



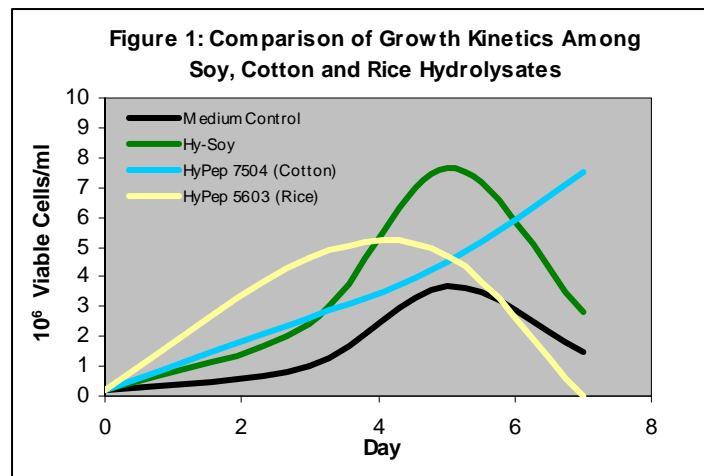
Cell Culture Application Notes

Application Note No. 2 – Key Considerations When Screening Hydrolysates

Introduction

Protein hydrolysates are routinely employed to enhance the overall performance of many biopharmaceutical production systems, providing a number of different benefits. Cell viability, cell proliferation and target protein production all may be improved. However, these effects might not be observed concurrently in a given system. As every cell culture platform is unique, so are the protein hydrolysates used to supplement the culture medium. Each may have its own distinctive effect on the growth and productivity of transfected cells. The contribution of protein hydrolysates to the system performance can be influenced by a number of factors including the specific cell line being employed, the raw material used to manufacture the hydrolysate, the manufacturing process, the hydrolysate dosage, and the composition of the basal growth medium. The following figures illustrate this important point.

Figure 1 demonstrates the influence of the hydrolysate raw material on the growth kinetics of cells grown in the same basal medium. Each hydrolysate was dosed at the same final concentration, however the CHO-K1 cells responded differently to each hydrolysate.

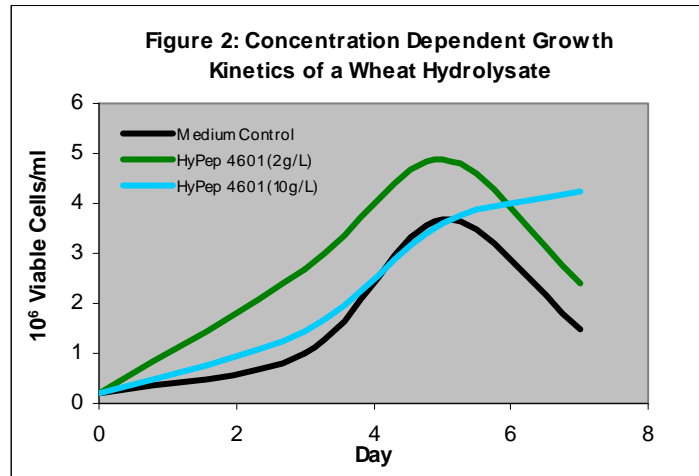


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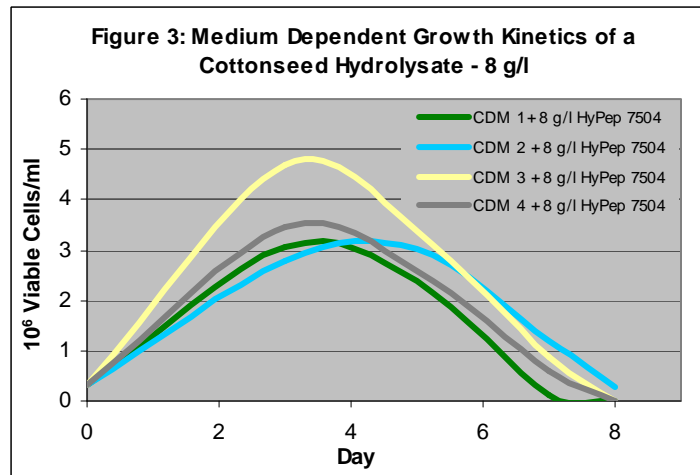


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Figure 2 demonstrates the influence of hydrolysate dosage on the growth kinetics of cells grown in the same basal medium. The CHO-K1 cells responded differently to each dosage of the same hydrolysate.



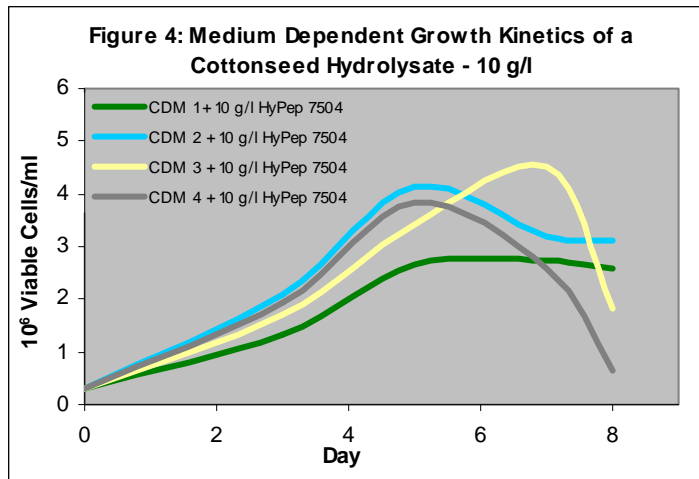
Figures 3 and 4 demonstrate the effect of four different chemically defined basal media formulations, each supplemented with either 8- or 10- g/l of HyPep 7504, on the growth kinetics of CHO-K1 cells. The cells responded differently to each individual basal medium formulation, independent of the hydrolysate supplementation. Both the basal medium formulation and the hydrolysate dosage influenced the results. At 8 g/l growth peaked earlier and declined more rapidly than at 10



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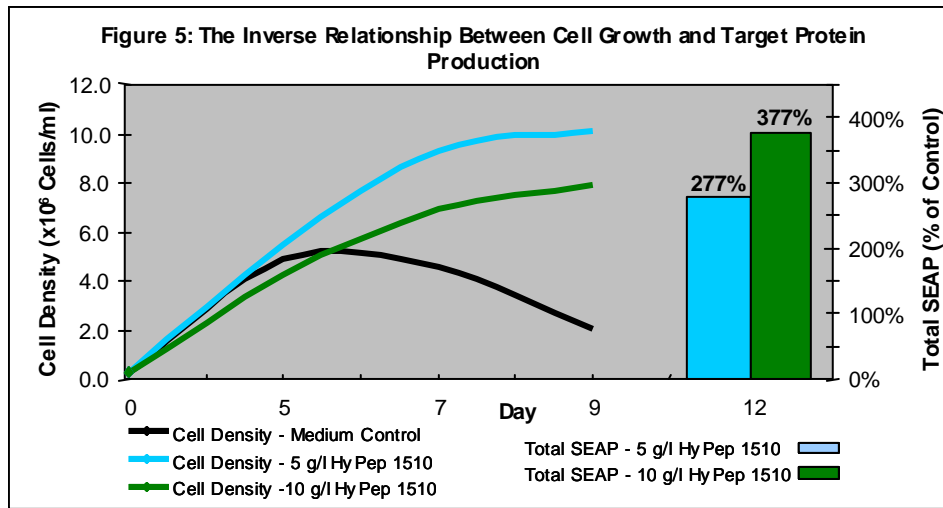
There are implications to this variety of growth curves generated through hydrolysate supplementation in terms of the effects on target protein production. When cell proliferation and target protein production of CHO-K1 cells cultivated in a hydrolysate-supplemented medium are compared with an un-supplemented control medium, an inverse relationship becomes apparent. If cell proliferation is enhanced by the hydrolysate supplement, target protein production is often inhibited. If cell proliferation is inhibited, target protein production is often enhanced. In all cases, cell viability is comparable or improved in the hydrolysate-supplemented media. This has been observed over a range of supplementation schemes, but further experimentation will be required to determine whether this inverse relationship is unique to CHO-K1 cells, or if this phenomenon occurs across a range of cell lines, expression systems, and culture platforms.

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Figure 5 illustrates the manifestation of this inverse relationship, which should be taken into account when evaluating data from medium optimization experiments. It may be necessary to select a dosage or hydrolysate that is less than optimal for growth promotion in order to achieve the maximum benefit for target protein production.



It has been shown that supplementation of a growth medium with plant-derived protein hydrolysates can enhance the performance of various aspects of a biopharmaceutical production system in a variety of ways. To achieve optimal results, it is necessary to experimentally determine the proper hydrolysate dosage for a given hydrolysate-medium combination which provides the desired effect, be it better growth promotion, enhanced cell viability or increased target protein production, or a combination of all three, as determined by the requirements for a particular production system.

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