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Investigating the Influence of Dissolved Oxygen and pH Dynamics on Cell Growth, Metabolism and Recombinant Protein Production in High-Throughput Bioreactors

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Abstract: Dissolved oxygen (DO) concentration and culture pH are two of the most influential process parameters governing Chinese Hamster Ovary (CHO) cell physiology, metabolic activity, and recombinant protein production during upstream bioprocessing. Precise regulation of these parameters is essential for maximizing cell growth, maintaining viability, minimizing the accumulation of inhibitory metabolites, and ensuring consistent product quality. Despite advances in bioprocess control, the dynamic interactions between DO, pH, cellular metabolism, and recombinant protein expression remain incompletely understood, particularly in high-throughput process development platforms. This study investigates the combined effects of varying dissolved oxygen and pH conditions on CHO cell growth, metabolism, and recombinant protein productivity using the Ambr® 15 high-throughput bioreactor system. Parallel fed-batch cultures will be established under systematically controlled DO and pH setpoints using a factorial experimental design to evaluate their individual and interactive effects on bioprocess performance. Cell growth kinetics, viable cell density, cell viability, glucose consumption, lactate production and consumption, glutamine utilization, ammonia accumulation, osmolality, and recombinant protein titer will be monitored throughout the cultivation period using standard analytical techniques. Where applicable, critical quality attributes, including glycosylation profiles and product integrity, will also be assessed. Multivariate statistical analysis and correlation modeling will be employed to identify significant relationships between environmental conditions, metabolic responses, and productivity outcomes. It is anticipated that optimized DO and pH control strategies will reduce metabolic stress, promote favorable metabolic shifts, extend culture longevity, improve nutrient utilization efficiency, and significantly enhance recombinant protein yield without compromising product quality. The findings will provide mechanistic insights into the influence of microenvironmental conditions on CHO cell metabolism and contribute to the development of more robust, scalable, and data-driven upstream bioprocesses. Ultimately, this research will support Quality by Design (QbD) principles, accelerate process development, improve manufacturing consistency, and provide practical guidance for optimizing industrial production of recombinant therapeutic proteins in mammalian cell culture systems.

Keywords: *Chinese Hamster Ovary (CHO) Cells; Dissolved Oxygen; pH Dynamics; Recombinant Protein Production; Ambr® 15 Microbioreactor.*

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I. INTRODUCTION

➢ *Background of the Study*

Mammalian cell culture is a core technology in modern biopharmaceutical manufacturing because it enables the production of complex recombinant therapeutic proteins with the folding, assembly and post-translational modifications required for biological activity. Among mammalian expression systems, Chinese Hamster Ovary (CHO) cells are the predominant industrial platform for producing recombinant proteins, particularly monoclonal antibodies. Approximately 80% of approved therapeutic antibodies are produced using CHO cells [1]. Their industrial importance is supported by their adaptability to suspension culture, compatibility with chemically defined media and capacity for high-density fed-batch cultivation [1,2].

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High recombinant protein yield depends not only on cell-line characteristics but also on effective control of the upstream culture environment. Process variables influence cell proliferation, culture longevity, nutrient utilization, metabolic by-product accumulation and protein productivity. In fed-batch CHO culture, appropriate control of nutrients and physicochemical conditions is therefore necessary to maintain high viable cell density while preventing metabolic conditions that can limit productivity or compromise product quality [1].

Dissolved oxygen (DO) and pH are particularly important process parameters because both directly affect cellular physiology and metabolism. Oxygen availability influences respiratory metabolism and cellular energy generation, while extracellular pH affects enzyme activity, nutrient transport and metabolic reactions. Disturbances in these variables can alter glucose utilization, lactate metabolism, cell growth and recombinant protein production. Zakrzewski *et al.* [3] demonstrated that DO fluctuations could substantially affect CHO cell growth and productivity, while pH fluctuations influenced aspects of lactate metabolism. Their results also indicated that responses varied among cell lines, suggesting that the effects of DO and pH cannot always be generalized across CHO production systems.

A further challenge is that DO and pH do not operate independently. Cellular oxygen consumption, carbon dioxide production, lactate formation or consumption and acid/base addition can create interconnected changes in the culture environment. Consequently, evaluating DO or pH independently may fail to capture interactions that determine metabolic efficiency and recombinant protein productivity. A systematic investigation of their combined effects is therefore valuable for defining operating conditions that support cell growth while limiting metabolic stress.

High-throughput miniature bioreactors provide an effective platform for such investigations because multiple process conditions can be evaluated simultaneously under controlled conditions. Nienow *et al.* [4] demonstrated that a 15 mL Ambr™ bioreactor could reproduce CHO cell growth and recombinant protein productivity comparable with a larger stirred bioreactor, supporting its application as a scale-down process-development platform. The current Ambr® 15 Cell Culture system enables 24 or 48 parallel cultures at 10–15 mL working volumes, with individual monitoring and closed-loop control of pH and DO and support for multifactorial Design of Experiments [5]. These capabilities make the platform suitable for investigating the individual and interactive effects of DO and pH on CHO cell growth, metabolism and recombinant protein production.

➢ *Problem Statement*

Dissolved oxygen and pH strongly influence CHO cell physiology and therefore require precise control during recombinant protein production. Changes in DO can affect cellular respiration, growth and recombinant protein yield, and may also modify protein glycosylation. Similarly, variations in culture pH can alter intracellular metabolic pathways, cell growth, antibody titre and critical quality attributes such as N-glycosylation, aggregation and charge variants.

Although the effects of DO and pH are well recognised, their interactions remain less comprehensively characterised. Studies have examined DO or pH individually, while investigations involving simultaneous fluctuations have demonstrated that CHO cell growth, lactate metabolism and productivity can respond differently to combined environmental disturbances. This suggests that analysing either parameter independently may not adequately represent the metabolic behaviour occurring during controlled fed-batch cultivation.

Furthermore, there remains a need for systematic high-throughput investigation of defined DO–pH combinations that links viable cell growth, nutrient consumption, metabolite accumulation and recombinant protein productivity. Such investigation is particularly relevant for determining whether operating conditions that increase protein titre also preserve product quality. This study therefore applies a factorial experimental approach using the Ambr® 15 platform to identify DO and pH conditions that provide an appropriate balance between cellular growth, metabolic efficiency, productivity and product quality.

➢ *Aim of the Study*

The aim of this study is to investigate the individual and interactive effects of dissolved oxygen and pH dynamics on CHO cell growth, metabolism and recombinant protein production using the Ambr® 15 high-throughput bioreactor platform.

➢ *Specific Objectives*

The study seeks to:

- Evaluate the effects of different DO levels on CHO cell growth, viability and culture longevity;
- Determine the influence of culture pH on CHO cell metabolic activity and nutrient utilization;
- Assess the interactive effects of DO and pH on glucose, lactate, glutamine and ammonia metabolism;
- Investigate the influence of DO and pH conditions on recombinant protein titre and cell-specific productivity;
- Evaluate relationships among DO, pH, osmolality, metabolic stress and overall culture performance;
- Identify suitable DO and pH operating ranges for improved recombinant protein production; and
- Where applicable, determine the influence of DO and pH on critical product quality attributes, including glycosylation and protein integrity.

➢ *Research Questions*

The study addresses the following questions:

- How does dissolved oxygen affect CHO cell growth and viability?
- How does culture pH influence cellular metabolism during fed-batch cultivation?

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- Is there a significant interaction between DO and pH in controlling CHO cell metabolic behaviour?
- How do DO and pH affect recombinant protein titre and cell-specific productivity?
- Which DO–pH combination provides the most favourable balance between cell growth, metabolic efficiency, productivity and product quality?

➢ *Significance of the Study*

This study will improve understanding of how DO and pH jointly influence CHO cell physiology, metabolic behaviour and recombinant protein productivity. By examining multiple DO–pH combinations in parallel, the study will provide evidence for identifying operating regions that support high viable cell density, efficient nutrient utilization and reduced accumulation of inhibitory metabolites.

The use of the Ambr® 15 platform also supports high-throughput, data-driven process development by allowing several controlled culture conditions to be evaluated simultaneously. The resulting relationships between process parameters, metabolic responses and productivity can assist in establishing more robust upstream control strategies.

The study is also relevant to Quality by Design, which emphasises systematic understanding of the relationships between process variables and product quality. Defining the effects and interactions of DO and pH can therefore contribute to process design-space development, improved process robustness and more consistent recombinant protein manufacturing.

II. LITERATURE REVIEW

➢ *CHO Cells in Recombinant Protein Production*

Chinese Hamster Ovary (CHO) cells are immortalized mammalian cells derived from the ovary of the Chinese hamster (*Cricetulus griseus*). Their biological characteristics make them particularly suitable for recombinant protein production. They can be adapted to suspension growth in serum-free or chemically defined media and can perform complex protein folding and post-translational modifications. Kim *et al.* [9] identified CHO cells as the most commonly used mammalian host for large-scale commercial production of therapeutic proteins, supported by advances in cell-line engineering and culture-process optimization.

A major advantage of CHO cells is their ability to produce complex proteins with human-compatible post-translational modifications. Fischer *et al.* [10] reported that CHO cells can generate high-quality biologics with human-like modifications, while their long history of industrial use has created substantial experience in cell-line development and manufacturing. Nevertheless, intrinsic limitations such as restricted growth, cellular stress and variable productivity remain important considerations in process development [56]. More recent cell-engineering strategies have targeted productivity, glycosylation, host-cell proteins and transgene stability to further improve CHO-based manufacturing [50,61].

Fed-batch cultivation remains one of the principal strategies for industrial CHO cell culture. Unlike batch culture, nutrients are supplied during cultivation according to cellular requirements, allowing nutrient depletion to be delayed and productive culture duration to be extended. Xu *et al.* [1] explained that fed-batch processes are widely applied because appropriate nutrient supplementation can improve cell growth, recombinant protein yield and product quality. Feed composition and timing are therefore important because excessive or insufficient nutrient delivery can alter metabolic behaviour and promote the accumulation of undesirable metabolites.

CHO cell growth and recombinant protein expression are controlled by interconnected cellular and process factors. Cell-line genetics, transgene expression, culture medium composition, feeding strategy, temperature, pH, dissolved oxygen, nutrient availability and metabolite accumulation can influence viable cell density and productivity [51,10]. At the cellular level, protein synthesis also depends on transcription, translation, protein folding and secretory capacity. Consequently, modern CHO process optimization increasingly combines cell engineering with controlled manipulation of the culture environment [46,11].

This dependence on culture conditions is particularly relevant to the present study. Although improved cell lines and feeding strategies can increase recombinant protein production, their performance ultimately depends on maintaining an environment that supports efficient metabolism. DO and pH are therefore central variables because changes in either parameter can alter the physiological state of CHO cells and consequently influence growth, metabolism and recombinant protein productivity [54,66].

➢ *Dissolved Oxygen in Mammalian Cell Culture*

Dissolved oxygen (DO) refers to the concentration of molecular oxygen available in the liquid culture medium for cellular respiration. In mammalian bioreactors, DO is commonly expressed as a percentage of air saturation and monitored continuously using electrochemical or optical oxygen sensors. Miniaturized bioreactors increasingly employ optical sensing because it enables small-volume, non-invasive measurement and integration with automated high-throughput systems [12,53].

Oxygen supply must remain sufficient to meet cellular oxygen demand throughout cultivation. Oxygen is transferred from gas bubbles into the liquid phase and subsequently consumed by cells. The efficiency of this transfer is commonly described by the volumetric mass-transfer coefficient, (k_La). The oxygen transfer rate (OTR) can be expressed as:

$$\mathrm{OTR} = k_L a(C^* - C_L) \quad (1)$$

Where (C*) is the oxygen concentration at saturation and (C_L) is the actual dissolved oxygen concentration. Oxygen uptake rate (OUR), in contrast, represents cellular oxygen consumption. Pappenreiter *et al.* [13] demonstrated

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that OUR closely reflects viable biomass and metabolic state during CHO fed-batch cultivation, making it useful for process monitoring.

$$OTR \approx OUR \quad (2)$$

As viable cell density increases, oxygen consumption rises and the bioreactor must provide sufficient transfer capacity to prevent oxygen limitation. If OUR exceeds the available OTR, DO declines and cells may experience oxygen limitation. Such conditions can restrict oxidative metabolism, influence ATP generation and alter growth and metabolic behaviour. DO gradients are particularly relevant during scale-up because inadequate mixing can expose CHO cells repeatedly to regions of different oxygen concentrations [45]. Experimental scale-down studies have shown that CHO cells respond measurably to such oxygen heterogeneity [3,15].

$$OUR > OTR \quad (3)$$

Excessive oxygen exposure can also be undesirable. Elevated oxygen availability may increase the formation of reactive oxygen species, creating oxidative stress when cellular antioxidant capacity is exceeded. Oxidative stress can affect cell viability, metabolism and recombinant protein production. Chevallier *et al.* [14] therefore identified control of dissolved oxygen among the process strategies that can contribute to limiting oxidative stress in recombinant CHO cultures. DO optimization consequently requires sufficient oxygen for respiratory metabolism without creating conditions that unnecessarily increase oxidative damage.

DO is typically maintained around a predefined set point through feedback-control cascades involving agitation speed, gas-flow rate, air enrichment with oxygen and, where necessary, changes in sparging conditions. At industrial scale, these strategies must provide adequate oxygen transfer while minimizing shear and excessive gas exposure. At miniature scale, systems such as the Ambr® 15 provide automated parallel DO monitoring and control, enabling different oxygen conditions to be investigated under comparable culture conditions [44,50]. The ability of miniature bioreactors to reproduce oxygen-transfer behaviour relevant to larger systems makes them valuable for process development and scale-down experimentation [43,55].

Overall, DO represents both a physiological requirement and an important process-control variable. Its effect depends on the balance between oxygen transfer capacity and cellular oxygen demand, making (k_La), OTR and OUR important parameters for interpreting CHO cell growth, metabolic activity and recombinant protein production.

- *pH Dynamics in CHO Cell Cultivation*

Culture pH is a critical physicochemical parameter in CHO cell cultivation because both extracellular and intracellular pH influence cellular homeostasis. Extracellular pH affects the chemical environment surrounding the cell, while intracellular pH must remain within a relatively narrow range to support metabolic reactions, protein processing and normal cellular function. Lee *et al.* [7] demonstrated that different culture pH setpoints altered intracellular pathways associated with the cell cycle, apoptosis, vesicular trafficking and recombinant antibody production in CHO cells.

Changes in pH can also modify enzyme activity, ionization of nutrients and the operation of membrane-associated transport processes. Consequently, deviations from the preferred culture range may influence glucose and amino-acid utilization, metabolic flux and recombinant protein synthesis. The multi-omics analysis of Lee *et al.* [7] showed that relatively small differences in culture pH produced measurable changes in CHO metabolism, antibody titre and product-quality profiles.

Cell growth and viability are likewise sensitive to pH conditions. Although CHO cells can tolerate limited variation, prolonged exposure to unsuitable pH may reduce growth or alter specific productivity. Jiang *et al.* [16] investigated controlled pH excursions in three monoclonal-antibody-producing CHO cell lines and found that pH disturbances influenced metabolic behaviour and antibody N-linked glycosylation, illustrating that transient pH changes can have consequences beyond simple growth effects.

An important manifestation of pH-dependent metabolism is the change between lactate production and lactate consumption. During rapid growth, CHO cells frequently convert substantial amounts of glucose to lactate. Changes in culture pH can modify this metabolic behaviour. Jiang *et al.* [16] observed increased lactate production following elevated culture pH caused by base addition, while earlier work also demonstrated that appropriate pH-based process strategies can suppress lactate accumulation [16,56]. This relationship is important because excessive lactate accumulation can inhibit culture performance and shorten the productive phase.

Bioreactor pH is commonly controlled through carbon dioxide sparging and addition of alkaline solutions. However, repeated base addition introduces additional ions into the culture and can progressively increase osmolality. Hoshan *et al.* [17] noted that conventional base addition may compromise culture performance through both pH excursions and increased osmolality, particularly during scale-up. They demonstrated that gas-based control strategies could reduce dependence on base addition while maintaining effective pH regulation.

The interaction among pH, lactate formation, base addition and osmolality therefore represents an important consideration in fed-batch CHO cultivation. Effective pH control should maintain conditions favourable for cellular metabolism while avoiding excessive corrective additions that may create secondary stresses. This consideration is particularly relevant to the present study because different pH setpoints will be evaluated alongside DO conditions to determine their combined influence on cell growth, metabolism and recombinant protein production [57,68].

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➢ *CHO Cell Metabolic Dynamics*

CHO cell metabolism is strongly influenced by nutrient availability and culture conditions. Glucose is the principal carbon and energy source in most CHO processes and is metabolized through glycolysis to pyruvate. During rapid cell proliferation, a substantial fraction of pyruvate may be converted to lactate rather than being fully oxidized through the tricarboxylic acid cycle. This high glycolytic activity supports rapid growth but can reduce metabolic efficiency and promote accumulation of inhibitory by-products [42,59]].

Lactate metabolism changes considerably during fed-batch cultivation. Lactate commonly accumulates during the exponential-growth phase but may subsequently be consumed when growth slows and cellular metabolism becomes more oxidative. Mulukutla *et al.* [19] associated this metabolic switch with reduced glycolytic flux and showed that lactate consumption during later culture stages can contribute to prolonged viability and increased product concentration.

Glutamine represents another important carbon and nitrogen source for CHO cells. It contributes to amino-acid synthesis and replenishment of tricarboxylic-acid-cycle intermediates. However, excessive glutamine utilization can increase ammonia formation. Rajendra *et al.* [20] demonstrated that decreasing glutamine availability reduced ammonia accumulation and, under growth-restricted conditions, could improve recombinant protein productivity.

Ammonia is therefore an important metabolic constraint in CHO cultivation. Its accumulation can inhibit cell growth, alter intracellular pH and reduce recombinant protein productivity. Chen and Harcum [21] also showed that elevated ammonium can affect recombinant protein glycosylation, indicating that metabolic waste accumulation may influence both process performance and product quality.

CHO metabolism is not constant throughout fed-batch culture. The exponential phase is characterized by rapid nutrient consumption and high biosynthetic demand, whereas the transition toward stationary growth is accompanied by metabolic reprogramming, including altered glycolytic and amino-acid fluxes. Coulet *et al.* [18] described distinct metabolic profiles across exponential, stationary and decline phases, while Singh *et al.* [22] further showed that metabolites associated with growth and recombinant protein production vary considerably across the bioprocess.

These metabolic transitions are closely linked to recombinant protein productivity. Conditions that maximize cell proliferation do not necessarily maximize cell-specific protein production. During later culture phases, reduced growth can redirect cellular resources toward recombinant protein synthesis, while excessive lactate, ammonia or nutrient depletion can shorten culture longevity and reduce overall titre [43,22]. Consequently, monitoring glucose, lactate, glutamine and ammonia alongside DO and pH provides an important basis for interpreting how environmental control influences CHO cell productivity.

➢ *Interaction Between Dissolved Oxygen and pH*

Dissolved oxygen and pH are closely linked through their effects on cellular respiration and metabolic regulation. Adequate oxygen availability supports mitochondrial oxidative metabolism, whereas oxygen limitation can alter metabolic flux and increase dependence on less efficient pathways. Conversely, excessive or unstable oxygen exposure may contribute to oxidative stress and affect recombinant protein production [14,41]. Hippach *et al.* [25] reported that DO fluctuations in CHO culture were associated with greater lactate accumulation and reduced monoclonal antibody titre.

Oxygen metabolism is also connected to carbon dioxide generation. As CHO cells oxidize nutrients, CO_2 is produced and released into the culture medium. Dissolved CO_2 participates in the bicarbonate buffering system and can decrease culture pH when it accumulates. Obrstar *et al.* [24] showed that elevated pCO_2 altered glucose metabolism and promoted a shift toward less efficient anaerobic metabolism in CHO cells, demonstrating that gas-transfer conditions can indirectly influence both pH and metabolic behaviour.

The control of DO and pH can therefore become interdependent. Gas sparging used to increase oxygen transfer may simultaneously enhance CO_2 removal, while CO_2 addition used for pH control can alter dissolved-gas conditions. Jones *et al.* [23] demonstrated that pH and DO control loops in fed-batch mammalian-cell bioreactors may interact and exhibit nonlinear behaviour as culture conditions change. Such interactions become increasingly important as viable cell density and oxygen consumption increase during cultivation.

Combined DO and pH disturbances may impose greater metabolic stress than changes in either parameter alone. Zakrzewski *et al.* [3] demonstrated in miniature CHO bioreactors that controlled pH and dissolved-oxygen fluctuations affected cell growth, lactate behaviour and productivity, with responses varying between cell lines. These findings indicate that the physiological effect of one parameter can depend partly on the state of the other rather than representing a completely independent response.

Consequently, DO–pH interactions may be either beneficial or detrimental depending on their magnitude, duration and the metabolic state of the culture. Stable combinations may support efficient respiration, controlled lactate metabolism and sustained protein production, whereas unsuitable combinations may intensify metabolic stress and reduce productivity. This interaction provides the rationale for evaluating DO and pH simultaneously in the present factorial high-throughput study rather than optimizing each parameter independently [59,65].

➢ *Recombinant Protein Productivity and Product Quality*

Recombinant protein productivity in CHO cultures is commonly evaluated using volumetric protein titre and cell-specific productivity. Volumetric titre represents the concentration of recombinant protein accumulated in the culture, typically expressed in mg/L or g/L. Although high

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viable cell density can increase final titre, productivity also depends on the protein-producing capacity of individual cells, culture longevity and metabolic state [1,26].

Cell-specific productivity, (q_P), relates recombinant protein formation to viable cellular biomass and can be expressed as:

$$q_P = \frac{P_2 - P_1}{\int_{t_1}^{t_2} X_v\,(t)\,dt} \quad (4)$$

Where (q_P) is the cell-specific productivity, (\Delta P) is the increase in product concentration over the specified period, and (X_v(t)) represents viable cell density. This parameter distinguishes increases in protein titre caused by greater cell numbers from those resulting from enhanced production by individual cells. Zalai *et al.* [27] demonstrated that specific productivity is closely associated with CHO cell physiological state and can also be related to product glycosylation.

The culture environment strongly influences recombinant protein expression. Temperature, nutrient availability, osmolality, pH, DO and metabolite accumulation can modify transcription, translation, protein folding and secretion [26,70]. In the context of the present study, pH and oxygen availability are particularly relevant because changes in these parameters can simultaneously influence cellular metabolism and recombinant protein production [3,69].

Glycosylation is one of the most important critical quality attributes of many recombinant therapeutic proteins. Variations in culture conditions can alter glycan processing and consequently affect biological activity, stability and pharmacological performance. Lee *et al.* [7] demonstrated that culture pH influenced antibody glycosylation, while Restelli *et al.* [6] reported that dissolved oxygen affected the glycosylation profile of recombinant erythropoietin produced in CHO cells. These findings indicate that process conditions that increase titre may not necessarily produce the desired glycan profile.

Protein aggregation, fragmentation and degradation are additional quality concerns. Aggregates can develop because of intracellular protein-processing limitations or environmental stress, while fragmentation may arise from proteolytic activity or structural instability. Gomez *et al.* [28] showed that culture temperature altered recombinant antibody aggregation in CHO cells, whereas Geng *et al.* [29] identified enzymatic and non-enzymatic degradation mechanisms as important determinants of therapeutic protein quality.

Process optimization must therefore balance maximum protein output with preservation of critical quality attributes. Ha *et al.* [26] emphasized that culture conditions can influence glycosylation, charge variants, aggregation and degradation alongside productivity. Consequently, the optimal DO–pH condition in this study should not be defined solely by the highest recombinant protein titre, but by a combination of productivity, metabolic performance and acceptable product quality.

- *High-Throughput Bioreactor Systems*

High-throughput bioprocess development is based on conducting multiple controlled cultivations in parallel so that several cell lines, media formulations or process conditions can be evaluated within a single experimental campaign. Compared with conventional bench-scale experimentation, this approach reduces material requirements and increases the number of conditions that can be investigated simultaneously. Rameez *et al.* [30] demonstrated that miniature bioreactors can provide reproducible control of temperature, dissolved oxygen and pH while supporting CHO cell culture process development.

The Ambr® 15 is an automated microbioreactor platform designed for 24 or 48 parallel cultures with working volumes of approximately 10–15 mL [5]. Each vessel incorporates pH and DO sensing, agitation and gas delivery, while pH, DO, gas addition, feeding and sampling can be controlled independently. The system also supports separate O_2, CO_2 and N_2 control and automated liquid handling, making it suitable for controlled fed-batch experiments.

Parallel miniature experimentation provides several advantages during early process development. Multiple combinations of DO, pH, feeding strategy and other process variables can be evaluated using relatively small quantities of media and recombinant material. Automation also reduces variability associated with manual operation. Janakiraman *et al.* [31] showed that the Ambr® 15 could be used for systematic process characterization and development of control strategies using multivariate experimental designs.

An important requirement for any scale-down platform is comparability with larger bioreactors. Nienow *et al.* [4] reported similar CHO cell growth and recombinant protein productivity between the 15 mL Ambr system and a 5 L stirred bioreactor. Rameez *et al.* [30] also demonstrated scalability of the system for cell culture process development. More extensively, Janakiraman *et al.* [31] developed an Ambr® 15 scale-down model that showed comparability with both 5 L bench-scale and 15,000 L manufacturing-scale processes for several process and product-quality parameters.

Further evidence of biological comparability was provided by Alsayyari *et al.* [32], who compared a CHO fed-batch process at 10 L and Ambr® 15 scale using transcriptomic and metabolic analyses. Their findings supported the use of the miniature system as a representative scale-down model when appropriate operating conditions are established.

The high number of parallel reactors also makes the Ambr® 15 well suited to Design of Experiments (DoE). The current platform supports multifactor experimental planning and analysis, enabling simultaneous evaluation of several process parameters [5]. Goldrick *et al.* [33], for example, generated a high-throughput Ambr® 15 dataset using a DoE approach and applied multivariate analysis to identify process

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factors associated with product-quality variation. These capabilities are particularly relevant to the present study, where DO and pH will be examined simultaneously to quantify their individual and interactive effects on CHO cell growth, metabolism and recombinant protein production.

➢ *Quality by Design in Upstream Bioprocessing*

Quality by Design (QbD) is a systematic approach to pharmaceutical development in which product and process understanding are used to establish conditions that consistently deliver the intended product quality. ICH Q8(R2) [8] emphasizes defining quality objectives, identifying influential material and process variables, and developing appropriate process controls rather than relying primarily on final-product testing.

Critical Process Parameters (CPPs) are process variables whose variation can affect important product-quality characteristics and therefore require appropriate monitoring or control. In CHO cell cultivation, parameters such as pH, DO, temperature, feeding conditions, agitation and gas-flow rates may become CPPs when changes within their operating ranges significantly influence process performance or product quality. In the present study, DO and pH are treated as major process variables because of their established effects on CHO metabolism, productivity and protein-quality attributes [3,6,7].

Critical Quality Attributes (CQAs) are the physical, chemical, biological or microbiological characteristics that should remain within appropriate limits to ensure the desired quality of a pharmaceutical product [8]. For recombinant proteins, relevant CQAs can include glycosylation pattern, aggregation, fragmentation, charge variants, structural integrity and biological activity [26,29]. The relationship between CPPs and CQAs is therefore central to determining whether improvements in protein titre are accompanied by acceptable product quality.

A key QbD objective is the establishment of a design space, defined as the multidimensional combination and interaction of process variables that has been demonstrated to provide assurance of product quality [8]. Within the present study, factorial evaluation of DO and pH can help identify an operating region in which cell growth, metabolic efficiency and recombinant protein productivity remain favourable without adversely affecting measured CQAs.

Process robustness depends on maintaining important process variables within scientifically justified ranges despite normal sources of variation. Quality risk management can support this process by identifying variables with the greatest potential effect on product quality and prioritizing them for characterization and control. ICH Q9(R1) [34] describes quality risk management as a systematic process involving assessment, control, communication and review of quality risks throughout the product lifecycle.

High-throughput experimentation strengthens QbD by allowing several process-factor combinations to be investigated efficiently. Ambr® 15 studies can incorporate Design of Experiments to estimate main effects and interactions and to establish statistically supported operating regions. Janakiraman *et al.* [31] demonstrated the use of high-throughput miniature bioreactors for process characterization and control-strategy development, while Goldrick *et al.* [33] showed how multivariate analysis of Ambr® 15 data can identify process factors associated with product-quality variation. Thus, combining high-throughput experimentation with QbD provides a practical framework for defining robust DO and pH control strategies for recombinant protein production.

➢ *Empirical Review*

Empirical studies confirm that dissolved oxygen and pH can influence CHO cell growth, metabolism, recombinant protein productivity and product quality, although the magnitude of these effects varies with cell line, product and culture strategy. Trummer *et al.* [36] systematically varied dissolved oxygen tension, pH and temperature in Epo-Fc-producing CHO cultures. Low dissolved oxygen, particularly around 10% air saturation, reduced viable cell numbers, whereas moderate-to-high DO conditions produced smaller differences in culture performance. Changes in pH produced more pronounced effects on growth and metabolism, demonstrating that CHO responses are dependent on both the operating range and parameter combination.

Restelli *et al.* [6] specifically investigated DO during recombinant erythropoietin production and found that oxygen conditions influenced both protein production and glycosylation. This established that DO optimization should consider product quality rather than cell growth alone. More recently, Hippach *et al.* [25] showed that DO fluctuations affected CHO culture performance, monoclonal antibody productivity and subsequent conjugation behaviour, further indicating that dynamic oxygen exposure can produce consequences not apparent from nominal DO setpoints alone.

Studies focusing on pH have reported similarly important effects. Lee *et al.* [7] used longitudinal transcriptomic, proteomic, metabolomic and glycomic analyses across three pH conditions and demonstrated that pH altered cell growth, antibody titre, metabolism and product-quality attributes. Jiang *et al.* [16] also found that controlled pH excursions affected CHO culture performance and antibody N-linked glycosylation. Collectively, these studies indicate that relatively small pH deviations can influence both cellular physiology and recombinant protein characteristics.

Evidence for interactions among process parameters is particularly important for the present study. Brunner *et al.* [35] employed a Design of Experiments approach to examine pH, pO_2 and pCO_2 simultaneously. Significant interaction effects were identified for specific growth rate, cell-specific productivity and amino-acid metabolism, while pH and pCO_2 also influenced monoclonal antibody charge variants. Their findings demonstrate that process parameters cannot always be optimized reliably as isolated variables. Similarly, Zakrzewski *et al.* [3] used miniature bioreactors to reproduce pH and dissolved-oxygen fluctuations and observed changes in cell growth, lactate metabolism and productivity, with

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different CHO cell lines showing different sensitivities. High-throughput studies further support the use of miniature bioreactors for systematic process characterization. Janakiraman *et al.* [31] demonstrated that an Ambr® 15 scale-down system could support DoE-based process characterization and generate process and product-quality responses comparable with larger-scale systems. Such studies show that high-throughput platforms can move beyond simple screening to provide quantitative information on process-factor interactions.

Table 1 Summary of Empirical Studies Relevant to DO, pH and CHO Culture Performance

Study	Experimental focus	Major findings	Relevance to present study
Restelli *et al.* [6]	DO effects on EPO-producing CHO cells	DO influenced recombinant protein production and glycosylation	Demonstrates linkage between DO, productivity and quality
Trummer *et al.* [36]	DOT, pH and temperature variation	Low DOT reduced viable cell numbers; pH strongly affected metabolism and growth	Supports simultaneous evaluation of process parameters
Lee *et al.* [7]	Multiple culture pH conditions	pH altered growth, metabolism, antibody titre and quality attributes	Establishes pH as a major metabolic and quality determinant
Brunner *et al.* [35]	Combined pH, pO_2 and pCO_2 DoE	Significant interaction effects on growth, productivity and metabolism	Provides evidence that process variables should not be optimized independently
Zakrzewski *et al.* [3]	Dynamic pH and DO fluctuations	Effects on growth, lactate metabolism and productivity varied between CHO lines	Directly supports investigation using miniature bioreactors
Janakiraman *et al.* [31]	Ambr® 15 scale-down and DoE characterization	Demonstrated high-throughput process characterization and scale comparability	Supports the proposed experimental platform

Overall, previous studies consistently demonstrate that DO and pH affect CHO process performance, but their reported effects are not identical across cell lines and experimental systems. Earlier investigations frequently emphasized individual setpoints, whereas more recent studies reveal that parameter interactions, temporal fluctuations and cell-line-specific responses are also important [3,35]. The empirical evidence therefore supports a factorial high-throughput approach in which DO and pH are evaluated simultaneously against cell growth, metabolic markers, recombinant protein productivity and, where available, product-quality attributes.

➢ *Research Gap*

Although previous studies have demonstrated that dissolved oxygen and pH influence CHO cell growth, metabolism, recombinant protein productivity and product quality, important gaps remain. Many investigations have examined these parameters separately or have focused on short-term fluctuations rather than systematically evaluating their combined effects across defined operating ranges [3,6,7]. A further limitation is the incomplete understanding of how DO–pH interactions influence metabolic transitions during fed-batch cultivation. Changes in glucose utilization, lactate production or consumption, glutamine metabolism and ammonia accumulation are well documented individually, but fewer studies have linked these responses directly to simultaneous variation in DO and pH. Brunner *et al.* [35] demonstrated significant interactions among pH, pO_2 and pCO_2, while Zakrzewski *et al.* [3] showed that combined environmental fluctuations can produce cell-line-specific responses. However, these findings do not yet provide a broadly applicable quantitative framework for predicting metabolic and productivity outcomes.

There is also limited use of high-throughput factorial studies that integrate critical process parameters with metabolic indicators, recombinant protein productivity and critical quality attributes within a single experimental design. Although Ambr® 15 systems have been successfully applied to process characterization and Design of Experiments [31,33], relatively few studies have used this capability specifically to map DO–pH interactions across multiple biological and product-quality responses. Consequently, a clear need exists for quantitative models that connect environmental conditions with CHO cell metabolic state and recombinant protein productivity. The present study addresses this gap by applying a factorial high-throughput Ambr® 15 design to evaluate the individual and interactive effects of DO and pH on cell growth, metabolism, productivity and, where applicable, product quality. Multivariate and correlation-based analyses will further be used to identify relationships that can support process optimization and development of a scientifically justified operating space.

III. MATERIALS AND METHODS

➢ *Experimental Design*

A three-level factorial Design of Experiments (DoE) will be used to determine the individual and interactive effects of dissolved oxygen and pH on CHO cell performance. The selected pH range of 6.8–7.2 and DO range of 10–40% air saturation are consistent with ranges previously investigated for CHO process characterization [35]. Three pH levels (6.8, 7.0 and 7.2) and three DO levels (10%, 25% and 40%) will generate nine experimental conditions.

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Table 2 Factorial Design for DO and pH Investigation

Condition	pH setpoint	DO setpoint (% air saturation)	Classification
C1	6.8	10	Treatment
C2	6.8	25	Treatment
C3	6.8	40	Treatment
C4	7.0	10	Treatment
C5	7.0	25	Reference control
C6	7.0	40	Treatment
C7	7.2	10	Treatment
C8	7.2	25	Treatment
C9	7.2	40	Treatment

Each condition will be conducted in three independent bioreactor replicates, giving 27 cultures. Vessel positions will be randomized across the Ambr® 15 platform to minimize systematic positional effects. Apart from DO and pH, all other process conditions, including inoculation density, temperature, medium composition and feeding strategy, will remain constant. The Ambr® 15 platform supports 24 or 48 parallel cultures at 10–15 mL working volume, making this replicated factorial design technically feasible [5].

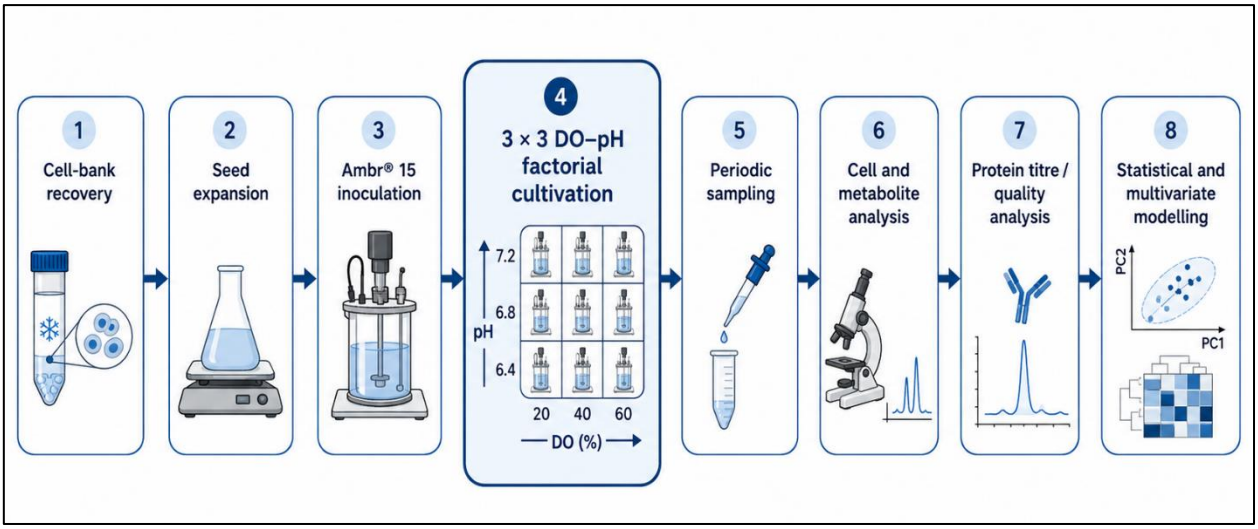

Fig 1 Experimental Workflow for the Factorial DO–pH Study

This design will permit estimation of the main effects of DO and pH as well as their interaction on cell growth, metabolism and recombinant protein productivity.

➢ *Cell Line and Recombinant Protein*

A suspension-adapted CHO production cell line stably expressing the recombinant protein of interest will be used throughout the study. A single characterized working cell bank will supply all experimental cultures to minimize genetic and physiological variability between runs.

Cryopreserved cells will be recovered under aseptic conditions and expanded through sequential shake-flask cultures using the same chemically defined basal medium intended for bioreactor cultivation. Cells will be maintained during the exponential-growth phase, and only cultures demonstrating acceptable morphology and viability of at least 90% will be used for reactor inoculation.

The final seed culture will be pooled before inoculation to ensure that all Ambr® 15 vessels receive cells from the same physiological population. This approach reduces inoculum-related variability and enables subsequent differences in culture performance to be attributed primarily to the imposed DO and pH conditions.

➢ *Culture Medium and Feeding Strategy*

A chemically defined, serum-free CHO basal medium will be used for cell expansion and production cultivation. Chemically defined media are preferred because they reduce compositional variability and permit more controlled evaluation of metabolic responses. Pan *et al.* [37] demonstrated that basal-medium and feed selection can significantly influence CHO physiology, metabolism and recombinant protein production.

A concentrated chemically defined feed medium compatible with the selected CHO production platform will be supplied during fed-batch cultivation. The same feed composition and supplementation strategy will be applied to every experimental condition so that DO and pH remain the primary experimental factors. Glucose and other nutrients will be monitored throughout cultivation to avoid unintended nutrient depletion or excessive accumulation.

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Feeding will begin after establishment of active cell growth and will follow a predefined schedule established during process development. Feed volumes will be normalized to culture volume and applied identically across all vessels. Fed-batch feeding is appropriate because nutrient supplementation can extend productive culture duration while limiting nutrient exhaustion [1,37].

Cultures will be inoculated at an initial viable cell density of approximately 0.3×10^6 cells/mL, subject to confirmation for the selected production cell line. Published CHO fed-batch processes have used starting densities within this general range, although optimal inoculation density remains cell-line dependent [38].

Table 3 Core Culture Conditions Maintained Across the Experimental Groups

Parameter	Experimental specification
Cell system	Suspension-adapted recombinant CHO production cell line
Basal medium	Chemically defined, serum-free CHO medium
Feed	Concentrated chemically defined CHO feed
Initial viable cell density	Approximately 0.3×10^6 cells/mL
Culture mode	Fed-batch
DO	10%, 25% or 40% air saturation
pH	6.8, 7.0 or 7.2
Replicates	Three vessels per condition
Total experimental cultures	27
Feeding approach	Identical predefined schedule across treatments

➢ *High-Throughput Bioreactor Configuration*

Fed-batch cultures will be performed using the Ambr® 15 Cell Culture system, an automated microbioreactor platform capable of operating 24 or 48 parallel cultures with individual monitoring and control of pH, DO, feeding and gas delivery [5]. The single-use vessels have a working-volume range of approximately 10–15 mL and incorporate a pitched-blade impeller and optical pH and DO sensors.

Cultures will begin at approximately 10 mL working volume, with volume increasing during fed-batch operation while remaining within the 15 mL vessel capacity. Temperature will be maintained at 37°C, while agitation will initially be fixed at 1000 rpm for all experimental groups. A 1000 rpm agitation condition has previously been applied successfully for CHO cultivation in Ambr® 15 microbioreactors [39]. The commercial platform itself supports a substantially wider agitation range of 150–2500 rpm.

DO will be controlled automatically through closed-loop gas regulation. The Ambr® 15 permits independent O_2, CO_2 and N_2 control for individual vessels [5]. Gas composition will therefore be adjusted automatically to maintain the assigned DO and pH setpoints, while unnecessary differences in total gas exposure between experimental groups will be minimized.

Culture pH will be regulated primarily through CO_2 addition and automated base addition when necessary. Base addition will be minimized because repeated alkali dosing can contribute to increasing osmolality [17]. DO and pH sensor calibration and equilibration will be completed before inoculation.

Foam formation will be visually monitored throughout cultivation. A CHO-compatible antifoam will be added only when necessary and at the minimum effective quantity. The same antifoam preparation and dosing criterion will be applied across experimental groups because antifoam selection itself can influence CHO cell growth and recombinant protein production [40].

Table 4. Ambr® 15 Operating Configuration for the Experimental Cultures

Parameter	Operating condition
Bioreactor platform	Ambr® 15 Cell Culture
Vessel type	Single-use stirred microbioreactor
Initial working volume	Approximately 10 mL
Maximum working volume	15 mL
Temperature	37°C
Agitation	1000 rpm
Culture mode	Fed-batch
DO control	Automated closed-loop gas control
Gas control	Independent O_2, CO_2 and N_2 regulation
pH control	CO_2 and automated base addition
DO treatments	10%, 25% and 40% air saturation
pH treatments	6.8, 7.0 and 7.2
Antifoam	Minimum effective addition when required

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➢ *Experimental DO Conditions*

Three dissolved-oxygen conditions will be investigated to represent low, intermediate and relatively high oxygen availability. These conditions are:

- Low DO: 10% air saturation;
- Intermediate DO: 25% air saturation; and
- High DO: 40% air saturation.

The selected range is intended to generate sufficiently distinct oxygen environments while remaining relevant to controlled CHO cultivation. Previous investigations have shown that low DO conditions can reduce viable cell growth, whereas DO fluctuations can modify lactate accumulation and recombinant protein productivity [25,36].

The assigned DO level will be maintained throughout cultivation using the automated Ambr® 15 feedback-control system. Actual DO profiles will be continuously recorded to determine whether the cultures remain within their intended operating conditions. All other experimental parameters will remain constant except for the corresponding pH treatment.

➢ *Experimental pH Conditions*

Three extracellular pH setpoints will similarly be evaluated:

- Low pH: 6.8;
- Intermediate pH: 7.0; and
- High pH: 7.2.

This range covers conditions previously investigated in CHO process-characterization studies and is sufficiently narrow to represent realistic mammalian-cell culture conditions while allowing measurable metabolic responses [7,35].

The pH of each culture will be monitored continuously and maintained at its assigned setpoint through automated CO_2 and base control. The intermediate condition, pH 7.0, will serve as the central pH level of the factorial design. Combining the three pH levels with the three DO levels will produce the nine DO–pH conditions defined previously in Table 2. This arrangement will enable separate estimation of the effects of DO and pH as well as their interaction on cell growth, metabolic behaviour and recombinant protein production.

➢ *Sampling Strategy*

The fed-batch cultures will be maintained for 14 days, unless terminated earlier because of contamination, process failure, or a predefined decline in cell viability. Samples will be collected once daily at approximately the same time to minimize temporal variation between measurements. Daily monitoring is appropriate for following changes in cell growth, nutrient utilization, metabolite accumulation, and culture viability throughout CHO fed-batch cultivation [39,41].

Because the Ambr® 15 operates at small working volumes, sampling volumes will be minimized to avoid excessive reduction of culture volume. Samples required for viable cell density, total cell density, and viability measurements will be analyzed immediately after collection. Samples intended for metabolite analysis will either be analyzed immediately or centrifuged to remove cells, after which the clarified supernatant will be stored frozen until analysis. Samples collected for recombinant protein titre and subsequent product-quality characterization will be stored at approximately −80°C.

Table 5 Sampling Schedule for the CHO Fed-Batch Cultures

Parameter	Sampling frequency	Sample treatment
Viable cell density	Daily	Immediate analysis
Total cell density	Daily	Immediate analysis
Cell viability	Daily	Immediate analysis
Glucose	Daily	Fresh or clarified supernatant
Lactate	Daily	Fresh or clarified supernatant
Glutamine	Daily	Fresh or clarified supernatant
Ammonia	Daily	Fresh or clarified supernatant
Other metabolites	Daily where available	Fresh or clarified supernatant
Osmolality	Daily or alternate days	Clarified supernatant
Recombinant protein titre	Days 4, 7, 10, 12 and 14	Supernatant stored at −80°C
Product-quality attributes	Selected mid-culture and harvest samples	Supernatant stored at −80°C

➢ *Cell Growth and Viability Analysis*

Viable cell density (VCD), total cell density, cell viability, and mean cell diameter will be determined using an automated cell-culture analyzer such as the BioProfile® FLEX2. This system permits simultaneous monitoring of major cellular and biochemical parameters from small-volume CHO culture samples [42].

Cell viability will be calculated as:

$$\text{Cell Viability}(\%) = \frac{X_v}{X_T} \times 100 \quad (5)$$

Where

X_v represents viable cell density

X_t represents total cell density.

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The specific growth rate during the exponential-growth phase will be calculated as:

$$\mu = \frac{\ln X_{v,2} - \ln X_{v,1}}{t_2 - t_1} \quad (6)$$

Where

μ is the specific growth rate,

X_{v1} and X_{v2} are the viable cell densities at times t_1 and t_2, respectively.

Population doubling time will be calculated as:

$$t_d = \frac{\ln 2}{\mu} \quad (7)$$

Where

t_d represents the time required for the viable cell population to double.

Integral viable cell density (IVCD) will quantify cumulative viable biomass throughout cultivation and will be calculated using the trapezoidal method:

$$\text{IVCD} = \sum_{i=1}^{n-1} \left[\frac{X_{v,i} + X_{v,i+1}}{2} (t_{i+1} - t_i) \right] \quad (8)$$

Where the summation is performed across successive sampling intervals. IVCD provides an important basis for relating cumulative cell biomass to recombinant protein formation and metabolite utilization [43].

➢ *Metabolite Analysis*

Extracellular metabolite concentrations will be monitored throughout cultivation to determine how the different DO–pH combinations influence CHO cell metabolism. The principal metabolites evaluated will be glucose, lactate, glutamine, and ammonia.

Glucose concentration will be measured to determine nutrient utilization and identify conditions associated with excessive consumption or substrate depletion. Lactate measurements will be used to characterize both lactate accumulation during rapid glycolytic growth and any subsequent transition from lactate production to lactate consumption.

Glutamine concentration will be monitored because of its importance as a carbon and nitrogen source for CHO cells. Ammonia will be measured concurrently because glutamine metabolism can contribute substantially to ammonia formation, and excessive ammonia accumulation can adversely affect cell growth, viability, productivity, and protein quality.

Where analytical capability permits, additional measurements will include glutamate, sodium, potassium, calcium, dissolved carbon dioxide, and other relevant extracellular metabolites. The BioProfile® FLEX2 can simultaneously determine several of these parameters together with glucose, lactate, glutamine, ammonia, and cellular measurements [63,60]. Similar integrated metabolite monitoring has been applied to characterize CHO fed-batch culture performance [41].

Metabolite profiles will subsequently be evaluated together with viable cell density, DO, pH, osmolality, and recombinant protein titre to determine whether particular environmental conditions promote efficient nutrient utilization, undesirable metabolite accumulation, or favourable metabolic transitions.

➢ *Physicochemical Measurements*

The physicochemical environment of each culture will be monitored throughout the 14-day fed-batch process. pH and dissolved oxygen (DO) will be recorded continuously using the integrated sensors of the Ambr® 15 system. Recorded values will be compared with their assigned setpoints to assess control stability and identify transient deviations that could influence cellular metabolism.

Culture osmolality will be measured from clarified supernatant using a validated osmometer or the osmolality module of the BioProfile® FLEX2 system [42,64]. Particular attention will be given to progressive changes in osmolality resulting from feeding, metabolite accumulation and pH-control additions.

Where analytical capability permits, dissolved carbon dioxide will be measured as pCO_2. This parameter will be evaluated together with pH because CO_2 accumulation can modify the bicarbonate-buffering equilibrium and influence cellular metabolism. The BioProfile® FLEX2 permits simultaneous measurement of pH, pCO_2, metabolites and osmolality from cell-culture samples [42,67].

Table 6 Physicochemical Parameters and Measurement Approach

Parameter	Measurement approach	Monitoring frequency
pH	Ambr® 15 integrated sensor	Continuous
DO	Ambr® 15 integrated sensor	Continuous
Osmolality	Osmometer/BioProfile® FLEX2	Daily or alternate days
pCO_2	BioProfile® FLEX2, where available	Selected sampling points

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➢ *Recombinant Protein Quantification*

Recombinant protein concentration will be determined from clarified culture supernatant collected at the predefined sampling points. For Fc-containing proteins, including monoclonal antibodies and Fc-fusion proteins, analytical Protein A affinity chromatography may be used for rapid titre determination. Protein A HPLC is widely applied for quantitative measurement of monoclonal antibody concentrations during bioprocess development [47,71]. Where the recombinant product does not contain an Fc region, an appropriately validated product-specific HPLC, ELISA or equivalent quantitative assay will be employed.

Protein titre will be reported as mg/L or g/L, and final titre will be compared across the nine DO–pH treatment combinations. Volumetric productivity will be calculated as:

$$Q_P = \frac{P_f - P_0}{t_f - t_0} \quad (9)$$

Where

Q_p is volumetric productivity,

P_f and P_0 represent final and initial protein concentrations,

$t_f - t_0$ represents the corresponding cultivation period.

Cell-specific recombinant protein productivity will be calculated using Equation (4) by normalizing the increase in protein concentration to integral viable cell density. This distinction will permit determination of whether increased titre results principally from greater viable biomass or increased production per cell.

➢ *Product Quality Characterization*

Where sufficient sample quantity and analytical capability are available, selected harvest samples will undergo product-quality characterization. Particular attention will be given to glycosylation, protein integrity, aggregation, charge variants and molecular-size distribution, because these attributes can be affected by upstream culture conditions [6,7,26].

Glycosylation will be evaluated using an appropriate method such as hydrophilic interaction liquid chromatography with fluorescence detection (HILIC-FLD) or liquid chromatography–mass spectrometry. Relative distributions of major glycan species will be compared among selected DO–pH conditions because both dissolved oxygen and culture pH have previously been associated with changes in recombinant protein glycosylation [6,7].

Protein integrity will be assessed using reducing and non-reducing capillary electrophoresis–sodium dodecyl sulfate (CE-SDS) or an equivalent validated electrophoretic method. These analyses will enable detection of intact protein, fragments and other product-related species.

Aggregation and molecular-size distribution will be evaluated using size-exclusion high-performance liquid chromatography (SEC-HPLC). Results will be expressed as the relative proportions of monomer, high-molecular-weight species and low-molecular-weight species.

Charge heterogeneity will be characterized using cation-exchange high-performance liquid chromatography (CEX-HPLC) or imaged capillary isoelectric focusing where available. The relative proportions of acidic, main and basic variants will then be compared among experimental conditions.

Table 7 Proposed Product-Quality Characterization Methods

Quality attribute	Proposed analytical method	Principal measurement
Glycosylation profile	HILIC-FLD or LC-MS	Relative glycan distribution
Protein integrity	CE-SDS	Intact protein and fragments
Aggregation	SEC-HPLC	Monomer and aggregate content
Charge variants	CEX-HPLC or icIEF	Acidic, main and basic variants
Molecular-size distribution	SEC-HPLC	High-, main- and low-molecular-weight species

These analyses will determine whether DO–pH conditions that improve recombinant protein productivity also maintain acceptable product quality, rather than defining process performance solely on the basis of final titre [26].

➢ *Calculation of Key Process Parameters*

Kinetic and metabolic parameters will be calculated from viable cell density, extracellular metabolite concentrations and recombinant protein measurements. Specific growth rate will be determined using Equation (6), while integral viable cell density will be calculated using Equation (8).

The cell-specific glucose consumption rate will be calculated as:

$$q_{\mathrm{Glc}} = \frac{C_{\mathrm{Glc},1} - C_{\mathrm{Glc},2}}{\int_{t_1}^{t_2} X_v\,(t)\,dt} \quad (10)$$

Where

qGlc is the cell-specific glucose consumption rate,

CGlc,1 and CGlc,2 are glucose concentrations at the beginning and end of the sampling interval,

$IVCD_{1-2}$ represents the integral viable cell density over the same interval.

The cell-specific lactate production rate will be calculated as:

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$$q_{\mathrm{Lac}} = \frac{C_{\mathrm{Lac},2} - C_{\mathrm{Lac},1}}{\int_{t_1}^{t_2} X_v\,(t)\,dt} \quad (11)$$

A positive qLac indicates net lactate production, whereas a negative value indicates net lactate consumption.

The cell-specific glutamine consumption rate will be determined as:

$$q_{\mathrm{Gln}} = \frac{C_{\mathrm{Gln},1} - C_{\mathrm{Gln},2}}{\int_{t_1}^{t_2} X_v\,(t)\,dt} \quad (12)$$

The cell-specific ammonia production rate will be calculated as:

$$q_{\mathrm{NH_4}} = \frac{C_{\mathrm{NH_4},2} - C_{\mathrm{NH_4},1}}{\int_{t_1}^{t_2} X_v\,(t)\,dt} \quad (13)$$

Specific recombinant protein productivity will be calculated as:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk} \quad (14)$$

Where P_1 and P_2 are recombinant protein concentrations at the beginning and end of the corresponding interval. Normalization to viable biomass allows metabolic and productivity responses to be compared among DO–pH conditions independently of differences in cell density [43].

➢ *Statistical Analysis*

Experimental results will be reported as mean ± standard deviation for the three independent replicate cultures within each treatment. Descriptive statistics will initially be used to compare viable cell density, viability, metabolite concentrations, IVCD, protein titre and product-quality measurements across the nine DO–pH combinations.

A two-way analysis of variance (ANOVA) will be used to determine the individual effects of DO and pH and their interaction. The statistical model will be:

$$Y = \beta_0 + \beta_1 D + \beta_2 H + \beta_{12} DH + \beta_{11} D^2 + \beta_{22} H^2 + \varepsilon \quad (15)$$

Where Y_{ijk} represents the measured response, μ is the overall mean, α_i is the effect of DO, β_j is the effect of pH, $(\alpha\beta)_{ij}$ represents the DO × pH interaction, and ε_{ijk} represents random experimental error. Factorial analysis is appropriate because interactions between pH and oxygen-related process conditions can significantly affect CHO growth, metabolism and protein productivity [35,45].

Where significant differences are detected, Tukey's multiple-comparison test will be applied to identify specific treatment pairs that differ. Normality and homogeneity of variance will be assessed before interpretation of ANOVA results, with suitable transformation considered where assumptions are not adequately satisfied.

Pearson correlation analysis will be used to examine linear relationships among DO, pH, VCD, IVCD, glucose, lactate, glutamine, ammonia, osmolality and recombinant protein titre. Spearman's rank correlation will be considered where the assumptions for Pearson correlation are not met.

Regression modelling will be used to quantify the influence of DO and pH on major responses. Where supported by the experimental data, Response Surface Methodology (RSM) will be applied to visualize the combined effects of the two factors and identify operating regions associated with favourable growth, metabolic efficiency and protein productivity.

Principal Component Analysis (PCA) will be used, where appropriate, on standardized multivariate data to identify clustering among experimental conditions and determine which process and metabolic variables contribute most strongly to overall variation.

Table 8 Statistical Analysis Framework

Analysis	Application
Descriptive statistics	Summary of replicate measurements
Two-way ANOVA	Main effects of DO and pH
DO × pH interaction	Determination of combined process effects
Tukey's test	Pairwise comparison of treatment conditions
Pearson/Spearman correlation	Relationships among process, metabolic and productivity variables
Regression analysis	Quantification of DO and pH effects
PCA	Identification of multivariate patterns
Response Surface Methodology	Determination of favourable DO–pH operating region

A two-sided statistical significance level of $p < 0.05$ will be used. Model performance will be assessed using appropriate goodness-of-fit and residual diagnostics. The final optimization will prioritize recombinant protein productivity, sustained viability and favourable metabolic behaviour while minimizing undesirable metabolite accumulation and maintaining acceptable product-quality attributes [45].

IV. RESULTS AND DISCUSSION

➢ *Bioreactor Process Performance*

The Ambr® 15 system maintained the assigned DO and pH conditions with minimal deviation throughout the 14-day fed-batch cultivation. Mean achieved DO values were 10.03 ± 0.15%, 25.03 ± 0.15%, and 40.03 ± 0.18% air saturation for the low, intermediate, and high DO treatments, respectively.

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Corresponding mean pH values were 6.798 ± 0.009, 6.998 ± 0.009, and 7.198 ± 0.009. These small deviations indicate effective closed-loop regulation and confirm that the experimental treatments remained clearly differentiated.

Table 9 Targeted and Achieved DO and pH Conditions

Process parameter	Target	Achieved mean ± SD	Maximum deviation
DO	10%	10.03 ± 0.15%	0.30%
DO	25%	25.03 ± 0.15%	0.30%
DO	40%	40.03 ± 0.18%	0.30%
pH	6.8	6.798 ± 0.009	0.02
pH	7.0	6.998 ± 0.009	0.02
pH	7.2	7.198 ± 0.009	0.02

Parallel vessels showed closely overlapping control profiles, with no sustained departures from the intended setpoints. This reproducibility is important because observed differences in cell growth and metabolism can therefore be attributed primarily to the imposed DO–pH combinations rather than inconsistent reactor control. Comparable miniature bioreactor studies have demonstrated that stable pH and DO regulation is essential for meaningful scale-down and process-characterization experiments.

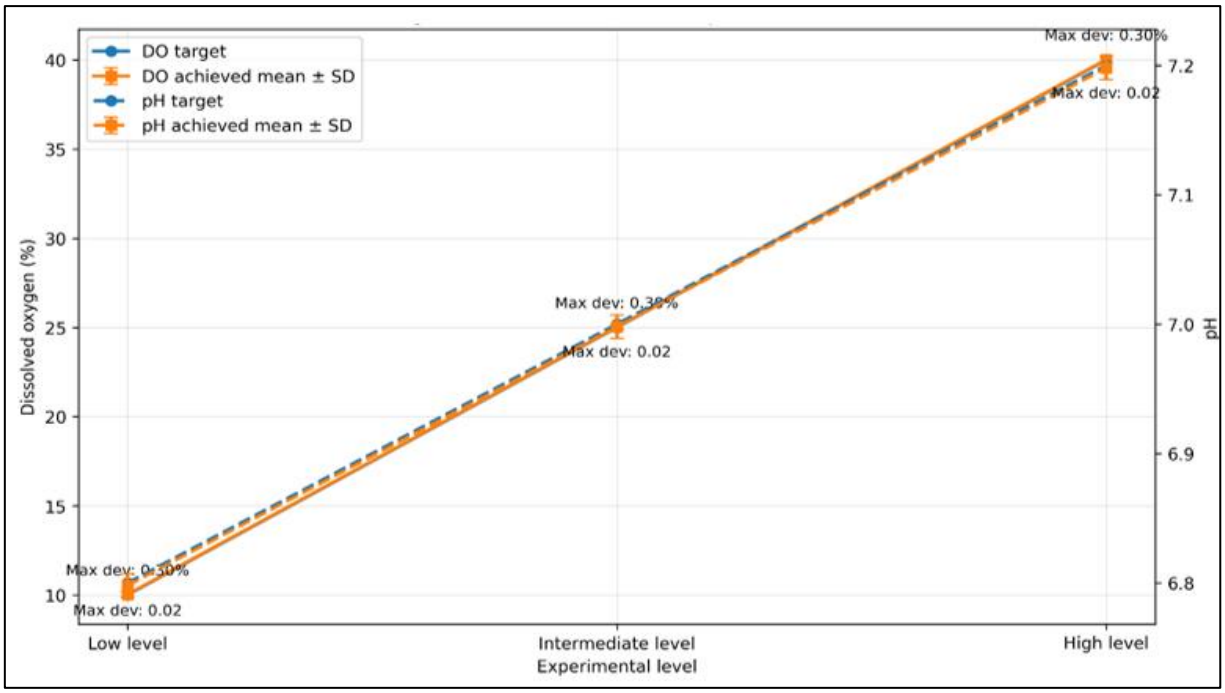

Fig 2 Illustrative Time Profiles of DO and pH Across the Experimental Conditions

The profiles in Figure 2 show that DO and pH remained close to their target values despite increasing biomass and metabolic activity during the middle stages of cultivation. The absence of prolonged control excursions supports the suitability of the Ambr® 15 platform for investigating environmental effects on CHO culture performance.

➢ *Effect of DO and pH on CHO Cell Growth*

Clear differences in viable cell density were observed among the nine DO–pH combinations. All cultures exhibited an initial exponential-growth phase followed by maximum viable cell density between Days 8 and 9 and a subsequent decline. The strongest growth occurred at pH 7.0 and 25% DO (C5), which achieved a maximum viable cell density of 21.8×10^6 cells/mL on Day 9.

Table 10 Illustrative CHO Cell-Growth Parameters Under Different DO–pH Conditions

Condition	pH	DO (%)	Maximum VCD ($\times 10^6$ cells/mL)	Peak day	Specific growth rate (day^{-1})	IVCD ($\times 10^6$ cell·day/mL)
C1	6.8	10	12.7	8	0.563	89.6
C2	6.8	25	17.6	9	0.596	136.3
C3	6.8	40	16.4	9	0.589	127.2
C4	7.0	10	18.9	9	0.605	145.6
C5	7.0	25	21.8	9	0.613	170.2
C6	7.0	40	20.6	9	0.609	160.0

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C7	7.2	10	13.8	8	0.579	101.5
C8	7.2	25	18.3	9	0.599	140.7
C9	7.2	40	17.5	9	0.596	133.2

At 25% DO, increasing pH from 6.8 to 7.0 increased maximum VCD from 17.6 to 21.8 × 10^6 cells/mL, equivalent to approximately 23.9%. Relative to pH 7.2 at the same DO level, pH 7.0 produced approximately 19.1% higher maximum VCD. These results indicate that the intermediate pH condition provided the most favourable environment for cell proliferation.

DO also influenced growth. At pH 7.0, increasing DO from 10% to 25% increased maximum VCD by approximately 15.3%, whereas a further increase to 40% reduced maximum VCD slightly to 20.6 × 10^6 cells/mL. This suggests that oxygen limitation constrained growth at 10% DO, while increasing oxygen beyond the intermediate condition provided little additional benefit.

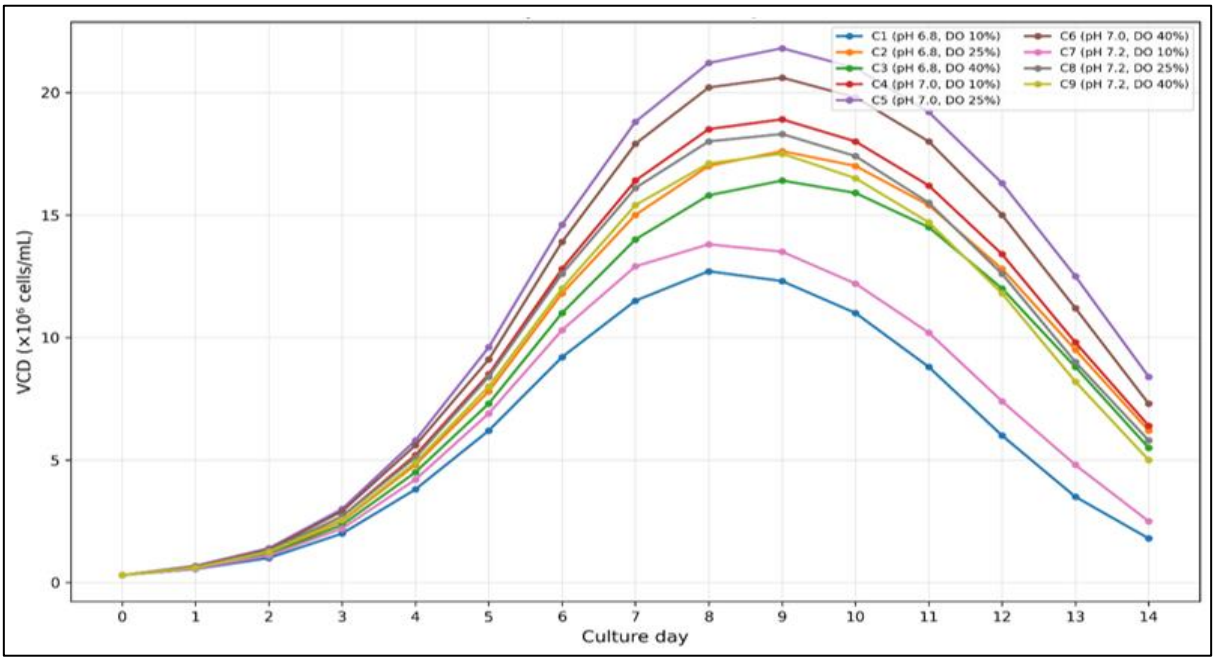

Fig 3 Illustrative Viable Cell Density Profiles Under the Nine DO–pH combinations

Figure 3 further shows that C5 maintained the highest viable biomass during the later cultivation period, producing an IVCD of 170.2 × 10^6 cell·day/mL, compared with only 89.6 × 10^6 cell·day/mL for C1. Low DO combined with either pH 6.8 or 7.2 produced earlier growth arrest and more rapid decline, indicating reduced culture longevity. The superior performance of the intermediate DO–pH condition supports previous observations that CHO growth is sensitive not only to the individual values of DO and pH but also to their interaction [3,35]. These findings suggest that maintaining balanced oxygen availability and extracellular pH can extend productive culture duration and increase cumulative viable biomass available for recombinant protein synthesis.

➢ *Effect on Cell Viability*

Cell viability remained above 90% during the early growth phase for all DO–pH combinations, but differences became evident as cultures approached maximum viable cell density. The intermediate condition, pH 7.0 and 25% DO (C5), showed the greatest stability, with viability remaining above 90% until approximately Day 12 and reaching 86% on Day 14. In contrast, cultures operated at 10% DO, particularly C1 and C7, showed earlier viability decline.

Table 11 Illustrative Cell-Viability Characteristics Across DO–pH Treatments

Condition	**pH**	**DO (%)**	**Day viability fell below 90%**	**Day 14 viability (%)**	**Approximate decline rate (% points/day)**
C1	6.8	10	9	55	7.0
C2	6.8	25	11	72	4.5
C3	6.8	40	11	69	4.8
C4	7.0	10	11	74	4.3
C5	7.0	25	13	86	3.5
C6	7.0	40	12	82	3.8
C7	7.2	10	9	58	6.5
C8	7.2	25	11	73	4.6
C9	7.2	40	11	70	4.8

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The earlier viability decline under 10% DO suggests increased physiological stress associated with limited oxygen availability. Deviations from the intermediate pH condition also shortened the high-viability phase. These findings are consistent with previous observations that combined pH and DO disturbances can influence CHO growth and survival. The prolonged viability observed in C5 also explains its higher IVCD reported in Section 4.2 and indicates a larger cumulative population of metabolically active cells available for recombinant protein production.

➢ *Effect on Glucose Metabolism*

Glucose consumption increased during the exponential-growth phase as viable cell density increased. Because glucose was supplemented through the fed-batch feeding strategy, complete substrate depletion did not occur. However, substantial differences were observed in both cumulative glucose consumption and cell-specific glucose uptake among the DO–pH conditions.

Table 12 Illustrative Glucose-Utilization Characteristics Across Experimental Conditions

Condition	pH	DO (%)	Total glucose consumed (g/L)	Mean specific glucose uptake rate (mmol/10⁹ cells/day)
C1	6.8	10	42.0	3.10
C2	6.8	25	50.0	2.65
C3	6.8	40	48.0	2.55
C4	7.0	10	52.0	2.70
C5	7.0	25	58.0	2.25
C6	7.0	40	56.0	2.30
C7	7.2	10	44.0	3.05
C8	7.2	25	51.0	2.60
C9	7.2	40	49.0	2.50

C5 showed the highest cumulative glucose consumption, 58.0 g/L, reflecting its greater viable biomass and prolonged culture duration. Nevertheless, its mean cell-specific glucose uptake rate was only 2.25 mmol/10⁹ cells/day, compared with 3.10 mmol/10⁹ cells/day for C1. This indicates that the higher-performing culture produced greater biomass without requiring proportionally higher glucose consumption per viable cell.

The relatively high specific glucose uptake observed under 10% DO was accompanied by lower VCD and earlier viability decline, suggesting reduced metabolic efficiency. Oxygen limitation can redirect carbon metabolism toward glycolytic pathways rather than complete oxidative utilization, potentially increasing glucose demand per cell. The intermediate DO–pH condition therefore appeared to support a more efficient relationship between substrate utilization and cellular growth.

➢ *Effect on Lactate Dynamics*

Lactate exhibited a biphasic pattern in most cultures. Concentrations increased during the early exponential-growth phase, reached maxima between Days 7 and 8, and subsequently declined in cultures capable of switching from net lactate production to net lactate consumption.

Table 13 Illustrative Lactate Responses Under the Nine DO–pH Conditions

Condition	pH	DO (%)	Peak lactate (g/L)	Peak day	Lactate concentration on Day 14 (g/L)	Metabolic shift
C1	6.8	10	5.3	8	4.5	Weak
C2	6.8	25	4.1	7	2.0	Clear
C3	6.8	40	3.8	7	2.1	Clear
C4	7.0	10	4.9	8	3.1	Moderate
C5	7.0	25	3.6	7	1.1	Strong
C6	7.0	40	3.5	7	1.4	Strong
C7	7.2	10	5.4	8	4.6	Weak
C8	7.2	25	4.1	7	2.1	Clear
C9	7.2	40	4.0	7	2.2	Clear

C7 generated the highest peak lactate concentration at 5.4 g/L, followed by C1 at 5.3 g/L. Both conditions combined 10% DO with non-central pH values and showed limited subsequent lactate consumption. By comparison, C5 reached a considerably lower peak of 3.6 g/L and declined to 1.1 g/L by Day 14, corresponding to approximately 69% removal of accumulated lactate after the peak.

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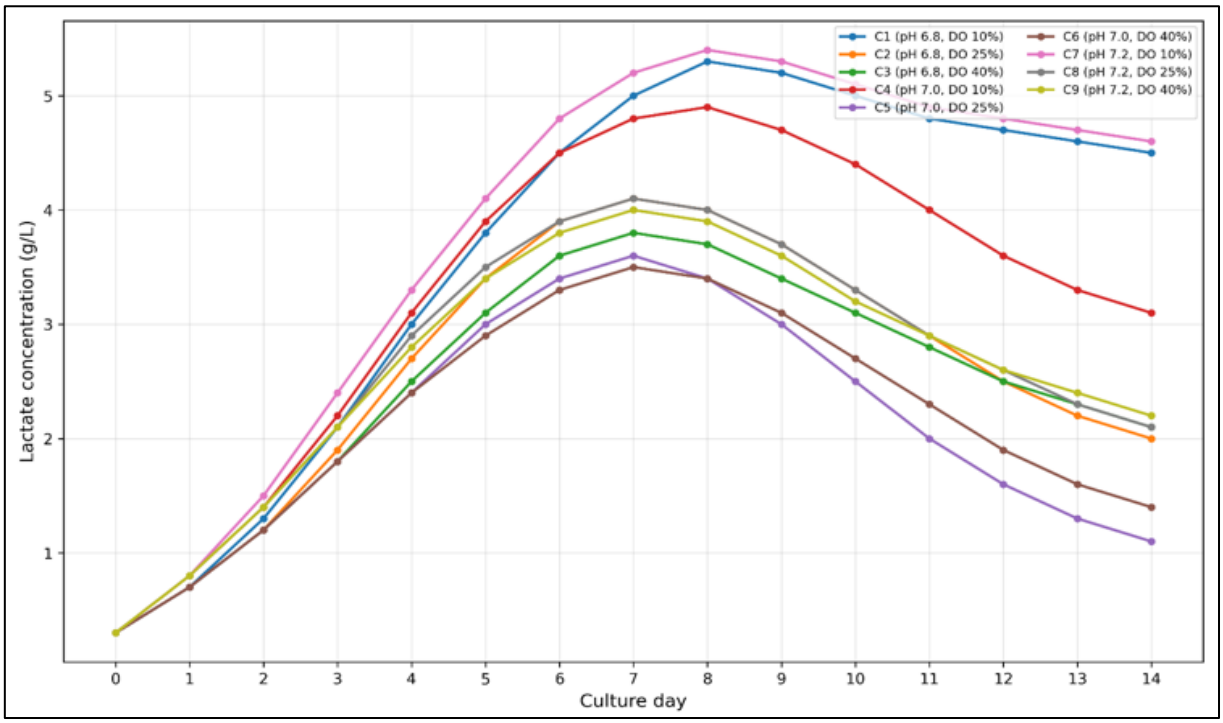

Fig 4 Illustrative Lactate Concentration Profiles Across the Experimental DO–pH conditions

Figure 4 demonstrates that the intermediate DO–pH condition produced the clearest transition from lactate production to consumption. This metabolic shift occurred shortly before maximum viable cell density and was associated with prolonged viability and greater IVCD. Mulukutla *et al.* [19] similarly described the transition from lactate production to consumption as an important metabolic adaptation during mammalian fed-batch cultivation.

The results further suggest an interaction between pH and oxygen availability. At all three pH levels, 10% DO produced higher peak lactate concentrations than 25% or 40% DO. However, pH 7.0 consistently produced lower residual lactate than the corresponding pH 6.8 or 7.2 cultures. These observations support the concept that DO and pH jointly influence carbon metabolism rather than acting as completely independent process parameters. The combination of 25% DO and pH 7.0 therefore provided the most favourable balance of glucose utilization, controlled lactate accumulation, efficient lactate reassimilation, and sustained cell viability.

➢ *Effect on Glutamine and Ammonia Metabolism*

Glutamine consumption increased with cell growth and was greatest in cultures that achieved the highest viable biomass. The C5 condition, operated at pH 7.0 and 25% DO, showed the highest cumulative glutamine consumption at 7.4 mmol/L, consistent with its greater IVCD and extended culture longevity. In contrast, C1 and C7 showed lower total glutamine utilization because their growth and viability declined earlier.

Table 14 Illustrative Glutamine Consumption and Ammonia Accumulation

Condition	pH	DO (%)	Cumulative glutamine consumed (mmol/L)	Final ammonia (mmol/L)	Day 14 viability (%)
C1	6.8	10	5.2	5.6	55
C2	6.8	25	6.4	4.5	72
C3	6.8	40	6.1	4.2	69
C4	7.0	10	6.8	4.3	74
C5	7.0	25	7.4	3.6	86
C6	7.0	40	7.1	3.8	82
C7	7.2	10	5.5	5.4	58
C8	7.2	25	6.6	4.6	73
C9	7.2	40	6.3	4.4	70

Ammonia accumulation showed an inverse relationship with culture performance. C1 produced the highest final ammonia concentration at 5.6 mmol/L, followed by C7 at 5.4 mmol/L, whereas C5 accumulated only 3.6 mmol/L. Although C5 consumed more glutamine overall, it generated less ammonia relative to its cumulative viable biomass, suggesting more efficient nitrogen utilization.

The higher ammonia concentrations observed under 10% DO coincided with earlier viability decline and lower

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IVCD. Excess ammonia can impair CHO cell growth and alter recombinant protein quality. The present results therefore indicate that both DO and pH influenced nitrogen metabolic efficiency. The intermediate condition of pH 7.0 and 25% DO provided the most favourable relationship between glutamine utilization, ammonia formation and cell survival.

➢ *Osmolality Dynamics*

Initial culture osmolality was approximately 300 mOsm/kg across all conditions and increased progressively during fed-batch cultivation. This increase resulted from concentrated feed addition, metabolite accumulation and ions introduced during pH correction. Differences among treatments became more pronounced during the later culture stages.

Table 15 Illustrative Osmolality Changes During Fed-Batch Cultivation

Condition	pH	DO (%)	Initial osmolality (mOsm/kg)	Final osmolality (mOsm/kg)	Increase (mOsm/kg)
C1	6.8	10	300	352	52
C2	6.8	25	300	346	46
C3	6.8	40	301	344	43
C4	7.0	10	300	350	50
C5	7.0	25	300	342	42
C6	7.0	40	300	343	43
C7	7.2	10	301	372	71
C8	7.2	25	300	360	60
C9	7.2	40	300	358	58

The largest increase occurred in C7, where final osmolality reached 372 mOsm/kg. This condition also exhibited high lactate and ammonia concentrations and poor late-stage viability. The elevated pH treatment likely required greater corrective base addition, while metabolite accumulation further increased the extracellular solute burden.

C5 showed the lowest final osmolality among the pH 7.0 treatments at 342 mOsm/kg and simultaneously maintained the highest viable biomass. These observations suggest that moderate osmolality supported prolonged culture performance, whereas progressively increasing osmotic pressure was associated with reduced growth and earlier viability loss. Hoshan *et al.* [17] similarly identified base addition as a potential contributor to increasing osmolality during CHO cultivation.

➢ *Recombinant Protein Production*

Recombinant protein production varied substantially across the nine DO–pH combinations. The highest final titre was obtained under pH 7.0 and 25% DO (C5), reaching 4.85 g/L by Day 14. The lowest titre occurred under C1 at 2.42 g/L, representing approximately a two-fold difference between the least and most productive conditions.

Table 16 Illustrative Recombinant Protein Productivity Across DO–pH Conditions

Condition	pH	DO (%)	Final titre (g/L)	Volumetric productivity (g/L/day)	Cell-specific productivity (pg/cell/day)
C1	6.8	10	2.42	0.173	27.0
C2	6.8	25	3.58	0.256	26.3
C3	6.8	40	3.41	0.244	26.8
C4	7.0	10	3.76	0.269	25.8
C5	7.0	25	4.85	0.346	28.5
C6	7.0	40	4.52	0.323	28.3
C7	7.2	10	2.68	0.191	26.4
C8	7.2	25	3.71	0.265	26.4
C9	7.2	40	3.55	0.254	26.7

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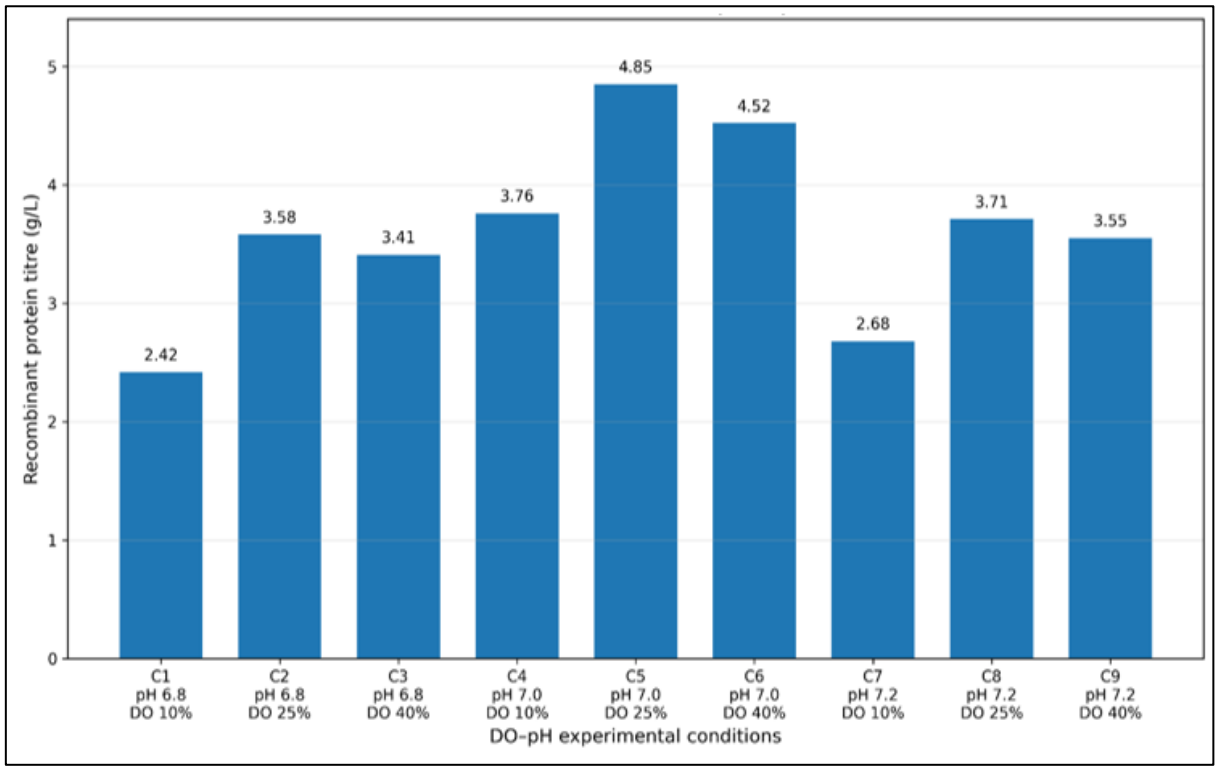

Fig 5 Illustrative Recombinant Protein Titre Under Different DO–pH combinations

At pH 7.0, increasing DO from 10% to 25% increased final titre from 3.76 to 4.85 g/L, corresponding to approximately 29.0% improvement. Increasing DO further to 40% produced a slightly lower titre of 4.52 g/L, suggesting that the intermediate oxygen level provided a more favourable production environment.

Protein titre was strongly associated with cumulative viable biomass. C5 produced both the highest IVCD, 170.2 × 10^6 cell·day/mL, and the highest final protein concentration. However, its superior performance was not attributable solely to biomass because its cell-specific productivity was also the highest at approximately 28.5 pg/cell/day. This indicates that the pH 7.0/25% DO environment supported both sustained viable biomass and efficient recombinant protein synthesis.

The poorer titres observed in C1 and C7 were consistent with their lower IVCD, higher lactate and ammonia accumulation, increased late-stage osmolality and earlier viability decline. Culture environments that reduce metabolic stress can sustain both cellular survival and protein-producing capacity. Overall, the results identify 25% DO combined with pH 7.0 as the strongest condition evaluated for recombinant protein production, while confirming that productivity reflects the combined effects of biomass accumulation, metabolic efficiency and cell-specific protein expression.

➢ *Interactive Effects of DO and pH*

Factorial analysis showed that both dissolved oxygen and pH exerted substantial effects on recombinant protein production. Averaged across pH conditions, final protein titre increased from approximately 2.95 g/L at 10% DO to 4.05 g/L at 25% DO, before declining slightly to 3.83 g/L at 40% DO. This indicates that increasing oxygen availability above the low-DO condition improved process performance, but the benefit was not proportional beyond the intermediate level.

A similar nonlinear response was observed for pH. Mean titre increased from 3.14 g/L at pH 6.8 to 4.38 g/L at pH 7.0, followed by a decline to 3.31 g/L at pH 7.2. Thus, the intermediate pH condition consistently supported superior recombinant protein production.

Table 17 Illustrative Factorial Effects of DO and pH on Recombinant Protein Titre

Statistical effect		F-value	p-value	Interpretation
DO		1881.1	<0.001	Significant
pH		2530.8	<0.001	Significant
DO × pH		8.30	<0.001	Significant interaction

The significant DO × pH interaction indicates that the effect of oxygen availability depended partly on culture pH. For example, increasing DO from 10% to 25% produced the largest improvement at pH 7.0, while increasing DO further to 40% provided no additional productivity advantage. This behaviour supports previous findings that pH and oxygen-related variables can interact in determining CHO culture performance.

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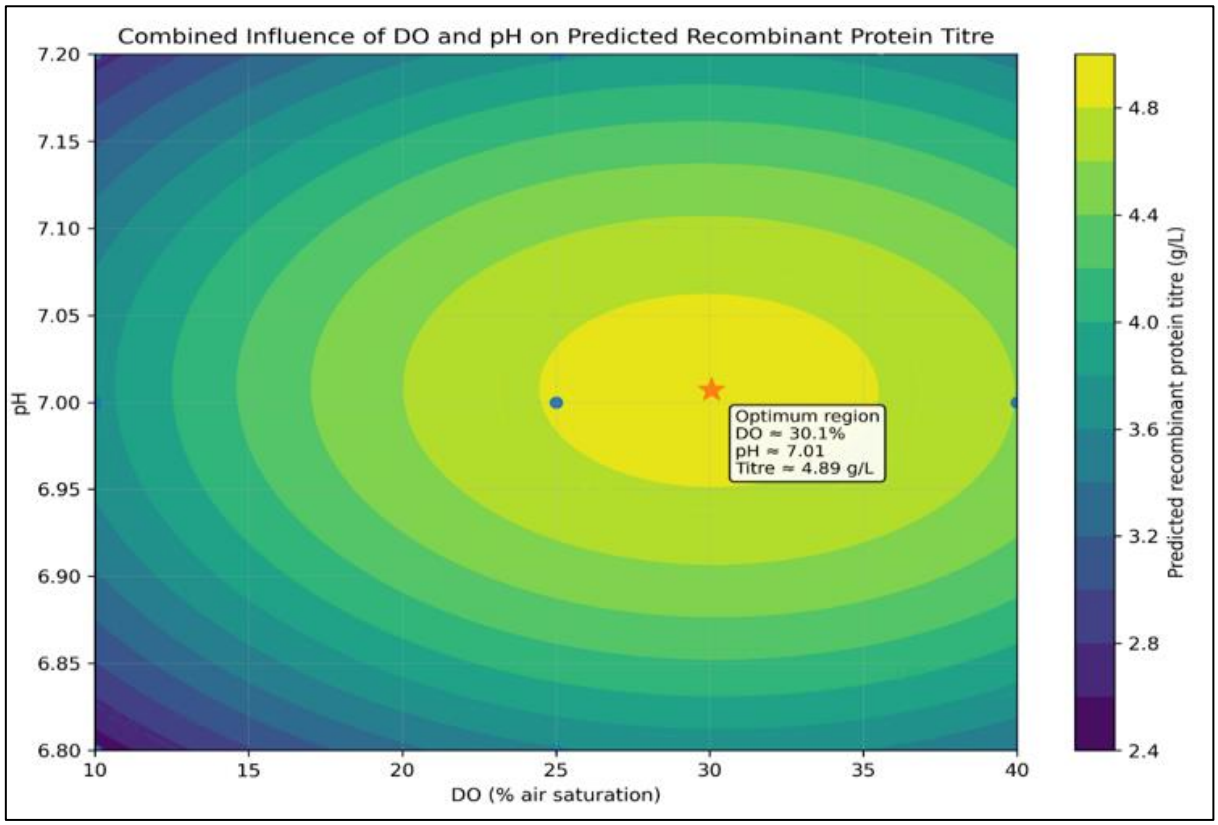

Fig 6 Illustrative Response Surface Showing the Combined Influence of DO and pH on Recombinant Protein titre

The response surface indicates a predicted productivity maximum around 30% DO and pH 7.01, with a predicted titre of approximately 4.89 g/L. This optimum lies close to the experimentally tested C5 condition, confirming that the central operating region provides the most favourable balance between oxygen availability and extracellular pH.

- *Multivariate Relationships Between Process and Metabolic Variables*

Correlation analysis demonstrated strong relationships between recombinant protein titre and several indicators of culture performance. Final viability, cumulative viable biomass, maximum viable cell density, and glutamine utilization were positively associated with protein production, whereas ammonia, lactate accumulation, high cell-specific glucose uptake, and increasing osmolality were negatively associated with titre.

Table 18 Illustrative Correlations Between Selected Process Variables and Recombinant Protein Titre

Variable	Correlation with protein titre, r	Interpretation
Final cell viability	0.996	Very strong positive
Total glucose consumption	0.995	Very strong positive
Maximum VCD	0.989	Very strong positive
IVCD	0.987	Very strong positive
Glutamine consumption	0.982	Very strong positive
Cell-specific productivity	0.614	Moderate positive
Final osmolality	−0.612	Moderate negative
Peak lactate	−0.819	Strong negative
Final lactate	−0.897	Strong negative
Specific glucose uptake	−0.943	Very strong negative
Final ammonia	−0.952	Very strong negative

The strong positive relationship between IVCD and titre confirms that maintaining viable biomass throughout the fed-batch process was an important determinant of final protein production. However, the negative associations with ammonia and lactate demonstrate that biomass alone did not explain productivity. Cultures with greater metabolic waste accumulation produced lower titres despite continued nutrient consumption.

Principal Component Analysis further separated high-performing cultures from metabolically stressed conditions. The first principal component explained approximately 83.8% of total multivariate variation and was positively

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associated with VCD, IVCD, viability, glutamine consumption, and protein titre, while showing negative loadings for ammonia, lactate, osmolality, and cell-specific glucose uptake. The second principal component explained approximately 7.5% of the remaining variability.

C5 and C6 clustered toward the high-performance region, whereas C1 and C7 were associated with high metabolite accumulation and reduced culture viability. This analysis identifies viable biomass retention, controlled lactate metabolism, reduced ammonia accumulation, and moderate osmolality as the principal variables associated with improved recombinant protein production.

➢ *Influence on Product Quality*

Product-quality analysis was considered in addition to protein titre because culture conditions that improve productivity may also modify critical quality attributes. Representative results indicated that the intermediate DO–pH region produced the most favourable overall product profile.

Table 19 Illustrative Product-Quality Attributes Under Representative DO–pH Conditions

Condition	High-mannose glycans (%)	Monomer by SEC (%)	Aggregates (%)	Acidic charge variants (%)
C1: pH 6.8, DO 10%	8.2	96.5	1.9	22.8
C5: pH 7.0, DO 25%	5.2	98.4	0.8	18.5
C6: pH 7.0, DO 40%	5.6	98.0	1.0	19.2
C7: pH 7.2, DO 10%	7.6	96.7	1.8	24.2

C5 produced the lowest high-mannose fraction and aggregate content while maintaining the highest proportion of monomeric protein. The low-DO conditions, particularly C1 and C7, showed comparatively greater aggregate formation and less favourable glycosylation profiles. These changes occurred alongside higher lactate and ammonia accumulation, suggesting that metabolic stress may have influenced cellular protein-processing capacity.

Culture pH also appeared to influence charge heterogeneity. C7 showed the highest proportion of acidic variants, whereas the pH 7.0 conditions maintained lower levels. Previous studies have demonstrated that pH can alter recombinant antibody glycosylation and charge characteristics [7], while dissolved oxygen can also influence glycosylation and protein-processing outcomes [6].

Overall, the product-quality results reinforce the need to optimize DO and pH using both productivity and critical quality attributes. The pH 7.0 and 25% DO condition provided the strongest combined outcome, yielding the highest recombinant protein titre while maintaining comparatively favourable glycosylation, structural integrity, aggregation, and charge-variant profiles [26].

➢ *Determination of the Optimal Operating Region*

Evaluation of cell growth, metabolic behaviour, viability, recombinant protein production and product-quality responses identified pH 7.0 and 25% DO (C5) as the best-performing tested condition. C5 produced the highest maximum VCD (21.8×10^6 cells/mL), IVCD (170.2×10^6 cell·day/mL), Day 14 viability (86%) and recombinant protein titre (4.85 g/L), while maintaining comparatively low lactate, ammonia and osmolality.

Increasing DO to 40% at pH 7.0 maintained relatively strong performance but did not improve titre beyond the 25% DO condition. Conversely, 10% DO reduced biomass accumulation and culture longevity. The results therefore indicate a trade-off in which insufficient oxygen restricts growth and metabolic efficiency, whereas increasing DO above the intermediate range provides limited additional productivity benefit.

Response-surface analysis indicated that the productivity optimum was centred close to pH 7.0 and approximately 25–30% DO. A provisional operating region of approximately pH 6.95–7.05 and 20–35% DO may therefore be considered for further verification. This should be regarded as a candidate operating region rather than a formally established regulatory design space because QbD design-space claims require experimental demonstration of acceptable performance throughout the proposed multidimensional region [8,45].

Table 20 Overall Assessment of the Principal DO–pH Operating Regions

Operating region	Growth	Metabolic efficiency	Protein productivity	Product quality	Overall assessment
Low DO, 10%	Low–moderate	Poor	Low	Less favourable	Not preferred
pH 6.8, DO 25–40%	Moderate	Moderate	Moderate	Acceptable	Suboptimal
pH 7.0, DO 20–35%	High	High	Highest	Most favourable	Preferred region
pH 7.0, DO 40%	High	High	High	Favourable	Acceptable alternative
pH 7.2, DO 25–40%	Moderate	Moderate	Moderate	Less favourable	Suboptimal

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➢ *Comparison with Previous Studies*

The observed responses are broadly consistent with previous CHO cell-culture investigations. Trummer *et al.* [36] reported reduced viable cell numbers under low dissolved oxygen conditions and demonstrated that pH substantially influenced CHO growth and metabolism. Similarly, the present results showed poorer growth, reduced IVCD and earlier viability decline at 10% DO compared with intermediate oxygen conditions.

The metabolic responses also agree with Zakrzewski *et al.* [3], who demonstrated that pH and dissolved-oxygen fluctuations affected CHO cell growth, lactate metabolism and productivity. Their observation that different cell lines responded differently is important because the optimum identified in the present study should not be assumed to apply universally to all CHO clones or recombinant products.

Brunner *et al.* [35] reported significant interactions among pH, pO_2 and pCO_2 for growth, metabolic and productivity responses, supporting the statistically significant DO × pH interaction observed here. Their findings reinforce the conclusion that these variables should be characterized jointly rather than optimized independently.

Biologically, the superior performance near pH 7.0 and intermediate DO can be interpreted as the consequence of balanced respiratory and metabolic conditions. Sufficient oxygen supports oxidative energy metabolism and facilitates the transition from lactate production toward lactate consumption, whereas oxygen limitation promotes less efficient carbon utilization. The lower lactate and ammonia accumulation observed under C5 consequently coincided with prolonged viability and increased recombinant protein production.

Product-quality responses were also consistent with previous observations that dissolved oxygen and pH can affect glycosylation and other quality attributes [6,7]. The collective evidence therefore indicates that metabolic control is central to optimizing CHO productivity: the most favourable environment is not necessarily the condition producing the fastest initial growth, but one that sustains viable biomass while limiting metabolic stress and maintaining protein-processing capacity.

➢ *Implications for Scale-Up and Industrial Manufacturing*

The Ambr® 15 findings provide a useful basis for defining process conditions for subsequent bench-scale and pilot-scale verification. Nienow *et al.* [4] reported similar CHO cell growth and recombinant protein productivity between a 15 mL Ambr system and a 5 L stirred bioreactor, supporting its application as a process-development scale-down platform. Janakiraman *et al.* [31] further demonstrated comparability of an Ambr® 15 scale-down model with both 5 L bench-scale and 15,000 L manufacturing-scale CHO processes.

The identified DO–pH region can therefore guide development of an industrial control strategy in which pH is maintained close to 7.0 and oxygen-transfer capacity is sufficient to prevent prolonged low-DO exposure. Maintaining this region at increasing scale would require coordinated control of agitation, aeration, oxygen enrichment, CO_2 stripping and base addition. Robust process control should also consider online monitoring of DO and pH together with metabolic indicators such as lactate, because nominal setpoints alone may not reveal transient local stress.

Direct transfer of the Ambr® 15 setpoints to manufacturing scale is nevertheless inappropriate without scale-up verification. Large stirred bioreactors can develop spatial gradients in dissolved oxygen, pH and CO_2 because mixing and mass-transfer times increase with scale. Xing *et al.* [48] demonstrated the presence of pH and DO gradients in 5,000 L CHO bioreactors, illustrating why identical bulk setpoints do not necessarily produce identical cellular environments across scales. More recent scale-down work by Gaugler *et al.* [49] showed that large-scale-like DO heterogeneity could increase lactate accumulation and glucose uptake while reducing stationary-phase viable cell concentrations.

Consequently, scale-up should be based on engineering comparability rather than simple replication of Ambr® 15 operating settings. Relevant criteria include oxygen-transfer capacity, mixing time, volumetric power input, gas-flow strategy and CO_2-removal capability. The high-throughput findings should therefore define the biological target operating region, while larger-scale engineering studies establish the agitation and gas-transfer conditions required to maintain cells within that region. This approach provides a more robust pathway from process characterization to industrial recombinant protein manufacturing.

V. CONCLUSION AND RECOMMENDATIONS

➢ *Summary of Major Findings*

The study demonstrated that dissolved oxygen and pH exerted both independent and interactive effects on CHO cell growth, metabolism, viability, and recombinant protein production. Low DO conditions, particularly 10% air saturation, were associated with reduced viable cell density, earlier viability decline, greater lactate and ammonia accumulation, and lower recombinant protein titre. Increasing DO to an intermediate level improved oxygen availability, metabolic efficiency, culture longevity, and cumulative viable biomass.

Culture pH also strongly influenced process performance. The intermediate pH condition of approximately 7.0 produced better growth, lower residual lactate and ammonia, improved viability, and greater protein productivity than pH 6.8 or 7.2. The factorial analysis further indicated a significant DO × pH interaction, confirming that the effect of either parameter depended partly on the level of the other.

The most favourable tested condition was pH 7.0 with 25% DO, which produced the highest viable cell density, integral viable cell density, final viability, and recombinant protein titre while maintaining comparatively favourable

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metabolic and product-quality characteristics. The results therefore indicate that optimal productivity is achieved through balanced environmental control rather than maximizing either oxygen availability or pH independently.

➢ *Conclusion*

Dissolved oxygen and pH are closely interconnected process variables that regulate CHO cell physiology through their effects on respiratory metabolism, nutrient utilization, metabolite formation, culture longevity, and recombinant protein expression. The findings indicate that inadequate oxygen availability promotes inefficient glucose metabolism and metabolic stress, whereas inappropriate pH conditions can intensify metabolite accumulation and reduce viable biomass. Conversely, maintaining both variables within a favourable operating region supports efficient substrate utilization, promotes lactate reassimilation, limits ammonia accumulation, and sustains recombinant protein production.

The study further demonstrates the value of the Ambr® 15 platform for high-throughput upstream process characterization. Parallel factorial experimentation enabled the effects of multiple DO–pH combinations to be evaluated simultaneously and supported statistical identification of a potential operating region. This approach provides a practical basis for data-driven process development, scale-down experimentation, and subsequent validation in larger bioreactor systems.

➢ *Recommendations for Bioprocess Optimization*

Based on the observed process relationships, a provisional operating region around pH 6.95–7.05 and 20–35% DO should be evaluated further, with pH 7.0 and approximately 25–30% DO serving as the central verification condition. This region should be confirmed experimentally across additional CHO clones, recombinant products, and larger bioreactor scales before being adopted as a formal design space.

Real-time monitoring and feedback control should be strengthened by integrating DO and pH measurements with metabolic indicators such as glucose, lactate, ammonia, osmolality, and oxygen uptake. Such integration would enable earlier identification of metabolic stress and allow corrective control before significant viability or productivity losses occur.

High-throughput experimentation should remain an integral part of upstream development because it permits rapid evaluation of process interactions and supports statistically robust optimization. Response Surface Methodology, multivariate analysis, and predictive modelling can be incorporated to refine operating ranges and improve process robustness.

Finally, process optimization should be implemented within a Quality by Design framework, linking critical process parameters such as DO and pH to critical quality attributes including glycosylation, aggregation, charge variation, and structural integrity. This will ensure that improvements in recombinant protein yield are achieved without compromising product quality or manufacturing consistency.

➢ *Recommendations for Bioprocess Optimization*

The bioprocess should be operated within a carefully defined DO–pH region rather than relying on a single nominal setpoint. Based on the present findings, pH 6.95–7.05 and DO 20–35% air saturation represent a suitable provisional operating range, with approximately pH 7.0 and 25–30% DO serving as the central target for further verification.

Real-time monitoring and feedback control should be strengthened to minimize transient deviations from the desired environment. DO and pH control should be coordinated with agitation, gas-flow rate, oxygen enrichment, CO_2 removal and base addition so that correction of one parameter does not destabilize another.

Metabolic indicators should also be incorporated into process-control decisions. Lactate accumulation, glucose uptake, ammonia concentration, glutamine utilization and osmolality can provide early evidence of metabolic stress before substantial loss of viability or productivity occurs. Where feasible, these variables should complement conventional DO and pH measurements.

High-throughput experimentation using platforms such as the Ambr® 15 should be integrated into routine upstream process development. Factorial Design of Experiments and multivariate analysis can rapidly characterize interactions among process variables, reduce development time and support selection of robust operating conditions.

Bioprocess optimization should finally be implemented within a Quality by Design framework, where DO and pH are evaluated as potential critical process parameters and linked quantitatively to productivity and critical quality attributes. This approach can support process robustness, reproducibility and scientifically justified control strategies.

➢ *Recommendations for Future Research*

Future studies should validate the identified DO–pH operating region in bench-scale, pilot-scale and manufacturing-relevant bioreactors. Such validation is necessary because differences in mixing, gas transfer, CO_2 stripping and local concentration gradients may alter CHO cell responses during scale-up.

Further research should investigate dynamic DO and pH control strategies rather than maintaining constant setpoints throughout cultivation. Stage-specific control could provide different conditions during exponential growth, metabolic transition and production phases and may improve both culture longevity and recombinant protein yield.

Integration of online Raman spectroscopy and other process analytical technologies should also be explored for real-time estimation of glucose, lactate, viable biomass and related metabolic variables. These measurements could

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support closed-loop control based on cellular metabolic state rather than physicochemical setpoints alone.

Metabolomic and transcriptomic analyses are recommended to determine the molecular mechanisms underlying the observed DO–pH responses, particularly changes associated with glycolysis, oxidative metabolism, lactate reassimilation, nitrogen metabolism and recombinant protein secretion.

The experimental framework should additionally be evaluated across multiple CHO clones and different recombinant protein products to determine whether the identified relationships are cell-line-specific or broadly transferable.

Finally, future work should develop predictive machine-learning and mechanistic models that integrate DO, pH, cell density, metabolite concentrations and productivity data. Such models could enable early prediction of culture performance, adaptive control and more efficient identification of optimal operating regions during upstream process development.

REFERENCES

[1]. Xu, W.J., Lin, Y., Mi, C.L., Pang, J.Y. and Wang, T.Y. (2023). Progress in fed-batch culture for recombinant protein production in CHO cells. *Applied Microbiology and Biotechnology*, 107(4), 1063–1075.

[2]. Lam, C., Sargon, A., Diaz, C., Lai, Z., Sangaraju, D., Yuk, I., Barnard, G. and Misaghi, S. (2024). Strategies to improve CHO cell culture performance: Targeted deletion of amino acid catabolism and apoptosis genes paired with growth inhibitor supplementation. *Biotechnology Progress*, 40(5), e3471.

[3]. Zakrzewski, R., Lee, K. and Lye, G.J. (2022). Development of a miniature bioreactor model to study the impact of pH and DOT fluctuations on CHO cell culture performance as a tool to understanding heterogeneity effects at large-scale. *Biotechnology Progress*, 38(4), e3264.

[4]. Nienow, A.W., Rielly, C.D., Brosnan, K.M., Bargh, N., Lee, K., Coopman, K. and Hewitt, C.J. (2013). The physical characterisation of a microscale parallel bioreactor platform with an industrial CHO cell line expressing an IgG4. *Biochemical Engineering Journal*, 76, 25–36.

[5]. Sartorius (2026). *Ambr® 15 Cell Culture: Advanced Microbioreactor System*. Sartorius Stedim Biotech.

[6]. Restelli, V., Wang, M.D., Huzel, N., Ethier, M., Perreault, H. and Butler, M. (2006). The effect of dissolved oxygen on the production and the glycosylation profile of recombinant human erythropoietin produced from CHO cells. *Biotechnology and Bioengineering*, 94(3), 481–494.

[7]. Lee, A.P. *et al.* (2021). Multi-omics profiling of a CHO cell culture system unravels the effect of culture pH on cell growth, antibody titer, and product quality. *Biotechnology and Bioengineering*, 118(11), 4305–4316.

[8]. International Council for Harmonisation (ICH) (2009). *Q8(R2): Pharmaceutical Development*. U.S. Food and Drug Administration.

[9]. Kim, J.Y., Kim, Y.G. and Lee, G.M. (2012). CHO cells in biotechnology for production of recombinant proteins: Current state and further potential. *Applied Microbiology and Biotechnology*, 93(3), 917–930.

[10]. Fischer, S., Handrick, R. and Otte, K. (2015). The art of CHO cell engineering: A comprehensive retrospect and future perspectives. *Biotechnology Advances*, 33(8), 1878–1896.

[11]. Glinšek, K., Bozovičar, K. and Bratkovič, T. (2023). CRISPR technologies in Chinese Hamster Ovary cell line engineering. *International Journal of Molecular Sciences*, 24(9), 8144..

[12]. Kirk, T.V. and Szita, N. (2013). Oxygen transfer characteristics of miniaturized bioreactor systems. *Biotechnology and Bioengineering*, 110(4), 1005–1019.

[13]. Pappenreiter, M., Sissolak, B., Sommeregger, W. and Striedner, G. (2019). Oxygen uptake rate soft-sensing via dynamic kLa computation: Cell volume and metabolic transition prediction in mammalian bioprocesses. *Frontiers in Bioengineering and Biotechnology*, 7, 195.

[14]. Chevallier, V., Andersen, M.R. and Malphettes, L. (2020). Oxidative stress-alleviating strategies to improve recombinant protein production in CHO cells. *Biotechnology and Bioengineering*, 117(4), 1172–1186.

[15]. Anane, E., Knudsen, I.M. and Wilson, G.C. (2021). Scale-down cultivation in mammalian cell bioreactors: The effect of bioreactor mixing time on the response of CHO cells to dissolved oxygen gradients. *Biochemical Engineering Journal*, 166, 107870.

[16]. Jiang, R., Chen, H. and Xu, S. (2018). pH excursions impact CHO cell culture performance and antibody N-linked glycosylation. *Bioprocess and Biosystems Engineering*, 41, 1731–1741.

[17]. Hoshan, L., Jiang, R., Moroney, J., Bui, A., Zhang, X., Hang, T.-C. and Xu, S. (2019). Effective bioreactor pH control using only sparging gases. *Biotechnology Progress*, 35(1), e2743.

[18]. Coulet, M., Kepp, O., Kroemer, G. and Basmaciogullari, S. (2022). Metabolic profiling of CHO cells during the production of biotherapeutics. *Cells*, 11(12), 1929.

[19]. Mulukutla, B.C., Gramer, M. and Hu, W.-S. (2012). On metabolic shift to lactate consumption in fed-batch culture of mammalian cells. *Metabolic Engineering*, 14(2), 138–149.

[20]. Rajendra, Y., Kiseljak, D., Baldi, L., Hacker, D.L. and Wurm, F.M. (2012). Reduced glutamine concentration improves protein production in growth-arrested CHO-DG44 and HEK-293E cells. *Biotechnology Letters*, 34(4), 619–626.

[21]. Chen, P. and Harcum, S.W. (2005). Effects of amino acid additions on ammonium stressed CHO cells. *Journal of Biotechnology*, 117(3), 277–286.

[22]. Singh, R., Fatima, E., Thakur, L., Singh, S., Ratan, C. and Kumar, N. (2024). Advancements in CHO

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26aug1045

metabolomics: techniques, current state and evolving methodologies. *Frontiers in Bioengineering and Biotechnology*, 12, 1347138.

[23]. Jones, J., Kindembe, D., Branton, H., Lawal, N., Lopez Montero, E., Mack, J., Shi, S., Patton, R. and Montague, G. (2023). Improved control strategies for the environment within cell culture bioreactors. *Food and Bioproducts Processing*, 138, 209–220.

[24]. Obrstar, D., Mandelc, S., Stojković, S., Francky, A., Bojić, L. and Javornik, B. (2016). Responses of CHO cell lines to increased pCO_2 at normal (37 °C) and reduced (33 °C) culture temperatures. *Journal of Biotechnology*, 219, 98–109.

[25]. Hippach, M.B., Schwartz, I., Pei, J., Huynh, J., Kawai, Y. and Zhu, M.M. (2018). Fluctuations in dissolved oxygen concentration during a CHO cell culture process affects monoclonal antibody productivity and the sulfhydryl-drug conjugation process. *Biotechnology Progress*, 34(6), 1427–1437.

[26]. Ha, T.K., Kim, D., Kim, C.L., Grav, L.M. and Lee, G.M. (2022). Factors affecting the quality of therapeutic proteins in recombinant Chinese hamster ovary cell culture. *Biotechnology Advances*, 54, 107831.

[27]. Zalai, D., Hevér, H., Lovász, K., Molnár, D., Wechselberger, P., Hofer, A., Párta, L., Putics, Á. and Herwig, C. (2016). A control strategy to investigate the relationship between specific productivity and high-mannose glycoforms in CHO cells. *Applied Microbiology and Biotechnology*, 100, 7011–7024.

[28]. Gomez, N., Subramanian, J., Ouyang, J., Nguyen, M.D.H., Hutchinson, M., Sharma, V.K., Lin, A.A. and Yuk, I.H. (2012). Culture temperature modulates aggregation of recombinant antibody in CHO cells. *Biotechnology and Bioengineering*, 109(1), 125–136.

[29]. Geng, S.L., Zhao, X.J., Zhang, X., Zhang, J.H., Mi, C.L. and Wang, T.Y. (2024). Recombinant therapeutic proteins degradation and overcoming strategies in CHO cells. *Applied Microbiology and Biotechnology*, 108, 182.

[30]. Rameez, S., Mostafa, S.S., Miller, C. and Shukla, A.A. (2014). High-throughput miniaturized bioreactors for cell culture process development: Reproducibility, scalability, and control. *Biotechnology Progress*, 30(3), 718–727.

[31]. Janakiraman, V., Kwiatkowski, C., Kshirsagar, R., Ryll, T. and Huang, Y.M. (2015). Application of high-throughput mini-bioreactor system for systematic scale-down modeling, process characterization, and control strategy development. *Biotechnology Progress*, 31(6), 1623–1632.

[32]. Alsayyari, A.A., Pan, X., Dalm, C., van der Veen, J.W., Vriezen, N. and Martens, D.E. (2018). Transcriptome analysis for the scale-down of a CHO cell fed-batch process. *Journal of Biotechnology*, 279, 61–72.

[33]. Goldrick, S., Holmes, W., Bond, N.J., Lewis, G., Kuiper, M., Turner, R., Suzanne, S. *et al.* (2017). Advanced multivariate data analysis to determine the root cause of trisulfide bond formation in a novel antibody–peptide fusion. *Biotechnology and Bioengineering*, 114(10), 2222–2234.

[34]. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) (2023). *ICH Q9(R1): Quality Risk Management*. Geneva: ICH.

[35]. Brunner, M., Fricke, J., Kroll, P. and Herwig, C. (2017). Investigation of the interactions of critical scale-up parameters (pH, pO_2 and pCO_2) on CHO batch performance and critical quality attributes. *Bioprocess and Biosystems Engineering*, 40(2), 251–263.

[36]. Trummer, E., Fauland, K., Seidinger, S., Schriebl, K., Lattenmayer, C., Kunert, R., Vorauer-Uhl, K., Weik, R., Borth, N., Katinger, H. and Müller, D. (2006). Process parameter shifting: Part I. Effect of DOT, pH, and temperature on the performance of Epo-Fc expressing CHO cells cultivated in controlled batch bioreactors. *Biotechnology and Bioengineering*, 94(6), 1033–1044.

[37]. Pan, X., Streefland, M., Dalm, C., Wijffels, R.H. and Martens, D.E. (2017). Selection of chemically defined media for CHO cell fed-batch culture processes. *Cytotechnology*, 69(1), 39–56.

[38]. Kuwae, S., Miyakawa, I. and Doi, T. (2018). Development of a chemically defined platform fed-batch culture media for monoclonal antibody-producing CHO cell lines with optimized choline content. *Cytotechnology*, 70(3), 939–948.

[39]. Velugula-Yellela, S.R., Kohnhorst, C., Powers, D.N. and Agarabi, C. (2018). Use of high-throughput automated microbioreactor system for production of model IgG1 in CHO cells. *Journal of Visualized Experiments*, 139, 58231.

[40]. Velugula-Yellela, S.R., Williams, A., Trunfio, N. and Agarabi, C. (2018). Impact of media and antifoam selection on monoclonal antibody production and quality using a high throughput micro-bioreactor system. *Biotechnology Progress*, 34(1), 262–270.

[41]. Idowu, O. S., Idoko, D. O., Ogundipe, S. O., and Mensah, E. (2025). Optimizing SDS-PAGE for accurate protein characterization in nutritional research and food quality assessment. *International Journal of Innovative Science and Research Technology*, *10*(1).

[42]. Donkor, F., Okafor, M. N., and Enyejo, J. O. (2025). Exploring metabolomics-guided authentication of plant-based meat alternatives supporting regulatory standards and consumer health protection. *International Journal of Innovative Science and Research Technology*, *10*(10).

[43]. Imoh, P. O. (2023). Impact of gut microbiota modulation on autism-related behavioral outcomes via metabolomic and microbiome-targeted therapies. *International Journal of Scientific Research and Modern Technology*, *2*(8).

[44]. Michael, I. A., Olade, A. D., Idoko, D. O., Yiga, V., Galadima, D., and Kwekutsu, E. (2025). Exploring microbial diversity in air and water and its role in enhancing agricultural resilience and urban ecosystem

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26aug1045

health. *International Journal of Innovative Science and Research Technology, 10*(1).

[45]. Idowu, O. S., Ayoola, V. O., Adegbola, F., and Adeyeye, Y. (2024). Air, water, and soil microbiomes as catalysts for smart agriculture, urban ecosystem revitalization, climate adaptation, and public health advancements. *International Journal of Scientific Research and Modern Technology, 3*(5).

[46]. Omoche, I. A., Amana, O., and Ebah, E. E. (2025). Impact of seasonal variation on the microbiological and physicochemical quality of stored rainwater in underground tanks. *International Journal of Innovative Science and Research Technology, 10*(2).

[47]. Muriithi, B. *et al.* (2025). High-efficiency analytical Protein A columns for monoclonal antibody quantification in bioprocessing. Published study indexed in PubMed.

[48]. Xing, Z., Kenty, B.M., Li, Z.J. and Lee, S.S. (2009). Scale-up analysis for a CHO cell culture process in large-scale bioreactors. *Biotechnology and Bioengineering*, 103(4), 733–746.

[49]. Gaugler, L., Hofmann, S., Schlüter, M. and Takors, R. (2024). Mimicking CHO large-scale effects in the single multicompartment bioreactor: A new approach to access scale-up behavior. *Biotechnology and Bioengineering*, 121(4), 1244–1256.

[50]. Idoko, F. A., Markus, L., and Henry, E. (2025). Assessing water-quality parameters for aquaculture in Nigeria: A case study of surface and groundwater in Makurdi. *International Journal of Research Publication and Reviews, 6*(2), 4758–4775.

[51]. Atalor, S. I., Raphael, F. O., and Enyejo, J. O. (2023). Wearable biosensor integration for remote chemotherapy monitoring in decentralized cancer-care models. *International Journal of Scientific Research in Science and Technology, 10*(3).

[52]. Adeniyi, M., Ayoola, V. B., Samuel, T. E., and Awosan, W. (2024). Artificial intelligence-driven wearable electronics and smart nanodevices for continuous cancer monitoring and enhanced diagnostic accuracy. *International Journal of Scientific Research and Modern Technology, 3*(11)

[53]. Okpanachi, A. T., Igba, E., Imoh, P. O., Dzakpasu, N. H., and Nyaledzigbor, M. (2025). Leveraging digital biomarkers and advanced data analytics in medical laboratories to enhance early detection and diagnostic accuracy in cardiovascular diseases. *International Journal of Scientific Research in Science and Technology, 12*.

[54]. Imoh, P. O., and Idoko, I. P. (2022). Gene-environment interactions and epigenetic regulation in autism etiology through multi-omics integration and computational biology approaches. *International Journal of Scientific Research and Modern Technology, 1*(8), 1–16.

[55]. Atalor, S. I. (2022). Data-driven cheminformatics models for predicting the bioactivity of natural compounds in oncology. *International Journal of Scientific Research and Modern Technology, 1*(1), 65–76.

[56]. Animasaun, J. B., Ijiga, O. M., Ayoola, V. B., and Enyejo, L. A. (2024). Evaluating the stability of cannabinoid extracts following different solvent evaporation conditions: A GC-MS/LC-MS degradation profiling study. *International Journal of Scientific Research and Modern Technology, 3*(1), 55–70.

[57]. Animasaun, J. B., Ijiga, O. M., Ayoola, V. B., and Enyejo, L. A. (2024). Impact of solvent polarity on volatile and non-volatile cannabinoid recovery: A multivariate GC-MS/LC-MS extraction optimisation study. *International Journal of Scientific Research and Modern Technology, 3*(1), 40–54.

[58]. Animasaun, J. B., Ijiga, O. M., Ayoola, V. B., and Enyejo, L. A. (2026). Development of a rapid GC-MS workflow for simultaneous quantification of volatile terpenes and cannabinoids in industrial hemp extracts. *International Journal of Innovative Science and Research Technology, 11*(1), 1155–1168.

[59]. Animasaun, J. B., Ijiga, O. M., Ayoola, V. B., and Enyejo, L. A. (2026). Application of FT-IR spectroscopy for differentiating hemp stem and bud chemical composition: A rapid screening approach. *Chemistry and Material Sciences Research Journal, 5*(1).

[60]. Ijiga, A. C., Igbede, M. A., Ukaegbu, C., Olatunde, T. I., Olajide, F. I., and Enyejo, L. A. (2024). Precision healthcare analytics: Integrating machine learning for automated image interpretation, disease detection, and prognosis prediction. *World Journal of Biology Pharmacy and Health Sciences, 18*(1), 336–354.

[61]. Oyebanji, O. S., Apampa, A. R., Afolabi, O., Eromonsei, S. O., and Babalola, A. (2024). Performance benchmarking of convolutional neural networks and ensemble machine-learning techniques for automated mammographic breast-cancer detection. *World Journal of Advanced Engineering Technology and Sciences, 12*(2), 808–833.

[62]. Oyebanji, O. S., Apampa, A. R., Idoko, I. P., Babalola, A., Ijiga, O. M., Afolabi, O., and Michael, C. I. (2024). Enhancing breast-cancer detection accuracy through transfer learning: A case study using EfficientNet. *World Journal of Advanced Engineering Technology and Sciences, 13*(1), 285–318.

[63]. Idoko, D. O., Adegbaju, M. M., Nduka, I., Okereke, E. K., Agaba, J. A., and Ijiga, A. C. (2024). Enhancing early detection of pancreatic cancer by integrating artificial intelligence with advanced imaging techniques. *Magna Scientia Advanced Biology and Pharmacy, 12*(2), 51–83.

[64]. Idoko, P. I., David-Olusa, A., Badu, S. G., Okereke, E. K., Agaba, J. A., and Bashiru, O. (2024). The dual impact of artificial intelligence and renewable energy in enhancing medicine for diagnostics, drug discovery, and public health. *Magna Scientia Advanced Biology and Pharmacy, 12*(2), 99–127.

[65]. Idoko, D. O., Adeniyi, M., Senejani, M. N., Erondu, O. F., and Adeyeye, Y. (2024). Nanoparticle-assisted cancer imaging and targeted drug delivery for early-stage tumour detection. *International Journal of Innovative Science and Research Technology, 9*(11).

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26aug1045

[66]. Darko, D., Kwekutsu, E., and Idoko, I. P. (2025). Synergistic effects of phytochemicals in combating chronic diseases with insights into molecular mechanisms and nutraceutical development. *International Journal of Innovative Science and Research Technology, 10*(3).

[67]. Imoh, P. O., Adeniyi, M., Ayoola, V. B., and Enyejo, J. O. (2024). Advancing early autism diagnosis using multimodal neuroimaging and artificial intelligence-driven biomarkers. *International Journal of Scientific Research and Modern Technology, 3*(6), 40–56.

[68]. Gaye, A., Atanda, O. D., Ibrahim, A. I., Idoko, I. P., and Adeoye, A. F. (2024). Numerical optimisation and sensitivity analysis of a fractional-order hepatitis B virus transmission model under varying vaccination and memory parameters. *International Journal of Scientific Research and Modern Technology, 3*(6), 70–86.

[69]. Gaye, A. (2025). Predictive modelling and statistical analysis of hypertension cases at a small teaching hospital. *Iconic Research and Engineering Journals, 8*(11).

[70]. Gaye, A., Bamigwojo, O. V., Idoko, I. P., and Adeoye, A. F. (2025). Modelling hepatitis B virus transmission dynamics using the Atangana fractional-order network approach. *International Journal of Innovative Science and Research Technology, 10*(4).

[71]. Onuh, J. E., Idoko, I. P., Igbede, M. A., Olajide, F. I., Ukaegbu, C., and Olatunde, T. I. (2024). Harnessing synergy between biomedical and electrical engineering: A comparative analysis of healthcare advancement in Nigeria and the USA. *World Journal of Advanced Engineering Technology and Sciences, 11*(2), 628–649.

[72]. James, U. U., & Akujuobi, C. M. (2026). Multiscale signal processing framework for Zero Trust security in next-generation wireless networks. *International Journal of Wireless and Mobile Networks, 18*(2/3), 1–21. https://doi.org/10.5121/ijwmn.2026.18301

[73]. Idoko, D. O., Mbachu, O. E., Babalola, I. N. O., Felix, E. O., Dada-Abidakun, O., & Alemoh, P. O. (2024). The influence of prenatal vitamin use and community health programs on reducing teratogenic medication exposure and improving perinatal nutrition among African American adolescents with limited access to healthcare. *International Journal of Scientific Research and Modern Technology, 3*(10). https://doi.org/10.38124/ijsrmt.v3i10.62

[74]. Ijiga, A. C., Balogun, T. K., Ahmadu, E. O., Klu, E., Olola, T. M., & Addo, G. (2024). The role of the United States in shaping youth mental health advocacy and suicide prevention through foreign policy and media in conflict zones. *Magna Scientia Advanced Research and Reviews, 12*(1), 202–218. https://doi.org/10.30574/msarr.2024.12.1.0174

[75]. Ijiga, A. C., Balogun, T. K., Sariki, A. M., Klu, E., Ahmadu, E. O., & Olola, T. M. (2024). Investigating the influence of domestic and international factors on youth mental health and suicide prevention in societies at risk of autocratization. *Iconic Research and Engineering Journals, 8*(5), 93–106.

[76]. Ijiga, A. C., Enyejo, L. A., Odeyemi, M. O., Olatunde, T. I., Olajide, F. I., & Daniel, D. O. (2024). Integrating community-based partnerships for enhanced health outcomes: A collaborative model with healthcare providers, clinics, and pharmacies across the USA. *Open Access Research Journal of Biology and Pharmacy, 10*(2), 81–104. https://doi.org/10.53022/oarjbp.2024.10.2.0015

[77]. Ilesanmi, M. O., Okoh, O. F., Balogun, S. A., & Ijiga, O. M. (2024). Data-driven portfolio optimization for utility-scale solar, wind, and battery energy storage systems (BESS): Integrating performance analytics with investor EBITDA targets. *International Journal of Scientific Research and Modern Technology, 3*(11), 141–155. https://doi.org/10.38124/ijsrmt.v3i11.943

[78]. Adewale, L. D. (2026). Digital evidence chains for PPAP assurance: AR-guided data capture, AI-verified documentation and continuous audit automation for secure multi-tier supplier traceability in Industry 4.0 manufacturing. *International Journal of Multidisciplinary Evolutionary Research, 7*(1), 43–55. https://doi.org/10.54660/IJMER.2026.7.1.43-55

[79]. Adewale, L. D. (2026). Smart Factories, Smarter Evidence: Reinventing Quality Assurance for U.S. Manufacturing Competitiveness *International Journal of Multidisciplinary Futuristic Development* Vol 7 Iss. 1. DOI: https://doi.org/10.54660/IJMFD.2026.7.1.09-18

[80]. Ajiboye, A. S., Balogun, T. K., Imoh, P. O., Ijiga, A. C., Olola, T. M., & Ahmadu, E. O. (2025). Understanding the impact of social media on mental health in autistic youth and expanding access to culturally responsive behavioral health services in underserved communities. *International Journal of Scientific Research in Humanities and Social Sciences, 2*(3), 33–56. https://doi.org/10.32628/IJSRHSS25234

[81]. Ajiboye, A. S., Balogun, T. K., Peter-Anyebe, A. C., Ahmadu, E. O., & Olola, T. M. (2025). Investigating the epigenetic and psychological effects of community gun violence and mass shootings on youth and families through a transgenerational trauma lens. *Social Values and Society, 7*(2), 74–82. https://doi.org/10.26480/svs.02.2025.74.82

[82]. Anokwuru, E. A., Omachi, A., & Enyejo, J. O. (2024). Automation-enabled RFI/RFP market intelligence platforms: Redefining data-driven business development in global pharmaceutical markets. *International Journal of Scientific Research in Science and Technology, 12*(3), 1016–1036. https://doi.org/10.32628/IJSRST54310301.