framework diagram illustrating the significance of unconventional yeast consuming gibberellin fermentation waste liquid to produce single-cell proteins

PROMOTE THE RESOURCE UTILIZATION AND POLLUTION CONTROL OF GIBBERELLIN FERMENTATION WASTE LIQUID

The industrial fermentation of gibberellin produces waste liquid containing high concentrations of organic matter, free amino acids and ammonium nitrogen. The chemical oxygen demand is high. If it is discharged directly, it will cause serious eutrophication pollution to the receiving water body. Traditional physicochemical treatment methods are costly and have low resource utilization, making it difficult to truly achieve high-value conversion of waste liquid [1]. Using microbial fermentation to convert organic carbon and nitrogen in waste liquid into high-protein bacterial biomass can reduce the pollution level of waste liquid and produce single-cell protein with feed industry value at the same time. It is a green technology route that combines pollution control and resource recycling, which is in line with the circular economy and sustainable development strategy [2].

EXPANDING THE SOURCES OF RAW MATERIALS AND BIOTRANSFORMATION PATHWAYS FOR SINGLE-CELL PROTEIN PRODUCTION

Single-cell proteins have good application prospects in solving the global protein supply and demand contradiction due to their balanced amino acid composition, short production cycle and no dependence on arable land resources. At present, large-scale industrial production mainly uses glucose as raw material, which is costly. This study demonstrates that by achieving a specific C/N balance of 18:1 through controlled glucose supplementation in the waste liquid, the metabolic efficiency of unconventional yeast is significantly enhanced compared to traditional substrates. Some studies have pointed out that there are a large amount of degradable organic matter and multiple nitrogen sources in the gibberellin fermentation residue, which can be used as a low-cost alternative matrix, which is not only conducive to energy conservation and emission reduction, but also opens up a new way for waste recycling [3]. Compared with traditional Saccharomyces cerevisiae, unconventional yeasts such as N. rattus exhibit non-canonical metabolic traits, including the ability to utilize a wider range of non-hexose carbon sources and superior tolerance to high osmotic pressure and organic acid inhibition. Unlike S. cerevisiae, which primarily relies on the Crabtree effect, N. rattus maintains high biomass productivity in complex gibberellin waste liquids by avoiding ethanol overflow and demonstrating enhanced nitrogen assimilation efficiency under industrial stress [4].

PROVIDING THEORETICAL BASIS AND TECHNICAL SUPPORT FOR UNCONVENTIONAL YEAST INDUSTRIAL APPLICATIONS

Unconventional yeasts generally possess the ability to withstand extreme environmental conditions such as high os-

motic pressure, acid and alkali resistance, and high salinity, and have broad application prospects in the fields of industrial wastewater treatment and biological resource utilization. However, current research on the growth characteristics, pollutant assimilation mechanisms, and protein metabolism patterns of unconventional yeasts in complex industrial waste liquids is still insufficient [5]. Using gibberellin fermentation by-products as an experimental model, in-depth research on the growth kinetics and metabolic regulation patterns of N. rattus in this medium can not only provide theoretical support for its large-scale application, but also provide technical experience for other atypical yeast strains to adapt to similar working conditions [6].

III. PRACTICAL PROBLEMS IN THE PRODUCTION OF SINGLE-CELL PROTEIN FROM GIBBERELLIN FERMENTATION WASTE LIQUID USING UNCONVENTIONAL YEAST

Currently, this technical approach faces three types of mutually restrictive core problems in its actual implementation, and their logical relationship is shown in Figure 2.

THE GIBBERELLIN FERMENTATION WASTE LIQUID HAS A COMPLEX COMPOSITION, MAKING YEAST STRAIN SCREENING AND ADAPTATION DIFFICULT

The chemical composition of gibberellin fermentation waste liquid is very complex. It contains a variety of macromolecular organic acids, unconsumed fermentation substrates, pigments, and metabolic byproducts in the gibberellin fermentation process. The concentration of each component will fluctuate greatly due to different batches and processes. This highly heterogeneous characteristic makes it difficult to screen yeast strains in a targeted manner. Traditional screening methods are difficult to screen out dominant strains that are highly adaptable to gibberellin fermentation waste liquid in a short period of time. The osmotic pressure stress caused by high concentration of organic acids in the waste liquid, the low dissolved oxygen environment, and the fluctuation of pH value will affect the normal metabolic activities of yeast, making the cells unable to grow normally during the lag phase [7]. At present, the work on the screening of tolerant yeasts is mostly focused on single waste liquid types such as brewing wastewater and molasses waste liquid, while there are few systematic strain adaptability evaluations and domestication studies for gibberellin fermentation waste liquid, and the available adaptive strain resources are very limited [8].

THE SINGLE-CELL PROTEIN CONVERSION EFFICIENCY IS LOW, AND THERE IS A LACK OF SYSTEMATIC RESEARCH ON THE OPTIMIZATION OF FERMENTATION PROCESS PARAMETERS

In the single-cell protein production system constructed using gibberellin fermentation waste liquid as raw material, the biomass accumulation efficiency of the cells is regulated by a variety of factors. The main factors such as substrate nutrient composition, dilution ratio, added carbon source concentration, aeration rate and pH adjustment will affect it. Existing studies have shown that when the original liquid is used directly as a culture medium, the growth of yeast will be greatly inhibited. Appropriate dilution can improve the initial growth conditions, but the low substrate concentration limits the potential for the generation of the target product. While general wastewater treatment protocols are well-established, there is a lack of specialized research on the synergistic metabolic flux optimization between gibberellin-specific waste liquid dilution ratios and supplemental carbon sources for protein synthesis. Current literature often treats these as isolated environmental remediation steps rather than an integrated bio-manufacturing system designed to maximize amino acid profiles [9]. The response surface has not been systematically studied, nor has the global optimal solution been explored. During the engineering scale-up process, the stability of the tank reactor is often compromised by significant variations in oxygen transfer rates (OTR) compared to bench-top bottle cultures. While bottle cultures are limited by surface aeration, the mechanical agitation and sparging in tank reactors alter the metabolic flux toward biomass synthesis; therefore, a systematic comparison of volumetric oxygen mass transfer coefficients across scales is essential to ensure consistent dry matter yields and prevent shifts toward fermentative rather than oxidative pathways [10].

THE PRODUCT QUALITY EVALUATION SYSTEM IS INCOMPLETE, WHICH RESTRICTS SAFETY AND INDUSTRIALIZATION PROMOTION

Single-cell protein prepared from industrial waste liquid is stable and safe, but its metabolites, heavy metals and trace metal ions will be enriched and absorbed by microorganisms during fermentation, which may affect the food safety of downstream products. Therefore, there is a lack of systematic detection and evaluation system to ensure the food safety of such products, specifically regarding the potential bioaccumulation of residual gibberellin—a potent plant growth regulator—and its secondary metabolites within the yeast biomass. Assessing the hormonal disruption risks and long-term toxicity of these trace residues in the food chain is a critical prerequisite for industrial application [11]. There is little research data on the residual level of harmful substances and long-term health effects. There is also little information on the amino acid composition distribution, nutritional characteristics and digestibility of such products, which cannot meet the needs of large-scale promotion in the fields of animal husbandry and aquaculture [12].

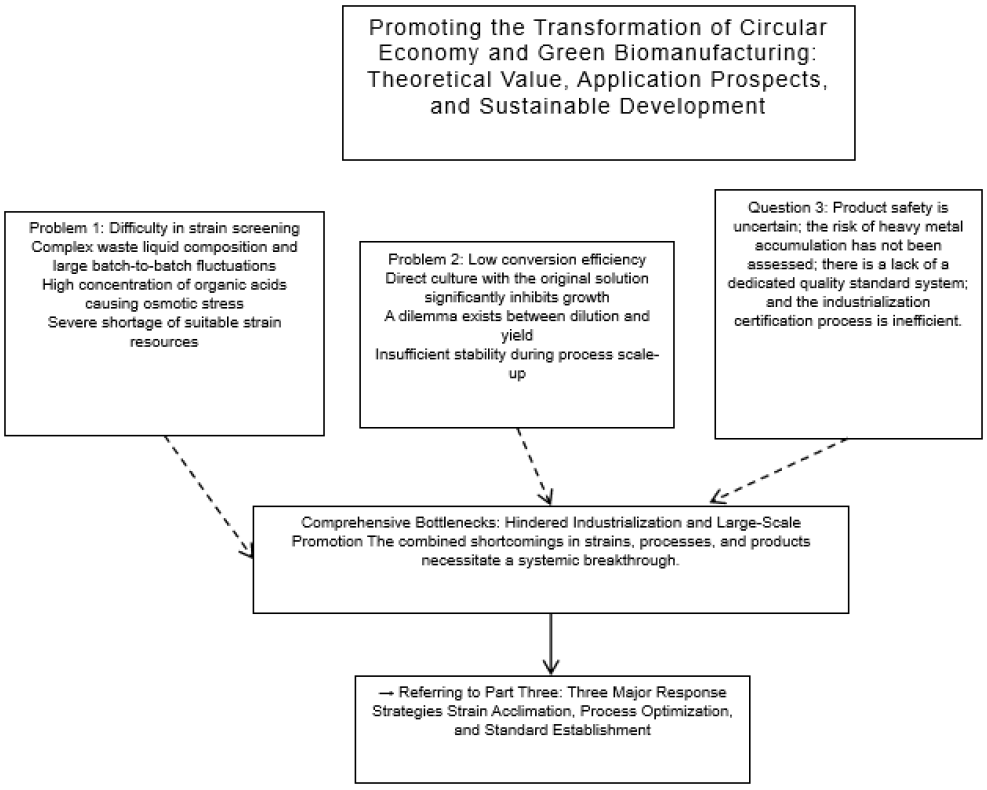

FIGURE 2. Relationship between practical problems in the production of single-cell protein from gibberellin fermentation waste liquid by unconventional yeast

IV. STRATEGIES FOR UNCONVENTIONAL YEAST TO UTILIZE GIBBERELLIN FERMENTATION WASTE LIQUID TO PRODUCE SINGLE-CELL PROTEIN

TARGETED SCREENING AND DOMESTICATION OF UNCONVENTIONAL YEAST STRAINS WITH TOLERANCE BASED ON WASTE LIQUID CHARACTERISTICS

Since the gibberellin fermentation waste liquid has a complex composition and contains a variety of inhibitory factors, the strain screening work should be carried out in combination with its actual physicochemical properties and a corresponding stress gradient screening system should be established. The main physicochemical parameters (pH value, conductivity, osmotic pressure, total organic carbon content, etc.) in the waste liquid were comprehensively measured to draw the fingerprint spectrum of potential inhibitory substances and provide data support for experimental design. On this basis, the waste liquid diluted stepwise was used as a selective culture medium and the proportion of waste liquid was gradually increased to adapt and domesticate the target strains, enriching the dominant individuals that can grow and metabolize normally in high concentration environments. During domestication, attention should be paid to the metabolic activation efficiency of the strains in the lag phase, the specific growth rate in the logarithmic growth phase and the maximum biomass in the stationary phase. Transcriptomics technology was used to analyze the gene expression of tolerant strains in the face of waste liquid stress, find the key functional genes involved in osmotic pressure response and nitrogen metabolism regulation, and provide targeted modification molecular target support, which greatly improves the accuracy and prospectiveness of the screening work [13]. The specific operation procedures of each stage are shown in Table 1.

TABLE 1. Targeted Screening and Domestication Strategies for Unconventional Yeast Tolerant Strains

Stage	Core Practices	Key Indicators to Watch
Waste liquid characterization	The system measures physicochemical parameters and plots fingerprints of the waste liquid.	pH, osmotic pressure, TOC, types of inhibitors
Gradient stress screening	Candidate strains were screened in parallel using serially diluted waste liquid as culture medium.	Lag period duration, specific growth rate
Adaptive domestication	Gradually increase the proportion of waste liquid for subculture domestication.	Upper limit of biomass CDW, ammonia nitrogen assimilation efficiency
Molecular mechanism analysis	Transcriptome analysis of gene expression changes under wastewater stress.	Key genes in osmotic response and nitrogen metabolism

OPTIMIZATION OF FERMENTATION CONDITIONS AND CONSTRUCTION OF A HIGH-EFFICIENCY BIOTRANSFORMATION PROCESS SYSTEM

After determining the appropriate strain, the optimization of the fermentation process system becomes the key link to improve the yield of single-cell protein, which mainly includes three aspects: waste liquid pretreatment, culture medium formulation design and process parameter control. In the waste liquid pretreatment stage, it is necessary to comprehensively analyze the effects of technologies such

TABLE 2. Multi-level Optimization Framework for Gibberellin Waste Liquid Single-cell Protein Fermentation Process

Optimization level	Key variables	Optimization methods	Target Indicators
Waste liquid pretreatment	Filtration method, pH adjustment range, dilution ratio	Single-factor comparison experiment	Improvement in bacterial growth
Culture medium ratio	C/N ratio, type and concentration of exogenous carbon source	Orthogonal or response surface design	CDW maximize
Process parameter control	Temperature, aeration rate, stirring speed, inoculum size	Multiparameter response surface model	The optimal synergy between protein yield and ammonia nitrogen removal rate
Tank enlargement	Dissolved oxygen feedback strategy, dynamic pH control, and feeding sequence	Online monitoring and process control	Batch stability and process repeatability

as centrifugation filtration, pH adjustment and appropriate dilution on the growth performance of microorganisms, and then establish a standard pretreatment process based on batch characteristics to reduce the risk of fermentation instability caused by raw material fluctuations. For the culture medium preparation problem, the focus is on studying the mechanism of the carbon-nitrogen ratio on the synthesis efficiency of the target product, and the optimal C/N balance is achieved by supplementing external nutrients or adjusting the substrate composition. The optimization of fermentation process parameters should focus on core variables such as temperature, aeration rate, stirring speed and inoculation amount, and use a multi-factor interactive response surface model to obtain the global optimal configuration. For large-scale fermentation in tanks, the balance control of dissolved oxygen transfer and microbial oxygen consumption characteristics should be considered, and the culture

conditions should be adjusted at any time in combination with the dynamic change law of pH value to avoid damage to cell activity due to excessive acidification [14]. The important factors and objective functions at each level are shown in Table 2.

ESTABLISHMENT OF SINGLE-CELL PROTEIN QUALITY STANDARDS AND IMPROVEMENT OF INDUSTRIALIZATION SAFETY EVALUATION MECHANISM

To promote the industrialization of gibberellin fermentation waste liquid for single-cell protein production, it is necessary to carry out quality control and safety evaluation at the same time. The establishment of quality standards should refer to the current national standards for feed and food protein products, design a technical parameter system including core indicators such as total protein content, amino acid composition ratio, digestibility and absorption performance, and ash content, and determine the minimum quality requirements under each application scenario. Safety hazard prevention and control should establish a comprehensive risk factor screening mechanism, mainly focusing on metabolite residue levels, heavy metal exceedance, nucleic acid component variation and other hidden dangers, and use standardized animal model experiments to explore the characteristics of its long-term impact on human health. During the industrial transformation and upgrading, the focus should be on promoting the core process links to

TABLE 3. Indicator System for Single-Cell Protein Quality and Safety Evaluation of Gibberellin Waste Liquid as Substrate

Evaluation Dimensions	Testing items	Reference	Applicable Scenarios
Basic nutrition	Crude protein content, amino acid composition, digestibility, and ash content	National Standards for Feed Ingredients	Livestock and aquatic feed
Safety risk factors	Gibberellin metabolite residues and heavy metal accumulation	Feed Hygiene Standard GB13078	Universal for all scenarios
Nucleic acid content	Total nucleic acid content	Single-cell protein nucleic acid limits	Human/Feed Grades
Animal feeding verification	Palatability, growth performance, organ pathology	OECD Feed Safety Assessment Procedures	Industrialization Access
Industrialization Certification	Clean production certification, quality traceability and grading system	Clean Production Audit Guidelines	Large-scale promotion

obtain clean production certification, establishing a product traceability system and hierarchical quality control mechanism, relying on the government-industry-academia-research collaborative innovation model, building a product evaluation and verification network for the entire industrial chain, and accelerating the transformation of scientific and technological achievements into actual productivity [15]. Table 3 gives the important evaluation indicators and specific standards for product quality and safety.

V. CONCLUSION

The resource utilization of gibberellin fermentation waste liquid represents a critical pathway for the green development of the biomanufacturing industry, particularly in providing sustainable chemical inputs and treated water for the textile industry. Optimizing this recovery process ensures that the textile industry reduces its environmental footprint while maintaining the high-performance material standards required for modern production. The technical route of using unconventional yeast waste liquid to produce single-cell proteins meets the dual objectives of pollution control and resource recovery, possessing significant theoretical value and application prospects. This study systematically investigates the growth characteristics and transformation patterns of Nectaromyces rattus in gibberellin fermentation waste liquid. The results show that the CDW of this strain can reach 192.336 g/L under a 12-fold dilution and 10 g/L glucose addition, and the ammonia nitrogen degradation rate can reach 85.54% in tank fermentation. While these metrics demonstrate high biotransformation efficiency, the ultimate feasibility of this technical route as a commercial feed source remains provisional, pending comprehensive toxicity assessments and heavy metal enrichment analysis to ensure compliance with global food safety standards. However, further work is needed on aspects such as strain adaptability, process parameter optimization, product safety evaluation, and standardization. Future research should focus on optimizing waste liquid nutrient structure and strain metabolic enhancement to provide the textile industry with high-quality recycled water and bio-based auxiliaries, advancing unconventional yeast resource utilization technology toward greater efficiency and safety. By perfecting process scale-up control, these scientific advancements will provide robust technical support for the green and low-carbon transformation of both the biomanufacturing and textile industries.

AUTHOR CONTRIBUTIONS

Yiheng Li designed, collected and analyzed the data, and drafted the manuscript. Yiheng Li conducted the study, critically revised the manuscript for important intellectual content, and gave final approval of the version to be published. Yiheng Li participated fully in the work, take public responsibility for appropriate portions of the content, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONFLICTS OF INTEREST

The author declares no conflict of interest.

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