CLAIMS

What is claimed is:

1. A method of culturing microorganisms in non-axenic mixotrophic conditions, comprising:
    a. Inoculating an aqueous culture medium with a culture of microorganisms comprising at least some contaminating bacteria in a culturing vessel;
    b. Supplying the culture of microorganisms with at least some light;
    c. Supplying the culture of microorganisms with an organic carbon source comprising an organic acid; and
    d. Wherein the culture of microorganisms maintains a level of contaminating bacteria below 25% of a total cell count of the culture and a microorganism yield of at least 50 g/m^2 day.

2. The method of claim 1, wherein the microorganism comprises at least one microorganism of the genus selected from the group consisting of: *Chlorella*, *Anacystis*, *Synechococcus*, *Synechocystis*, *Neospongiococcum*, *Chlorococcum*, *Phaeodactylum*, *Spirulina*, *Micractinium*, *Haematococcus*, *Nannochloropsis*, and *Brachiomonas*.

3. The method of claim1, wherein the contaminating bacteria comprises at least one selected from the group consisting of: *Achromobacter* sp., *Acidovorax* sp., *Aeromonas* sp., *Agrobacterium* sp., *Alteromonas* sp., *Aquaspirillum* sp., *Azospirillum* sp., *Azotobacter* sp., *Bergeyella* sp., *Brochothrix* sp., *Brumimicrobium* sp., *Burkholderia* sp., *Caulobacter* sp., *Cellulomonas* sp., *Chryseobacterium* sp., *Curtobacterium* sp., *Delftia* sp., *Empedobacter* sp., *Enterobacter* sp., *Escherichia* sp., *Flavobacterium* sp., *Marinobacter* sp., *Microbacterium* sp., *Myroides* sp., *Paracoccus* sp., *Pedobacter* sp., *Phaeobacter* sp., *Pseudoalteromonas* sp., *Pseudomonas* sp., *Rahnella* sp., *Ralstonia* sp., *Rhizobium* sp., *Rhodococcus* sp., *Roseomonas* sp., *Staphylococcus* sp., *Stenotrophomonas* sp., *Vibrio* sp., and *Zobelliae* sp.

4. The method of claim 1, wherein the organic acid of the organic carbon source comprises 0.5-50% acetic acid.

5. The method of claim 1, wherein the culture of microorganisms maintains a level of contaminating bacteria below 20% of a total cell count of the culture.

6. The method of claim1, wherein the culture of microorganisms maintains a level of contaminating bacteria below 10% of a total cell count of the culture.

7. The method of claim 1, wherein the culture of microorganisms maintains a level of contaminating bacteria below 5% of a total cell count of the culture.

8. The method of claim 1, wherein the organic acid is combined with at least one other nutrient and supplied to the culture medium in combination, the at least one other nutrient comprising at least one selected from the group consisting of: nitrates, phosphates, iron, cobalt, copper, sodium, molybdenum, manganese, zinc, salts, and silica.

9. The method of claim 1, further comprising supplying a least one oxidizing agent to the microorganism culture, the at least one oxidizing agent comprising at least one selected from the group consisting of: ozone, hydrogen peroxide, chlorine, chlorite, chlorate, hypochlorite, nitric acid, chromium, permanganate, silver oxide, and bromine.

10. The method of claim 1, wherein the organic carbon source is supplied to the culture by a pH auxostat system when a measured pH level of the culture reaches a threshold level.

11. The method of claim 10, wherein the pH threshold level is 7.5.

12. The method of claim 10, wherein the pH auxostat system maintains a substantially constant pH level in the microorganism culture.

13. The method of claim 10, wherein the pH auxostat system maintains a pH level within a defined hysteresis range that inhibits proliferation of contaminating bacteria in the microorganism culture.

14. The method of claim 1, wherein the organic carbon source is supplied to the culture until a measured dissolved oxygen level of the culture reaches a critical level below 2 mg O_2/L.

15. The method of claim 1, wherein the aqueous culture medium comprises an initial concentration of sodium acetate, sodium hydroxide, or potassium hydroxide.

16. The method of claim 15, wherein the initial concentration of sodium acetate, sodium hydroxide, or potassium hydroxide is between 0.1 and 6 g/L.

17. The method of claim 1, wherein the at least some light is supplied to the microorganism culture for a photoperiod of 10-16 hours per day.

18. The method of claim 1, wherein the at least some light is supplied to the microorganism culture for a photoperiod of less than 15 hours per day.

19. The method of claim 1, wherein the at least some light is supplied for more than 15 hours per day.

20. The method of claim 1, wherein the at least some light is natural light.

21. The method of claim 1, wherein the at least some light is artificial light.

22. The method of claim 1, wherein the at least some light is a combination of natural and artificial light.

23. The method of claim 1, wherein the at least some light comprises at least one specific wavelength spectrum from the group consisting of violet (about 380-450 nm), blue (about 450-495 nm), green (about 495-570 nm), yellow (570-590 nm), orange (about 590-620 nm), red (about 620-750 nm), and far red (about 700-800 nm).

24. The method of claim 1, wherein the culturing vessel is an open vessel disposed outdoors.

25. A method of controlling bacterial contamination in a mixotrophic culture of microorganisms in non-axenic conditions, comprising:

    a. Inoculating an aqueous culture medium with a culture of microorganisms comprising at least some contaminating bacteria in a culturing vessel;

    b. Supplying the culture of microorganisms with at least some light;

c. Supplying the culture of microorganisms with an organic carbon source;

d. Supplying the culture of microorganisms with an oxidizing agent; and

e. Wherein the culture of microorganisms maintains a level of contaminating bacteria below 25% of total cell count of the microorganism culture.

26. The method of claim 25, wherein the oxidizing agent comprises at least one selected from the group consisting of: ozone, hydrogen peroxide, chlorine, chlorite, chlorate, hypochlorite, nitric acid, chromium, permanganate, silver oxide, and bromine.

27. The method of claim 26, wherein the ozone is supplied in concentrations between 0.01 and 2.0 mg/L.

28. The method of claim 27, wherein the ozone is supplied in concentrations between 0.01 and 0.50 mg/L.

29. The method of claim 25, wherein the oxidizing agent is supplied to the culture of microorganisms through at least one of a sparger and a venturi injector.

30. The method of claim 25, wherein the microorganism comprises at least one microorganism of a genus selected from the group consisting of: *Agmenellum, Amphora, Anabaena, Anacystis, Apistonema, Arthrospira (Spirulina), Botryococcus, Brachiomonas, Chlamydomonas, Chlorella, Chloroccum, Cruciplacolithus, Cylindrotheca, Coenochloris, Cyanophora, Cyclotella, Dunaliella, Emiliania, Euglena, Extubocellulus, Fragilaria, Galdieria, Goniotrichium, Haematococcus, Halochlorella, Isochyrsis, Leptocylindrus, Micractinium, Melosira, Monodus, Nostoc, Nannochloris, Nannochloropsis, Navicula, Neospongiococcum, Nitzschia., Odontella, Ochromonas, Ochrosphaera, Pavlova, Picochlorum, Phaeodactylum, Pleurochyrsis, Porphyridium, Poteriochromonas, Prymnesium, Rhodomonas, Scenedesmus, Skeletonema, Spumella, Stauroneis, Stichococcus, Auxenochlorella, Cheatoceros, Neochloris, Ochromonas, Porphiridium, Synechococcus, Synechocystis, Tetraselmis Thraustochytrias* and *Thalassiosira*.

31. The method of claim 25, wherein the contaminating bacteria comprises at least one selected from the group consisting of: *Achromobacter* sp., *Acidovorax* sp., *Aeromonas* sp., *Agrobacterium* sp., *Alteromonas* sp., *Aquaspirillum* sp., *Azospirillum* sp., *Azotobacter* sp., *Bergeyella* sp., *Brochothrix* sp., *Brumimicrobium* sp., *Burkholderia* sp., *Caulobacter* sp., *Cellulomonas* sp., *Chryseobacterium* sp., *Curtobacterium* sp., *Delftia* sp., *Empedobacter* sp., *Enterobacter* sp., *Escherichia* sp., *Flavobacterium* sp., *Marinobacter* sp., *Microbacterium* sp., *Myroides* sp., *Paracoccus* sp., *Pedobacter* sp., *Phaeobacter* sp., *Pseudoalteromonas* sp., *Pseudomonas* sp., *Rahnella* sp., *Ralstonia* sp., *Rhizobium* sp., *Rhodococcus* sp., *Roseomonas* sp., *Staphylococcus* sp., *Stenotrophomonas* sp., *Vibrio* sp., and *Zobelliae* sp.

32. The method of claim 25, wherein the culture of microorganisms maintains a level of contaminating bacteria below 20% of a total cell count of the culture.

33. The method of claim 25, wherein the culture of microorganisms maintains a level of contaminating bacteria below 10% of a total cell count of the culture.

34. The method of claim 25, wherein the culture of microorganisms maintains a level of contaminating bacteria below 5% of a total cell count of the culture.

35. The method of claim 25, wherein the organic carbon source comprises at least one selected from the group consisting of: acetate, acetic acid, ammonium linoleate, arabinose, arginine, aspartic acid, butyric acid, cellulose, citric acid, ethanol, fructose, fatty acids, galactose, glucose, glycerol, glycine, lactic acid, lactose, maleic acid, maltose, mannose, methanol, molasses, peptone, plant based hydrolysate, proline, propionic acid, ribose, sacchrose, partial or complete hydrolysates of starch, sucrose, tartaric, TCA-cycle organic acids, thin stillage, urea, industrial waste solutions, and yeast extract.

36. The method of claim 25, wherein the organic carbon source is combined with at least one other nutrient and supplied to the culture medium in combination, the at least one other nutrient comprising at least one selected from the group consisting of: nitrates, phosphates, iron, cobalt, copper, sodium, molybdenum, manganese, zinc, salts, and silica.

37. The method of claim 25, wherein the at least some light is supplied to the microorganism culture for a photoperiod of 10-16 hours per day.

38. The method of claim 25, wherein the at least some light is supplied to the microorganism culture for a photoperiod of less than 15 hours per day.

39. The method of claim 25, wherein the at least some light is supplied for more than 15 hours per day.

40. The method of claim 25, wherein the at least some light is natural light or artificial light.

41. The method of claim 25, wherein the at least some light is a combination of natural and artificial light.

42. The method of claim 25, wherein the light comprises at least one specific wavelength spectrum from the group consisting of: violet (about 380-450 nm), blue (about 450-495 nm), green (about 495-570 nm), yellow (570-590 nm), orange (about 590-620 nm), red (about 620-750 nm), and far red (about 700-800 nm).

43. The method of claim 25, wherein the culturing vessel is an open vessel disposed outdoors.

44. A method of culturing microorganisms in non-axenic mixotrophic conditions, comprising:

    a. Inoculating an aqueous culture medium with a culture of microorganisms comprising at least some contaminating bacteria in a culturing vessel;

    b. Supplying the culture of microorganisms with at least some light;

    c. Supplying the culture of microorganisms with an organic carbon source;

    d. Supplying the culture with a gas comprising oxygen; and

    e. Wherein the gas is supplied to the culture at a k_La of at least $2.40X10^{-3}$ s^{-1}, and results in a productivity rate for the microorganisms of at least 0.4 g/L d.

45. The method of claim 44, wherein the k_La is between $2.70X10^{-3}$ s^{-1} and 21 s^{-1}.

46. The method of claim 44, wherein the productivity rate for the microorganisms is between 0.5 g/L d and 50 g/L d.

47. The method of claim 44, wherein the productivity rate for the microorganisms is between 0.5 g/L d and 5 g/L d.

48. The method of claim 44, wherein the productivity rate for the microorganism is between 0.5 g/L d and 2.5 g/L d.

49. The method of claim 44, wherein the contaminating bacteria comprises at least one selected from the group consisting of: *Achromobacter* sp., *Acidovorax* sp., *Aeromonas* sp., *Agrobacterium* sp., *Alteromonas* sp., *Aquaspirillum* sp., *Azospirillum* sp., *Azotobacter* sp., *Bergeyella* sp., *Brochothrix* sp., *Brumimicrobium* sp., *Burkholderia* sp., *Caulobacter* sp., *Cellulomonas* sp., *Chryseobacterium* sp., *Curtobacterium* sp., *Delftia* sp., *Empedobacter* sp., *Enterobacter* sp., *Escherichia* sp., *Flavobacterium* sp., *Marinobacter* sp., *Microbacterium* sp., *Myroides* sp., *Paracoccus* sp., *Pedobacter* sp., *Phaeobacter* sp., *Pseudoalteromonas* sp., *Pseudomonas* sp., *Rahnella* sp., *Ralstonia* sp., *Rhizobium* sp., *Rhodococcus* sp., *Roseomonas* sp., *Staphylococcus* sp., *Stenotrophomonas* sp., *Vibrio* sp., and *Zobelliae* sp.

50. The method of claim 44, wherein the microorganism comprises at least one microorganism of a genus selected from the group consisting of: *Agmenellum, Amphora, Anabaena, Anacystis, Apistonema, Arthrospira (Spirulina), Botryococcus, Brachiomonas, Chlamydomonas, Chlorella, Chloroccum, Cruciplacolithus, Cylindrotheca, Coenochloris, Cyanophora, Cyclotella, Dunaliella, Emiliania, Euglena, Extubocellulus, Fragilaria, Galdieria, Goniotrichium, Haematococcus, Halochlorella, Isochyrsis, Leptocylindrus, Micractinium, Melosira, Monodus, Nostoc, Nannochloris, Nannochloropsis, Navicula, Neospongiococcum, Nitzschia., Odontella, Ochromonas, Ochrosphaera, Pavlova, Picochlorum, Phaeodactylum, Pleurochyrsis, Porphyridium, Poteriochromonas, Prymnesium, Rhodomonas, Scenedesmus, Skeletonema, Spumella, Stauroneis, Stichococcus, Auxenochlorella, Cheatoceros, Neochloris, Ocromonas, Porphiridium, Synechococcus, Synechocystis, Tetraselmis Thraustochytrids,* and *Thalassiosira.*

51. The method of claim 44, wherein the gas is supplied to the culture of microorganisms by at least one selected from the group consisting of a gas injector, porous diffuser, micropore diffuser, gas permeable membrane, microbubble generator, venturi injection, and microbubble fluidic oscillator.

52. The method of claim 44, wherein the organic carbon source comprises at least one selected from the group consisting of: acetate, acetic acid, ammonium linoleate, arabinose, arginine, aspartic acid, butyric acid, cellulose, citric acid, ethanol, fructose, fatty acids, galactose, glucose, glycerol, glycine, lactic acid, lactose, maleic acid, maltose, mannose, methanol, molasses, peptone, plant based hydrolyzate, proline, propionic acid, ribose, sacchrose, partial or complete hydrolysates of starch, sucrose, tartaric, TCA-cycle organic acids, thin stillage, urea, industrial waste solutions, and yeast extract.

53. The method of claim 44, wherein the organic carbon source is combined with at least one other nutrient and supplied to the culture medium in combination, the at least one other nutrient comprising at least one selected from the group consisting of: nitrates, phosphates, iron, cobalt, copper, sodium, molybdenum, manganese, zinc, salts, and silica.

54. The method of claim 44, wherein the organic carbon source comprises acetic acid and the aqueous culture medium comprises an initial concentration of sodium acetate, sodium hydroxide, or potassium hydroxide.

55. The method of claim 54, wherein the initial concentration of sodium acetate, sodium hydroxide, or potassium hydroxide is between 0.1 and 6 g/L.

56. The method of claim 54, wherein the concentration of sodium acetate, sodium hydroxide, or potassium hydroxide is supplied for the first one to five days of microorganism growth.

57. The method of claim 44, wherein the vessel is an open vessel disposed outdoors.

58. A method of growing microorganisms mixotrophically, comprising:

a. Providing a culture of microorganisms in an aqueous culture medium in a culturing vessel, the microorganisms being capable of utilizing light and organic carbon as energy sources;

b. Supplying at least some light to the culture of microorganisms; and

c. Supplying an organic carbon source comprising an organic acid and an oxaloacetate promoter to the culture of microorganisms.

59. The method of claim 58, wherein the organic acid comprises at least one from the group consisting of acetic acid, acetate, and acetic anhydride.

60. The method of claim 58, wherein the oxaloacetate promoter comprises at least one from the group consisting of propionic acid, valine, isoleucine, threonine, and methionine.

61. The method of claim 58, wherein the ratio of organic acid to oxaloacetate promoter ranges from 10:0.01 to 10:2.

62. The method of claim 58, wherein the organic carbon source is supplied using a pH-auxostat device.

63. The method of claim 58, wherein the microorganisms comprises at least one selected from the group consisting of microalgae and cyanobacteria.

CLAIMS

What is claimed is:

1. A method of culturing *Chlorella* in non-axenic mixotrophic culture conditions, comprising inoculating an aqueous culture medium with a culture comprising *Chlorella* in an open culturing vessel in the presence of:
    a. light comprising photosynthetically active radiation (PAR);
    b. an organic carbon source comprising at least one selected from the group consisting of acetic acid, acetate, and acetic anhydride, at a concentration in the range of 15-50% (v/v);
    c. a pH in the range of 6-9;
    d. an initial concentration of sodium acetate, sodium hydroxide, or potassium hydroxide in the range of 0.05-10 g/L; and
    e. a dissolved oxygen (DO) concentration in the range of 1-6 mg O_2/Liter of culture.
2. The method of claim 1, the method further comprising controlling temperature of the culture with heating and cooling to maintain the temperature within the range of 10-30°C.
3. The method of claim 1, wherein the organic carbon source comprises acetic acid and further comprises a second organic carbon component comprising propionic acid.
4. The method of claim 3, wherein the organic carbon source comprises acetic acid and propionic acid in an acetic acid:propionic acid ratio in the range of 10:0.01 to 10:2.
5. The method of claim 1, wherein the organic carbon source further comprises the organic carbon source in combination with at least one additional nutrient comprising at least one selected from the group consisting of nitrates and phosphates.
6. The method of claim 5, wherein the organic carbon source in combination with at least one additional nutrient comprises acetic acid and NO_3 in an acetic acid: NO_3 ratio of 10:0.5 to 10:2.
7. The method of claim 1, wherein the inoculation density of *Chlorella* in the culture comprises 0.3-0.5 g/L.
8. The method of claim 1, wherein the volume of the culture is at least 500 liters.
9. The method of claim 1, the method further comprising harvesting *Chlorella* from the culture on a periodic basis once the density of *Chlorella* in the culture reaches a threshold density of 5 g/l.

10. The method of claim 1, wherein the initial concentration of sodium acetate, sodium hydroxide, or potassium hydroxide is in the range of 1-6 g/l.
11. The method of claim 1, wherein the organic carbon is supplied by a pH auxostat system with a set point in the range of 6-9.
12. The method of claim 1, wherein the initial concentration of sodium acetate, sodium hydroxide, or potassium hydroxide is supplied for the first 1-5 days after the culture is inoculated.