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Published:

- with international search report (Art. 21(3))
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(54) Title: FERMENTATION PROCESS FOR ANTIBODY PRODUCTION

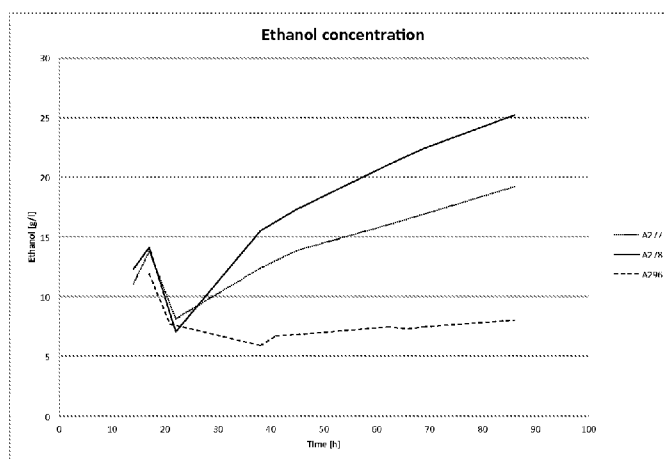


Figure 8: Ethanol concentration profile for 3 RQ control set points indicating effect of RQ control on maintaining non toxic ethanol levels for mAb1 antibody Strain A in 20L fermentors

(57) Abstract: A feedback control mechanism for a fermentation of yeast cells to make recombinant proteins uses a respiratory quotient measurement which adjusts the levels of oxygenation and/or fermentable sugar feed. The feedback control mechanism permits well controlled cultures that produce good amounts of product while avoiding toxic accumulation of ethanol. Additionally, recombinant proteins so produced have excellent qualitative properties, such as excellent homogeneity and proper inter-subunit assembly.

WO 2014/145521 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 14/30311

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 1/15, C12N 1/19, C12P 21/08 (2014.01)

CPC - C12N 1/16, C12N 15/81, C12P 21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): C12N 1/15, C12N 1/19, C12P 21/08 (2014.01)

CPC: C12N 1/16, C12N 15/81, C12P 21/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

CPC: C12N 15/815

(keyword limited; terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatBase, PubWest, Google Scholar, Google Patent: Fermentation, process, antibody, yeast, production, glyceraldehyde-3-phosphate, transcription, promoter, sugar feed rate, oxygen, transfer, harvest, culture medium, respirat* quotient, RQ, polypeptide, glucose, oxygen, ethanol, exhaust gas

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2010/0112639 A1 (CARLSON et al.) 06 May 2010 (06.05.2010); para [0012], [0021], [0029]	1-22
Y	EP 0,537,897 A1 (BOECK) 03 April 1996 (03.04.1996); p 3, ln 57- p 4, ln 4; p4, ln 18-24; p 5, ln 58- p6, ln 8; p 5, ln 55-56; p 5, ln 56- p 6, ln 1; p 6, ln 3	1-22
Y	US 6,220,076 B1 (LAYZELL et al.) 24 April 2001 (24.04.2001); abstract, col 5, ln 59-61; col 6, ln 22-29	6
Y	US 7,892,825 B2 (BARR et al.) 22 February 2011 (22.02.2011); col 17, ln 48-67; col 33, ln 60-65; col 34, ln 14	8, 11-13, 16, 18
Y	WO 2012/049179 A2 (WISSELINK et al.) 19 April 2012 (19.04.2012); pg 55, ln 2-6	10
Y	JP 2006/288201 A (KIMURA et al.) 26 October 2006 (26.10.2006); Abstract	14-15
Y	US 2011/0014650 A1 (YOUNG et al.) 20 January 2011 (20.01.2011); para [0070], [0078]	20, 22

 Further documents are listed in the continuation of Box C.


* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/30311

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

- 1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

- 2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

- 3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-22, drawn to a method for producing a recombinant protein in yeast cells

Group II: Claims 23-25, drawn to a method for producing an antibody comprising two heavy chains and two light chains or an antibody fragment in Pichia yeast cells

Group III: Claims 26-64, drawn to a large scale fermentation process

--continued in extra sheet--

- 1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
- 2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
- 3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
- 4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-22

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

Continuation of:

Box No: III Observations where unity of invention is lacking

The inventions listed as Groups I, II, and III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

Group I requires measuring respiratory quotient (RQ) of the population, wherein the desired predetermined range of RQ at about 20 - 40 hours after initiation of the culturing is between about 1.08 and about 1.35 and adjusting one or both of the fermentable sugar feed rate to a second feed rate or the oxygen transfer rate to a second oxygen transfer rate, when the RQ is outside of a desired predetermined range, not required by Groups II and III.

Group II requires production of antibody comprising two heavy chains and two light chains or an antibody fragment in *Pichia* yeast cells by culturing under hypoxic conditions, not required by Groups I and III.

Group III requires large-scale fermentation conditions, not required by Groups I and II.

Common Technical Features

The feature shared by Groups I, II, and III are the method steps of: culturing yeast cells under fed-batch fermentation conditions, wherein said cultured yeast cells are engineered to express a recombinant protein; and harvesting the yeast cells or the culture medium and recovering the recombinant protein from the harvested cells or culture medium. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is taught by US 2010/0112639 A1 to Carlson et al. (hereinafter 'Carlson'). Carlson discloses culturing yeast cells under fed-batch fermentation conditions, wherein said cultured yeast cells are engineered to express a recombinant protein (para [0029] - "The production of brazzein by *Pichia* yeast engineered to produce brazzein proteins under control of the pGAP promoter can, surprisingly, be regulated by controlling the availability of oxygen in the fermentation reactor. In general, fermentation cultures of *Pichia* are grown as fed batch cultures"); and harvesting the yeast cells or the culture medium (para [0012] - "removing the cells from the pH adjusted fermentation medium") and recovering the recombinant protein from the harvested cells or culture medium (para [0012] - "c) subjecting the cell-free fermentation medium to ion-exchange chromatography and eluting the protein as a solution; d) passing the eluted protein solution through a first membrane to remove unwanted high molecular weight solutes; and e) concentrating and diafiltering the protein permeate on a second membrane to remove low molecular weight solutes"). As the technical feature was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

The feature shared by Groups I and II is a DNA segment encoding a polypeptide, wherein the DNA segment is operably linked to a glyceraldehyde-3-phosphate (GAP) transcription promoter and a transcription terminator. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is taught by Carlson. Carlson discloses a DNA segment encoding a brazzein polypeptide, wherein the DNA segment is operably linked to a glyceraldehyde-3-phosphate (GAP) transcription promoter and a transcription terminator (para [0021] - "Bam H1 gene cassettes are designed to contain, in order from 5' to 3', the *P. pastoris* pGAP promoter ... either the type II or type III brazzein gene and the AOX1 terminator"). As the technical feature was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

The feature shared by Groups I and III are monitoring RQ values of the yeast population during the batch fermentation and determining whether the RQ value falls within a specified range, and adjusting at least one culture parameter so as to adjust or maintain the RQ value of the fed-batch yeast culture. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is taught by EP 0537897 A1 to (Boeck). Boeck discloses measuring respiratory quotient (RQ) of a cell population during fermentation (p 5, ln 55-56 gauge respiratory quotient during fermentation) and determining if it is within a desired predetermined range, wherein the desired predetermined range of RQ at about 20-40 hours after initiation of the culturing is between about 1.08 and about 1.35 (p 5, ln 56 -- p 6, ln 1 - "respiration quotient of the organism... stabilizes at about 1.0.. a respiratory quotient of 1.0 denotes...initiation of biosynthesis.. under optimum conditions, this frequency occurs at about 40-50 hours after initiation"); and adjusting a fermentable sugar feed rate to a second feed rate when the RQ is outside of a desired predetermined range (p 6, ln 3 - "the glucose feed rate should be adjusted to maintain a respiration quotient"). As the technical feature was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Groups I, II, and III therefore lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.