the mannose-5 glycostructure amount in the glycosylation pattern can be obtained. The DGL value or respectively the amount of glucose available per time unit has to be kept constant and at a defined reduced value per time unit.

The present disclosure relates to~~A first aspect as reported herein is~~ a method for the production of a polypeptide, in one embodiment of an immunoglobulin, in a eukaryotic cell, comprising the following steps

a) providing a eukaryotic cell comprising a nucleic acid encoding the polypeptide,

b) cultivating the cell under conditions wherein the degree of glucose limitation (DGL) is kept constant and wherein the DGL is less than 0.8, and

c) recovering the polypeptide from the culture,

wherein the fraction of the polypeptide with a mannose-5 glycostructure is 10 % or less of the sum comprising the amount of the polypeptide with a mannose-5 glycostructure, the amount of the polypeptide G(0) isoform, the amount of the polypeptide G(1) isoform, and the amount of the polypeptide G(2) isoform.

In one embodiment the DGL is kept constant in the range of from 0.8 to 0.2. In a further embodiment the DGL is kept constant in the range of from 0.6 to 0.4. In another embodiment the fraction of the polypeptide with a mannose-5 glycostructure is 8 % or less of the sum comprising the polypeptide with a mannose-5 glycostructure, the polypeptide G(0) isoform, the polypeptide G(1) isoform, and the polypeptide G(2) isoform. In still another embodiment the polypeptide is an immunoglobulin, in one embodiment an immunoglobulin of class G or E.

The present disclosure also relates to~~Another aspect as reported herein is~~ a method for the production of an immunoglobulin comprising the following steps:

a) providing a mammalian cell comprising a nucleic acid encoding the immunoglobulin,

b) cultivating the cell in a cultivation medium wherein the amount of glucose available in the cultivation medium per time unit is kept constant

and limited to less than 80 % of the amount that could maximally be utilized by the cells in the cultivation medium per time unit, and

c) recovering the immunoglobulin from the cells or the cultivation medium.

In a first aspect, there is provided the use of a method comprising

(a) cultivating a eukaryotic cell comprising a nucleic acid encoding the immunoglobulin in a cultivation medium wherein the ratio of the current specific glucose consumption rate of a single cell in a cultivation medium to the maximum known specific glucose consumption rate of the kind of cell is kept constant, and

(b) recovering the immunoglobulin from the culture,

for decreasing the amount of an immunoglobulin with a mannose-5 glycostructure in an immunoglobulin preparation.

In a second aspect, there is provided the use of a method comprising

(a) cultivating a eukaryotic cell comprising a nucleic acid encoding the immunoglobulin in a cultivation medium wherein the amount of glucose available in the cultivation medium per time unit is kept constant and limited to a constant value of less than 80% of the amount that could maximally be utilized by the cells in the cultivation medium per time unit, and

(b) recovering the immunoglobulin from the culture.for decreasing the amount of an immunoglobulin with a mannose-5 glycostructure in an immunoglobulin preparation.

In one embodiment the amount of glucose available in the cultivation medium per time unit is kept constant and limited to a value in the range of from 80 % to 20 %. In a further embodiment the range is of from 60 % to 40 %. In another embodiment the cells in the cultivation medium are the viable cells in the cultivation medium.

In one embodiment ~~of the aspects~~ as reported herein the eukaryotic cell is selected from CHO cells, NS0 cells, HEK cells, BHK cells, hybridoma cells, PER.C6® cells, insect cells, or Sp2/0 cells. In one embodiment the eukaryotic cell is a Chinese Hamster Ovary (CHO) cell. In another embodiment ~~of the aspects~~ as reported herein the cultivating is at a pH value in the range of from about pH 7.0 to about pH 7.2.

In still another embodiment ~~of the aspects~~ as reported herein the cultivating is a continuous or a fed-batch cultivating. The methods may comprise in another embodiment a final step of purifying the polypeptide. In still another embodiment the cell is cultivated for six to twenty days or for six to fifteen days. In a further embodiment the cell is cultivated for six to eight days.

The present disclosure also relates to ~~Another aspect as reported herein is~~ a composition comprising an immunoglobulin, wherein the composition has been prepared with a method as reported herein.

In one embodiment the immunoglobulin is an anti-IL-6R antibody. In a further embodiment the anti-IL-6R antibody comprises Tocilizumab. In another embodiment the mannose-5 glycostructure attached to the anti-IL-6R antibody is 8 % or less. In still a further embodiment the mannose-5 glycostructure is 6 % or less. In another embodiment the mannose-5 glycostructure is 4 % or less. In a further embodiment the G(0) glycostructure attached to the anti-IL-6R antibody is in the range from 40 % to 46 % and the G(2) glycostructure attached to the anti-IL-6R antibody is in the range from 9 % to 11 %.