

(43) International Publication Date
23 July 2009 (23.07.2009)(10) International Publication Number
WO 2009/090480 A4(51) International Patent Classification:
C12P 7/06 (2006.01) *C12P 7/10* (2006.01)(21) International Application Number:
PCT/IB2008/003536(22) International Filing Date:
18 December 2008 (18.12.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PA 2007 01862 21 December 2007 (21.12.2007) DK
61/015,688 21 December 2007 (21.12.2007) US(71) Applicant (for all designated States except US): **INBI-
CON A/S** [DK/DK]; Kraftværksvej 53, DK-7000 Fred-
ericia (DK).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **LARSEN, jan**
[DK/DK]; Osterbro 28, DK-5690 Tommerup (DK).(74) Agent: **BUDDE SCHOU A/S**; Vester Søgade 10,
DK-1601 Copenhagen V (DK).(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ,
CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ,EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,
HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR,
KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME,
MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO,
NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG,
SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA,
UG, US, UZ, VC, VN, ZA, ZM, ZW.(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ,
TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,
MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI
(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
NE, SN, TD, TG).

Declarations under Rule 4.17:

— of inventorship (Rule 4.17(iv))

Published:

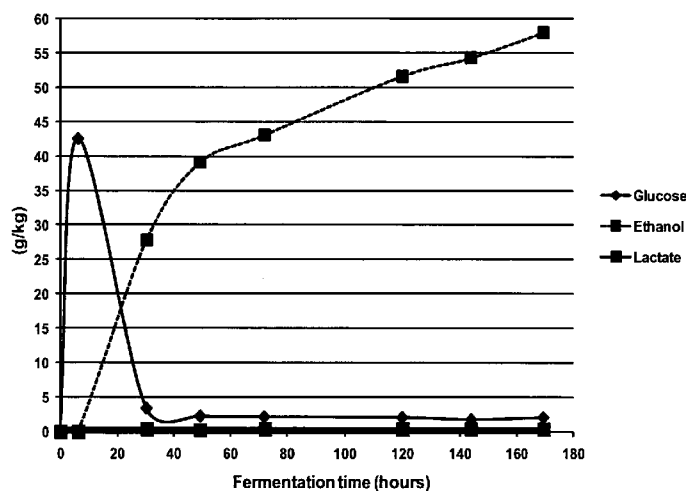
— with international search report (Art. 21(3))

— with amended claims (Art. 19(1))

(88) Date of publication of the international search report:
3 September 2009

[Continued on next page]

(54) Title: NON-STERILE FERMENTATION OF BIOETHANOL

FIG. 2. Non-sterile pre-hydrolysis and SSF of pretreated wheat
straw having a water/biomass ratio of 5:1.(57) Abstract: A range of concentrations exists
in which fermentation inhibitors derived from
pretreatment of lignocellulosic feed stocks inhib-
it growth of lactic acid bacteria without affecting
fermentive yeast. By optimizing levels of fer-
mentation inhibitors to fall within this range,
yeast fermentations of lignocellulosic biomass
can be conducted under non-sterile conditions
with ethanol yields comparable to those
achieved under sterile conditions. Optimised in-
hibitor levels can be achieved by controlling the
water/biomass ratio of a lignocellulosic biomass
during and after pretreatment, for example by
washing the fiber fraction of a previously pre-
treated lignocellulosic biomass with a pre-de-
fined amount of fresh water or recycled process
solutions. Crude extracts of liquid fraction or
process solutions from pretreatment of lignocel-
lulosic biomass can also provide an effective
anti-bacterial treatment for first generation starch
fermentations.



Date of publication of the amended claims: 12 November
2009

received by the International Bureau on 21 September 2009 (21.09.09)

Claims:

1. A method of processing lignocellulosic biomass comprising preparing a non-sterile yeast fermentation mixture comprising the fiber fraction of a pretreated
5 lignocellulosic biomass and optimised levels of fermentation inhibitors achieved by controlling the water/biomass ratio of a pretreated biomass or by otherwise adjusting the amounts present in a pretreated lignocellulosic biomass and/or in a fermentation mixture in such manner that when the pretreated lignocellulosic biomass is
10 fermented in a fermentation mixture or when the fermentation mixture containing optimised levels is fermented, lactate yield in the fermentation mixture after the desired part of fermentable sugars have been converted is lower than about 4 g/ kg and ethanol yield is higher than about 75% theoretical yield.

2. The method of claim 1 further comprising fermentation of said non-sterile yeast
15 fermentation mixture.

3. The method of claim 1 or 2 wherein optimised levels of fermentation inhibitors are achieved by controlling the water/biomass ratio in the pretreatment and in any post pretreatment processing of the pretreated lignocellulosic biomass.
20

4. The method of claim 3 wherein the pretreated lignocellulosic biomass is subject to washing and pressing steps.

5. The method of claim 4 wherein the pretreated lignocellulosic biomass is washed
25 with water to achieve a water/biomass ratio in the pretreatment and in any post pretreatment processing to about 5:1.

6. The method of claim 4 wherein the pretreated lignocellulosic biomass is washed with water to achieve a water/biomass ratio of between about 4:1 and about 8:1.
30

7. The method of claim 4 wherein the lignocellulosic biomass is pretreated to severity index at least about 3.5.

8. The method of claim 4 wherein the lignocellulosic biomass comprises wheat straw, bagasse, or corn stover.

9. The method of claim 4 wherein the lignocellulosic biomass comprises rice straw, bagasse, corn fiber, hardwood bulk, softwood bulk, nut shells, corn cobs, grass, paper, or cotton seed hairs.

10. The method of claim 1 or 2 wherein the fiber fraction of the pretreated lignocellulosic biomass comprises dry matter mass of at least 40 kg.

11. The method of claim 1 or 2 wherein the fermentation mixture is a continuous culture.

12. The method of claim 1 or 2 wherein the fermentation mixture is a batch or fed-batch culture.

13. The method of claim 1 or 2 wherein the fermentation mixture is prepared from a pre-hydrolysed fiber fraction of a pretreated lignocellulosic biomass.

14. An anti-bacterial composition comprising a crude extract of liquid fraction from steam pretreated lignocellulosic biomass adapted for use as an alternative to hops or other anti-bacterial or aseptic agents in yeast fermentation mixtures.

15. A method of fermentation to produce ethanol or other yeast fermentation product comprising addition of the anti-bacterial composition of 14 to the fermentation mixture.

16. A method of fermentation of starch biomass comprising the steps of

- preparing a non-sterile fermentation mixture comprising a starch biomass and optimised levels of fermentation inhibitors that inhibit contaminating bacterial growth and/or lactate production while having substantially no affect on ethanol fermentive organisms;
- fermenting said fermentation mixture.