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(54) **SECRETION OF FATTY ACIDS BY PHOTOSYNTHETIC MICROORGANISMS**

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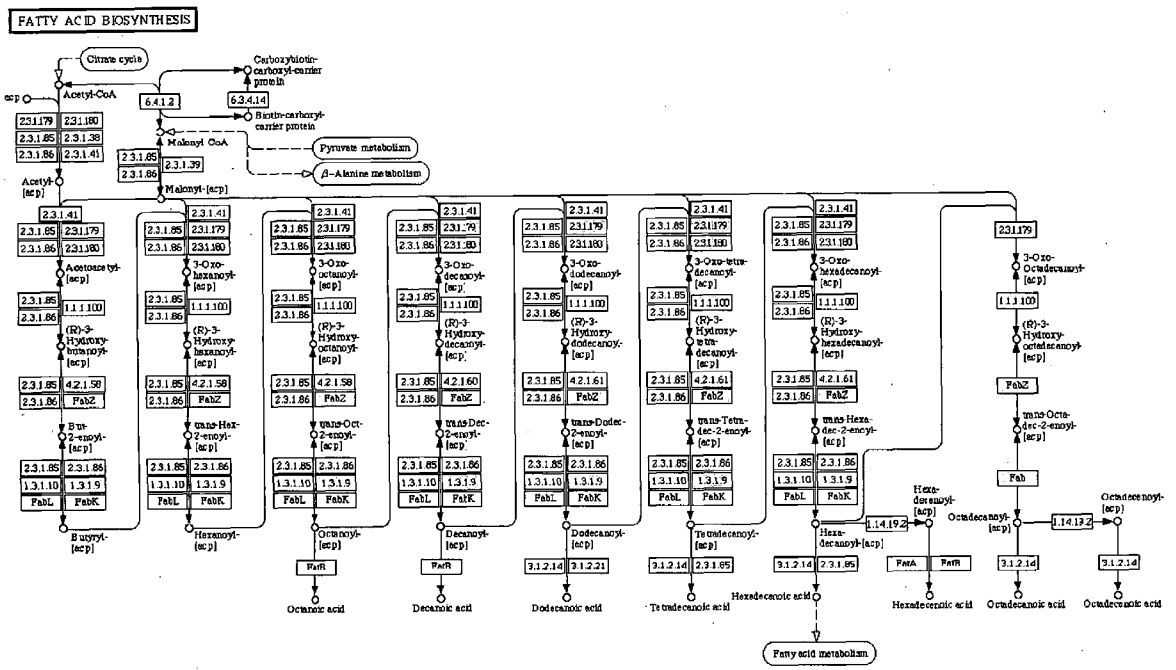
(57) **ABSTRACT**

Recombinant photosynthetic microorganisms that convert inorganic carbon to secreted fatty acids are described. Methods to recover the secreted fatty acids from the culture medium without the need for cell harvesting are also described.



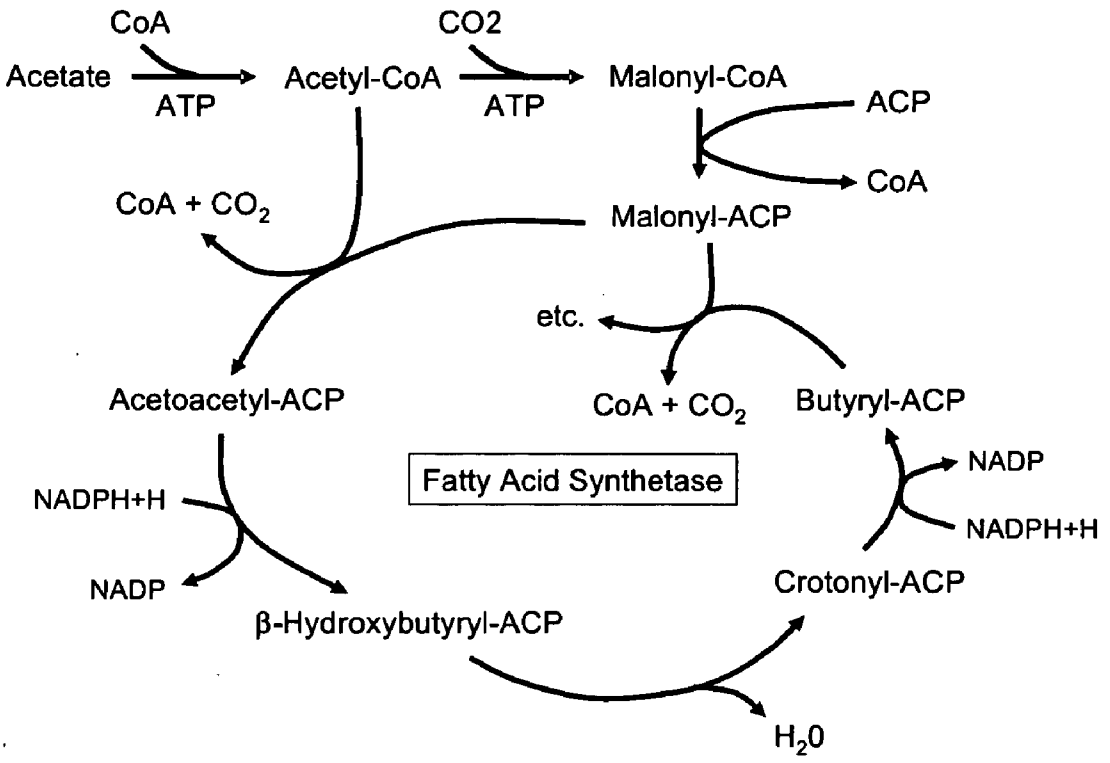

CoA
CO2
Acetate
ATP
Acetyl-CoA
ATP
Malonyl-CoA
ACP
CoA
CoA + CO_2
Malonyl-ACP
etc.
Acetoacetyl-ACP
CoA + CO_2
Butyryl-ACP
Fatty Acid Synthetase
NADPH+H
NADP
NADP
NADPH+H
Crotonyl-ACP
β-Hydroxybutyryl-ACP
H_20

Figure 1

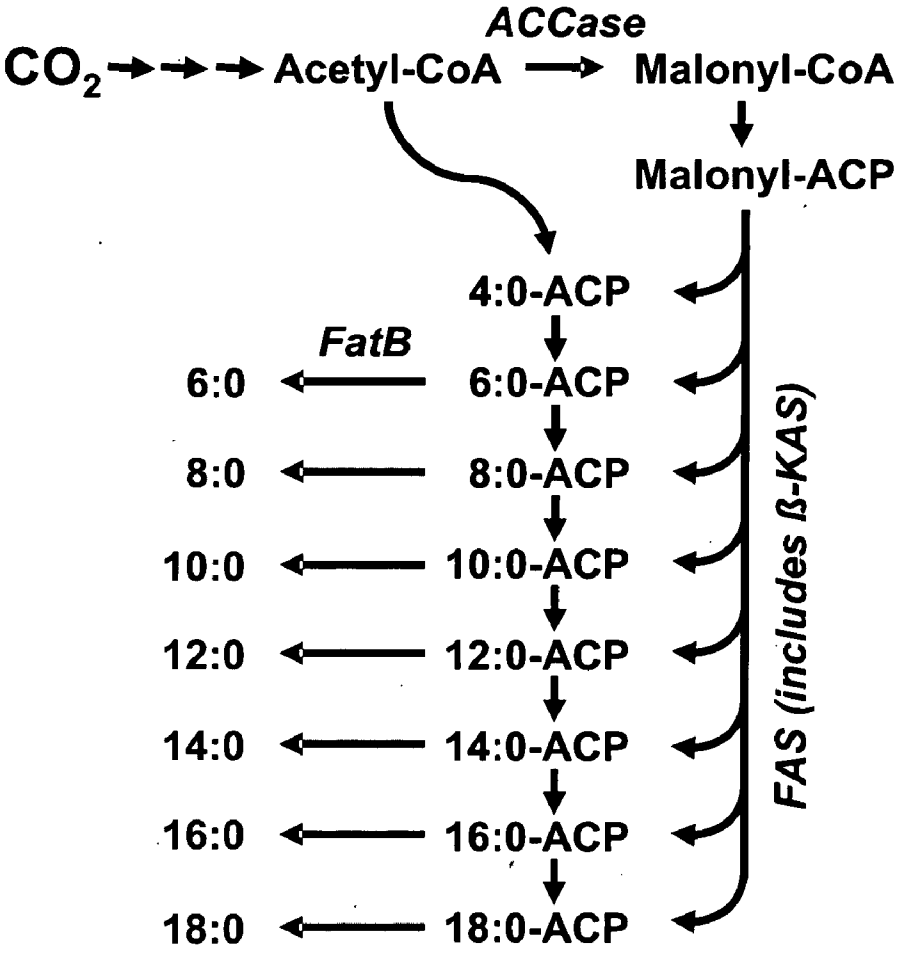

ACCase
CO2
Acetyl-CoA
Malonyl-CoA
Malonyl-ACP
4:0-ACP
FatB
6:0
6:0-ACP
8:0
8:0-ACP
10:0
10:0-ACP
12:0
12:0-ACP
14:0
14:0-ACP
16:0
16:0-ACP
18:0
18:0-ACP
FAS (includes ß-KAS)

Figure 2

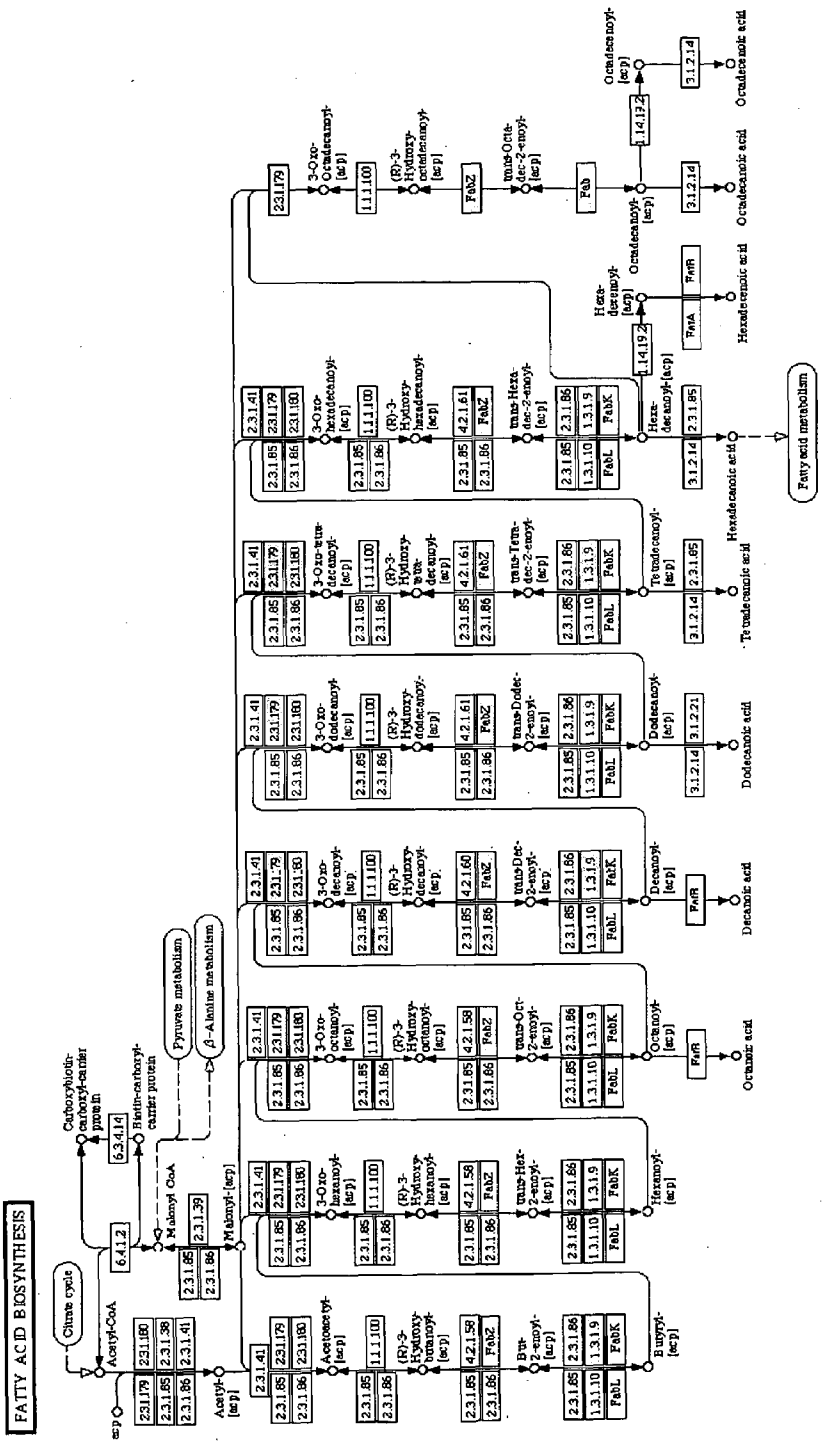
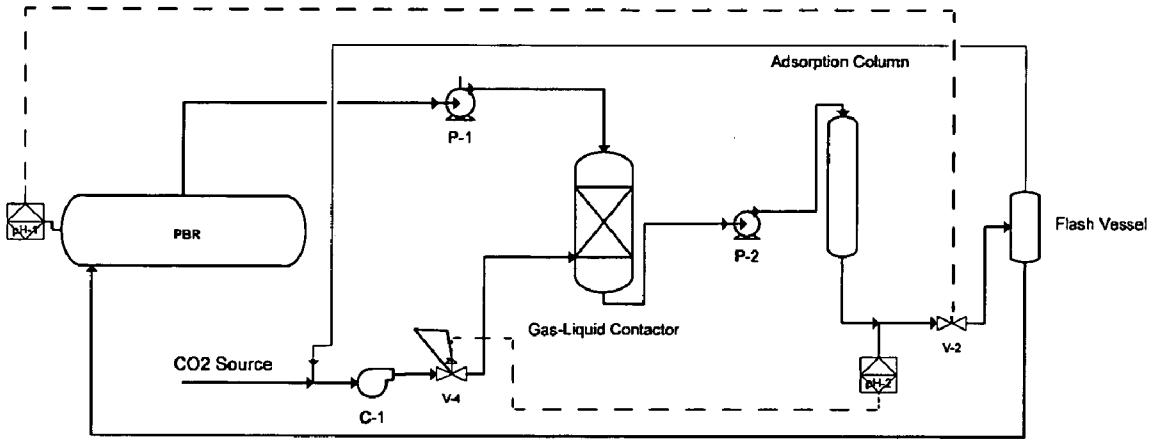
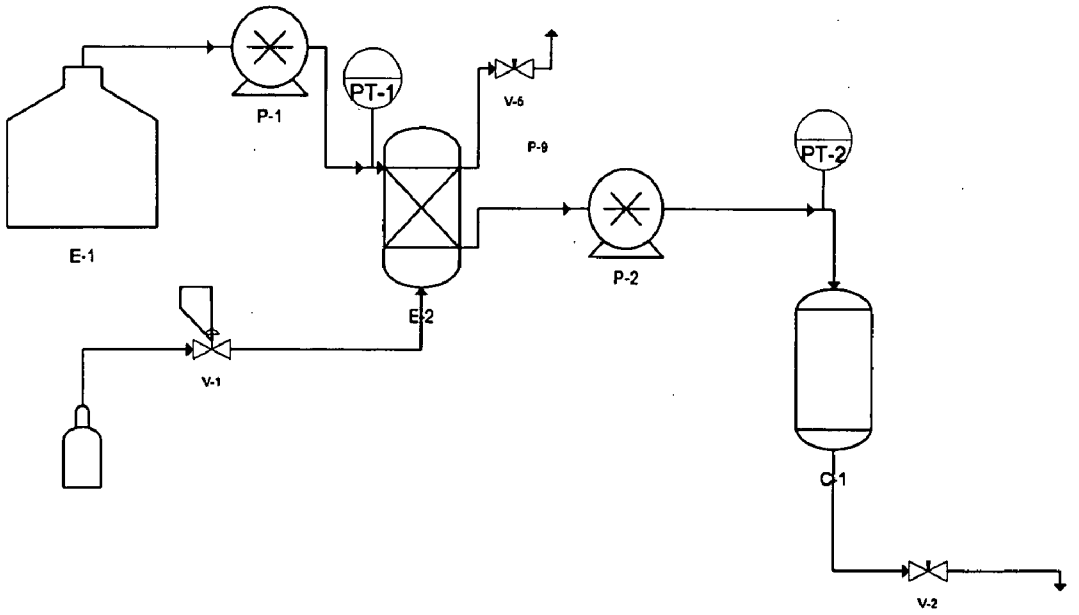

FATTY ACID BIOSYNTHESIS
Citrate cycle
Pyruvate metabolism
β-Alanine metabolism
Fatty acid metabolism
Octanoic acid
Decanoic acid
Dodecanoic acid
Tetradecanoic acid
Hexadecenoic acid
Hexadecanoic acid
Octadecenoic acid
Octadecanoic acid
FabH
FabG
FabZ
FabI
FabK
FabL
FabA
FabB
FabF
FabR

Figure 3



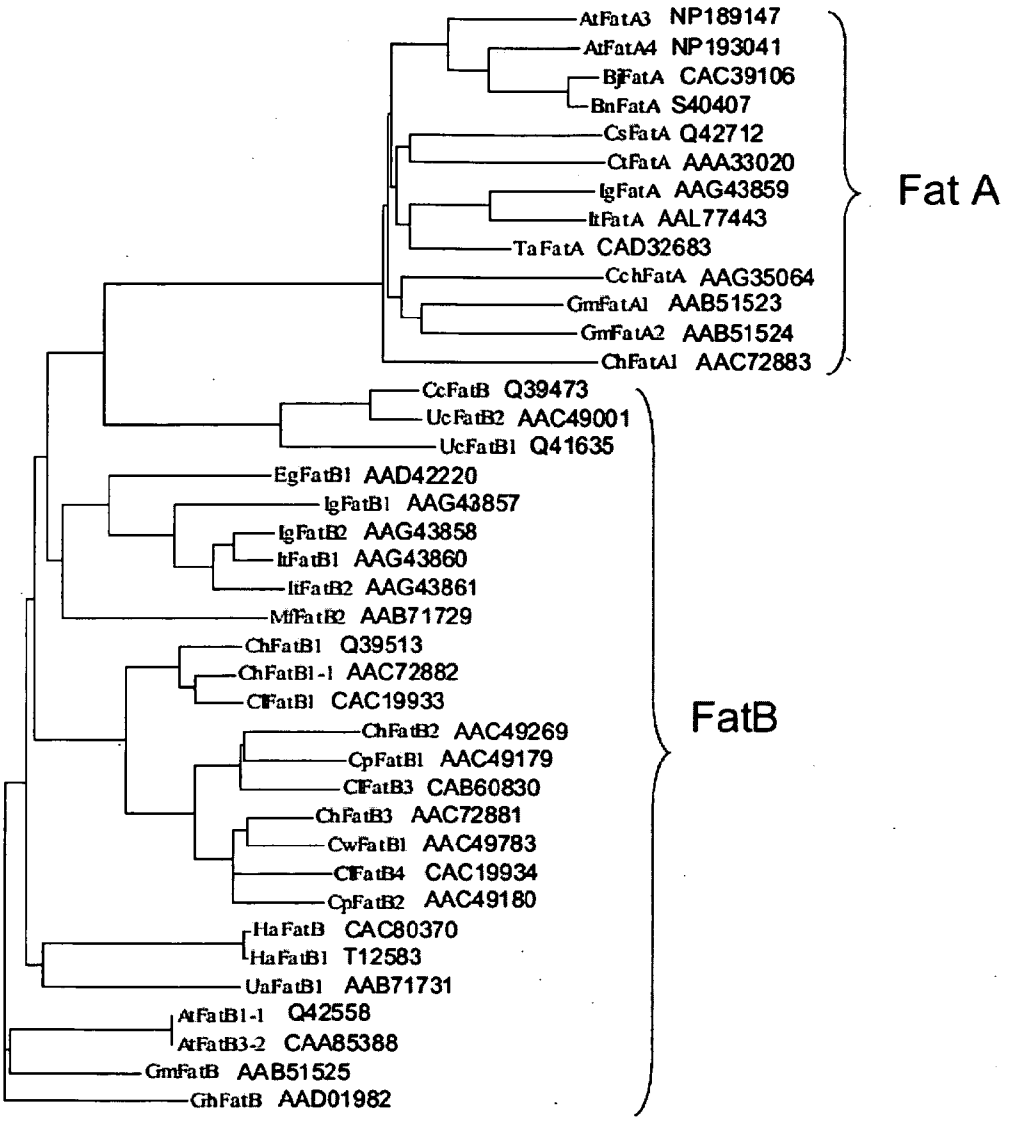
Integrated CO_2 delivery and product recovery schematic.

Figure 4



CO_2-mediated acidification of hydrophobic adsorption column load

Figure 5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AtFatA4 NP193041
BjFatA CAC39106
BnFatA S40407
CsFatA Q42712
CtFatA AAA33020
IgFatA AAG43859
ItFatA AAL77443
TaFatA CAD32683
CchFatA AAG35064
GmFatA1 AAB51523
GmFatA2 AAB51524
ChFatA1 AAC72883
Fat A
CcFatB Q39473
UcFatB2 AAC49001
UcFatB1 Q41635
EgFatB1 AAD42220
IgFatB1 AAG43857
IgFatB2 AAG43858
ItFatB1 AAG43860
ItFatB2 AAG43861
MfFatB2 AAB71729
ChFatB1 Q39513
ChFatB1-1 AAC72882
CtFatB1 CAC19933
ChFatB2 AAC49269
CpFatB1 AAC49179
CtFatB3 CAB60830
ChFatB3 AAC72881
CwFatB1 AAC49783
CtFatB4 CAC19934
CpFatB2 AAC49180
HaFatB CAC80370
HaFatB1 T12583
UaFatB1 AAB71731
AtFatB1-1 Q42558
AtFatB3-2 CAA85388
GmFatB AAB51525
GhFatB AAD01982
FatB

Figure 6

SECRETION OF FATTY ACIDS BY PHOTOSYNTHETIC MICROORGANISMS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of provisional application 61/007,333 filed 11 Dec. 2007. The contents of this application are incorporated herein by reference.

TECHNICAL FIELD

[0002] This invention relates to photosynthetic microorganisms that convert inorganic carbon to fatty acids and secrete them into the culture medium, methods of production of fatty acids using such organisms, and uses thereof. The fatty acids may be used directly or may be further modified to alternate forms such as esters, reduced forms such as alcohols, or hydrocarbons, for applications in different industries, including fuels and chemicals.

BACKGROUND ART

[0003] Photosynthetic microorganisms, including eukaryotic algae and cyanobacteria, contain various lipids, including polar lipids and neutral lipids. Polar lipids (e.g., phospholipids, glycolipids, sulfolipids) are typically present in structural membranes whereas neutral lipids (e.g., triacylglycerols, wax esters) accumulate in cytoplasmic oil bodies or oil globules. A substantial research effort has been devoted to the development of methods to produce lipid-based fuels and chemicals from photosynthetic microorganisms. Typically, eukaryotic microalgae are grown under nutrient-replete conditions until a certain cell density is achieved, after which the cells are subjected to growth under nutrient-deficient conditions, which often leads to the accumulation of neutral lipids. The cells are then harvested by various means (e.g., settling, which can be facilitated by the addition of flocculants, followed by centrifugation), dried, and then the lipids are extracted from the cells by the use of various non-polar solvents. Harvesting of the cells and extraction of the lipids are cost-intensive steps. It would be desirable to obtain lipids from photosynthetic microorganisms without the requirement for cell harvesting and extraction.

[0004] PCT publication numbers WO2007/136762 and WO2008/119082 describe the production of biofuel components using microorganisms. These documents disclose the production by these organisms of fatty acid derivatives which are, apparently, short and long chain alcohols, hydrocarbons, fatty alcohols and esters including waxes, fatty acid esters or fatty esters. To the extent that fatty acid production is described, it is proposed as an intermediate to these derivatives, and the fatty acids are therefore not secreted. Further, there is no disclosure of converting inorganic carbon directly to secreted fatty acids using a photosynthetic organism grown in a culture medium containing inorganic carbon as the primary carbon source. The present invention takes advantage of the efficiency of photosynthetic organisms in secreting fatty acids into the medium in order to recover these valuable compounds.

[0005] The invention includes the expression of heterologous acyl-ACP thioesterase (TE) genes in photosynthetic microbes. Many of these genes, along with their use to alter lipid metabolism in oilseeds, have been described previously. Genes encoding the proteins that catalyze various steps in the synthesis and further metabolism of fatty acids have also been extensively described.

[0006] The two functional classes of plant acyl-ACP thioesterases (unsaturated fatty acid-recognizing FatA versus saturated fatty acid-recognizing FatB) can be clustered based on amino acid sequence alignments as well as function. FatAs show marked preference for 18:1-ACP with minor activity towards 18:0- and 16:0-ACPs, and FatBs hydrolyze primarily saturated acyl-ACPs with chain lengths that vary between 8-16 carbons. Several studies have focused on engineering plant thioesterases with perfected or altered substrate specificities as a strategy for tailoring specialty seed oils.

[0007] As shown in FIG. **1**, fatty acid synthetase catalyzes a repeating cycle wherein malonyl-acyl carrier protein (ACP) is condensed with a substrate, initially acetyl-CoA, to form acetoacetyl-ACP, liberating CO_2. The acetoacetyl-ACP is then reduced, dehydrated, and reduced further to butyryl-ACP which can then itself be condensed with malonyl-ACP, and the cycle repeated, adding a 2-carbon unit at each turn. The production of free fatty acids would therefore be enhanced by a thioesterase that would liberate the fatty acid itself from ACP, breaking the cycle. That is, the acyl-ACP is prevented from reentering the cycle. Production of the fatty acid would also be encouraged by enhancing the levels of fatty acid synthetase and inhibiting any enzymes which result in degradation or further metabolism of the fatty acid.

[0008] FIG. **2** presents a more detailed description of the sequential formation of acyl-ACPs of longer and longer chains. As shown, the thioesterase enzymes listed in FIG. **2** liberate the fatty acid from the ACP thioester.

[0009] Taking advantage of this principle, Dehesh, K., et al., *The Plant Journal* (1996) 9:167-172, describe "Production of high levels of octanoic (8:0) and decanoic (10:0) fatty acids in transgenic canola by overexpression of ChFatB2, a thioesterase cDNA from *Cuphea hookeriana*." Dehesh, K., et al., *Plant Physiology* (1996) 110:203-210, and report "Two novel thioesterases are key determinants of the bimodal distribution of acyl chain length of *Cuphea palustris* seed oil."

[0010] Voelker, T., et al., *Science* (1992) 257:72-74, describe "Fatty acid biosynthesis redirected to medium chains in transgenic oilseed plants." Voelker, T., and Davies, M., *Journal of Bacteriology* (1994) 176:7320-7327, describe "Alteration of the specificity and regulation of fatty acid synthesis of *Escherichia coli* by expression of a plant medium-chain acyl-acyl carrier protein thioesterase."

DISCLOSURE OF THE INVENTION

[0011] The present invention is directed to the production of recombinant photosynthetic microorganisms that are able to secrete fatty acids derived from inorganic carbon into the culture medium. Methods to remove the secreted fatty acids from the culture medium without the need for cell harvesting are also provided. It is anticipated that these improvements will lead to lower costs for producing lipid-based fuels and chemicals from photosynthetic microorganisms. In addition, this invention enables the production of fatty acids of defined chain length, thus allowing their use in the formulation of a variety of different products, including fuels and chemicals.

[0012] Carbon dioxide (which, along with carbonic acid, bicarbonate and/or carbonate define the term "inorganic carbon") is converted in the photosynthetic process to organic compounds. The inorganic carbon source includes any way of delivering inorganic carbon, optionally in admixture with any

other combination of compounds which do not serve as the primary carbon feedstock, but only as a mixture or carrier (for example, emissions from biofuel (e.g., ethanol) plants, power plants, petroleum-based refineries, as well as atmospheric and subterranean sources).

[0013] One embodiment of the invention relates to a culture of recombinant photosynthetic microorganisms, said organisms comprising at least one recombinant expression vector encoding at least one exogenous acyl-ACP thioesterase, wherein the at least one exogenous acyl-ACP thioesterase preferentially liberates fatty acid chains containing 6 to 20 carbons from these ACP thioesters. The fatty acids are formed from inorganic carbon as their carbon source and the culture contains substantially only inorganic carbon as a carbon source. The presence of the exogenous thioesterase will increase the secretion levels of desired fatty acids by at least 2-4 fold.

[0014] Specifically, in one embodiment, the invention is directed to a cell culture of a recombinant photosynthetic microorganism where the microorganism has been modified to contain a nucleic acid molecule comprising at least one recombinant expression system that produces at least one exogenous acyl-ACP thioesterase, wherein said acyl-ACP thioesterase preferentially liberates a fatty acid chain that contains 6-20 carbons, and wherein the culture medium provides inorganic carbon as substantially the sole carbon source and wherein said microorganism secretes the fatty acid liberated by the acyl-ACP thioesterase into the medium. In alternative embodiments, the thioesterase preferentially liberates a fatty acid chain that contains 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 carbons.

[0015] In other aspects, the invention is directed to a method to produce fatty acids of desired chain lengths by incubating these cultures and recovering these secreted fatty acids from the cultures. In one embodiment, the recovery employs solid particulate adsorbents to harvest the secreted fatty acids. The fatty acids thus recovered can be further modified synthetically or used directly as components of biofuels or chemicals.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] FIG. **1** is a diagram of the pathway of fatty acid synthesis as is known in the art.

[0017] FIG. **2** is a more detailed diagram of the synthesis of fatty acids of multiple chain lengths as is known in the art.

[0018] FIG. **3** is an enzymatic overview of fatty acid biosynthesis identifying enzymatic classes for the production of various chain length fatty acids.

[0019] FIG. **4** is a schematic diagram of a recovery system for fatty acids from the medium.

[0020] FIG. **5** shows an experimental system based on the principles in FIG. **4**.

[0021] FIG. **6** shows representative acyl-ACP thioesterase from a variety of organisms.

MODES OF CARRYING OUT THE INVENTION

[0022] The present invention provides photosynthetic microorganisms that secrete fatty acids into the culture medium, along with methods to adsorb the fatty acids from the culture medium and collect them for processing into fuels and chemicals. The invention thereby eliminates or greatly reduces the need to harvest and extract the cells, resulting in substantially reduced production costs.

[0023] FIG. **2** is an overview of one aspect of the invention. As shown in FIG. **2**, carbon dioxide is converted to acetyl-CoA using the multiple steps in the photosynthetic process. The acetyl-CoA is then converted to malonyl-CoA by the action of acetyl-CoA carboxylase. The malonyl-CoA is then converted to malonyl-ACP by the action of malonyl-CoA: ACP transacylase which, upon progressive action of fatty acid synthetase, results in successive additions of two carbon units. In one embodiment of the invention, the process is essentially halted at carbon chain lengths of 6 or 8 or 10 or 12 or 14 or 16 or 18 carbons by supplying the appropriate thioesterase (shown in FIG. **2** as FatB). To the extent that further conversions to longer chain fatty acids occur in this embodiment, the cell biomass can be harvested as well. The secreted fatty acids can be converted to various other forms including, for example, methyl esters, alkanes, alkenes, alpha-olefins and fatty alcohols.

[0024] Thioesterases (Acyl-ACP TEs)

[0025] In order to effect secretion of the free fatty acids, the organism is provided at least one expression system for at least one thioesterase that operates preferentially to liberate fatty acids of the desired length. Many genes encoding such thioesterases are available in the art. Some of these are subjects of U.S. patents as follows:

[0026] Examples include U.S. Pat. No. 5,298,421, entitled "Plant medium-chain-preferring acyl-ACP thioesterases and related methods," which describes the isolation of an acyl-ACP thioesterase and the gene that encodes it from the immature seeds of *Umbellularia californica*. Other sources for such thioesterases and their encoding genes include U.S. Pat. No. 5,304,481, entitled "Plant thioesterase having preferential hydrolase activity toward C12 acyl-ACP substrate," U.S. Pat. No. 5,344,771, entitled "Plant thioesterases," U.S. Pat. No. 5,455,167, entitled "Medium-chain thioesterases in plants," U.S. Pat. No. 5,512,482, entitled "Plant thioesterases," U.S. Pat. No. 5,530,186, entitled "Nucleotide sequences of soybean acyl-ACP thioesterase genes," U.S. Pat. No. 5,639,790, entitled "Plant medium-chain thioesterases," U.S. Pat. No. 5,667,997, entitled "C8 and C10 medium-chain thioesterases in plants," U.S. Pat. No. 5,723,761, entitled "Plant acyl-ACP thioesterase sequences," U.S. Pat. No. 5,807,893, entitled "Plant thioesterases and use for modification of fatty acid composition in plant seed oils," U.S. Pat. No. 5,850,022, entitled "Production of myristate in plant cells," U.S. Pat. No. 5,910,631, entitled "Middle chain-specific thioesterase genes from *Cuphea lanceolata*," U.S. Pat. No. 5,945,585, entitled "Specific for palmitoyl, stearoyl and oleoyl-alp thioesters nucleic acid fragments encoding acyl-ACP thioesterase enzymes and the use of these fragments in altering plant oil composition," U.S. Pat. No. 5,955,329, entitled "Engineering plant thioesterases for altered substrate specificity," U.S. Pat. No. 5,955,650, entitled "Nucleotide sequences of canola and soybean palmitoyl-ACP thioesterase genes and their use in the regulation of fatty acid content of the oils of soybean and canola plants," and U.S. Pat. No. 6,331,664, entitled "Acyl-ACP thioesterase nucleic acids from maize and methods of altering palmitic acid levels in transgenic plants therewith."

[0027] Others are described in the open literature as follows:

[0028] Dörmann, P. et al., *Planta* (1993) 189:425-432, describe "Characterization of two acyl-acyl carrier protein thioesterases from developing *Cuphea* seeds specific for medium-chain and oleoyl-acyl carrier protein." Dörmann, P.,

et al., *Biochimica Biophysica Acta* (1994) 1212:134-136, describe "Cloning and expression in *Escherichia coli* of a cDNA coding for the oleoyl-acyl carrier protein thioesterase from coriander (*Coriandrum sativum* L.)." Filichkin, S., et al., *European Journal of Lipid Science and Technology* (2006) 108:979-990, describe "New FATB thioesterases from a high-laurate *Cuphea* species: Functional and complementation analyses." Jones, A., et al., *Plant Cell* (1995) 7:359-371, describe "Palmitoyl-acyl carrier protein (ACP) thioesterase and the evolutionary origin of plant acyl-ACP thioesterases." Knutzon, D. S., et al., *Plant Physiology* (1992) 100:1751-1758, describe "Isolation and characterization of two safflower oleoyl-acyl carrier protein thioesterase cDNA clones." Slabaugh, M., et al., *The Plant Journal* (1998)13:611-620, describe "Condensing enzymes from *Cuphea wrightii* associated with medium chain fatty acid biosynthesis."

[0029] Additional genes, not previously isolated, that encode these acyl-ACP TEs can be isolated from plants that naturally contain large amounts of medium-chain fatty acids in their seed oil, including certain plants in the Lauraceae, Lythraceae, Rutaceae, Ulmaceae, and Vochysiaceae families. Typically, the fatty acids produced by the seeds of these plants are esterified to glycerol and retained inside the cells. The seeds containing the products can then be harvested and processed to isolate the fatty acids. Other sources of these enzymes, such as bacteria may also be used.

[0030] The known acyl-ACP TEs from plants can be divided into two main classes, based on their amino acid sequences and their specificity for acyl-ACPs of differing chain lengths and degrees of unsaturation. The "FatA" type of plant acyl-ACP TE has preferential activity on oleoyl-ACP, thereby releasing oleic acid, an 18-carbon fatty acid with a single double bond nine carbons distal to the carboxyl group. The "FatB" type of plant acyl-ACP TE has preferential activity on saturated acyl-ACPs, and can have broad or narrow chain length specificities. For example, FatB enzymes from different species of *Cuphea* have been shown to release fatty acids ranging from eight carbons in length to sixteen carbons in length from the corresponding acyl-ACPs. Listed below in Table 1 are several plant acyl-ACP TEs along with their substrate preferences. (Fatty acids are designated by standard shorthand notation, wherein the number preceding the colon represents the acyl chain length and the number after the colon represents the number of double bonds in the acyl chain.)

TABLE 1

Plant Acyl-ACP Thioesterase	
Garcinia mangostana FatA	18:1 and 18:0
Carthamus tinctorius FatA	18:1
Coriandrum sativum FatA	18:1
Cuphea hookeriana FatB1	16:0
Cuphea hookeriana FatB2	8:0 and 10:0
Cuphea wrightii FatB1	12:0 to 16:0
Cuphea palustris FatB1	8:0 and 10:0
Cuphea palustris FatB2	14:0 and 16:0
Cuphea calophylla FatB1	12:0 to 16:0
Umbellularia californica FatB1	12:0
Ulmus americana FatB1	8:0 and 10:0

[0031] The enzymes listed in Table 1 are exemplary and many additional genes encoding acyl-ACP TEs can be isolated and used in this invention, including but not limited to genes such as those that encode the following acyl-ACP TEs (referred to by GenPept Accession Numbers):

[0032] CAA52069.1, CAA52070.1, CAA54060.1, CAA85387.1, CAA85388.1, CAB60830.1, CAC19933.1, CAC19934.1, CAC39106.1, CAC80370.1, CAC80371.1, CAD32683.1, CAL50570.1, CAN60643.1, CAN81819.1, CAO17726.1, CAO42218.1, CAO65585.1, CAO68322.1, AAA33019.1, AAA33020.1, AAB51523.1, AAB51524.1, AAB51525.1, AAB71729.1, AAB71730.1, AAB71731.1, AAB88824.1, AAC49001.1, AAC49002.1, AAC49179.1, AAC49180.1, AAC49269.1, AAC49783.1, AAC49784.1, AAC72881.1, AAC72882.1, AAC72883.1, AAD01982.1, AAD28187.1, AAD33870.1, AAD42220.2, AAG35064.1, AAG43857.1, AAG43858.1, AAG43859.1, AAG43860.1, AAG43861.1, AAL15645.1, AAL77443.1, AAL77445.1, AAL79361.1, AAM09524.1, AAN17328.1, AAQ08202.1, AAQ08223.1, AAX51636.1, AAX51637.1, ABB71579.1, ABB71581.1, ABC47311.1, ABD83939.1, ABE01139.1, ABH11710.1, ABI18986.1, ABI20759.1, ABI20760.1, ABL85052.1, ABU96744.1, EAY74210.1, EAY86874.1, EAY86877.1, EAY86884.1, EAY99617.1, EAZ01545.1, EAZ09668.1, EAZ12044.1, EAZ23982.1, EAZ37535.1, EAZ45287.1, NP_001047567.1, NP_001056776.1, NP_001057985.1, NP_001063601.1, NP_001068400.1, NP_172327.1, NP_189147.1, NP_193041.1, XP_001415703.1, Q39473, Q39513, Q41635, Q42712, Q9SQI3, NP_189147.1, AAC49002, CAA52070.1, CAA52069.1, 193041.1, CAC39106, CAO17726, AAC72883, AAA33020, AAL79361, AAQ08223.1, AAB51523, AAL77443, AAA33019, AAG35064, and AAL77445.

Additional sources of acyl-ACP TEs that are useful in the present invention include: *Arabidopsis thaliana* (At); *Bradyrhizobium japonicum* (Bj); *Brassica napus* (Bn); *Cinnamonum camphorum* (Cc); *Capsicum chinense* (Cch); *Cuphea hookeriana* (Ch); *Cuphea lanceolata* (Cl); *Cuphea palustris* (Cp); *Coriandrum sativum* (Cs); *Carthamus tinctorius* (Ct); *Cuphea wrightii* (Cw); *Elaeis guineensis* (Eg); *Gossypium hirsutum* (Gh); *Garcinia mangostana* (Gm); *Helianthus annuus* (Ha); *Iris germanica* (Ig); *Iris tectorum* (It); *Myristica fragrans* (Mf); *Triticum aestivum* (Ta); *Ulmus Americana* (Ua); and *Umbellularia californica* (Uc). Exemplary TEs are shown in FIG. **6** with corresponding NCBI accession numbers.

[0033] In one embodiment, the present invention contemplates the specific production of an individual length of medium-chain fatty acid, for example, predominently producing C8 fatty acids in one culture of recombinant photosynthetic microorganisms. In another embodiment, the present invention contemplates the production of a combination of two or more different length fatty acids, for example, both C8 and C10 fatty acids in one culture of recombinant photosynthetic microorganisms.

[0034] Illustrated below are manipulations of these art-known genes to construct suitable expression systems that result in production of effective amounts of the thioesterases in selected recombinant photosynthetic organisms. In such constructions, it may be desirable to remove the portion of the gene that encodes the plastid transit peptide region, as this region is inappropriate in prokaryotes. Alternatively, if expression is to take place in eukaryotic cells, the appropriate

plastid transit peptide encoding region to the host organism may be substituted. Preferred codons may also be employed, depending on the host.

[0035] Other Modifications

[0036] In addition to providing an expression system for one or more appropriate acyl-ACP TE genes, further alterations in the photosynthetic host may be made. For example, the host may be modified to include an expression system for a heterologous gene that encodes a β-ketoacyl synthase (KAS) that preferentially produces acyl-ACPs having medium chain lengths. Such KAS enzymes have been described from several plants, including various species of *Cuphea*. See Dehesh, K., et al., *The Plant Journal* (1998) 15:383-390, describe "KAS IV: a 3-ketoacyl-ACP synthase from *Cuphea* sp. is a medium chain specific condensing enzyme."; Slabaugh, M., et al., *The Plant Journal* (1998) 13:611-620), and would serve to increase the availability of acyl-ACP molecules of the proper length for recognition and cleavage by the heterologous medium-chain acyl-ACP TE. Another example is that the photosynthetic host cell containing a heterologous acyl-ACP TE gene may be further modified to include an expression system for a heterologous gene that encodes a multifunctional acetyl-CoA carboxylase or a set of heterologous genes that encode the various subunits of a multi-subunit type of acetyl-CoA carboxylase. Other heterologous genes that encode additional enzymes or components of the fatty acid biosynthesis pathway could also be introduced and expressed in acyl-ACP TE-containing host cells.

[0037] The photosynthetic microorganism may also be modified such that one or more genes that encode beta-oxidation pathway enzymes have been inactivated or downregulated, or the enzymes themselves may be inhibited. This would prevent the degradation of fatty acids released from acyl-ACPs, thus enhancing the yield of secreted fatty acids. In cases where the desired products are medium-chain fatty acids, the inactivation or downregulation of genes that encode acyl-CoA synthetase and/or acyl-CoA oxidase enzymes that preferentially use these chain lengths as substrates would be beneficial. Mutations in the genes encoding medium-chain-specific acyl-CoA synthetase and/or medium-chain-specific acyl-CoA oxidase enzymes such that the activity of the enzymes is diminished would also be effective in increasing the yield of secreted fatty acids. An additional modification inactivates or down-regulates the acyl-ACP synthetase gene or inactivates the gene or protein. Mutations in the genes can be introduced either by recombinant or non-recombinant methods. These enzymes and their genes are well known, and may be targeted specifically by disruption, deletion, generation of antisense sequences, generation of ribozymes or other recombinant approaches known to the practitioner. Inactivation of the genes can also be accomplished by random mutation techniques such as UV, and the resulting cells screened for successful mutants. The proteins themselves can be inhibited by intracellular generation of appropriate antibodies or intracellular generation of peptide inhibitors.

[0038] The photosynthetic microorganism may also be modified such that one or more genes that encode storage carbohydrate or polyhydroxyalkanoate (PHA) biosynthesis pathway enzymes have been inactivated or down-regulated, or the enzymes themselves may be inhibited. Examples include enzymes involved in glycogen, starch, or chrysolaminarin synthesis, including glucan synthases and branching enzymes. Other examples include enzymes involved in PHA biosynthesis such as acetoacetyl-CoA synthase and PHA synthase.

[0039] Expression Systems

[0040] Expression of heterologous genes in cyanobacteria and eukaryotic algae is enabled by the introduction of appropriate expression vectors. For transformation of cyanobacteria, a variety of promoters that function in cyanobacteria can be utilized, including, but not limited to the lac, tac, and trc promoters and derivatives that are inducible by the addition of isopropyl β-D-1-thiogalactopyranoside (IPTG), promoters that are naturally associated with transposon- or bacterial chromosome-borne antibiotic resistance genes (neomycin phosphotransferase, chloramphenicol acetyltransferase, spectinomycin adenyltransferase, etc.), promoters associated with various heterologous bacterial and native cyanobacterial genes, promoters from viruses and phages, and synthetic promoters. Promoters isolated from cyanobacteria that have been used successfully include the following:

[0041] secA (secretion; controlled by the redox state of the cell)

[0042] rbc (Rubisco operon)

[0043] psaAB (PS I reaction center proteins; light regulated)

[0044] psbA (D1 protein of PSII; light-inducible)

[0045] Likewise, a wide variety of transcriptional terminators can be used for expression vector construction. Examples of possible terminators include, but are not limited to, psbA, psaAB, rbc, secA, and T7 coat protein.

[0046] Expression vectors are introduced into the cyanobacterial strains by standard methods, including, but not limited to, natural DNA uptake, conjugation, electroporation, particle bombardment, and abrasion with glass beads, SiC fibers, or other particles. The vectors can be: 1) targeted for integration into the cyanobacterial chromosome by including flanking sequences that enable homologous recombination into the chromosome, 2) targeted for integration into endogenous cyanobacterial plasmids by including flanking sequences that enable homologous recombination into the endogenous plasmids, or 3) designed such that the expression vectors replicate within the chosen host.

[0047] For transformation of green algae, a variety of gene promoters and terminators that function in green algae can be utilized, including, but not limited to promoters and terminators from *Chlamydomonas* and other algae, promoters and terminators from viruses, and synthetic promoters and terminators.

[0048] Expression vectors are introduced into the green algal strains by standard methods, including, but not limited to, electroporation, particle bombardment, and abrasion with glass beads, SiC fibers, or other particles. The vectors can be 1) targeted for site-specific integration into the green algal chloroplast chromosome by including flanking sequences that enable homologous recombination into the chromosome, or 2) targeted for integration into the cellular (nucleus-localized) chromosome.

[0049] For transformation of diatoms, a variety of gene promoters that function in diatoms can be utilized in these expression vectors, including, but not limited to: 1) promoters from *Thalassiosira* and other heterokont algae, promoters from viruses, and synthetic promoters. Promoters from *Thalassiosira pseudonana* that would be suitable for use in expression vectors include an alpha-tubulin promoter (SEQ ID NO:1), a beta-tubulin promoter (SEQ ID NO:2), and an

actin promoter (SEQ ID NO:3). Promoters from *Phaeodactylum tricornutum* that would be suitable for use in expression vectors include an alpha-tubulin promoter (SEQ ID NO:4), a beta-tubulin promoter (SEQ ID NO:5), and an actin promoter (SEQ ID NO:6). These sequences are deduced from the genomic sequences of the relevant organisms available in public databases and are merely exemplary of the wide variety of promoters that can be used. The terminators associated with these and other genes, or particular heterologous genes can be used to stop transcription and provide the appropriate signal for polyadenylation and can be derived in a similar manner or are known in the art.

[0050] Expression vectors are introduced into the diatom strains by standard methods, including, but not limited to, electroporation, particle bombardment, and abrasion with glass beads, SiC fibers, or other particles. The vectors can be 1) targeted for site-specific integration into the diatom chloroplast chromosome by including flanking sequences that enable homologous recombination into the chromosome, or 2) targeted for integration into the cellular (nucleus-localized) chromosome.

[0051] Host Organisms

[0052] The host cells used to prepare the cultures of the invention include any photosynthetic organism which is able to convert inorganic carbon into a substrate that is in turn converted to fatty acid derivatives. These organisms include prokaryotes as well as eukaryotic organisms such as algae and diatoms.

[0053] Host organisms include eukaryotic algae and cyanobacteria (blue-green algae). Representative algae include green algae (chlorophytes), red algae, diatoms, prasinophytes, glaucophytes, chlorarachniophytes, euglenophytes, chromophytes, and dinoflagellates. A number of cyanobacterial species are known and have been manipulated using molecular biological techniques, including the unicellular cyanobacteria *Synechocystis* sp. PCC6803 and *Synechococcus elongates* PCC7942, whose genomes have been completely sequenced.

[0054] The following genera of cyanobacteria may be used: one group includes

Chamaesiphon
Chroococcus
Cyanobacterium
Cyanobium
Cyanothece
Dactylococcopsis
Gloeobacter
Gloeocapsa
Gloeothece
Microcystis
Prochlorococcus
Prochloron
Synechococcus
Synechocystis

[0055] Another group includes

Cyanocystis
Dermocarpella
Stanieria
Xenococcus
Chroococcidiopsis

-continued

Myxosarcina
Pleurocapsa

[0056] Still another group includes

Arthrospira
Borzia
Crinalium
Geitlerinema
Halospirulina
Leptolyngbya
Limnothrix
Lyngbya
Microcoleus
Oscillatoria
Planktothrix
Prochlorothrix
Pseudanabaena
Spirulina
Starria
Symploca
Trichodesmium
Tychonema

[0057] Still another group includes

Anabaena
Anabaenopsis
Aphanizomenon
Calothrix
Cyanospira
Cylindrospermopsis
Cylindrospermum
Nodularia
Nostoc
Rivularia
Scytonema
Tolypothrix

[0058] And another group includes

Chlorogloeopsis
Fischerella
Geitleria
Iyengariella
Nostochopsis
Stigonema

[0059] In addition, various algae, including diatoms and green algae can be employed.

[0060] Desirable qualities of the host strain include high potential growth rate and lipid productivity at 25-50° C., high light intensity tolerance, growth in brackish or saline water, i.e., in wide range of water types, resistance to growth inhibition by high O_2 concentrations, filamentous morphology to aid harvesting by screens; resistance to predation, ability to be flocculated (by chemicals or 'on-demand autoflocculation'), excellent inorganic carbon uptake characteristics, virus or cyanophage-resistance, tolerance to free fatty acids or other compounds associated with the invention method, and ability to undergo metabolic engineering.

[0061] Metabolic engineering is facilitated by the ability to take up DNA by electroporation or conjugation, lack of a restriction system and efficient homologous recombination in the event gene replacement or gene knockouts are required.

[0062] Fatty Acid Adsorption, Removal, and Recovery

[0063] The fatty acids secreted into the culture medium by the recombinant photosynthetic microorganisms described above can be recovered in a variety of ways. A straightforward isolation method by partition using immiscible solvents may be employed. In one embodiment, particulate adsorbents can be employed. These may be lipophilic particulates or ion exchange resins, depending on the design of the recovery method. They may be circulating in the separated medium and then collected, or the medium may be passed over a fixed bed column, for example, a chromatographic column containing these particulates. The fatty acids are then eluted from the particulate adsorbents by the use of an appropriate solvent. Evaporation of the solvent, followed by further processing of the isolated fatty acids and lipids can then be carried out to yield chemicals and fuels that can be used for a variety of commercial purposes.

[0064] The particulate adsorbents may have average diameters ranging from 0.5 mm to 30 mm which can be manufactured from various materials including, but not limited to, polyethylene and derivatives, polystyrene and derivatives, polyamide and derivatives, polyester and derivatives, polyurethane and derivatives, polyacrylates and derivatives, silicone and derivatives, and polysaccharide and derivatives. Certain glass and ceramic materials can also be used as the solid support component of the fat adsorbing objects. The surfaces of the particulate adsorbents may be modified so that they are better able to bind fatty acids and lipids. An example of such modification is the introduction of ether-linked alkyl groups having various chain lengths, preferably 8-30 carbons. In another example, acyl chains of various lengths can be attached to the surface of the fat adsorbing objects via ester, thioester, or amide linkages.

[0065] In one embodiment, the particulate adsorbents are coated with inorganic compounds known to bind fatty acids and lipids. Examples of such compounds include but are not limited to aluminum hydroxide, graphite, anthracite, and silica.

[0066] The particles used may also be magnetized or otherwise derivatized to facilitate recovery. For instance the particles may be coupled to one member of a binding pair and the adsorbed to a substrate containing the relevant binding partner.

[0067] The fatty acids may be eluted from the particulate adsorbents by the use of an appropriate solvent such as hexane or ethanol. The particulate adsorbents may be reused by returning them to the culture medium or used in a regenerated column. The solvent containing the dissolved fatty acids is then evaporated, leaving the fatty acids in a purified state for further conversion to chemicals and fuels. The particulate adsorbents can be designed to be neutrally buoyant or positively buoyant to enhance circulation in the culture medium. A continuous cycle of fatty acid removal and recovery can be implemented by utilizing the steps outlined above. The recovered fatty acids may be converted to alternative organic compounds, used directly, or mixed with other components. Chemical methods for such conversions are well understood in the art, and developments of biological methods for such conversions are also contemplated

[0068] The present invention further contemplates a variety of compositions comprising the fatty acids produced by the recombinant photosynthetic microorganisms described herein, and uses thereof. The composition may comprise the fatty acids themselves, or further derivatives of the fatty acids, such as alcohols, alkanes, and alkenes which can be generated from the fatty acids produced by the microorganisms by any methods that are known in the art, as well as by development of biological methods of conversion. For examples, fatty acids may be converted to alkenes by catalytic hydrogenation and catalytic dehydration.

[0069] The composition may serve, for example, as a biocrude. The biocrude can be processed through refineries that will convert the composition compounds to various petroleum and petrochemical replacements, including alkanes, olefins and aromatics through processes including hydrotreatment, decarboxylation, isomerization and catalytic cracking and reforming. The biocrude can be also converted to ester-based fuels, such as fatty acid methyl ester (commercially known as biodiesel), through established chemical processes including transesterification and esterification.

[0070] In addition, one of skill in the art could contemplate a variety of other uses for the fatty acids of the present invention, and derivatives thereof, that are well known in the art, for example, the production of chemicals, soaps, surfactants, detergents, lubricants, nutraceuticals, pharmaceuticals, cosmetics, etc. For example, derivatives of the fatty acids of the present invention include C8 chemicals, such as octanol, used in the manufacture of esters for cosmetics and flavors as well as for various medical applications, and octane, used primarily as a co-monomer in production of polyethylene. Derivatives of the fatty acids of the present invention may also include C10 chemicals, such as decanol, used in the manufacture of plasticizers, surfactants and solvents, and decene, used in the manufacture of lubricants.

[0071] Biocrudes are biologically produced compounds or a mix of different biologically produced compounds that are used as a feedstock for refineries in replacement of, or in complement to, crude oil or other forms of petroleum. In general, but not necessarily, these feedstocks have been preprocessed through biological, chemical, mechanical or thermal processes in order to be in a liquid state that is adequate for introduction in a petroleum refinery.

[0072] The fatty acids of the present invention can be a biocrude, and further processed to a biofuel composition. The biofuel can then perform as a finished fuel or a fuel additive.

[0073] "Finished fuel" is defined as a chemical compound or a mix of chemical compounds (produced through chemical, thermochemical or biological routes) that is in an adequate chemical and physical state to be used directly as a neat fuel or fuel additive in an engine. In many cases, but not always, the suitability of a finished fuel for use in an engine application is determined by a specification which describes the necessary physical and chemical properties that need to be met. Some examples of engines are: internal combustion engine, gas turbine, steam turbine, external combustion engine, and steam boiler. Some examples of finished fuels include: diesel fuel to be used in a compression-ignited (diesel) internal combustion engine, jet fuel to be used in an aviation turbine, fuel oil to be used in a boiler to generate steam or in an external combustion engine, ethanol to be used in a flex-fuel engine. Examples of fuel specifications are ASTM standards, mainly used ion the US, and the EN standards, mainly used in Europe.

[0074] "Fuel additive" refers to a compound or composition that is used in combination with another fuel for a variety of reasons, which include but are not limited to complying with mandates on the use of biofuels, reducing the consumption of fossil fuel-derived products or enhancing the performance of a fuel or engine. For example, fuel additives can be used to alter the freezing/gelling point, cloud point, lubricity, viscosity, oxidative stability, ignition quality, octane level, and flash point. Additives can further function as antioxidants, demulsifiers, oxygenates, thermal stability improvers, cetane improvers, stabilizers, cold flow improvers, combustion improvers, anti-foams, anti-haze additives, icing inhibitors, injector cleanliness additives, smoke suppressants, drag reducing additives, metal deactivators, dispersants, detergents, demulsifiers, dyes, markers, static dissipaters, biocides, and/or corrosion inhibitors.

[0075] The following examples are offered to illustrate but not to limit the invention.

Example 1

Secretion of Fatty Acids by Strains Derived from the Unicellular Photoautotrophic Cyanobacterium *Synechococcus elongatus* PCC 7942

[0076] The *Cuphea hookeriana* FatB2 gene encoding an acyl-ACP thioesterase (ChFatB2) enzyme was modified for optimized expression in *Synechococcus elongatus* PCC 7942. First, the portion of the gene that encodes the plastid transit peptide region of the native ChFatB2 protein was removed. The remainder of the coding region was then codon-optimized using the "Gene Designer" software program (version 1.1.4.1) provided by DNA2.0, Inc. The nucleotide sequence of this derivative of the ChFatB2 gene (hereafter ChFatB2-7942) is provided as SEQ ID NO:7. The protein sequence encoded by this gene is provided in SEQ ID NO:8.

[0077] Two different versions of the trc promoter, trc (Egon, A., et al., *Gene* (1983) 25:167-178) and "enhanced trc" (hereafter trcE, from pTrcHis A, Invitrogen) were used to drive the expression of ChFatB2-7942 in *S. elongatus* PCC 7942. The trc promoter is repressed by the Lac repressor protein encoded by the lacIq gene and can be induced by the addition of isopropyl β-D-1-thiogalactopyranoside (IPTG). The trcE promoter is a derivative of trc designed to facilitate expression of eukaryotic proteins in *E. coli* and is also inducible by IPTG.

[0078] The fusion fragments of ChFatB2-7942 operably linked to trc or trcE, together with the lacIq gene, were cloned into the shuttle vector pAM2314 (Mackey, S. R., et al., Methods Mol. Biol. (2007) 362:115-129), which enables transformation of *S. elongatus* PCC 7942 via double homologous recombination-mediated integration into the "NS1" site of the chromosome. The constructed plasmid containing the trcE::ChFatB2-7942 expression cassette and lacIq gene is designated pSGI-YC01. SEQ ID NO:9 represents the sequence between and including the NS1 recombination sites of pSGI-YC01. The constructed plasmid containing the trc::ChFatB2-7942 expression cassette and lacIq gene is designated pSGI-YC09. SEQ ID NO: 10 represents the sequence between and including the NS1 recombination sites of pSGI-YC09.

[0079] Each of the plasmids pSGI-YC01 and pSGI-YC09, along with the control vector pAM2314, were introduced into wild-type *S. elongatus* PCC 7942 cells as described by Golden and Sherman (*J. Bacteriol.* (1984) 158:36-42). Both recombinant and control strains were pre-cultivated in 100 mL of BG-11 medium supplied with spectinomycin (5 mg/L) to late-log phase ($OD_{730\,nm}$=1.0) on a rotary shaker (150 rpm) at 30° C. with constant illumination (60 μE m^{-2} sec^{-1}). Cultures were then subcultured at initial $OD_{730\,nm}$=0.4-0.5 in BG-11 and cultivated overnight to $OD_{730\,nm}$=0.7-0.9. For time-course study, 60 mL aliquots of the culture were transferred into 250-mL flasks and induced by adding IPTG (final conc.=1 mM) if applicable. Cultures were sampled 0, 48, 96, and 168 hours after IPTG induction and then filtered through Whatman® GF/F filters using a Millipore vacuum filter manifold. Filtrates were collected in screw top culture tubes for gas chromatographic (GC) analysis.

[0080] Free fatty acids (FFAs) were separated from filtered cell cultures using liquid-liquid extraction. Five mL of the filtrate were mixed with 125 μL of 1 M H_3PO_3 and 0.25 mL of 5 M NaCl, followed by addition of 2 mL of hexane and thorough mixing. For GC-FID analyze, a 0.2 μl sample of the hexane was injected using a 40:1 split ratio onto a DB-FFAP column (J&W Scientific, 15 m×250 μm×0.25 μm), with a temperature profile starting at 150° C. for 0.5 min, then heating at 15° C./min to 230° C. and holding for 7.1 min (1.1 mL/min He).

[0081] GC analysis results indicating the levels of medium-chain FFAs (8:0 and 10:0) in cultures containing various *Synechococcus elongatus* strains 168 hours after IPTG induction are shown in Table 1-1.

TABLE 1-1

Medium-chain fatty acid secretion in various strains of *S. elongatus*

Strain	Parent Strain	Plasmid Added	Transgenes	Fatty Acids (mg/L) 8:0	10:0
SGC-YC2-5	PCC 7942	pAM2314	none	ND	ND
SGC-YC1-2	PCC 7942	pSGI-YC01	trcE::ChFatB2-7942	1.5	3.5
SGC-YC14-4	PCC 7942	pSGI-YC09	trc::ChFatB2-7942	5.1	10.1

Note:
ND represents "not detected" (<1 mg/L).

Example 2

Secretion of Fatty Acids by Strains Derived from the Unicellular Photoheterotrophic Cyanobacterium *Synechocystis* sp. PCC 6803

[0082] The trcE::ChFatB2-7942 and trc::ChFatB2-7942 fusion fragments, together with the lacIq gene, were cloned into the shuttle vector pSGI-YC03 (SEQ ID NO:11), which enables transformation of *Synechocystis* sp. PCC 6803 via double homologous recombination-mediated integration into the "RS1" site of the chromosome (Williams, Methods Enzymol. (1988) 167:766-778). The constructed plasmid containing the trcE::ChFatB2-7942 expression cassette and lacIq gene is designated pSGI-YC08. SEQ ID NO:12 represents the sequence between and including the RS1 recombination sites of pSGI-YC08. The constructed plasmid containing the trc::ChFatB2-7942 expression cassette and lacIq gene is des-

ignated pSGI-YC14. SEQ ID NO:13 represents the sequence between and including the RS1 recombination sites of pSGI-YC14.

[0083] Each of the plasmids pSGI-YC08, pSGI1-YC14, and the control vector pSGI-YC03, was introduced into wild-type *Synechocystis* PCC 6803 cells, as described by Zang, X. et al., *J. Microbiol.* (2007) 45:241-245. Both recombinant and control strains were pre-cultivated in 100 mL of BG-11 medium supplied with kanamycin (10 mg/L) to late-log phase ($OD_{730\ nm}$=1.0) on a rotary shaker (150 rpm) at 30° C. with constant illumination (60 $\mu E \cdot m^{-2}$ sec^{-1}). Cultures were then subcultured at initial $OD_{730\ nm}$=0.4-0.5 in BG-11 and cultivated overnight to $OD_{730\ nm}$=0.7-0.9. For time-course studies, 60-mL aliquots of the culture were transferred into 250-mL flasks and induced by adding IPTG (final conc.=1 mM) when applicable. Cultures were sampled 0, 72, and 144 hours after IPTG induction and then filtered through Whatman® GF/B filters using a Millipore vacuum filter manifold. Filtrates were collected in screw top culture tubes for gas chromatographic (GC) analysis. Free fatty acids (FFA) were separated from the filtered culture supernatant solutions by liquid-liquid extraction. For each sample, 2 mL filtered culture was extracted with a mixture of 50 μl phosphoric acid (1 M), 100 μl NaCl (5 M) and 2 mL hexane. A 0.2 μl sample was injected using a 40:1 split ratio on to a DB-FFAP column (J&W Scientific, 15 m×250 μm×0.25 μm), with a temperature profile starting at 150° C. for 0.5 min, then heating at 15° C./min to 230° C. and holding for 7.1 min (1.1 mL/min He).

[0084] GC analysis results indicating the levels of medium-chain FFAs (8:0 and 10:0) in cultures 144 hours after IPTG induction are shown in Table 2-1.

TABLE 2-1

Medium-chain fatty acid secretion in various strains of *Synechocystis*.

	Parent	Plasmid		Fatty Acids (mg/L)	
Strain	Strain	Added	Transgenes	8:0	10:0
SGC-YC9-8	PCC 6803	pSGI-YC03	none	ND	ND
SGC-YC10-5	PCC 6803	pSGI-YC08	trcE::ChFatB2-7942	61.3	52.7
SGC-YC16-2	PCC 6803	pSGI-YC14	trc::ChFatB2-7942	2.7	5.8

Note:
ND represents "not detected" (<1 mg/L).

Example 3

Secretion of Fatty Acids by Strains Derived from the Filamentous Cyanobacterium *Anabaena variabilis* ATCC 29413

[0085] The trc::ChFatB2-7942 and trcE::ChFatB2-7942 fusion fragments, together with the lacIq gene, were PCR amplified using primers RS3-3F (SEQ ID NO:14) and 4YC-rrnBter-3 (SEQ ID NO:15) from pSG1-YC14 and pSGI-YC08, respectively, and then cloned into the shuttle vector pEL17, which enables transformation of *A. variabilis* ATCC 29413 via double homologous recombination-mediated integration into the nifU1 locus of the chromosome (Lyons and Thiel, J. Bacteriol. (1995) 177:1570-1575). The constructed plasmids are designated pSG1-YC69 and pSG1-YC70 for trc::ChFatB2-7942 and trcE::ChFatB2-7942, respectively.

[0086] Each of the plasmids pSG1-YC69, pSG1-YC70, along with the control vector pEL17, are introduced into wild-type *A. variabilis* ATCC 29413 cells via tri-parental conjugation, as described by Elhai and Wolk (*Methods Enzymol.* (1988) 167:747-754). Both recombinant and control strains are pre-cultivated in 100 mL of BG-11 medium supplied with 5 mM NH_4Cl and spectinomycin (3 mg/L) to late-log phase ($OD_{730\ nm}$=1.0) on a rotary shaker (150 rpm) at 30° C. with constant illumination (60 $\mu E \cdot m^{-2} \cdot sec^{-1}$). Cultures are then subcultured at initial $OD_{730\ nm}$=0.4-0.5 in BG-11 and cultivated overnight to $OD_{730\ nm}$=0.7-0.9. For time-course studies, 60-mL aliquots of the culture are transferred into 250 mL flasks and induced by adding IPTG (final conc. =1 mM) if applicable. Cultures are sampled every 72 hours and then filtered through Whatman® GF/F filters using a Millipore vacuum filter manifold. Filtrates are collected in screw top culture tubes for gas chromatographic (GC) analysis as described in Example 1.

Example 4

Secretion of Fatty Acids in Strains Derived from *Synechococcus elongatus* PCC 7942 Containing an Inactivated Acyl-ACP Synthetase Gene

[0087] A putative acyl-ACP synthetase gene in *S. elongatus* PCC 7942, synpcc7942_0918 (Cyanobase gene designation), was disrupted via replacing of an internal 422-bp portion of its coding region with a 1,741-bp DNA sequence carrying the chloramphenicol resistance marker gene, cat (which encodes chloramphenicol acetyltransferase). Primer pairs 918-15 (SEQ ID NO: 16)/918-13 (SEQ ID NO: 17) and 918-25 (SEQ ID NO:18)/918-23 (SEQ ID NO:19) were used to amplify two DNA fragments corresponding to a 5' portion (1-480 bp) and a 3' portion (903-1521 bp) of the coding region of synpcc7942_0918, respectively. The cat fragment was amplified from plasmid pAM1573 (Mackey et al., Methods Mol. Biol. 362:115-29) using PCR with primers NS21-3 Cm (SEQ ID NO:20) and ter-3 Cm (SEQ ID NO:21), which overlap primers 918-13 and 918-25, respectively. The recombinant chimeric PCR technique was then used to amplify the complete disruption cassette with the three aforementioned PCR fragments, as well as primers 918-15 and 918-23. The resulting 2,840-bp blunt-end PCR fragment (SEQ ID NO:22) was then ligated into pUC19 (Yanisch-Perron et al., Gene 33:103-119), which has been digested with both HindIII and EcoRI to remove the multiple cloning sites and subsequently blunted with T4 DNA polymerase, to yield plasmid pSGI-YC04.

[0088] Plasmid pSG1-YC04 was introduced into *S. elongatus* strain SGC-YC1-2, which harbors a copy of trcE::ChFatB2-7942 integrated into NS1 (see Example 1). The resulting strain was designated SGC-YC4-7. Fatty acid production assays and GC analyses were performed as described in Example 1. The results of GC analyses indicating the levels of FFAs in cultures of various *S. elongatus* strains 168 hours after IPTG induction are shown in Table 4-1. It is possible that inactivation of the acyl-ACP synthetase gene has a larger impact on secretion of long-chain fatty acids than on secretion of medium-chain fatty acids.

TABLE 4-1

Medium-chain fatty acid secretion in various strains of *S. elongatus*.

		Plasmid			Fatty Acids (mg/L)			
Strain	Parent Strain	Added	Transgenes	Deletions	8:0	10:0	16:0	16:1
SGC-YC2-5	PCC 7942	pAM2314	none	none	ND	ND	ND	1.4
SGC-YC1-2	PCC 7942	pSGI-YC01	trcE::ChFatB2-7942	none	1.4	4.2	ND	1.6
SGC-YC4-7	SGC-YC1-2	pSGI-YC04	trcE::ChFatB2-7942	synpcc7942_0918	1.0	3.1	1.1	3.9

Note:
ND represents "not detected" (<1 mg/L).

Example 5

Secretion of Fatty Acids in Strains Derived from *Synechocystis* sp. PCC6803 Containing an Inactivated Acyl-ACP Synthetase Gene

[0089] A ~b **1**.7-kbp DNA fragment spanning an area upstream and into the coding region of the acyl-ACP synthetase-encoding gene, slr1609 (Cyanobase gene designation), from *Synechocystis* sp. PCC 6803 was amplified from genomic DNA using PCR with primers NB001 (SEQ ID NO:23) and NB002 (SEQ ID NO:24). This fragment was cloned into the pCR2.1 vector (Invitrogen) to yield plasmid pSG1-NB3 and subsequently cut with the restriction enzyme Mfe1. A chloramphenicol resistance marker cassette containing the cat gene and associated regulatory control sequences was amplified from plasmid pAM1573 (Andersson, et al., *Methods Enzymol*. (2000) 305:527-542) to contain flanking Mfe1 restriction sites using PCR with primers NB010 (SEQ ID NO:25) and NB011 (SEQ ID NO:26). The cat gene expression cassette was then inserted into the MfeI site of pSG1-NB3 to yield pSG1-NB5 (SEQ ID NO:27).

[0090] The pSGI-NB5 vector was transformed into trcE::ChFatB2-7942-containing *Synechocystis* strain SGC-YC10^{-5} (see Example 1) according to Zang et al., *J Microbiology* (2007) 45:241-245. Insertion of the chloramphenicol resistance marker into the Slr1609 gene through homologous recombination was verified by PCR screening of insert and insertion site. The resulting strain was designated SGC-NB10-4, which was tested in liquid BG-11 medium for fatty acid secretion. All liquid medium growth conditions used a rotary shaker (150 rpm) at 30° C. with constant illumination (60 $\mu E \cdot m^{-2} \cdot sec^{-1}$). Cultures were inoculated in 25 mL of BG-11 medium containing chloramphenicol and/or kanamycin (5 μg/mL) accordingly and grown to a sufficient density (minimal $OD_{730\ nm}$=1.6–2). Cultures were then used to inoculate 100 mL BG-11 medium in 250 mL polycarbonate flasks to $OD_{730\ nm}$=0.4–0.5 and incubated overnight. 45 mL of overnight culture at $OD_{730\ nm}$=0.7–0.9 were added to new 250 mL flasks, inducing with 1 mM IPTG or using as uninduced controls. 5 mL samples were taken at 0, 72 and 144 hours post induction and processed as described in Example 2.

[0091] Free fatty acids (FFA) were separated from the filtered culture supernatant solutions by liquid-liquid extraction for GC/FID (flame ionization detector) analysis. For each sample, 2 mL filtered culture was extracted with a mixture of 50 μl phosphoric acid (1 M), 100 μl NaCl (5 M) and 2 mL hexane. A 0.2 μl sample was injected using a 40:1 split ratio on to a DB-FFAP column (J&W Scientific, 15 m×250 μm×0.25 μm), with a temperature profile starting at 150° C. for 0.5 min, then heating at 15° C./min to 230° C. and holding for 7.1 min (1.1 mL/min He).

[0092] GC results indicating secreted levels of free fatty acids after 144 hours are shown in Table 5-1.

TABLE 5.1

Medium-chain fatty acid secretion in various strains of *Synechocystis*.

		Plasmid			Fatty Acids (mg/L)	
Strain	Parent Strain	Added	Transgenes	Deletions	8:0	10:0
SGC-YC10-5	PCC 6803	pSGI-YC08	trcE::ChFatB2-7942	none	58.3	67.7
SGC-NB10-4	SGC-YC10-5	pSGI-NB5	trcE::ChFatB2-7942	slr1609	57.7	73.7

Note:
ND represents "not detected" (<1 mg/L).

Example 6

Expression of *Cuphea lanceolata* Kas-IV and *Helianthus annuus* Kas-III genes in *Synechocystis* sp.

[0093] A DNA fragment comprising a functional operon was synthesized such that it contained the following elements in the given order: the trc promoter, the *Cuphea lanceolata* 3-ketoacyl-acyl carrier protein synthase IV gene (ClKas-IV, GenBank Accession No. CAC59946) codon-optimized for expression in *Synechococcus elongatus* PCC 7942, and the rps14 terminator (SEQ ID NO:28) from *Synechococcus* sp. WH8102. The nucleotide sequence of this entire functional

operon, along with various flanking restriction enzyme recognition sites, is provided in SEQ ID NO:29.

[0094] Another DNA fragment comprising a functional operon was synthesized such that it contained the following elements in the given order: the trc promoter, the *Helianthus annuus* 3-ketoacyl-acyl carrier protein synthase III gene (HaKas-III, GenBank Accession No. ABP93352) codon-optimized for expression in both *Synechococcus elongatus* PCC 7942 and *Synechocystis* sp. PCC 6803, and rps14 terminator from *Synechococcus* sp. WH8102. The nucleotide sequence of this functional operon, along with various flanking restriction enzyme recognition sites, is provided in SEQ ID NO:30.

[0095] Codon optimization was performed by the use of the "Gene Designer" (version 1.1.4.1) software program provided by DNA2.0, Inc. The functional operon (expression cassette) containing the codon-modified ClKas-IV gene as represented in SEQ ID NO:29 was digested by the restriction enzymes SpeI and XbaI and inserted into plasmid pSGI-YC39 between the restriction sites SpeI and XbaI to form plasmid pSGI-BL26, which enables integration of the functional operon into the *Synechocystis* sp. PCC 6803 chromosome at the "RS2" recombination site (Aoki, et al., *J. Bacteriol* (1995) 177:5606-5611). The plasmid pSGI-BL27 containing the DNA fragment represented in SEQ ID NO:30 was constructed in the same way.

[0096] Plasmid pSGI-BL43 contains the trcE promoter, the codon-optimized ClKas-IV gene, and the rps14 terminator as represented in SEQ ID NO:31 and was made by inserting a SpeI/NcoI trcE fragment from pTrcHis A (Invitrogen) into SpeI/NcoI-digested pSGI-BL26. An additional plasmid, pSGI-BL44, contains the trcE promoter, the optimized ClKas-IV gene, the *S. elongatus* PCC 7942 kaiBC intergenic region, the optimized HaKas-III gene, and the rps14 terminator as represented in SEQ ID NO:32 and was made by inserting a BamHI/SacI fragment (containing the *S. elongatus* kaiBC intergenic region, the HaKas-III gene, and the rps14 terminator) generated via PCR amplification into BglII/SacI-digested pSGI-BL43. The PCR primers used to generate the DNA fragment containing the kaiBC region, HaKas-III, and rps14 terminator are provided as SEQ ID NO:33 and SEQ ID NO:34.

[0097] Wild-type *Synechocystis* PCC 6803 cells and transgenic *Synechocystis* strain SGC-YC10-5, which contains the ChFatB2-7942 gene, were transformed with plasmids pSG1-BL26, pSG1-BL27, pSG1-BL43 and pSG1-BL44 as described by Zang, X. et al. J. Microbiol. (2007) 45:241-245. Both recombinant and wild-type control strains were precultivated in 20 mL of BG-11 medium to mid-log phase ($OD_{730\ nm}$=0.7–0.9) on a rotary shaker (150 rpm) at 30° C. with constant illumination (60 $\mu E \cdot m^{-2} \cdot sec^{-1}$). Kanamycin (5 μg/mL) and/or spectinomycin (10 μg/mL) were included in recombinant cultures as appropriate. Cultures were then subcultured at initial $OD_{730\ nm}$=0.4–0.5 in BG-11 and cultivated overnight to $OD_{730\ nm}$=0.7–0.9. For a time-course study, 45-mL aliquots of the culture were transferred into 250 mL flasks and induced by adding IPTG (final conc.=1 mM) when applicable. Cultures were sampled 0, 72, and 144 hours after IPTG induction and then filtered through Whatman® GF/B filters using a Millipore vacuum filter manifold. Filtrates were collected in screw top culture tubes for gas chromatographic (GC) analysis as described in Example 2.

[0098] Results indicating the levels of secreted octanoic acid and decanoic acid in culture supernatants 144 hours after culture inoculation are shown in Table 6-1. The ClKas-IV and HaKas-III genes present in the indicated strains were under the control of the trc promoter.

TABLE 6-1

Medium-chain fatty acid secretion in (in mg/L) various *Synechocystis* sp. strains

	Parent	Plasmid		Fatty Acids (mg/L)	
Strain	Strain	Added	Transgenes	8:0	10:0
PCC 6803	n/a	n/a	None	ND	ND
SGC-YC10-5	PCC 6803	pSGI-YC08	trcE-ChFatB2-7942	69.8	68.4
SGC-BL26-3	PCC 6803	pSGI-BL26	trc-ClKas-IV	ND	ND
SGC-BL26-5	SGC-YC10-5	pSGI-BL26	trcE-ChfatB2-7942 trc-ClKas-IV	69.5	71.9
SGC-BL27-1	PCC 6803	pSGI-BL27	trc-HaKas-III	ND	ND
SGC-BL27-2	SGC-YC10-5	pSGI-BL27	trcE-ChFatB2-7942 trc-HaKas-III	65.7	66.6

Note:
ND represents "not detected" (<1 mg/L).

[0099] For a more optimized measurement of fatty acid secretion in these strains, the fatty acid secretion data shown in Table 6-1 were normalized to cell culture density, measured as optical density at 730 nm ($OD_{730\ nm}$); these data are presented in Table 6-2. Other experiments described in this application could be normalized in a similar fashion.

TABLE 6-2

Normalized medium-chain fatty acid secretion (mg/L/$OD_{730\ nm}$) in various *Synechocystis* sp. strains

	Parent	Plasmid		Fatty Acids	
Strain	Strain	Added	Transgenes	8:0	10:0
PCC 6803	n/a	n/a	None	ND	ND
SGC-YC10-5	PCC 6803	pSGI-YC08	trcE-ChFatB2-7942	11.7	11.4
SGC-BL26-3	PCC 6803	pSGI-BL26	trc-ClKas-IV	ND	ND
SGC-BL26-5	SGC-YC10-5	pSGI-BL26	trcE-ChfatB2-7942 trc-ClKas-IV	11.7	12.1
SGC-BL27-1	PCC 6803	pSGI-BL27	trc-HaKas-III	ND	ND
SGC-BL27-2	SGC-YC10-5	pSGI-BL27	trcE-ChFatB2-7942 trc-HaKas-III	12.2	12.3

Note:
ND represents "not detected" (<1 mg/L).

[0100] Results indicating the levels of secreted octanoic acid and decanoic acid in culture supernatants of additional strains 120 hours after culture inoculation are shown in Table 6-3. The ClKas-IV and HaKas-III genes present in the indicated strains were under the control of the trcE promoter.

TABLE 6-3

Medium-chain fatty acid secretion (in mg/L) in various *Synechocystis* sp. strains

Strain	Parent Strain	Plasmid Added	Transgenes	Fatty Acids (mg/L) 8:0	10:0
SGC-YC10-5	PCC 6803	pSGI-YC08	trcE-ChFatB2-7942	34.8	43.5
SGC-BL44	PCC 6803	pSGI-BL44	trcE-ClKAS-IV + HaKAS-III	ND	ND
SGC-YC10-5-BL43	SGC-YC10-5	pSGI-BL43	trcE-ChFatB2-7942 trcE-ClKas-IV	40.0	48.1
SGC-YC10-5-BL44	SGC-YC10-5	pSGI-BL44	trcE-ChfatB2-7942 trcE-ClKAS-IV + HaKAS-III	38.5	47.1

Note:
ND represents "not detected" (<1 mg/L).

[0101] For a more optimized measurement of fatty acid secretion in these strains, the fatty acid secretion data shown in Table 6-1 were normalized to cell culture density, measured as optical density at 730 nm ($OD_{730\ nm}$); these data are presented in Table 6-4.

TABLE 6-4

Normalized medium-chain fatty acid secretion (mg/L/$OD_{730\ nm}$) in various *Synechocystis* sp. strains

Strain	Parent Strain	Plasmid Added	Transgenes	Fatty Acids 8:0	10:0
SGC-YC10-5	PCC 6803	pSGI-YC08	trcE-ChFatB2-7942	6.8	8.5
SGC-BL44	PCC 6803	pSGI-BL44	trcE-ClKAS-IV + HaKAS-III	ND	ND
SGC-YC10-5-BL43	SGC-YC10-5	pSGI-BL43	trcE-ChFatB2-7942 trcE-ClKas-IV	7.4	8.9
SGC-YC10-5-BL44	SGC-YC10-5	pSGI-BL44	trcE-ChfatB2-7942 trcE-ClKAS-IV + HaKAS-III	8.3	10.2

Example 7

Introduction of a Heterologous Acyl-ACP Thioesterase Gene into a Diatom

[0102] A synthetic gene that encodes a derivative of the ChFatB2 enzyme with specificity for medium-chain (8:0-10:0) acyl-ACPs is expressed in various diatoms (Bacillariophyceae) by constructing and utilizing expression vectors comprising the ChFatB2 gene operably linked to gene regulatory regions (promoters and terminators) that function in diatoms. In a preferred embodiment, the gene is optimized for expression in specific diatom species and the portion of the gene that encodes the plastid transit peptide region of the native ChFatB2 protein is replaced with a plastid transit peptide that functions optimally in diatoms. The nucleotide sequence provided as SEQ ID NO:35 represents a synthetic derivative of the ChFatB2 gene that has been optimized for expression in *Thalassiosira pseudonana* and in which the native plastid transit peptide-encoding region of the gene has been replaced with the plastid transit peptide (including coupled signal sequence) associated with the gamma subunit of the coupling factor portion (CF1) of the chloroplast ATP synthase from *T. pseudonana* (JGI Identifier=jgi/Thaps3/40156/est Ext_gwp_gwl.C_chr_40019). The protein encoded by this gene, referred to hereafter as ChFatB2-Thal,) is provided in SEQ ID NO:36.

[0103] To produce an expression vector for *T. pseudonana*, the ChFatB2-Thal gene was placed between the *T. pseudonana* alpha-tubulin promoter and terminator regulatory sequences. The alpha-tubulin promoter was amplified from genomic DNA isolated from *T. pseudonana* CCMP 1335 by use of primers PR1 (SEQ ID NO:37) and PR3 (SEQ ID NO:38), whereas the alpha-tubulin terminator was amplified by use of primers PR4 (SEQ ID NO:39) and PR8 (SEQ ID NO:40). The KpnI/BamHI fragment from the alpha-tubulin promoter amplicon, the BamHI/XbaI fragment from the alpha-tubulin terminator and the large fragment from KpnI/XbaI-cut pUC118 (Vieira and Messing, *Meth. Enzymol.* (1987) 153:3-11) were then combined to form pSG1-PR5. The NcoI/BamHI fragment from ChFatB2-Thal gene was then inserted into NcoI/BamHI-digested pSG1-PR5 to form pSG1-PR16. In addition, a codon-optimized gene that encodes the nourseothricin acetyltransferase (NAT) enzyme from *Streptomyces noursei* (SEQ ID NO:41) (Krugel, et al., *Gene* (1993) 127:127-131) was synthesized and the NcoI/BamHI fragment from this NAT-encoding DNA molecule was inserted into the large NcoI/BamHI fragment from pSG1-PR5 to form pSG1-PR7, which upon introduction into *T. pseudonana* and other diatoms can provide resistance to the antibiotic nourseothricin.

[0104] pSGI-PR16 and pSGI-PR7 were co-transformed into *T. pseudonana* CCMP 1335 by means of particle bombardment essentially as described by Poulsen, et al., (*J. Phycol.* (2006) 42:1059-1065). Transformed cells were selected on agar plates in the presence of 100 mg/L nourseothricin (ClonNAT, obtained from Werner BioAgents, Germany). The presence of the ChFatB2-Thal gene in cells was confirmed by

the use of PCR. Transformants were grown in ASW liquid medium (Darley and Volcani, *Exp. Cell Res.* (1964) 58:334) on a rotary shaker (150 rpm) at 18° C. with constant illumination (60 $\mu E \cdot m^{-2} \cdot sec^{-1}$). Samples were removed seven days after inoculation and the culture medium was tested for the presence of FFAs as described in Example 1.

[0105] Although no fatty acid secretion was detected under these particular experimental conditions, optimization of the ChFatB2-Thal gene and diatom host strain can be performed to achieve fatty acid secretion in diatoms, which are known to have relatively impervious cell walls.

Example 8

Secretion of Fatty Acids by Green Algae

[0106] A synthetic gene that encodes a derivative of the ChFatB2 enzyme with specificity for medium-chain (8:0-10:0) acyl-ACPs is expressed in green algae (Chlorophyceae) by constructing and utilizing expression vectors comprising the ChFatB2 gene operably linked to gene regulatory regions (promoters and terminators) that function in green algae. The gene is optimized for expression in specific green algal species and the portion of the gene that encodes the plastid transit peptide region of the native ChFatB2 protein is replaced with a plastid transit peptide that functions optimally in green algae. The nucleotide sequence provided as SEQ ID NO:42 represents a derivative of the ChFatB2 gene optimized for expression in *Chlamydomonas reinhardtii* and in which the native plastid transit peptide-encoding region of the gene has been replaced with the plastid transit peptide associated with the gamma subunit of the coupling factor portion (CF1) of the chloroplast ATP synthase from *C. reinhardtii* (GenPept Accession No. XP 001696335). The protein encoded by this gene is provided in SEQ ID NO:43.

Example 9

Secretion of Fatty Acids in Strains of *Synechocystis* sp. Containing a Disrupted 1,4-alpha-Glucan Branching Enzyme Gene

[0107] A 1.4-kbp DNA fragment spanning an area upstream and into the coding region of the 1,4-alpha-glucan branching enzyme gene (glgB, Cyanobase gene designation=s110158) from *Synechocystis* sp. PCC6803 was amplified from genomic DNA using PCR with primers glgB-5 (SEQ ID NO:44) and glgB-3 (SEQ ID NO:45). This fragment was cloned into the pCR4-Topo vector (Invitrogen) to yield plasmid pSGI-BL32 and subsequently cut with the restriction enzyme AvaI. A spectinomycin resistance marker cassette containing the aadA gene and associated regulatory control sequences was digested by HindIII from plasmid pSGI-BL27. Both of the linear fragments were treated with the Quick Blunting™ Kit (New England Biolabs). The aadA gene expression cassette was then inserted into the AvaI site of pSGI-BL32 to yield pSGI-BL33. The portion of pSGI-BL33 that inserts into and inactivates the glgB gene is provided as SEQ ID NO:46).

[0108] The pSGI-BL33 vector was transformed into wild-type *Synechocystis* PCC 6803 and into trcE::ChFatB2-7942-containing *Synechocystis* strain SGC-YC10-5 (see Example 1) according to Zang, et al., *J. Microbiology* (2007) 45:241-245. Insertion of the spectinomycin resistance marker into the S110158 (glgB) gene via homologous recombination was verified by PCR screening of insert and insertion site. Verified knockout strains were tested in liquid BG-11 medium for secretion of fatty acids. All liquid medium growth conditions used a rotary shaker (150 rpm) at 30° C. with constant illumination (60 $\mu E \cdot m^{-2} \cdot sec^{-1}$). Cultures were inoculated in 25 mL of BG-11 medium containing spectinomycin (10 μg/mL) and/or kanamycin (5 μg/mL) accordingly and grown to a sufficient density (minimal $OD_{730\ nm}$=1.6–2). Cultures were then used to inoculate 100 mL BG-11 medium in 250-mL polycarbonate flasks to $OD_{730\ nm}$=0.4–0.5 and incubated overnight. Forty-five mL of overnight culture at $OD_{730\ nm}$=0.5 were added to new 250-mL flasks; some cultures were induced with 1 mM IPTG or used as uninduced controls. Samples (0.5 mL) were taken at 0, 72, 144, and 216 hours post induction and processed as described in Example 2.

[0109] Free fatty acids (FFA) were separated from the filtered culture supernatant solutions by liquid-liquid extraction for GC/FID analysis. For each sample, 2 mL of filtered culture were extracted with a mixture of 50 μL phosphoric acid (1 M), 100 μL NaCl (5 M) and 2 mL hexane. A 0.2 μl sample was injected using a 40:1 split ratio on to a DB-FFAP column (J&W Scientific, 15 m×250 μm×0.25 μm), with a temperature profile starting at 150° C. for 0.5 min, then heating at 15° C./min to 230° C. and holding for 7.1 min (1.1 mL/min He).

[0110] GC results indicating secreted levels of free fatty acids after 216 hours are shown in Table 9-1.

TABLE 9-1

Medium-chain Fatty Acid Secretion (in mg/L) in Various *Synechocystis* sp. Strains

		Plasmid			Fatty Acids	
Strain	Parent Strain	Added	Deletion	Transgenes	8:0	10:0
PCC 6803	n/a	n/a	None	None	ND	ND
SGC-BL33-1	PCC 6803	pSGI-BL33	Sll0158 (glgB)	None	ND	ND
SGC-YC10-5	PCC 6803	pSGI-YC08	None	trcE-ChFatB2-7942	70.0	68.7
SGC-BL33-2	SGC-YC10-5	pSGI-BL33	Sll0158 (glgB)	trcE-ChFatB2-7942	66.2	68.1

Note:
ND represents "not detected" (<1 mg/L).

[0111] For a more optimized measurement of fatty acid secretion in these strains, the fatty acid secretion data shown in Table 9-1 were normalized to cell culture density, measured as optical density at 730 nm ($OD_{730\ nm}$); these data are presented in Table 9-2. Other experiments described in this application could be normalized in a similar fashion.

TABLE 9-2

Normalized Medium-chain Fatty Acid Secretion (mg/L/$OD_{730\ nm}$) in Various *Synechocystis* sp. Strains

		Plasmid			Fatty Acids	
Strain	Parent Strain	Added	Deletion	Transgenes	8:0	10:0
PCC 6803	n/a	n/a	None	None	ND	ND
SGC-BL33-1	PCC 6803	pSGI-BL33	Sll0158 (glgB)	None	ND	ND
SGC-YC10-5	PCC 6803	pSGI-YC08	None	trcE-ChFatB2-7942	9.8	9.7
SGC-BL33-2	SGC-YC10-5	pSGI-BL33	Sll0158 (glgB)	trcE-ChFatB2-7942	10.4	10.7

Note:
ND represents "not detected" (<1 mg/L).

Example 10

Capture of Free Fatty Acids from Model Solutions with Hydrophobic Adsorbent Resins

[0112] A spike solution was formulated by dissolving 75 mg/L octanoic acid and 75 mg/L decanoic acid in BG-11 medium supplemented with 300 mM NaCl and adjusting the pH to 5.8. 50 mg of each of the resins listed in Table 1 were weighed into a 50 mL centrifuge tube and combined with 1.0 mL of methanol and shaken gently. The excess methanol was decanted and the resins were dried under a 25 in Hg vacuum, room temperature, overnight. 50 mL of the spike solution was then added to each of the resins and incubated with gentle shaking at 31° C. for 24 hours. Following incubation, the resins were removed by filtering over a Whatman® GF/F glass fiber filter and the filtrates were analyzed for octanoic acid and decanoic acid content by gas chromatography as described in Example 2. The capacity of each resin for octanoic and decanoic acid could then be determined by the difference in the concentration of each fatty acid before and after incubation with each resin. The results are shown in Table 10-1 below.

TABLE 10-1

Adsorption capacities of several commercially-available adsorbents

		Adsorption Capacity (mg/g)		
Description	Resin type	Octanoic Acid	Decanoic acid	Total free fatty acids
Dowex Optipore ® V503 (Dow Chemical)	Post cross-linked macroporous polystyrene divinyl benzene	26.3	69.8	96.0
Lewatit 1064 MD (LanXess)	Macroporous polystyrene divinyl benzene	1.1	46.7	47.8
Zeolyst CBV 28014 (Zeolyst)	Very low-alumina zeolite	17.4	74.7	92.0
Zeolyst CBV 901 (Zeolyst)	Low-alumina zeolite	5.4	64.8	70.1
Hisiv 3000 Silicalite (UOP Honeywell)	Hydrophobic silicalite	15.3	23.7	39.1
Lipidex 5000 (Packard Instrument Co.)	Alkylated sephadex gel	0.00	18.6	18.6
Norit ROW 0.8 (Fluka)	Extruded activated charcoal	40.2	71.8	112.1

[0113] Elution of free fatty acids from the hydrophobic adsorbents was also investigated. Dowex® Optipore® V503, Zeolyst CBV 28014, Zeolyst CBV 901, and Norit® ROW were incubated with 1.0 mL of spike solution per mg of adsorbent as described above. After the incubation period, the adsorbents were rinsed and combined with 0.1, 0.5, or 1.0 mL methanol per mg of adsorbent and shaken gently at room temperature for 4 hours. The methanol eluates and post-adsorption spikes were analyzed for free fatty acid concentration by gas chromatography. The results are listed in Table 10-2 below.

TABLE 10-2

Desorption of free fatty acids in methanol

	% Desorption		
mL MeOH/mg Resin	0.1 mL/mg	0.5 mL/mg	1.0 mL/mg
Dowex Optipore ® V503	92%	84%	100%
CBV 28014	53%	76%	84%
CBV 901	78%	76%	57%
Norit ® ROW	44%	85%	77%

[0114] The effect of pH on adsorbent capacity was studied utilizing Dowex® Optipore® V503. 40 mg of the resin were combined with 40 mL of BG-11 media spiked with 150 mg/L of octanoic and decanoic acid and adjusted to a pH of 10.0, 7.5, 4.8, or 2.8. The pH 10 spike was buffered with 5 mM CAPS. The pH 7.5 and 2.8 spikes were buffered with 5 mM phosphate, and the pH 4.8 was buffered naturally by the dissolved fatty acids, with 5 mM NaCl added to maintain consistent conductivity. The spikes were incubated with resin as described above. Free fatty acid concentrations were measured with an enzymatic assay purchased from Zen-bio. The

results are displayed in Table 10-3 below. From these results, it is clear that hydrophobic adsorption of free fatty acids is possible over a wide range of pH.

TABLE 10-3

Adsorption capacity of Dowex ® Optipore ® V503 at various pH values

pH	Adsorption Capacity (mg FFA/g resin)
10	42 ± 13
7.5	64 ± 4
4.8	172 ± 4
2.8	259 ± 1

[0115] Reported values are the mean of two experimental replicates, +/−one standard deviation.

Example 11

In Vivo Capture of Free Fatty Acids from Cultures of *Synechocystis* Strain SGC-YC10-5

[0116] *Synechocystis* sp. strain SGC-YC10-5, which contains the ChFatB2-7942 gene as described in Example 1, was cultured in BG-11 with and without Dowex® Optipore® V503 resin. 400 mL of fresh culture was induced with 5 mM IPTG and incubated at room temperature for 1 hour to allow for uptake of the inducer. The culture was then divided into four 1,000 mL baffled Erlenmeyer flasks with PTFE vent caps. To two of the flasks, approximately 400 mg of Dowex® Optipore® V503 were added. The adsorbent resin in the test flasks was recovered and exchanged for fresh resin daily for 10 days. The recovered resin was washed liberally with deionized water and eluted with 2 mL of methanol. Samples of culture medium from the test flasks and control flasks were also taken daily. The samples were measured for $OD_{730\ nm}$ and filtered over a Whatman® GF/B glass fiber filter and analyzed for octanoic acid and decanoic acid content by gas chromatography as previously described in Example 2. The results are presented in Table 11-1.

TABLE 11-1

In vivo capture of free fatty acids from *Synechocystis* SGC-YC10-5 cultures

	Avg. Specific Growth Rate (d^{-1})	Average Free Fatty Acid Productivity ($mg\ L^{-1}\ d^{-1}$)
Without Dowex	0.090 ± 0.005	16 ± 0.8
With Dowex	0.090 ± 0.010	31 ± 3

[0117] Reported values are the mean of two biological duplicates +/−one standard deviation.

Example 12

Integration of CO_2 Delivery and Product Recovery as a Means for Enhancing the Efficiency and Economy of Both

[0118] Table 10-3 above reveals a clear relationship between free fatty acid adsorption capacity and pH. This relationship results from the inefficiency of extraction of the ionized form of the free fatty acids. Many potential production hosts require a pH significantly higher than the pKa of free fatty acids in order to survive and reproduce. An extreme example of this would be the alkalophilic cyanobacteria such as those belonging to the genera Synechococcus, *Synechocystis, Spirulina*, and many others, which prefer a pH between 9 and 11 for optimum growth. FIG. **5** outlines an embodiment of the invention wherein this problem is solved by recycling a portion of the culture first through a vessel where it is contacted with concentrated CO_2 gas to lower the pH, then through a stationary adsorbent column wherein the protonated free fatty acids are captured.

[0119] The CO_2-enriched, free fatty acid-depleted suspension is then returned to the bulk culture. The pressure inside the gas-liquid contactor can be controlled independently to provide a constant pH in the stream exiting the adsorption column. Further, the pressure of the post-column flash vessel can be controlled so as to provide a supply of CO_2 which is titrated to the CO_2 consumption rate of the bulk culture through PID control of pH, dissolved CO_2, off-gas CO_2, or any combination of the three. The excess CO_2 can then be recycled.

[0120] In order to demonstrate proof of concept for the invention described above, an experimental system was constructed as displayed in FIG. **5**.

[0121] Vessel E-1 was filled with 4L of a spike solution containing 700 mg/L octanoic acid dissolved in 100 mM NaCl, pH 11.1. Column C1 was filled with 45.2 g of Dowex® Optipore® V503 polymeric resin. The resin was activated with two column volumes of methanol, followed by a wash of three column volumes of 100 mM NaCl, pH 11.1. Liquid-gas contact vessel E2 was then filled with 200 mL of spike solution and 34.7 psia of CO_2. When the pH of the spike solution inside E-2 had decreased to between 5 and 6 (as determined by a slip of pH paper contained within E-2) peristaltic pumps P-1 and P-2 were set to the same flow rate and column loading was initiated. Valve V-2 was adjusted as needed to increase the column pressure and prevent the formation of gas bubbles.

[0122] Fractions of the flow through were taken at periodic intervals of 70-100 mL and assayed for octanoic acid by a commercially-available free fatty acid assay purchased from Zen-Bio. Two superficial linear flow rates were evaluated: 16.3 cm/min and 6.1 cm/min. For both flow rates, a control run was performed whereby vessel E-2 was bypassed and the column was loaded directly at a pH of 11.1. Table 12-1 below displays the results of this experiment. For both flow rates, column dynamic binding capacity was approximately 4-fold greater when CO_2 was used to lower the pH of the load.

TABLE 12-1

Dynamic binding capacity with and without CO_2-mediated load acidification

	Dynamic Binding Capacity (mg/g)	
Flow velocity (cm/min)	+34.7 psia CO_2	Control (pH 11.1) 0 psia CO_2
6.1	43.5	10.5
16.3	7.2	1.9

Example 13

Secretion of Oleic Acid by Photosynthetic Microorganisms

[0123] A synthetic gene that encodes a derivative of a FatA-type plant acyl-ACP TE enzyme with specificity for oleoyl-ACP is expressed in various photosynthetic microorganisms by constructing and utilizing expression vectors comprising a

FatA gene operably linked to gene regulatory regions (promoters and terminators) that function in the host photosynthetic microorganism. The gene is optimized for expression in the host photosynthetic microorganism and the portion of the gene that encodes the plastid transit peptide region of the native FatA protein is removed for expression in cyanobacteria or replaced with a plastid transit peptide that functions effectively in the host eukaryotic photosynthetic microorganisms.

[0124] Genes that could be used for this purpose include, but are not limited to, those that encode the following acyl-ACP TEs (referred to by GenPept Accession Numbers): NP_189147.1, AAC49002, CAA52070.1, CAA52069.1, 193041.1, CAC39106, CAO17726, AAC72883, AAA33020, AAL79361, AAQ08223.1, AAB51523, AAL77443, AAA33019, AAG35064, and AAL77445.

[0125] The following is a sequence listing of all sequences referred to above. SEQ ID NO:1

SEQUENCE LISTING

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Val Phe Arg Gln Ser Phe Ser Ile Arg Ser Tyr Glu Ile Gly Thr Asp
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Arg Thr Ala Ser Ile Glu Thr Leu Met Asn His Leu Gln Glu Thr Ser
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Ala Met Asp Pro Ser Lys Val Gly Val Arg Ser Gln Tyr Gln His Leu
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<220> FEATURE:
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<400> SEQUENCE: 16

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<400> SEQUENCE: 18

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<400> SEQUENCE: 19

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 20

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<210> SEQ ID NO 21
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<220> FEATURE:
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<400> SEQUENCE: 21

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<212> TYPE: DNA
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<223> OTHER INFORMATION: Synthetic construct

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 23

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<210> SEQ ID NO 24
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<400> SEQUENCE: 24

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<220> FEATURE:
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<400> SEQUENCE: 25

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<210> SEQ ID NO 26
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<212> TYPE: DNA
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gcgaagaggc ccgcaccgat cgcccttccc aacagttgcg cagcctgaat ggcgaatgga 3540

cgcgccctgt agcggcgcat taagcgcggc gggtgtggtg gttacgcgca gcgtgaccgc 3600

tacacttgcc agcgccctag cgcccgctcc tttcgctttc ttcccttcct ttctcgccac 3660

gttcgccggc tttccccgtc aagctctaaa tcgggggctc cctttagggt tccgatttag 3720

tgctttacgg cacctcgacc ccaaaaaact tgattagggt gatggttcac gtagtgggcc 3780

atcgccctga tagacggttt ttcgcccttt gacgttggag tccacgttct ttaatagtgg 3840

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agggattttg ccgatttcgg cctattggtt aaaaaatgag ctgatttaac aaaaatttaa 3960

cgcgaatttt aacaaaattc agggcgcaag ggctgctaaa ggaagcggaa cacgtagaaa 4020

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gccagtccgc agaaacggtg ctgaccccgg atgaatgtca gctactgggc tatctggaca 4080

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tggcgcaggg gatcaagatc tgatcaagag acaggatgag gatcgtttcg catgattgaa 4320

caagatggat tgcacgcagg ttctccggcc gcttgggtgg agaggctatt cggctatgac 4380

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tttttgcggc attttgcctt cctgtttttg ctcacccaga aacgctggtg aaagtaaaag 5220

atgctgaaga tcagttgggt gcacgagtgg gttacatcga actggatctc aacagcggta 5280

agatccttga gagttttcgc cccgaagaac gttttccaat gatgagcact tttaaagttc 5340

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ccaacttact tctgacaacg atcggaggac cgaaggagct aaccgctttt ttgcacaaca 5580

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ctggcgaact acttactcta gcttcccggc aacaattaat agactggatg gaggcggata 5760

aagttgcagg accacttctg cgctcggccc ttccggctgg ctggtttatt gctgataaat 5820

ctggagccgg tgagcgtggg tctcgcggta tcattgcagc actggggcca gatggtaagc 5880

cctcccgtat cgtagttatc tacacgacgg ggagtcaggc aactatggat gaacgaaata 5940

gacagatcgc tgagataggt gcctcactga ttaagcattg gtaactgtca gaccaagttt 6000

actcatatat actttagatt gatttaaaac ttcattttta atttaaaagg atctaggtga 6060

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gttcgtgcac acagcccagc ttggagcgaa cgacctacac cgaactgaga tacctacagc 6540

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gcggcagggt cggaacagga gagcgcacga gggagcttcc agggggaaac gcctggtatc 6660

tttatagtcc tgtcgggttt cgccacctct gacttgagcg tcgatttttg tgatgctcgt 6720

caggggggcg gagcctatgg aaaaacgcca gcaacgcggc ctttttacgg ttcctggcct 6780

tttgctggcc ttttgctcac atgttctttc ctgcgttatc ccctgattct gtggataacc 6840

gtattaccgc ctttgagtga gctgataccg ctcgccgcag ccgaacgacc gagcgcagcg 6900

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ggccgattca ttaatgcagc tggcacgaca ggtttcccga ctggaaagcg ggcagtgagc 7020

gcaacgcaat taatgtgagt tagctcactc attaggcacc ccaggcttta cactttatgc 7080

ttccggctcg tatgttgtgt ggaattgtga gcggataaca atttcacaca ggaaacagct 7140

atgaccatga ttacgccaag cttggtaccg agctcggatc cactagtaac ggccgccagt 7200

gtgctggaat tcgcccttct cgag 7224

<210> SEQ ID NO 28
<211> LENGTH: 79
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 28

gatccgctgt tgacccaaca gcatgagtcg ttatccaagg ggagcttcgg ctccctttt 60

tcatgcgcgg atgcggtga 79

<210> SEQ ID NO 29
<211> LENGTH: 1503
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 29

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gtgagcggat aacaatttca cacaggaaac agaccatggc cgtcgcactg caaccagctc 120

aagaagtcgc aactaagaaa aagcctgcaa tcaaacagcg gcgcgtggtg gttaccggca 180

tgggtgtggt gactcccctc gggcatgaac cggatgtgtt ttacaacaat ctcctggatg 240

gcgtgagcgg cattagtgag atcgagaatt ttgactcgac gcagtttccc actcgcattg 300

ccggcgaaat caagagtttc agcaccgacg gctgggtcgc gcccaaattg agcaaacgga 360

tggataaatt gatgctgtat ctgctcaccg caggcaagaa agcgctggcc gatgcgggca 420

tcacggatga tgtgatgaaa gagctggata aacgcaaatg tggagttctg attggcagtg 480

gcatgggcgg catgaagctg ttctacgatg cgctcgaagc cctgaagatt tcgtatcgaa 540

agatgaaccc attctgtgtg ccttttgcga ccacgaatat gggtagcgcc atgctggcta 600

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tggatttggg gtggatgggg ccgaattata gtatttccac cgcgtgcgca acctcgaact 660

tctgcatctt gaacgcggct aaccacatta tccgtggtga agcagacatg atgctctgcg 720

gcggctccga tgcggtcatt atcgctatcg gtttgggcgg ctttgttgct tgccgcgcct 780

tgagccaacg caataacgac ccaaccaagg catcgcgccc gtgggacagc aatcgcgatg 840

gcttcgtcat gggcgaggga gccggggtgc tgctgttgga ggagctggaa cacgcgaaaa 900

agcgaggcgc gacaatctat gctgagttct tgggagggtc ctttacatgc gatgcctacc 960

acatgacgga gcctcaccca gagggcgcag gcgtgatctt gtgtatcgag aaggcaatgg 1020

ctcaggcagg agtctctcgc gaggatgtta actacattaa tgctcacgca acgtccacgc 1080

cggctggtga catcaaggaa taccaagctc tcgcccattg tttcggccag aactcggagc 1140

tgcgggtcaa tagtacaaag tccatgatcg gtcatctgct gggtgctgcc ggtggcgtcg 1200

aagctgtgac agtcattcaa gccatccgca ccggctggat tcaccctaat ctgaacctgg 1260

aagacccgga caaggccgtt gacgcaaaat tcctcgtcgg accggagaaa gaacgtctca 1320

acgttaaagt cggattgagc aatagtttcg gttttggtgg ccataactct agtatcctgt 1380

ttgcacccta taattgataa tagatctgat ccgctgttga cccaacagca tgagtcgtta 1440

tccaagggga gcttcggctc ccttttttca tgcgcggatg cggtgagagc tcacgtgtct 1500

aga 1503

<210> SEQ ID NO 30
<211> LENGTH: 1224
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 30

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gtgagcggat aacaatttca cacaggaaac agaccatggc aagccgtgtt gttggtaaag 120

gttgtaaact cgttggatgt ggtagtgccg tcccgaagtt ggaggtgagt aacgacgacc 180

tcagtaagat cgtggatact tccgatgaat ggatttctgt tcggacggga atccgcaacc 240

ggcgggtgat tactggtaag gataagatga cggggctggc ggtcgaggca gcccagaaag 300

ccctggaaat ggctgaagtc gatgctgacg atgtggactt gctcctgttg tgcacctcca 360

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accctctggc atttgatatt acagccgctt gtagcggctt cgttctgggt ctggtgagtg 480

cttcctgcta tatccgcggc ggcgggttca agaacgtcct ggttatcggc gcggacgcac 540

tgagccgcta cgtcgattgg actgaccgcg gcacatgcat tctctttggt gacgccgctg 600

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ggacgaacgg ttccgttgtg gattttccgc ccaagcgcag cagctactct tccatccaaa 780

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cactcgagaa cgcgggcctc acacgttcta gcattgattg gctgctgctc caccaagcaa 900

accaacgaat cttggatgcc gtcgcaacgc gtctggaaat tcccgcagac cgcgtgatta 960

gtaacttggc taattacggc aatacttctg ccgccagcat tccgttggca ctggatgaag 1020

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ccgtgcgcag cggtaaggtc aaacccggtc agactatcgc aacttcgggg tttggagcag 1080

gcttgacatg gggcagcgcg atcattcgct ggaattaatg atagatctga tccgctgttg 1140

acccaacagc atgagtcgtt atccaagggg agcttcggct ccctttttc atgcgcggat 1200

gcggtgagag ctcacgtgtc taga 1224

<210> SEQ ID NO 31
<211> LENGTH: 1613
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 31

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acacaggaaa cagcgccgct gagaaaaagc gaagcggcac tgctctttaa caatttatca 120

gacaatctgt gtgggcactc gaccggaatt atcgattaac tttattatta aaaattaaag 180

aggtatatat taatgtatcg attaaataag gaggaataaa ccatggccgt cgcactgcaa 240

ccagctcaag aagtcgcaac taagaaaaag cctgcaatca aacagcggcg cgtggtggtt 300

accggcatgg gtgtggtgac tcccctcggg catgaaccgg atgtgtttta caacaatctc 360

ctggatggcg tgagcggcat tagtgagatc gagaattttg actcgacgca gtttcccact 420

cgcattgccg gcgaaatcaa gagtttcagc accgacggct gggtcgcgcc caaattgagc 480

aaacggatgg ataaattgat gctgtatctg ctcaccgcag gcaagaaagc gctggccgat 540

gcgggcatca cggatgatgt gatgaaagag ctggataaac gcaaatgtgg agttctgatt 600

ggcagtggca tgggcggcat gaagctgttc tacgatgcgc tcgaagccct gaagatttcg 660

tatcgaaaga tgaacccatt ctgtgtgcct tttgcgacca cgaatatggg tagcgccatg 720

ctggctatgg atttggggtg gatggggccg aattatagta tttccaccgc gtgcgcaacc 780

tcgaacttct gcatcttgaa cgcggctaac cacattatcc gtggtgaagc agacatgatg 840

ctctgcggcg gctccgatgc ggtcattatc cctatcggtt tgggcggctt tgttgcttgc 900

cgcgccttga gccaacgcaa taacgaccca accaaggcat cgcgcccgtg ggacagcaat 960

cgcgatggct tcgtcatggg cgagggagcc ggggtgctgc tgttggagga gctggaacac 1020

gcgaaaaagc gaggcgcgac aatctatgct gagttcttgg gagggtcctt tacatgcgat 1080

gcctaccaca tgacggagcc tcacccagag ggcgcaggcg tgatcttgtg tatcgagaag 1140

gcaatggctc aggcaggagt ctctcgcgag gatgttaact acattaatgc tcacgcaacg 1200

tccacgccgg ctggtgacat caaggaatac caagctctcg cccattgttt cggccagaac 1260

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aacctggaag acccggacaa ggccgttgac gcaaaattcc tcgtcggacc ggagaaagaa 1440

cgtctcaacg ttaaagtcgg attgagcaat agtttcggtt ttggtggcca taactctagt 1500

atcctgtttg caccctataa ttgataatag atctgatccg ctgttgaccc aacagcatga 1560

gtcgttatcc aaggggagct tcggctccct tttttcatgc gcggatgcgg tga 1613

<210> SEQ ID NO 32
<211> LENGTH: 2698

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 32

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caatttcaca caggaaacag cgccgctgag aaaaagcgaa gcggcactgc tctttaacaa 120

tttatcagac aatctgtgtg ggcactcgac cggaattatc gattaacttt attattaaaa 180

attaaagagg tatatattaa tgtatcgatt aaataaggag gaataaacca tggccgtcgc 240

actgcaacca gctcaagaag tcgcaactaa gaaaaagcct gcaatcaaac agcggcgcgt 300

ggtggttacc ggcatgggtg tggtgactcc cctcgggcat gaaccggatg tgttttacaa 360

caatctcctg gatggcgtga gcggcattag tgagatcgag aattttgact cgacgcagtt 420

tcccactcgc attgccggcg aaatcaagag tttcagcacc gacggctggg tcgcgcccaa 480

attgagcaaa cggatggata aattgatgct gtatctgctc accgcaggca agaaagcgct 540

ggccgatgcg ggcatcacgg atgatgtgat gaaagagctg gataaacgca aatgtggagt 600

tctgattggc agtggcatgg gcggcatgaa gctgttctac gatgcgctcg aagccctgaa 660

gatttcgtat cgaaagatga acccattctg tgtgcctttt gcgaccacga atatgggtag 720

cgccatgctg gctatggatt tggggtggat ggggccgaat tatagtattt ccaccgcgtg 780

cgcaacctcg aacttctgca tcttgaacgc ggctaaccac attatccgtg gtgaagcaga 840

catgatgctc tgcggcggct ccgatgcggt cattatccct atcggtttgg gcggctttgt 900

tgcttgccgc gccttgagcc aacgcaataa cgacccaacc aaggcatcgc gcccgtggga 960

cagcaatcgc gatggcttcg tcatgggcga gggagccggg gtgctgctgt tggaggagct 1020

ggaacacgcg aaaaagcgag gcgcgacaat ctatgctgag ttcttgggag ggtcctttac 1080

atgcgatgcc taccacatga cggagcctca cccagagggc gcaggcgtga tcttgtgtat 1140

cgagaaggca atggctcagg caggagtctc tcgcgaggat gttaactaca ttaatgctca 1200

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taatctgaac ctggaagacc cggacaaggc cgttgacgca aaattcctcg tcggaccgga 1440

gaaagaacgt ctcaacgtta aagtcggatt gagcaatagt ttcggttttg gtggccataa 1500

ctctagtatc ctgtttgcac cctataattg ataatagatc ctgtcgttaa ctgctttgtt 1560

ggtactacct gacttcaccc tcttttaaga tggcaagccg tgttgttggt aaaggttgta 1620

aactcgttgg atgtggtagt gccgtcccga agttggaggt gagtaacgac gacctcagta 1680

agatcgtgga tacttccgat gaatggattt ctgttcggac gggaatccgc aaccggcggg 1740

tgattactgg taaggataag atgacggggc tggcggtcga ggcagcccag aaagccctgg 1800

aaatggctga agtcgatgct gacgatgtgg acttgctcct gttgtgcacc tccaccccag 1860

atgatctctt tggaagtgcg ccgcaaatcc aggcggcact cggctgcaaa ggaaaccctc 1920

tggcatttga tattacagcc gcttgtagcg gcttcgttct gggtctggtg agtgcttcct 1980

gctatatccg cggcggcggg ttcaagaacg tcctggttat cggcgcggac gcactgagcc 2040

gctacgtcga ttggactgac cgcggcacat gcattctctt tggtgacgcc gctggcgctg 2100

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tgttggtcca ggcgtgtgag agcgaggacg acggcgtctt cgggtttgat ctgcatagcg 2160

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acggttccgt tgtggatttt ccgcccaagc gcagcagcta ctcttccatc caaatgaatg 2280

ggaaagaagt gttccgtttc gcctgccgcg tcgtgcccca gtctattgag atcgcactcg 2340

agaacgcggg cctcacacgt tctagcattg attggctgct gctccaccaa gcaaaccaac 2400

gaatcttgga tgccgtcgca acgcgtctgg aaattcccgc agaccgcgtg attagtaact 2460

tggctaatta cggcaatact tctgccgcca gcattccgtt ggcactggat gaagccgtgc 2520

gcagcggtaa ggtcaaaccc ggtcagacta tcgcaacttc ggggtttgga gcaggcttga 2580

catggggcag cgcgatcatt cgctggaatt aatgatagat ctgatccgct gttgacccaa 2640

cagcatgagt cgttatccaa ggggagcttc ggctcccttt tttcatgcgc ggatgcgg 2698

<210> SEQ ID NO 33
<211> LENGTH: 89
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 33

gtacgggatc cctgtcgtta actgctttgt tggtactacc tgacttcacc ctcttttaag 60

atggcaagcc gtgttgttgg taaaggttg 89

<210> SEQ ID NO 34
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 34

cacgtgagct ctcaccgcat ccgcgcatg 29

<210> SEQ ID NO 35
<211> LENGTH: 1252
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 35

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gtcctgcctc tttcaccact gtcagcagtc cttcggtgaa caatgtgttc ggacaggagg 120

gaaatgctca caggaacagg agagctacca ttgtcatgga tggagctaac ggaagtgcag 180

tcagtttgaa aagtgggtca ttgaatacgc aggaggacac aagttcgtcg ccaccgcccc 240

gtacattcct tcaccaactc cctgattgga gcagattgct cactgccatc acaaccgttt 300

ttgttaaaag taagcgtccg gatatgcatg atcgtaagtc gaaaaggccg gacatgctcg 360

tggatagttt cgggttggag agtaccgttc aggatggact cgtgttccgt caaagctttt 420

cgatccgttc atatgagatt ggaactgatc gtacggcttc cattgagact ttgatgaacc 480

atcttcagga gacttccctc aaccattgta agagtacagg aattttgttg gatggattcg 540

gacgcacact cgaaatgtgt aagcgcgatt tgatttgggt cgtcattaaa atgcagatca 600

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aggttaatag atacccggcc tggggcgata cagtagaaat caatactagg ttcagcagac 660

ttggtaagat cggcatgggt cgagattggc tcattagcga ctgcaatacc ggtgagatcc 720

tcgtcagggc aaccagcgcc tacgccatga tgaatcagaa gacccgaaga ctctcgaagc 780

ttccgtacga ggtccaccaa gagattgtcc ccctttttgt cgactccccc gtaattgaag 840

attcggatct caaggtccac aaattcaaag ttaaaacggg tgacagcatc cagaagggac 900

ttactcctgg ttggaacgac ctcgatgtga accaacatgt ttcgaacgtg aaatatatcg 960

gctggattct tgagagtatg ccaaccgagg tacttgagac gcaggaattg tgctcgttgg 1020

cattggagta tcgtcgtgag tgtgggcgag actcagtcct cgaaagtgta acagcaatgg 1080

acccaagcaa agttggtgtt cgttcacagt atcaacacct cctccgtctc gaggatggaa 1140

cagccattgt gaacggggcc acagagtgga ggccaaagaa cgctggcgct aacggagcta 1200

tctccacagg aaagaccagc aatggtaact ctgtgagtta atgataggat cc 1252

<210> SEQ ID NO 36
<211> LENGTH: 412
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 36

Met Lys Phe Leu Val Val Ala Val Ser Ala Leu Ala Thr Ala Ser Ala
1 5 10 15

Phe Thr Thr Ser Pro Ala Ser Phe Thr Thr Val Ser Ser Pro Ser Val
20 25 30

Asn Asn Val Phe Gly Gln Glu Gly Asn Ala His Arg Asn Arg Arg Ala
35 40 45

Thr Ile Val Met Asp Gly Ala Asn Gly Ser Ala Val Ser Leu Lys Ser
50 55 60

Gly Ser Leu Asn Thr Gln Glu Asp Thr Ser Ser Ser Pro Pro Pro Arg
65 70 75 80

Thr Phe Leu His Gln Leu Pro Asp Trp Ser Arg Leu Leu Thr Ala Ile
85 90 95

Thr Thr Val Phe Val Lys Ser Lys Arg Pro Asp Met His Asp Arg Lys
100 105 110

Ser Lys Arg Pro Asp Met Leu Val Asp Ser Phe Gly Leu Glu Ser Thr
115 120 125

Val Gln Asp Gly Leu Val Phe Arg Gln Ser Phe Ser Ile Arg Ser Tyr
130 135 140

Glu Ile Gly Thr Asp Arg Thr Ala Ser Ile Glu Thr Leu Met Asn His
145 150 155 160

Leu Gln Glu Thr Ser Leu Asn His Cys Lys Ser Thr Gly Ile Leu Leu
165 170 175

Asp Gly Phe Gly Arg Thr Leu Glu Met Cys Lys Arg Asp Leu Ile Trp
180 185 190

Val Val Ile Lys Met Gln Ile Lys Val Asn Arg Tyr Pro Ala Trp Gly
195 200 205

Asp Thr Val Glu Ile Asn Thr Arg Phe Ser Arg Leu Gly Lys Ile Gly
210 215 220

Met Gly Arg Asp Trp Leu Ile Ser Asp Cys Asn Thr Gly Glu Ile Leu

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225                 230                 235                 240

Val Arg Ala Thr Ser Ala Tyr Ala Met Met Asn Gln Lys Thr Arg Arg
                245                 250                 255

Leu Ser Lys Leu Pro Tyr Glu Val His Gln Glu Ile Val Pro Leu Phe
            260                 265                 270

Val Asp Ser Pro Val Ile Glu Asp Ser Asp Leu Lys Val His Lys Phe
        275                 280                 285

Lys Val Lys Thr Gly Asp Ser Ile Gln Lys Gly Leu Thr Pro Gly Trp
    290                 295                 300

Asn Asp Leu Asp Val Asn Gln His Val Ser Asn Val Lys Tyr Ile Gly
305                 310                 315                 320

Trp Ile Leu Glu Ser Met Pro Thr Glu Val Leu Glu Thr Gln Glu Leu
                325                 330                 335

Cys Ser Leu Ala Leu Glu Tyr Arg Arg Glu Cys Gly Arg Asp Ser Val
            340                 345                 350

Leu Glu Ser Val Thr Ala Met Asp Pro Ser Lys Val Gly Val Arg Ser
        355                 360                 365

Gln Tyr Gln His Leu Leu Arg Leu Glu Asp Gly Thr Ala Ile Val Asn
    370                 375                 380

Gly Ala Thr Glu Trp Arg Pro Lys Asn Ala Gly Ala Asn Gly Ala Ile
385                 390                 395                 400

Ser Thr Gly Lys Thr Ser Asn Gly Asn Ser Val Ser
                405                 410


<210> SEQ ID NO 37
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 37

caggatccgg ggaggtgtgg tgtagt                                        26


<210> SEQ ID NO 38
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 38

taggatccag tggtgcccat ggtactttgt taggggagga tag                     43


<210> SEQ ID NO 39
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 39

caggatcctc actctgtcgc gctgttg                                       27


<210> SEQ ID NO 40
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 40

catctagaga ggattgattt ccgagtc 27

<210> SEQ ID NO 41
<211> LENGTH: 573
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 41

atgggcacca ctctcgacga cacggcttac cgctaccgca ccagtgtgcc gggggacgcc 60

gaggccatcg aggcactgga tgggtccttc accaccgaca ccgtcttccg cgtcaccgcc 120

accggggacg gcttcaccct gcgggaggtg ccggtggacc cgcccctgac caaggtgttc 180

cccgacgacg agtcggacga cgagtcggac gacggggagg acggcgaccc ggactcccgg 240

acgttcgtcg cgtacgggga cgacggcgac ctggcgggct tcgtggtcgt ctcgtactcc 300

ggctggaacc gccggctgac cgtcgaggac atcgaggtcg ccccggagca ccgggggcac 360

ggggtcgggc gcgcgctgat ggggctcgcg acggagttcg cccgcgagcg gggtgccggg 420

cacctctggc tggaggtcac caacgtcaac gcaccggcga tccacgcgta ccggcggatg 480

gggttcaccc tctgcggcct ggacaccgcc ctgtacgacg gcaccgcctc ggacggcgag 540

caggcgctct acatgtccat gccctgcccc taa 573

<210> SEQ ID NO 42
<211> LENGTH: 1198
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 42

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ccgcccccaa gggcgtcgcc agccgcggct ccctgcaggt ggtggccggc gccaacggca 120

gcgcggtgag cctgaagtcg ggttccctca acactcagga ggacacctcg tcctcgcccc 180

cgccgcgcac gttcctgcac cagctgccgg actggtcccg cctgctgacg gctattacga 240

ccgtgttcgt gaagtcgaag cgccccgaca tgcacgaccg caagagcaag cggcctgata 300

tgctggtgga cagctttggc ctggagtcca cggtgcagga cggcctcgtg ttccggcaaa 360

gcttcagcat ccgcagctac gagatcggca cggaccgcac cgcgtcgatc gagacgctca 420

tgaaccacct ccaggagacg tcgctcaacc actgcaagtc caccggtatc ctgctggacg 480

gctttggccg caccctggag atgtgcaagc gggatctgat ctgggtggtg atcaagatgc 540

agatcaaggt gaaccgctat cccgcctggg gtgacaccgt cgagattaac acccgcttct 600

cgcgcctggg caagatcggc atggggcgcg actggctgat ctcggactgc aacactggcg 660

agatcctggt ccgggccacg tcggcctacg ccatgatgaa ccagaagact cggcggctga 720

gcaagctgcc ttacgaggtg catcaggaga tcgtgccgct cttcgtggac agccccgtga 780

tcgaggacag cgatctgaag gtgcacaagt tcaaggtcaa gaccggcgac agcatccaga 840

agggcctgac tcccggctgg aacgacctgg acgtgaacca gcacgtctcg aacgtgaagt 900

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acatcggctg gattctggag tcgatgccca ccgaggtgct ggagacgcag gagctgtgct 960

ccctggcgct ggagtatcgc cgcgagtgcg gccgcgactc cgtgctggag tccgtcaccg 1020

cgatggaccc gtcgaaggtg ggtgtccgca gccagtacca acacctgctg cgcctcgagg 1080

acggcaccgc cattgtgaac ggcgcgacgg agtggcggcc gaagaacgcg ggcgctaacg 1140

gcgccatctc cacgggcaag acctccaacg gcaactcggt gagctaatga taggatcc 1198

<210> SEQ ID NO 43
<211> LENGTH: 394
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 43

Met Ala Ala Met Leu Ala Ser Lys Gln Gly Ala Phe Met Gly Arg Ser
1 5 10 15

Ser Phe Ala Pro Ala Pro Lys Gly Val Ala Ser Arg Gly Ser Leu Gln
20 25 30

Val Val Ala Gly Ala Asn Gly Ser Ala Val Ser Leu Lys Ser Gly Ser
35 40 45

Leu Asn Thr Gln Glu Asp Thr Ser Ser Ser Pro Pro Pro Arg Thr Phe
50 55 60

Leu His Gln Leu Pro Asp Trp Ser Arg Leu Leu Thr Ala Ile Thr Thr
65 70 75 80

Val Phe Val Lys Ser Lys Arg Pro Asp Met His Asp Arg Lys Ser Lys
85 90 95

Arg Pro Asp Met Leu Val Asp Ser Phe Gly Leu Glu Ser Thr Val Gln
100 105 110

Asp Gly Leu Val Phe Arg Gln Ser Phe Ser Ile Arg Ser Tyr Glu Ile
115 120 125

Gly Thr Asp Arg Thr Ala Ser Ile Glu Thr Leu Met Asn His Leu Gln
130 135 140

Glu Thr Ser Leu Asn His Cys Lys Ser Thr Gly Ile Leu Leu Asp Gly
145 150 155 160

Phe Gly Arg Thr Leu Glu Met Cys Lys Arg Asp Leu Ile Trp Val Val
165 170 175

Ile Lys Met Gln Ile Lys Val Asn Arg Tyr Pro Ala Trp Gly Asp Thr
180 185 190

Val Glu Ile Asn Thr Arg Phe Ser Arg Leu Gly Lys Ile Gly Met Gly
195 200 205

Arg Asp Trp Leu Ile Ser Asp Cys Asn Thr Gly Glu Ile Leu Val Arg
210 215 220

Ala Thr Ser Ala Tyr Ala Met Met Asn Gln Lys Thr Arg Arg Leu Ser
225 230 235 240

Lys Leu Pro Tyr Glu Val His Gln Glu Ile Val Pro Leu Phe Val Asp
245 250 255

Ser Pro Val Ile Glu Asp Ser Asp Leu Lys Val His Lys Phe Lys Val
260 265 270

Lys Thr Gly Asp Ser Ile Gln Lys Gly Leu Thr Pro Gly Trp Asn Asp
275 280 285

Leu Asp Val Asn Gln His Val Ser Asn Val Lys Tyr Ile Gly Trp Ile

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    290                 295                 300

Leu Glu Ser Met Pro Thr Glu Val Leu Glu Thr Gln Glu Leu Cys Ser
305                 310                 315                 320

Leu Ala Leu Glu Tyr Arg Arg Glu Cys Gly Arg Asp Ser Val Leu Glu
                325                 330                 335

Ser Val Thr Ala Met Asp Pro Ser Lys Val Gly Val Arg Ser Gln Tyr
            340                 345                 350

Gln His Leu Leu Arg Leu Glu Asp Gly Thr Ala Ile Val Asn Gly Ala
        355                 360                 365

Thr Glu Trp Arg Pro Lys Asn Ala Gly Ala Asn Gly Ala Ile Ser Thr
    370                 375                 380

Gly Lys Thr Ser Asn Gly Asn Ser Val Ser
385                 390


<210> SEQ ID NO 44
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 44

ggtggaaaat gcctatgtgt taacg                                          25


<210> SEQ ID NO 45
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 45

cgtaggcagt gtgcaaccag gagcc                                          25


<210> SEQ ID NO 46
<211> LENGTH: 3418
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 46

ggtggaaaat gcctatgtgt taacggatct acaaaccagc accaaactct attacgaacc     60

ccacggtttc cactctcccc aactgcaaga cttggggccc attgatgtgg ttttaacccc    120

cgtcattggc atcaatatcc tcggattcct gccggtgctc aatggccaga aaaccaccct    180

ggagctttgt cgcactgtcc atccccaggc gatcgtcccc acctctggag ccgcagaatt    240

gaactatagc ggtttactaa ctaaagtttt acgtttagac ggcgatctca gtcaatttcg    300

ccagtcccta attgacgaag ggatacaagc ttccctatgg gaaccccagg tgggagtgcc    360

cctcaatgtg ccccaatcca ccgttggcta ggttggaatg ttcaaatcac tgtgcggtgt    420

gatgcttgat aaatacagtg agccagggaa aactgcaaaa aagtgtataa agtaggttta    480

acttgaatca aaatcctttc tccgcagtca tagccaggag taggaagatt accagcgaag    540

caagttgtct tcccctagct ttgggcgggc aaaccccttg cagtattgcc aacgtcaaaa    600

aatcaccata gccgaatgac ctacaccatc aacgctgacc aagtccatca gattgtccat    660
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aatcttcacc acgatccctt tgaagtgttg ggctgccatc ccctcggagc tttatgcttg 720

taaaccgttt tgtgaaaaaa tttttaaaat aaaaaagggg acctctaggg tccccaatta 780

attagtaata taatctatta aaggtcattc aaaaggtcat ccaccggatc agcttagtaa 840

agccctcgct agattttaat gcggatgttg cgattacttc gccaactatt gcgataacaa 900

gaaaaagcca gcctttcatg atatatctcc caatttgtgt agggcttatt atgcacgctt 960

aaaaataata aaagcagact tgacctgata gtttggctgt gagcaattat gtgcttagtg 1020

catctaacgc ttgagttaag ccgcgccgcg aagcggcgtc ggcttgaacg aattgttaga 1080

cattatttgc cgactacctt ggtgatctcg cctttcacgt agtggacaaa ttcttccaac 1140

tgatctgcgc gcgaggccaa gcgatcttct tcttgtccaa gataagcctg tctagcttca 1200

agtatgacgg gctgatactg ggccggcagg cgctccattg cccagtcggc agcgacatcc 1260

ttcggcgcga ttttgccggt tactgcgctg taccaaatgc gggacaacgt aagcactaca 1320

tttcgctcat cgccagccca gtcgggcggc gagttccata gcgttaaggt ttcatttagc 1380

gcctcaaata gatcctgttc aggaaccgga tcaaagagtt cctccgccgc tggacctacc 1440

aaggcaacgc tatgttctct tgcttttgtc agcaagatag ccagatcaat gtcgatcgtg 1500

gctggctcga agatacctgc aagaatgtca ttgcgctgcc attctccaaa ttgcagttcg 1560

cgcttagctg gataacgcca cggaatgatg tcgtcgtgca caacaatggt gacttctaca 1620

gcgcggagaa tctcgctctc tccaggggaa gccgaagttt ccaaaaggtc gttgatcaaa 1680

gctcgccgcg ttgtttcatc aagccttacg gtcaccgtaa ccagcaaatc aatatcactg 1740

tgtggcttca ggccgccatc cactgcggag ccgtacaaat gtacggccag caacgtcggt 1800

tcgagatggc gctcgatgac gccaactacc tctgatagtt gagtcgatac ttcggcgatc 1860

accgcttccc tcatgatgtt taactttgtt ttagggcgac tgccctgctg cgtaacatcg 1920

ttgctgctcc ataacatcaa acatcgaccc acggcgtaac gcgcttgctg cttggatgcc 1980

cgaccgaggc atagactgta ccccaaaaaa acagtcataa caagccatga aaaccgccac 2040

tgcgccgtta ccaccgctgc gttcggtcaa ggttctggac cagttgcgtg agcgcatacg 2100

ctacttgcat tacagcttac gaaccgaaca ggcttatgtc cactgggttc gtgccttcat 2160

ccgtttccac ggtgtgcgtc acccggcaac cttgggcagc agcgaagtcg aggcatttct 2220

gtcctggctg gcgaacgagc gcaaggtttc ggtctccacg catcgtcagg cattggcggc 2280

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aagacctcgg ccgtcgcggc gcttgccggt ggtgctgacc ccggatgaag tggttcgcat 2400

cctcggtttt ctggaaggcg agcatcgttt gttcgcccag cttctgtatg gaacgggcat 2460

gcggatcagt gagggtttgc aactgcgggt caaggatctg gatttcgatc acggcacgat 2520

catcgtgcgg gagggcaagg gctccaagga tcgggcctgg cacccagcct gcgcgagcag 2580

gggaattgat ccggtggatg acctttttgaa tgacctttaa tagattatat tactaattaa 2640

ttggggaccc tagaggtccc ctttttttatt ttaaaaattt tttcacaaaa cggtttacaa 2700

gcataaagct tcggggacca cggcaaggtc aatcaatggg tcattcgtgc ctatttaccc 2760

acggctgaag cggtaacggt gttgcttccc accgatcgcc gggaagtgat tatgaccacg 2820

gtccaccatc ccaacttttt tgaatgcgtg ttggagttgg aagaaccgaa gaattatcaa 2880

ttaagaatta ccgaaaatgg ccacgaaagg gtaatttatg acccctatgg ttttaaaact 2940

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cccaaactga cggattttga cctccatgtg tttggggaag gcaaccacca ccgtatttac   3000

gaaaaactcg gtgctcacct gatgacggtg gatggagtta aaggggttta ttttgctgtg   3060

tgggccccca atgcccgcaa cgtttccatt ttgggggatt tcaacaactg ggacggcaga   3120

ttgcaccaaa tgcggaaacg caacaacatg gtgtgggaat tatttatccc tgagttgggg   3180

gtgggcactt cttataagta tgagattaaa aactgggaag ggcacatcta cgaaaagact   3240

gacccctacg gtttttacca agaagtacgc cccaaaaccg cttccattgt ggcagacttg   3300

gacggttacc aatggcacga cgaagattgg ttggaagcta ggcgcaccag cgatcccctg   3360

agcaaacccg tttccgttta cgaactccat ttaggctcct ggttgcacac tgcctacg      3418
```

1. A cell culture of a recombinant photosynthetic microorganism, said microorganism modified to contain a nucleic acid molecule comprising at least one recombinant expression system that produces at least one exogenous acyl-ACP thioesterase,

wherein said acyl-ACP thioesterase preferentially liberates a fatty acid chain that contains 6-20 carbons, and

wherein the culture medium provides inorganic carbon as substantially the sole carbon source and

wherein said microorganism secretes the fatty acid liberated by the acyl-ACP thioesterase into the culture medium.

2. The culture of claim **1**, wherein the at least one exogenous acyl-ACP thioesterase is a Fat B thioesterase.

3. The culture of claim **1**, wherein the at least one exogenous acyl-ACP thioesterase is a Fat B thioesterase derived from the genus *Cuphea*.

4. The culture of claim **1**, wherein the at least one exogenous acyl-ACP thioesterase is ChFatB2.

5. The culture of claim **1**, wherein the recombinant photosynthetic microorganism has further been modified to produce an exogenous β-ketoacyl synthase (KAS).

6. The culture of claim **5**, wherein the exogenous KAS preferentially produces acyl-ACPs having the chain length for which the thioesterase has preferred activity.

7. The culture of claim **1**, wherein the recombinant photosynthetic microorganism is further modified so that one or more genes encoding beta-oxidation pathway enzymes are inactivated or downregulated, or said enzymes are inhibited.

8. The culture of claim **1**, wherein the recombinant photosynthetic microorganism is further modified so that one or more genes encoding acyl-ACP synthetases are inactivated or downregulated, or said synthetases are inhibited.

9. The culture of claim **1**, wherein the recombinant photosynthetic microorganism is further modified so that one or more genes encoding an enzyme involved in carbohydrate biosynthesis are inactivated or downregulated, or said enzymes are inhibited.

10. The culture of claim **9**, wherein the enzyme involved in carbohydrate biosynthesis is a branching enzyme.

11. A method to convert inorganic carbon to fatty acids, said method comprising:

incubating the culture of claim **1** such that the recombinant photosynthetic microorganism therein secretes the fatty acid into the culture medium; and

recovering the secreted fatty acids from the culture medium.

12. The method of claim **11**, wherein the fatty acids are recovered from the culture by contacting the medium with particulate adsorbents.

13. The method of claim **12**, wherein the particulate adsorbents circulate in the medium.

14. The method of claim **12**, wherein the particulate absorbents are contained in a fixed bed column.

15. The method of claim **14**, wherein the pH of the medium is lowered during said contacting.

16. The method of claim **15**, wherein said pH lowering process comprises adding CO_2.

17. The method of claim **16**, wherein the medium is recirculated to the culture.

18. The method of claim **12**, wherein the particulate adsorbents are lipophilic.

19. The method of claim **12**, wherein the particulate adsorbents are ion exchange resins.

20. A composition comprising a fatty acid produced by the culture of claim **1**.

21. The composition of claim **20**, wherein the composition is used to produce another compound.

22. The composition of claim **20**, wherein the composition is a biocrude.

23. A composition comprising a derivative of a fatty acid produced by the culture of claim **1**.

24. The composition of claim **23**, wherein the composition is a finished fuel or fuel additive.

25. The composition of claim **23**, wherein the composition is a biological substitute for a petrochemical product.

26. The composition of claim **23**, wherein the derivative is an alcohol, an alkane, or an alkene.

* * * * *