Optimization of Culture Medium with the Use of Protein Hydrolysates

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Abstract: In this study the effect of protein hydrolysates in order to increase cell viability, cumulative cell cell-days number, volumetric and specific productivity with respect to product quality was examined. 10 different complete non-animal protein hydrolysates (wheat gluten, rice, soy, pea, cotton, corn) were tested in standard grade roller bottles. The concentration levels ranged from $1g \cdot l^{-1}$ to $5g \cdot l^{-1}$. The experiments revealed a 30% – 50% increase of volumetric productivity compared to standard cultures.

The effect based mainly on the extended culture duration of the bioprocess. For characterizing product quality the techniques of anti-idiotypic ELISA with target antigen, complement dependent cytotoxicity, antibody dependant cellular cytotoxicity and isoelectric focusing were employed. It could be shown that the use of peptones did not change the product quality, potency or specificy. As conclusion, addition of peptones might be a successful strategy to enhance the performance of biological production processes.

Key words: peptones, protein hydrolysates, media optimization, hybridoma, cell growth, productivity, cell culture, serum-free, monoclonal antibodies, Animals, growth substances, cost-benefit, cancer, immuno therapeutic, economic

1. INTRODUCTION

The increasing awareness of the risk of contamination with mycoplasma, viruses and TSE caused by animal-derived components has led to serum-free media formulations. With the elimination of serum, e.g. Fetal Calf Serum or

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other animal derived additives for biological production processes, cell culture performance often decreases. Lots of different agents such as amino acids, trace elements, vitamins or complete vegetarian protein hydrolysates are used to overcome this problem. Especially small peptides like protein hydrolysates, which are also called peptones, protein fission products, peptides and hydrolyzed proteins, seem to be a viable option for the replacement of serum. In addition, peptones provide a stable source of glutamine and other amino acids.

The aim of this study was to show the effect of 10 different protein hydrolysates, which are using soy, rice, corn, wheat gluten, cotton or pea as raw material, added to a chemical defined medium on a recombinant SP2/0 hybridoma cell line in order to increase cell viability, cumulative cell number, volumetric and specific productivity with respect to product quality.

2. MATERIAL AND METHODS

The used cell line was a recombinant SP2/0 producing a humanized anti-Lewis Y antibody. As standard medium for all experiments the commercial available Ex-Cell™-Sp2/0 (JRH, UK) supplemented with 8mM L-glutamine 200mM (100x) (Gibco, UK) was used. Ex-Cell™-Sp2/0 is an animal-component-free, protein-free, and chemically defined liquid medium. The content of protein hydrolysate ranged between $1 g \cdot l^{-1}$ and $5 g \cdot l^{-1}$.

Cell culture was performed at 37°C in standard grade polystyrene cell culture flasks (Nunc, Denmark) in a humidified incubator with 7% CO_2. Tests were performed in 850 cm² standard roller bottles (Corning, Germany) with 200 to 450 ml medium in duplicates. The inoculation densities varied from $1 \cdot 10^5$ to $2.5 \cdot 10^5$ cells·ml^{-1}. Sampling was performed on a one to three day basis. The cell number of the supernatant was determined with the trypan blue dye exclusion method and the concentration of produced antibodies were measured with size exclusion chromatography (SEC). The cultures were terminated, if viability dropped below 40%.

The quality of the produced antibody IGN311 was examined with the following assays: Lewis Y ELISA, IEF-Page, CDC and ADCC. The LewisY ELISA was used to evaluate the binding activity of antibodies to the carbohydrate antigen LewisY. Specifically bound immunoglobulins were detected with an anti-human immunoglobulin-enzyme conjugate which was inducing a photometrically measurable color change. For the IEF Page an Ampholine PAGplate™ was used with pH values between 3.5 and 9.5. ADCC against SKBR 3 cell line was analyzed in a ^{51}Cr release assay. CDC against SKBR 3 cell line was analyzed in a caspase enzyme assay.

3. RESULTS

3.1 Growth Characteristics

As it can be seen in Figure 1, HyPep4601 had an positive effect on the cumulative cell-days number Hence, HyPep4601 increased cumulative cell-days number by 25%. HyPep5603 had no significant influence on cumulative cell-days number (Figure 1) and viable cell number.

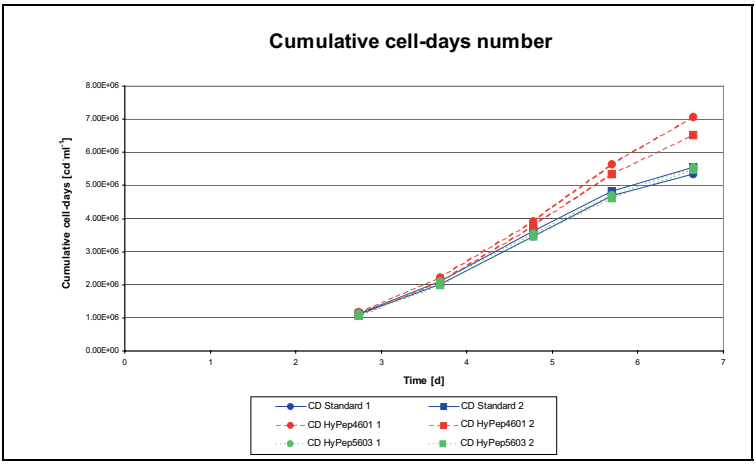

Figure 1. Cumulative cell-days number of standard cultures, cultures supplemented with HyPep4601 and cultures supplemented with HyPep5603.

6 out of 10 protein hydrolysates (HyPep1510, HyPep4601, PCE80B, CNE50M, WGE80M-UF and SE50MAF-UF) had an positive effect on the growth characteristics, which was characterized by the parameters maximum viable cell number, cumulative cell-days number, growth rate and cell culture duration, in comparison to the standard culture.

3.2 Productivity

Figure 2 depicts an increase of 56% in final volumetric productivity by the addition of HyPep4601 in comparison to the standard culture. The final mean endtiter of cultures supplemented with HyPep4601 was 323 $mg \cdot l^{-1}$. The standard cultures only had a final mean endtiter of 206 $mg \cdot l^{-1}$. HyPep5603 showed no influence on the cell culture in terms of volumetric productivity (211 $mg \cdot l^{-1}$).

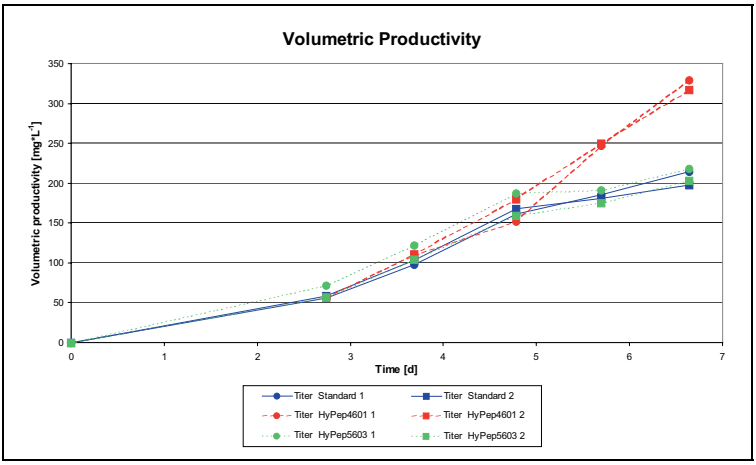

Figure 2. Volumetric Productivities of standard cultures and cultures supplemented with HyPep4601 and HyPep5603.

In our study a significant and reproducible increase in specific productivity by the use of protein hydrolysates could not been shown. Hence, the main effect of protein hydrolysates must be based on nutritional benefit.

3.3 Product Quality

The IGN311 produced under serum free conditions with the use of peptones had the same antigen binding activity and a slight increase in effector functions as measured by ADCC- and CDC-assays in comparison to the reference standard. The Lewis Y ELISA showed no significant differences between IGN311 ref. and the tested AB's.

The IEF Page of the antibody produced under serum containing conditions had one band less than the antibodies produced under serum free conditions and the use of protein hydrolysates. However, the cell line is robust with regards to productivity and product quality under the tested conditions (medium change, up-scaling).

4. CONCLUSION

7 out of 10 protein hydrolysates improved volumetric productivity (see Table 1). The effect based mainly on the extended culture duration of the bioprocess. The experiments revealed an increase up to 56% in terms of

Table 1. Summary selected parameters of all batch cultures.

Name	**Growth rate [d^{-1}]**	**Cumulative cell-days [$cd \cdot ml^{-1}$]**	**Duration [d]**	**Specific productivity [$pg \cdot cd^{-1}$]**	**Volumetric productivity [$mg \cdot l^{-1}$]**
HyPep1510	0.41 / R=0.98	$4.7\,10^6$	7	51 / R=0.99	264
HyPep4601	0.36 / R=0.97	$6.8\,10^6$	7	46 / R=0.98	323
HyPep4602	0.36 / R= 0.99	$4.3\,10^6$	7	32 / R= 0.97	211
HyPep5603	0.26 / R= 0.93	$5.5\,10^6$	7	32 / R= 0.93	162
HyPep7401	n.a.	n.a.	n.a.	n.a.	n.a.
HyPep7504	0.28 / R=0.97	$4.0\,10^6$	7	53 / R= 0.99	288
SE50MAF-UF	0.44 / R=0.96	$8.0\,10^6$	8	27 / R= 0.95	241
WGE80M-UF	0.40 / R=0.98	$8.2\,10^6$	8	28 / R=0.93	263
CNE50M	0.51 / R= 0.95	$9.3\,10^6$	9	33 / R= 0.97	319
PCE80B	0.43 / R=0.96	$8.7\,10^6$	10	43 / R= 0.96	321
Standard	0.40	$5.2\,10^6$	7	40	209

volumetric productivity compared to the standard culture in a chemically defined medium. The best results could be obtained with HyPep4601. This hydrolysate, based on wheat gluten, increased volumetric productivity by 56%, cumulative cell-days by 25% and specific productivity by 36%.

The quality of the monoclonal antibody produced in peptone-supplemented cell cultures, analyzed by ELISA and IEF was not affected. In CDC-tests a slightly positive influence on the potency of the antibody produced in protein-hydrolysate containing medium could be revealed.

As conclusion, addition of peptones might be a successful strategy to replace animal-derived components and to enhance the performance of biological production processes.