

## CLAIMS

1. A composition suitable for use in the degradation of synthetic plastic polyalkene polymers, said composition comprising a KatG/EC 1.11.1.21 polyalkenase (PAase) enzyme and a suitable excipient.
2. A composition as claimed in claim 1, wherein the KatG/EC 1.11.1.21 PAase is a KatG enzyme.
3. A composition as claimed in claim 1 or 2, wherein the KatG enzyme comprises, consists of, or consists essentially of, the amino acid sequence of B2UBU5 KatG<sup>N</sup>, or a variant thereof which comprises, consists of, or consists essentially of, an amino acid sequence which is at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to B2UBU5 KatG<sup>N</sup>.
4. A composition as claimed in 1 or 2, wherein the KatG enzyme comprises, consists of, or consists essentially of, the amino acid sequence of B2UBU5, or a variant thereof which comprises, consists of, or consists essentially of, an amino acid sequence which is at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to B2UBU5.
5. A composition as claim 1 to 4, wherein the KatG enzyme is a B2UBU5 orthologue.
6. A composition as claimed in claim 5, wherein the B2UBU5 orthologue is selected from:
  - A0A1I5SK77 (A0A1I5SK77\_9RALS)
  - A0A0F0E7J1 (A0A0F0E7J1\_9BURK)
  - A0A0J9DTW2 (A0A0J9DTW2\_9RALS)
  - S9RSJ5 (S9RSJ5\_9RALS)
  - A0A191ZZS8 (A0A191ZZS8\_9RALS)
  - A0A5C6Y6C3 (A0A5C6Y6C3\_9RALS)
  - C6BD71 (C6BD71\_RALP1)
  - A0A2P4RN88 (A0A2P4RN88\_RALPI)
  - U3GIS7 (U3GIS7\_9RALS)
  - A0A1C0XEH7 (A0A1C0XEH7\_RALPI)
  - ROCJV6 (ROCJV6\_RALPI)
  - A0A2N4TPC1 (A0A2N4TPC1\_RALPI)
  - A0A2N5LD46 (A0A2N5LD46\_9RALS)
  - A0A291EI92 (A0A291EI92\_RALPI)
  - A0A401K9C7 (A0A401K9C7\_9RALS)

UPI00046A45B6

UPI000DD31867

UPI000CEF361E

UPI00073F3865

5 UPI00046AAE20

UPI0012AE63A9

UPI000D5F3903

UPI0004803C34

UPI000CEDCA44

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7. A composition as claimed in any one of claims 1 to 6, wherein the KatG enzyme is a hyperactive KatG enzyme.

8. A composition, of any one of claims 1 to 7, wherein the KatG enzyme is a non-catalatic KatG variant.

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9. A composition, of claim 8, wherein the non-catalatic KatG variant comprises a disrupted tripeptide adduct.

10. A composition of either claims 8 or 9, wherein the non-catalatic KatG variant is a non-catalatic B2UBU5 variant or a non-catalatic variant of any one of the orthologues of claim 6.

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11. A composition, of claims 8 to 10, wherein the non-catalatic KatG variant comprises one or more of the following amino acid substitutions: M248I, M248V, Y221F, Y221A, Y221L, W98A, W98F and/or R410N.

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12. A composition as claimed in any of claims 1 to 11, wherein the KatG enzyme is a single domain KatG enzyme.

13. A composition as claimed in claim 1 to 12 further comprising an enzyme capable of generating a peroxide.

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14. A composition as claimed in claim 1 or 13 further comprising a plastic binding agent.

15. A composition as claimed in claim 13, wherein the hydrogen peroxide generating enzyme is selected from the group; formate oxidase, formate dehydrogenase, formaldehyde dismutase, methanol oxidase or combinations thereof.

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16. A composition as claimed in any preceding claim, further comprising one or more adjunctive plasticase enzymes.
- 5 17. A composition as claimed in claim 16, wherein the adjunctive plasticase enzyme is selected from the group: (a) a laccase (EC 1.10.3.2.); (b) a manganese peroxidase (MnP, EC 1.11.1.13); (c) a lignin peroxidase (LiP, EC 1.11.1.14); and (d) combinations of two or more of the foregoing.
- 10 18. A composition as claimed in claim 17, wherein said one or more adjunctive plasticase enzymes comprise a complementary plasticase.
- 15 19. A composition as claimed in claim 18, wherein the complementary plasticase is selected from: (a) a laccase (EC 1.10.3.2.); (b) a manganese peroxidase (MnP, EC 1.11.1.13); (c) a lignin peroxidase (LiP, EC 1.11.1.14); (d) a versatile peroxidase; (e) a protease; (f) a lipase; (g) a carboxylesterases; (h) an esterase; and (i) combinations of two or more of the foregoing.
- 20 20. A composition of claim 19, wherein complementary plasticase comprises a cutinase and/or a PETase.
21. A composition of any one of the preceding claims, further comprising a waste remediation enzyme cocktail comprising one or more ancillary non-plasticase bioremediation enzymes.
- 25 22. A composition of claim 21, wherein said one or more ancillary bioremediation enzymes are selected from: (a) oxidoreductases; (b) oxygenases; (c) monooxygenases; (d) dioxygenases; (e) laccases; (f) peroxidases; (g) a hydrolase; (h) a peroxygenase, for example from *Agrocybe aegerita*; and (i) combinations of two or more of the foregoing.
- 30 23. A composition of claim 21 or claim 22, wherein the ancillary bioremediation enzymes comprise a hydrolase selected from: (a) a lipase; (b) a cellulase; (c) a protease; and (d) combinations of two or more of the foregoing.
24. A composition of any one of the preceding claims, further comprising one or more ancillary cellulose, hemicellulose and/or ligninase enzymes.
- 35 25. A composition of any one of the preceding claims, further comprising one or more cofactors.

26. A composition of claim 25, wherein said one or more cofactors comprises haem and/or iron and/or flavin mononucleotide (FMN) and/or FAD(H<sub>2</sub>) and/or NAD(H).
27. A composition of any preceding claim, wherein one or more the enzymes of the composition are produced in situ, by a recombinant host expressing and secreting said enzyme.
28. A composition as claimed in claim 27, wherein two or more enzymes are produced by expression and secretion from the host.
29. A composition as claimed in claim 27 or 28, wherein the host is selected from: *Escherichia coli*; *Bacillus subtilis*; *Bacillus megaterium*; *Bacillus licheniformis*; *Lactococcus lactis*; *Ralstonia eutropha*; *Pseudomonas* spp; *Ralstonia pickettii*; *Corynebacterium* spp; *Saccharomyces cerevisiae*; *Hansenula polymorpha*; *Komagatella phaffii*; *Candida biodini*; *Schizosaccharomyces pombe* a filamentous fungus, for example *Aspergillus* spp. and *Trichoderma* spp., and *Myceliophthora thermophile*, optionally in the form of a C1 expression system.
30. A composition of any one of the preceding claims, further comprises one or more microorganisms selected from the following genera: *Achromobacter*, *Acinetobacter*, *Actinomadura*, *Alcaligenes*, *Alcanivorax*, *Amycolatopsis*, *Aneurinibacillus*, *Archaeoglobus*, *Aromatoleum*, *Arthrobacter*, *Aspergillus*, *Azoarcus*, *Actinomadura*, *Bionectria*, *Isaria*, *Bacillus*, *Pseudomonas*, *Fusarium*, *Beauveria*, *Brevibacillus*, *Brevibacterium*, *Brevundimonas*, *Candida*, *Chaetomium*, *Citrobacter*, *Cladosporium*, *Comamonas*, *Coriolus*, *Coryneformes*, *Corynebacterium*, *Cunninghamella*, *Curvularia*, *Cycloclasticus*, *Delftia*, *Desulfococcus*, *Desulfosarcina*, *Dictyoglomus*, *Diplococcus*, *Engyodontium*, *Enterobacter*, *Exiguobacterium*, *Flavobacterium*, *Gliocladium*, *Gordonia*, *Halomonas*, *Hansenula*, *Kibdelosporangium*, *Klebsiella*, *Kluyveromyces*, *Leptothrix*, *Listeria*, *Marinobacter*, *Microbacterium*, *Micrococcus*, *Moraxella*, *Mortierella*, *Mucor*, *Mycobacterium*, *Nocardia*, *Ochrobactrum*, *Oleispira*, *Paecylomyces*, *Paenibacillus*, *Penicillium*, *Phanerochaete*, *Pleurotus*, *Proteobacterium*, *Proteus*, *Pseudomonas*, *Pseudozyma*, *Pullularia*, *Rahnella*, *Ralstonia*, *Rhizopus*, *Rhodococcus*, *Sphingomonas*, *Saccharomyces*, *Serratia*, *Sphingobacterium*, *Sphingomonas*, *Streptomyces*, *Staphylococcus*, *Stenotrophomonas*, *Streptococcus*, *Talaromyces*, *Thalassolituus*, *Thermomonospora*, *Trametes*, *Trichoderma*, *Tritirachium*, *Vibrio*, *Xanthomonas* and combinations of two or more of the foregoing.
31. A composition as claimed in any preceding claim, wherein one or more of the enzymes are immobilised on a support.
32. A composition as claimed in any preceding claim, wherein the KatG enzyme is B2UBU5 or M1FBG9.

33. A composition as claimed in any preceding claim in the form of an aqueous solution, emulsion, suspension or dispersion.
- 5 34. A composition as claimed in any preceding claim for use in the degradation of a PA polymer.
35. A composition as claimed in claim any preceding claim for use in the bioremediation of material contaminated with a PA polymer.
- 10 36. A method for the enzymatic degradation of a PA polymer, the method comprising contacting the PA polymer with a composition of any one of claims 1 to 33.
37. A method as claimed in claim 35, wherein the enzymatic degradation of the PA polymer occurs in a reactor.
- 15 38. A method for producing fragments, oligomers, isolated monomers or repeating units, monomer or repeating unit fragments and/or monomer or repeating unit derivatives of a PA polymer, the method comprising the step of degrading said polymer by contacting it with a composition according to any of claims 1 to 33.
- 20 39. A method for producing a microbial biomass comprising the steps of: (a) providing a seed culture comprising at least one microorganism expressing a KatG/EC 1.11.1.21 PAase; (b) providing a feedstock comprising a PA polymer; (c) mixing said seed culture with said feedstock to form a seeded feedstock; and (d) incubating the seeded feedstock under conditions whereby C-C bonds in the PA polymer are cleaved to produce degradation products of said polymer which are metabolized by said microorganism to yield said microbial biomass.
- 25 40. A method for producing a bioproduct comprising the steps of: (a) providing a seed culture comprising at least one microorganism expressing a KatG/EC 1.11.1.21 PAase; (b) providing a feedstock comprising a PA polymer; (c) mixing said seed culture with said feedstock to form a seeded feedstock; and (d) incubating the seeded feedstock under conditions whereby C-C bonds in the PA polymer are cleaved by said enzyme to produce degradation products of said polymer which are metabolized by said microorganism to yield said bioproduct.
- 30 41. A method of claim 40, wherein the bioproduct comprises a biofuel.
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42. A method of claim 40 or 41, wherein the bioproduct comprises one or more carboxylic acids, aldehydes, or dicarboxylic acids.
43. A method of claims 40 to 42, wherein the bioproduct comprises one or more compounds selected from the group: oxalic (ethanedioic), malonic (propanedioic), succinic (butanedioic), glutaric (pentanedioic), adipic(hexanedioic), pimelic (heptanedioic), suberic (octanedioic), azelaic (nonanedioic), sebacic (decanedioic), undecanedioic and dodecanedioic acids, glycolic acid, glyoxylic acid, citric acid, furoic acid and phenylpyruvic acids.
44. A method as claimed in claim 40 to 43, wherein the PA polymer is pre-treated abiotically.
45. A method as claimed in claim 44, wherein the abiotic pre-treatment is selected from mechanical, physical and chemical treatments or a combination of two or more thereof.
46. Use of a composition as claimed in any of claims 1 to 33, for the degradation of a PA polymer.
47. Use of a composition as claimed in any of claims 1 to 33, for producing fragments, oligomers, isolated monomers or repeating units, monomer or repeating unit fragments and/or monomer or repeating unit derivatives of a PA polymer.
48. Use of a composition as claimed in any of claims 1 to 33, for the bioremediation of material contaminated with a PA polymer.
49. A KatG prodegradant comprising a KatG/EC 1.11.1.21 polyalkenase enzyme (and/or a microorganism expressing and/or secreting a KatG/EC 1.11.1.21 polyalkenase enzyme), for use as a biotic prodegradant plastic additive, optionally wherein the PAase is present in the form of an inactive proenzyme.
50. A composite KatG plastic comprising a plastic polymer in combination with the KatG prodegradant of claim 50.
51. An autodegradative PA polymer comprising a PA polymer in combination with the KatG prodegradant of claim 50.
52. A composite KatG plastic or autodegradative polymer of claim 51 or 52, wherein the KatG prodegradant is contained within or disposed on the surface of the polymer.

53. A plastic article comprising the KatG prodegradant, composite KatG plastic or autodegradative PA polymer of any one of claims 51 to 53.
- 5 54. A KatG EC 1.11.1.21 enzyme comprising one or more of the following amino acid substitutions: M248I, M248V, Y221F, Y221A, Y221L, W98A, W98F and/or R410N.
55. A composition as claimed herein wherein the KatG/EC1.11.1.21 enzyme is the enzyme of claim 53.