Form 6 (Non-Convention)

Regulation 12(1)

COMMONWEALTH OF AUSTRALIA

Patents Act 1952

DECLARATION IN SUPPORT OF AN APPLICATION FOR A PATENT

In support of the Application
made by UNIVERSITY OF QUEENSLAND
for a patent for an invention entitled:
"CONVERSION OF SUCROSE TO ETHANOL USING THE BACTERIUM ZYMOMONAS MOBILIS"

I, DOUGLAS PORTER
of St. Lucia, Queensland, 4067, Australia
do solemnly and sincerely declare as follows:

1. I am authorized by the UNIVERSITY OF QUEENSLAND the Applicant for the patent to make this declaration on its behalf.

2. HORST WERNER DOELLE, of Belsize Street, Kenmore, Queensland, 4069, Australia, is the actual inventor of the invention and the facts upon which the said Applicant is entitled to make the application are as follows:

"The said Applicant is the assignee of the said invention from the said inventor."

DECLARED at Brisbane, Queensland, Australia
this 9th day of July 1987.

UNIVERSITY OF QUEENSLAND,

by Douglas Porter
(Douglas Porter Registrar)

GRANT ADAMS & COMPANY,
333 Adelaide Street,
BRISBANE. QUEENSLAND. 4000
AUSTRALIA.

TO: The Commissioner of Patents,
COMMONWEALTH OF AUSTRALIA

(12) PATENT ABRIDGMENT (11) Document No. AU-B-53970/86
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 603333

(54) Title
CONVERSION OF SUCROSE TO ETHANOL USING THE BACTERIUM ZYMOMONAS MOBILIS

International Patent Classification(s)
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(71) Applicant(s)
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(56) Prior Art Documents
WO 01563/82
AU 29530/84
AU 59181/80

(57) A "single-stage process" is defined as a process whereby growth and the ethanol production phase occur in the same fermenter vessel. Initiation of the process can be done either be a seed culture containing Zymomonas mobilis added to the fermenter vessel containing the fermentation medium or by adding the fermentation medium to the fermenter which contains a portion of the fermented medium from a previous fermentation run, the fermented medium containing Zymomonas mobilis.

"Microaerophilic conditions" are defined as conditions whereby no gas (oxygen, air, nitrogen, etc.) is added to the fermenter and the surface of

.../2

the fermentation medium is exposed to atmosphere. The Zymomonas mobilis organism does not require air or oxygen (aerobic) or nitrogen (anaerobic) for growth and production of ethanol, but can tolerate the presence of air on the surface of the fermentation medium.

CLAIM

1. A method for the production of ethanol from sucrose in a fermentation medium in a fermenter vessel characterized by:

fermenting the sucrose with a strain of the micro-organism Zymomonas mobilis, the fermentation being carried out under microaerophilic conditions (as herein-before defined), in a single-stage process where the growth of the micro-organism and the fermentation of the sucrose to ethanol by the micro-organism occur in the same fermenter vessel, the sucrose concentration in the fermentation medium being in the range of 10-30% (w/v).

9. A method according to any one of Claims 1 to 8 characterized in that the pH of the fermentation medium is maintained in the range 4.0 to 7.0.

11. A method according to any one of Claims 1 to 10 characterized in that the temperature in the fermenter is maintained in the range 34° to 40°C.

AU-AI 53970/86

PCT

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(81) Designated States: AT (European patent), AU, BB, BE (European patent), BR, CH (European patent), DE (European patent), FR (European patent), GB, GB (European patent), HU, IT (European patent), JP, KR, LK, LU (European patent), NL (European patent), SD, SE (European patent), SU, US.

Published
With international search report.

(54) Title: CONVERSION OF SUCROSE TO ETHANOL USING THE BACTERIUM *ZYMOMONAS MOBILIS*

(57) Abstract

A new single-stage fermentation process for the commercial production of ethanol from refined sucrose, raw sugar, sugar cane juice, sugar cane syrup, sucrose hydrolysates and invert sugars has been developed using *Zymomonas mobilis*. The process gives a 94-98% sucrose hydrolysis efficiency and a 95-98% ethanol conversion efficiency. Within 24-30 hours; 200 g/L sucrose is converted to produce 95.5 g/L ethanol. Reinoculation is carried out from the fermented broth without the need for centrifugation or membrane filtration.

Title: "CONVERSION OF SUCROSE TO ETHANOL USING THE BACTERIUM ZYMOMONAS MOBILIS"

BACKGROUND OF THE INVENTION

(1) Field of Invention

This invention relates to a method for converting sucrose to ethanol in a single stage fermentation process using high efficiency strains of the bacterium Zymomonas mobilis in microaerophilic conditions.

(2) Prior Art

The traditional process of ethanol production is carried out in a two-stage batch process using yeast, whereby the first stage involves an aerobic propagation of the yeast referred to as the growth stage and the second stage involves the anaerobic process of ethanol production in the presence or absence of small amounts of oxygen. In order to further propagate yeast during the ethanol producing second stage, a slight addition of air or oxygen is required. The latter is required if the efficiency of the total process is to be increased using the occasional recycling of yeast cells by systems such as sedimentation or centrifugation. Since yeast fermentation is inherently dependent on coupling of growth with rate of ethanol production, to optimize ethanol production the medium must either be supplemented with growth enhancing substances or with finely controlled aeration.

The traditional yeast fermentation process (stage 2) is therefore dependent on large inoculum size of approximately 5 to 10 million cells per ml. The preferred optimal temperature of fermentation is 30° C and heat produced has to be controlled through the use of cooling equipment. The fermentation time for obtaining between 9 and 11% (v/v) ethanol is 30 to 70 hours with stage 2 batch fermentation. The time of this fermentation can be reduced to 10 hours by

increasing the inoculum density by 80-100 fold through cell recycling.

A second process for ethanol production is known, which utilizes the bacterium Zymomonas mobilis (see European Patent No. 0047641 - George Weston Ltd.). This process is also a two-stage process as was described above for yeast, but the bacterium does not require the addition of air for its growth stage (stage 1). Instead, an adequate supply of nitrogen is required to keep conditions anaerobic. During the second stage of the process for the production of ethanol, the sugar concentration must never exceed 6% (w/v) and thus the stage requires a stepwise or continuous addition of a concentrated sugar solution. The preferred temperature is 28° C to 33° C and the preferred pH is about 5.5. This process may also require a supply of nitrogen as well as additional nutrients.

A third process for ethanol production has been described, which utilizes immobilized yeast or strains of Zymomonas in a two-stage process, each with a limited amount of sugar (i.e. 10% w/v) present (see British Patent No. 2055121 - Tanabe Sugaku Co. Ltd.).

In the case of yeast fermentation the examples for carbon source conversion are known to be sucrose, glucose, molasses and sugar cane juice, whereas in the case of the two-stage process utilizing Zymomonas the examples are limited to glucose, and in the case of the immobilized cells, to glucose and molasses.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a method for producing ethanol from sucrose in a single-stage process using the bacterium Zymomonas mobilis.

It is a preferred object of the present invention to provide such a method using single-stage

batch fermentation, or if required, adjustments to this culturing method, e.g. fed-batch, continuous or multi-stage system, where the energy input is low.

It is a further preferred object to provide a method whereby the purity of the sucrose is not vital to the method.

In one aspect the present invention resides in a method for the production of ethanol from sucrose in a fermenter vessel characterized by:

fermenting the sucrose with a strain of the micro-organism Zymomonas mobilis, the fermentation being carried out under microaerophilic conditions (as herein-before defined), in a single-stage process where the growth of the micro-organism and the fermentation of the sucrose to ethanol by the micro-organism occur in the same fermenter vessel, the sucrose concentration in the fermentation medium being in the range of 10-30% (w/v).

A "single-stage process" is defined as a process whereby growth and the ethanol production phase occur in the same fermenter vessel. Initiation of the process can be done either be a seed culture containing Zymomonas mobilis added to the fermenter vessel containing the fermentation medium or by adding the fermentation medium to the fermenter which contains a portion of the fermented medium

from a previous fermentation run, the fermented medium containing Zymomonas mobilis.

"Microaerophilic conditions" are defined as conditions whereby no gas (oxygen, air, nitrogen, etc.) is added to the fermenter and the surface of the fermentation medium is exposed to atmosphere. The Zymomonas mobilis organism does not require air or oxygen (aerobic) or nitrogen (anaerobic) for growth and production of ethanol, but can tolerate the presence of air on the surface of the fermentation medium.

The preferred strain of the micro-organism Zymomonas mobilis has been deposited in the culture collection of the University of Queensland, Microbiology Department, St.Lucia, Queensland, 4067, Australia, under Deposit No. UQM 2716 and in the American Type Culture Collection (ATCC) 12301 Parklawn Drive, Rockville, Maryland, 20852, U.S.A. on 24th April, 1984 under Deposit No. 39676.

This strain was derived by selection using continuous cultivation techniques from the strain deposited under Deposit No. NCIB 11199 at the National Collection of Industrial Bacteria, Torrey Research Station, Abbey Road, Aberdeen, AB9 8DG, United Kingdom and under Deposit No. 29191 at the ATCC. The selection was determined on improved performance and metabolic rate of sucrose conversion

and these features are the only difference in the taxonomic description of the parent strain ATCC No. 29191 set out at pages 576-580 of "Bergey's Manual of Determinative Bacteriology" (8th Edition) (1975). The parent strain NCIB 11199/ATCC No. 29191 and other strains of Zymomonas mobilis may also be used.

Preferably the sucrose is obtained from sugar cane or sugar beet and may be supplied to the fermenter in the form of refined sugar, raw sugar, crushed sugar cane juice, sugar beet juice, molasses or like or in combination of any other named substrates.

Preferably the sucrose concentration is in the range of 15-25% (w/v), a concentration of 20% being most preferred for maximum ethanol yield.

In the case of refined or raw sugar, preferably the medium includes any one or more of the following components: yeast extract, peptone (casein hydrolysate);

potassium dihydrogen phosphate; ammonium sulphate ($(NH_4)_2SO_4$) or urea; and magnesium sulphate ($MgSO_4 . 7 H_2O$). Preferably the components are provided in the concentration range of 0.01 to 0.5% each, with approximately 0.2% being preferred.

The abovenamed medium components may be replaced by the addition of sugar cane juice, sugar beet juice or molasses in appropriate concentrations as the medium components may be found in the juice or molasses.

In the case of any of the following; sugar cane juice or concentrate, sugar beet juice or concentrate and molasses as fermentation substrates, no additional supplements of any kind may be required.

Preferably the pH of the fermentation process is maintained in the range of 4.0 to 7.0. Preferably the pH is initially set in the range of 6.5 to 7.0. As the fermentation process proceeds the pH drops and then after e.g. 1-2 hours, the pH is maintained in the range of 5.0 to 6.0. The pH range may be controlled by the addition of NaOH or other suitable alkali.

Preferably the temperature in the fermenter is maintained in the range of 34° C to 40° C, with a constant temperature control at 35° C being preferred.

Preferably, when the fermentation has been completed, the micro-organism is separated from the fermentation medium, e.g. by filtration, centrifugation etc., and the ethanol is distilled off.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

To enable the invention to be fully understood, preferred examples of the method will now be described. "In all Examples, percentages are expressed as "% (w/v)" where 1% corresponds to 10 g/L."

EXAMPLE 1: (Initial growth and production phases -

Laboratory Scale)

Sugar Cane Juice as Substrates: 1800 mL of sugar cane juice containing 12.1% sucrose was placed in a 2.0 L fermentation vessel.

200 mL of a 12-24 hours seed culture of Zymomonas mobilis (ATCC No. 39676) grown under micro-aerophilic condition in a medium containing 10% sucrose, 0.2% yeast extract, 0.2% casein hydrolysate (peptone), 0.2% KH_2PO_4, 0.2% $MgSO_2 \cdot 7\ H_2O$, 0.2% $((NH_4)_2SO_4)$ at 37° was added to the fermenter vessel. The pH of the fermentation process was brought to 6.8 before initiation. Over the first 1-2 hours, the pH dropped to 6.0 and was thereafter controlled at 6.0 by the addition of 2N NaOH (80 g/L). Cultivation was carried out at a temperature of 35° C with a stirring rate of 100 rpm.

After 24 hours the utilization of the sugar was complete giving an ethanol concentration of 60.8 g/L or 6.08% (w/v).

EXAMPLE 2: (Growth and production phases - Laboratory Scale)

Sugar Cane Juice as Substrate: 90 L of sugar cane juice containing 15.2% sucrose was placed into a 100 L fermentation vessel.

10 L of a 12-24 hours seed culture of Zymomonas mobilis (ATCC 39676) grown in the same medium as in Example 1 was added to the fermentation vessel. The initial pH was brought to 6.8 and pH was then maintained during the fermentation at 6.0 or 5.0 by the addition of 8 N NaOH. Cultivation was carried out at a temperature of 35° C with a stirring rate of 250 rpm.

After 26 hours the utilization of the sugar was complete giving an ethanol concentration of 85 g/L or 8.5% (w/v).

EXAMPLE 3: (Growth and production phases - Laboratory Scale)

Refined Sugar as Substrate: 1800 mL of a fermenation medium containing 9.69% (96.94 g/L) refined sucrose, 0.2% yeast extract, 0.2% casein hydrolysate (peptone), 0.2% $(NH_4)_2SO_4$, 0.2% $MgSO_4$. 7 H_2O and 0.2% KH_2PO_4 were placed into a 2.0 L fermentation vessel.

200 mL of a 12-24 hours seed culture of Zymomonas mobilis (ATCC 39676) grown in the same medium as described in Example 1 was added to the fermenter vessel. The initial pH was brought to 6.8 and pH was then maintained at 5.0 or 6.0 by the addition of 2 N NaOH. Cultivation was carried out at a temperature of 35° C with a stirring rate of 100 rpm.

After 10 hours the fermentation was complete giving an ethanol concentration of 4.7% (w/v) or 47.22 g/L.

EXAMPLE 4: (Growth and production phases - Laboratory Scale)

Refined Sugar as Substrate: 1800 ml of a fermentation medium containing 18.25% (182.54 g/L) refined sucrose plus the media components described in Example 3 were placed into a 2.0 L fermentation vessel.

200 mL of a 12-25 hours seed culture Zymomonas mobilis (ATCC 39676) grown in the same medium as described in Example 1 was added to the fermentation vessel. The initial pH was brought to 6.8 and pH was then maintained at 5.0 or 6.0 by the addition of 2 N NaOH. Cultivation was carried out at a temperature of 35° C with a stirring rate of 100 rpm.

After 19 hours the fermentation was complete giving an ethanol concentration of 8.94% (w/v) or 89.44 g/L.

EXAMPLE 5: (Production phase - Laboratory Scale)

Refined Sugar as Substrate: This example is concerned with the continuous use of bacterial

fermentation broth from one fermenter to be used for the subsequent batch process as seed culture.

200 mL of fermentation broth from Example 4 was used as seed culture for 1800 mL fermentation medium of the same composition. Under identical cultivation conditions, the fermentation was complete after 19 hours giving the same ethanol concentration.

This reuse does not require any filtration, concentration or centrifugation and has been carried out successfully up to 6 times.

EXAMPLE 6: (Production phase - Laboratory Scale)

Mill Raw Sugar (Sucrose) as Substrate: The fermentation was carried out using mill raw sugar and the fermentation broth of Example 4 and the only supplementation needed was 0.05% calcium pantothenate, 0.02% $MgSO_4 \cdot 7\ H_2O$ and 0.02% KH_2PO_4. (No supplementation is required for sugar cane juice or concentrate as the substrate.)

EXAMPLE 7: (Production phase - Laboratory Scale)

Refined Sugar as Substrate: 1800 mL of a fermentation medium containing 15% (150.0 g/L) refined sucrose, 0.2% yeast extract, 0.2% casein hydrolysate (peptone), 0.2% $(NH_4)_2SO_4$, 0.2% $MgSO_4 \cdot 7\ H_2O$, and 0.2% KH_2PO_4 were placed into a 3.0 L fermentation vessel.

200 mL of a 12-24 hours seed culture of Zymomonas mobilis (NCIB 11199/ATCC 29191) grown in the same medium was added to the fermenter vessel. The pH was brought to 6.8 at the start of the fermentation, and after the culture decreased the pH to 5.0 or 6.0, the pH was maintained at 5.0 or 6.0 by the addition of 2 N NaOH. Cultivation was carried out at a temperature of 35° C with a stirring rate of 100 rpm.

After 45 hours the fermentation was complete giving an ethanol concentration of 6.1% (w/v) or 61.21 g/L.

EXAMPLE 8: (Growth and production phases - Commercial Scale)

100 L of the fermentation broth of Example 4 was used as a seed culture for 3,000 L of a fermentation vessel. The initial pH was brought to 6.8, and after the culture decreased the pH to 5.0, it was maintained at 5.0 by the addition of 2 NaOH.

After 12-24 hours, 27,000 L of the fermentation medium was added to the vessel to fill the vessel and the fermentation conditions above were repeated.

After 24-30 hours, 27,000 L of the fermented medium was pumped from the vessel with an ethanol concentration of 9.5% (w/v).

To the 3,000 L of the fermented medium remaining in the vessel was added 27,000 L of the fermentation medium and the fermentation process was repeated.

The fermentation process, using fermented medium from a preceding process as an inoculum for the Zymomonas mobilis, was repeated several times and the ethanol concentration remained in the range of 9.5 to 10% (w/v). It was observed that the Zymomonas mobilis cells grew rapidly in the fermentation medium and both growth and production phases occurred simultaneously after the initial growth phase on addition of the fresh fermentation medium.

EXAMPLE 9: (Growth and production phases - Laboratory Scale)

9.0 L of a fermentation medium containing 1600 g of sucrose, 500 g molasses, 5.0 g $(NH_4)_2SO_4$, 5 g $MgSO_4 . 7 H_2O$ (total of 1800 g sucrose) were added to a 14.0 L fermentation vessel.

1.0 L of a seed culture of Zymomonas mobilis (ATCC 39676) grown in a medium as described in earlier examples was added. The pH was adjusted to 7.0 and the pH control was maintained at 6.0. The temperature of

incubation was 35° C and the stirring rate 100 rpm.

After 36 hours, the sucrose was utilized and 89.6 g/L (8.96% w/v)) (or 11.3% v/v)) ethanol was produced.

Four (4.0) litres of the culture medium was centrifuged for 10 minutes at 4,000 rpm and used as seed culture for the next fermentation run.

9.0 L of a fermentation medium containing 1500 g sucrose, 500 g molasses, 5 g $(NH_4)_2SO_4$, 5 g $MgSO_4$. 7 H_2O (total of 1600 g sucrose) were added to the 14.0 L fermentation vessel.

1.0 L of the resuspended culture from 4.0 L of the previous run was added. The pH was adjusted to 7.0 and the pH control was maintained at 5.0. The temperature of incubation was 35° C and the stirring rate 100 rpm.

After 17 hours the fermentation was complete and 76.69 g/L (7.67% (w/v)) (or 9.7% (v/v)) ethanol was produced.

TABLE 1 gives the results of fermentation experiments with the 2.0 L bench-top and 100 L pilot plant fermenters using a variety of refined sugar (sucrose) concentrations.

The fermentation process can be carried out using other strains of Zymomonas mobilis, including parent strain NCIB 11199/ATCC 29191 but the best results are produced using the ATCC 39676 strain.

The ethanol produced has commercial value as a component for gasohol or as a base product in the chemical industry e.g. for the production of ethylene, while the other by-product, carbon dioxide, may be used for dry ice or as a carbon source for the growth of algae biomass.

The fermentation process requires only a low energy input as the micro-organism produces a fair amount

of heat during the fermentation process. In addition, the fermentation is carried out in microaerophilic conditions, avoiding the need for aerating or addition of nitrogen pumps, the fermentation components and products only requiring little mechanical stirring and pH control.

Experiments have shown that the success of the fermentation process is not wholly dependent on the quality of the sucrose as substrate. Successful tests have been carried out even on rotting cane which indicates that the process is particularly suited for industrial application and the fermenter can be adjacent a sugar mill to reduce transport costs. As the sugar cane juice does not have to be sterilized, the energy input can be kept low.

The invention is not limited to the specific examples described and various changes and modifications may be made to the examples without departing from the scope of the present invention defined in the appended claims.

TABLE 1

Sucrose initial	Sucrose residual	Sucrose hydrolysis efficiency	Ethanol	Ethanol theoret.	Conversion efficiency (Hydrolysed sucrose to ethanol	Fermentation time
g/L	g/L	%	g/L	g/L	%	h
96.9	4.85	95.0	47.0	49.52	94.9	20
121.0*	3.63	97.0	60.8	63.14	96.2	29
152.0	6.94	95.4	75.0	78.1	96.1	24
179.7	11.26	93.7	83.7	90.62	92.4	24
182.5	7.3	96.0	89.4	94.25	94.8	24
200.0	11.25	94.0	95.5	101.54	94.1	32
229.2	7.63	96.6	96.3	119.20	80.8	32
250.0	7.64	96.9	96.5	130.4	74.0	32
266.2	25.44	90.4	87.0	129.5	67.2	32
268.9	25.75	90.4	105.4	130.81	80.57	32

* Pilot plant fermentation with total volume of 100 litres.

Ethanol production from sucrose Zymomonas mobilis using refined sugar (sucrose) as substrate.

International Application No: PCT/ /

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 4, line 1-22 of the description [4]

A. IDENTIFICATION OF DEPOSIT [5]

Further deposits are identified on an additional sheet ☐ [5]

Name of depositary institution [6]

AMERICAN TYPE CULTURE CENTRE (ATCC)

Address of depositary institution (including postal code and country) [6]

12301 Parklawn Drive,
Rockville, Maryland, 28852, U.S.A.

Date of deposit [5]	Accession Number [6]
24 April, 1984 (24-04-84)	ATCC No. 39676

B. ADDITIONAL INDICATIONS [7] (leave blank if not applicable). This information is continued on a separate attached sheet ☐

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE [8] (if the indications are not for all designated States)

D. SEPARATE FURNISHING OF INDICATIONS [9] (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later [9] (Specify the general nature of the indications e.g., "Accession Number of Deposit")

E. ☐ This sheet was received with the international application when filed (to be checked by the receiving Office)

_____
(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau [10]

was

_____
(Authorized Officer)

Form PCT/RO/134 (January 1981)

SUBSTITUTE SHEET

CLAIMS

1. A method for the production of ethanol from sucrose in a fermentation medium in a fermenter vessel characterized by:

fermenting the sucrose with a strain of the micro-organism Zymomonas mobilis, the fermentation being carried out under microaerophilic conditions (as herein-before defined), in a single-stage process where the growth of the micro-organism and the fermentation of the sucrose to ethanol by the micro-organism occur in the same fermenter vessel, the sucrose concentration in the fermentation medium being in the range of 10-30% (w/v).

2. A method according to Claim 1 characterized in that the Zymomonas mobilis is a strain deposited in the ATCC under Deposit No. 39676 or a mutant or variant of ATCC 39676.

3. A method according to Claim 1 characterized in that the Zymomonas mobilis is a strain deposited in the ATCC under Deposit No. 29191.

4. A method according to any one of Claims 1 to 3 characterized in that the sucrose is added to the fermentation medium in the form of refined sugar, raw sugar, crushed sugar cane juice, sugar beet juice, molasses or a combination of two or more of these.

5. A method according to any of Claims 1 to 4 characterized in that the sucrose concentration is in the range 15-25% (w/v).

6. A method according to Claim 5 characterized in that the sucrose concentration is 20% (w/v).

7. A method according to any one of Claims 1 to 6 characterized in that the fermentation medium contains one or more of the following components: peptone, yeast extract, potassium dihydrogenphosphate, ammonium sulphate, urea or magnesium sulphate, the concentration of each component being in the range 0.01 to 0.5% (w/v).

8. A method according to Claim 7 characterized in that the concentration of each component is 0.2% (w/v).

9. A method according to any one of Claims 1 to 8 characterized in that the pH of the fermentation medium is maintained in the range 4.0 to 7.0.

10. A method according to Claim 10 characterized in that the pH is initially in the range 6.5 to 7.0, allowed to fall as fermentation commences and then maintained in the range 5.0 to 6.0.

11. A method according to any one of Claims 1 to 10 characterized in that the temperature in the fermenter is maintained in the range 34° to 40°C.

12. A method according to Claim 11 characterized in that the temperature is maintained at 35°C.

13. A method according to any one of Claims 1 to 12 characterized in that, when the fermentation is completed, the micro-organism Zymomonas mobilis is separated from the fermentation medium and the ethanol is distilled off.

14. A method according to Claim 1 characterized in that the Zymomonas mobilis comprises Zymomonas mobilis bacterial cells suspended in the fermentation medium.

15 A method according to Claim 14 characterized in that a portion of the fermented medium from a preceding fermentation is added to the fermenter as the inoculum of the Zymomonas mobilis bacterial cells for the succeeding fermentation.

16. Ethanol produced from sucrose by the method according to any one of Claims 1 to 15.

INTERNATIONAL SEARCH REPORT

International Application No PCT/AU 86/00015

I CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl. C12P 007/06, C12N 001/20

II FIELDS SEARCHED

Minimum Documentation Searched

Classification System	Classification Symbols
IPC :	C12G 003/02, C12P 007/06, 7/08, 7/10; Chemical Abstracts
Keyword :	Zymomonas mobilis

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched

III DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X,P	AU,A, 29530/84 (UNIVERSITY OF QUEENSLAND) 4 April 1985 (04.04.85)	1-4,8-19
X	AU,B, 78199/81 (540186) (UNISEARCH LTD) 17 June 1982 (17.06.82)	1,4-6,8,15, 16,19
X	WO,A, 82/01563 (B. MATTIASSON, B. HAGERDAL, P. ALBERTSSON) 13 May 1982 (13.05.82)	1,5-7,10,19
X	AU,B, 67697/81 (537029) (UNISEARCH LTD) 19 November 1981 (19.11.81)	1,4,5-8,10, 15,16,19
X	AU,B, 59181/80 (531176) (TANABE SEIYAKU CO. LTD) 18 December 1980 (18.12.80)	1,5,6,10,19
X	Advances in Biochemical Engineering, Volume 23, issued 1982 (Heidelberg, West Germany) P.L. Rogers, K.J. Lee, M.L. Skotnicki and D.E. Tribe, 'Ethanol Production by Zymomonas mobilis', see pages 34-84	1,3,5-11,15, 16,19

Continued

* 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 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

"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

IV CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
22 April 1986 (22.04.86)	(07-05-86) 7 MAY 1986
International Searching Authority	**Signature of Authorized Officer**
Australian Patent Office	J.W. ASHMAN

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category*	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	European Journal of Applied Biotechnology, Volume 11, issued 1981 (Heidelberg, West Germany) S. Cromie and H.W. Doelle, 'Nutritional Effects on the Kinetics of Ethanol Production from Glucose by Zymomonas mobilis', see pages 116-119	1,8-13,15, 16,19
X	Biotechnology Letters, Volume 4 No. 4, issued April 1982 (Surrey, England) H.J.J. Van Vuuren and L. Meyer, 'Production of Ethanol from Sugar Cane Molasses', see pages 253-256	1,4,10,12,15 16,19
X	Biotechnology Letters, Volume 3 No. 5, issued May 1981 (Surrey, England) E. Lyness and H.W. Doelle, 'Ethanol Production from Cane Juice by Zymomonas mobilis', see pages 257-260	1,3,4,10,12, 13,15,16,19
X	Biotechnology Letters, Volume 2 No. 12, issued December 1980 (Surrey, England) E. Lyness and H.W. Doelle, 'Effect of Temperature on Sucrose to Ethanol Conversion by Zymomonas mobilis Strains', see pages 549-554	1,3,5-8,10, 12,13,15,16, 19
X	Biotechnology Letters, Volume 1 No. 10, issued October 1979 (Heidelberg, West Germany) K.J. Lee, D.E. Tribe, P.L. Rogers, 'Ethanol Production by Zymomonas mobilis in Continuous Culture at High Glucose Concentrations', see pages 421-426	1,5-7,10,15, 16,19

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU **86/00015**

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Members		
AU 29530/84	BR 8404428	EP 142230	ES 531928
	JP 60214887	ZA 8402294	
AU 78199/81	BR 8107912	DE 3148329	FR 2495637
	US 4443543		
WO 8201563	EP 63146	AU 77269/81	
AU 67697/81	BR 8101334	BR 8101335	CA 1173381
	CA 1174191	DE 3108384	DE 3108386
	FR 2477571	FR 2477572	GB 2074188
	JP 56164790	NZ 196399	US 4403034
	US 4443544	ZA 8101434	ZA 8101435
	PH 17444		
AU 59181/80	BR 8003540	DE 3022063	ES 492372
	FI 801829	FR 2458586	GB 2055121
	IN 154144	JP 55165796	PH 16533
	SE 8004386	SU 1181555	US 4350765
	ZA 8003474	CA 1143307	JP 56048887

END OF ANNEX

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

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

1. [X] Claim numbers 17-18 because they relate to subject matter not required to be searched by this Authority, namely:

Plant or animal varieties

2. [ ] Claim numbers ..... 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. [ ] Claim numbers ....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] 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 claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.