

Use of plant hydrolysates in animal cell culture media formulations

Introduction

The aim of this work was to evaluate the influence of different plant hydrolysates in cell culture media formulations on the growth of CHO cells cultivated in suspension. Cultures were performed in 96 well plates (200 μ L) and the determination of total cell number and cell morphology were carried out by image analyses using the fully-automated Cellscreen system.

Material and Methods

CHO cell propagation was realized in 25 cm² T-flasks in reference medium. This cell line was adapted to culture in suspension. The adequate initial cell density for the growth in micro-wells was determined previously as 0.8 10^5 cells / mL. T-flasks and plates were incubated at 37 °C in a humidified atmosphere containing 5 % CO₂. Eight wells were filled with the cell suspension for each plant hydrolysate tested in media formulations and compared to a reference (cells cultivated under the same conditions but with reference medium and with the same volume of water).

Results

About one hundred different medium formulations were tested and the reproducibility of the observed effects concerning different additives was verified. In the following parts, we present some results and their validation at different scales (microplate, Erlenmeyer flasks, spinner, bioreactor).

Reproducibility: The same experiments were realized in triplicates to compare the cell growth in the reference medium with the growth in a medium formulation containing the additive 1. Figure 1 shows results of the cell growth kinetics determined by the Cellscreen system.

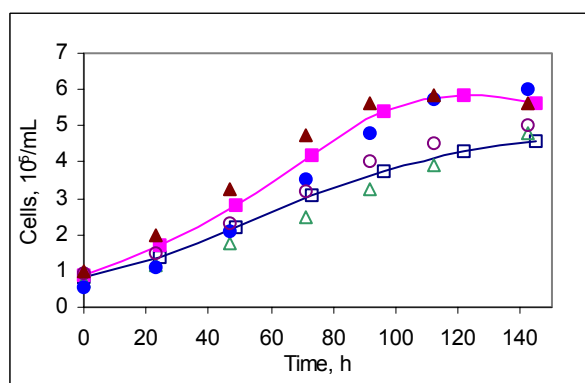


Figure 1: Reproducibility study : Influence of the additive 1 on the total cell concentration. Open symbols correspond to the reference culture and filled symbols to cells cultivated in presence of the additive 1.

These results indicate a good reproducibility of the system used and confirm the positive effect of additive 1 on cell growth: This effect was previously observed with cells growing in T-flasks (data not shown). Furthermore, the concentration of total cells reached in the culture with the additive 1 (about 6.10⁵ cells/mL) appeared to be the maximal limit of detection for

the cell counting system due to cell confluence. An underestimation of cell concentration must be taken into account in this case.

Validation of Cellscreen results at different higher scales: The influence of media supplemented with different additives on cell growth has been evaluated at different scales, either grown in microplates and analysed with the Cellscreen system, or grown in spinners or bioreactors and counted with a hemacytometer. Results are shown in figure 2 for three additives presenting opposite effects.

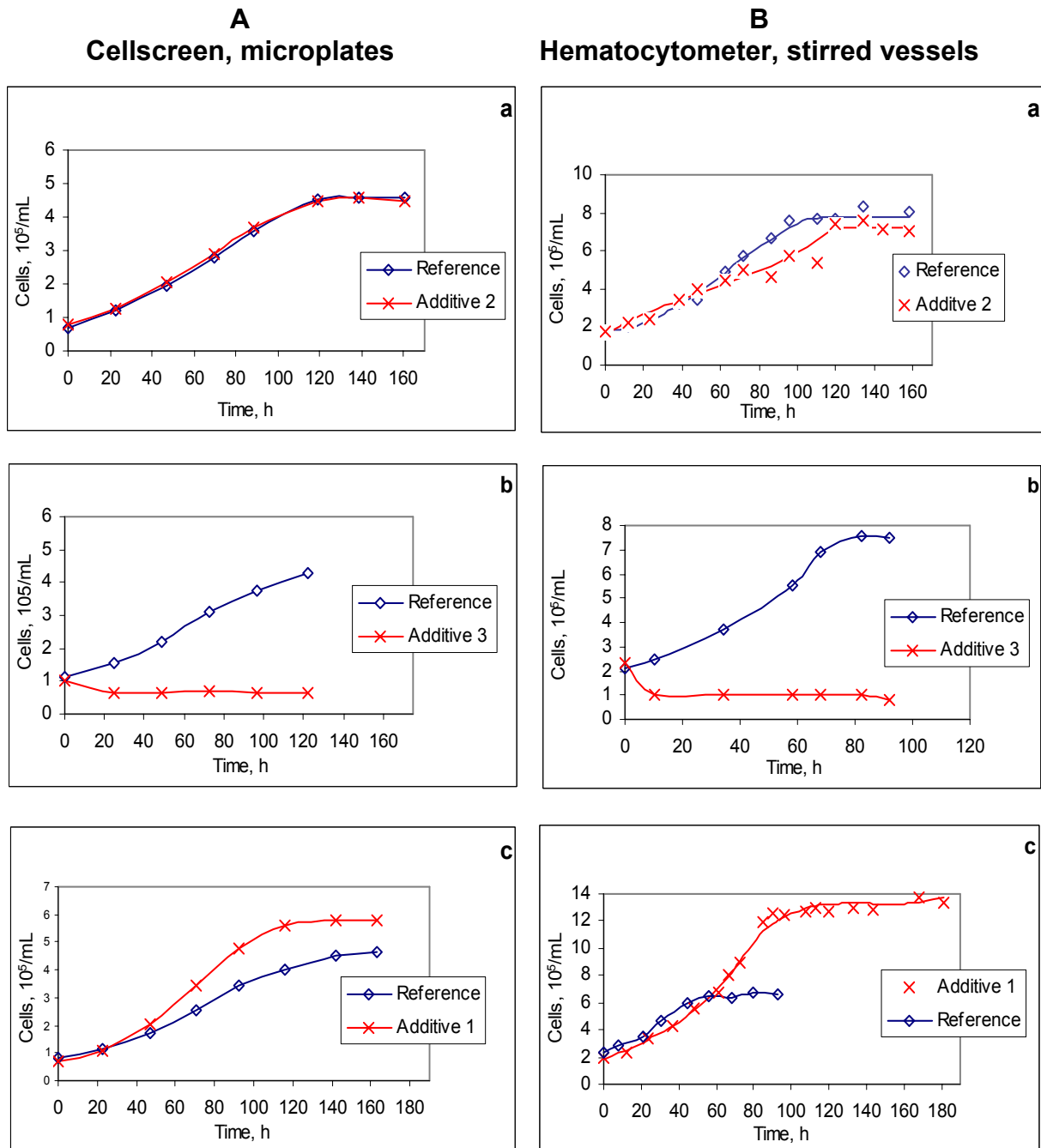


Figure 2: Comparison of different additives effect on cell growth. A) Cells cultures were realized in microplates and cell counts were performed with the Cellscreen. B) Total cells were cultivated in suspension in stirred vessels and were counted using a haematocytometer. a) spinner 150 mL ; b) stirred erlen 50 mL ; c) bioreactor 1200 mL.



The effects of additives detected by analysing microplates with the Cellscreen were verified in different stirred systems. The inhibitory or neutral effects of additives 3 and 2 respectively were confirmed with a similar growth behaviour in both systems. However, the effect of additive 1 is quite different depending on the culture system used. Thus, in a bioreactor where pH, temperature and PO₂ were controlled (figure 2, B-c), the growth of cells in presence of the additive 1 was maintained for 100 h, while cells growing in the reference medium reached the maximal concentration after 60 h. When cells growing in microplates were analysed by the Cellscreen, a positive effect of the additive 1 was also identified, but more in terms of an increase of the growth rate.

Conclusion

The use of the Cellscreen Proliferation Studies module allowed the screening of many different substances to optimize formulations of cell culture media.

In the present study, various effects of plant hydrolysates observed at this microscale have been validated in different stirred culture systems at higher scale. The main advantage of this micro-technology is to require small few quantities of potential bioactive molecules to evaluate their effects on the cell proliferation. The evolution of cell morphology can also be followed precisely during the cultures course. The Cellscreen is a time and cost reducing system that can be applied in the development of high-throughput screening technology.

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