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(54) Title: FERMENTATION MEDIUM COMPRISING BORON SUITABLE FOR THE PRODUCTION OF ALCOHOL

(57) Abstract: The present invention relates to a fermentation medium comprising: (a) Magnesium at a concentration greater than 40mg/L of the fermentation medium, (b) Sulphur at a concentration greater than 85mg/L of the fermentation medium, (c) Aluminium at a detectable concentration, (d) Boron at a detectable concentration, (e) Potassium at a concentration greater than 100mg/L of the fermentation medium, or combinations thereof and a method of using this formation medium for producing at least one alcohol from a carbon source.



FERMENTATION MEDIUM COMPRISING BORON SUITABLE FOR THE PRODUCTION OF ALCOHOL

FIELD OF THE INVENTION

5 The present invention relates to a biotechnological method for producing alcohols in a suitable fermentation medium. In particular, the fermentation medium comprises specific essential elements and specific concentrations for alcohol production from a carbon source lacking in carbon monoxide.

BACKGROUND OF THE INVENTION

Biotechnological production of alcohols is of great interest and use in the current world, especially in the production of biofuels. These alcohols may also be used in downstream processes to produce organic compounds.

15 Numerous conventional methods exist for sustaining microorganism culture that is capable of producing alcohols. However, these methods suffer from numerous inefficiencies. Several of these microorganisms that produce alcohol for example acetogenic bacteria are delicate by nature and susceptible to slight changes in the surrounding conditions in the cultural medium. This reduces the efficiency of production of alcohols from a suitable carbon source. Further, the amount of alcohols
20 produced varies depending on the carbon source. For example, acetogenic bacteria are known to grow and produce alcohols, in particular ethanol, when CO is present in the carbon source. However, in the absence of CO, acetogenic bacteria are not capable of producing alcohols so efficiently. There thus remains a need for additional more effective methods for sustaining acetogenic microorganism cultures in an aqueous medium for producing alcohols when the carbon
25 source lacks CO.

In particular, there remains a need in the art for preserving acetogenic cells in a carbon source deficient in carbon monoxide in suitable conditions so that the bacteria will function at its optimised level. There is thus a need in using a suitable fermentation medium that may be capable of
30 maintaining the efficiency of these acetogenic cells in a CO lacking carbon source in the event of carbon source lacking in CO is to be used.

DESCRIPTION OF THE INVENTION

The present invention attempts to solve the problems above by providing a fermentation medium
35 comprising specific elements at suitable concentrations such that a cell may be able to produce at least one C₁-C₈ alcohol from a carbon source that is carbon monoxide (CO) free and comprises at least carbon dioxide (CO₂) and hydrogen (H₂). This fermentation medium comprising specific components at specific concentrations allow for the cell to produce alcohol, in particular ethanol, efficiently. In particular, the fermentation medium allows for a larger amount of alcohol to be

produced from the same amount of carbon source when the specific component is present at a suitable concentration in the fermentation medium relative to a medium without the specific component at the suitable concentration. The method thus allows for an efficient production of alcohol at a lower cost as more alcohol is produced per gram of the component present in the fermentation medium. The efficiency of production and the cost effectiveness allow for the fermentation medium according to any aspect of the present invention to be preferred over the mediums known in the art. Further, the carbon source used according to any aspect of the present invention may not comprise CO, or comprises CO at undetectable amounts. The specific fermentation medium according to any aspect of the present invention thus allows for a versatile range of substrates to be used that do not necessarily need CO in the substrate medium.

According to one aspect of the present invention, there is provided a fermentation medium comprising:

- (a) Magnesium at a concentration greater than 40mg/L of the fermentation medium,
 - (b) Sulphur at a concentration greater than 85mg/L of the fermentation medium,
 - (c) Aluminium at a detectable concentration,
 - (d) Boron at a detectable concentration,
 - (e) Potassium at a concentration greater than 100mg/L of the fermentation medium,
- or combinations thereof.

According to another aspect of the present invention, there is provided a fermentation medium comprising:

- (c) Boron at a concentration of 0.02 to 1.00mg/L of the fermentation medium.

In particular, boron may be at a concentration of at least 0.5mg/L of the fermentation medium.

In one example, the fermentation medium according to any aspect of the present invention may further comprise at least one of the components selected from the group consisting of:

- (a) Magnesium at a concentration greater than 40mg/L of the fermentation medium,
- (b) Sulphur at a concentration greater than 85mg/L of the fermentation medium,
- (c) Aluminium at a detectable concentration,
- (e) Potassium at a concentration greater than 100mg/L of the fermentation medium, or combinations thereof.

In another example, the fermentation medium according to any aspect of the present invention comprises all the components:

- (a) Magnesium at a concentration greater than 40mg/L of the fermentation medium,
- (b) Sulphur at a concentration greater than 85mg/L of the fermentation medium,
- (c) Aluminium at a detectable concentration, and

- (e) Potassium at a concentration greater than 100mg/L of the fermentation medium.

In particular, the fermentation medium according to any aspect of the present invention may comprise at least one of the components selected from the group consisting of:

- 5 (a) Magnesium at a concentration of 40- 70 mg/L of the fermentation medium,
 (b) Sulphur at a concentration of 85- 110mg/L of the fermentation medium,
 (c) Aluminium at a concentration of 1 to 15µg/L of the fermentation medium, and
 (e) Potassium at a concentration of 100- 300mg/L of the fermentation medium.

- 10 More in particular, the fermentation medium according to any aspect of the present invention may comprise the components:

- (a) Magnesium at a concentration of 40- 70 mg/L of the fermentation medium,
 (b) Sulphur at a concentration of 85- 110mg/L of the fermentation medium,
 (c) Aluminium at a concentration of 1 to 15µg/L of the fermentation medium, and
15 (e) Potassium at a concentration of 100- 300mg/L of the fermentation medium.

In particular, the medium according to any aspect of the present invention may comprise:

- (b) Sulphur at a concentration greater than 85mg/L of the fermentation medium.

- 20 More in particular, the sulphur may be at a concentration of 85-110 mg/L of the fermentation medium.

In one example, the fermentation medium according to any aspect of the present invention comprises at least one of the components selected from the group consisting of:

- 25 (a) Magnesium at a concentration of about 62.5 mg/L of the fermentation medium,
 (b) Sulphur at a concentration of about 100mg/L of the fermentation medium,
 (c) Aluminium at a concentration of about 11 µg/L of the fermentation medium,
 (e) Potassium at a concentration of about 260mg/L of the fermentation medium, or
 combinations thereof.

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In particular, the fermentation medium according to any aspect of the present invention comprises

- (a) Magnesium at a concentration of about 62.5 mg/L of the fermentation medium,
 (b) Sulphur at a concentration of about 100mg/L of the fermentation medium,
 (c) Aluminium at a concentration of about 11 µg/L of the fermentation medium, and
35 (e) Potassium at a concentration of about 260mg/L of the fermentation medium.

In particular, the medium may comprise magnesium at a concentration greater than 40mg/L of the fermentation medium. More in particular, the cell is able to produce at least 1000mg of ethanol per mg of Mg and per gram of cell present in the fermentation medium. The method thus allows for an

efficient production of alcohol at a lower cost as more alcohol is produced per gram of the Mg and per gram of cells present in the fermentation medium when the magnesium is present at a concentration greater than 45mg/L in the fermentation medium relative to a medium without Mg at a concentration greater than 45mg/L. The Mg may be present in the medium at a concentration
5 greater than 40mg/L of the fermentation medium. The term 'greater than 40mg/L' includes 40mg/L. There may be at least 40mg/L of Mg thus present in the fermentation medium.

In one example, the fermentation medium may comprise 40-70mg/L of magnesium. More in particular, the fermentation medium may comprise 45-55, 45-50, 50-60 or 50-65mg/L of
10 magnesium. Even more in particular, the fermentation medium may comprise about 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70 mg/L of magnesium. In one example, the fermentation medium may comprise about 63 mg/L of magnesium.

15 The term 'about' as used herein refers to a variation within 20 percent. In particular, the term "about" as used herein refers to +/- 20%, more in particular, +/-10%, even more in particular, +/- 5% of a given measurement or value.

The source of magnesium may be any magnesium salt known in the art. In particular, the source of
20 magnesium may be selected from the group consisting of magnesium acetate, magnesium aspartate, magnesium bicarbonate, magnesium carbonate, magnesium chloride, magnesium citrate, magnesium diglutamate, magnesium fluoride, magnesium formate, magnesium gluconate, magnesium glycinate, magnesium hydroxide, magnesium iodide, magnesium lactate, magnesium malate, magnesium nitrate, magnesium oxalate, magnesium phosphate, magnesium phosphate
25 tribasic, magnesium sulphate, magnesium sulfide and the like. More in particular, the source of magnesium may be selected from the group consisting magnesium carbonate, magnesium chloride, magnesium phosphate, magnesium sulphate, magnesium sulfide and combinations thereof. Even more in particular, the source of magnesium may be magnesium chloride (MgCl₂) or magnesium sulphate (MgSO₄).

30 In one example, the medium may comprise sulphur (S) at a concentration greater than 85mg/L of the fermentation medium. More in particular, the cell is able to produce at least 618mg of ethanol per mg of S and per gram of cell present in the fermentation medium. The method thus allows for an efficient production of alcohol at a lower cost as more alcohol is produced per gram of S and per
35 gram of cells present in the fermentation medium when the sulphur is present at a concentration greater than 85mg/L in the fermentation medium relative to a medium without S at a concentration greater than 85mg/L.. The S may be present in the medium at a concentration greater than 85mg/L of the fermentation medium. The term 'greater than 85mg/L' includes 85mg/L. There may be at least 85mg/L of S thus present in the fermentation medium.

In one example, the fermentation medium may comprise 85-110mg/L of sulphur. More in particular, the fermentation medium may comprise 85-105, 85-100, 90-110 or 90-100 mg/L of sulphur. Even more in particular, the fermentation medium may comprise about 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110 mg/L of sulphur. In one example, the fermentation medium may comprise about 102 mg/L of sulphur.

The source of sulphur may be a sulphur compound known in the art. In particular, the source of sulphur may be a compound in which sulphur has an oxidation state below 6. More in particular, the source of sulphur may be selected from the group consisting of SO₂, H₂SO₃, Na₂S₂O₄, S₈, Na₂S, NaHS, cysteine, NH₄HSO₃, NaHSO₃ (NH₄)₂SO₃.bisulfites, polysulfides, disulphur dioxide, disulphur monoxide, sulphur dioxide, sulphur monoxide, ammonium sulphite, barium sulphite, beryllium sulphite, calcium sulphite, lithium sulphite, magnesium sulphite, potassium sulphite, silver sulphite, sodium sulphite, sulphurous acid, aluminium sulfide, ammonium hydrosulfide, ammonium sulfide, barium sulfide, boron sulfide, cadmium sulfide, calcium sulfide, carbon disulfide, carbon monosulfide, carbonyl sulfide, chromium(III) sulfide, cobalt sulfide, copper monosulfide, copper sulfide, copper(I) sulfide, iron sulfide, iron(II,III) sulfide, iron(II) sulfide, iron(III) sulfide, lead(II) sulfide, lead(IV) sulfide, magnesium sulfide, manganese(II) sulfide, mercury sulfide, nickel sulfide, phosphorus sulfide, potassium sulfide, sodium hydrosulfide, sodium sulfide, zinc cadmium sulfide, zinc sulfide and the like. In particular, the source of sulphur may be selected from the group consisting of cysteine, sodium sulfide and combinations thereof.

In one example, the medium may comprise aluminium (Al) at a detectable concentration in the fermentation medium. More in particular, the cell is able to produce at least 5525mg of ethanol per µg of Al and per gram of cell present in the fermentation medium. This medium allows for a larger amount of alcohol to be produced from the same amount of carbon source when the aluminium is present at a detectable concentration in the fermentation medium relative to a medium without detectable Al. The Al may be present in the medium at a detectable concentration. The term 'detectable concentration' refers to the concentration of Al that is in the fermentation medium that may be measured using any instrument known in the art. The detectable concentration of Al refers to a concentration that is above 0mg/L that may be the minimum concentration of Al that may be measured in a liquid medium by any method known in the art. In one example, the concentration of Al in a medium may be calculated based on the amount of Al compound that is added to the medium. In another example, the amount of Al in the fermentation medium may be measured using ICP-OES (Inductively Coupled Plasma- Optical Emission Spectrometry). In particular, the detectable concentration of aluminium may at least be 0.0005 mg/L in the fermentation medium.

In one example, the fermentation medium may comprise aluminium at a concentration of 1 to 15 µg/L of the fermentation medium. In particular, the fermentation medium may comprise 1 to 14, 1-13, 1-12, 1-11, 1-10, 2-15, 2-14, 2-13, 2-12, 5-15, 5-14, 1-13, 5-12, 5-11, 5-10 µg/L of Al in the fermentation medium. More in particular, the fermentation medium may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 µg/L of Al in the fermentation medium. In one example, the fermentation medium may comprise about 11 µg/L of Al.

The source of aluminium may be any aluminium salt known in the art. In particular, the source of aluminium may be selected from the group consisting of alumide, aluminium acetate, aluminium acetoacetate, aluminium acetotartrate, aluminium acetylacetonate, aluminium antimonide, aluminium arsenate, aluminium arsenide, aluminium borohydride, aluminium bromide, aluminium carbide, aluminium carbonate, aluminium chloride, aluminium chlorohydrate, aluminium clofibrate, aluminium diacetate, aluminium diboride, aluminium diethyl phosphinate, aluminium dodecaboride, aluminium fluoride, aluminium formate, aluminium gallium arsenide, aluminium gallium indium phosphide, aluminium gallium nitride, aluminium gallium phosphide, aluminium glycinate, aluminium hydride, aluminium hydroxide, aluminium hydroxide oxide, aluminium indium arsenide, aluminium iodide, aluminium isopropoxide, aluminium magnesium boride, aluminium molybdate, aluminium monobromide, aluminium monochloride, aluminium monofluoride, aluminium monoiodide, aluminium monostearate, aluminium nicotinate, aluminium nitrate, aluminium nitride, aluminium oxide, aluminium oxynitride, aluminium phosphate, aluminium phosphide, aluminium selenide, aluminium silicate, aluminium sulphate, aluminium sulfide, aluminium triethoxide, aluminium(I) oxide, aluminium(II) oxide, aluminoxane, ammonium aluminium sulphate, ammonium hexafluoroaluminate and the like.

In one example, the medium may comprise boron (B) at a detectable concentration in the fermentation medium. More in particular, the cell is able to produce at least 12000mg of ethanol per mg of boron and per gram of cell present in the fermentation medium. This medium allows for a larger amount of alcohol to be produced from the same amount of carbon source when the boron is present at a detectable concentration in the fermentation medium relative to a medium without detectable boron. The method thus allows for an efficient production of alcohol at a lower cost as more alcohol is produced per gram of the boron present in the fermentation medium. The boron may be present in the medium at a detectable concentration. The term 'detectable concentration' refers to the concentration of boron that is in the fermentation medium that may be measured using any instrument known in the art. The detectable concentration of boron refers to a concentration that is above 0mg/L that may be the minimum concentration of boron that may be measured in a liquid medium by any method known in the art. In one example, the concentration of boron in a medium may be calculated based on the amount of boron compound that is added to the medium. In another example, the amount of boron in the fermentation medium may be measured using ICP-

OES (Inductively Coupled Plasma- Optical Emission Spectrometry). In particular, the detectable concentration of boron may at least be 0.0005 mg/L in the fermentation medium.

In one example, the fermentation medium may comprise boron at a concentration of 0.02 to 1.00mg/L or 0.05 to 1.00mg/L of the fermentation medium. In particular, the fermentation medium may comprise 0.10-1.00, 0.15-1.00, 0.20-1.00, 0.25-1.00, 0.30-1.00, 0.35-1.00, 0.40-1.00, 0.45-1.00, 0.50-1.00, 0.55-1.00, 0.60-1.00, 0.20-0.90, 0.20-0.80, 0.20-0.70, 0.30-0.90 mg/L of boron in the fermentation medium. More in particular, the fermentation medium may comprise 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.55, 0.60, 0.65, 0.70, 0.75, 0.80, 0.85, 0.90, 0.95, 1.00 mg/L of boron in the fermentation medium. In one example, the fermentation medium may comprise about 0.5 mg/L of boron.

The source of boron may be any boron salt known in the art. In particular, the source of boron may be selected from the group consisting of, boron monofluoride, boron monoxide, , boron oxide, boron phosphate, boron suboxide, boron sulfide, boron tribromide, boron trichloride, boron trifluoride, boron triiodide, boron trioxide, sodium borate, boric acid, sodium tetraborate decahydrate (borax) and the like.

In one example, the fermentation medium may further comprise at least potassium. In particular, the fermentation medium may comprise potassium at a concentration greater than 100mg/L of the fermentation medium. More in particular, the cell is able to produce at least 240mg of ethanol per mg of potassium and per gram of cell present in the fermentation medium. The method thus allows for an efficient production of alcohol at a lower cost as more alcohol is produced per gram of the potassium and per gram of cells present in the fermentation medium when the potassium is present at a concentration greater than 100mg/L in the fermentation medium relative to a medium with less than 100mg/L potassium.. The potassium may be present in the medium at a concentration of 85 mg/L to 300 mg/L of potassium. More in particular, the fermentation medium may comprise 90 mg/L to 300 mg/L, 100 mg/L to 300 mg/L, , 110 mg/L to 300 mg/L, 120 mg/L to 300 mg/L, 130 mg/L to 300 mg/L, 140 mg/L to 300 mg/L, 150 mg/L to 300 mg/L, 160 mg/L to 300 mg/L, 180 mg/L to 300 mg/L, 190 mg/L to 200 mg/L, 210 mg/L to 300 mg/L, 220 mg/L to 300 mg/L, 230mg/L to 300 mg/L, 240 mg/L to 300 mg/L, 250 mg/L to 300 mg/L, 100 mg/L to 270 mg/L of potassium. Even more in particular, the fermentation medium may comprise about 200, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300 mg/L of potassium. In one example, the fermentation medium may comprise about 262 mg/L of potassium.

The source of potassium may be any potassium salt known in the art. In particular, the source of potassium may be selected from the group consisting of Potassium Permanganate (KMnO_4),

Potassium Hypochlorite (KClO_9), Potassium Phosphate (K_3PO_4), Potassium Oxalate ($\text{K}_2\text{C}_2\text{O}_4$), Potassium Chromate (K_2CrO_4), Potassium Hydrogen Phthalate ($\text{KHC}_8\text{H}_4\text{O}_4$), Potassium Dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$), Potassium Chloride (KCl), Potassium Nitrate (KNO_3), Potassium Carbonate (K_2CO_3), Potassium Hydroxide (KOH), Potassium Sulphate (K_2SO_4), Potassium Hydrogen Phosphate (K_2HPO_4), Potassium Hydrogen Carbonate (KHCO_3), Potassium Iodide (KI), Monopotassium Phosphate (KH_2PO_4), Potassium Chlorate (KClO_3), Potassium Hypiodite (KIO), Potassium Oxide (K_2O), Potassium Hydrogen Sulphate (KHSO_4), Potassium Bromite (KBrO_2), Potassium Perchlorate (KClO_4), Potassium Bromide (KBr), Potassium Hypobromite (KBrO), Potassium Perbromate (KBrO_4), Potassium Sulfite (K_2SO_3), Potassium Phosphide (K_3P), Potassium Sulfide (K_2S), Potassium Acetate (KCH_3COO), Potassium Dihydrogen Phosphite (KH_2PO_3), Potassium Iodite (KIO_2) and the like. More in particular, the source of potassium may be KCl .

The fermentation medium may comprise any one of the elements boron, potassium, aluminium, sulphur, magnesium at the concentrations mentioned above. In particular, the fermentation medium according to any aspect of the present invention may comprise combination of 1, 2, 3, 4 or 5 of the elements selected from the group consisting of boron, potassium, aluminium, sulphur, magnesium.

In one example, the fermentation medium according to any aspect of the present invention may comprise a combination of at least two of the elements boron, potassium, aluminium, sulphur, magnesium at the concentrations mentioned above. In particular, the fermentation medium may comprise a combination of two elements selected from the group consisting of boron and potassium; boron and aluminium; boron and sulphur; boron and magnesium; potassium and aluminium; potassium and sulphur; potassium and magnesium; aluminium and sulphur; aluminium and magnesium and sulphur and magnesium.

In another example, the fermentation medium according to any aspect of the present invention may comprise a combination of at least three of the elements boron, potassium, aluminium, sulphur, magnesium at the concentrations mentioned above. In particular, the fermentation medium may comprise a combination of three elements selected from the group consisting of boron, potassium and aluminium; boron, potassium and sulphur; boron, potassium and magnesium; boron, aluminium and sulphur, boron, aluminium and magnesium; boron, sulphur and magnesium; aluminium, sulphur and potassium; aluminium, sulphur and magnesium; aluminium, potassium and magnesium; aluminium, potassium and sulphur; and sulphur, potassium and magnesium.

In a further example, the fermentation medium according to any aspect of the present invention may comprise a combination of at least four of the elements boron, potassium, aluminium, sulphur, magnesium at the concentrations mentioned above. In particular, the fermentation medium may comprise a combination of four elements selected from the group consisting of boron, aluminium,

sulphur and potassium; boron, aluminium, sulphur and magnesium; boron, sulphur, potassium and magnesium; and aluminium, sulphur, potassium and magnesium.

In yet another example, the fermentation medium according to any aspect of the present invention may comprise all five elements boron, potassium, aluminium, sulphur, magnesium at the concentrations mentioned above.

The term 'combinations thereof' as used herein, refers to a combination of 1, 2, 3, 4 or 5 of the elements selected from the group consisting of boron, potassium, aluminium, sulphur, magnesium in the specific concentrations mentioned above.

In particular, the fermentation medium according to any aspect of the present invention may comprise

- (a) Magnesium at a concentration greater than 40mg/L of the fermentation medium,
- (b) Sulphur at a concentration greater than 85mg/L of the fermentation medium,
- (c) Aluminium at a detectable concentration,
- (d) Boron at a detectable concentration, and
- (e) Potassium at a concentration greater than 100mg/L of the fermentation medium.

More in particular, the fermentation medium according to any aspect of the present invention may comprise

- (a) Magnesium at a concentration of 40- 70 mg/L of the fermentation medium,
- (b) Sulphur at a concentration of 85- 110mg/L of the fermentation medium,
- (c) Aluminium at a concentration of 1 to 15µg/L of the fermentation medium,
- (d) Boron at a concentration of 0.05 to 1.00mg/L of the fermentation medium,
- (e) Potassium at a concentration of 100- 300mg/L of the fermentation medium, or combinations thereof.

Even more in particular, the fermentation medium according to any aspect of the present invention may comprise:

- (a) Magnesium at a concentration of 40- 70 mg/L of the fermentation medium,
- (b) Sulphur at a concentration of 85- 110mg/L of the fermentation medium,
- (c) Aluminium at a concentration of 1 to 15µg/L of the fermentation medium,
- (d) Boron at a concentration of 0.05 to 1.00mg/L of the fermentation medium, and
- (e) Potassium at a concentration of 100- 300mg/L of the fermentation medium.

The fermentation medium to any aspect of the present invention may comprise:

- (a) Magnesium at a concentration of about 62.5 mg/L of the fermentation medium,
- (b) Sulphur at a concentration of about 100mg/L of the fermentation medium,

- (c) Aluminium at a concentration of about 11 µg/L of the fermentation medium,
- (d) Boron at a concentration of 0.5mg/L of the fermentation medium,
- (e) Potassium at a concentration of about 260mg/L of the fermentation medium, or combinations thereof.

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In particular, the fermentation medium according to any aspect of the present invention may comprise

- (a) Magnesium at a concentration of about 62.5 mg/L of the fermentation medium,
- (b) Sulphur at a concentration of about 100mg/L of the fermentation medium,
- 10 (c) Aluminium at a concentration of about 11 µg/L of the fermentation medium,
- (d) Boron at a concentration of 0.5mg/L of the fermentation medium, and
- (e) Potassium at a concentration of about 260mg/L of the fermentation medium.

15 In one example, the fermentation medium may further comprise calcium. The fermentation medium may comprise 10 mg/L to 70 mg/L of calcium. More in particular, the fermentation medium may comprise 30-40mg/L. Even more in particular, the fermentation medium according to any aspect of the present invention may comprise about 35, 36, 37, 38, or 39 mg/L of calcium.

20 In another example, the fermentation medium may further comprise iron. The fermentation medium may comprise 2 mg/L to 5 mg/L of iron. More in particular, the fermentation medium may comprise 3-5mg/L of iron. Even more in particular, the fermentation medium according to any aspect of the present invention may comprise about 3, 4.5, or 5 mg/L of iron.

25 In yet another example, the fermentation medium according to any aspect of the present invention may comprise both calcium and iron.

30 In one example, the fermentation medium may further comprise cobalt. The fermentation medium may comprise 480 µg/L to 500 µg/L of cobalt. More in particular, the fermentation medium may comprise 490-500 µg/L of cobalt. Even more in particular, the fermentation medium according to any aspect of the present invention may comprise about 495, 495.5 or 496 µg/L of cobalt.

35 In one example, the fermentation medium may further comprise nickel. The fermentation medium may comprise 40 µg/L to 55 µg/L of nickel. More in particular, the fermentation medium may comprise 45-50 µg/L of nickel. Even more in particular, the fermentation medium according to any aspect of the present invention may comprise about 49, 49.5 or 50 µg/L of nickel.

In one example, the fermentation medium may further comprise calcium. The fermentation medium may comprise 30 mg/L to 50 mg/L of calcium. More in particular, the fermentation medium may comprise 35-40 mg/L of calcium. Even more in particular, the fermentation medium according to any aspect of the present invention may comprise about 35, 36 or 37
5 mg/L of calcium.

In one example, the fermentation medium may further comprise manganese. The fermentation medium may comprise 0.5 mg/L to 2.5 mg/L of manganese. More in particular, the fermentation medium may comprise 1.0-2.0 mg/L of manganese. Even more in
10 particular, the fermentation medium according to any aspect of the present invention may comprise about 1.5, 1.6, 1.8 mg/L of manganese.

In one example, the fermentation medium may further comprise zinc. The fermentation medium may comprise 0.3 mg/L to 0.5 mg/L of zinc. More in particular, the fermentation
15 medium may comprise 0.45 mg/L of zinc.

In one example, the fermentation medium may further comprise molybdenum. The fermentation medium may comprise 100-200 µg/L of molybdenum. More in particular, the fermentation medium may comprise 110-120 µg/L of molybdenum. Even more in particular, the fermentation medium
20 according to any aspect of the present invention may comprise about 118, 119, or 120 µg/L of molybdenum.

In one example, the fermentation medium may further comprise selenium. The fermentation medium may comprise 85-110 µg/L of selenium. More in particular, the fermentation medium may
25 comprise 90-100 µg/L of selenium. Even more in particular, the fermentation medium according to any aspect of the present invention may comprise about 90, 91, or 92 µg/L of selenium.

In one example, the fermentation medium may further comprise tungsten. The fermentation medium may comprise 100-120 µg/L of tungsten. More in particular, the fermentation medium may
30 comprise 110-115 µg/L of tungsten. Even more in particular, the fermentation medium according to any aspect of the present invention may comprise about 111, 112, or 113 µg/L of tungsten.

The fermentation medium according to any aspect of the present invention may comprise any element selected from the group consisting of magnesium, sulphur, aluminium, boron, potassium,
35 cobalt, nickel, calcium, manganese, iron, zinc, molybdenum, selenium, and tungsten. In particular, the fermentation medium according to any aspect of the present invention may comprise at least one element selected from the group consisting of magnesium, sulphur, aluminium, boron and potassium. The fermentation medium according to any aspect of the present invention may comprise a mixture of any one of the elements selected from the group consisting of magnesium,

sulphur, aluminium, boron, potassium, cobalt, nickel, calcium, manganese, iron, zinc, molybdenum, selenium, and tungsten.

- In one example, the fermentation medium according to any aspect of the present invention may
5 comprise the following elements in the following concentrations as shown in Table 1.

Elements	Concentration	Unit
Mg	62.7	mg/L
Co	495.4	µg/L
Ni	49.4	µg/L
K	262.2	mg/L
Ca	36.8	mg/L
Mn	1.6	mg/L
Fe	4.8	mg/L
Zn	0.45	mg/L
Mo	119.0	µg/L
Se	91.3	µg/L
W	111.5	µg/L
S	101.7	mg/L
B	0.52	mg/L
Al	11.4	µg/L

Table 1. Elements and concentrations thereof in one example of the fermentation medium according to any aspect of the present invention.

In one example, the sources of the elements may be according to Table 2.

Compound	Concentration	
MgCl ₂ x 6 H ₂ O	0.5	g/L
MgSO ₄ x 7 H ₂ O	0.03	g/L
CoCl ₂ x 6 H ₂ O	2	mg/L
NiCl ₂ x 6 H ₂ O	0.2	mg/L
KCl	0.5	g/L
CaCl ₂ x 2 H ₂ O	0.135	g/L
MnSO ₄ x H ₂ O	5	mg/L
FeCl ₃	10	mg/L
FeSO ₄ x 7 H ₂ O	1	mg/L
Fe(SO ₄) ₂ (NH ₄) ₂ x 6 H ₂ O	8	mg/L
ZnSO ₄ x 7 H ₂ O	2	mg/L
Na ₂ MoO ₄ x 2 H ₂ O	0.3	mg/L
Na ₂ SeO ₃	0.2	mg/L

Na ₂ WO ₄ x 2 H ₂ O	0.2	mg/L
L-Cystein HCl	500	mg/L
H ₃ BO ₃	3.0	mg/L
AlK(SO ₄) ₂ x 12 H ₂ O	0.2	mg/L

Table 2. Specific compounds of the elements and concentrations thereof in one example of the fermentation medium according to any aspect of the present invention.

In yet another example, the fermentation medium according to any aspect of the present invention may comprise the following elements in the following concentrations as shown in Table 3.

Elements	Concentration	Unit
Mg	48.6	mg/L
Co	117.9	µg/L
Ni	293.5	µg/L
K	977.5	mg/L
Ca	80.2	mg/L
Mn	0.11	mg/L
Fe	5.6	mg/L
Zn	0.33	mg/L
Mo	191.9	µg/L
Se	157.9	µg/L
W	367.7	µg/L
S	101.7	mg/L
B	0.02	mg/L

Table 3. Elements and concentrations thereof in another example of the fermentation medium according to any aspect of the present invention.

In one example, the sources of the elements may be according to Table 4.

Compound	Concentration	
MgCl ₂ x 6 H ₂ O	0.41	g/L
CoCl ₂ x 6 H ₂ O	0.48	mg/L
NiCl ₂ x 6 H ₂ O	1.19	mg/L
KCl	1.86	g/L
CaCl ₂ x 2 H ₂ O	0.29	g/L
MnCl ₂ x 4 H ₂ O	0.4	mg/L
FeCl ₂ x 4 H ₂ O	19.9	mg/L
ZnCl ₂	0.68	mg/L
Na ₂ MoO ₄ x 2 H ₂ O	0.48	mg/L
Na ₂ SeO ₃	0.35	mg/L

Na ₂ WO ₄ x 2 H ₂ O	0.66	mg/L
L-Cystein HCl	500	mg/L
H ₃ BO ₃	0.12	mg/L

Table 4. Specific compounds of the elements and concentrations thereof in another example of the fermentation medium according to any aspect of the present invention.

According to a further aspect of the present invention there is provided a method of producing at least one C₁ to C₈ alcohol from a carbon source in a fermentation medium, wherein the method comprises,

- contacting at least one acetogenic cell to the carbon source,

wherein the fermentation medium comprises:

- (a) Magnesium at a concentration greater than 40mg/L of the fermentation medium, or
- (b) Sulphur at a concentration greater than 85mg/L of the fermentation medium, or
- (c) Aluminium at a detectable concentration, or
- (d) Boron at a detectable concentration, or
- (e) Potassium at a concentration greater than 100mg/L of the fermentation medium, or combinations thereof, and

the carbon source is carbon monoxide free and comprises hydrogen and carbon dioxide.

In a further aspect, there is provided a method of producing at least one C₁ to C₈ alcohol from a carbon source in the fermentation medium according to any aspect of the present invention wherein the method comprises,

- contacting at least one acetogenic cell to the carbon source, and

the carbon source is carbon monoxide free and comprises hydrogen and carbon dioxide.

The term "acetogenic bacteria" as used herein refers to a microorganism which is able to perform the Wood-Ljungdahl pathway and thus is able to convert CO, CO₂ and/or hydrogen to acetate.

These microorganisms include microorganisms which in their wild-type form do not have a Wood-Ljungdahl pathway, but have acquired this trait as a result of genetic modification. Such microorganisms include but are not limited to E. coli cells. These microorganisms may be also known as carboxydophilic bacteria. Currently, 21 different genera of the acetogenic bacteria are known in the art (Drake et al., 2006), and these may also include some clostridia (Drake & Kusel, 2005). These bacteria are able to use carbon dioxide or carbon monoxide as a carbon source with hydrogen as an energy source (Wood, 1991). Further, alcohols, aldehydes, carboxylic acids as well as numerous hexoses may also be used as a carbon source (Drake et al., 2004). The reductive pathway that leads to the formation of acetate is referred to as acetyl-CoA or Wood-Ljungdahl pathway.

In particular, the acetogenic bacteria may be selected from the group consisting of *Acetoanaerobium* sp., *Acetonema* sp., *Acetobacterium* sp., *Alkalibaculum* sp., *Archaeoglobus* sp., *Blautia* sp., *Butyribacterium* sp., *Clostridium* sp., *Desulfotomaculum* sp., *Eubacterium* sp., *Methanosarcina* sp., *Moorella* sp., *Oxobacter* sp., *Sporomusa* sp., *Thermoanaerobacter* sp. and the like. More in particular, the acetogenic bacteria may be selected from the group consisting of *Acetoanaerobium notera* (ATCC 35199), *Acetonema longum* (DSM 6540), *Acetobacterium carbinolicum* (DSM 2925), *Acetobacterium malicum* (DSM 4132), *Acetobacterium species* no. 446 (Morinaga et al., 1990, J. Biotechnol., Vol. 14, p. 187-194), *Acetobacterium wieringae* (DSM 1911), *Acetobacterium woodii* (DSM 1030), *Alkalibaculum bacchi* (DSM 22112), *Archaeoglobus fulgidus* (DSM 4304), *Blautia producta* (DSM 2950, formerly *Ruminococcus productus*, formerly *Peptostreptococcus productus*), *Butyribacterium methylotrophicum* (DSM 3468), *Clostridium aceticum* (DSM 1496), *Clostridium autoethanogenum* (DSM 10061, DSM 19630 and DSM 23693), *Clostridium carboxidivorans* (DSM 15243), *Clostridium coskatii* (ATCC no. PTA-10522), *Clostridium drakei* (ATCC BA-623), *Clostridium formicoaceticum* (DSM 92), *Clostridium glycolicum* (DSM 1288), *Clostridium ljungdahlii* (DSM 13528), *Clostridium ljungdahlii* C-01 (ATCC 55988), *Clostridium ljungdahlii* ERI-2 (ATCC 55380), *Clostridium ljungdahlii* O-52 (ATCC 55989), *Clostridium mayombeii* (DSM 6539), *Clostridium methoxybenzovorans* (DSM 12182), *Clostridium ragsdalei* (DSM 15248), *Clostridium scatologenes* (DSM 757), *Clostridium species* ATCC 29797 (Schmidt et al., 1986, Chem. Eng. Commun., Vol. 45, p. 61-73), *Desulfotomaculum kuznetsovii* (DSM 6115), *Desulfotomaculum thermobezoicum* subsp. *thermosyntrophicum* (DSM 14055), *Eubacterium limosum* (DSM 20543), *Methanosarcina acetivorans* C2A (DSM 2834), *Moorella* sp. HUC22-1 (Sakai et al., 2004, Biotechnol. Lett., Vol. 29, p. 1607-1612), *Moorella thermoacetica* (DSM 521, formerly *Clostridium thermoaceticum*), *Moorella thermoautotrophica* (DSM 1974), *Oxobacter pfennigii* (DSM 322), *Sporomusa aerivorans* (DSM 13326), *Sporomusa ovata* (DSM 2662), *Sporomusa silvacetica* (DSM 10669), *Sporomusa sphaeroides* (DSM 2875), *Sporomusa termitida* (DSM 4440) and *Thermoanaerobacter kivui* (DSM 2030, formerly *Acetogenium kivui*).

More in particular, the acetogenic bacteria may be selected from the group consisting of *Acetobacterium woodii*, *Alkalibaculum bacchi*, *Blautia producta*, *Clostridium aceticum*, *Clostridium autoethanogenum*, *Clostridium carboxidivorans*, *Clostridium drakei*, *Clostridium formicoaceticum*, *Clostridium ljungdahlii*, *Clostridium magnum*, *Butyribacterium methylotrophicum*, *Clostridium scatologenes*, *Eubacterium limosum*, *Moorella thermoacetica*, *Sporomusa ovate*, *Sporomusa silvacetica*, *Sporomusa sphaeroides*, *Oxobacter pfennigii*, and *Thermoanaerobacter kivui*. More in particular, the acetogenic bacteria may be selected from the group consisting of *Clostridium autoethanogenum* and *Clostridium ljungdahlii*. Even more in particular, the acetogenic bacteria may be *Clostridium autoethanogenum*.

The term "fermentation" as used herein refers to a process for the production of one of more alcohols by the anaerobic metabolism of the acetogenic bacterium in a medium suitable for the

growth of the bacterium. This medium suitable for the growth of the bacterium refers to a fermentation medium comprising the ingredients necessary for the anaerobic bacterial growth and production of the alcohol. The medium will usually include carbon, nitrogen, phosphorus and sulphur sources, nutrients, trace elements, salts vitamins and so forth. In particular, the carbon source for fermentation by acetogenic bacteria for the production of alcohol usually requires the presence of CO as the carbon source. However, the inventors have surprisingly found that where the carbon source in the fermentation medium is carbon monoxide free and comprises hydrogen and carbon dioxide, varying the fermentation medium to comprise at least one ingredient at a specific concentration may enable the acetogenic bacteria to produce at least one alcohol from the carbon source. In particular, the fermentation medium according to any aspect of the present invention may comprise magnesium, potassium, sulphur, aluminium and/or boron at suitable concentrations as mentioned above.

The fermentation may be carried out under suitable conditions. The term "under suitable conditions" as used herein refers to the physical and chemical parameters of the fermentation medium necessary for the growth of the acetogenic bacteria and/or production of the desired alcohol, comprising pH, temperature, salinity, pressure, dissolved oxygen concentration, nitrogen requirements and substrate, nutrient and trace element concentrations and the like.

A skilled person would understand the suitable conditions necessary to carry out the method according to any aspect of the present invention. In particular, the conditions in the container (e.g. fermenter) may be varied depending on the acetogenic bacteria used. The varying of the conditions to be suitable for the optimal functioning of the microorganisms is within the knowledge of a skilled person.

In one example, the method according to any aspect of the present invention may be carried out in an aqueous medium with a pH between 5 and 8, 5.5 and 7. The pressure may be between 1 and 10 bar.

The alcohol produced according to any aspect of the present invention may be at least one C₁-C₈ alcohol. In particular, the alcohol may be selected from the group consisting of methanol, ethanol, propanol, butanol, pentanol, hexanol, heptanol, octanol and combinations thereof. More in particular, the alcohol may be ethanol, butanol or combinations thereof.

The carbon source used according to any aspect of the present invention may be CO free. In particular, the carbon source comprises carbon dioxide. A skilled person would understand that many possible sources for the provision of CO₂ as a carbon source exist. It can be seen that in practice, as the carbon source according to any aspect of the present invention any gas or any gas

mixture can be used which is able to supply the microorganisms with sufficient amounts of carbon, so that at least one desired alcohol, may be formed from the source of CO₂.

5 Generally, the carbon source comprises at least 50% by volume, at least 70% by volume, particularly at least 90% by volume of CO₂, wherein the percentages by volume - % relate to all carbon sources that are available to the acetogenic bacteria in the fermentation medium.

10 Examples of carbon sources in gas forms include exhaust gases such as synthesis gas, flue gas and petroleum refinery gases produced by yeast fermentation or clostridial fermentation. These exhaust gases are formed from the gasification of cellulose-containing materials or coal gasification. In one example, these exhaust gases may not necessarily be produced as by-products of other processes but can specifically be produced for use with the mixed culture according to any aspect of the present invention.

15 According to any aspect of the present invention, the carbon source may be waste gases comprising at least CO₂. CO₂ can for example be produced as a by-product of coal gasification. Accordingly, the acetogenic cell according to any aspect of the present invention may be capable of converting a substance which is a waste product into a valuable resource. In another example, CO₂ may be a by-product of gasification of widely available, low-cost agricultural raw materials for
20 use with the mixed culture of the present invention to produce at least one alcohol.

There are numerous examples of raw materials that can be converted into CO₂, as almost all forms of vegetation can be used for this purpose. In particular, raw materials are selected from the group consisting of perennial grasses such as miscanthus, corn residues, processing waste such as
25 sawdust and the like.

In general, CO₂ may be obtained in a gasification apparatus of dried biomass, mainly through pyrolysis, partial oxidation and steam reforming.

30 Mixtures of sources can be used as a carbon source.

According to any aspect of the present invention, a reducing agent, for example hydrogen may be supplied together with the carbon source. In particular, this hydrogen may be supplied when the C and/or CO₂ is supplied and/or used. In one example, the hydrogen gas is part of the gas present
35 according to any aspect of the present invention. In another example, where the hydrogen gas in the gas is insufficient for the method according to any aspect of the present invention, additional hydrogen gas may be supplied.

A 'CO free carbon source' as used herein refers to a carbon substrate that may comprise carbon in any form that may be used as a substrate outside of CO. In particular, the carbon source comprises none or an undetectable concentration of CO. The concentration of CO in the carbon substrate or fermentation medium may be measured using any method known in the art. In particular, the method for measuring CO may be selected from the group consisting of gas chromatography, mass spectrometry, Orsat chemical analysis, infrared and electrochemical analysis. Typically, methods for on-line analysis of CO may use infrared adsorption. The carbon source used according to any aspect of the present invention may be considered CO free as there may be no detectable amount of CO in the medium.

In particular, the aqueous medium may comprise a carbon source comprising CO₂. More in particular, the carbon source comprising CO₂ is provided to the aqueous medium in a continuous gas flow. In one example, the gases are part of the same flow/stream. In another example, each gas is a separate flow/stream provided to the aqueous medium. These gases may be divided for example using separate nozzles that open up into the aqueous medium, frits, membranes within the pipe supplying the gas into the aqueous medium and the like.

The term "contacting", as used herein, means bringing about direct contact between the acetogenic bacteria according to any aspect of the present invention and the carbon source. For example, the cell in the fermentation medium and the carbon source may be in different compartments. In particular, the carbon source may be in a gaseous state and added to the fermentation medium comprising the cells according to any aspect of the present invention.

According to yet another aspect of the present invention, there is provided a use of a fermentation medium according to any aspect of the present invention in a method for producing at least one C₁ to C₈ alcohol from a carbon source, wherein the carbon source is carbon monoxide free and comprises hydrogen and carbon dioxide.

EXAMPLES

The foregoing describes preferred embodiments, which, as will be understood by those skilled in the art, may be subject to variations or modifications in design, construction or operation without departing from the scope of the claims. These variations, for instance, are intended to be covered by the scope of the claims.

Example 1

Production of ethanol by Clostridium autoethanogenum on synthesis gas without carbon monoxide

For the biotransformation of hydrogen and carbon dioxide to ethanol the homoacetogenic bacterium *Clostridium autoethanogenum* was cultivated on synthesis gas without carbon

monoxide. All cultivation steps were carried out under anaerobic conditions in pressure-resistant glass bottles that were closed airtight with butyl rubber stoppers.

For the pre-culture 500 ml medium (ATCC 1754, pH = 6.0, 20 g L⁻¹ MES, 1 g L⁻¹ yeast extract, 0.8 g L⁻¹ NaCl, 1 g L⁻¹ NH₄Cl, 0.1 g L⁻¹ KCl, 0.1 g L⁻¹ KH₂PO₄, 0.2 g L⁻¹ MgSO₄ x 7 H₂O, 0.02 g L⁻¹ CaCl₂ x 2 H₂O, 20 mg L⁻¹ nitrilotriacetic acid, 10 mg L⁻¹ MnSO₄ x H₂O, 8 mg L⁻¹ (NH₄)₂Fe(SO₄)₂ x 6 H₂O, 2 mg L⁻¹ CoCl₂ x 6 H₂O, 2 mg L⁻¹ ZnSO₄ x 7 H₂O, 0.2 mg L⁻¹ CuCl₂ x 2 H₂O, 0.2 mg L⁻¹ Na₂MoO₄ x 2 H₂O, 0.2 mg L⁻¹ NiCl₂ x 6 H₂O, 0.2 mg L⁻¹ Na₂SeO₄, 0.2 mg L⁻¹ Na₂WO₄ x 2 H₂O, 20 µg L⁻¹ d-biotin, 20 µg L⁻¹ folic acid, 100 µg L⁻¹ pyridoxine-HCl, 50 µg L⁻¹ thiamine-HCl x H₂O, 50 µg L⁻¹ riboflavin, 50 µg L⁻¹ nicotinic acid, 50 µg L⁻¹ Ca-pantothenate, 1 µg L⁻¹ vitamin B₁₂, 50 µg L⁻¹ p-aminobenzoic acid, 50 µg L⁻¹ lipoic acid, approx. 67.5 mg L⁻¹ NaOH) with additional 400 mg L⁻¹ L-cysteine-hydrochloride and 400 mg L⁻¹ Na₂S x 9 H₂O were inoculated with 5 mL of a frozen cryo stock of *C. autoethanogenum*. The autotrophic cultivation was carried out in a 1L pressure-resistant glass bottle at 37°C, 100 rpm and a gassing rate of 3 L h⁻¹ with a synthesis gas consisting of 67% H₂ and 33% CO₂ in an open water bath shaker Innova 3100 from New Brunswick for 71 h. The gas was dispersed into the medium through a microbubble sparger with a pore size of 10 µm, which was mounted in the center of the reactors. The experiment was carried out without control of pH.

After the pre-cultivation, adequate amounts of cell suspension was centrifuged (10 min, 4200 rpm) in order to reach an OD_{600nm} of 0.2 in the main cultivation in 500 mL medium. The pellets were resuspended in fresh medium and transferred into the main cultivation vessels. A first main culture was carried out in medium ATCC 1754 with additional 400 mg L⁻¹ L-cysteine-hydrochloride and a second main culture was carried out in defined (mineral) medium 1 with additional 500 mg L⁻¹ L-cysteine-hydrochloride. The medium 1 is composed as follows (pH = 6.5) 1.3 g L⁻¹ KOH, 0.5 g L⁻¹ MgCl₂, 0.21 g L⁻¹ NaCl, 0.135 g L⁻¹ CaCl₂ x 2H₂O, 2.65 g L⁻¹ NaH₂PO₄ x 2H₂O, 0.5 g L⁻¹ KCl, 2.5 g L⁻¹ NH₄Cl, 15 mg L⁻¹ nitrilotriacetic acid, 30 mg L⁻¹ MgSO₄ x 7 H₂O, 5 mg L⁻¹ MnSO₄ x H₂O, 1 mg L⁻¹ FeSO₄ x 7 H₂O, 8 mg L⁻¹ Fe(SO₄)₂(NH₄)₂ x 6 H₂O, 2 mg L⁻¹ CoCl₂ x 6 H₂O, 2 mg L⁻¹ ZnSO₄ x 7 H₂O, 200 µg L⁻¹ CuCl₂ x 2 H₂O, 200 µg L⁻¹ KAl(SO₄)₂ x 12 H₂O, 3 mg L⁻¹ H₃BO₃, 300 µg L⁻¹ Na₂MoO₄ x 2 H₂O, 200 µg L⁻¹ Na₂SeO₃, 200 µg L⁻¹ NiCl₂ x 6 H₂O, 200 µg L⁻¹ Na₂WO₄ x 2 H₂O, 200 µg L⁻¹ d-biotin, 200 µg L⁻¹ folic acid, 100 µg L⁻¹ pyridoxine-HCl, 500 µg L⁻¹ thiamine-HCl, 500 µg L⁻¹ riboflavin, 500 µg L⁻¹ nicotinic acid, 500 µg L⁻¹ Ca-pantothenate, 500 µg L⁻¹ vitamin B₁₂, 500 µg L⁻¹ p-aminobenzoate, 500 µg L⁻¹ lipoic acid, 10 mg L⁻¹ FeCl₃ with additional 500 mg L⁻¹ L-cysteine-hydrochloride.

The autotrophic cultivations were carried out in a 1 L pressure-resistant glass bottle at 37°C, 150 min⁻¹ and a gassing rate of 1 L h⁻¹ with synthesis gas consisting of 67% H₂ and 33% CO₂ in an open water bath shaker Innova 3100 from New Brunswick for 162 h. The gas was dispersed into the medium through a microbubble sparger with a pore size of 10 µm, which was mounted in the center of the reactors. The pH was held constant between 4.5 and 6.5 by discontinuous additions

of a 100 g L⁻¹ NaOH solution (anaerobic). During the autotrophic cultivations, 5 mL samples were taken to determinate OD_{600nm}, pH und product formation. The determination of the product concentrations was performed by semi-quantitative ¹H-NMR spectroscopy. As an internal quantification standard sodium trimethylsilylpropionate (T(M)SP) was used.

5

During the cultivation in medium ATCC 1754 the concentration of ethanol only increased from 4 to 180 mg L⁻¹. In the defined medium 1, the concentration of ethanol increased during the same time from 1 to 3750 mg L⁻¹. Meanwhile, the concentration of acetate increased from 29 to 18400 mg L⁻¹ in medium ATCC 1754 and from 26 to 6950 mg L⁻¹ in medium 1. The average cell dry weight during the autotrophic cultivations was 0.26 g L⁻¹ in the medium ATCC 1754 and 0.06 g L⁻¹ in the medium 1.

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In the following, calculations were done for ethanol production on H₂/CO₂ without CO in relation to the consumption of dedicated medium components.

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In the case of magnesium, 1002 mg_(ethanol) mg_(magnesium)⁻¹ g_(cell dry weight)⁻¹ were produced in the medium 1, what is considerably more than 35 mg_(ethanol) mg_(magnesium)⁻¹ g_(cell dry weight)⁻¹ in the ATCC 1754.

Example 2

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Production of ethanol by Clostridium autoethanogenum on synthesis gas without boron

For the biotransformation of hydrogen, carbon monoxide and carbon dioxide to ethanol the homoacetogenic bacterium *Clostridium autoethanogenum* was cultivated on synthesis gas. All cultivation steps were carried out under anaerobic conditions in pressure-resistant glass bottles that can be closed airtight with a butyl rubber stopper.

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For the preculture 400 ml medium 2 (pH = 5.8; 0.407 g/L MgCl₂ * 6 H₂O, 0.117 g/L NaCl, 0.294 g/L CaCl₂ * 2 H₂O, 1.864 g/L KCl, 0.375 ml/L H₃PO₄, 19.8 mg/L FeCl₂ x 4 H₂O, 0.5 g/L cysteine-HCl, 3.92 g/L NH₄-acetate, 0.396 mg/L MnCl₂ x 4 H₂O, 0.476 mg/L CoCl₂ x 6 H₂O, 0.682 mg/L ZnCl₂, 0.124 mg/L H₃BO₃, 0.484 mg/L Na₂MoO₄ x 2 H₂O, 0.346 mg/L Na₂SeO₃ * 5 H₂O, 1.189 mg/L NiCl₂ x 6 H₂O, 0.660 mg/L Na₂WO₄ x 2 H₂O, 20 µg/L d-biotin, 20 µg/L folic acid, 10 µg/L pyridoxine-HCl, 50 µg/L thiamine-HCl x H₂O, 50 µg/L riboflavin, 50 µg/L nicotinic acid, 50 µg/L Ca-pantothenate, 50 µg/L vitamin B₁₂, 50 µg/L p-aminobenzoate, 50 µg/L lipoic acid) were inoculated with cells from a fresh culture of *C. autoethanogenum* to a start OD_{600nm} of 0.1. The chemolithoautotrophic cultivation was carried out in a 0.5L pressure-resistant glass bottle at 37°C, 150 rpm and a ventilation rate of 2.3 L/h with a premixed gas with 60% H₂, 20% CO₂ and 20% CO in an open water bath shaker for 476 h. The gas was discharged into the medium through an aeration membrane, which was mounted in the center of the reactors. The pH was hold at 5.5 by automatic

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addition of 2.5 M NH_3 solution. Fresh medium was continuously fed to the reactor and fermentation broth continuously removed from the reactor with a dilution rate of 1.0 d^{-1} .

After the precultivation, the cell suspension was centrifuged (10 min, 4200 rpm) and the pellet was resuspended in fresh main culture medium. For the main culture, as many cells from the preculture as necessary for an $\text{OD}_{600\text{nm}}$ of 1.0 were transferred in 400 mL medium. For the main culture also (mineral) medium 2 was used, but without H_3BO_3 . The chemolithoautotrophic cultivation was carried out in a 0.5L pressure-resistant glass bottle at 37°C , 150 rpm and a ventilation rate of 2.3 L/h with a premixed gas with 60% H_2 , 20% CO_2 and 20% CO in an open water bath shaker for 48 h. The gas was discharged into the medium through an aeration membrane, which was mounted in the center of the reactors. The pH was hold at 5.5 by automatic addition of 2.5 M NH_3 solution. Fresh medium was continuously feeded to the reactor and fermentation broth continuously removed from the reactor with a dilution rate of 1.0 d^{-1} . During cultivation several 5 mL samples were taken to determinate $\text{OD}_{600\text{nm}}$, pH und product formation. The determination of the product concentrations was performed by semiquantitative $^1\text{H-NMR}$ spectroscopy. As an internal quantification standard sodium trimethylsilylpropionate (T(M)SP) was used.

During the main cultivation in mineral medium 2, 2.99 g ethanol and 2.91 g acetate were produced.

Example 3

Production of ethanol by Clostridium autoethanogenum on synthesis gas with boron

For the biotransformation of hydrogen, carbon monoxide and carbon dioxide to ethanol the homoacetogenic bacterium *Clostridium autoethanogenum* was cultivated on synthesis gas. All cultivation steps were carried out under anaerobic conditions in pressure-resistant glass bottles that can be closed airtight with a butyl rubber stopper.

For the preculture 400 ml medium 2 ($\text{pH} = 5.8$; 0.407 g/L $\text{MgCl}_2 \cdot 6 \text{ H}_2\text{O}$, 0.117 g/L NaCl , 0.294 g/L $\text{CaCl}_2 \cdot 2 \text{ H}_2\text{O}$, 1.864 g/L KCl , 0.375 ml/L H_3PO_4 , 19.8 mg/L $\text{FeCl}_2 \cdot 4 \text{ H}_2\text{O}$, 0.5 g/L cysteine-HCl, 3.92 g/L NH_4 -acetate, 0.396 mg/L $\text{MnCl}_2 \cdot 4 \text{ H}_2\text{O}$, 0.476 mg/L $\text{CoCl}_2 \cdot 6 \text{ H}_2\text{O}$, 0.682 mg/L ZnCl_2 , 0.124 mg/L H_3BO_3 , 0.484 mg/L $\text{Na}_2\text{MoO}_4 \cdot 2 \text{ H}_2\text{O}$, 0.346 mg/L $\text{Na}_2\text{SeO}_3 \cdot 5 \text{ H}_2\text{O}$, 1.189 mg/L $\text{NiCl}_2 \cdot 6 \text{ H}_2\text{O}$, 0.660 mg/L $\text{Na}_2\text{WO}_4 \cdot 2 \text{ H}_2\text{O}$, 20 $\mu\text{g/L}$ d-biotin, 20 $\mu\text{g/L}$ folic acid, 10 $\mu\text{g/L}$ pyridoxine-HCl, 50 $\mu\text{g/L}$ thiamine-HCl $\cdot \text{H}_2\text{O}$, 50 $\mu\text{g/L}$ riboflavin, 50 $\mu\text{g/L}$ nicotinic acid, 50 $\mu\text{g/L}$ Ca-pantothenate, 50 $\mu\text{g/L}$ vitamin B_{12} , 50 $\mu\text{g/L}$ p-aminobenzoate, 50 $\mu\text{g/L}$ lipoic acid) were inoculated with cells from a fresh culture of *C. autoethanogenum* to a start $\text{OD}_{600\text{nm}}$ of 0.1. The chemolithoautotrophic cultivation was carried out in a 0.5L pressure-resistant glass bottle at 37°C , 150 rpm and a ventilation rate of 2.3 L/h with a premixed gas with 60% H_2 , 20% CO_2 and 20% CO in an open water bath shaker for 476 h. The gas was discharged into the medium through an aeration membrane, which was mounted in the center of the reactors. The pH was hold at 5.5 by automatic addition of 2.5 M NH_3 solution. Fresh medium was continuously fed to the reactor and fermentation broth continuously removed from the reactor with a dilution rate of 1.0 d^{-1} .

After the precultivation, the cell suspension was centrifuged (10 min, 4200 rpm) and the pellet was resuspended in fresh main culture medium. For the main culture, as many cells from the preculture as necessary for an OD_{600nm} of 1.0 were transferred in 400 mL medium. For the main culture also mineral medium 2 was used. The chemolithoautotrophic cultivation was carried out in a 0.5L
5 pressure-resistant glass bottle at 37°C, 150 rpm and a ventilation rate of 2.3 L/h with a premixed gas with 60% H₂, 20% CO₂ and 20% CO in an open water bath shaker for 45 h. The gas was discharged into the medium through an aeration membrane, which was mounted in the center of the reactors. The pH was hold at 5.5 by automatic addition of 2.5 M NH₃ solution. Fresh medium was continuously fed to the reactor and fermentation broth continuously removed from the reactor
10 with a dilution rate of 1.0 d⁻¹. During cultivation several 5 mL samples were taken to determinate OD_{600nm}, pH und product formation. The determination of the product concentrations was performed by semiquantitative 1H-NMR spectroscopy. As an internal quantification standard sodium trimethylsilylpropionate (T(M)SP) was used.

During the main cultivation in mineral medium 2, 3.22 g ethanol and 1.11 g acetate were produced.

CLAIMS

1. A fermentation medium comprising:
(c) Boron at a concentration of 0.05 to 1.00mg/L of the fermentation medium.
- 5 2. The fermentation medium according to claim 1, wherein the Boron concentration is at least 0.5mg/L of the fermentation medium.
3. The fermentation medium according to either claim 1 or 2, where the source of boron is selected from the group consisting of sodium borate, boric acid, sodium borate decahydrate
10 and combinations thereof.
4. The fermentation medium according to any one of the preceding claims, wherein the fermentation medium further comprises at least one of the components selected from the group consisting of:
15 (c) Magnesium at a concentration greater than 40mg/L of the fermentation medium,
(d) Sulphur at a concentration greater than 85mg/L of the fermentation medium,
(c) Aluminium at a detectable concentration,
(e) Potassium at a concentration greater than 100mg/L of the fermentation medium, or combinations thereof.
- 20 5. The fermentation medium according to any one of the preceding claims, wherein the fermentation medium comprises the components:
(c) Magnesium at a concentration greater than 40mg/L of the fermentation medium,
(d) Sulphur at a concentration greater than 85mg/L of the fermentation medium,
25 (c) Aluminium at a detectable concentration, and
(e) Potassium at a concentration greater than 100mg/L of the fermentation medium.
6. The fermentation medium according to any one of the preceding claims, wherein the medium comprises at least one of the components selected from the group consisting of:
30 (a) Magnesium at a concentration of 40- 70 mg/L of the fermentation medium,
(b) Sulphur at a concentration of 85- 110mg/L of the fermentation medium,
(c) Aluminium at a concentration of 1 to 15µg/L of the fermentation medium, and
(e) Potassium at a concentration of 100- 300mg/L of the fermentation medium.
- 35 7. The fermentation medium according to any one of the preceding claims, wherein the medium comprises the components:
(a) Magnesium at a concentration of 40- 70 mg/L of the fermentation medium,
(b) Sulphur at a concentration of 85- 110mg/L of the fermentation medium,
(c) Aluminium at a concentration of 1 to 15µg/L of the fermentation medium, and

- (e) Potassium at a concentration of 100- 300mg/L of the fermentation medium.
8. The fermentation medium according to any one of the preceding claims, wherein the medium comprises:
- 5 (c) Sulphur at a concentration greater than 85mg/L of the fermentation medium.
9. The fermentation medium according to any one of the preceding claims, wherein the medium comprises:
- 10 (b) Sulphur at a concentration of 85-110 mg/L of the fermentation medium.
10. The fermentation medium according to any one of the preceding claims, wherein the medium comprises at least one of the components selected from the group consisting of:
- 15 (c) Magnesium at a concentration of about 62.5 mg/L of the fermentation medium,
(d) Sulphur at a concentration of about 100mg/L of the fermentation medium,
(c) Aluminium at a concentration of about 11 µg/L of the fermentation medium,
(e) Potassium at a concentration of about 260mg/L of the fermentation medium, or combinations thereof.
- 20 11. The fermentation medium according to any one of the preceding claims, wherein the medium comprises:
- (a) Magnesium at a concentration of about 62.5 mg/L of the fermentation medium,
(b) Sulphur at a concentration of about 100mg/L of the fermentation medium,
(c) Aluminium at a concentration of about 11 µg/L of the fermentation medium, and
25 (e) Potassium at a concentration of about 260mg/L of the fermentation medium.
12. The fermentation medium according to any one of the preceding claims, wherein
- 30 (a) the source of magnesium is selected from the group consisting magnesium carbonate, magnesium chloride, magnesium phosphate, magnesium sulphate, magnesium sulfide and combinations thereof;
- (b) the source of sulphur is selected from the group consisting of sodium sulfide, cysteine, iron sulphate, Ammonium iron(II) sulphate hexahydrate, magnesium sulphate, zinc sulphate and combinations thereof;
- 35 (c) the source of aluminium is selected from the group consisting of aluminium bromide, aluminium carbonate, aluminium chloride, aluminium monochloride, aluminium nitrate, aluminium phosphate, aluminium sulphate, aluminium(I) oxide, aluminium(II) oxide and combinations thereof;

- (e) the source of potassium is selected from the group consisting of potassium permanganate potassium phosphate, potassium chloride, potassium nitrate, potassium hydroxide (KOH), potassium sulphate, potassium hydrogen phosphate and combinations thereof.
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13. A method of producing at least one C₁ to C₈ alcohol from a carbon source in the fermentation medium according to any one of claim 1 to 12, wherein the method comprises,
- 10
- contacting at least one acetogenic cell to the carbon source, and
- the carbon source is carbon monoxide free and comprises hydrogen and carbon dioxide.
14. The method according to claim 13, wherein the cell is selected from the group consisting of *Acetbacterium woodii*, *Alkalibaculum bacchi*, *Blautia producta*, *Clostridium aceticum*, *Clostridium autoethanogenum*, *Clostridium carboxidivorans*, *Clostridium drakei*, *Clostridium formicoaceticum*, *Clostridium ljungdahlii*, *Clostridium magnum*, *Butyribacterium methyotrphoicum*, *Clostridium scatologenes*, *Eubacterium limosum*, *Moorella thermoacetica*, *Sporomusa ovate*, *Sporomusa silvacetica*, *Sporomusa sphaeroides*, *Oxobacter pfennigii*, and *Thermoanaerobacter kiuvi*.
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- 20 15. Use of a fermentation medium according to any one of claims 1 to 12 in a method for producing at least one C₁ to C₈ alcohol from a carbon source, wherein the carbon source is carbon monoxide free and comprises hydrogen and carbon dioxide.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
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ADD.

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)
C12P 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 practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 3 050 967 A1 (EVONIK DEGUSSA GMBH [DE]) 3 August 2016 (2016-08-03) examples	1-15
X	WO 2016/108048 A1 (USW COMMERCIAL SERVICES LTD [GB]) 7 July 2016 (2016-07-07) claims; figure 11	1-3, 12-15
X	EP 2 460 883 A1 (AJINOMOTO KK [JP]) 6 June 2012 (2012-06-06) paragraphs [0229], [0250], [0256], [0262]	1-12
	----- -/-	



Further documents are listed in the continuation of Box C.



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INTERNATIONAL SEARCH REPORT

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DSMZ GmbH: "318. METHANOSARCINA THERMOPHILA (BCYT) MEDIUM", DSMZ GmbH, 2014, pages 1-2, XP055458377, Retrieved from the Internet: URL:https://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium318.pdf [retrieved on 2018-03-12] the whole document -----	1-3,12
X	WO 2010/064933 A1 (LANZATECH NEW ZEALAND LTD [NZ]; MIHALCEA CHRISTOPHE DANIEL [NZ]; FUNG) 10 June 2010 (2010-06-10) page 41; example 3 -----	1-4,8
E	WO 2018/019847 A1 (EVONIK DEGUSSA GMBH [DE]) 1 February 2018 (2018-02-01) page 28, line 27 - page 29, line 1 -----	1-6, 8-10, 12-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2017/084186

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 3050967	A1	03-08-2016	AR 103521 A1 17-05-2017
			BR 102016001728 A2 02-08-2016
			CA 2919028 A1 28-07-2016
			CN 105820971 A 03-08-2016
			EP 3050967 A1 03-08-2016
			EP 3050969 A1 03-08-2016
			KR 20160092931 A 05-08-2016
			RU 2016102684 A 01-08-2017
			US 2016215302 A1 28-07-2016
			ZA 201600520 B 26-04-2017
WO 2016108048	A1	07-07-2016	AU 2015373278 A1 03-08-2017
			EP 3240901 A1 08-11-2017
			US 2017314045 A1 02-11-2017
			WO 2016108048 A1 07-07-2016
EP 2460883	A1	06-06-2012	CN 102471790 A 23-05-2012
			EP 2460883 A1 06-06-2012
			JP WO2011013707 A1 10-01-2013
			US 2012202255 A1 09-08-2012
			US 2014363857 A1 11-12-2014
			WO 2011013707 A1 03-02-2011
WO 2010064933	A1	10-06-2010	US 2011300593 A1 08-12-2011
			US 2013230894 A1 05-09-2013
			WO 2010064933 A1 10-06-2010
WO 2018019847	A1	01-02-2018	WO 2018019841 A1 01-02-2018
			WO 2018019847 A1 01-02-2018