Novel Lipid Supplement Enhance Protein Production in Small Bioreactors

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Introduction

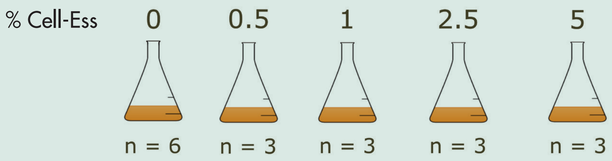
Biotherapeutics have been a growing area of medicine for many years. Manufacturing those therapies has been predominantly done by cell factories. The cost and consistency around that process has and continues to be an area for novel techniques used to improve bioprocessing. Small bioreactors have been used to model large scale bioreactors. There has been a move in the industry to single-use systems which are composed of plastic. To date there has not been a lipid supplement that functions well with plastic.

Abstract

Protein production is an ongoing process of optimizing cell lines in serum free conditions. There is a balance between cell proliferation and protein production. Many metrics like the metabolic profiles are used to analyze cell health and determine how to optimize health and productivity. We investigated the use of a lipid supplement using various strategies to improve protein yield. We found when adding the lipid supplements at the beginning of culture we were able to increase the yield in titer of antibody protein production by 30% from CHO cells without increasing proliferation. Furthermore when we examined the metabolic profile we discovered there were no differences in any of the metabolites we examined, including but not limited to, ammonia, glutamate, lactate, and glucose. We also tried using the supplement as a feed and found there were 2 notable effects. The first effect was increasing the titer yield by close to 25%. The second effect was extending the window for peak protein production from 1 day to 2 days. The supplement was also used in a single–use bioreactor. The supplement resulted in an increase of 27% in a 5 liter Wave bioreactor. These results show there are windows for further optimization of protein production using lipids. Furthermore, some of the standard metrics like metabolic profiles may not indicate an improved profile while not showing a detrimental effect despite increased protein yield. It is possible the use of lipids reduces the energy requirement for new cell formation and therefore can be used for protein production as evidenced by the scalable, small bioreactor results.

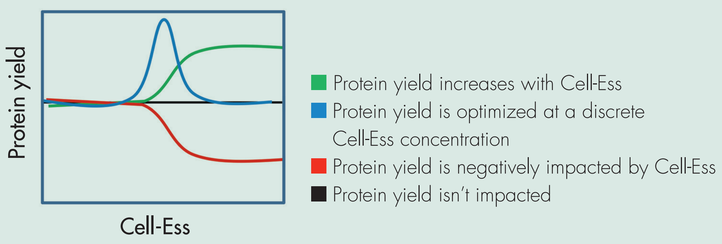
Methods

Goal 1: Identify if Cell-Ess can increase protein production as an initial supplement



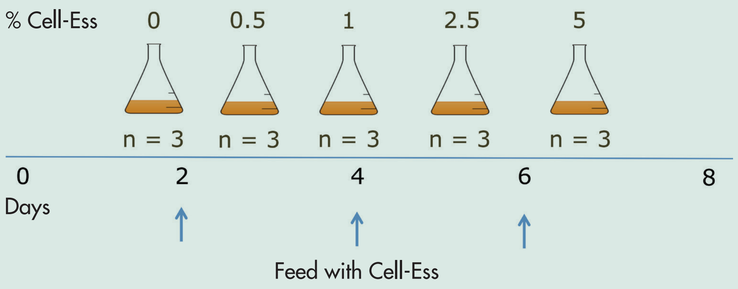
- Perform in triplicate (15 flasks total).
- Analytical determinations were performed termination (day 8).
- Balance of Cell-Ess Additive will be basal media (*SAFC Platform Media*).

Predicted potential analytical outcomes and downstream application



- Use optimal dose range for future fed batch studies.
- Fed batch studies anticipate following normal feeding regimen (approx 3, 6, 9), but substitute Cell-Ess at concentration defined during shake flask studies.
- Define cellular performance using same metrics used in shake flask but incorporate time component through regular sampling.

Goal 2: Identify if Cell-Ess can increase protein production as a feed



Shake Flask

- Perform in triplicate (15 flasks total).
- The experiment was terminated when cell viability reaches 50%.
- Cell-Ess Additive was used in conjunction with basal LifeTech Forti-Cho and LifeTech feed.
- Feed was given every 2 days starting on day 2.
- Endpoint ELISA was used to quantify mAb yield.

Bioreactor

- Two 5 liter Wave Bioreactors were run.
- The experiment was terminated when cell viability reaches 50%.
- Cell-Ess Additive was used in conjunction with basal LifeTech Forti-Cho and LifeTech feed.
- Feed was provided every 3 days starting on day 3.
- Endpoint ELISA was used to quantify mAb yield.

Lipid Supplement as an Initial Supplement

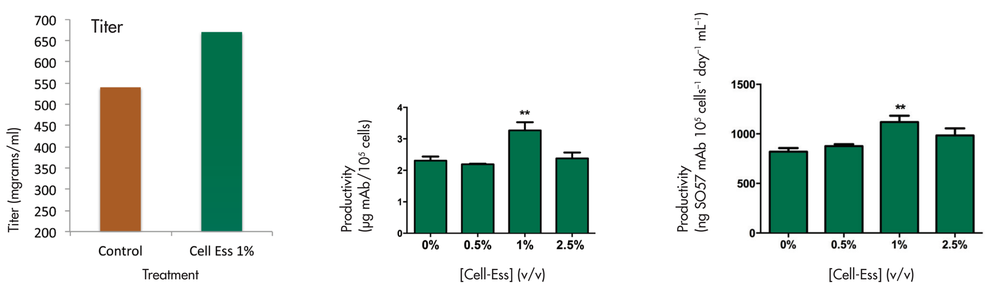


Figure 1: At 1% Cell-Ess there is a significant increase in mAb production. The amount of mAb produced on a per cell basis was measured. The date are represented as titer, a ratio titer to VCD and IVCD. The results are presented as a per 10^5 cells. The increase in titer, yield per cell, and productivity were then calculated and represented as a percent increase over control. These data are representative. This type of experiment was performed over 3 times and in triplicates in each run.

Effect of Cell-Ess on Metabolites

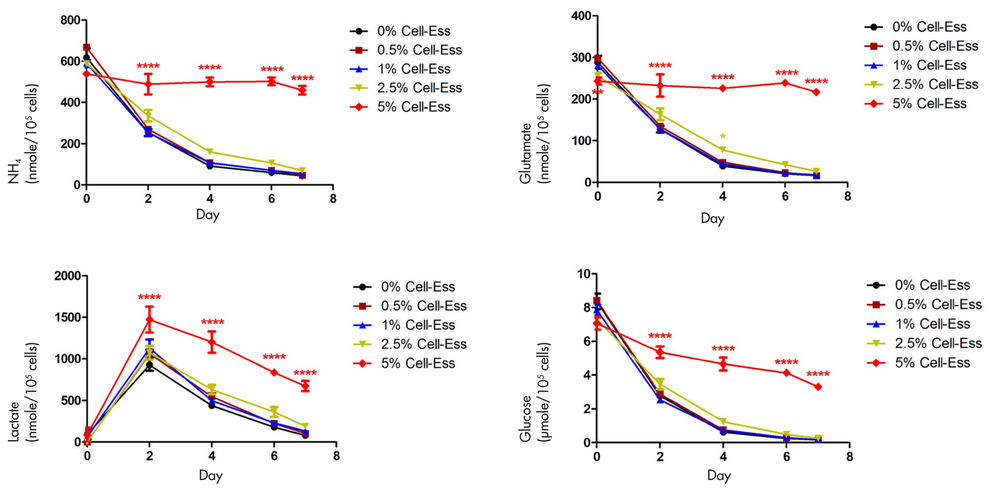


Figure 2: Cell-Ess was added as an initial supplement and samples were analyzed for their metabolic profile. With 1% addition of Cell-Ess there was a significant increase in protein production but there was no change in any of the metabolites examined. The metabolites pictured in the four panels are reflective of the overall analysis.

Lipid Supplement as a Feed in a Bioreactor

Shake Flask

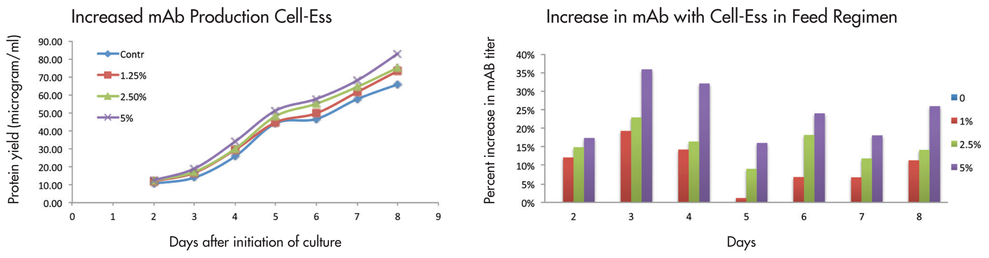


Figure 3: There is an increase in titer of mAb with the addition of Cell-Ess in the Forti-CHO system. Cell-Ess was used in conjunction with standard feed every 2 days for 8 days in a shake flask experiment. The amount of mAb was quantified using an ELISA. These data show the increase in protein over the course of the experiment with the addition of several different concentrations of Cell-Ess.

Bioreactor

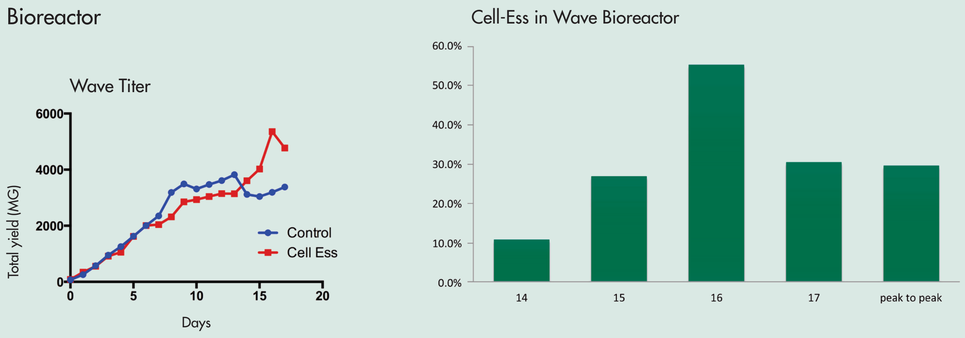


Figure 4: There is an increase in titer of mAb with the addition of Cell-Ess® in a Wave Bioreactor. Cell-Ess was used in conjunction with standard feed every 3 days, until the viability fell below 50%. In this experiment, the use of Cell-Ess extended the production to 17 days. The amount of mAb was quantified using an ELISA. These data show the increase in protein over the course of the experiment.

Conclusions

- A lipid supplement used as an initial supplement increases mAb production.
- The lipid supplement does not alter the metabolic profile while increasing the mAb titer.
- Cell-Ess as a feed increases the titer early and throughout a shake flask model.
- Cell-Ess as feed in a 5 liter Wave Bioreactor increased the production of mAb by over 25%.

Cell-Ess® Let it Grow