US009410177B2

(12) **United States Patent**
Šiurkus et al.

(10) **Patent No.: US 9,410,177 B2**
(45) **Date of Patent: Aug. 9, 2016**

(54) **PROTEIN PRODUCTION**

(75) Inventors: **Juozas Šiurkus**, Kaisiadorys (LT); **Peter Neubauer**, Berlin (DE)

(73) Assignee: **Thermo Fisher Scientific Baltics UAB**, Vilnius (LT)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 883 days.

(21) Appl. No.: **13/354,748**

(22) Filed: **Jan. 20, 2012**

(65) **Prior Publication Data**

US 2012/0196322 A1 Aug. 2, 2012

(30) **Foreign Application Priority Data**

Feb. 2, 2011 (GB) 1101794.4

(51) **Int. Cl.**
C12P 21/06 (2006.01)
C12P 21/02 (2006.01)

(52) **U.S. Cl.**
CPC ***C12P 21/02*** (2013.01)

(58) **Field of Classification Search**
CPC C12P 21/02; C12P 21/06
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

6,333,175 B1 12/2001 Glockshuber et al.
7,560,248 B1 7/2009 Raines
2004/0219529 A1 11/2004 Latham et al.
2005/0064545 A1 3/2005 DeMarco et al.
2010/0021964 A1 1/2010 Bornscheuer et al.

FOREIGN PATENT DOCUMENTS

EP	0422217	*	1/1998	..... C12P 21/00
WO	90/03430		4/1990	
WO	90/12881		11/1990	
WO	95/30428		11/1995	
WO	2006/014899		2/2006	
WO	2006/091892		8/2006	

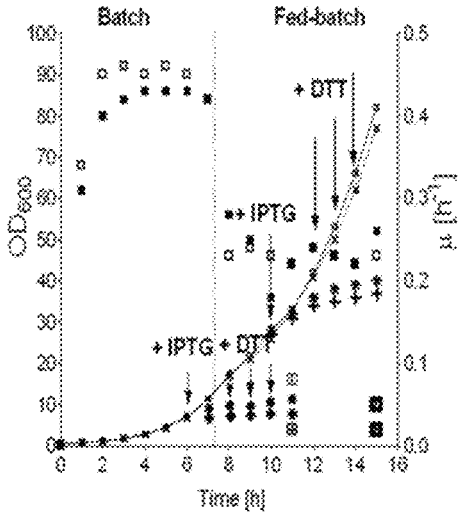
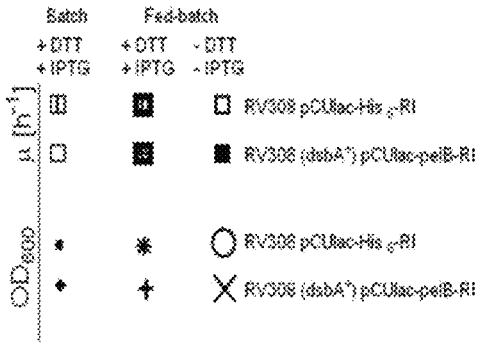
OTHER PUBLICATIONS

Garvie et al., The growth of *Escherichia coli* in buffer substrate and distilled water, J Bacteriol. Apr. 1955; 69(4): 393-398.*
Peterson et al., Bacterial Cell Surface Damage Due to Centrifugal Compaction, Applied and Environmental Microbiology, Jan. 2012 vol. 78 No. 1, p. 120-125.*
Nishihara et al., Chaperone Coexpression Plasmids: Differential and Synergistic Roles of DnaK-DnaJ-GrpE and GroEL-GroES in Assisting Folding of an Allergen of Japanese Cedar Pollen, Cryj2, in *Escherichia coli*, Appl Environ Microbiol. May 1998;64(5):1694-9.*

(Continued)

Primary Examiner — Reza Ghafoorian
(74) *Attorney, Agent, or Firm* — Thompson Hine LLP

(57) **ABSTRACT**

A method for producing from host cells a heterologous polypeptide or protein comprising at least one cysteine residue in a reduced state. The method cultured the host cells in a culture medium comprising a reducing agent; and recovered the heterologous polypeptide or protein comprising at least one cysteine residue in a reduced state from the host cells or from the culture medium, where the host cells comprise a nucleic acid encoding the heterologous protein or polypeptide, and where the reducing agent is capable of permeating the plasma membrane of the host cells.

21 Claims, 19 Drawing Sheets