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REVIEW ARTICLE

Escherichia coli Fermentation and GFP Production by using the Eppendorf BioFlo®120 Bioprocess Control Station

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ABSTRACT

This article is an examination of a bioprocess experiment involving the cultivation of a GFP-expressing strain of *Escherichia coli* using the BioFlo®120 bioprocess control station. The BioFlo 120 is a bench-scale bioreactor/fermentor system perfectly suited for all levels of research and development. The study highlights the capabilities of the BioFlo®120 in supporting high-density growth and protein production. There is two key parameters were monitored to assess the progress of the fermentation process, the optical density (OD_{600}) to estimate cell density and relative fluorescence units (RFU) to quantify the green fluorescent protein production. Additionally, it discusses the Auto Culture modes, which enable automatic process control of common microbial and mammalian cultures, making it accessible for users with varying levels of experience. The application note also delves into the differences between batch, fed-batch, and continuous fermentation processes, with a focus on *E. coli* fermentation at bench scale. It covers various aspects such as biomass and nutrient concentration analysis methods, including growth rate, productivity, yield, and process costs. This comprehensive approach provides valuable insights for bioprocess engineers in selecting the most suitable fermentation method for their specific requirements. This article explores the positive outcome for bioprocess engineers and researchers who aim to optimize bio-production systems for industrial or research purposes.

Keywords: *Escherichia coli*, Green fluorescent protein, BioFlo®120 bioprocess control station, Optical density, Culture feeding, Relative fluorescence units.

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INTRODUCTION

The BioFlo 120 is a bench-scale bioreactor/fermentor system perfectly suited for all levels of research and development. The BioFlo®120 bioprocess control station offers versatile capabilities for controlling both autoclavable and single-use vessels, making it suitable for various fermentation and cell culture applications, including batch, fed-batch, perfusion, and continuous cultures. Equipped with proprietary software, it supports mass flow-controlled gassing and automatic mixing of up to four gasses, enabling precise dissolved oxygen (DO) control in different applications, such as high-density mammalian cell culture and bacteria and yeast fermentations.

The Auto Culture modes streamline the operation of the BioFlo®120, providing users with pre-defined protocols for *E. coli* batch fermentation or CHO batch cell culture processes. These modes are designed with set points and cascades tested by experienced application development teams, minimizing the learning curve for users and ensuring reliable results. Users only need to select the vessel size and type and perform standard preparations, such as sensor and pump calibration, before initiating the run. The application note discusses the significance of fermentation in producing various products, such as biopharmaceuticals, food supplements, biofuels, and chemical building blocks. It emphasizes the importance of considering factors like media and supplement costs, process runtime, microbial growth, product titer, yield, and quality during process development. The concentrations of nutrients and by-products in the culture medium play a crucial role in optimizing the bioprocess.

Batch fermentation involves inoculating microorganisms into a fixed volume of medium, leading to changing culture conditions as nutrients are consumed and by-products accumulate. Fed-batch fermentation, a modified version of batch fermentation, involves the addition of nutrients in increments throughout the fermentation process to support extensive biomass accumulation and limit by-product

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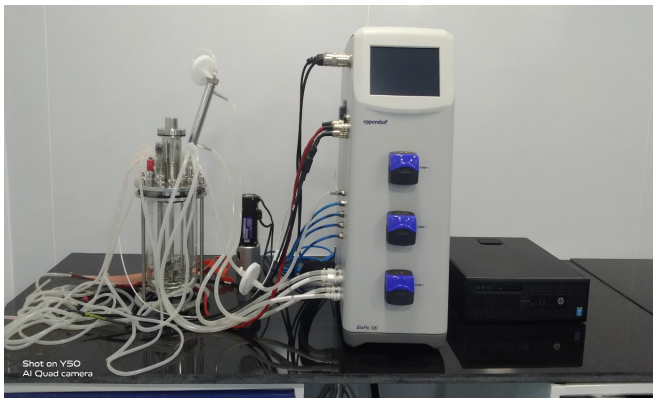

Figure 1: BioFlo®120

accumulation. Continuous fermentation entails the continuous addition of fresh medium while harvesting cells and used medium simultaneously, maintaining steady-state conditions to reduce downtime and enhance economic competitiveness. In the described application note, *Escherichia coli* was cultured in Eppendorf BioBLU®3f Single-Use Vessels, allowing for a direct comparison of batch, fed-batch, and continuous fermentation modes. This comprehensive review provides valuable insights for bioprocess engineers in selecting the optimal fermentation mode for their specific applications, considering factors like product yield, biomass density, and process economics.

Strain selection and Preculture preparation

The *E. coli* strain used in this study, ATCC® 25922GFP™, is engineered to express GFP, which facilitates easy monitoring of protein expression. The strain carries an ampicillin resistance gene on a plasmid, necessitating the continuous presence of ampicillin (0.1 g/L) to maintain plasmid stability. Preculture preparation involves growing the strain in terrific broth (TB) medium at 37°C and 200 rpm overnight. This process sets the stage for large-scale fermentation by ensuring a robust and active inoculum.[2,4]

Medium Preparation

Complex medium

Composed of TB-modified glycerol and Antifoam 204, this medium supports robust growth but may introduce variability in protein production due to its complex composition.[4]

Chemically defined medium

This medium is formulated with precise concentrations of phosphate/citrate buffer, trace elements, thiamine, magnesium sulfate, and glucose, allowing for better control of fermentation conditions and more consistent protein yields. For *E. coli* fermentation, a chemically defined medium at pH 7.0 was used.[3]

Fermentation

Fermentation was conducted in a heat-blanketed glass vessel controlled by a BioFlo 120 bioprocess control station. The process parameters included:

Table 1: BioFlo® 120 hardware configuration

Parameter	*Configuration*
Gas mix	Automatic gas mix
Gas flow control	One thermal mass flow controller (TMFC) with 0-20 standard Liters per minute (SLPM) flow range
Vessel	Heat-blanketed glass vessel with baffle assembly (maximum working volume of 2.2L)
Motor	Direct drive motor
Impeller	Two Rushton-type impellers
Sparger	Ring sparger (macro sparger)

Temperature

Maintained at 37°C to optimize growth conditions for *E. coli*.

pH control

The pH was tightly regulated at 7.0 using a 25% NH4OH solution. Proper pH control is essential for maintaining enzyme activity and overall cell health.

Dissolved oxygen (DO)

Set to 30% to ensure adequate oxygen supply for aerobic respiration.

Antifoam 204 was added as needed to control foam formation, which can interfere with the fermentation process.

Inoculum preparation

Inoculum preparation involved growing *E. coli* in TB modified supplemented with ampicillin. The inoculum was then transferred to the fermentation vessel to achieve a starting optical density (OD600) between 0.1 and 1.0. Preliminary studies indicated that varying the starting OD600 within this range did not significantly affect the fermentation outcome.

Feeding medium and fermentation

It elaborates on the preparation of a concentrated feeding medium, essential for providing additional nutrients during the fermentation process. It then describes the setup for the fermentation itself, highlighting the use of a heat-blanketed glass vessel connected to a BioFlo®120 bioprocess control station. It mentions the inoculation of the culture with the preculture and specifies the operating conditions, such as temperature, pH control, and dissolved oxygen (DO) level. Additionally, it notes the selective addition of antifoam as needed, indicating proactive management of potential foaming issues during fermentation.[4]

Measurement techniques

Various techniques were employed for measuring optical density, glucose concentration, and cell wet/dry weight to assess fermentation progress.

Automatic controls

The automation of pH and DO control without user intervention enhances process efficiency and consistency.

Table 2: BioFlo® 120 Bioprocess control station

Versatility	*Supports batch, fed-batch, and continuous cultures.*
Capacity	Up to 40 L with BioBLU® Single-Use Vessels, 10.5 L with glass autoclavable vessels.
Control features	Mass-flow-controlled gassing, automatic mixing of up to four gasses, DO control.
User-friendliness	Push-button bioprocess concept, recommended setpoints, and cascades for quick setup and optimization.
Performance	High cell densities and protein titers were demonstrated in *E. coli* fed-batch fermentation (OD600 of 191, 13,000 RFU of GFP).

DISCUSSION

The application note provides comprehensive insights into the experimental setup, process control, and analysis methods employed in the study. The results indicate successful fermentation with high cell densities and protein production using the BioFlo®120 system. The detailed parameter configuration, including pH and DO control strategies, enhances the reproducibility and scalability of the fermentation process. Moreover, the ability to use both autoclavable and single-use vessels expands the system's versatility and applicability in various bioprocessing applications. The integration of feeding strategies further optimizes the production process, ensuring sustained growth and productivity. Overall, the study highlights the BioFlo®120 capability as a reliable and efficient bioprocess control station for high-density *E. coli* fermentation and protein production, offering potential implications for biotechnological industries.

Performance of BioFlo 120

Analysis of the system's efficiency in maintaining optimal fermentation conditions.

Comparison with Other Systems

Advantages of using the BioFlo 120 over other bioprocess control systems.

Challenges and Solutions

Common issues encountered during high-density fermentation and how the BioFlo 120 addresses them.

CONCLUSION

The study successfully demonstrated the effectiveness of the BioFlo®120 bioprocess control station in cultivating a GFP-expressing strain of *E. coli*. The optimization of *E. coli* fermentation processes requires careful attention to strain selection, medium formulation, and process control. By employing both complex and chemically defined media and utilizing advanced fermentation technologies like single-use vessels, researchers can achieve high yields of GFP and other recombinant proteins. Future advancements in fermentation technology and media formulation will continue to enhance the efficiency and effectiveness of *E. coli*-based production systems. Moreover, the article provides valuable insights into the selection of appropriate fermentation methods based on factors like biomass concentration, nutrient management, and overall process efficiency. The BioFlo®120's ability to maintain controlled environments, automate critical processes, and ensure consistent results makes it a reliable choice for both industrial and research bioproduction applications.

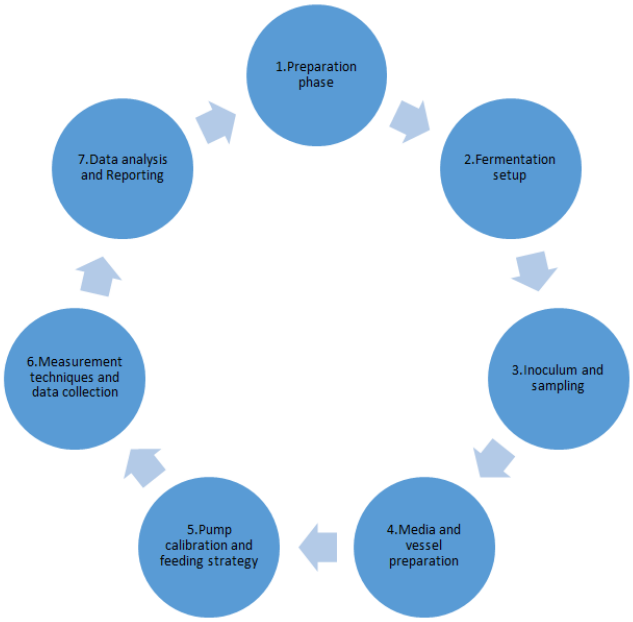

Figure 2: Flow chart of BioFlo®120

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