Kuschelewski et al., Biotechnol. Prog., **33**(3) (2017) 759-770 considered the effect of reactive species generated by cell culture media on the overall stability of the media and the behaviour of cells cultured *in vitro*. L-cysteine and other thiol-containing components of cell culture media are likely to be oxidised, forming H_2O_2 or free radical intermediates such as cysteinyl radicals, which can themselves form disulphides in the presence of metal catalysts such as copper or react with oxygen to form cys-SO_2H or react with H_2O_2 to form cys-SO_3H. The generation of oxidation products contributes to an increased cellular stress level.

There is a continuing need to optimise medium formulations and process parameters to improve cell viability, specific productivity and titre. Improvements to medium formulations and/or feeding strategies to result in desirable recombinant protein expression, titre, cell growth and/or cell viability can reduce the costs associated with manufacturing protein therapeutics.

SUMMARY ~~OF THE INVENTION~~

The method of the present disclosure entails implementing a conditioning period for a medium prior to inoculation with a recombinant protein-producing eukaryotic cell. Eukaryotic cells grown in the resulting medium show increased cell-specific productivity. Furthermore, hydrolytic activity and process specific oxygen demand is improved. Also, the recombinant protein generated by the cell cultured in the resulting medium comprises a decreased fraction of host cell protein impurities.

In one aspect, the present disclosure refers to a method for treatment of a cell culture medium, the method comprising: holding a cell culture medium in a vessel under conditions suitable for inoculation of the medium with a eukaryotic cell that has been engineered to recombinantly express an exogenous protein, characterized in that the medium comprises the following nutrients: one or more of cystine, cysteine and a cysteine derivative; and one or more redox active trace metals and in that the medium comprising the nutrients is held under said conditions suitable for inoculation for a period of at least 10 hours in the absence of cells prior to inoculation. In another aspect, the present disclosure refers to a cell culture medium obtainable by the method of the disclosure. In another aspect, the present disclosure refers to use of the medium of the disclosure in a eukaryotic cell fermentation process for production of recombinant protein. In yet another aspect, the present disclosure refers to a method of producing a recombinant protein by recombinant expression from a eukaryotic cell, the method comprising: subjecting a cell culture medium suitable for

cultivating said eukaryotic cell to the method according to the disclosure; inoculating the treated cell culture medium with a eukaryotic cell engineered to recombinantly express an exogenous protein to form a cell culture; cultivating the cell culture so that the recombinant protein is produced.

FIGURES

Figure 1: Cell specific productivity (qP) from Example 1 for clone 2. For each illustration, the data for the DoE cases “low TES, low CysCys” (A), “low TES, high CysCys” (B), and “high TES, high CysCys” (C) were normalized to the supplementation time “-0 h”.

Figure 2: CHO derived host cell protein (CHO HCP) concentration from Example 1 for clone 2. For each illustration, the data for the DoE cases “low TES, low CysCys” (A), “low TES, high CysCys” (B), and “high TES, high CysCys” (C) were normalized to the supplementation time “-0 h”.