

CLAIMS

1. A recombinant microorganism for producing hydrogen gas, wherein the microorganism comprises:

- 5 - exogenous nucleic acid sequences encoding one or more proteins for enabling the microorganism to produce hydrogen,
- wherein the one or more proteins comprise an Fe-Fe dependent hydrogenase, preferably HydA,
- wherein the nucleic acid sequences are operably linked to one or more promoters for enabling expression of the nucleic acid sequences in the
10 microorganism, and
- wherein the microorganism or cell comprises a genetic modification which promotes utilisation of carbon via the pentose phosphate pathway.

2. The recombinant microorganism of claim 1, wherein the nucleic acid sequences encode the proteins ferredoxin NADP reductase (FNR) and Ferredoxin.

15 3. The recombinant microorganism of claim 1 or 2, wherein the nucleic acid sequences encode at least one assembly protein for enabling maturation and activation of the hydrogenase.

4. The recombinant microorganism of any one of claims 1 to 3, wherein the genetic modification which promotes utilisation of carbon via the pentose phosphate
20 pathway, reduces or inhibits the activity or levels of one or more endogenous proteins of the microorganism selected from: phosphofructokinase, pyruvate kinase, glycerate mutase, 6-phosphogluconoate dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase.

5. The recombinant microorganism of any one of claims 1 to 4, wherein the
25 genetic modification which promotes utilisation of carbon via the pentose phosphate pathway, increases the level or activity of one or more proteins of the pentose phosphate pathway and NADPH regulating proteins, wherein the genetic modification is
a) a modification of the promoter region of the gene encoding the one or more proteins
or b) replacement of an endogenous gene encoding the one or more proteins, with a
30 heterologous gene sequence.

6. The recombinant microorganism of any one of claims 2 to 5, wherein the FNR and Ferredoxin proteins are from *Chlamydomonas reinhardtii* or are functionally equivalent homologs or derivatives of the FNR and Ferredoxin proteins from *Chlamydomonas reinhardtii*,
- 5 7. The recombinant microorganism of any one of claims 3 to 6, wherein the at least one assembly protein are selected from HydEF and HydG or functionally equivalent homologs or derivatives thereof.
8. The recombinant microorganism of claim 7, wherein the nucleic acid sequence encode HydEF and HydG from *Chlamydomonas reinhardtii* or functionally
10 equivalent homologs or derivatives of the HydEF, HydG proteins from *Chlamydomonas reinhardtii*.
9. The recombinant microorganism of any one of claims 1 to 8, wherein the Fe-Fe dependent hydrogenase is an HydA protein or a functionally equivalent homolog or derivative thereof, from a microorganism selected from the group consisting of:
15 *Chlamydomonas reinhardtii*, *Volvox carteri*, *Giardia lamblia*, *Entamoeba nuttalli*, *Ilyobacter polytrophus*, *Trichomonas vaginalis*, *Megasphaera micronuciformis*, *Veillonella parvula*, *Veillonella atypica*, and *Peptoclostridium bifermentans*.
10. The recombinant microorganism of claim 9, wherein the HydA protein, or functionally equivalent homolog or derivative thereof, is from *Chlamydomonas*
20 *reinhardtii*.
11. The recombinant microorganism of any one of claims 1 to 10, wherein the microorganism is a strain of *Escherichia coli* (*E coli*).
12. The recombinant microorganism of any one of claims 1 to 11, wherein the exogenous nucleic acid sequences are provided in a single polynucleotide construct.
- 25 13. The recombinant microorganism of any one of claims 1 to 12, wherein the exogenous nucleic acid sequences are codon optimised to provide for optimised expression in the microorganism

14. An *E. coli* cell comprising exogenous nucleic acids encoding proteins that enable the cell to produce hydrogen, wherein the proteins comprise, consist or consist essentially of the polypeptides HydEF, HydG, HydA, ferredoxin and FNR,

wherein the HydEF, HydG, Ferredoxin and FNR are from *Chlamydomonas reinhardtii*, or are functionally equivalent homologs or derivatives of the HydEF, HydG, Ferredoxin and FNR from *Chlamydomonas reinhardtii*, and

wherein a) the cell comprises a genetic modification which reduces or inhibits the activity or levels of one or more endogenous proteins selected from the group consisting of: phosphofructokinase, pyruvate kinase, glycerate mutase, 6-phosphogluconate dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase; and/or b) the cell comprises a genetic modification which increases the level or activity of one or more proteins of the pentose phosphate pathway and NADPH regulating proteins.

15. The cell of claim 14, wherein the HydA protein is an HydA protein or a functionally equivalent homolog or derivative thereof, from a microorganism selected from the group consisting of: *Chlamydomonas reinhardtii*, *Volvox carteri*, *Giardia lamblia*, *Entamoeba nuttalli*, *Ilyobacter polytrophus*, *Trichomonas vaginalis*, *Megasphaera micronuciformis*, *Veillonella parvula*, *Veillonella atypica*, and *Peptoclostridium bifermentans*.

16. The recombinant microorganism or cell of any one of the preceding claims, wherein the microorganism or cell microorganism comprises a genetic modification which partially or completely excises the nucleic acid sequence corresponding to one or more of the genes *pfkA*, *pps*, *gpmA/gpmM*, *edd* and *eda*, encoding phosphofructokinase, pyruvate kinase, glycerate mutase, 6-phosphogluconate dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase respectively.

17. The recombinant microorganism or cell of claim 16, wherein the genetic modification results in a partial loss-of-function mutation in the gene.

18. The recombinant microorganism or cell of claims 16 or 17 wherein the genetic modification reduces or inhibits the activity of phosphofructokinase and/or glycerate mutase.

19. The recombinant microorganism or cell of claim 5 or 14, wherein the one or more proteins of the pentose phosphate pathway and NADPH regulating proteins is selected from the group consisting of: phosphoglucomutase, glucose-6-phosphate dehydrogenase, 6-phosphogluconolactonase, glyceraldehyde-3-phosphate dehydrogenase 6-phosphogluconate dehydrogenase, transketolase, transldolase, NAD kinase and soluble pyridine nucleotide transhydrogenase.

20. The recombinant microorganism or cell of claim 5, wherein the promoter region of the gene encoding the one or more proteins is replaced with the *gapA* or *osmYp* promoter.

21. The recombinant microorganism or cell of claim 20, wherein the promoter of the *zwf* gene, encoding glucose-6-phosphate dehydrogenase, is replaced with the *gapA* or *osmY* promoter or the anaerobically induced *nar* or *nirB* promoters.

22. The recombinant microorganism or cell of claim 20 or 21 wherein the promoter of the *gnd* gene, encoding 6-phosphogluconate dehydrogenase is replaced with the *gapA* or *osmY* promoter.

23. The recombinant microorganism or cell of any one of claims 20 to 22, wherein the promoter of the *pgi* gene, encoding phosphoglucomutase, is replaced with the *gapA* or *osmY* promoter.

24. The recombinant microorganism or cell of any one of claims 20 to 23, wherein the promoter of the gene *pgl* encoding 6-phosphogluconolactonase is replaced with the *gapA* or *osmY* promoter.

25. The recombinant microorganism or cell of claim 19, wherein the gene encoding glucose-6-phosphate dehydrogenase (*zwf*), is replaced with the *zwf* gene from *Zygomonas mobilis*.

26. The recombinant microorganism or cell of claim 19, wherein, the gene encoding 6-phosphogluconate dehydrogenase (*gnd*) is replaced with the *gnd* gene from *Corynebacterium glutamicum*.

27. The recombinant microorganism or cell of claim 19, wherein the gene encoding glyceraldehyde-3-phosphate dehydrogenase (*gapA*) is replaced with the *gapC* gene from *Clostridium acetobutylicum*.

28. The recombinant microorganism or cell of any one of claims 1 to 27
5 wherein the recombinant microorganism comprises a nucleic acid construct encoding one or more proteins to enable the microorganism or cell to metabolise sucrose for energy consumption, preferably wherein the nucleic acid construct comprises the genes *cscA*, *cscB* and *sp* genes, encoding sucrose hydrolase, sucrose permease, and sucrose phosphorylase respectively.

10 29. A method for producing hydrogen gas, the method comprising:

providing a cell of any one of claims 1 to 28,

culturing the cell in a suitable culture medium and under suitable conditions for enabling the cell to produce hydrogen gas.

15 30. The method of claim 29, wherein the culturing of the cell is under anaerobic conditions.

31. The method of claim 29 or 30, wherein the culturing comprises contacting the cell with one or more factors for enabling maturation of the hydrogenase.

32. The method of any one of claims 29 to 31, wherein the culturing comprises supplementation of the culture medium with ferric (iron III) or ferrous (iron II), preferably
20 at a concentration equal to or greater than about 20 μ M.

33. The method of any one of claims 29 to 32, wherein the culturing is performed at no more than about 37 °C, more preferably at less than about 35 °C, less than about 32 °C, less than about 30 °C, less than about 25 °C, less than about 20 °C, preferably no less than about 10°C.

25 34. A device for producing electricity from hydrogen gas, comprising the microorganism or cell of any one of claims 1 to 28.