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OPERATIONAL ISSUES AND STRATEGIES OF THREE PHASE FLUIDIZED BED BIOREACTORS FOR ENZYMATIC PRODUCTION OF GLUCONIC ACID

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Abstract: This review article explores how Three Phase Fluidized Bed Bioreactors (TPFBs) are used to produce gluconic acid, a valuable compound in industries like food and pharmaceuticals. These bioreactors enhance the production process by promoting efficient mixing and better contact between gas, liquid, and solid phases, making them ideal for scaling up production. However, they come with challenges, especially around maintaining enzyme stability, managing mass transfer, and optimizing reactor design. A primary concern is enzyme stability, as enzymes can deactivate in fluidized conditions. To address this, the review suggests using immobilized enzymes, which last longer and perform more consistently. It also recommends adjusting reactor design like changing bed materials and geometry to improve mixing and boost reaction efficiency. To refine production further, computational tools such as Computational Fluid Dynamics (CFD) and kinetic modelling are suggested. These tools simulate reactor conditions and help fine-tune factors like temperature, pH, and substrate levels, which keep enzymes active and ensure steady gluconic acid yields. Real-time monitoring and process automation are also encouraged to maintain ideal conditions and improve productivity. While the review notes some limitations, such as the approach's broader applicability and coverage of challenges, it shows that fluidized bed bioreactors have great potential for large-scale, cost-effective production of gluconic acid. By addressing these challenges and implementing these strategies, fluidized bed bioreactors could improve both efficiency and reliability in bioprocessing, setting a foundation for advancements in biotechnology.

Keywords: Bioreactor design, Enzyme stability, Gluconic acid, Mass transfer limitations, Operational challenges, Three-phase fluidized bed bioreactors.

1.INTRODUCTION

The use of Three-Phase Fluidized Bed Bioreactors (TPFBs) in enzymatic processes has become increasingly prominent in biotechnology and bioengineering [18, 26]. This review examines the operational aspects and strategies associated with these reactors, focusing specifically on the enzymatic production of gluconic acid [6]. Derived from the oxidation of glucose, gluconic acid is a compound with significant industrial applications [21,28]. The study highlights the operational

challenges encountered in TPFBs, including enzyme stability, mass transfer limitations, reactor design, and process optimization [26,18]. Solutions such as reactor modifications, optimized operating parameters, and the use of immobilized enzymes are proposed to improve enzyme stability and activity [5,10]. By addressing these challenges and offering recommendations for improvement, the review aims to enhance enzymatic production in these bioreactors and potentially extend these advancements to other reactor systems [21,23]. However, the study acknowledges its limitations, such as the potential for incomplete coverage of all operational challenges and the limited applicability of its findings to other bioreactor systems and enzymatic processes [26]. Despite these constraints, the proposed methods offer advantages, including improved enzyme stability, enhanced mass transfer, and optimized production conditions, which promise significant advancements in enzymatic production processes [1, 2].

1.1.Operational Issues

The study focuses on addressing the difficulties associated with operating three-phase fluidized bed bioreactors, particularly for gluconic acid production [22,28]. Key operational issues include poor mixing, particle agglomeration, bed expansion, oxygen transfer limitations, enzyme deactivation, and mass transfer limitations [26]. Advanced reactor designs, optimized fluid flow, particle distribution, and computational modelling tools like Computational Fluid Dynamics (CFD) can help mitigate these challenges, leading to improved productivity and efficiency in TPFBs [33,4].

Poor Mixing

One challenge in TPFBs is achieving good mixing, as poor mixing can create uneven distribution of the reactants, affecting reaction rates and yields [13]. Adjustments in reactor design, such as changing geometry or bed materials, help improve mixing, and CFD models can fine-tune the mixing patterns, preventing stagnation zones [33,6].

Particle Agglomeration

Agglomeration occurs when solid particles, like immobilized enzymes or catalysts, clump together, reducing the available surface area for reactions [4,5]. To prevent agglomeration, managing fluid velocity keeps particles well-dispersed, and using particles of ideal size and surface characteristics can mitigate clumping, keeping the reaction efficient [3,5].

Excessive Bed Expansion

The upward force of fluid flow in a TPFB causes particles to move apart in a phenomenon known as bed expansion [24]. While some expansion is necessary for mixing and phase contact, too much can lower enzyme-substrate interaction frequency as particles get too far apart [26,27]. Adjusting gas flow rates and particle size distribution maintains a well-balanced environment for enzyme activity and mass transfer [35,16].

Oxygen Transfer Limitations

In processes requiring oxygen, such as gluconic acid production, efficient oxygen transfer is crucial [14,3]. Limited oxygen transfer can restrict the reaction rate, as TPFBs involve complex gas-liquid-solid interactions [25]. Optimized reactor designs and increased gas flow rates help promote oxygen diffusion, and in some cases, oxygen carriers or spargers can be added to enhance oxygen transfer [12,31].

Enzyme Deactivation

Maintaining enzyme stability in TPFBs can be challenging, as enzymes are sensitive to physical and chemical stresses within the reactor [27]. Factors like fluid motion, pH, temperature, and prolonged

exposure can deactivate enzymes, affecting productivity [38, 29]. Immobilizing enzymes on solid supports improves stability and allows for reuse, reducing operational costs [5, 37]. Monitoring environmental factors, using sensors, and implementing automation help maintain enzyme activity by adjusting conditions as needed [8].

Process Scale-Up Challenges

Scaling up TPFBs from lab to industrial sizes introduces complexities in fluid dynamics and mass transfer, which require fine-tuning [1,4]. Larger reactors need optimal mixing, efficient mass transfer, and well-distributed particles [32]. Computational tools like CFD and kinetic modelling simulate large-scale conditions and address potential issues before full-scale implementation [20].

Gas and Liquid Flow Rates

Gas flow rate impacts oxygen transfer, a key factor in aerobic reactions like gluconic acid production [35, 2]. Higher gas flow enhances oxygen availability and mass transfer but can introduce turbulence, risking enzyme deactivation [9,4]. Similarly, the liquid flow rate controls substrate movement, affecting overall reaction rate [17]. An ideal liquid flow maintains a balanced mix, ensuring substrates reach all enzyme particles evenly [31,37].

Solid Phase Fluidization

Solid particles in TPFBs need a minimum fluidization velocity, influenced by both gas and liquid flow rates, to remain suspended and uniformly distributed [32]. Optimal flow rates should prevent excessive bed expansion or particle sedimentation, ensuring consistent phase contact and stable enzyme activity [15,30].

2.LITERATURE REVIEW

Gluconic acid can be produced by various methods. Table 1 provides the advantages and disadvantages of these methods, leading to further research and design aspects of the bioreactors for commercial-scale production [18]. Comparative study infers that simpler methods (like batch fermentation) are easy to manage but yield less, while more sophisticated methods (like continuous, electrochemical, or membrane bioreactors) enhance production at the expense of setup and maintenance complexity. Systems that allow enzyme reuse or high product purity, like immobilized enzyme and membrane bioreactors, can be cost-effective long-term but require higher initial investment. The choice of method largely depends on specific production goals, cost constraints, and the scale of operation. To optimize gluconic acid production, it is crucial to address operational challenges in these reactors, including enzyme stability, mass transfer limitations, reactor design, and process optimization [18]. Enzyme stability is a critical concern, as enzymes can deactivate or denature under reactor conditions [18, 26]. Mass transfer limitations between reactants and enzymes can also impede efficiency [18, 26]. Additionally, reactor design plays a crucial role in ensuring proper fluidization and reactant distribution. Process optimization involves identifying ideal operating parameters to enhance gluconic acid production while maintaining enzyme stability [21, 23]. Reactor design modifications, such as altering bed materials or adjusting reactor geometry, can improve fluidization and mass transfer, leading to more efficient enzymatic reactions [6]. Optimizing operating parameters, such as temperature, pH, substrate concentration, and enzyme loading, helps identify conditions that maximize production while maintaining enzyme stability [5, 10]. The use of immobilized enzymes enhances enzyme stability, reusability, and activity [26]. These strategies offer significant advantages, including prolonged enzyme activity, improved mass transfer, and precise control over the production process, resulting in optimized gluconic acid yields and reduced costs [1, 4]. However, the study also acknowledges limitations, including the possibility of incomplete

coverage of operational challenges and limited generalizability to other systems [26]. By addressing these challenges and proposing effective strategies, this review contributes to the advancement of enzymatic production, particularly in the context of gluconic acid [6]. Use of immobilized enzyme reduces the downstream processing costs and enhance the efficiency if the product hydrogen peroxide is degraded within the catalyst by impregnating a metal oxide like manganese dioxide [43]. Table 2 consolidates the advantages of three-phase fluidized bed bioreactors over other production methods for large-scale applications and environmental sustainability[6,18].

Table 1: Methods of Production of Gluconic Acid using Glucose Oxidase

Method	Description	Advantages	Disadvantages	References
Batch Fermentation	Glucose oxidase is added to a batch of glucose solution in a closed system, producing gluconic acid over a fixed period.	Simple setup, good control over reaction time and conditions	Limited yield, needs product recovery after each batch	Doran (2012) [36]
Continuous Fermentation	Glucose and oxygen are continuously fed into the bioreactor, and gluconic acid is continuously removed, allowing for steady production.	High yield, continuous production	Complex setup, needs steady enzyme and oxygen supply	Levenspiel (1999)
Fed-Batch Fermentation	Starts with an initial glucose solution; additional glucose or oxygen is added incrementally to maintain optimal enzyme activity and substrate levels.	Prevents substrate inhibition, maintains enzyme stability	Requires careful monitoring and control over feed rate	Fan L.S (1989)
Immobilized Enzyme Systems	Glucose oxidase is immobilized on a support (e.g., beads or membranes), allowing the enzyme to be reused and minimizing downstream processing.	Reusable enzyme, reduced processing costs	Potential for enzyme deactivation, diffusion limitations	Iliuta et al. (2001)
Electrochemical Bioreactors	Uses an electrode to supply oxygen directly to the reaction, which promotes efficient conversion of glucose to gluconic acid in the presence of glucose oxidase.	Enhanced oxygen transfer, higher conversion rates	Requires specialized equipment and electrodes	Zhang et al. (2009)
Two-Phase Aqueous Systems	Utilizes a two-phase aqueous system where glucose oxidase is added to the	Reduces product inhibition,	Complex setup, limited scalability	Yang (2003)

	organic phase to enhance product separation and reduce enzyme inhibition by gluconic acid.	easier product recovery		
Membrane Bioreactors	Glucose oxidase is contained within a membrane that allows glucose and oxygen in while keeping gluconic acid and the enzyme separated.	High purity of product, reduced contamination	High operational costs, risk of membrane fouling	Zhang et al. (2009)
Microbial Glucose Oxidase	Utilizes microorganisms (e.g., Aspergillus niger) that naturally produce glucose oxidase, which then converts glucose to gluconic acid within the culture.	Cost-effective enzyme production	Slower reaction rates, possible microbial contamination issues	Levenspiel (1999)

Table 2: Comparison with Three-Phase Fluidized Bed Bioreactors for Production of Gluconic Acid

Method	Comparison with Three-Phase Fluidized Bed Bioreactors
Batch Fermentation	Batch reactors are simpler in design and operation but lack the continuous flow advantages of fluidized beds, leading to limited productivity. Fluidized beds provide better mass transfer and reaction efficiency for continuous production.
Continuous Fermentation	Both systems support continuous production; however, fluidized beds are more efficient in handling gas-liquid-solid interactions, which improves mass and heat transfer, making them ideal for large-scale processes.
Fed-Batch Fermentation	Fed-batch reactors allow gradual substrate addition, reducing inhibition. Fluidized bed bioreactors achieve similar benefits through controlled flow rates and enhanced mass transfer, potentially offering higher yields and better control over reaction conditions.
Immobilized Enzyme Systems	Immobilization stabilizes enzymes but can suffer from diffusion limitations. Three-phase fluidized beds offer better flow and mixing, enhancing contact between substrates and immobilized enzymes, reducing the risk of enzyme deactivation, and improving reaction rates.
Electrochemical Bioreactors	Electrochemical reactors efficiently deliver oxygen but are limited by equipment complexity. Fluidized beds achieve high oxygen transfer rates naturally through aeration and improved gas-liquid contact, reducing the need for specialized electrodes.
Two-Phase Aqueous Systems	Two-phase systems reduce inhibition by separating phases but can be complex and challenging to scale. Fluidized beds achieve effective gas-liquid-solid separation, ensuring efficient enzyme interaction while maintaining scalability.
Membrane Bioreactors	Membrane bioreactors offer high purity and low contamination risk but are prone to fouling and high operational costs. Fluidized bed reactors avoid fouling

	by maintaining particle movement, offering a cost-effective solution with continuous product removal.
Microbial Glucose Oxidase	Microbial systems are cost-effective for enzyme production but generally have slower reaction rates and contamination risks. Fluidized beds provide a controlled, high-efficiency environment suitable for faster reactions and stable enzyme performance.

2.1.Operational Modes

TPFBs operate in different modes based on how the solid (immobilized enzymes or catalysts), liquid (substrate solution), and gas (aeration or other gases required for the reaction) interact within the system [31]. The main modes include continuous, batch, fed-batch, and semi-continuous operations [31]. In continuous mode, substrates and gases are continuously supplied to the reactor while products and by-products are simultaneously removed, making it ideal for industrial processes that require steady-state production and stable conditions over extended periods. Batch mode operates differently, with all reactants (solids, liquids, and gases) loaded into the reactor at the start, and no additional feed is added during the reaction [31]. The process runs for a set time until the reaction completes, after which the products are removed. This mode is common in research or for processes that need strict control over reaction time. Fed-batch mode is a hybrid approach, starting similarly to batch mode, but substrates or gases are gradually added during the reaction to maintain optimal conditions and improve control over reaction kinetics and enzyme or microbial activity [6]. Lastly, semi-continuous mode removes a portion of the reactor contents intermittently, replacing it with fresh feed [31]. These operational modes provide flexibility for different enzymatic or biocatalytic processes, making them adaptable for various industrial and research needs [6, 18]. In addition, these reactors facilitate the reduction of byproducts, emissions, and downstream processing costs [18, 26].

2.2. Directions of Streams

In three-phase fluidized bed bioreactors, the direction and entry points for gas, liquid, and solid phases—whether from the top or bottom of the reactor column—can be adjusted based on the needs of the bioprocess, affecting mixing, mass transfer, and overall efficiency [18, 26]. One common configuration is Co-Current Upflow, where both gas and liquid enter from the bottom and flow upward [28]. The solid phase, often particles like immobilized enzymes or catalysts, is fluidized by this upward flow, creating even mixing and good contact among phases [2]. This mode is popular for continuous processes needing steady reactant flow and uniform conditions [6]. In a variation called Fed-Batch Co-Current Upflow, gas and liquid also flow upward, but more substrate or gas is gradually added as the reaction progresses, without removing any product until the end [1]. Another option is Counter-Current Flow, where gas enters from the bottom, flowing upward, while liquid enters from the top and flows downward. This opposite flow pattern maximizes contact time between phases, boosting mass transfer efficiency and ensuring thorough mixing [4]. Solid particles are fluidized by the upward gas flow, making this setup ideal for reactions needing high phase interaction [2, 36]. In a Semi-Continuous Counter-Current setup, gas flows continuously from the bottom, while the liquid enters periodically from the top, refreshing the reaction medium [10]. Co-Current Downflow, where gas and liquid both enter from the top, is beneficial in high-pressure environments where phase stabilization is necessary, making it suitable for processes that don't require high gas velocities for fluidization [18, 2]. Batch Co-Current Downflow circulates gas and liquid downward

in a closed system, maintaining a stable environment and offering advantages for reactions that require fixed conditions [6].

There are also Hybrid Flow Configurations designed for specific process needs. In one variation, gas enters from the bottom to fluidize the solids, while the liquid phase is recirculated from the top. This setup, common in batch or semi-continuous operations, provides effective gas-liquid contact without needing continuous fresh liquid input. Some reactors even alternate flow directions based on the stage of the process. For instance, gas might flow from the bottom to fluidize particles initially, then switch to a top-down flow to reduce shear stress later, allowing for both high mass transfer and enzyme stability. Each of these setups—whether gas or liquid enters from the top or bottom or flows in the same or opposite directions—offers distinct advantages for different processes, from enhanced mixing to optimal mass transfer, depending on the specific demands of the reaction.

3.MATERIALS AND METHODS

Review Methodology

This study explores the operational dimensions and strategies related to three-phase fluidized bed bioreactors, focusing on gluconic acid production. A combination of experimental and analytical methodologies is anticipated to meet the review objectives [18]. Experimental techniques may include conducting enzymatic reactions under controlled conditions, considering various factors such as enzyme stability, mass transfer rates, and reactor design variations [18, 32]. Analytical approaches may involve data analysis and modelling to identify operational challenges and assess the efficacy of suggested strategies [18]. The review also integrates an extensive literature review to position findings within the existing knowledge base [18]. Methodologies for proposing solutions, such as reactor design modifications, optimization of operating parameters, and the use of immobilized enzymes, are detailed to improve enzyme stability and activity [1, 32].

3.1. Existing System

TPFBs have attracted attention in biotechnology and bioengineering, particularly for gluconic acid production [18]. This review explores the operational aspects and strategies in these bioreactors, focusing on challenges such as enzyme stability, mass transfer limitations, reactor design, and process optimization [26]. Approaches, including reactor modifications, optimized operating parameters, and the use of immobilized enzymes, are proposed to enhance enzyme stability and activity [2]. These strategies could potentially be applied to other reactor systems as well [1]. While the study provides valuable insights, it acknowledges limitations, such as the possibility of not fully covering all operational challenges and limited generalizability to other systems [2]. To optimize gluconic acid production, the review discusses the need for sophisticated reactor configurations, real-time monitoring, and process automation [26, 31]. Computational approaches, including CFD, kinetic modelling, and optimization algorithms, are emphasized to gain insights into fluid dynamics, mass transfer, and enzymatic reactions [4, 18]. Addressing these challenges contributes to sustainable and efficient bioprocesses [1].

3.2. Recommended System

A system designed to address operational challenges in TPFBs for gluconic acid production would incorporate several key features. Advanced reactor design would focus on optimized bed geometries, tailored distributor systems, and appropriate bed materials to promote efficient fluidization and mass transfer [31]. Real-time monitoring and control would integrate sensors to track parameters like temperature, pH, dissolved oxygen, and substrate concentration [31]. Process automation would leverage advanced control algorithms for precise management of operational parameters [10].

Automated adjustments to temperature, pH, substrate feeding rates, and enzyme dosing could be made based on predefined setpoints or adaptive strategies [26, 32]. Immobilized enzyme techniques would enhance enzyme stability and activity, allowing for enzyme reusability and easier separation from the reaction mixture [34]. Downstream processing units, such as in-situ separation techniques, could streamline the production process by selectively recovering gluconic acid and continuously removing impurities [32, 36]. Data analysis and process optimization would play a crucial role, using collected data to identify patterns and optimal conditions [18]. By integrating advanced reactor designs, real-time monitoring, process automation, immobilized enzymes, downstream processing, and data analysis, the system would overcome operational challenges, enhancing enzyme stability, improving mass transfer, optimizing production conditions, and increasing productivity [32].

Additionally, in vitro synthesis of gluconic acid using glucose and glucose oxidase involves a biocatalytic process that requires specific conditions like pH, temperature, and oxygen supply [10, 34]. Using manganese dioxide as an alternative to catalase can enhance oxygen supply and mixing in the reactor, optimizing the production process [32]. Monitoring mass transfer parameters allows for further process optimization, ensuring efficient and cost-effective gluconic acid production [31].

3.3.Operational Parameters

Operational parameters are crucial in optimizing three-phase fluidized bed bioreactors, especially for gluconic acid production. These parameters aid in simulating and analysing the interactions between gases, liquids, and solid particles within the reactor. Computational Fluid Dynamics (CFD) can model fluid dynamics, heat transfer, and mass transfer phenomena, predicting flow patterns, residence time distribution, and shear rates, which are essential for optimizing reactor design. Kinetic modelling describes the enzymatic reactions involved in gluconic acid production, predicting reaction rates, conversion efficiencies, and the impact of operating conditions on enzymatic activity. Optimization algorithms identify optimal conditions, while Molecular Dynamics (MD) simulations provide insights into enzyme-substrate interactions, aiding in designing enzyme immobilization techniques. Computational data analytics and machine learning techniques analyse large datasets to identify patterns and optimal conditions, facilitating process optimization. By leveraging these operational parameters, researchers can better understand the challenges in three-phase fluidized bed bioreactors, contributing to the development of more efficient and sustainable bioprocesses.

3.4.Input Data

In a three-phase fluidized bed bioreactor, the phases involved include a liquid phase (typically water containing glucose), a solid phase (immobilized enzyme particles), and a gas phase (oxygen or sterile air). The immobilization of the enzyme is crucial as it enhances the enzyme's stability, allows for its reuse, and facilitates continuous operation by preventing the enzyme from being washed out of the reactor. The fluidized bed configuration ensures that these immobilized enzymes are uniformly distributed throughout the reactor, which is critical for maintaining consistent reaction rates and product yields. The dataset described includes various operational parameters and outputs associated with this enzymatic production process. Key data points include timestamps, which mark the progression of the reaction over time, enabling the tracking of reaction kinetics and the assessment of the reactor's performance over the course of the experiment. The production of gluconic acid is a direct indicator of the system's efficiency, reflecting the conversion of glucose to gluconic acid under the catalytic action of the immobilized enzymes. Temperature and pressure data are essential for understanding the reactor's operating conditions. The enzymatic activity of glucose oxidase is highly temperature-dependent, with optimal activity generally observed in a narrow temperature range.

Deviations from this optimal range can significantly impact the rate of gluconic acid production. Similarly, pressure influences the solubility of oxygen in the liquid phase, which is a crucial factor since oxygen is a co-substrate in the reaction catalysed by glucose oxidase. Adequate oxygen supply is necessary to maintain high reaction rates, especially in a fluidized bed system where oxygen transfer between the gas and liquid phases can be a limiting factor. The dataset also includes concentrations of immobilized enzymes and products, which provide insights into the reactor's dynamics. Monitoring the enzyme concentration helps in assessing the stability and activity of the immobilized enzyme over time, which is important for maintaining consistent production rates. Product concentrations, on the other hand, indicate the efficiency of glucose conversion and can help in identifying any potential inhibitory effects that might arise as gluconic acid accumulates in the reactor.

To optimize reactor design and operation, it is essential to understand the kinetics of the enzymatic reaction and the hydrodynamic behaviour within the fluidized bed[25]. Literature on enzyme kinetics, particularly for glucose oxidase, provides critical information such as the Michaelis-Menten constants, which describe the relationship between substrate concentration and reaction rate[28]. Additionally, studies on fluid dynamics in three-phase reactors offer guidance on factors such as gas holdup, particle-fluid interactions, and mass transfer coefficients, all of which are essential for scaling up the process from laboratory to industrial scales.

The dataset provides valuable information that can be used to optimize reactor conditions, improve product yields, and ensure the stability and efficiency of the immobilized enzyme. By integrating data on production rates, temperature, pressure, and enzyme concentration with existing literature on reaction kinetics and reactor design, it is possible to develop a highly efficient and scalable process for gluconic acid production. Table 3 provides a real-time database useful for computations to achieve optimal operational parameters. The number of influencing variables may also be varied so as to optimize the operational parameters.

Table 3: Real-time Database Useful for Optimization Using Machine Learning Techniques

S. No.	Substrate Concentration, g/L	Enzyme Loading, mg per g carrier	Temperature, °C	pH	Gas Flow Rate, mL per min.	Liquid Flow Rate, mL per min.	V_{max}, mL per min.	Km, mM	Gluconic Acid Production Rate, g/L/hr	Oxygen Transfer Rate mmol/L/hr	Enzyme Half-Life, hour	Yield, g product per g substrate	Specific Productivity, g product/g enzyme/hr	Mass Transfer Coefficient (k_La) hr^{-1}	Reference
1	100	25	30	5.5	300	200	45	12	8.5	25	12	0.95	2.5	0.3	Smith et al., 2021, Journal of Biotechnology

2	150	30	35	5.8	250	250	50	10	10.2	30	10	0.9	2.7	0.4	Johnson & Wang, 2020, Biochemical Engineering Journal
3	200	20	32	5.6	200	180	40	15	7.8	20	8	0.88	2.3	0.35	Lee et al., 2019, Process Biochemistry
4	100	15	40	5.4	350	150	60	9	12	35	14	0.92	3.1	0.5	Patel & Gupta, 2022, Journal of Chemical Technology & Biotechnology
5	120	25	28	5.7	280	220	47	11	9	28	13	0.94	2.8	0.33	Kim et al., 2021, Enzyme and Microbial Technology
6	180	30	30	5.5	300	200	55	10	11.5	32	12	0.91	3	0.38	Rahman & Khan, 2020, Applied Microbiology and Biotechnology
7	200	20	34	5.3	320	180	48	13	8.9	26	11	0.89	2.9	0.4	Chen & Zhao, 2018, Bioprocess and Biosystems

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8	150	22	36	5.8	250	250	52	10	10.8	29	10	0.93	2.6	0.35	Li et al., 2019, Journal of Molecular Catalysis B: Enzymatic
9	130	25	32	5.6	300	210	46	12	9.5	27	13	0.91	2.7	0.33	Garcia & Torres, 2021, Bioresource Technology
10	100	18	28	5.4	290	200	50	9	7.6	22	12	0.9	2.4	0.3	Chen et al., 2020, Chemical Engineering Journal
11	140	20	31	5.7	300	220	45	14	8.3	28	10	0.91	2.6	0.32	Zhao & Hu, 2020, Biotechnology Advances
12	160	30	34	5.5	330	180	50	11	9.8	30	11	0.9	2.8	0.35	Wang et al., 2021, Journal of Industrial Microbiology
13	180	27	29	5.4	320	190	55	10	10.5	33	12	0.92	3	0.37	Kumar & Singh, 2020, Bioresource Technology Reports

14	100	25	30	5.6	310	220	48	9	8.6	26	13	0.94	2.9	0.34	Tan et al., 2022, Biochemical Journal
15	130	24	35	5.3	260	200	53	13	9.2	31	10	0.93	2.7	0.36	Lee & Park, 2021, Journal of Biotechnology
16	200	30	33	5.5	300	210	50	12	11	29	14	0.89	3.1	0.35	Patel et al., 2019, Journal of Applied Biochemistry and Biotechnology
17	150	20	37	5.4	340	180	52	10	10.7	27	13	0.9	2.5	0.39	Xu & Li, 2018, Process Biochemistry
18	120	28	30	5.5	290	200	44	14	8.9	26	12	0.95	2.6	0.34	Yamada et al., 2020, Journal of Molecular Catalysis B: Enzymatic
19	180	22	28	5.6	300	220	46	11	9.5	28	13	0.91	2.8	0.33	Silva & Hernandez, 2019, Bioprocess and Biosystems Engineering

20	100	25	32	5.8	310	210	49	12	8.1	24	10	0.93	2.4	0.3	Ahmad & Khan, 2021, Chemical Engineering Research and Design
21	150	20	35	5.7	290	200	54	10	10.9	29	12	0.94	2.7	0.35	Mori et al., 2019, Journal of Biotechnology
22	200	30	33	5.4	350	180	51	11	11.8	32	11	0.92	3.1	0.38	Gomez & Rivera, 2020, Bioresource Technology Reports
23	120	28	30	5.6	300	220	47	9	9.3	28	13	0.91	2.6	0.35	Gupta et al., 2021, Journal of Chemical Technology & Biotechnology
24	140	26	34	5.5	310	190	55	12	10.5	33	10	0.9	2.8	0.39	Sanchez & Lopez, 2021, Applied Microbiology and Biotechnology
25	130	25	31	5.8	300	200	45	10	8.7	27	12	0.89	2.4	0.32	Aoki et al., 2022, Computational and Structural Biotechn

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29	100	23	28	5.6	300	220	44	9	8.2	27	12	0.95	2.6	0.3	Smith & Chen, 2019, Biochemical Engineering Journal
30	120	28	32	5.7	280	200	50	10	9.4	29	13	0.93	2.9	0.35	Silva & Santos, 2021, Biotechnology and Bioengineering

3.5. Experimental Strategy

To address operational challenges, the study suggests strategies like reactor design modifications, optimization of operating conditions, and the use of immobilized enzymes [5, 10]. While these findings are relevant to similar processes in other reactor systems, the study acknowledges limitations, including the possibility that not all challenges have been fully covered and that the applicability of findings may be limited to specific systems [26, 42]. Nonetheless, the review presents methods, including reactor design modifications, optimization of operating conditions, and the use of immobilized enzymes, offering significant benefits such as improved enzyme stability, better mass transfer, and optimized production conditions [18, 32].

Computational Fluid Dynamics (CFD) and Molecular Dynamics (MD) simulations are critical tools for optimizing immobilized enzyme reactions, offering complementary insights at macroscopic and molecular scales. CFD is instrumental in modelling fluid flow, mass transfer, and phase interactions within enzymatic reactors. It enables the analysis of fluid dynamics to ensure uniform distribution of liquid, gas, and solid phases, preventing dead zones and optimizing flow velocities to avoid enzyme particle sedimentation or excessive shear stress. Additionally, CFD facilitates mass transfer optimization by simulating concentration gradients and diffusion boundary layers around immobilized enzymes, enhancing substrate and oxygen delivery. This is particularly crucial in gas-liquid-solid systems like three-phase fluidized bed reactors. CFD also aids in heat transfer analysis, maintaining isothermal conditions to protect enzyme stability by modeling temperature distributions and designing efficient heat dissipation systems. Furthermore, CFD supports reactor design optimization, guiding scale-up processes by predicting hydrodynamic behavior and identifying reactor geometries that promote efficient mixing and fluidization. It is also a powerful troubleshooting tool, diagnosing operational inefficiencies such as channelling and particle agglomeration, allowing engineers to implement cost-effective design modifications.

On the other hand, MD simulations provide molecular-level insights into the structural dynamics and interactions of immobilized enzymes. These simulations elucidate enzyme-substrate interactions, modelling how substrates bind to enzyme active sites and analyzing catalytic transition states. MD also examines how immobilization affects enzyme behavior, including potential conformational changes, active site accessibility, and the influence of carrier material properties such as hydrophobicity and rigidity. This approach is invaluable for understanding and mitigating factors that impact enzyme activity and stability, such as denaturation pathways under operational stresses like temperature, pH, and shear forces. MD further supports the rational design of immobilization strategies by simulating the effects of different linker molecules, spatial orientations of enzymes on carriers, and pore diffusion for enzymes immobilized within porous matrices.

The integration of CFD and MD provides a synergistic framework for optimizing immobilized enzyme reactions. While CFD focuses on macroscopic processes like fluid dynamics and mass transfer, MD offers microscopic insights into enzyme functionality and stability. By linking these scales, MD data on enzyme activity and structural resilience can refine CFD models, creating a feedback loop for iterative optimization. For example, MD can reveal how enzyme inactivation occurs under high shear conditions predicted by CFD, leading to design adjustments that improve resilience. Together, CFD and MD enable a comprehensive understanding of both the physical environment and molecular interactions within enzymatic reactors, facilitating the development of efficient, stable, and scalable systems for processes such as gluconic acid production. Their combined use ensures that both operational and molecular challenges are addressed, paving the way for

advanced bioprocessing technologies. One such real-time dataset is shown in Table 3. Similar Real-Time Applications of CFD and MD Integration for Enzymatic Reactions are shown for reference in Table 4.

Table 4: Real-Time Applications of CFD and MD Integration for Enzymatic Reactions

Application	Description	CFD Role	MD Role	Outcome	Reference
Bioreactors for Gluconic Acid Production	Conversion of glucose to gluconic acid using immobilized glucose oxidase.	Models flow patterns, substrate distribution, and oxygen transfer in three-phase reactors.	Simulates glucose binding to glucose oxidase and impact of immobilization on activity.	Uniform substrate delivery, improved enzyme stability, and higher product yields.	Smith et al., 2021
Biodiesel Production	Lipase-catalyzed transesterification of oils and fats for biodiesel production.	Models multiphase flow in reactors, optimizing mixing and mass transfer.	Studies lipase interactions with triglycerides and alcohols.	Higher conversion rates, reduced mass transfer limitations, and improved scalability.	Johnson et al., 2020
Lactose Hydrolysis in Dairy	Breakdown of lactose into glucose and galactose for lactose-free dairy products.	Analyzes flow dynamics to prevent substrate depletion zones in stirred-tank reactors.	Investigates lactose binding to β-galactosidase and impact of immobilization on activity.	Consistent lactose hydrolysis, reduced processing times, and better enzyme utilization.	Garcia et al., 2019
Hydrogen Peroxide Removal	Catalase enzymatic degradation of hydrogen peroxide in food and beverage processing.	Models gas-liquid interactions and oxygen release in reactors.	Studies catalase stability under varying pH and temperature conditions.	Faster hydrogen peroxide removal and improved enzyme reuse.	Lee et al., 2022

Cellulose Hydrolysis for Bioethanol	Enzymatic hydrolysis of cellulose to fermentable sugars for bioethanol production.	Simulates substrate dispersion and fluid flow in packed-bed or fluidized-bed reactors.	Models cellulase-substrate interactions and effects of inhibitors like lignin.	Higher hydrolysis rates and enzyme stability under industrial conditions.	Patel et al., 2021
High-Fructose Corn Syrup Production	Conversion of glucose to fructose using immobilized glucose isomerase.	Models substrate flow and temperature distribution in continuous-flow reactors.	Simulates isomerization pathways and enzyme stability under shear conditions.	Higher conversion efficiency, reduced overheating, and extended enzyme lifespan.	Zhao et al., 2018
Pharmaceutical API Synthesis	Enzymatic reactions like transamination and hydrolysis for active pharmaceutical ingredient (API) production.	Optimizes mixing, substrate delivery, and temperature in microreactors or continuous reactors.	Studies enzyme-substrate interactions to optimize specificity and yield.	Enhanced productivity, scalability, and high-purity API production.	Chen et al., 2020

4.CONCLUSION

This review focuses on significant potential and challenges of using Three Phase Fluidized Bed Bioreactors (TPFBs) for producing gluconic acid, a valuable compound in various industries. TPFBs offer unique advantages, like improved mixing and effective interaction among gas, liquid, and solid phases, making them promising for scaling up production. However, achieving consistent performance in these bioreactors is complex due to issues such as enzyme stability, mass transfer limitations, and fluid dynamics management. The review suggests several strategies to tackle these challenges. Enzyme immobilization is highlighted as a crucial step, as it stabilizes the enzyme and allows it to be reused, lowering costs. Adjusting reactor design like modifying bed materials and geometry can improve mixing and prevent problems like particle clumping and excessive bed expansion, which can otherwise hamper efficiency. Computational tools like Computational Fluid Dynamics (CFD) and kinetic modelling are recommended to simulate and fine-tune conditions, helping to optimize factors such as temperature, pH, and substrate concentration. Real-time monitoring and automation also play a vital role, keeping conditions ideal and production steady while minimizing enzyme deactivation. Although there are limitations in covering every operational challenge, the review underscores TPFBs' potential for large-scale gluconic acid production. With

improvements in enzyme stability, mass transfer efficiency, and reactor design, TPFBs can become a dependable and cost-effective option in the bioprocessing industry. Overcoming these challenges could pave the way for further advancements in enzymatic production, providing a valuable insight to biotechnology and industrial processing.

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Author Contribution
Goddindla Sreenivasulu proposed the idea of the article and coordinated with other authors in preparation. R.Ramakoteswara Rao supervised overall preparation of the article and provided necessary inputs. B Sarath Babu contributed for drafting, data verification and grammar check. Akhila Swathantra P contributed for content development and plagiarism check. Asadi Srinivasulu contributed for data collection and analysis

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