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Scale-Up Challenges and Optimization Strategies for Green Fluorescent Protein Production in *E. coli* JM109: A Shake Flask and Batch Fermentation Study

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Abstract

Scale-up of recombinant protein production processes is a crucial step in biotechnology and involves the transition from small-scale shake flasks to large-scale bioreactors. This study aimed to detect errors and understand optimization strategies in the scale-up process of Green Fluorescent Protein (GFP) production using E. coli JM109 strain by comparing shake flask and batch fermentation approaches. Scale up process was inefficient (product yield efficiency was < 1). Shake flask cultures gave higher GFP production levels than the batch fermentation approach. The differences in GFP yield were attributed primarily to inconsistent KLa and OTR among other factors such as plasmid stability, cell density and scale-up parameters. Furthermore, the study investigated the impact of process parameters like wet cell weight, dry cell weight, and optical density on GFP productivity. It was observed that despite having a high cell density in the batch fermentation approach, the total protein yield in mg/mL was lower compared to the shake flask method values (200 F = 6.247, 250 F = 8.024, 200 SF = 9.948, 250 SF = 10.209). The influence of promoter regions on GFP expression and the potential benefits of protein engineering for enhancing protein production was also explored. The results underscores the importance of understanding and optimizing various factors during the scale-up process to ensure efficient GFP production and management of resources. By keeping OTR constant and addressing issues such as plasmid stability, lysis step, cell density, viability, scale-up parameters, and promoter activity, GFP yield can be improved in large-scale bioreactors. Additionally, protein engineering strategies could provide valuable tools for enhancing GFP expression and production, ultimately contributing to the development of more efficient protein production platforms in the biotechnology industry.

Keywords: Green Fluorescent Protein (GFP), protein expression and purification, E. coli JM109, scale up, shake flask culture, fed-batch fermentation

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Introduction

Green Fluorescent Protein (GFP) is naturally found in the bioluminescent jellyfish named Aequorea Victoria [1]. It was first discovered by Shimomura in 1960 and has emerged as an invaluable and prominent reporter protein in cell biology, biochemistry, and molecular biology largely because of its ability to fluoresce in vivo without the need for an exogenous cofactor [2]. The unique structure of GFP (Fig. 1); a 27 kDa monomer and a beta-barrel [3] comprises of eleven β-strands with chromophore 4-(p hydroxybenzylidene) imidazolidin-5-one (HBI) at the center of the alpha-helix [4] that plays a crucial role in its remarkable fluorescence properties. As described by Liu et al [4], it consists of 238 amino acids and exhibits a unique ability to retain fluorescence under various harsh conditions, including heat, pH extremes, and exposure to chaotropes and detergents. As a result of these characteristics, several studies have been able to visualize protein localization, protein-protein interactions, and gene expression in cells using the GFP.

Furthermore, the chromophore, generated from the tripeptide; Ser-Tyr-Gly (Fig. 1) in its protein sequence, only turns luminous when incorporated into the entire GFP structure. [5]. Formation of the chromophore, which involves a oxidation step and a cyclization reaction, is either autocatalytic or utilizes ubiquitous factors present in lots of organisms [6].

Shake flask and batch fermentation methods are commonly used for studying microbial growth and protein expression [7]. However, preferences are given depending on the scale of application. For example, shake flasks are favored for their simplicity and ease of use in smaller-scale applications, while

batch fermentation offers the potential for higher cell densities, leading to increased product yields. These two widely used methods will be juxtaposed in terms of scale for potential optimization strategies.

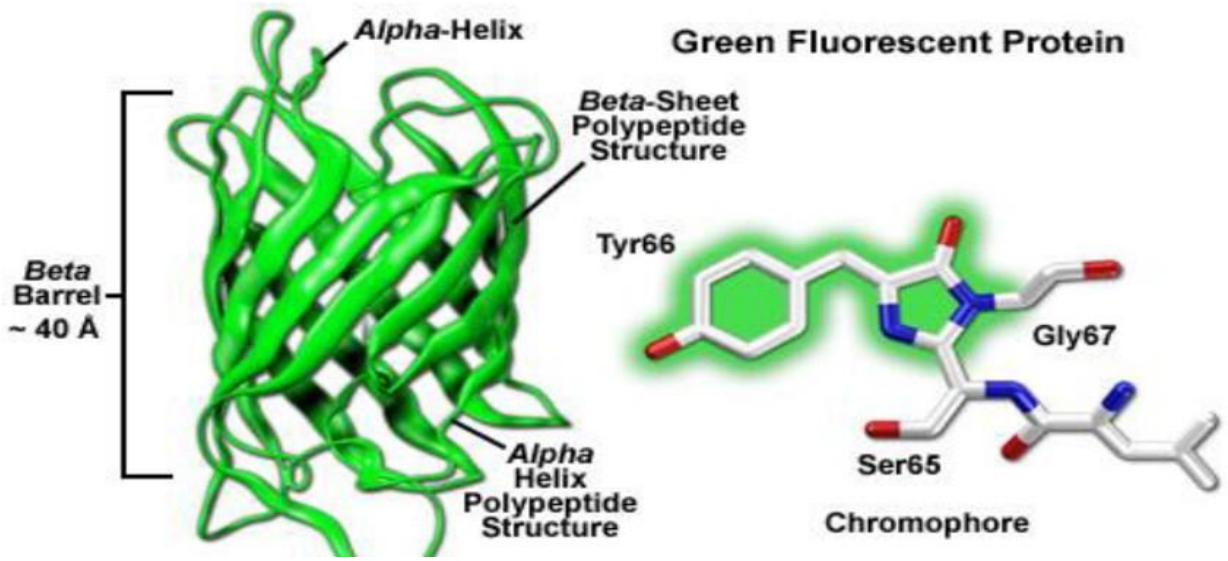

Figure 1: GFP model protein structure and its chromophore demonstrating its numerous advantages, including the ability to fluoresce without requiring cofactors, substrates, or additional gene products, and compatibility with both in vivo and fixed tissue assays [6].

Expression of recombinant GFP in bacterial systems like Escherichia coli has extensively been studied for different purposes not limited to understanding the functions of GFP as a reporter for protein localization [8], exploitation for production of high recombinant protein [9], investigating the antimicrobial activities of silver nanoparticles [10] to pilot and acknowledge the development of various strategies for scale up among and other applications like surveillance or reporters in biological systems. However, scale up comparison of GFP production between shake flask and batch fermentation approaches in E. coli JM109 strain is yet to be explored. This study will help to understand the complexities and challenges associated with scale-up process, as well as in guiding the development of successful scale-up strategies for bioreactor processes.

This study employs a comprehensive approach, starting with the cloning of the GFP gene into a suitable plasmid vector, pHG165, which allows for high-level expression of the target protein. Following the transformation of the E. coli JM109 cells, transformed cells were cultivated under controlled conditions to maximize GFP expression, including temperature, aeration, and nutrient availability. After cell growth and induction, the microbial cells was harvested and the GFP extracted for purification. Ion exchange chromatography was utilized for GFP purification, followed by a thorough analysis of the purified protein fractions using gel electrophoresis and Bradford protein quantification assay. Findings from this study will contribute to the growing body of knowledge on GFP expression and purification by giving an added understanding of the factors influencing its production, and ultimately enabling more efficient utilization of this vital reporter protein in various scientific applications like such as genomic tagging and live-cell imaging. Similarly, it may lead to the development of innovative and more efficient strategies for the expression and purification of GFP and other recombinant proteins in different vectors and/or bacterial cells.

Methods

Bacterial strain, plasmid construction, and expression

Escherichia coli strain JM109 was used for the expression of green fluorescent protein (GFP). The GFP gene from Aequorea victoria was cloned into the pHG165 (Fig. 2) expression vector as described by Stewart et al [11] using the EcoRI and PstI restriction enzymes. Ligation was performed at a 1:4 vector: insert ratio with T4 DNA ligase (this means that for every one molecule of the cut vector, there are four molecules of the cut insert present in the reaction mixture). This ratio was chosen to increase the chances of the vector and insert joining together, which facilitates the formation of the desired recombinant plasmid, this was followed by incubation at 15℃ overnight. The recombinant plasmid was transformed into E. coli JM109 competent cells. Transformants were selected on LB agar plates and confirmed by colony PCR and DNA sequencing.

Shake Flask Culture and inoculum preparation

A single colony of E. coli JM109 harboring the constitutively expressing GFP plasmid was inoculated into 50 mL of LB medium supplemented with 50 µg/mL kanamycin in a 250 mL shake flask (Table 1, 2, 3). The culture was incubated at 37℃ and 200 rpm, allowing for continuous GFP expression without the need for induction. After reaching the mid-exponential stage at an optical density at 600 nm (OD600) of 0.6, the culture was further incubated for 16 hours to give sufficient time for the expression of the GFP to reach its maximum level. The goal is to provide enough time for the bacterial cells to produce and accumulate the recombinant protein while maintaining the overall health and integrity of the culture.

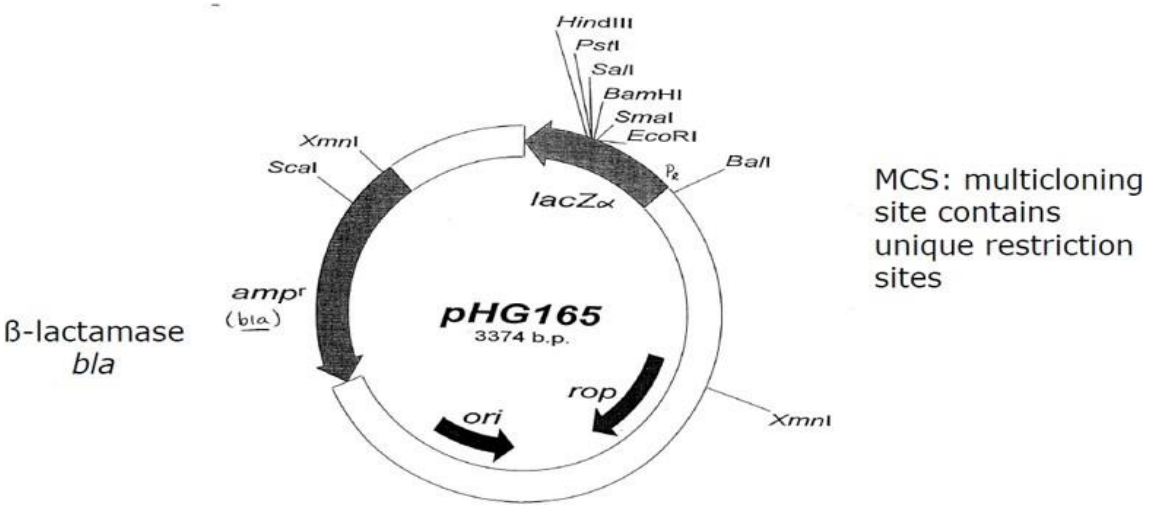

Figure 2: Plasmid/expression vector used (adapted from Stewart et al., (1986). The host organism can be any E coli JM series. Here, we used the strain JM109.

The cells were harvested by centrifugation at 4,000 x g for 20 minutes at 4℃, and the cell pellet was resuspended in lysis buffer (50 mM Tris- HCl, pH 8.0, 150 mM NaCl, 1% Triton X-100) for protein extraction. Subsequently, the seed culture was aseptically transferred to a 15 L fermenter, achieving a final OD of 0.1 to 0.5. The fermentation process took place at 37℃ under aerobic conditions, using nutrient rich, modified medium supplemented with 50 µg/mL kanamycin and 0.5% glucose.

Batch Fermentation

A seed culture was prepared by inoculating a single colony of E. coli JM109 into a 100 mL of LB medium containing 50 µg/mL kanamycin and incubating at 37℃ and 200 rpm overnight. The seed culture was then used to inoculate a 15 L bioreactor containing the modified medium supplemented with 50 µg/mL kanamycin and 0.5% glucose at an initial OD600 of 0.1 (Table 1, 2 and 3). The fermentation was carried out at 37℃ with a constant aeration rate of 1 vvm and an agitation speed of 600 rpm. Cells were harvested by continuous centrifugation at 4,000 x g for 20 minutes at 4℃, and the cell pellet was resuspended in lysis buffer for protein extraction.

Table 1: Process/control parameters in shake flask

Process Parameters	
Inoculation source	Shake flask
Inoculation medium	LB
Inoculation age (h)	24 h
Fermentation Medium	Complex
pH – controlled by 5 M NaOH and phosphoric acid	7
pO2 control (% air saturation) maintained by 1st agitation, 2nd airflow	30%

Table 2: Media composition for shake flask and fermentation

Component of medium	Amount required (per litre)	Amount added (in 15L)	
$(NH4)_2SO_4$	14g	210g	
Glycerol	20g	300g	
KH_2PO_4	2g	30g	
K_2HPO_4	16.5g	247.5g	
Citric acid	7.5g	112.5g	
Conc H_3PO_4	1.5 mL	22.5g	
Antifoam (AF204)	0.2 mL		
Yeast extract	40g	600g	
Deionized water	Top to volume		
*$MgSO_4.7H_2O$	10.4 mL	Concentration required g l^{-1}	Amount added (g) for 1000 mL
		240.0	240.0
*$CaCl_2H_2O$	1.7 mL	Concentration required g l^{-1}	Amount added (g) for 200 mL
		172	34.4
*Trace D solution	34 mL	___	
10 mg/mL Kanamycin**	5 mL	___	

*Added aseptically after basal medium sterilization when fermenter has cooled.

**Added aseptically just prior to inoculation of the fermenter

Protein Extraction and Purification

The resuspended cell pellets from both shake flask and batch fermentation cultures were lysed by bead beating with glass beads. The lysate was then centrifuged at 12,000 x g for 30 minutes at 4℃ to separate soluble and insoluble fractions. The soluble fraction was subjected to ion exchange chromatography using DEAE Sepharose. The column was equilibrated with 20 mM Tris-HCl, pH 7.4, and the protein sample was loaded onto the column. The column was washed with 20 mM Tris-HCl, pH 7.4, to remove any unbound proteins. To elute the bound proteins, a series of buffers with increasing NaCl concentrations (50 mM, 100 mM, 150 mM, 200 mM, and 250 mM NaCl in 20 mM Tris-HCl, pH 7.4) were used. Fractions were collected and examined for GFP fluorescence. The greenest fraction, indicating the highest concentration of GFP, was identified.

Table 3: Trace metals solution

Component	Concentration required (g l^{-1})	Amount for 2.0 (g)
Conc. Orthphosphoric acid	48.0 mL	96 mL
Iron sulphate. $7H_2O$	3.36	6.72
Zinc Sulphate.$7H_2O$	0.84	1.64
Manganese Sulphate.H_2O	0.51	1.02
Sodium Molybdate.$2H_2O$	0.25	0.50
Copper Sulphate.$5H_2O$	0.12	0.24
Boric acid	0.36	0.72

Protein purification

Preparation of protein extracts for gel electrophoresis

Selected fractions from the highest concentration of elution buffer i.e. the greenest fractions were chosen for analysis and further quantification. 10 µL of these fractions were mixed with 10 µL of 2X sample buffer and heated at 100°C for 5 minutes. Crude and purified samples were then centrifuged for 30 seconds and mixed before loading onto pre-cast gels. The gels were run at 200 V in 1X Tris glycine buffer for 45 minutes to 1 hour, followed by visualization under a gel doc system after staining and de-staining. Images were captured for further analysis.

Bradford Quantification

A standard curve of bovine serum albumin (BSA) was prepared by diluting the 0.1 mg/mL stock solution to different concentrations. Three steps of a 1:10 dilution series of both shake flask and fermentation samples were prepared, and 200 µL of Bradford assay reagent was added to all standard and GFP samples in the cuvettes. The samples were mixed and left to equilibrate for about 5 minutes before measuring the absorbance at 595 nm using a spectrophotometer. The protein concentrations in the samples were determined using the standard curve.

Data Analysis

A standard curve was plotted using the absorbance measurements of the standard protein, bovine serum albumin (BSA), at different concentrations. To ensure accurate quantification, the linear range of the calibration curve was identified, within which the protein concentration and absorbance at 595 nm exhibit a strong linear relationship. This is indicated by a linear correlation coefficient (R^2) above 0.99. From the standard curve, the minimum and maximum protein concentrations that fall within the linear range were determined. This range was used for the accurate estimation of the protein concentrations in the shake flask and fermentation samples.

Using the established linear range of the standard curve, the protein concentrations in the shake flask and fermentation samples were estimated. The absorbance values of the samples at 595 nm were compared to the standard curve, and the corresponding protein concentrations were determined.

Results

To evaluate the efficiency of green fluorescent protein (GFP) production during process scale-up, recombinant expression outcomes obtained from shake flask cultures were systematically compared with those from batch fermentation. Multiple parameters were assessed to capture both biomass accumulation and product formation across the two scales. These included qualitative indicators of GFP expression, such as pellet coloration and fluorescence intensity, as well as quantitative measurements of optical density, wet and dry cell weights, total protein concentration, and active GFP yield (Figures 3 – 8). In addition, purification efficiency and product quality were evaluated using ion exchange chromatography, SDS-PAGE analysis, and fluorescence-based assays. The results presented below collectively illustrate the differences in cellular growth dynamics, protein recovery, and overall scale-up performance between shake flask and fermenter systems, providing a basis for identifying key limitations affecting large-scale GFP production (Table 4 and 5)

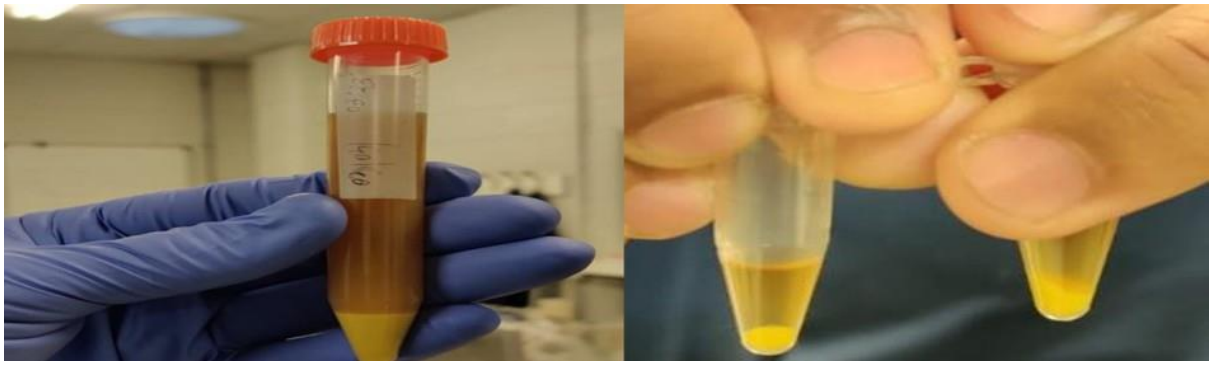

Figure 3: GFP production in both scales was successful.

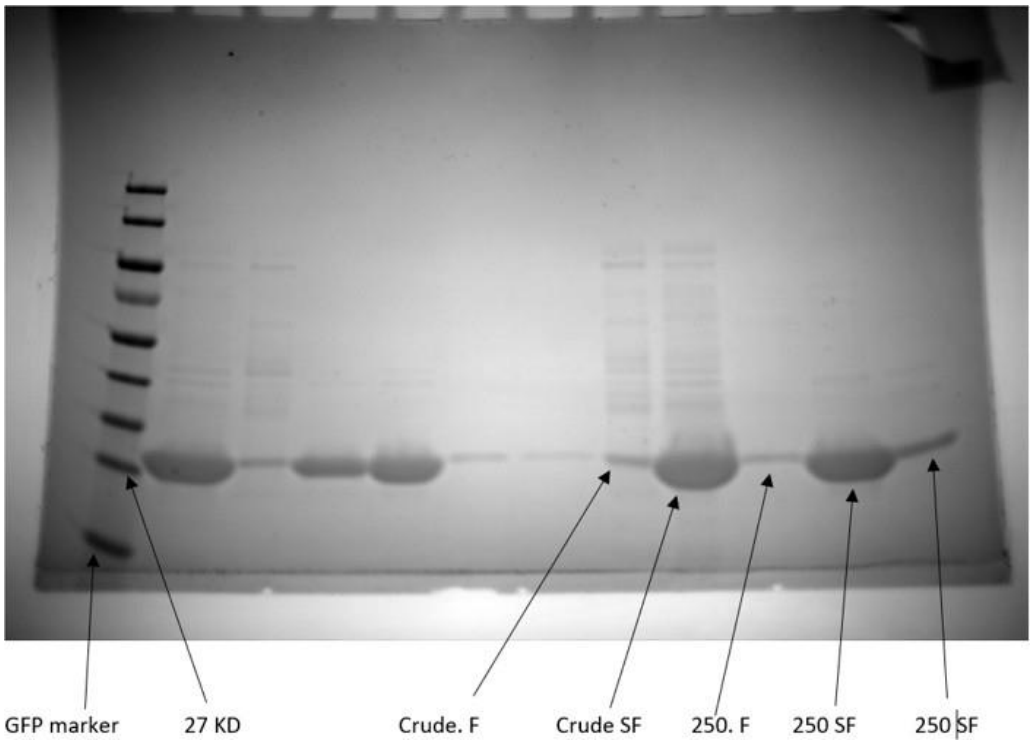

Figure 4: SDS-PAGE analysis demonstrating the successful expression and purification of GFP: lane 1 = GFP marker; lane 2 = crude lysate (fermentation); lane 3 = crude lysate (shake flask); lane 4 = 250 fermentation; lane 5= 250 shake flask; lane 6= 200 shake flask. The molecular weight of the band is approximately 27 kDa which is consistent with the band of GFP protein. The bands on the respective lanes suggests that shake flask had the most efficient purification compared to fermentation. 250 nM buffer fractions for shake flask had just one band indicating the required concentration for better GFP purification of the GFP.

Figure 5: Ion exchange purification fractions for fermentation and shake flask. S3 = fermentation; SF = Shake flask.

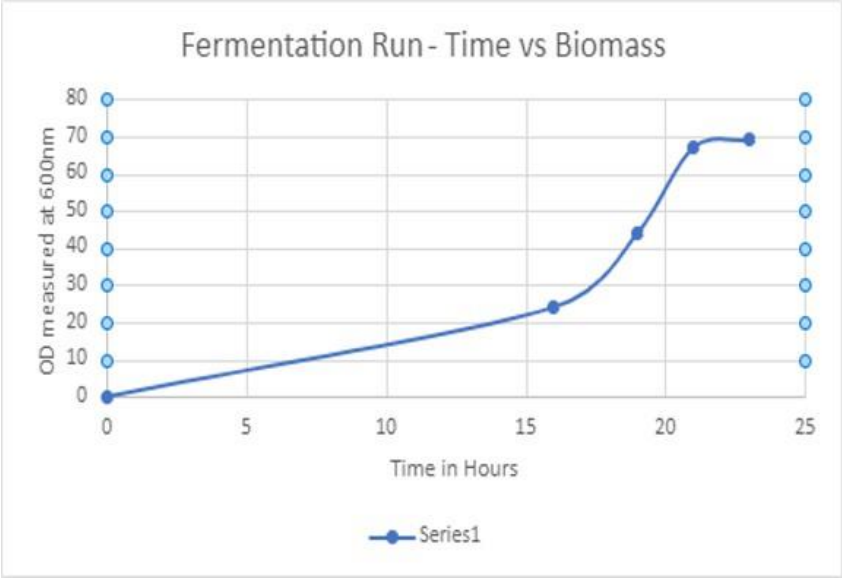

Figure 6: Bacterial biomass dynamics during fermentation. Based on these observations, the bacterial culture undergoes growth during the fermentation process, with the biomass increasing over time. However, the growth appears to slow down or reach a plateau between 21 and 23 hours. At 21 hours, the OD600 is 67, showing a continued increase in biomass. At 23 hours, the OD600 is 69, indicating that the biomass has reached a plateau or near-plateau phase, as the increase in biomass between 21 and 23 hours is minimal.

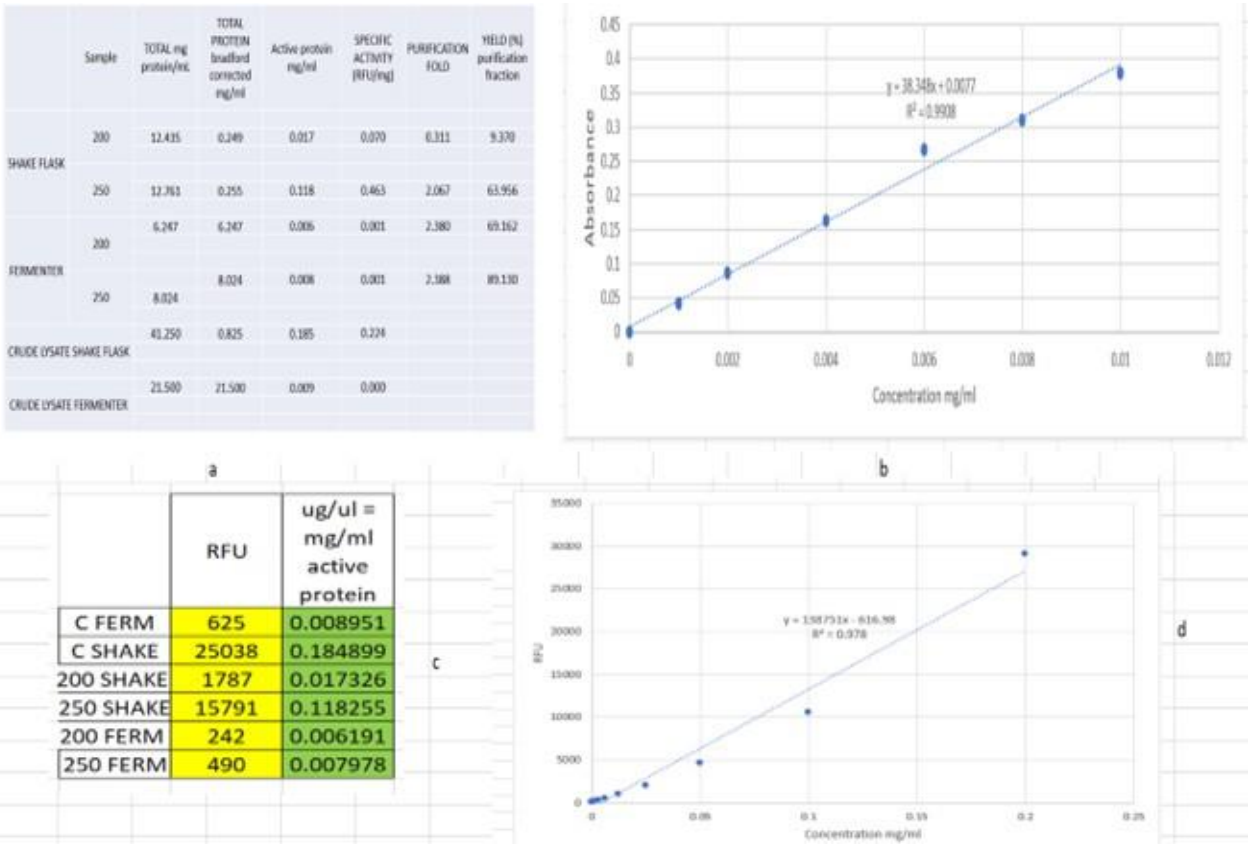

Figure 7: (a): Protein quantities and purification metrics for shake flask and fermenter samples, (b): Standard curve of Bovine Serum Albumin (BSA) correlating concentration and absorbance at 595 nm. The absorbance at 595 nm increases as the concentration increases, useful for quantifying unknown GFP protein from their absorbance alone at 595, (c): Relative fluorescence units (RFU) and active protein concentrations for shake flask and fermenter samples, (d): Relative fluorescence units and corresponding GFP concentrations.

The high R^2 value (0.9908) demonstrates the excellent linearity of the calibration curve, which suggests that the Bradford assay used for determining protein concentrations is reliable and robust. This strong correlation also highlights that the assay is

sensitive and able to distinguish between different BSA concentrations, which is essential for accurate quantification of protein samples in this study.

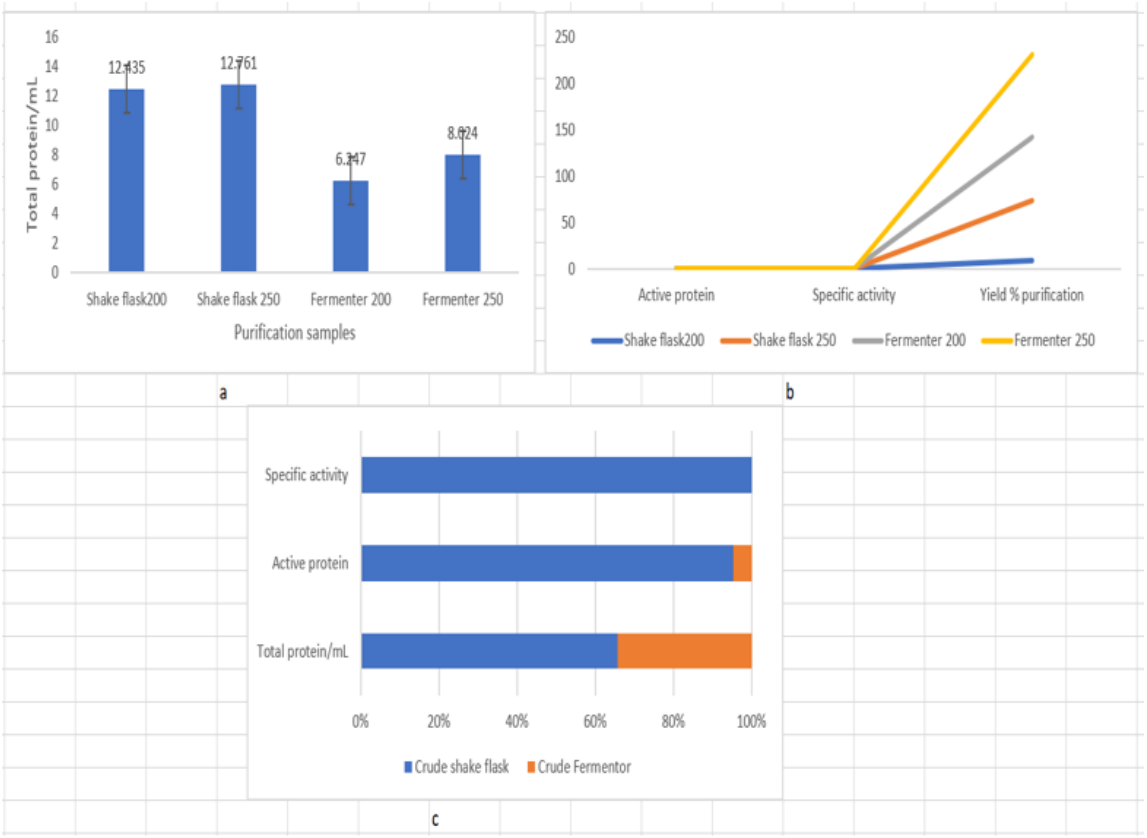

Figure 8: (a): Total protein concentration in purified GFP samples from shake flask and fermenter cultures (b): Active protein, specific activity, and yield percentage of purification for shake flask and fermenter samples (c): Comparison of total protein, active protein, and specific activity for crude shake flask and crude fermenter samples.

Table 4: Fermentation process parameters and cell growth measurements for GFP expressing E. coli cultures

Sample	Time	Run time	Temperature	pH	Dissolved Oxygen	Stirrer	Centrifugation (rpm)	OD 600	WCW (5mL)	WCW (g/mL)	DCW
S2	12:13	19 hrs	37.2	7.01	29.6	1495	13,000	44	0.68g	68	14
S3	14:00	21 hrs	37.1	7.08	9.22	1495	13,000	67	1.11g	110	16.7
S$	16:00	23 hrs	37	7.08	91	1000	13,000	69	0.77g	77	19

OD – Optical density
WCW – Wet cell weight
DCW – Dry cell weight

Evidenced by the increase in OD and WCW, the parameter depicts that the bacterial culture is growing over time. Doubling time for the 19 to 21- hour interval is approximately 3.53 hours. Biomass decreased in the second interval so doubling time cannot be calculated for this period. The high R^2 value (0.9908) demonstrates the excellent linearity of the calibration curve, which suggests that the Bradford assay used for determining protein concentrations is reliable and robust. This strong correlation also highlights that the assay is sensitive and able to distinguish between different BSA concentrations, which is essential for accurate quantification of protein samples in this study.

Product yield efficiency represents the ratio of the total protein obtained from LS fermentation to the total protein obtained for SS fermentation (Gameil et al. 2021). A value less than one indicates that the scale up has failed while a value of one or higher signifies that the scale-up process has been successful.

Protein yield scale-up success and efficiency

Table 5: Comparison of scale-up ratios and product yield efficiencies across samples

Samples	Scale ratio (Vt, LS /Vt, SS)	Protein production in large scale	Protein production in small scale	Protein yield efficiency for scale up (Total protein LS / Total protein SS)	Inference
200	30	6.247 mg/mL	12.435 mg/mL	0.502	< 1
250		8.024 mg/mL	12.761 mg/mL	0.629	< 1

Vt – Total volume of the vessel
SS – Small scale or shake flask
LS – Large scale or fermentation

Discussion

GFP production

GFP production was successfully produced in both shake flask and fermentation scales, as evidenced by the green color of the cell pellets shown in Fig. 3. The green color indicates the presence of GFP, a fluorescent protein that glows green when exposed to blue or ultraviolet light [5]. The production of green-colored cell pellets in both scales suggests that the expression of the GFP gene and the subsequent production of GFP protein was successful in both conditions. Similarly, the WCW and OD values in Table 4, 5 and figure 6 aligns with preliminary findings.

The color intensity appeared more in the pellets for shake flask (Fig. 5) suggesting that the parameters used were favorable compared to the fermentation system. The greener appearance of the GFP extract from the shake flask further supports the possibility of higher GFP expression in the shake flask culture also corroborated by the corresponding bands in the gel. As described by Alonso et al [12], we can also infer that there are more GFP in the shake flask as opposed to the fermentation from the densitometry of the SDS page.

GFP Quality Assessment

Gel electrophoresis results supports the qualitative findings from the GFP production steps. As shown in Fig. 4, the presence of fewer bands in the purified fractions compared to the crude protein extracts for both shake flask and fermentation samples indicates that the purification process was successful in removing most of the unwanted proteins and contaminants. Although, minor impurities are still present due to manual and precision errors during the purification process, the overall success of the purification is evident from the significantly reduced number of bands in the purified fractions.

Similar to Lee et al [13], the 250 nM buffer solution appears to be more effective in purifying the samples, as evidenced by the presence of one band in both the shake flask and fermentation samples, indicating minimal impurities. In contrast, the 200 nM solution resulted in two bands, suggesting a slightly less effective purification process. Based on these findings, we can recommend that the 250 nM buffer solution be adopted during the GFP scale-up process, as it leads to a higher level of purity in the final product.

Quantification

While the intensity of the green color provides a qualitative indication of GFP production, it is crucial to conduct quantitative analysis [14] to accurately determine the amount of GFP produced in each condition and compare the relative efficiency of shake flask versus fermentation for GFP production (Figs. 7 and 8). The estimation of fluorescence readings in Figs. 7 and 8 supports the observation that shake flask samples had better GFP production quality, as seen in the intensity of bands and the fluorescence readings (25038 = SF, 625 = F, 1787 = 200 SF, 250 = 15791, 242 = 200 F, 490 = 250 F). This is in line with the data generated for active protein and specific activity of protein, which were higher in shake flask samples (Figs. 7 and 8). Total protein in mg/mL was lower in the fermentation/large-scale samples, while shake flask/small-scale samples had higher total protein values (200 F = 6.247, 250 F = 8.024, 200 SF = 9.948, 250 SF = 10.209). Possible reasons for these differences could be due to plasmid stability, cell density and viability, and scale-up parameters.

Wet cell weight was 110 g/L, dry cell weight was 16.7, and the OD was 67. These process parameters could impact the productivity of GFP protein [15]. Also, factors such as cell viability and plasmid stability also play a crucial role in determining the actual GFP yield [16]. It is essential to maintain optimal growth conditions to ensure cell health and efficient protein production.

Promoter regions in plasmids can lead to low production of GFP protein if they are not at optimum function [17]. This is because they initiate transcription, which is the first step in gene expression. If there are deficiencies or mutations in the promoter region, the efficiency of transcription initiation may be affected, resulting in reduced gene expression and ultimately leading to lower production of the protein of interest [18]. Mutations can arise from unfavorable growth and environmental conditions of the bacteria. Therefore, choosing an appropriate promoter and ensuring its proper functioning in both systems is crucial for efficient production and scale up of GFP.

Scale up assessment

The scale-up of the bioprocess in this study was not efficient as the protein yield scale-up efficiency values were less than one (Table 5). Similar happenings have also been reported by Schirmer et al [19] where production reduction was experienced from fermentation process. There are multiple potential reasons for this failure, including inconsistencies in power per unit volume, constant oxygen transfer, constant aeration, constant DO levels, and the need to minimize shear effects. Gameil et al [15] also corroborated similar findings,

emphasizing the importance of maintaining constant values for these parameters during scale-up to ensure that the production of recombinant proteins in E. coli is successful.

In this study, we evaluated some of the most commonly assessed parameters, including sparger speed, dissolved oxygen concentration, OTR (oxygen transfer rates), and KLA (volumetric oxygen transfer co-efficient). While the speeds were kept relatively constant at 1500 and 1495 rpm, respectively (Table 4), the OTR values differed between the shake flask (3 L/min) and fermenter (9.2 L/min). This difference in OTR values indicates a change in oxygen transfer conditions between the two scales, which could contribute to the unsuccessful scale-up. Oxygen transfer rate is directly proportional to KLa, suggesting that the KLA values also differed between the scales [20].

To optimize the scale-up process, it is essential to maintain constant KLA values between shake flask and fermentation systems, as reported by Gameil et al [15]. Ensuring consistent KLA values will help improve oxygen transfer conditions and ultimately contribute to successful scale- up. This means that the efficiency of oxygen transfer in both the shake flask and the bioreactor remains the same. However, this does not necessarily mean that the oxygen levels themselves will be exactly the same, as other factors such as culture volume, cell density, and oxygen consumption rate can affect the overall oxygen levels in the systems. Proper agitation, aeration and monitoring controls are proposed.

Furthermore, the high OD values observed in the fermentation samples might have affected the protein lysis step. Denser cell populations require more efficient protein lysis strategies [21], which could be a factor in the low total protein observed for the fermentation samples. It is also possible that the ion exchange chromatography used for purification may have had a limited capacity for protein extraction, hindering protein binding to the column, thereby reducing the recovery of proteins from the fermentation samples. To address this issue, it might be necessary to optimize the protein lysis strategy by evaluating the capacity of the ion exchange column to accommodate a larger column. Preventing overloading of the column will also ensure proper extraction of proteins during the purification process.

Maintaining constant oxygen transfer in aerobic fermentation cannot be overemphasized. Factors for dissolved oxygen concentration are influenced by oxygen intake rates by microorganisms, oxygen transport rates from liquid to cells, and oxygen transfer rates from gas to liquid phase [20]. These factors are dependent on culture conditions and the bioreactor's physical attributes. As culture scale increases, maintaining dissolved oxygen availability becomes more challenging, potentially limiting the fermentation process [22]. To ensure oxygen levels stay above a critical threshold, it is essential to focus on oxygen transfer parameters during scale-up, often achieved using a constant kLA or controlling a minimum dissolved oxygen setpoint [23].

An alternative scale-up approach may involve focusing on constant aeration number, flow rate per unit volume or vessel volume or constant oxygen saturation concentration [24, 25]. However, this method has limitations. While a cascade can control dissolved oxygen (DO) in the bioreactor above a specific level, excessive agitation can lead to shear-related problems if not set correctly. During bioreactor scale-up, maintaining constant shear is also crucial, as it becomes more challenging to maintain homogeneity of cells and medium within the bioreactor. An impeller speed that balances sufficient mixing with shear effects on cells is highly required [20]. High shear forces can disrupt cells, cause morphological changes, and hinder product formation. Additionally, power consumption should be considered when increasing speed [26].

Controlling agitation speed during scale-up, while taking maximum allowable shear into account, ensures adequate mixing without cell damage. Using constant shear as a scale-up criterion has proven successful in achieving satisfactory yield values and effective bioreactor scale-up [15, 22]. Crude lysate data for shake flask and fermentation samples was interpolated from a different sample (S4) (Table 4) which was extracted, purified and analyzed by a different group of scientists, this could be a reason for some anomalies and variation in the results.

To improve the scale-up process and achieve higher yields of active protein, it is crucial to maintain constant KLA values, optimize protein lysis strategies, and evaluate the capacity of the purification system. Having established the criteria that need to be considered and kept constant during scale up process. The use of automation, robotics, or artificial intelligence in this process will be extremely beneficial.

Conclusion

This study demonstrates that effective scale-up of recombinant protein production requires a holistic process design approach that integrates upstream culture conditions with downstream recovery strategies. Maintaining constant KLa, optimizing cell lysis protocols, preventing column overloading during purification, and ensuring promoter stability are critical considerations for improving large-scale GFP production. These strategies are broadly applicable to other recombinant protein systems and provide a working system for diagnosing scale-up efficiency and guiding process optimization in industrial biotechnology.

Declarations

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No funding was received for this research work

Conflicts of Interest

The author declares no conflict of interest.

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