

Considerations for Selecting Hydrolysates for use in Cell Culture Process



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tion troduct

Hydrolysates have been used in cell culture media for cell growth and productivity enhancement. To finalize hydrolysate supplementation strategy, a comprehensive understanding of their impact on growth, productivity and product quality is required. This was achieved by performing structured performing

addition of hydrolysates in medium or feed without impacting



Results and conclusions



Observations:

- Cell growth improved by reducing CH concentration.
- Productivity is higher than control where CH added. Moderately higher productivity observed in SH conditions.
- Comparable fuscosylation in only CH condition compared to SH condition.
- Product purity is better in SH conditions compared to CH conditions.

ective

experiments at small-scale to identify top hydrolysates. To improve CHO cell growth and titer productivity with

product quality.

Screening identified hydrolysates with Additional Proof of concept dentification and experiments in AMBR250 different and consistency at Scale up selection of hydrolysates concentrations and 5L addition strategy in shake flasks

The One factor at a time design approach was implemented to screen two different hydrolysates and to determine the addition approach with media or feed. Screening and addition strategy experiments were conducted in shake flasks. After finalizing the process in SF and AMBR250, process was scaled-up to 5L.

Experiment objective: Objective is to screen two hydrolysates Soy (SH) and Cotton (CH).



CH in

medium +

CH in

feed

- Hydrolysates are proven to have impact on cell growth and productivity.
- The SH and CH hydrolysates are derived from SOY and Cotton by enzymatic hydrolysis. The enzymes used for digestion are AOF.

Conclusions:

- Addition of CH alone or with SH is yielding more product but with less purity.
- Addition of SH alone improved productivity by 26% with comparable purity to control.



Observations:

- Cell growth in bioreactors with SH and CH is better than control run.
- Viability is better in SH conditions compared to control and CH bioreactors. Due to better viability culture duration in SH bioreactor is increased.
- Productivity in SH and CH conditions is 25% more than control condition which is similar to observation on Ambr250 runs.
- The HMW and fragments are more in CH conditions compared to SH and control.
- Fucosylation is better in SH and CH compared to control.





These hydrolysates are ultrafiltered. (10 g/L)

- The SH and CH are rich in trace elements. carbohydrates and peptides.
- They don't have any residual enzyme activity

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Conclusion

Conclusion

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Selection of Hydrolysate

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Impact on

productivity

- In bioreactor runs also higher aggregates and fragments are observed with CH.
- Considering better product quality, 5 g/L SH in medium is finalized.
- Process consistency to be established with 5 g/L SOY.





Results and conclusions





<u>CH conditions</u>: Lower VCC with better viability and

Conclusions: 1) Further evaluation of SH without

2) Evaluation of lower CH concentration as there is



impact on growth.

viability and improved productivity.

CH in feed and different concentration.

specific productivity.



Factors to be considered for selection of Hydrolysates comprehensive approach * A with step yields evaluation wise the best Concentrat on of combination of hydrolysate with media hydrolysate or feed. Addition strategy

- Assessment of product quality and purity should be part of evaluation.
- Reproducibility of process is critical to finalize the process conditions.