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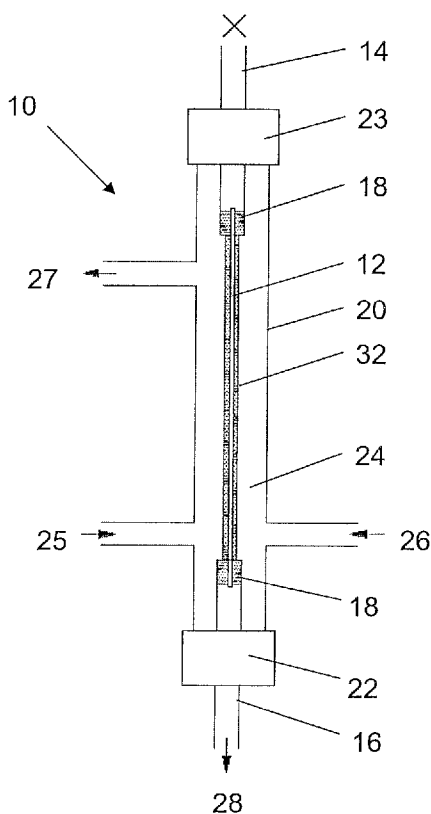
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[Continued on next page]

(54) Title: PRODUCTION OF SECONDARY METABOLITES USING CAPILLARY MEMBRANES



(57) Abstract: The invention provides methods of producing secondary metabolites and under recombinant products oxygen-limited or anaerobic culture conditions. The methods include providing a porous substrate having a first side and a second side and which has a biofilm of microorganisms attached to the first side thereof, and causing a nutrient solution to flow through the biofilm and the substrate in a direction from the first side thereof to the second side thereof under oxygen-limited or anaerobic culture conditions. The microorganisms include *Clostridium sp.*, *Bacillus sp.*, *Pseudomonas sp.*, *Vibrio sp.*, *Serratia sp.*, *Rhodopseudomonas sp.*, *Anaeromyxobacter sp.*, *Desulphovibrio sp.*, and *Candida sp.*, *Lactococcus lactis*, *Escherichia coli*, *Bacillus subtilis*, *Pichia sp.*, *Candida sp.*, *Hansenula polymorpha* and *Sacharomyces cerevisiae*.

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European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,  
FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT,  
RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA,  
GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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**INTERNATIONAL SEARCH REPORT**

International application No  
PCT/IB2006/052201

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> INV. C12N15/09				
According to International Patent Classification (IPC) or to both national classification and IPC				
<b>B. FIELDS SEARCHED</b>				
Minimum documentation searched (classification system followed by classification symbols) C12N				
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched				
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, COMPENDEX, WPI Data, INSPEC, EMBASE, BIOSIS				
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>				
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
X	EP 0 761 608 A2 (WATER RES COMMISSION [ZA]) 12 March 1997 (1997-03-12) claims 1-10; figures 1,2	1-30		
X	WO 02/094979 A2 (LEUKES WINSTON DANIEL [ZA]; GARCIN CLIVE [ZA] SYNEXA LIFE SCIENCES PRO) 28 November 2002 (2002-11-28) claims 1-14; figures 1-3	1-30		
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<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C.				
<input checked="" type="checkbox"/> See patent family annex.				
* Special categories of cited documents :				
<table style="width:100%; border: none;"> <tr> <td style="width:50%; border: none; vertical-align: top;">                     *A* document defining the general state of the art which is not considered to be of particular relevance                      *E* earlier document 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                 </td> <td style="width:50%; border: none; vertical-align: top;">                     *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.                      *&amp;* document member of the same patent family                 </td> </tr> </table>			*A* document defining the general state of the art which is not considered to be of particular relevance *E* earlier document 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
*A* document defining the general state of the art which is not considered to be of particular relevance *E* earlier document 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	Date of mailing of the international search report			
8 February 2007	27/04/2007			
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer  Stachowiak, Olaf			

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/IB2006/052201

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GOVENDER S ET AL: "Towards an optimum spore immobilisation strategy using Phanerochaete chrysosporium, reverse filtration and ultrafiltration membranes" JOURNAL OF MEMBRANE SCIENCE, ELSEVIER SCIENTIFIC PUBL.COMPANY. AMSTERDAM, NL, vol. 238, no. 1-2, 15 July 2004 (2004-07-15), pages 83-92, XP004513027 ISSN: 0376-7388 pages 83-86; figures 1,2,5	1-30
X	INLOES DOUGLAS S ET AL: "HOLLOW-FIBER MEMBRANE BIOREACTORS USING IMMOBILIZED E. COLI FOR PROTEIN SYNTHESIS" BIOTECHNOL BIOENG NOV 1983, vol. 25, no. 11, November 1983 (1983-11), pages 2653-2681, XP002406100 the whole document	1-30
X	US 4 804 628 A (CRACAUER RAY F [US] ET AL) 14 February 1989 (1989-02-14) abstract; claims 1-21	1-30
A	RAJAGOPALAN VENKATADRI ET AL: "CULTIVATION OF PHANEROCHAETE CHRYSOSPORIUM AND PRODUCTION OF LIGNIN PEROXIDASE IN NOVEL BIOFILM REACTOR SYSTEMS: HOLLOW FIBER REACTOR AND SILICONE MEMBRANE REACTOR" WATER RESEARCH, ELSEVIER, AMSTERDAM, NL, vol. 27, no. 4, 1 April 1993 (1993-04-01), pages 591-596, XP000356361 ISSN: 0043-1354 the whole document	1-30
P,X	CANOVAS ET AL: "Factors affecting the biotransformation of trimethylammonium compounds into l-carnitine by Escherichia coli" BIOCHEMICAL ENGINEERING JOURNAL, ELSEVIER, AMSTERDAM, NL, vol. 26, no. 2-3, 15 November 2005 (2005-11-15), pages 145-154, XP005097671 ISSN: 1369-703X paragraphs [02.2], [03.3]; figure 2	1-30

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/IB2006/052201

## Box 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 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:

see additional 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 an additional fee, this Authority did not invite payment of any additional fee.
  
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.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-14

Methods and devices for the production of secondary metabolites under oxygen-limited conditions comprising employing a porous substrate having first and second sides, a feeding device, and a biofilm across which a nutrient gradient is established.

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2. claims: 15-30

Methods and devices for the production of recombinant products under oxygen-limited conditions comprising employing a porous substrate having first and second sides, a feeding device, and a biofilm across which a nutrient gradient is established.

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No PCT/IB2006/052201
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Patent document cited in search report	Publication date	Publication date	Patent family member(s)	Publication date
EP 0761608	A2	12-03-1997	AT 199701 T	15-03-2001
			DE 69612048 D1	19-04-2001
			DE 69612048 T2	11-10-2001
			DK 761608 T3	16-07-2001
			GR 3036003 T3	28-09-2001
			US 5945002 A	31-08-1999
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US 4804628	A	14-02-1989	NONE	