mitochondrial compartment of a cultivated cell affecting the intracellular pH (pHi) and directly influencing important cellular processes.

Inside the cell, CO_2 reacts with H_2O and forms H_2CO_3. This reaction occurs spontaneously and is also catalyzed by carbonic anhydrase enzymes (CA, carbonate hydrolyase, EC 4.2.1.1) which are zinc metalloenzymes that catalyze the interconversion of carbon dioxide and water into carbonic acid, protons and bicarbonate ions. In mammals fourteen carbonic anhydrase isoforms have so far been identified and the predominant cytoplasmic isozyme is carbonic anhydrase II (CAII) [SLY, W.S. AND HU, P.Y., Ann. Rev. Biochem. 64 (1995) 375-601].

Carbonic anhydrase is known to play a central role in the regulation of intracellular [ROOS, A. AND BORON, W.F. Physiol. Rev. 61 (1981) 296-434] and extracellular pH [CHEN, J.C. AND CHESLER, M., Proc. Natl. Acad. Sci. USA 89 (1992) 7786-7790]. Carbonic anhydrase II consists of a single polypeptide chain with 260 amino acid residues corresponding to a molecular mass of about 29 kDa. It is present in the cytosol of most tissues. Overall, MOSTAFA AND GU [MOSTAFA, S.S. AND GU, X., Biotechnol. Prog. 19 (2003) 45-51] reported an improved productivity, culture time, and final titer.

De Zengotita, V.M., et al., performed a characterization of hybridoma cell responses to elevated pCO_2 and osmolality (Biotechnol. Bioeng. 77 (2002) 369-380). Effects of elevated pCO_2 and osmolality on growth of CHO cells and production of antibody-fusion protein B1 was reported by Zhu, M.M., et al. in Biotechnol. Prog. 21 (2005) 70-77. Gray, D.R., et al. reported CO_2 in large-scale and high-density CHO cell perfusion culture (Cytotechnol. 22 (1996) 65-78).

Summary ~~of the Invention~~

Herein is reported the generation of a cell line stably transfected with a structural gene encoding a carbonic anhydrase (CA) and its use in the recombinant production of a polypeptide. The expression of a carbonic anhydrase provides for an efficient control of pHi at alkaline levels, causing a cell cycle arrest in G0G1-phase and an increase in cell-specific productivity.

There is~~One aspect as~~ reported herein ~~is~~ a method for producing a polypeptide comprising the following steps

a) cultivating a mammalian cell comprising a first nucleic acid encoding a carbonic anhydrase and a second nucleic acid encoding the polypeptide in a cultivation medium, and

b) recovering the polypeptide from the cell or the cultivation medium and thereby producing the polypeptide.

One aspect as reported herein is a method for producing a polypeptide comprising the following steps

a) cultivating a mammalian cell comprising a first nucleic acid encoding a carbonic anhydrase and a second nucleic acid encoding the polypeptide, and

b) recovering the polypeptide from the cell or the cultivation medium and thereby producing the polypeptide,

whereby the first nucleic acid and the second nucleic acid are expressed.

In one embodiment the cultivating is for a first period of time with a first constant pCO_2 value, and thereafter for a second period of time with an increasing pCO_2 value from the first pCO_2 value to a second pCO_2 value.

In one embodiment the cultivating is further for a third period of time after the second period of time with the second pCO_2 value held constant.

In one embodiment the first constant pCO_2 value is of from about 4 % to about 9 %. In one embodiment the first constant pCO_2 value is about 5 %.

In one embodiment the second pCO_2 value is of from about 15 % to about 30 %. In one embodiment the second pCO_2 value is about 25 %.

In one embodiment the carbonic anhydrase is a carbonic anhydrase II or a carbonic anhydrase V.

In one embodiment the carbonic anhydrase is human carbonic anhydrase II or human carbonic anhydrase V.

In one embodiment the carbonic anhydrase has the amino acid sequence of SEQ ID NO: 03.

In one embodiment the first nucleic acid is stably integrated in the genome of the mammalian cell.

In one embodiment the polypeptide is an antibody, or an antibody conjugate, or an antibody fragment, or an Fc-region fusion polypeptide.

In one embodiment the mammalian cell is a CHO cell. In one embodiment the CHO cell is a CHO K1 cell.

There is~~One aspect as~~ reported herein ~~is~~ a CHO cell comprising a first nucleic acid encoding human carbonic anhydrase II or a variant thereof and a second nucleic

acid encoding an antibody, or an antibody conjugate, or an antibody fragment, or an Fc-region fusion polypeptide.

One aspect as reported herein is a CHO cell comprising a first nucleic acid encoding human carbonic anhydrase II or a S2A variant thereof and a second nucleic acid encoding an antibody, or an antibody conjugate, or an antibody fragment, or an Fc-region fusion polypeptide.

There is~~One aspect as~~ reported herein ~~is~~ a method for producing a polypeptide comprising the following steps

a) cultivating a mammalian cell comprising a nucleic acid encoding the polypeptide in a cultivation medium, and
b) recovering the polypeptide from the cell or the cultivation medium and thereby producing the polypeptide,

whereby the cultivating is for a first period of time with a first constant pCO_2 value, and thereafter for a second period of time with an increasing pCO_2 value from the first pCO_2 value to a second pCO_2 value.

In one embodiment the mammalian cell comprises a nucleic acid encoding human carbonic anhydrase. In one embodiment the carbonic anhydrase is human carbonic anhydrase II or human carbonic anhydrase V.

In one embodiment the cultivating is further for a third period of time after the second period of time with the second pCO_2 value held constant.

In one embodiment the first constant pCO_2 value is of from about 4 % to about 9 %. In one embodiment the first constant pCO_2 value is about 5 %.

In one embodiment the second pCO_2 value is of from about 15 % to about 30 %. In one embodiment the second pCO_2 value is about 25 %.

Detailed Description of the Invention

Herein is reported the generation of a cell line stably transfected with a structural gene encoding human carbonic anhydrase II (hCAII).

It has been found that the selection of a cell line producing a recombinant polypeptide can be made not only based on growth, productivity and metabolic data, but also on the expression and enzyme kinetics of the hCAII enzyme.

It has also been found that a cell stably transfected with a nucleic acid encoding and expressing hCAII has different characteristics when cultivated under different pCO2 profiles, such as a constant pCO_2 and a slowly increasing pCO_2.