CLAIMS

1. A process of producing methacrylic acid and/or methacrylic acid esters comprising the following steps:
    (a) biologically converting isobutyryl-CoA into methacrylyl-CoA by the action of an acyl-CoA oxidase; and
    (b) converting methacrylyl-CoA into methacrylic acid and/or methacrylic acid esters.

2. A process according to claim 1, wherein step (b) may be conducted biologically or chemically.

3. A process according to any of claims 1 or 2, wherein the methacrylic acid esters are C1 to C20 alkyl esters, most preferably C1 to C12 alkyl esters, especially C1 to C4 alkyl esters or C4 to C12 alkyl esters.

4. A process according to claim 3, wherein the methacrylic acid esters are butyl methacrylates, for example n-butylmethacrylate.

5. A process according to claim 3 or 4, wherein the methacrylic acid esters are formed biologically by the action of a transferase.

6. A process according to claim 5, wherein the transferase is an alcohol acyltransferase, suitably under EC group number 2.3.1.84.

7. A process according to claim 6, wherein the alcohol acyltransferase is derived from a fruit origin such as apple, melon or tomato origin.

8. A process according to any of claims 6 or 7, wherein the alcohol acyltransferase acts in the presence of an alcohol, for example a C1 to C12 alcohol, such as a C1 to C4 alcohol or a C4 to C12 alcohol to form the corresponding alkyl ester.

9. A process according to claim 8, wherein the alcohol is butanol.

10. A process according to any of claims 1 or 2, wherein the process comprises a further step (c) of converting methacrylic acid formed in step (b) into a methacrylic acid ester.

11. A process according to claim 10, wherein step (c) may be conducted biologically or chemically.

12. A process according to claims 10 or 11, wherein step (c) is conducted biologically by the action of an esterase or hydrolase.

13. A process according to any of claims 1-11, wherein the acyl-CoA oxidase is under EC group number 1.3.3.6.

14. A process according to claim 13, wherein the acyl-CoA oxidase is selected from any of the following enzymes: ACX4 from *Arabidopsis thaliana*, short chain acyl-CoA oxidase from *Arthrobacter Nicotianae*, a peroxisomal acyl-CoA oxidase from *Vigna radiate*., acyl-CoA oxidase from *Candida sp.* and acyl-CoA oxidase 4 from *Candida tropicalis*.

15. A process according to any of claims 13 or 14, wherein the acyl-CoA oxidase is ACX4 from *Arabidopsis thaliana.*

16. A process according to any of claims 1, 2 or 10-15, wherein methacrylyl-CoA is converted into methacrylic acid by the action of a thioesterase, transferase, synthetase, and/or a phosphotransacylase and a short chain fatty acid kinase.

17. A process according to claim 16, wherein methacrylyl-CoA is converted into methacrylic acid by the action of a thioesterase.

18. A process according to claim 16 or 17, wherein the thioesterase is a 4-hydroxybenzoyl-CoA thioesterase, suitably under EC group 3.1.2.23.

19. A process according to claim 16 or 17, wherein the thioesterase is under EC group number 3.1.2.X, the transferase is a CoA transferase under EC group number 2.8.3.X, the synthetase is an acid-thiol synthetase under EC group number 6.2.1.X, the phosphotransacylase is under EC group number 2.3.1.X, and the short chain fatty acid kinase is under EC group number 2.7.2.X.

20. A process according to any of claims 16-19 , wherein the thioesterase is selected from any of the following enzymes: acyl-CoA thioesterase 4HBT from *Arthrobacter sp*., acyl-CoA thioesterase 4HBT from *Arthrobacter globiformis*, 4HBT from *Pseudomonas sp.* strain CBS-3, EntH from *Escherichia coli*, YciA from *Escherichia coli* or *Haemophilus influenzae*, TesA or TesB from *Escherichia coli* and FcoT from *Mycobacterium tuberculosis;* the CoA transferase is selected from any of the following enzymes: butyryl-CoA:acetoacetate CoA transferase from *Clostridium sp.* SB4, butyryl-CoA:acetoacetate CoA transferase from *Clostridium sticklandii*, butyrate:acetoacetate CoA-transferase from *Clostridium acetobutylicum* ATCC824, and acetate coenzyme A transferase ydiF from *Escherichia coli*; the phosphotransacylase is selected from any of the following enzymes: Phosphotransbutyrylase from *Clostridium acetobutylicum* ATCC824; and the short chain fatty acid kinase is selected from any of the following enzymes: branched chain fatty acid kinase from *Spirochete MA-2*, butyrate kinase from *Thermotoga maritima*, and butyrate kinase from *Clostridium butyricum.*

21. A process according to any of claims 16 to 20, wherein methacrylyl-CoA is converted into methacrylic acid by the action of a thioesterase and the thioesterase is acyl-CoA thioesterase 4HBT from *Arthrobacter sp*. Strain SU.

22. A process according to any preceding claim, wherein the biological conversions of the process are conducted by enzymes in one or more host microorganism/s.

23. A microorganism for use in producing methacrylic acid and/or methacrylic acid esters according to a process comprising the following steps:
    (a) biologically converting isobutyryl-CoA into methacrylyl-CoA by the action of an acyl-CoA oxidase; and
    (b) biologically converting methacrylyl-CoA into methacrylic acid and/or methacrylic acid esters by the action of a 4-hydroxybenzoyl-CoA thioesterase (4HBT).

24. A recombinant microorganism adapted to conduct the following steps:
    (a) biologically converting isobutyryl-CoA into methacrylyl-CoA by expression of an acyl-CoA oxidase; and
    (b) biologically converting methacrylyl-CoA into methacrylic acid by expression of a 4-hydroxybenzoyl-CoA thioesterase (4HBT);
    wherein the recombinant microorganism is *Escherichia coli.*

25. A microorganism modified by one or more heterologous nucleic acids to conduct the following steps:
    (a) biologically converting isobutyryl-CoA into methacrylyl-CoA by expression of an acyl-CoA oxidase; and
    (b) biologically converting methacrylyl-CoA into methacrylic acid by expression of a 4-hydroxybenzoyl-CoA thioesterase (4HBT).

26. A microorganism adapted to conduct the following steps:
    (a) biologically converting isobutyryl-CoA into methacrylyl-CoA by expression of an acyl-CoA oxidase; and
    (b) biologically converting methacrylyl-CoA into methacrylic acid and/or methacrylic acid esters by expression of a 4-hydroxybenzoyl-CoA thioesterase (4HBT).

27. A microorganism according to claim 23 or 26, wherein the methacrylic acid esters are C1 to C20 alkyl esters, most preferably, C1 to C12 alkyl esters, especially C1 to C4 alkyl esters or C4 to C12 alkyl esters.

28. A process of production of methacrylic acid using a microorganism according to any of claims 23-27.

29. The microorganism according to any of claims 23-27, wherein the microorganism expresses the following enzymes:
    (a) ACX4; and

(b) 4HBT.

30. The microorganism according to any of claims 23-27 or 29, wherein the microorganism expresses the following enzymes:

(a) ACX4 from *Arabidopsis thaliana*; and

(b) 4HBT from *Arthrobacter sp*.

31. The microorganism according to any of claims 23-27 or 29-30, wherein the microorganism expresses the one or more enzymes endogenously or the microorganism expresses the one or more enzymes heterologously, or the microorganism expresses a combination of endogenous and heterologous enzymes.

32. The microorganism according to any of claims 23-27 or 29-31, wherein the microorganism may be selected from wild type or recombinant microorganism/s, for example, bacteria, archeae, yeast, fungus, algae or any of a variety of other microorganism/s applicable to fermentation processes.

33. The microorganism according to claim 32, wherein the microorganism is a bacterium selected from: enterobacteria belonging to proteobacteria of the genus *Escherichia, Enterobacter, Pantoea, Klebsiella, Serratia, Erwinia, Salmonella, Morganella,* or the like, so-called coryneform bacteria belonging to the genus *Brevibacterium, Corynebacterium* or *Microbacterium* and bacteria belonging to the genus *Alicyclobacillus, Bacillus, Hydrogenobacter, Methanococcus, Acetobacter, Acinetobacter, Agrobacterium, Axorhizobium, Azotobacter, Anaplasma, Bacteroides, Bartonella, Bordetella, Borrelia, Brucella, Burkholderia, Calymmatobacterium, Campylobacter, Chlamydia, Chlamydophila, Clostridium, Coxiella, Ehrlichia, Enterococcus, Francisella, Fusobacterium, Gardnerella, Haemophilus, Helicobacter, Kelbsiella, Methanobacterium, Micrococcus, Moraxella, Mycobacterium, Mycoplasma, Neisseria, Pasteurella, Peptostreptococcus, Porphyromonas, Prevotella, Pseudomonas, Rhizobium, Rickettsia, Rochalimaea, Rothia, Shigella, Staphylococcus, Stenotrophomonas, Streptococcus, Treponema, Vibrio, Wolbachia, Yersinia*.

34. The microorganism according to any of claims 23-27 or 29-33, wherein the microorganism is genetically modified to enhance production of methacrylic acid and/or methacrylic acid esters.

35. The microorganism according to any of claim 23-27 or 29-34, wherein the microorganism may be genetically modified by modifications which decrease or eliminate the activity of an enzyme that catalyses synthesis of a compound other than methacrylic acid and/or methacrylic acid esters by competing for the same substrates and/or intermediates, by modifications that decrease or eliminate the activity of an enzyme which metabolises methacrylic acid or metabolises an intermediate in the production of methacrylic acid, and/or by modifications which decrease or eliminate the activity of proteins involved in other cellular functions that remove intermediates in the production of methacrylic acid and/or methacrylic acid esters.

36. A microorganism according to any of claims 34 or 35, wherein the methacrylic acid esters are C1 to C20 alkyl esters, most preferably, C1 to C12 alkyl esters, especially C1 to C4 alkyl esters or C4 to C12 alkyl esters.

37. A process of fermentation comprising culturing one or more microorganism/s of any of claims 23-27 or 29-36 in a fermentation medium to produce methacrylic acid and/or methacrylic acid esters.

38. A process according to claim 37, wherein the methacrylic acid esters are C1 to C20 alkyl esters, most preferably, C1 to C12 alkyl esters, especially C1 to C4 alkyl esters or C4 to C12 alkyl esters.

39. A fermentation medium comprising one or more microorganism/s of any of claims 23-27 or 29-36.

40. A fermentation medium according to claim 39, wherein the medium further comprises methacrylic acid and/or methacrylic acid esters.

41. A fermentation medium according to claim 40, wherein the methacrylic acid esters are C1 to C20 alkyl esters, most preferably, C1 to C12 alkyl esters, especially C1 to C4 alkyl esters or C4 to C12 alkyl esters.

42. A bioreactor comprising one or more microorganism/s of any of claims 23-27 or 29-36 and/or the fermentation medium of any of claims 39-41.

43. A method of preparing polymers or copolymers of methacrylic acid or methacrylic acid esters, comprising the steps of:
    (i) preparation of methacrylic acid in accordance with any of claims 1-22 or 28;
    (ii) optional esterification of the methacrylic acid prepared in (i) to produce the methacrylic acid ester;
    (iii) polymerisation of the methacrylic acid prepared in (i) and/or, if present, the ester prepared in (ii), optionally with one or more comonomers, to produce polymers or copolymers thereof.

44. A method according to claim 43, wherein the methacrylic acid esters are C1 to C20 alkyl esters, most preferably, C1 to C12 alkyl esters, especially C1 to C4 alkyl esters or C4 to C12 alkyl esters.

45. A process of producing methacrylic acid comprising the following steps:
    (a) converting isobutyryl-CoA into methacrylyl-CoA by the action of an acyl-CoA oxidase; and
    (b) biologically converting methacrylyl-CoA into methacrylic acid by the action of a 4-hydroxybenzoyl-CoA thioesterase (4HBT), synthetase, and/or a phosphotransacylase and a short chain fatty acid kinase.

46. A microorganism for use in producing methacrylic acid according to a process comprising the following steps:

    (a) converting isobutyryl-CoA into methacrylyl-CoA by the action of an acyl-CoA oxidase; and

    (b) biologically converting methacrylyl-CoA into methacrylic acid by the action of a 4-hydroxybenzoyl-CoA thioesterase (4HBT).