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Animal Origin-free Yeast and Soytone-based Fed Batch Fermentation as a Versatile Platform for High-Yield Plasmid Production

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ABSTRACT

The production of plasmids, crucial for applications in gene therapy, vaccine development, and biotechnology, often faces yield limitations during the upstream fermentation. Fermentation media design and nutrients screening have been a point of focus in improving overall yield. This study investigates the use of Animal Origin Free (AOF) yeast and soytone to enhance cell growth and plasmid production across various cell lines and plasmid constructs including plasmids destined for viral vector and in-vitro transcription applications. The impact of yeast and soytone-based feed approach on plasmid yield was tested under controlled and identical media formulation (Bacto™ CD media), fermentation temperatures, pH, Agitation, and dissolved oxygen conditions across *E. Coli* strains and constructs. The process demonstrated a consistent improvement in both cell growth and plasmid yield, particularly in conditions where traditional media fall short. The findings suggest that AOF yeast and soytone feed are an effective supplement to standard fermentation media, offering a cost-effective solution to optimize plasmid production and increase yields up to 1 g/L of culture. Data showed that under optimal fermentation conditions the use of yeast and soytone minimizes the accumulation of byproducts such as acetate, regardless of the *E.Coli* strain.

BACKGROUND

- Akron Bio is an agile manufacturing partner to the cell and gene therapy industry, leveraging its portfolio of cytokines, media supplements, plasmid and endonuclease manufacturing services to provide advanced therapy developers the scale, compliance, and regulatory support necessary to drive novel treatments from discovery to commercialization.
- Akron develops robust platform processes to support high cell density cultures and high research grade or cGMP plasmid yield.
- Gibco Bacto™ CD Supreme Fermentation Medium has been used as base media to develop a new generation production platform that fits multiple cell lines and plasmid constructs.

MATERIALS & METHODS

Major Materials & Equipment

Bacto™ CD Supreme Fermentation Production Medium
Bacto™ Yeast Extract and Difco™ Soytone
AMBR 250M ; Biostat Twin DCU, 5 L Univessel
Roche Cedex Bioanalyzer, Qiagen Miniprep

Methods

Assessment of microbial growth by Absorbance at 600 nm spectrophotometry, plasmid production measurement using Qiagen Minprep kit, metabolite measurement using Roche Cedex Bioanalyzer. Culture supplementation with carbon source at various concentration or with yeast extract and soytone. Scale-up assessment from 250 mL Ambr250M scale to 5 L Biostat fermenter scale. Fermentation profile with Batch, Fed – Batch, and Cool Down phases.

Cell lines and Plasmids:

Cell Line	Plasmid ID	Plasmid Size (kbp)	Application
DH5α	9.1kb-AAV RepCapF	9.1	AAV Vector production
	9.2kb-AAV RepCapN	9.2	AAV Vector production
	11.3kb-AAV HelperH1	11.3	AAV Vector production
NEB Stable	6.3kb-mRNA IVTp6	6.3	mRNA IVT Template
	6.1kb-AAV RepCapX	6.1	AAV Vector production
	5.7kb-AAV ITR52	5.7	AAV Vector production
	8.3kb-mRNA IVTp3	8.3	mRNA IVT Template
NEB5α	6.9kb-mRNA IVTp4	6.9	mRNA IVT Template
	6.8kb-mRNA IVTp7	6.8	mRNA IVT Template
NEB 10β	9.2kb-AAV-RepCapS	9.2	AAV Vector production
	11.3kb-AAV HelperH2	11.3	AAV Vector production

RESULTS

DISCOVERY AND DEVELOPMENT

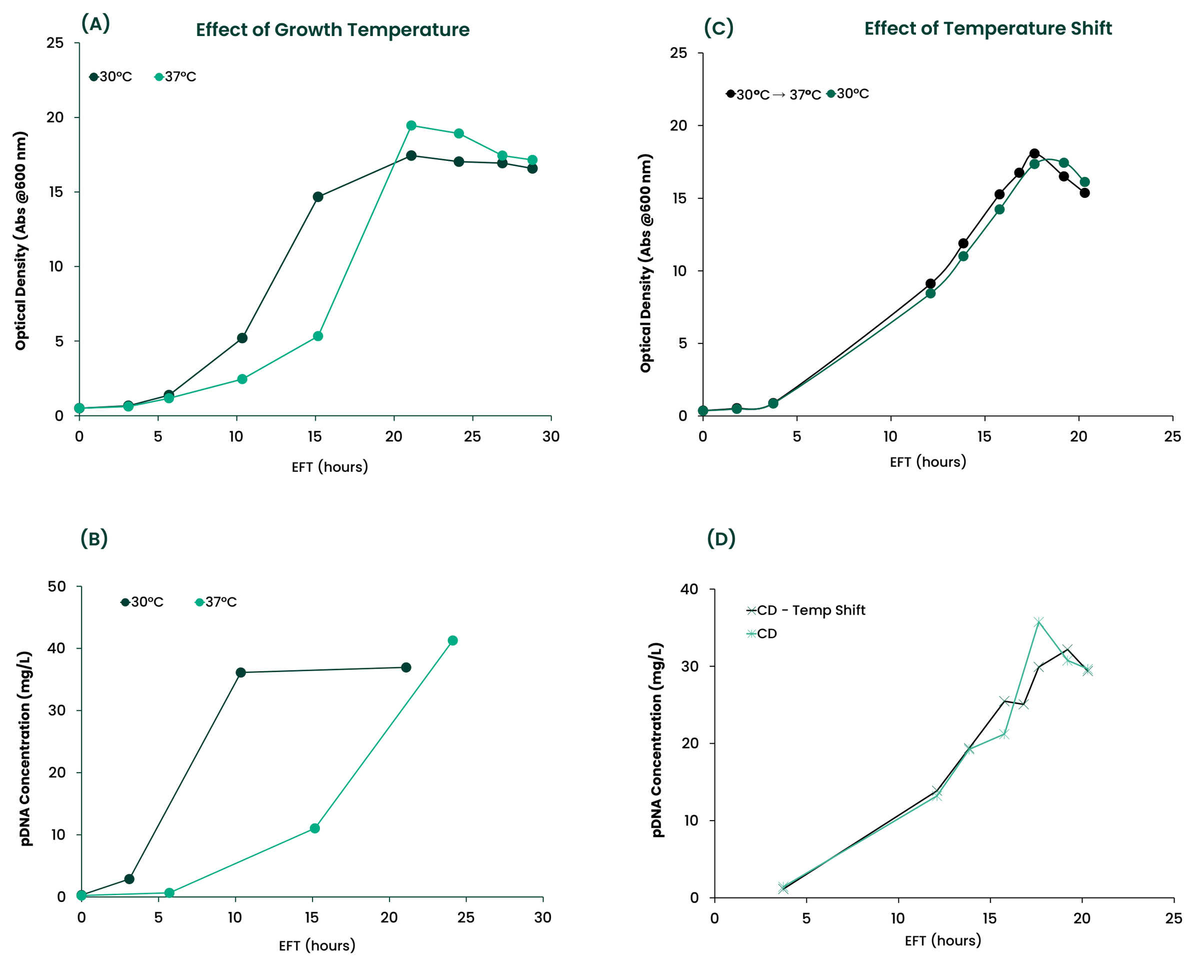

Figure 1: Effect of temperature on (A) Cell growth and (B) pDNA yield using Gibco Bacto™ CD Media. Cultures were incubated at 30°C or 37°C in Shake Flasks for the duration of the experiment. Results showed that 30°C promotes early cell growth with stagnant plasmid productivity from EFT 10 hours, while 37°C shows a delayed cell growth but a potential for higher plasmid yield. Therefore, the impact of a 30°C to 37°C temperature shift mid-process using the same Gibco Bacto™ CD Media was studied in Ambr250M vessels. Temperature shift experiments demonstrate that transitioning from 30°C to 37°C mid-process does not impact cell growth (C) as compared with the growth at constant 30°C and no significant difference in pDNA production.

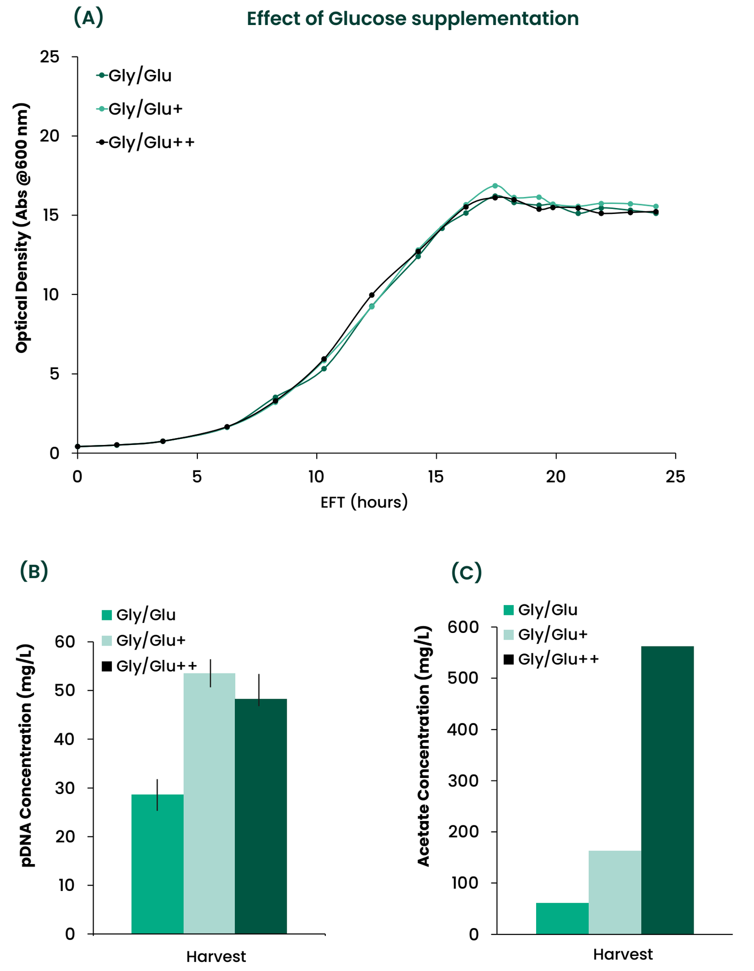

Figure 2: Effect of Gibco Bacto™ CD Media supplementation with Glucose as additional carbon source in Shake Flasks, on (A) Cell growth, (B) pDNA yield and (C) Acetate byproduct accumulation. Cultures were incubated at 30°C for growth and subject to temperature shift at EFT 14 hours. Results showed that although increasing the glucose concentration was associated a higher plasmid yield, a significant acetate build up is observed at the highest glucose feed concentration of 10 g/L.

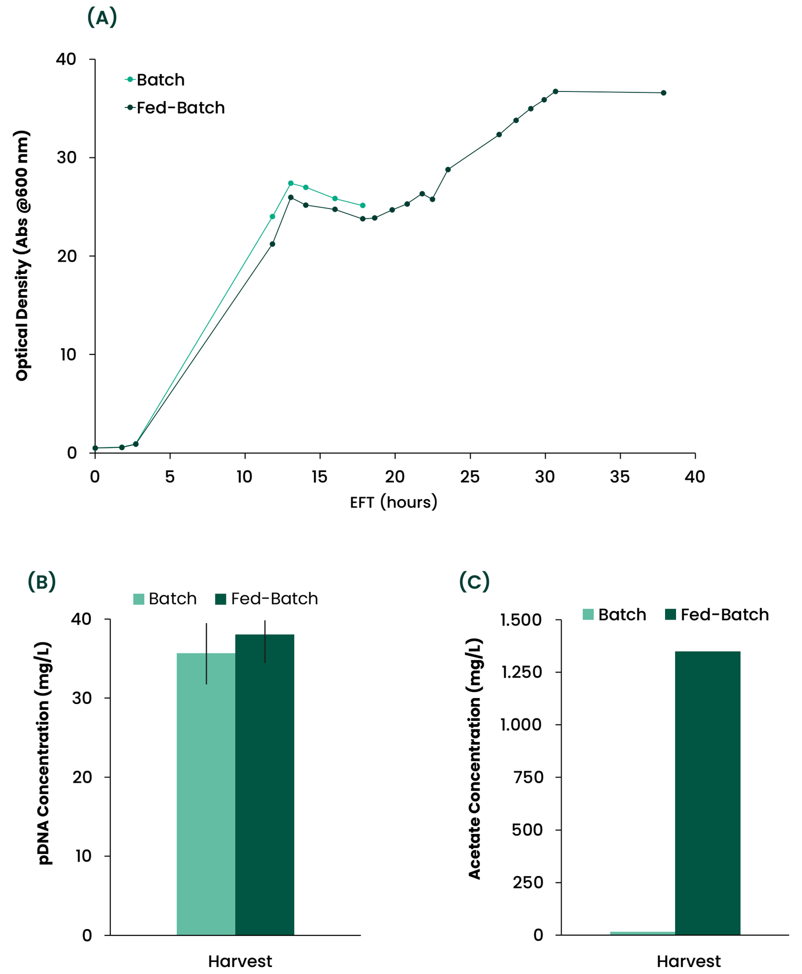

Figure 3: Effect of Gibco Bacto™ CD Media supplementation with Glucose as additional carbon source, in fed-batch mode, using in Ambr250M vessels, on (A) Cell growth, (B) pDNA yield and (C) Acetate byproduct accumulation. Cultures were incubated at 30°C for growth and subject to temperature shift at EFT 14 hours. Supplementation of the culture in fed-batch mode with 400 g/L of glycerol did not impact cell growth and overall plasmid yield, however glycerol feeding was associated with a significant Acetate build up.

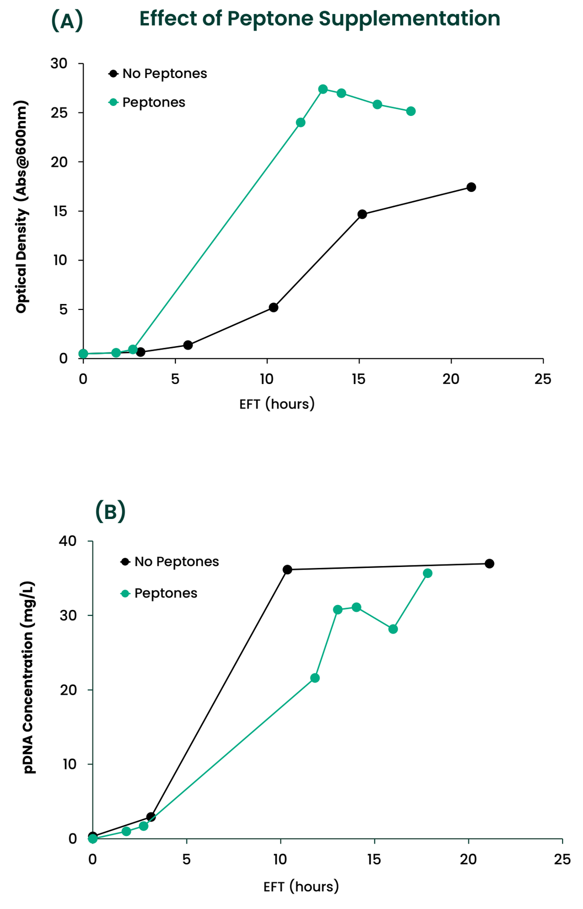

Figure 4: Effect Bacto™ CD Media supplementation with Soytone and Yeast (Peptones) in Shake Flasks, on (A) Cell growth and (B) pDNA yield. Cultures were incubated at 30°C for growth and subject to temperature shift at EFT 14 hours. Results showed that Peptone supplementation supports better cell growth with a gradual plasmid accumulation.

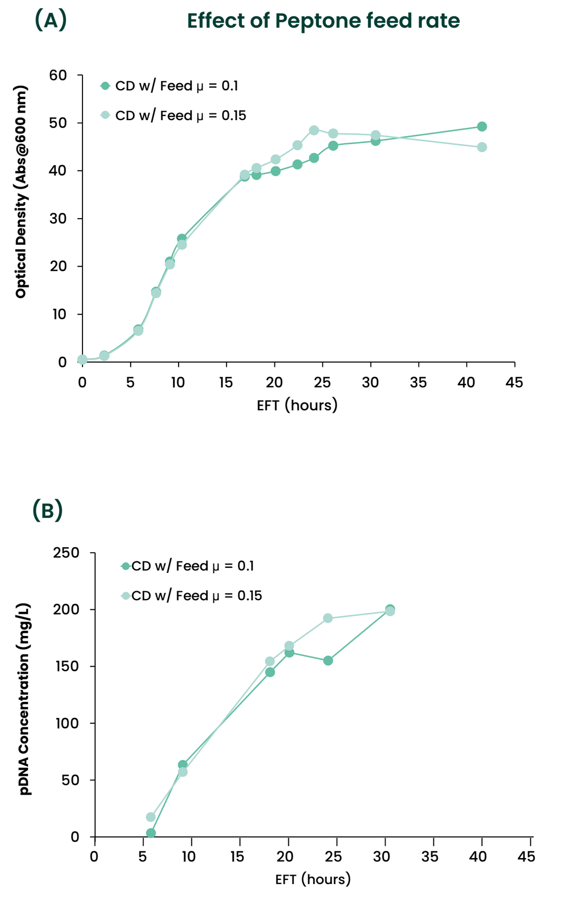

Figure 5: Effect of Glycerol and peptone mixture supplementation of BactoTM CD Media on (A) cell growth, (B) pDNA yield. Cultures were incubated at 30°C for growth in Ambr250M vessels and fed with Glycerol at μ of 0.10 and with Glycerol/Peptones mixture at μ of 0.15. Cultures were subject to temperature shift at EFT 25 hours. Results showed that feeding with Glycerol and Peptone mixture at higher rate does not promote higher cell growth nor plasmid yield

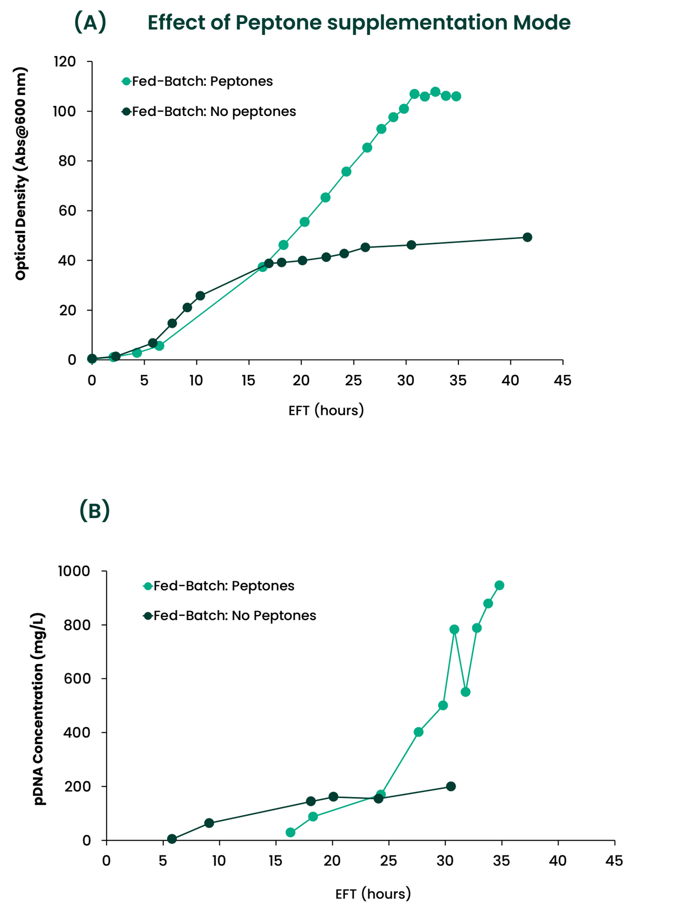

Figure 6: Effect Bacto™ CD Media supplementation with Peptones in Ambr250M vessels, in Fed-batch mode on (A) cell growth and (B) pDNA yield. Cultures were incubated at 30°C for growth and subject to temperature shift at EFT 25 hours. Cultures were fed at a μ of 0.10. Results showed that the use of Peptone in fed-batch mode significantly impact cell growth reaching more than 100 OD600nm. The increase in cell density was also correlated with an increase in plasmid concentration to up to 946 mg/L.

APPLICATION AT 5L FERMENTATION SCALE

Cell Line	Plasmid ID	Short
DH5α	9.1kb-AAV RepCapF	PLA001
	9.2kb-AAV RepCapN	PLA002
	11.3kb-AAV HelperH1	PLA003
NEB Stable	6.3kb-mRNA IVTp6	PLA004
	6.1kb-AAV RepCapX	PLA005
	5.7kb-AAV ITR52	PLA006
NEB5α	6.9kb-mRNA IVTp4	PLA007
	6.8kb-mRNA IVTp7	PLA008
NEB 10β	9.2kb-AAV-RepCapS	PLA009
	11.3kb-AAV HelperH2	PLA010

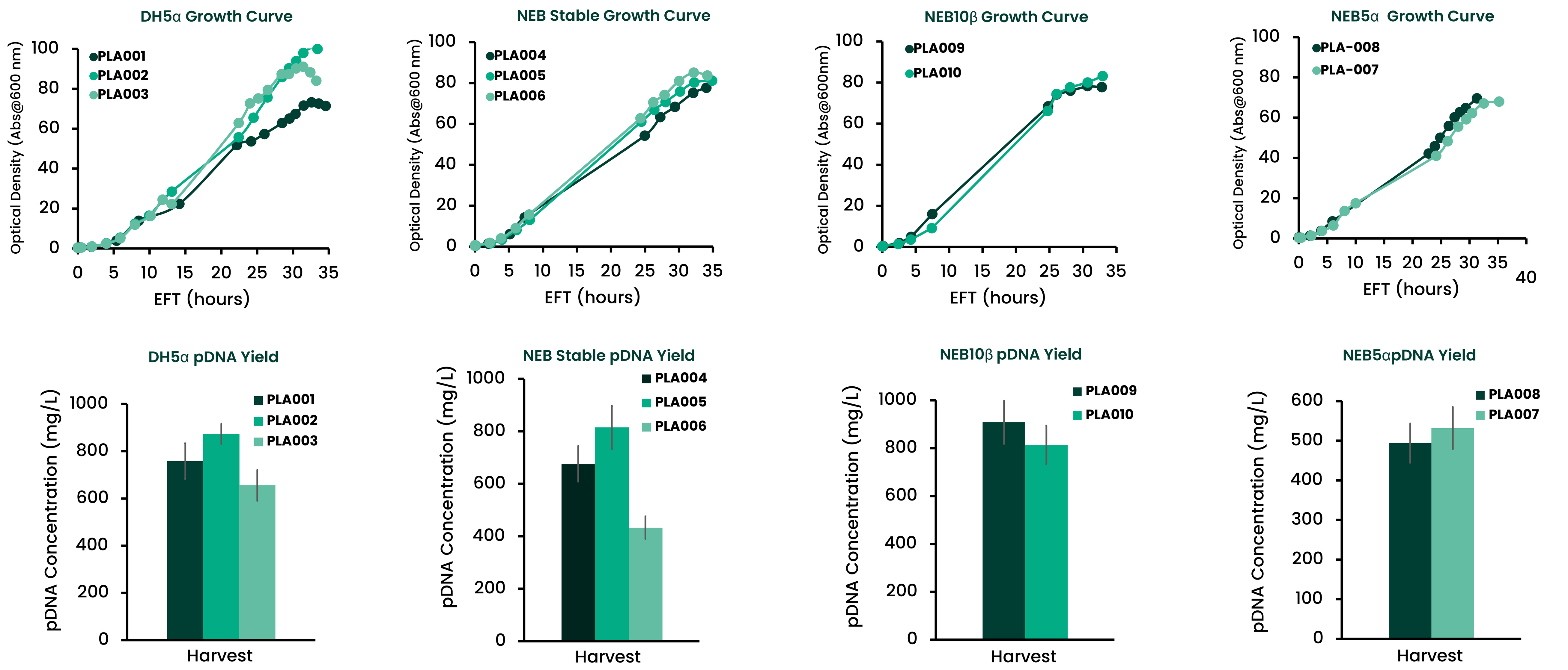

Figure 7: Growth curves and harvest plasmid yields showing application of the developed Peptone-based fed batch fermentation process using Gibco Bacto™ CD media with (A, B) DH5a-derived strain containing three different plasmid constructs; (C, D) NEB Stable strain containing three different plasmid constructs, (E, F) NEB10b strain containing two plasmids of 9kb and 11kb, respectively, and (G,H) NEBa strain containing a polyA containing plasmid. Growth curves for DH5α-derived strains and NEB5α show that while all strains grow steadily, certain variants demonstrate slightly higher cell density. NEB Stable strains exhibit consistent growth patterns across multiple isolates, indicating robustness of the NEB stable background. NEB10β strains also display reliable growth kinetics, with minimal variability between isolates. pDNA yield comparisons at harvest show notable strain-to-strain variability, with DH5α and NEB10β derivatives producing the highest pDNA concentrations. NEB5α-derived strains show moderate pDNA yields, whereas NEB Stable backgrounds yield lower amounts relative to other strains.

CONCLUSIONS

The incorporation of peptones into *Escherichia coli* cultures has been shown to significantly enhance both optical density and plasmid yield across a variety of cell lines. This effect is especially pronounced when peptones are supplemented into chemically defined media, where their complex composition of peptides, amino acids, vitamins, and trace elements compensates for the minimal nutritional profile of the base medium. By enriching the culture environment, peptones facilitate faster cell growth, improve metabolic efficiency, and support more robust plasmid replication. These enhancements are not only critical for maximizing biomass and plasmid output but also help maintain plasmid stability during high-density fermentation. Importantly, their consistent efficacy across genetically diverse *E. coli* strains highlights their versatility of the peptone-based fed batch platform. Altogether, peptones represent a powerful component for optimizing microbial cultures in both research and industrial-scale bioprocesses, particularly in systems reliant on chemically defined formulations.

REFERENCES/ACKNOWLEDGEMENTS

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