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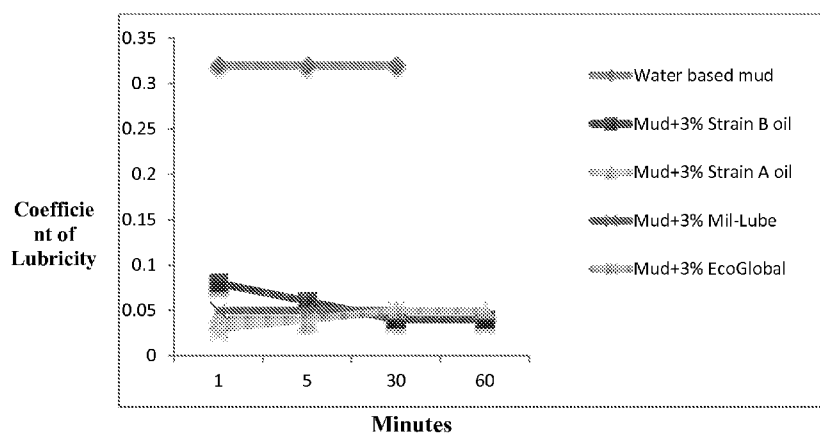
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(54) Title: LUBRICANTS AND WELLBORE FLUIDS

Figure 1



(57) Abstract: Provided are wellbore fluids comprising an oleaginous microbial cell and methods of using and making such fluids.

LUBRICANTS AND WELLBORE FLUIDS

CROSS REFERENCE TO RELATED APPLICATIONS

5 This application claims the benefit under 35 U.S.C. 119(e) of US Provisional Patent Application No. 62/020,921, filed July 3, 2014, US Provisional Patent Application No. 62/022,565, filed July 9, 2014, US Provisional Patent Application No. 62/048,740, filed September 10, 2014, and US Provisional Patent Application No. 62/156, 752, filed May 4, 2015, each of which is incorporated herein by reference in
10 its entirety.

BACKGROUND

 In the use of a cutting tool on a workpiece, friction between the tool and the workpiece can cause wear on the tool, hinder the cutting process, lead to slow manufacturing cycles, and negatively affect the quality and finish of the workpiece.
15 Lubricants are typically used to overcome these undesirable effects. In choosing the appropriate lubricants, consideration needs to be given to the compatibility of the lubricant with both the tool and the workpiece and whether the lubricant can operate efficiently under the conditions of the cutting process. One must also consider the environmental impact of the lubricant in its use and disposal, and on the health of
20 workers using the lubricant.

 When drilling subterranean formations, drilling fluids serve, in part, to cool and lubricate the drill bit. Drill bits often encounter increasing downhole friction that arise from changes in downhole pressures, changes in the geological makeup of the formation, and changes in the direction of the drilling, especially when drilling a
25 horizontal well. The increases in friction can lead to a reduced rate of penetration and can limit the ability of the drill bit to reach its target destination with accuracy and efficiency. For example, increasing the rotational torque of the drill bit to address increasing frictional changes can lead to corkscrewing of the drill bit from its intended drilling path and can also cause pipe buckling (both helical and sinusoidal). The
30 increase in friction can also accelerate wear on the drill bit, thus resulting in down

time and expensive equipment repair and replacement. Accordingly, the performance demands required of the drilling fluid to provide lubricity to the bit increases over the time course of drilling.

However, current methodologies for reducing downhole friction in lateral wells generally involve reactive addition of lubricant products that are broadly acting, that may adversely affect the rheology of the fluid system, or that may dissipate or degrade over time. Lubricity additives to water based muds range from liquid lubricants (e.g., biodiesel, fatty acid ester, polyalpha-olefins) to mechanical lubricants (e.g., glass beads, copolymer beads, graphite). Adding concentrated “pills” of lubricants have a tendency to lose efficacy over time (e.g., due to dilution, sticking to cuttings, loss to the formation). Mechanical lubricants are effective at reducing friction, but may also create issues in data transmission when using mud pulse telemetry systems for measurement while drilling (MWD) tools if they plug the MWD valve. Additionally, recovery and reuse of beads can also be an issue, particularly if they are broken in use.

SUMMARY

In one aspect, provided is a fluid for use in a wellbore operation, the fluid comprising water, an oleaginous microbial cell, and a solvent, and optionally one or more of a surfactant, alcohol, demulsifier, or combinations thereof. In some embodiments, the fluid comprises a surfactant and an alcohol. In some embodiments, solvent is an ester or a terpene. In some embodiments, the solvent is ethyl lactate. In some embodiments, the solvent is d-limonene. In some embodiments, the surfactant is a fatty acid soap. In some embodiments, the surfactant is ethoxylated castor oil, polyoxyethylene sorbitan monopalmitate, or polyethylene glycol or combinations thereof. In some embodiments, the alcohol is isopropanol. In some embodiments, the surfactant and solvent together form a microemulsion.

In one aspect, provided is a drilling fluid for providing delay-released lubrication to a drill bit in a drilling operation, the fluid comprising:

- a) a drilling mud and
- b) an oleaginous microbial cell;

said fluid capable of providing increasing lubricity during drilling and one or more of

i) at least a 5% reduction, *e.g.*, at least a 10%, 15%, 20%, 25% reduction, in torque to the drill string;

ii) at least a 5% increase in rate of penetration; or

5 iii) at least a 5% reduction in drag.

In one aspect, provided is a drilling fluid for providing delay-released lubrication to a drill bit in a drilling operation, the fluid comprising:

a) a drilling mud and

b) an oleaginous microbial cell;

10 said fluid capable of providing increasing lubricity during drilling and one or more of

i) at least a 5% reduction, *e.g.*, at least a 10%, 15%, 20%, 25% reduction, in torque to the drill bit;

ii) at least a 5% increase in rate of penetration; or

iii) at least a 5% reduction in drag.

15 In one aspect, provided is a drilling fluid for providing delay-released lubrication to a drill bit in a drilling operation comprising a drilling mud and an oleaginous microbial cell. In varying embodiments, the fluid is capable of providing or provides increasing lubricity during drilling and at least a 5% reduction, *e.g.*, at least a 10%, 15%, 20%, 25% reduction, in torque to the drill bit.

20 In some embodiments, the fluid is capable of providing or provides increasing lubricity over at least a 5, 15, 30, 45, or 60 minute time period.

In some embodiments, the fluid is capable of providing or provides at least a 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75% reduction in torque.

25 In some embodiments, the fluid is capable of providing or provides at least a 60%, 65%, 70%, or 75% reduction in torque.

In one aspect, provided is a method for preparing a drilling fluid for providing delay-released lubrication to a drill bit in a drilling operation, the method comprising mixing a drilling mud with an oleaginous microbial cell to form a drilling fluid capable of increasing lubricity during drilling and one or more of

30 i) at least a 5% reduction, *e.g.*, at least a 10%, 15%, 20%, 25% reduction, in torque to the drill bit;

ii) at least a 5% increase in rate of penetration; or

iii) at least a 5% reduction in drag.

In one aspect, provided is a method for providing delay-released lubrication to a drill bit in a drilling operation, the method comprising mixing a drilling mud with an oleaginous microbial cell to form a drilling fluid capable of increasing lubricity during
5 drilling and reducing torque at the drill bit by at least 20%.

In one aspect, provided is a method for drilling a wellbore in a drilling operation, the method comprising circulating a drilling fluid provided herein through the wellbore.

In some embodiments, the microbial cell is in an amount that is 40%, 35%,
10 30%, 25%, 20%, 15%, 10%, 5%, 3%, 2%, or 1% or less by volume of the drilling fluid.

In some embodiments, the microbial cell is in an amount that is 10% or less by volume of the drilling fluid.

In some embodiments, the microbial cell is in an amount that is 6% or less by
15 volume of the drilling fluid.

In some embodiments, the microbial cell comprises a microalgal cell containing at least 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, or 85% oil.

In some embodiments, the microbial cell comprises a whole cell.

In some embodiments, the microbial cell comprises a lysed cell. In some
20 embodiments the oil has been extracted from the lysed cell to give a de-fatted cell. In some embodiments the lysed, de-fatted cells contain less than 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% oil. In some embodiments the lysed, de-fatted cells are mixed with whole cells. In some embodiments provided is a drilling fluid comprising a mixture lysed, de-fatted cells and whole cells. In some
25 embodiments the amount by weight of lysed, de-fatted cells in the mixture is less than the amount of whole cells. In some embodiments the weight ratio of lysed, de-fatted cells to whole cells in the mixture is no more than 1:30, 1:25, 1:20 1:10, 1:9, 1:8:1, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, or 1:1. In other embodiments the amount by weight of lysed, de-fatted cells in the mixture is greater than the amount of whole cells. In
30 other embodiments the weight ratio of lysed, de-fatted cells to whole cells in the mixture is at least 20:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, or 2:1.

In some embodiments, the microbial cell comprises an oleaginous bacteria, yeast, or microalgae.

In some embodiments, the microbial cell is obtained from a heterotrophic oleaginous microalgae.

- 5 In some embodiments, the microbial cell is obtained from microalgae cultivated with sugar from corn, sorghum, sugar cane, sugar beet, or molasses as a carbon source.

In some embodiments, the microbial cell is obtained from microalgae cultivated on sucrose.

- 10 In some embodiments, the microbial cell is obtained from *Parachlorella*, *Prototheca*, or *Chlorella*.

In some embodiments, the microbial cell is obtained from *Prototheca moriformis*.

- 15 In some embodiments, the microbial cell is an oleaginous microalgae having a fatty acid profile of at least 60% C18:1; or at least 50% combined total amount of C10, C12, and C14; or at least 70% combined total amount of C16:0 and C18:1.

In some embodiments, the drilling mud is a water-based mud, a synthetic-based mud, or an oil-based mud.

- 20 In some embodiments, the drilling operation is a land-based or an off-shore drilling operation.

In some embodiments, the drilling operation is selected from the group consisting of completion operations, sand control operations, workover operations, and hydraulic fracturing operations.

- 25 In some embodiments, the wellbore is a vertical, horizontal, or deviated wellbore. In some embodiments, the wellbore is a vertical or horizontal wellbore.

In one aspect, provided is a drilling rig containing a drilling fluid provided herein.

In some embodiments, the fluid is in a drill pipe or mud tank.

In some embodiments, a lubricant comprises an oleaginous microbial cell, and

the cell containing at least 45% oil by dry cell weight. In some embodiments, the cell contains or comprises at least 50%, 55%, 60%, 65%, 70%, 75%, or 80% oil by dry cell weight.

In some embodiments, the lubricant is capable of providing or provides at least a 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75% reduction in torque. In some embodiments, the lubricant is capable of providing at least a 60%, 65%, 70%, or 75% reduction in torque.

In some embodiments, the lubricant is capable of providing or provides at least a 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, or 45% increase in rate of penetration. In some embodiments the lubricant is capable of providing at least a 20% increase in rate of penetration.

In some embodiments, the lubricant is capable of providing or at least provides at least a 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, or 45% reduction in drag. In some embodiments the lubricant is capable of providing at least a 32% reduction in drag.

In some embodiments, the microbial cell comprises a whole cell. In some embodiments, the microbial cell comprises a lysed cell. In some embodiments, the microbial cell comprises an oleaginous bacteria, yeast, or microalgae. In some embodiments, the microbial cell is obtained from a heterotrophic oleaginous microalgae. In some embodiments, the microbial cell is obtained from microalgae cultivated with sugar from corn, sorghum, sugar cane, sugar beet, or molasses as a carbon source. In some embodiments, the microbial cell is obtained from microalgae cultivated on sucrose.

In some embodiments, the microbial cell is obtained from *Parachlorella*, *Prototheca*, or *Chlorella*. In some embodiments, the microbial cell is obtained from *Prototheca moriformis*.

In some embodiments, the cells are in powdered form. The powdered cells can be in a dry powder form.

In some embodiments provided is a biodegradable lubricant or drilling fluid.

In some embodiments, the microbial cell contains or comprises an oleaginous microalgae having a fatty acid profile of at least 60% C18:1; or at least 50%

combined total amount of C10, C12, and C14; or at least 70% combined total amount of C16:0 and C18:1.

In some embodiments, the microbial oil provided herein is a microalgal oil comprising C29 and C28 sterols, wherein the amount of C28 sterols is greater than
5 C29 sterols.

In some embodiments, the microbial oil provided herein is a microalgal oil comprising one or more of: at least 10% ergosterol; ergosterol and β -sitosterol, wherein the ratio of ergosterol to β -sitosterol is greater than 25:1; ergosterol and brassicasterol; ergosterol, brassicasterol, and poriferasterol, and wherein the oil is
10 optionally free from one or more of β -sitosterol, campesterol, and stigmasterol.

In some embodiments, the lubricant is an extreme pressure lubricant.

In some embodiments, provided is a metal working fluid comprising a lubricant provided herein.

In some embodiments, the lubricant is in an amount that is 90%, 80%, 70%,
15 60%, 50%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5%, 3%, 2%, or 1% or less by volume of the fluid.

In some embodiments, the metal working fluid is an insoluble oil, soluble oil, semisynthetic, or synthetic metal working fluid.

In some embodiments, the metal working fluid further comprises one or more
20 of a surfactant, emulsifier, defoamer, alkaline reserve, anti-mist agent, corrosion inhibitor, biocide, extreme pressure additive, coupling agent, thickener, chelating agent, lubricity agent, humectant, odorant, or dye.

In some embodiments, the surfactant comprises an ether, an alkoxyated nonylphenol, or mixtures thereof. In some embodiments, the emulsifier comprises a
25 hexahydrobenzoic acid, naphthenate, sulfonate, soap, amide, nonionic ethoxylate, an amphoteric, or mixtures thereof. In some embodiments, the defoamer comprises a silicone, waxy, calcium nitrite, acetate, or mixtures thereof. In some embodiments, the alkaline reserve comprises an alkanolamine, an alkali hydroxide, or mixtures thereof. In some embodiments, the anti-mist agent comprises a polybutene,
30 polyacrylate, polyethylene oxide, or mixtures thereof. In some embodiments, the corrosion inhibitor comprises an amine carboxylate, amine dicarboxylate, boramide,

arylsulfonamido acid, sodium borate, sodium molybdate, sodium metasilicate, succinic acid derivative, tolyltriazole, benzotriazole, benzothiazole, thiadiazole, diethanolamine, triethanolamine, nitrite, chlorophenols, cresol, formaldehyde formalin, iodine, phosphate, organic mercurials, phenols, quarternary ammonium compounds, oxammonium, S-triazine compounds, tris-hydroxymethylnitromethane, 5 or mixtures thereof. In some embodiments, the biocide comprises a triazine, nitromorpholine, polymeric quat, bromonirile, phenol, halogenated carbamate, isothiazolone, or mixtures thereof. In some embodiments, the extreme pressure additive comprises a sulfurized hydrocarbon, sulfurized fatty acid ester, chlorinated paraffin, chlorinated acid, chlorinated ester, phosphate ester, or mixtures thereof. In 10 some embodiments, the coupling agent comprises an alcohol, ether, glycol ether, hexylene glycol, or mixtures thereof. In some embodiments, the thickener comprises a polyether, a polyvinyl alcohol, or mixtures thereof. In some embodiments, the chelating agent comprises sodium EDTA, a phosphonate, gluconate, or mixtures thereof. In some embodiments, the lubricity agent comprises an aromatic oil, esters, 15 naphthenic oil, paraffinic oil, polyether glycol, ester, fatty acid ester, glycol ester, block copolymer, or mixtures thereof. In some embodiments, the humectant comprises a polymeric ether, an ester, or mixtures thereof. In some embodiments, the odorant comprises an aldehyde. In some embodiments, the dye comprises an azo dye, 20 a fluorescein, or mixtures thereof.

In some embodiments the oil encapsulated cells provided herein have an average diameter of about 5 to 10 microns.

In some embodiments the lubricants (e.g. encapsulated cells) provided herein are used as a lubricant in trenchless tunneling operations. Trenchless tunneling 25 methods are desirable for underground installation of utilities such as sewer, water, gas, electricity, and telecommunications in congested areas such as under roadways and city streets, or in soft soils, environmentally sensitive or contaminated areas, or near water crossings, where open cut trench excavation, pipe installation, and subsequent backfill are inconvenient or difficult.

30 Trenchless tunneling can be performed with a tunnel boring machine.

In some embodiments, the lubrication provided herein is in used in a microtunneling operation. In some embodiments, provided is a microtunneling boring

machine (MTBM) comprising a lubricant provided herein. In some embodiments the lubricant is for lubricating the interface between the earth and the cutting wheel or between the earth and the pipe section.

5 In microtunneling, an entry pit is prepared to receive a steerable MTBM that is advanced horizontally towards a receiving pit. The MTBM typically bores tunnels ranging from 1 to 10 feet in diameter, more commonly from 1 to 3 feet. Because of this small diameter, the MTBM is guided by remote control and follows a projected laser beam. The MTBM houses a cutting wheel and optionally a trailing component engaged with a jacking frame. The pipes that are to be installed are positioned behind
10 the cutting wheel or, when present, behind the trailing component. This assembly is pushed by hydraulic jacks mounted on the jacking frame. Slurry feed and discharge lines are connected to the MTBM to allow for removal of cuttings. In some embodiments, the slurry comprises a lubricant provided herein to lubricate the cutting wheel. In some embodiments the slurry further comprises bentonite.

15 The diameter of the cutting wheel used is typically slightly greater than the diameter of the pipes to create an overcut resulting in an annular space around the pipes. This space reduces frictional forces on the pipes as they are being advanced. Lubricants from the MTBM can be injected into the annular space to further reduce the frictional forces on the pipe/pipestring and to reduce the jacking forces required to
20 advance the pipe/pipestring. Typical lubricants include bentonite, and chemical polymers can also be used. In some embodiments, provided is a lubricant comprising bentonite and an oil encapsulated cell provided herein. Once the pipes have been installed the annular space can be filled with cement grout.

In other embodiments, trenchless tunneling applications benefiting from the
25 encapsulated lubricants disclosed herein include tunnels ranging from a diameter of six inches to diameters of over sixty feet. In some embodiments, the lubricants facilitate and increase drilling efficiency when cutting through a variety of soil, clay, and rock formations. These formations can include sandy to hard rock formations. The length of the tunnel can range from a few feet to over fifty miles. The lubricants
30 provided herein are particularly useful in drilling through problem areas that otherwise would have a low rate of penetration.

Large diameter tunnels include those that are cut with a tunnel boring

machine. These machines utilize a rotating cutting wheel (cutter head) to bore through the ground formation. In some embodiments, the lubricants provide lubrication between the cutter head and the ground formation. In other embodiments, the lubricants facilitate the removal of the drill cuttings (also known as muck) through the tunnel boring machine. The lubricants prevent and/or minimize muck from partially or completely occluding the cutter head openings through which the muck enters the tunnel boring machine.

In some embodiments, a slurry containing a lubricant provided herein acts to lubricate the drilling assembly as it contacts and moves against the earth, counterbalances the earth pressures resulting from the excavation, forms a filter cake against the earth to limit fluid losses, facilitates removal of the cuttings from the well/tunnel, and/or facilitates separation of the solid components from the liquid components as the slurry is circulated from the well/tunnel to a separation plant for recycling. In some embodiments the liquid component of the slurry is water. In some embodiments the water has a pH of between 8.0 and 10. In some embodiments the slurry contains bentonite, a bentonite salt, or a combination of the two. In some embodiments the slurry contains sodium montmorillonite. Bentonite containing slurries are particularly beneficial when used in sandy or coarse grained soils with fines content of 50% or less as defined by ASTM D-2487, while non-bentonite based slurries are recommended when fines content are greater than 50%. In some embodiments the slurry is substantially free from bentonite. In some embodiments the slurry contains polymers and/or inert solids.

In some embodiments the drilling fluids provided herein contain oils encapsulated in microbial cells wherein the oils are released when microbial cells when exposed to conditions favorable to cell lysis. Such conditions include temperature, pressure, shear and friction; in the absence of lysing conditions, the cells recirculate through the mud system. The cells are thus able to release its cellular contents and deliver the lubricating oil directly to the area in need of lubrication. The precise delivery of the lubricant at the appropriate time and place maximizes the effectiveness of the lubricant and minimizes waste. In some embodiments the cells encapsulating the oils contain a polysaccharide rich shell. In some embodiments the reduction in friction to the drill string provided by the lubricant allows for improved directional control of the drill bit and for drilling cleaner and straighter holes. The

reduction in friction also allows for the drill bit to be drilled further and faster, while reducing stuck pipe instances, tool maintenance, and interval changes.

In some embodiments, the fluids provided herein provide lubrication between metal surfaces and/or pipes or tubing. These fluids reduce friction and wear between
5 surfaces that rub against each other. Friction and wear can arise from inserting or removing metal pipes or metal equipment or from their use. In some embodiments, the fluids reduce friction and/or wear between metals of differing hardness. In some embodiments, the fluids reduce friction and/or wear between chrome and steel, such as between chrome tubulars and steel pipes.

10 In some embodiments, the fluids provide lubrication to a coiled tubing used in wellbore operations. Coiled tubing is a continuous metal tubing that is spooled on a reel and inserted down hole *via* an injector head. Coiled tubing are commonly used in fracking and well clean outs, and increasingly in drilling operations. Coiled tubing also finds use in deviated wells including horizontal wells.

15 In some embodiments, the fluids provided herein provide lubrication to the external surface of the coiled tubing. The fluids reduce friction and/or wear such as when the tubing is inserted or removed from a metal pipe or casing in the wellbore. In some embodiments, the coiled tubing is a 16Cr or 13Cr chrome tubular or a carbon steel tubular. In other embodiments, the fluids provided herein provide lubrication
20 between a coiled tubing and a steel casing. In still other embodiments, the steel casing is an L-80 carbon steel.

In some embodiments, the fluids provided herein provide lubrication to the internal surface of the coiled tubing. In a fracking operation, proppants such as sand are pumped down hole under high pressures. When performed using a coiled tubing,
25 centrifugal forces resulting from pumping abrasive proppants through the spool portion of the tubing results in non-uniform wear on the interior of the tubing. Proppants are available in a range of mesh sizes but are still considerably larger than the microbial cells and thus can rupture the cells to release lubricating oil. In some embodiments, the fluids provided herein reduce wear and increase the operational life
30 of a coiled tubing.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates coefficients of lubricity of water based mud containing oil from Strains A and B as a function of time and in comparison to mud containing industrial lubricants.

5 Figure 2 illustrates coefficients of lubricity of water based mud containing lysed or whole cells from Strains A and B as a function of time and in comparison to mud containing industrial lubricants.

 Figure 3 illustrates coefficients of lubricity of synthetic based mud containing lysed or whole cells from Strains A and B as a function of time and in
10 comparison to mud containing industrial lubricants.

 Figure 4 illustrates coefficients of lubricity of salty water based mud containing lysed or whole cells from Strains A and B as a function of time and in comparison to mud containing industrial lubricants.

 Figure 5 illustrates cell lysis of Strain B cells isolated from broth or that
15 were further drum dried.

 Figure 6 illustrates the drill path for a field trial using water based mud with whole microalgal cells from Strain A in comparison to using water based mud alone.

 Figure 7 illustrates hook weights (lb) of drill bottom housing assemblies provided with water based mud containing whole cells from Strain A as a function of
20 bit height (ft) and in comparison to water based mud alone when tripping out at 1,110-1170 and 1,285-1,330 feet (measured distance) corresponding to 45 and 60 degree portions of the curve.

 Figure 8 illustrates drag measurements at the 60 degree portion of the curve.

 Figure 9 illustrates the rotational torque required to rotate the drill string and
25 bottom hole assembly in the presence and absence of encapsulated oil.

 Figure 10 illustrates rate of penetration observed when drilling laterally in the presence and absence of encapsulated oil.

 Figure 11 illustrates the interaction the encapsulated oils with the bottom hole assembly and formation. 11A) Encapsulated oil is added and circulates

throughout the drilling fluid system. 11B) Under the appropriate stimulus (high friction, shear, extreme pressure, etc.) cells containing the oil rupture and oil is released. 11C) Oil is delivered at high effective concentration to lubricate and coat where it is needed. 11D) Unbroken cells are re-circulated throughout the system.

5 Figure 12 illustrates the percent cell lysis based on free oil release of microalgal and yeast strains in water at increasing pressures.

Figure 13 illustrates the reductions in torque observed in water containing microalgal or yeast cells or free oil compared to a petroleum based lubricant (Stabil Lube).

10 Figure 14 illustrates the increased rate of penetration (mm/min) by a tunnel boring machine using encapsulated oil as compared to traditional bentonite based fluids.

Figure 15 illustrates the distribution of rate of penetration readings by five feet ring sections in a tunnel boring operation.

15 Figure 16 illustrates the increased productivity of ring installation and time savings in a tunnel boring operation.

DETAILED DESCRIPTION

Definitions

20 Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

25 “Bottom hole assembly” or “BHA” refers to the portion of the drill string attached to the drill pipe that includes the drill bit as well as drill collar(s) and related assemblies that assist, in part, to provide weight to the bit.

“Biomass” is material produced by growth and/or propagation of cells. Biomass may contain cells and/or intracellular contents as well as extracellular material, includes, but is not limited to, compounds secreted by a cell. Biomass

isolated from fermentation broth may include nutrients and feedstock used to grow the cells.

“Bridging material” is material added to a fluid that prevents or decreases loss of the fluid through geologic formations that have pores that are greater than 1
5 millidarcy.

“Bioreactor” and “fermentor” mean an enclosure or partial enclosure, such as a fermentation tank or vessel, in which cells are cultured, typically in suspension.

“Cellulosic material” includes the product of digestion of cellulose, including glucose and xylose, and optionally additional compounds such as disaccharides,
10 oligosaccharides, lignin, furfurals and other compounds. Nonlimiting examples of sources of cellulosic material include sugar cane bagasses, sugar beet pulp, corn stover, wood chips, sawdust and switchgrass.

“Cultivated”, and variants thereof such as “cultured” and “fermented”, refer to the intentional fostering of growth (increases in cell size, cellular contents, and/or
15 cellular activity) and/or propagation (increases in cell numbers via mitosis) of one or more cells by use of selected and/or controlled conditions. The combination of both growth and propagation is termed proliferation. Examples of selected and/or controlled conditions include the use of a defined medium (with known characteristics such as pH, ionic strength, and carbon source), specified temperature, oxygen tension,
20 carbon dioxide levels, and growth in a bioreactor. Cultivate does not refer to the growth or propagation of microorganisms in nature or otherwise without human intervention; for example, natural growth of an organism that ultimately becomes fossilized to produce geological crude oil is not cultivation.

“Dry weight” and “dry cell weight” mean weight determined in the relative
25 absence of water. For example, reference to oleaginous yeast biomass as comprising a specified percentage of a particular component by dry weight means that the percentage is calculated based on the weight of the biomass after substantially all water has been removed.

“Exogenous gene” is a nucleic acid that codes for the expression of an RNA
30 and/or protein that has been introduced (“transformed”) into a cell. A transformed cell may be referred to as a recombinant cell, into which additional exogenous gene(s)

may be introduced. The exogenous gene may be from a different species (and so heterologous), or from the same species (and so homologous), relative to the cell being transformed. Thus, an exogenous gene can include a homologous gene that occupies a different location in the genome of the cell or is under different control,
5 relative to the endogenous copy of the gene. An exogenous gene may be present in more than one copy in the cell. An exogenous gene may be maintained in a cell as an insertion into the genome or as an episomal molecule.

“Fixed carbon source” is a molecule(s) containing carbon, typically an organic molecule, that is present at ambient temperature and pressure in solid or liquid
10 form in a culture media that can be utilized by a microorganism cultured therein.

“Fluid loss control agent” is material added to a fluid that prevents or decreases loss of the liquid component of the fluid through geologic formations that have pores that are less than 1 millidarcy.

“Growth” means an increase in cell size, total cellular contents, and/or cell
15 mass or weight of an individual cell, including increases in cell weight due to conversion of a fixed carbon source into intracellular oil.

“Homogenate” is biomass that has been physically disrupted.

“Limiting concentration of a nutrient” is a concentration of a compound in a culture that limits the propagation of a cultured organism. A “non-limiting
20 concentration of a nutrient” is a concentration that supports maximal propagation during a given culture period. Thus, the number of cells produced during a given culture period is lower in the presence of a limiting concentration of a nutrient than when the nutrient is non-limiting. A nutrient is said to be “in excess” in a culture, when the nutrient is present at a concentration greater than that which supports
25 maximal propagation.

“Lipids” are a class of molecules that are soluble in nonpolar solvents (such as ether and chloroform) and are relatively or completely insoluble in water. Lipid molecules have these properties, because they consist largely of long hydrocarbon chains which are hydrophobic in nature. Examples of lipids include fatty acids
30 (saturated and unsaturated); glycerides or glycerolipids (such as monoglycerides, diglycerides, triglycerides or neutral fats, and phosphoglycerides or

glycerophospholipids); nonglycerides (sphingolipids, sterol lipids including cholesterol and steroid hormones, prenol lipids including terpenoids, fatty alcohols, waxes, and polyketides); and complex lipid derivatives (sugar-linked lipids, or glycolipids, and protein-linked lipids). “Fats” or “triglyceride oils” are a subgroup of lipids called “triacylglycerides.” The fatty acids are conventionally named by the notation that recites number of carbon atoms and the number of double bonds separated by a colon. For example oleic acid can be referred to as C18:1 and capric acid can be referred to as C10:0. As used herein, the term “triacylglycerides” and “triglycerides” are interchangeable.

“Lubricity” refers to the ability of a lubricant to reduce frictional forces such as torque and drag forces acting on a drill bit or drill string. The lubricity of a lubricant is measured by its coefficient of friction, which is defined as the ratio of the force required to move an object to the force applied perpendicular to the object. A low coefficient of friction corresponds to high lubricity.

“Lysate” is a solution containing the contents of lysed cells.

“Lysis” is the breakage of the plasma membrane and optionally the cell wall of a biological organism sufficient to release at least some intracellular content, often by mechanical, viral or osmotic mechanisms that compromise its integrity.

“Lysing” is disrupting the cellular membrane and optionally the cell wall of a biological organism or cell sufficient to release at least some intracellular content.

“Microorganism” and “microbe” are microscopic unicellular organisms.

“Mud” or “drilling fluid” is a generic term used to refer to a fluid used in drilling operations. Drilling fluids typically perform a number of functions, including cooling and lubricating the drill bit and drill string, transporting cuttings from the drill bit to the surface, and controlling downhole pressures to prevent blow-outs.

Examples of drilling fluids include water based drilling fluids and non-aqueous based systems such as oil based and synthetic based drilling fluids.

“Oil” means any triacylglyceride (or triglyceride oil), produced by organisms, including oleaginous yeast, plants, and/or animals. “Oil,” as distinguished from “fat,” refers, unless otherwise indicated, to lipids that are generally liquid at ordinary room temperatures and pressures. For example, “oil” includes vegetable or seed oils

derived from plants, including without limitation, an oil derived from soy, rapeseed, canola, palm, palm kernel, coconut, corn, olive, sunflower, cotton seed, cuphea, peanut, camelina sativa, mustard seed, cashew nut, oats, lupine, kenaf, calendula, hemp, coffee, linseed, hazelnut, euphorbia, pumpkin seed, coriander, camellia, sesame, safflower, rice, tung oil tree, cocoa, copra, opium poppy, castor beans, pecan, jojoba, jatropha, macadamia, Brazil nuts, and avocado, as well as combinations thereof.

“Oleaginous microorganism”, “oleaginous microbe”, and “oleaginous microbial cell” refers to a microorganism or cell producing at least 20 % lipid by dry cell weight. The microorganisms include wild-type, genetically engineered, or mutated microorganisms. In particular embodiments, the microorganism yields cells that are at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, or at least 70% or more lipid. “Oleaginous yeast” means yeast that can naturally accumulate more than 20% of their dry cell weight as lipid and are of the *Dikarya* subkingdom of fungi. Oleaginous yeast includes organisms such as *Yarrowia lipolytica*, *Rhodotorula glutinis*, *Cryptococcus curvatus* and *Lipomyces starkeyi*.

“Polysaccharides” or “glycans” are carbohydrates made up of monosaccharides joined together by glycosidic linkages. Cellulose is a polysaccharide that makes up certain plant cell walls. Cellulose can be depolymerized by enzymes to yield monosaccharides such as xylose and glucose, as well as larger disaccharides and oligosaccharides.

“Predominantly encapsulated” means that more than 50% of a referenced component, e.g., algal oil, is sequestered in an oleaginous microbe cell or cells.

“ppb” refers to pounds per barrel. 1 ppb is equivalent to 1 gram material per 350mL base fluid.

“Predominantly intact cells” and “predominantly intact biomass” mean a population of cells that comprise more than 50% intact cells. “Intact”, in this context, means that the physical continuity of the cellular membrane and/or cell wall enclosing the intracellular components of the cell has not been disrupted in any manner that would release the intracellular components of the cell to an extent that exceeds the permeability of the cellular membrane in culture.

“Predominantly lysed” means a population of cells in which more than 50%, of the cells have been disrupted such that the intracellular components of the cell are no longer completely enclosed within the cell membrane.

A “fatty acid profile” is the distribution of fatty acyl groups in the triglycerides of the oil without reference to attachment to a glycerol backbone. Fatty acid profiles are typically determined by conversion to a fatty acid methyl ester (FAME), followed by gas chromatography (GC) analysis with flame ionization detection (FID). The fatty acid profile can be expressed as one or more percent of a fatty acid in the total fatty acid signal determined from the area under the curve for that fatty acid. FAME-GC-FID measurement approximate weight percentages of the fatty acids. A “sn-2 profile” is the distribution of fatty acids found at the sn-2 position of the triacylglycerides in the oil. A “regiospecific profile” is the distribution of triglycerides with reference to the positioning of acyl group attachment to the glycerol backbone without reference to stereospecificity. In other words, a regiospecific profile describes acyl group attachment at sn-1/3 vs. sn-2. Thus, in a regiospecific profile, POS (palmitate-oleate-stearate) and SOP (stearate-oleate-palmitate) are treated identically. A “stereospecific profile” describes the attachment of acyl groups at sn-1, sn-2 and sn-3. Unless otherwise indicated, triglycerides such as SOP and POS are to be considered equivalent. A “TAG profile” is the distribution of fatty acids found in the triglycerides with reference to connection to the glycerol backbone, but without reference to the regiospecific nature of the connections. Thus, in a TAG profile, the percent of SSO in the oil is the sum of SSO and SOS, while in a regiospecific profile, the percent of SSO is calculated without inclusion of SOS species in the oil. In contrast to the weight percentages of the FAME-GC-FID analysis, triglyceride percentages are typically given as mole percentages; that is the percent of a given TAG molecule in a TAG mixture.

“Proliferation” means a combination of both growth and propagation.

“Propagation” means an increase in cell number via mitosis or other cell division.

“Renewable diesel” is a mixture of alkanes (such as C10:0, C12:0, C14:0, C16:0 and C18:0) produced through hydrogenation and deoxygenation of lipids.

“Spent biomass” and variants thereof such as “delipidated meal” and “defatted biomass” is microbial biomass after oil (including lipids) and/or other components have been extracted or isolated from it; e.g., through the use of mechanical (i.e., exerted by an expeller press) or solvent extraction or both. Such delipidated meal has
5 a reduced amount of oil/lipids as compared to before the extraction or isolation of oil/lipids from the microbial biomass but typically contains some residual oil/lipid.

“Sonication” is a process of disrupting biological materials, such as a cell, by use of sound wave energy.

“Viscosity modifying agent” is an agent that modifies the rheological
10 properties of a fluid. The viscosity of a fluid is the measure of the resistance of a fluid to flow. The viscosity modifying agent is used to increase or decrease the viscosity of a fluid used in oil field chemical applications

“V/V” or “v/v”, in reference to proportions by volume, means the ratio of the volume of one substance in a composition to the volume of the composition. For
15 example, reference to a composition that comprises 5% v/v yeast oil means that 5% of the composition's volume is composed of oil (e.g., such a composition having a volume of 100 mm³ would contain 5 mm³ of oil), and the remainder of the volume of the composition (e.g., 95 mm³ in the example) is composed of other ingredients.

“W/V” or “w/v”, in reference to a concentration of a substance means grams
20 of

“W/W” or “w/w”, in reference to proportions by weight, means the ratio of the weight of one substance in a composition to the weight of the composition. For example, reference to a composition that comprises 5% w/w oleaginous yeast biomass means that 5% of the composition's weight is composed of oleaginous yeast biomass
25 (e.g., such a composition having a weight of 100 mg would contain 5 mg of oleaginous yeast biomass) and the remainder of the weight of the composition (e.g., 95 mg in the example) is composed of other ingredients.

Oleaginous Microbes and Heterotrophic Culture Conditions

The triacylglycerides used in the preparation of the triacylglyceride mixtures
30 can be obtained from any organism producing triacylglycerides with C18:1 or saturated C:4-C24 fatty acids. Production of hydrocarbons by microorganisms is

reviewed by Metzger *et al.*, *Appl Microbiol Biotechnol* (2005) 66: 486-496 and A Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae, NREL/TP-580-24190, John Sheehan, Terri Dunahay, John Benemann and Paul Roessler (1998), incorporated herein by reference.

5 The triacylglycerides used in the preparation of the triacylglyceride mixtures can be obtained from any organism producing triacylglycerides with C18:1 or saturated C4-C24 fatty acids. Production of hydrocarbons by microorganisms is reviewed by Metzger *et al.*, *Appl Microbiol Biotechnol* (2005) 66: 486-496 and A Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel
10 from Algae, NREL/TP-580-24190, John Sheehan, Terri Dunahay, John Benemann and Paul Roessler (1998), incorporated herein by reference.

 In particular embodiments, the microorganism yields cells that are at least: about 40%, to 60% or more (including more than 70%) lipid when harvested for oil extraction. For many applications, organisms that grow heterotrophically (on sugar or
15 a carbon source other than carbon dioxide in the absence of light) or can be engineered to do so, are useful in the methods and drilling fluids provided herein. See PCT Publication Nos. 2010/063031; 2010/063032; 2008/151149, each of which is incorporated herein by reference in their entireties.

 Naturally occurring and genetically engineered microalgae are suitable
20 microorganisms as sources of C18:1 or saturated C4-C24 triacylglycerides suitable for use in the methods and materials provided herein. Thus, in various embodiments, the microorganism from which the triacylglyceride is obtained is a microalgae. Examples of genera and species of microalgae include, but are not limited to, the following genera and species microalgae in Table 1.

25

Table 1. Microalgae

Achnanthes orientalis, *Agmenellum*, *Amphiprora hyaline*, *Amphora coffeiformis*, *Amphora coffeiformis linea*, *Amphora coffeiformis punctata*, *Amphora coffeiformis taylori*, *Amphora coffeiformis tenuis*, *Amphora delicatissima*, *Amphora delicatissima capitata*, *Amphora sp.*, *Anabaena*, *Ankistrodesmus*, *Ankistrodesmus falcatus*, *Boekelovia hooglandii*, *Borodinella sp.*, *Botryococcus braunii*, *Botryococcus sudeticus*, *Bracteococcus aerius*, *Bracteococcus sp.*, *Bracteococcus grandis*, *Bracteococcus cinnabarinus*, *Bracteococcus minor*, *Bracteococcus medionucleatus*, *Carteria*, *Chaetoceros gracilis*, *Chaetoceros muelleri*, *Chaetoceros muelleri subsalsum*, *Chaetoceros sp.*, *Chlorella anitrata*, *Chlorella Antarctica*, *Chlorella aureoviridis*, *Chlorella candida*, *Chlorella capsulate*, *Chlorella desiccata*, *Chlorella ellipsoidea*, *Chlorella emersonii*, *Chlorella fusca*, *Chlorella fusca var. vacuolata*, *Chlorella glucotropha*, *Chlorella infusionum*, *Chlorella infusionum var. actophila*, *Chlorella infusionum var. auxenophila*, *Chlorella kessleri*, *Chlorella lobophora* (strain SAG 37.88), *Chlorella luteoviridis*, *Chlorella luteoviridis var. aureoviridis*, *Chlorella luteoviridis var. lutescens*, *Chlorella miniata*, *Chlorella cf. minutissima*, *Chlorella minutissima*, *Chlorella mutabilis*, *Chlorella nocturna*, *Chlorella ovalis*, *Chlorella parva*, *Chlorella photophila*, *Chlorella pringsheimii*, *Chlorella protothecoides* (including any of UTEX strains 1806, 411, 264, 256, 255, 250, 249, 31, 29, 25), *Chlorella protothecoides var. acidicola*, *Chlorella regularis*, *Chlorella regularis var. minima*, *Chlorella regularis var. umbricata*, *Chlorella reisigii*, *Chlorella saccharophila*, *Chlorella saccharophila var. ellipsoidea*, *Chlorella salina*, *Chlorella simplex*, *Chlorella sorokiniana*, *Chlorella sp.*, *Chlorella sphaerica*, *Chlorella stigmatophora*, *Chlorella vanniellii*, *Chlorella vulgaris*, *Chlorella vulgaris f. tertia*, *Chlorella vulgaris var. autotrophica*, *Chlorella vulgaris var. viridis*, *Chlorella vulgaris var. vulgaris*, *Chlorella vulgaris var. vulgaris f. tertia*, *Chlorella vulgaris var. vulgaris f. viridis*, *Chlorella xanthella*, *Chlorella zofingiensis*, *Chlorella trebouxiioides*, *Chlorella vulgaris*, *Chlorococcum infusionum*, *Chlorococcum sp.*, *Chlorogonium*, *Chroomonas sp.*, *Chrysosphaera sp.*, *Cricosphaera sp.*, *Cryptocodinium cohnii*, *Cryptomonas sp.*, *Cyclotella cryptica*, *Cyclotella meneghiniana*, *Cyclotella sp.*, *Dunaliella sp.*, *Dunaliella bardawil*, *Dunaliella bioculata*, *Dunaliella granulate*, *Dunaliella maritime*, *Dunaliella minuta*, *Dunaliella parva*, *Dunaliella peircei*, *Dunaliella primolecta*, *Dunaliella salina*, *Dunaliella terricola*, *Dunaliella tertiolecta*, *Dunaliella viridis*, *Dunaliella tertiolecta*, *Eremosphaera viridis*, *Eremosphaera sp.*, *Ellipsoidon sp.*, *Euglena*,

Franceia sp., *Fragilaria crotonensis*, *Fragilaria* sp., *Gleocapsa* sp., *Gloeothamnion* sp., *Hymenomonas* sp., *Isochrysis* aff. *galbana*, *Isochrysis galbana*, *Lepocinclis*, *Micractinium*, *Micractinium* (UTEX LB 2614), *Monoraphidium minutum*, *Monoraphidium* sp., *Nannochloris* sp., *Nannochloropsis salina*, *Nannochloropsis* sp., *Navicula acceptata*, *Navicula biskanterae*, *Navicula pseudotenelloides*, *Navicula pelliculosa*, *Navicula saprophila*, *Navicula* sp., *Neochloris oleabundans*, *Nephrochloris* sp., *Nephroselmis* sp., *Nitzschia communis*, *Nitzschia alexandrina*, *Nitzschia communis*, *Nitzschia dissipata*, *Nitzschia frustulum*, *Nitzschia hantzschiana*, *Nitzschia inconspicua*, *Nitzschia intermedia*, *Nitzschia microcephala*, *Nitzschia pusilla*, *Nitzschia pusilla elliptica*, *Nitzschia pusilla monoensis*, *Nitzschia quadrangular*, *Nitzschia* sp., *Ochromonas* sp., *Oocystis parva*, *Oocystis pusilla*, *Oocystis* sp., *Oscillatoria limnetica*, *Oscillatoria* sp., *Oscillatoria subbrevis*, *Parachlorella beijerinckii*, *Parachlorella kessleri*, *Pascheria acidophila*, *Pavlova* sp., *Phagus*, *Phormidium*, *Platymonas* sp., *Pleurochrysis carterae*, *Pleurochrysis dentate*, *Pleurochrysis* sp., *Prototheca stagnora*, *Prototheca portoricensis*, *Prototheca moriformis*, *Prototheca wickerhamii*, *Prototheca zopfii*, *Pseudochlorella aquatica*, *Pyramimonas* sp., *Pyrobotrys*, *Sarcinoid chrysophyte*, *Scenedesmus armatus*, *Scenedesmus rubescens*, *Schizochytrium*, *Spirogyra*, *Spirulina platensis*, *Stichococcus* sp., *Synechococcus* sp., *Tetraedron*, *Tetraselmis* sp., *Tetraselmis suecica*, *Thalassiosira weissflogii*, and *Viridiella fridericiana*.

The microorganisms can be genetically engineered to metabolize an alternative sugar source such as sucrose or xylose and/or produce an altered fatty acid profile. Where the microorganism can be grown heterotrophically, it can be an
 5 organism that is a permissive or obligate heterotroph. In a specific embodiment, the organism is *Prototheca moriformis*, an obligate heterotrophic oleaginous microalgae. In a further specific embodiment, the *Prototheca moriformis*, has been genetically engineered to metabolize sucrose or xylose.

In various embodiments, the microorganism is an organism of a species of the
 10 genus *Chlorella*. In some embodiments, the microalgae is *Chlorella protothecoides*, *Chlorella ellipsoidea*, *Chlorella minutissima*, *Chlorella zofinienesi*, *Chlorella luteoviridis*, *Chlorella kessleri*, *Chlorella sorokiniana*, *Chlorella fusca* var. *vacuolata* *Chlorella* sp., *Chlorella* cf. *minutissima* or *Chlorella emersonii*. *Chlorella* is a genus of single-celled green algae, belonging to the phylum Chlorophyta. It is spherical in

shape, about 2 to 10 μm in diameter, and is without flagella. Some species of *Chlorella* are naturally heterotrophic.

Chlorella, for example, *Chlorella protothecoides*, *Chlorella minutissima*, or *Chlorella emersonii*, can be genetically engineered to express one or more
 5 heterologous genes (“transgenes”). Examples of expression of transgenes in, e.g., *Chlorella*, can be found in the literature (see for example PCT Patent Publication Nos. 2010/063031, 2010/063032, and 2008/151149; *Current Microbiology* Vol. 35 (1997), pp. 356–362; Sheng Wu Gong Cheng Xue Bao. 2000 Jul;16(4):443-6; *Current Microbiology* Vol. 38 (1999), pp. 335–341; *Appl Microbiol Biotechnol* (2006) 72:
 10 197–205; *Marine Biotechnology* 4, 63–73, 2002; *Current Genetics* 39:5, 365–370 (2001); *Plant Cell Reports* 18:9, 778–780, (1999); *Biologia Plantarum* 42(2): 209–216, (1999); *Plant Pathol.* J 21(1): 13–20, (2005)), and such references teach various methods and materials for introducing and expressing genes of interest in such organisms. Other lipid-producing microalgae can be engineered as well, including
 15 prokaryotic Microalgae (see Kalscheuer et al., *Applied Microbiology and Biotechnology*, Volume 52, Number 4 / October, 1999).

With regard to the alga species recited herein, it is noted that the taxonomy of algal species is in constant flux. Therefore it is possible that genera, species, and strains will change their names as time progresses. Where possible, alternative strain
 20 names are provided. However, it is anticipated that the current status of genus and species designations will change over time and the invention will maintain its relevance to the strains whatever their eventual designation. A current example is the renaming of *Chlorella protothecoides* as *Auxenochlorella protothecoides*. For the purposes of this disclosure they should be treated as the same organism.

Prototheca is a genus of single-cell microalgae believed to be a non-photosynthetic mutant of *Chlorella*. While *Chlorella* can obtain its energy through photosynthesis, species of the genus *Prototheca* are obligate heterotrophs. *Prototheca* are spherical in shape, about 2 to 15 micrometers in diameter, and lack flagella. In various embodiments, the microalgae used to generate the triacylglycerides is selected
 30 from the following species of *Prototheca*: *Prototheca stagnora*, *Prototheca portoricensis*, *Prototheca moriformis*, *Prototheca wickerhamii* and *Prototheca zopfii*.

In addition to *Prototheca* and *Chlorella*, other microalgae can be used to as sources of triacylglycerides. In various preferred embodiments, the microalgae is selected from a genus or species from any of the following genera and species:

- Parachlorella kessleri*, *Parachlorella beijerinckii*, *Neochloris oleabundans*,
5 *Bracteacoccus grandis*, *Bracteacoccus cinnabarinus*, *Bracteococcus aerius*,
Bracteococcus sp. or *Scenedesmus rebescens*.

Genetically modified microalgae include those described in US provisional applications 61/887,268 filed 04-Oct-2013, 61/892,399 filed 17-Oct-2013, 61/895,355 filed 24-Oct-2013, 61/923,327 filed 03-Jan-2014, and 62/023,109 filed 10-Jul-2014.

- 10 These applications are incorporated herein by reference in their entireties. In some embodiments, the microalgal cells are genetically modified to down regulate a FATA1 gene, overexpress a KASII gene, and express a hairpin RNA to knockdown a delta 12 fatty acid desaturase as described in Example 57 of these applications. The fatty acid profile of triglyceride oils produced by these cells were found to have
15 greater than 90% C18:1 and less than 1% C18:2.

The oils produced according to the above methods in some cases are made using a microalgal host cell. As described above, the microalga can be, without limitation, fall in the classification of Chlorophyta, Trebouxioophyceae, Chlorellales, Chlorellaceae, or Chlorophyceae. It has been found that microalgae of

- 20 Trebouxioophyceae can be distinguished from vegetable oils based on their sterol profiles. Oil produced by *Chlorella protothecoides* was found to produce sterols that appeared to be brassicasterol, ergosterol, campesterol, stigmasterol, and β -sitosterol, when detected by GC-MS. However, it is believed that all sterols produced by *Chlorella* have C24 β stereochemistry. Thus, it is believed that the molecules
25 detected as campesterol, stigmasterol, and β -sitosterol, are actually 22,23-dihydrobrassicasterol, proferasterol and clionasterol, respectively. Thus, the oils produced by the microalgae described above can be distinguished from plant oils by the presence of sterols with C24 β stereochemistry and the absence of C24 α stereochemistry in the sterols present. For example, the oils produced may contain
30 22,23-dihydrobrassicasterol while lacking campesterol; contain Clionasterol, while lacking in β -sitosterol, and/or contain poriferasterol while lacking stigmasterol.

Alternately, or in addition, the oils may contain significant amounts of Δ^7 -poriferasterol.

In other embodiments, the oils provided herein are not vegetable oils. Vegetable oils are oils extracted from plants and plant seeds. Vegetable oils can be distinguished from the non-plant oils provided herein on the basis of their oil content. A variety of methods for analyzing the oil content can be employed to determine the source of the oil or whether adulteration of an oil provided herein with an oil of a different (e.g. plant) origin has occurred. The determination can be made on the basis of one or a combination of the analytical methods. These tests include but are not limited to analysis of one or more of free fatty acids, fatty acid profile, total triacylglycerol content, diacylglycerol content, peroxide values, spectroscopic properties (e.g. UV absorption), sterol profile, sterol degradation products, antioxidants (e.g. tocopherols), pigments (e.g. chlorophyll), $\delta^{13}\text{C}$ values and sensory analysis (e.g. taste, odor, and mouth feel). Many such tests have been standardized for commercial oils such as the Codex Alimentarius standards for edible fats and oils.

In some embodiments, the oil content of an oil provided herein comprises, as a percentage of total sterols, less than 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1% 24-ethylcholest-5-en-3-ol. In some embodiments, the 24-ethylcholest-5-en-3-ol is clionasterol. In some embodiments, the oil content of an oil provided herein comprises, as a percentage of total sterols, at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% clionasterol.

In some embodiments, the oil content of an oil provided herein contains, as a percentage of total sterols, less than 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1% 24-methylcholest-5-en-3-ol. In some embodiments, the 24-methylcholest-5-en-3-ol is 22,23-dihydrobrassicasterol. In some embodiments, the oil content of an oil provided herein comprises, as a percentage of total sterols, at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% 22,23-dihydrobrassicasterol.

In some embodiments, the oil content of an oil provided herein contains, as a percentage of total sterols, less than 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1% 5,22-cholestadien-24-ethyl-3-ol. In some embodiments, the 5,22-cholestadien-24-ethyl-3-ol is poriferasterol. In some embodiments, the oil content of an oil provided herein

comprises, as a percentage of total sterols, at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% poriferasterol.

In some embodiments, the oil content of an oil provided herein contains ergosterol or brassicasterol or a combination of the two. In some embodiments, the oil content contains, as a percentage of total sterols, at least 5%, 10%, 20%, 25%, 35%, 40%, 45%, 50%, 55%, 60%, or 65% ergosterol. In some embodiments, the oil content contains, as a percentage of total sterols, at least 25% ergosterol. In some embodiments, the oil content contains, as a percentage of total sterols, at least 40% ergosterol. In some embodiments, the oil content contains, as a percentage of total sterols, at least 5%, 10%, 20%, 25%, 35%, 40%, 45%, 50%, 55%, 60%, or 65% of a combination of ergosterol and brassicasterol.

In some embodiments, the oil content contains, as a percentage of total sterols, at least 1%, 2%, 3%, 4% or 5% brassicasterol. In some embodiments, the oil content contains, as a percentage of total sterols less than 10%, 9%, 8%, 7%, 6%, or 5% brassicasterol.

In some embodiments the ratio of ergosterol to brassicasterol is at least 5:1, 10:1, 15:1, or 20:1.

In some embodiments, the oil content contains, as a percentage of total sterols, at least 5%, 10%, 20%, 25%, 35%, 40%, 45%, 50%, 55%, 60%, or 65% ergosterol and less than 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1% β -sitosterol. In some embodiments, the oil content contains, as a percentage of total sterols, at least 25% ergosterol and less than 5% β -sitosterol. In some embodiments, the oil content further comprises brassicasterol.

Sterols contain from 27 to 29 carbon atoms (C27 to C29) and are found in all eukaryotes. Animals exclusively make C27 sterols as they lack the ability to further modify the C27 sterols to produce C28 and C29 sterols. Plants however are able to synthesize C28 and C29 sterols, and C28/C29 plant sterols are often referred to as phytosterols. The sterol profile of a given plant is high in C29 sterols, and the primary sterols in plants are typically the C29 sterols β -sitosterol and stigmasterol. In contrast, the sterol profile of non-plant organisms contain greater percentages of C27 and C28 sterols. For example the sterols in fungi and in many

microalgae are principally C28 sterols. The sterol profile and particularly the striking predominance of C29 sterols over C28 sterols in plants has been exploited for determining the proportion of plant and marine matter in soil samples (Huang, Wen-Yen, Meinschein W. G., "Sterols as ecological indicators"; *Geochimica et*

5 *Cosmochimia Acta*. Vol 43. pp 739-745).

In some embodiments the primary sterols in the microalgal oils provided herein are sterols other than β -sitosterol and stigmasterol. In some embodiments of the microalgal oils, C29 sterols make up less than 50%, 40%, 30%, 20%, 10%, or 5% by weight of the total sterol content.

10 In some embodiments the microalgal oils provided herein contain C28 sterols in excess of C29 sterols. In some embodiments of the microalgal oils, C28 sterols make up greater than 50%, 60%, 70%, 80%, 90%, or 95% by weight of the total sterol content. In some embodiments the C28 sterol is ergosterol. In some embodiments the C28 sterol is brassicasterol.

15 In addition to microalgae, oleaginous yeast can accumulate more than 20% of their dry cell weight as lipid and so are useful sources of triglycerides. Examples of oleaginous yeast include, but are not limited to, the oleaginous yeast listed in Table 2.

Table 2. Oleaginous Yeast.

<i>Candida apicola</i>, <i>Candida sp.</i>, <i>Cryptococcus curvatus</i>, <i>Cryptococcus terricolus</i>, <i>Debaromyces hansenii</i>, <i>Endomycopsis vernalis</i>, <i>Geotrichum carabidarum</i>, <i>Geotrichum cucujoidarum</i>, <i>Geotrichum histeridarum</i>, <i>Geotrichum silvicola</i>, <i>Geotrichum vulgare</i>, <i>Hyphopichia burtonii</i>, <i>Lipomyces lipofer</i>, <i>Lypomyces orientalis</i>, <i>Lipomyces starkeyi</i>, <i>Lipomyces tetrasporous</i>, <i>Pichia mexicana</i>, <i>Rodosporidium sphaerocarpum</i>, <i>Rhodosporidium toruloides</i>, <i>Rhodotorula aurantiaca</i>, <i>Rhodotorula dairenensis</i>, <i>Rhodotorula diffluens</i>, <i>Rhodotorula glutinus</i>, <i>Rhodotorula glutinis var. glutinis</i>, <i>Rhodotorula gracilis</i>, <i>Rhodotorula graminis</i> <i>Rhodotorula minuta</i>, <i>Rhodotorula mucilaginosa</i>, <i>Rhodotorula mucilaginosa var. mucilaginosa</i>, <i>Rhodotorula terpenoidalis</i>, <i>Rhodotorula toruloides</i>, <i>Sporobolomyces alborubescens</i>, <i>Starmerella bombicola</i>, <i>Torulaspora delbrueckii</i>, <i>Torulaspora pretoriensis</i>, <i>Trichosporon behrend</i>, <i>Trichosporon brassicae</i>, <i>Trichosporon domesticum</i>, <i>Trichosporon laibachii</i>, <i>Trichosporon loubieri</i>, <i>Trichosporon loubieri var. loubieri</i>, <i>Trichosporon montevidense</i>, <i>Trichosporon</i>

pullulans, *Trichosporon* sp., *Wickerhamomyces Canadensis*, *Yarrowia lipolytica*, and *Zygoascus meyeræ*.

Examples of oleaginous microbes include fungi such as the fungi are listed in Table 3.

Table 3. Oleaginous Fungi.

Mortierella, *Mortierella vinacea*, *Mortierella alpine*, *Pythium debaryanum*, *Mucor circinelloides*, *Aspergillus ochraceus*, *Aspergillus terreus*, *Penicillium lilacinum*, *Hansenula*, *Chaetomium*, *Cladosporium*, *Malbranchea*, *Rhizopus*, and *Pythium*

5

In one embodiment, the microorganism used for the production of triacylglycerides for use in drilling fluids provided herein is a fungus. Examples of suitable fungi (e.g., *Mortierella alpine*, *Mucor circinelloides*, and *Aspergillus ochraceus*) include those that have been shown to be amenable to genetic manipulation, as described in the literature (see, for example, *Microbiology*, Jul; 153(Pt.7): 2013-25 (2007); *Mol Genet Genomics*, Jun; 271(5): 595-602 (2004); *Curr Genet*, Mar;21(3):215-23 (1992); *Current Microbiology*, 30(2):83-86 (1995); Sakuradani, NISR Research Grant, "Studies of Metabolic Engineering of Useful Lipid-producing Microorganisms" (2004); and PCT/JP2004/012021).

15 In other embodiments, a microorganism producing a triglyceride is an oleaginous bacterium. Oleaginous bacteria are bacteria that can accumulate more than 20% of their dry cell weight as lipid. Species of oleaginous bacteria for use in the present methods include species of the genus *Rhodococcus*, such as *Rhodococcus opacus* and *Rhodococcus* sp. Methods of cultivating oleaginous bacteria, such as
20 *Rhodococcus opacus*, are known in the art (see Waltermann, et al., (2000) *Microbiology*, 146: 1143-1149).

The oleaginous microorganisms can be cultured for production of triglycerides. This type of culture is typically first conducted on a small scale and, initially, at least, under conditions in which the starting microorganism can grow.
25 Culture for purposes of hydrocarbon production is preferentially conducted on a large

scale and under heterotrophic conditions. Preferably, a fixed carbon source such as glucose or sucrose, for example, is present in excess. The culture can also be exposed to light some or all of the time, if desired or beneficial.

Microalgae and most other oleaginous microbes can be cultured in liquid media. The culture can be contained within a bioreactor. Optionally, the bioreactor does not allow light to enter. Alternatively, microalgae can be cultured in photobioreactors that contain a fixed carbon source and/or carbon dioxide and allow light to strike the cells. For microalgae cells that can utilize light as an energy source, exposure of those cells to light, even in the presence of a fixed carbon source that the cells transport and utilize (*i.e.*, mixotrophic growth), nonetheless accelerates growth compared to culturing those cells in the dark. Culture condition parameters can be manipulated to optimize total oil production, the combination of hydrocarbon species produced, and/or production of a particular hydrocarbon species. In some instances, it is preferable to culture cells in the dark, such as, for example, when using extremely large (40,000 liter and higher) fermentors that do not allow light to strike a significant proportion (or any) of the culture.

Culture medium typically contains components such as a fixed nitrogen source, trace elements, optionally a buffer for pH maintenance, and phosphate. Components in addition to a fixed carbon source, such as acetate or glucose, may include salts such as sodium chloride, particularly for seawater microalgae. Examples of trace elements include zinc, boron, cobalt, copper, manganese, and molybdenum, in, for example, the respective forms of ZnCl_2 , H_3BO_3 , $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ and $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$. Other culture parameters can also be manipulated, such as the pH of the culture media, the identity and concentration of trace elements and other media constituents.

For organisms able to grow on a fixed carbon source, the fixed carbon source can be, for example, glucose, fructose, sucrose, galactose, xylose, mannose, rhamnose, N-acetylglucosamine, glycerol, floridoside, glucuronic acid, and/or acetate. The one or more exogenously provided fixed carbon source(s) can be supplied to the culture medium at a concentration of from at least about 50 μM to at least 500 mM, and at various amounts in that range (*i.e.*, 100 μM , 500 μM , 5 mM, 50 mM).

Some microalgae species can grow by utilizing a fixed carbon source, such as glucose or acetate, in the absence of light. Such growth is known as heterotrophic growth. For *Chlorella protothecoides*, for example, heterotrophic growth can result in high production of biomass and accumulation of high lipid content. Thus, an
5 alternative to photosynthetic growth and propagation of microorganisms is the use of heterotrophic growth and propagation of microorganisms, under conditions in which a fixed carbon source provides energy for growth and lipid accumulation. In some embodiments, the fixed carbon energy source comprises cellulosic material, including depolymerized cellulosic material, a 5-carbon sugar, or a 6-carbon sugar.

10 Methods for the growth and propagation of *Chlorella protothecoides* to high oil levels as a percentage of dry weight have been reported (see for example Miao and Wu, *J. Biotechnology*, 2004, 11:85-93 and Miao and Wu, *Biosource Technology* (2006) 97:841-846, reporting methods for obtaining 55% oil dry cell weight).

PCT Publication WO2008/151149, incorporated herein by reference, describes
15 preferred growth conditions for microalgae such as *Chlorella*. Multiple species of *Chlorella* and multiple strains within a species can be grown in the presence of glycerol. The aforementioned patent application describes culture parameters incorporating the use of glycerol for fermentation of multiple genera of microalgae. Multiple *Chlorella* species and strains proliferate very well on not only purified
20 reagent-grade glycerol, but also on acidulated and non-acidulated glycerol byproduct from biodiesel transesterification. In some instances, microalgae, such as *Chlorella* strains, undergo cell division faster in the presence of glycerol than in the presence of glucose. In these instances, two-stage growth processes in which cells are first fed glycerol to increase cell density, and are then fed glucose to accumulate lipids can
25 improve the efficiency with which lipids are produced.

Other feedstocks for culturing microalgae under heterotrophic growth conditions include mixtures of glycerol and glucose, mixtures of glucose and xylose, mixtures of fructose and glucose, sucrose, glucose, fructose, xylose, arabinose, mannose, galactose, acetate, and molasses. Other suitable feedstocks include corn
30 stover, sugar beet pulp, and switchgrass in combination with depolymerization enzymes. In various embodiments, a microbe that can utilize sucrose as a carbon source under heterotrophic culture conditions is used to generate the microbial

biomass. PCT Publication Nos. 2012/106560, 2011/150410, 2011/150411, 2010/063032, and 2008/151149 which are herein incorporated by reference describe recombinant organisms, including but not limited to *Prototheca* and *Chlorella* microalgae, that have been genetically engineered to utilize sucrose as a carbon
5 source. In various embodiments, these or other organisms capable of utilizing sucrose as a carbon source under heterotrophic conditions are cultured in media in which the sucrose is provided in the form of a crude, sucrose-containing material, including but not limited to, sugar cane juice (*e.g.*, thick cane juice) and sugar beet juice.

For lipid and oil production, cells, including recombinant cells, are typically
10 fermented in large quantities. The culturing may be in large liquid volumes, such as in suspension cultures as an example. Other examples include starting with a small culture of cells which expand into a large biomass in combination with cell growth and propagation as well as lipid (oil) production. Bioreactors or steel fermentors can be used to accommodate large culture volumes. For these fermentations, use of
15 photosynthetic growth conditions may be impossible or at least impractical and inefficient, so heterotrophic growth conditions may be preferred.

Appropriate nutrient sources for culture in a fermentor for heterotrophic growth conditions include raw materials such as one or more of the following: a fixed carbon source such as glucose, corn starch, depolymerized cellulosic material,
20 sucrose, sugar cane, sugar beet, lactose, milk whey, molasses, or the like; a nitrogen source, such as protein, soybean meal, cornsteep liquor, ammonia (pure or in salt form), nitrate or nitrate salt; and a phosphorus source, such as phosphate salts. Additionally, a fermentor for heterotrophic growth conditions allows for the control of culture conditions such as temperature, pH, oxygen tension, and carbon dioxide
25 levels. Optionally, gaseous components, like oxygen or nitrogen, can be bubbled through a liquid culture. Other starch (glucose) sources include wheat, potato, rice, and sorghum. Other carbon sources include process streams such as technical grade glycerol, black liquor, and organic acids such as acetate, and molasses. Carbon sources can also be provided as a mixture, such as a mixture of sucrose and
30 depolymerized sugar beet pulp.

A fermentor for heterotrophic growth conditions can be used to allow cells to undergo the various phases of their physiological cycle. As an example, an inoculum

of lipid-producing cells can be introduced into a medium followed by a lag period (lag phase) before the cells begin to propagate. Following the lag period, the propagation rate increases steadily and enters the log, or exponential, phase. The exponential phase is in turn followed by a slowing of propagation due to decreases in nutrients
5 such as nitrogen, increases in toxic substances, and quorum sensing mechanisms. After this slowing, propagation stops, and the cells enter a stationary phase or steady growth state, depending on the particular environment provided to the cells.

In one heterotrophic culture method, microorganisms are cultured using depolymerized cellulosic biomass as a feedstock. As opposed to other feedstocks that
10 can be used to culture microorganisms, such as corn starch or sucrose from sugar cane or sugar beets, cellulosic biomass (depolymerized or otherwise) is not suitable for human consumption. Cellulosic biomass (*e.g.*, stover, such as corn stover) is inexpensive and readily available.

Suitable cellulosic materials include residues from herbaceous and woody
15 energy crops, as well as agricultural crops, *i.e.*, the plant parts, primarily stalks and leaves typically not removed from the fields with the primary food or fiber product. Examples include agricultural wastes such as sugarcane bagasse, rice hulls, corn fiber (including stalks, leaves, husks, and cobs), wheat straw, rice straw, sugar beet pulp, citrus pulp, citrus peels; forestry wastes such as hardwood and softwood thinnings,
20 and hardwood and softwood residues from timber operations; wood wastes such as saw mill wastes (wood chips, sawdust) and pulp mill waste; urban wastes such as paper fractions of municipal solid waste, urban wood waste and urban green waste such as municipal grass clippings; and wood construction waste. Additional cellulose include dedicated cellulosic crops such as switchgrass, hybrid poplar
25 wood, and miscanthus, fiber cane, and fiber sorghum. Five-carbon sugars that are produced from such materials include xylose.

Some microbes are able to process cellulosic material and directly utilize cellulosic materials as a carbon source. However, cellulosic material may need to be treated to increase the accessible surface area or for the cellulose to be first broken
30 down as a preparation for microbial utilization as a carbon source. PCT Patent Publication Nos. 2010/120939, 2010/063032, 2010/063031, and PCT 2008/151149,

incorporated herein by reference, describe various methods for treating cellulose to render it suitable for use as a carbon source in microbial fermentations.

Bioreactors can be employed for heterotrophic growth and propagation methods. As will be appreciated, provisions made to make light available to the cells in photosynthetic growth methods are unnecessary when using a fixed-carbon source in the heterotrophic growth and propagation methods described herein.

In certain embodiments, the oleaginous microbe is cultured mixotrophically. Mixotrophic growth involves the use of both light and fixed carbon source(s) as energy sources for cultivating cells. Mixotrophic growth can be conducted in a photobioreactor. Microalgae can be grown and maintained in closed photobioreactors made of different types of transparent or semitransparent material. Such material can include Plexiglass[®] enclosures, glass enclosures, bags made from substances such as polyethylene, transparent or semi-transparent pipes and other material. Microalgae can be grown and maintained in open photobioreactors such as raceway ponds, settling ponds and other non-enclosed containers. The following discussion of photobioreactors useful for mixotrophic growth conditions is applicable to photosynthetic growth conditions as well.

Microorganisms useful in accordance with the methods provided herein are found in various locations and environments throughout the world. As a consequence of their isolation from other species and their resulting evolutionary divergence, the particular growth medium for optimal growth and generation of oil and/or lipid from any particular species of microbe may need to be experimentally determined. In some cases, certain strains of microorganisms may be unable to grow on a particular growth medium because of the presence of some inhibitory component or the absence of some essential nutritional requirement required by the particular strain of microorganism. There are a variety of methods known in the art for culturing a wide variety of species of microalgae to accumulate high levels of lipid as a percentage of dry cell weight, and methods for determining optimal growth conditions for any species of interest are also known in the art.

Solid and liquid growth media are generally available from a wide variety of sources, and instructions for the preparation of particular media that is suitable for a wide variety of strains of microorganisms can be found, for example, online at

utex.org/, a site maintained by the University of Texas at Austin for its culture collection of algae (UTEX). For example, various fresh water and salt water media include those shown in Table 4.

Table 4. Algal Media.

Fresh Water Media	Salt Water Media
1/2 CHEV Diatom Medium	1% F/2
1/3 CHEV Diatom Medium	1/2 Enriched Seawater Medium
1/5 CHEV Diatom Medium	1/2 Erdschreiber Medium
1:1 DYIII/PEA + Gr+	1/2 Soil+Seawater Medium
2/3 CHEV Diatom Medium	1/3 Soil+Seawater Medium
2X CHEV Diatom Medium	1/4 ERD
Ag Diatom Medium	1/4 Soil+Seawater Medium
Allen Medium	1/5 Soil+Seawater Medium
BG11-1 Medium	2/3 Enriched Seawater Medium
Bold 1NV Medium	20% Allen + 80 % ERD
Bold 3N Medium	2X Erdschreiber's Medium
Botryococcus Medium	2X Soil+Seawater Medium
Bristol Medium	5% F/2 Medium
CHEV Diatom Medium	5/3 Soil+Seawater Agar Medium
Chu's Medium	Artificial Seawater Medium
CR1 Diatom Medium	BG11-1 + .36% NaCl Medium
CR1+ Diatom Medium	BG11-1 + 1% NaCl Medium
CR1-S Diatom Medium	Bold 1NV:Erdschreiber (1:1)
Cyanidium Medium	Bold 1NV:Erdschreiber (4:1)
Cyanophycean Medium	Bristol-NaCl Medium
Desmid Medium	Dasycladales Seawater Medium
DYIII Medium	Enriched Seawater Medium
Euglena Medium	Erdschreiber's Medium
HEPES Medium	ES/10 Enriched Seawater Medium
J Medium	ES/2 Enriched Seawater Medium
Malt Medium	ES/4 Enriched Seawater Medium
MES Medium	F/2 Medium
Modified Bold 3N Medium	F/2+NH ₄
Modified COMBO Medium	LDM Medium
N/20 Medium	Modified 2 X CHEV
Ochromonas Medium	Modified 2 X CHEV + Soil
P49 Medium	Modified Artificial Seawater Medium
Polytomella Medium	Modified CHEV

Fresh Water Media	Salt Water Media
Proteose Medium	Porphridium Medium
Snow Algae Media	Soil+Seawater Medium
Soil Extract Medium	SS Diatom Medium
Soilwater: BAR Medium	
Soilwater: GR- Medium	
Soilwater: GR-/NH ₄ Medium	
Soilwater: GR+ Medium	
Soilwater: GR+/NH ₄ Medium	
Soilwater: PEA Medium	
Soilwater: Peat Medium	
Soilwater: VT Medium	
Spirulina Medium	
Tap Medium	
Trebouxia Medium	
Volvocacean Medium	
Volvocacean-3N Medium	
Volvox Medium	
Volvox-Dextrose Medium	
Waris Medium	
Waris+Soil Extract Medium	

A medium suitable for culturing *Chlorella protothecoides* comprises Proteose Medium. This medium is suitable for axenic cultures, and a 1L volume of the medium (pH ~6.8) can be prepared by addition of 1g of proteose peptone to 1 liter of
5 Bristol Medium. Bristol medium comprises 2.94 mM NaNO₃, 0.17 mM CaCl₂·2H₂O, 0.3 mM MgSO₄·7H₂O, 0.43 mM, 1.29 mM KH₂PO₄, and 1.43 mM NaCl in an aqueous solution. For 1.5% agar medium, 15 g of agar can be added to 1 L of the solution. The solution is covered and autoclaved, and then stored at a refrigerated temperature prior to use.

10 Other suitable media for use with the methods provided herein can be readily identified by consulting the URL identified above, or by consulting other organizations that maintain cultures of microorganisms, SAG the Culture Collection of Algae at the University of Göttingen (Göttingen, Germany), CCAP the culture collection of algae and protozoa managed by the Scottish Association for Marine

Science (Scotland, United Kingdom), and CCALA the culture collection of algal laboratory at the Institute of Botany (Třeboň, Czech Republic).

Process conditions can be adjusted to increase the percentage weight of cells that is lipid. For example, in certain embodiments, a microbe (*e.g.*, a microalgae) is
5 cultured in the presence of a limiting concentration of one or more nutrients, such as, for example, nitrogen and/or phosphorous and/or sulfur, while providing an excess of fixed carbon energy such as glucose. Nitrogen limitation tends to increase microbial lipid yield over microbial lipid yield in a culture in which nitrogen is provided in excess. In particular embodiments, the increase in lipid yield is from at least about
10 10% to 100% to as much as 500% or more. The microbe can be cultured in the presence of a limiting amount of a nutrient for a portion of the total culture period or for the entire period. In particular embodiments, the nutrient concentration is cycled between a limiting concentration and a non-limiting concentration at least twice during the total culture period. In one embodiment, the C10-C14 content of the
15 microbial biomass used in the methods comprises at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, or at least about 60%, or at least 70% of the lipid content of the biomass. In another aspect, the saturated lipid content of the microbial biomass is at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90% of the lipid of the microbial
20 biomass.

To increase lipid as a percentage of dry cell weight, acetate can be employed in the feedstock for a lipid-producing microbe (*e.g.*, a microalgae). Acetate feeds directly into the point of metabolism that initiates fatty acid synthesis (*i.e.*, acetyl-CoA); thus providing acetate in the culture can increase fatty acid production.
25 Generally, the microbe is cultured in the presence of a sufficient amount of acetate to increase microbial lipid yield, and/or microbial fatty acid yield, specifically, over microbial lipid (*e.g.*, fatty acid) yield in the absence of acetate. Acetate feeding is a useful component of the methods provided herein for generating microalgal biomass that has a high percentage of dry cell weight as lipid.

30 In a steady growth state, the cells accumulate oil (lipid) but do not undergo cell division. In one embodiment, the growth state is maintained by continuing to provide all components of the original growth media to the cells with the exception of

a fixed nitrogen source. Cultivating microalgae cells by feeding all nutrients originally provided to the cells except a fixed nitrogen source, such as through feeding the cells for an extended period of time, can result in a high percentage of dry cell weight being lipid. In some embodiments, the nutrients, such as trace metals, phosphates, and other components, other than a fixed carbon source, can be provided at a much lower concentration than originally provided in the starting fermentation to avoid “overfeeding” the cells with nutrients that will not be used by the cells, thus reducing costs.

In other embodiments, high lipid (oil) biomass can be generated by feeding a fixed carbon source to the cells after all fixed nitrogen has been consumed for extended periods of time, such as from at least 8 to 16 or more days. In some embodiments, cells are allowed to accumulate oil in the presence of a fixed carbon source and in the absence of a fixed nitrogen source for over 30 days. Preferably, microorganisms grown using conditions described herein and known in the art comprise lipid in a range of from at least about 10% lipid by dry cell weight to about 75% lipid by dry cell weight. Such oil rich biomass can be used directly as a fluid loss control agent in drilling fluids, but often, the spent biomass remaining after lipid has been extracted from the microbes will be used as the fluid loss control agent.

Another tool for allowing cells to accumulate a high percentage of dry cell weight as lipid involves feedstock selection. Multiple species of *Chlorella* and multiple strains within a species of *Chlorella* accumulate a higher percentage of dry cell weight as lipid when cultured in the presence of biodiesel glycerol byproduct than when cultured in the presence of equivalent concentrations of pure reagent grade glycerol. Similarly, *Chlorella* can accumulate a higher percentage of dry cell weight as lipid when cultured in the presence of an equal concentration (weight percent) mixture of glycerol and glucose than when cultured in the presence of only glucose.

Another tool for allowing cells to accumulate a high percentage of dry cell weight as lipid involves feedstock selection as well as the timing of addition of certain feedstocks. For example, *Chlorella* can accumulate a higher percentage of dry cell weight as lipid when glycerol is added to a culture for a first period of time, followed by addition of glucose and continued culturing for a second period of time, than when the same quantities of glycerol and glucose are added together at the beginning of the

fermentation. See PCT Publication No. 2008/151149, incorporated herein by reference.

Triglycerides can be isolated from oleaginous microbes by mechanical pressing with pressure sufficient to extract oil. In various embodiments, the pressing
5 step will involve subjecting the oleaginous microbes to at least 10,000 psi of pressure. In various embodiments, the pressing step involves the application of pressure for a first period of time and then application of a higher pressure for a second period of time. This process may be repeated one or more times (“oscillating pressure”). In various embodiments, moisture content of the oleaginous microbes is controlled
10 during the pressing step. In various embodiments, the moisture is controlled in a range of from 0.1% to 3% by weight.

Expeller presses (screw presses) are routinely used for mechanical extraction of oil from soybeans and oil seeds. Generally, the main sections of an expeller press include an intake, a rotating feeder screw, a cage or barrel, a worm shaft and an oil
15 pan. The expeller press is a continuous cage press, in which pressure is developed by a continuously rotating worm shaft. An extremely high pressure, approximately 10,000-20,000 pounds per square inch, is built up in the cage or barrel through the action of the worm working against an adjustable choke, which constricts the discharge of the pressed cake (spent biomass) from the end of the barrel. In various
20 embodiments, screw presses from the following manufacturers are suitable for use : Anderson International Corp. (Cleveland, OH), Allico (Santa Fe, Argentina), De Smet Rosedowns (Humberside, UK), The Dupps Co. (Germantown, Ohio), Grupo Tecnal (Sao Paulo, Brazil), Insta Pro (Des Moines, Iowa), French Oil Mill (Piqua, OH), Harburg Freudenberger (previously Krupp Extraktionstechnik) (Hamburg,
25 Germany), Maschinenfabrik Reinartz (Neuss, Germany), Shann Consulting (New South Wales, Australia) and SKET (Magdeburg, Germany).

Drilling, Production, and Pumping-Services Fluids

Due to the protection afforded by encapsulation, the encapsulated oils provided herein can be proactively added to drilling fluid systems (e.g. water-based
30 systems), where it circulates through the system until conditions are met to break the encapsulation and release the oil lubricant.

The fluids provided herein include aqueous and non-aqueous drilling fluids and other well-related fluids including those used for production of oil or natural gas, for completion operations, sand control operations, workover operations, and for pumping-services such as cementing, hydraulic fracturing, and acidification. In one embodiment, a fluid includes a fluid loss control agent that is biomass from an oleaginous microbe. In one embodiment, the biomass comprises intact, lysed or partly lysed cells with greater than 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% oil. In another embodiment, the biomass is spent biomass from which oil has been removed. For example, the oil may be removed by a process of drying and pressing and optionally solvent-extracting with hexane or other suitable solvent. In a specific embodiment, the biomass is dried to less than 6% moisture by weight, followed by application of pressure to release more than 25% of the lipid. Alternately, the cells may be intact, which, when used in a drilling fluid, may impart improved fluid-loss control in certain circumstances. Generally, the drilling fluid can contain about 0.1% to about 20% by weight of said biomass, but in various embodiments, this amount may range from about 0.1% to about 10% by weight of said biomass; from about 0.1% to about 5% by weight of said biomass; from about 0.5% to about 4% by weight of said biomass; and from about 1% to about 4% by weight of said biomass.

In various embodiments, the fluid comprises a fluid loss control agent that is not derived from oleaginous microbial biomass. Suitable fluid loss control agents may include, but are not limited to, unmodified starch, hydroxypropyl starch, carboxymethyl starch, unmodified cellulose, carboxymethylcellulose, hydroxyethyl cellulose, and polyanionic cellulose.

The fluid can include an aqueous or non-aqueous solvent. The fluid can also optionally include one or more additional components so that the fluid is operable as a drilling fluid, a drill-in fluid, a workover fluid, a spotting fluid, a cementing fluid, a reservoir fluid, a production fluid, a fracturing fluid, or a completion fluid.

In various embodiments, the fluid is a drilling fluid and the added biomass from the oleaginous microbe serves to help transport cuttings, lubricate and protect the drill bit, support the walls of the well bore, deliver hydraulic energy to the

formation beneath the bit, and/or to suspend cuttings in the annulus when drilling is stopped.

When used in a drilling fluid, the biomass may operate to occlude pores in the formation, and to form or promote the formation of a filter cake.

5 In various embodiments, the fluid is a production fluid and the biomass serves to inhibit corrosion, separate hydrocarbons from water, inhibit the formation of scale, paraffin, or corrosion (e.g., metal oxides), or to enhance production of oil or natural gas from the well. In an embodiment, the biomass is used to stimulate
methanogenesis of microbes in the well. The biomass may provide nutrients and/or
10 bind inhibitors so as to increase production of natural gas in the well. In this embodiment, the well can be a coal seam having methane generating capacity. See, for example, US Patent Application Nos. 2004/0033557, 2012/0021495, 2011/0284215, US2010/0248322, 2010/0248321, 2010/0035309, and 2007/0248531.

 In various embodiments, the fluid comprises a viscosifier. Suitable
15 viscosifiers include, but are not limited to, an alginate polymer selected from the group consisting of sodium alginate, sodium calcium alginate, ammonium calcium alginate, ammonium alginate, potassium alginate, propyleneglycol alginate, and mixtures thereof. Other suitable viscosifiers include organophillic clay, polyacrylamide, xanthan gum, and mixtures of xanthan gum and a cellulose
20 derivative, including those wherein the weight ratio of xanthan gum to cellulose derivative is in the range from about 80:20 to about 20:80, and wherein the cellulose derivative is selected from the group consisting of hydroxyethylcellulose, hydroxypropylcellulose, carboxymethylcellulose and mixtures thereof. Other suitable viscosifiers include a biopolymer produced by the action of bacteria, fungi, or other
25 microorganisms on a suitable substrate.

 Mixtures of a bentonitic clay and additives can also be used as viscosifiers. The additives used in such mixtures can comprise, for example: (a) a nonionic, water-soluble polysaccharide selected from the group consisting of a non-ionic, water-soluble cellulosic derivative and a non-ionic water-soluble guar derivative; (b) an
30 anionic water-soluble polysaccharide selected from the group consisting of a carboxymethyl cellulose and *Xanthomonas campestris* polysaccharide or a combination thereof; (c) an intermediate molecular weight polyglycol, i.e., selected

from the group consisting of polyethylene glycol, polypropylene glycol, and poly-(alkanediol), having an average molecular weight of from about 600 to about 30,000; and (5) compatible mixtures thereof. Components of the mixtures may be added individually to the fluid to enhance the low shear rate viscosity thereof.

- 5 In some embodiments, the drilling fluid comprises one or more additives selected from the group consisting of an aphron, polymer particle, a thermoset polymer particle, and a nanocomposite particulate.

 Aphrons can be used as additives to drilling fluids and other fluids used in creating or maintaining a borehole. Aphrons can concentrate at the fluid front and act
10 as a fluid loss control agent and/or bridging agent to build an internal seal of the pore network along the side walls of a borehole. It is believed that aphrons deform during the process of sealing the pores and gaps encountered while drilling a borehole. Aphrons useful in the present methods are typically 50-100 μM , 25-100 μM , 25-50 μM , 5-50, 5-25 μM , 7-15 μM or about 10 μM .

- 15 In one embodiment, a drilling fluid comprises aphrons, microbial biomass in which the oil has not been extracted (unextracted microbial biomass), spent biomass or a combination of aphrons, unextracted microbial biomass, and spent biomass.

 Where an aphron is used, the aphron can have an average diameter of 5 to 50 micrometers and can make up about 0.001% to 5% by mass of the fluid.

- 20 The use of drilling fluids containing polymer particle additives has several applications in construction, drilling, completion, and fracture simulation of oil and natural gas wells. These particles are generally spherical in shape, solid, and have a specific gravity of 1.06. The use of these particles provides several advantages, such as increasing mechanical lubrication, reducing equipment wear, and aiding in
25 directional changes during sliding. These particles are generally resistant to deformation loads of up to >25,000 psi hydrostatic, and they display excellent resistance and thermal stability even at temperatures greater than 450⁰F in a drilling environment. These particles can also be manufactured in fine or coarse grades, depending on the requirements of a particular drilling operation.

- 30 Polymer particles are easily added to drilling fluid through a mud-mixing hopper machine. When used to control torque and drag, these beads can be applied at

concentrations of 2-8 ppb (5.71–22.87 kilograms/m³). For spotting in wire-line operations and running casing, the polymer beads may be added to concentrations of 8-12 ppb (22.87–34.31 kilograms/m³).

In some embodiments, the drilling fluid comprises a thermoset polymer particle such as those disclosed in US 8,088,718. In some embodiments, the drilling fluid comprises a nanocomposite particulate such as those disclosed in US 2005/0272611. In some embodiments, the drilling fluid comprises a co-polymer bead such as Alpine Drill Beads commercially available from Alpine Specialty Chemicals (Houston, Texas).

Examples of other additives that may be used in drilling applications include, but are not limited to: alkalinity agents, corrosion inhibitors, defoamers, dispersants, emulsifiers, fluid loss control agents, foaming agent for gas-based fluids, intermediates for corrosion inhibitor, lubricants, misting agents, oxygen scavengers, hydrosulfite scavengers, biocides, scale inhibitors, scale removers, shale inhibitors, solvents, specialty surfactants, thermal stabilizers, viscosifiers, and water purifiers.

The additives disclosed herein, *e.g.*, including the polymeric and glass bead additives, can contribute to bursting and releasing oil from the microbial cells. In such instances the additives work in concert with the cells to provide delay-released lubrication to the drill bit. Though not intended to be limited by the following mechanism, in one aspect this application is directed to a pressure sensitive lubricant that allows for time-delayed release of a lubricating oil by virtue of the oil being encapsulated within a cell. In instances when the lubricant is used in a drilling fluid, the pressure that triggers the oil to be released is provided by the drill string and/or drill bit. The oil is released only when sufficient downhole pressure and/or friction is present. Such pressure and friction is provided by the drill string and/or drill bit in its interaction with the well formation, such as when it is dragged along the well-bore (particularly in the non-vertical portions of the well-bore) or during the rotational motion of the drill string/drill bit during drilling.

Additives and lubricants to be used in combination with the oleaginous cells and oils provided herein include commercially available lubricants. These lubricants can be blended with oleaginous cells and oils produced by these cells. The commercially available lubricants include those marketed by Baker Hughes (RHEO-

LOGIC, MAGMA-TEQ, CARBO-DRILL, MPRESS, PERFORMAX, PERFLEX, TERRA-MAX, PYRO-DRILL, MAX-BRIDGE, CHEK-LOSS, LC-LUBE, MIL-CARB, SOLUFLAKE, FLOW-CARB, X-LINK crosslinked composition, and SOLU-SQUEEZE LCM), Haliburton (BAROID, BOREMAX, PERFORMADRIL, SHALEDRIL, SUPER-SAT, and BaraECD) and Schlumberger (DRILPLEX, DURATHERM, ENVIROTHERM NT, GLYDRIL, K-MAG, KLA-SHIELD, SAGDRIL, ULTRADRIL, ECOGREEN, MEGADRIL, NOVAPLUS, PARADRIL, PARALAND, PARATHERM, RHADIANT, VERSACLEAN, VERSADRIL, and WARP fluids).

10 In various embodiments, the fluid comprises a density modifier, also known as a weighting agent or a weighting additive. Suitable density modifiers include, but are not limited to, barite, hematite, manganese oxide, calcium carbonate, iron carbonate, iron oxide, lead sulfide, siderite, and ilmenite.

In various embodiments, the fluid comprises an emulsifier. Suitable emulsifiers may be nonionic, including ethoxylated alkylphenols and ethoxylated linear alcohols, or anionic, including alkylaryl sulfonates, alcohol ether sulfonates, alkyl amine sulfonates, petroleum sulfonates, and phosphate esters.

In various embodiments, the fluid comprises a lubricant. Non-limiting, suitable lubricants may include fatty acids, tall oil, sulphonated detergents, phosphate esters, alkanolamides, asphalt sulfonates, graphite, and glass beads.

The fluid can be a drilling fluid with a low shear rate viscosity as measured with a Brookfield viscometer at 0.5 rpm of at least 20,000 centipoise. In some embodiments, the low shear rate viscosity is at least about 40,000 centipoise.

Biomass added to fluid can be chemically modified prior to use. Chemical modification involves the formation or breaking of covalent bonds. For example, the biomass may be chemically modified by transesterification, saponification, crosslinking or hydrolysis. The biomass may be treated with one or more reactive species so as to attach desired moieties. The moieties may be hydrophobic, hydrophilic, amphiphilic, ionic, or zwitterionic. For example, the biomass may anionized (e.g., carboxymethylated), or acetylated. Methods for covalent modification including carboxymethylation and acetylation of biomass from oleaginous microbes are disclosed in U.S. Provisional Patent Application No.

61/615,832, filed on March 26, 2012 for “Algal Plastics and Absorbants”, incorporated herein by reference in relevant part. US Patent No. 3,795,670 describes an acetylation process that can be used to increase the hydrophobicity of the biomass by reaction with acetic anhydride. Carboxymethylation of the biomass can be
5 performed by treatment with monochloroacetic acid. See, e.g., US Patent No, 3,284,441. US Patents No. 2,639,239; 3,723,413; 3,345,358; 4,689,408, 6,765,042, and 7,485,719, which disclose methods for anionizing and/or cross-linking.

The fluid can include one or more additives such as bentonite, xanthan gum, guar gum, starch, carboxymethylcellulose, hydroxyethyl cellulose, polyanionic
10 cellulose, a biocide, a pH adjusting agent, polyacrylamide, an oxygen scavenger, a hydrogen sulfide scavenger, a foamer, a demulsifier, a corrosion inhibitor, a clay control agent, a dispersant, a flocculant, a friction reducer, a bridging agent, a lubricant, a viscosifier, a salt, a surfactant, an acid, a fluid loss control additive, a gas, an emulsifier, a density modifier, diesel fuel, and an aphron.

15 Other additives for use in the fluids disclosed herein include those described in US 6,776,234, US 7,199,085, US 7,231,976, US 7,312,184, US 7,373,977, US 7,380,606, US 7,392,844, US 7,544,639, US 7,998,911, and US 8,148,303. These patents are incorporated by reference herein in their entirety and, in particular, for the description of additives. Fluids containing these additives can be used in various
20 wellbore operations such as drilling, completion operations, stimulation operations, hydraulic fracturing, acidification, sand control operations, and workover operations.

Fluids may be mixed or sheared for times appropriate to achieve a homogenous mixture.

Fluids may be subject to aging prior to testing or use. Aging may be
25 performed under conditions that vary from static to dynamic and from ambient (20-25°C) to highly elevated temperatures (>250°C).

Fluids can be described as Newtonian or non-Newtonian depending on their response to shearing. The shear stress of a Newtonian fluid is proportional to the shear rate. For non-Newtonian fluids, viscosity decreases as shear rate increases.
30 One classification of non-Newtonian fluid behavior, pseudoplastic behavior, refers to a general type of shear-thinning that may be desirable for drilling fluids. Several mathematical models known in that art have been developed to describe the shear

stress/shear rate relationship of non-Newtonian fluids. These models, including the Bingham plastic model, the Power Law model, and the Herschel-Buckley Model are described in “*The Drilling Fluids Processing Handbook*, Shale Shaker Committee of the American Society of Mechanical Engineers eds, Gulf Professional Publishing, 5 2004”. Additionally, see reference manuals including “Drilling Fluids Reference Manual, 2006” available from Baker Hughes.

Examples

Example 1

10 Metal to metal lubricity tests were conducted using hot rolled lab formulated drilling fluids. The fluids were prepared by mixing a water-based, synthetic based, or oil based mud with microalgal cells and/or free oil extracted from the cells. The drilling fluids were hot rolled for 16 hours at atmospheric pressure and standard temperatures (150°F for oil-based mud, 120 °F for water-based mud and synthetic-
15 based mud).

The muds were prepared using the formulations provided in Tables 5-7. Strain A was derived from UTEX 1435 by classical mutagenesis and screened for high oil production. Strain B was also derived from UTEX 1435 by classical mutagenesis and screened for high oil production, and was further transformed
20 according to WO 2010/063031 to express a yeast sucrose invertase. The fatty acid profiles of oil from Strains A and B are given in Table 5.

Table 5. Fatty acid profile of oil from Strains A and B

Fatty acid	Strain A	Strain B
C10:0	0.0	0.0
C12:0	0.1	0.0
C14:0	2.3	0.7
C16:0	27.5	13.2
C18:0	2.03	5.2
C18:1	59.0	71.8
C18:2	6.2	6.4
C18:3	0.2	0.1
C20:0	0.2	0.4

The strains were cultured under heterotrophic conditions such as those described in WO2008/151149, WO2010/063031, WO2010/063032, WO2011/150411, and WO2013/158938. Upon cultivation, broth from lots corresponding to different fermentation runs were dried using a drum dryer.

- 5 Resulting solid biomass are shown in Table 6 below, and are identified according to strain (A or B) and, where applicable, lot number (1-4). A2 was prepared by taking the drum dried biomass and resuspending to 20% solids in deionized water, treating twice with a homogenizer at 1250 bar, and freeze drying.

10 **Table 6. Biomass**

Biomass	Processing
A1	Drum dried
A2	Drum dried and homogenized (2x)
A3	Drum dried
A4	Drum dried
B	Drum dried

Water based, synthetic based, or salty water based mud containing 3% or 6% by volume of the biomass were prepared as described in Tables 7-10.

Table 7. Mud A: Water-based drilling fluid

Component	Amount	Comments
Tap Water	4 liters	Add to mixing container
Sodium Bicarbonate	5 grams	Slowly add and mix for 5 minutes
Bentonite	56 grams	Slowly add and mix for a minimum of 16 hours
Low Viscosity CMC	14 grams	Slowly add and mix for 5 minutes
Xanthum Gum	14 grams	Slowly add and mix for 5 minutes
Barite	18 grams	Slowly add and mix for 30 minutes; hot roll mixture for 16 hours at 120°C

Table 8. Mud B: Synthetic based drilling fluid

Component	Amount	Comments
Escaid 110	1698 grams	Add to mixing container
EZ-Mul	60 grams	Slowly add and mix for 5 minutes
Tap Water	728 grams	Slowly add and mix for 5 minutes
Calcium chloride	254 grams	Slowly add and mix for 5 minutes
Lime	30 grams	Slowly add and mix for 5 minutes
Gel-tone II	80 grams	Slowly add and mix for 5 minutes

Duratone HT	60 grams	Slowly add and mix for 5 minutes
RM-63	6 grams	Slowly add and mix for 5 minutes
Barite	1210 grams	Slowly add and mix for 30 minutes; hot roll mixture for 16 hours at 150°C

Table 9. Mud C: Salty water-based drilling fluid

Component	Amount	Comments
Tap Water	1383.2	Add to mixing container
Quick Thin	50 grams	Slowly add and mix for 5 minutes
Aquagel	200 grams	Slowly add and mix for 5 minutes
PAC LV	5 grams	Slowly add and mix for 5 minutes
Seawater salts	1483 grams	Slowly add and mix for 5 minutes
Carbonox LV-CMC	30 grams	Slowly add and mix for 5 minutes
Sodium hydroxide	20 grams	Slowly add and mix for 5 minutes
Soda Ash	10 gram	Slowly add and mix for 5 minutes
Rev Dust	500 grams	Slowly add and mix for 5 minutes
Barite	1785 grams	Slowly add and mix for 30 minutes; hot roll mixture for 16 hours at 120°C

5

Table 10. Mud D: Water-based drilling fluid

Component	Amount	Comments
Tap Water	337.97	Add to mixing container
Bentonite	20 grams	Slowly add and allow bentonite to hydrate for minimum of 16 hours
Lignite	2 grams	Slowly add and mix for 5 minutes
Chrome free lignosulfonate	0.5 grams	Slowly add and mix for 5 minutes
Polyanionic cellulose	1.5 grams	Slowly add and mix for 5 minutes
Xanthum gum	0.75 grams	Slowly add and mix for 30 minutes; hot roll mixture for 16 hours at 120°C

The metal to metal lubricity coefficients (coefficients of friction) were determined using a Fann EP/Lubricity Tester Model 21200. In this test, 150 inch-pounds of force was applied between two hardened steel surfaces, a block and a rotating ring, at 60 RPM with readings taken at the indicated time points in Tables 11-15.

Table 11. Metal to metal lubricity with water based drilling mud

Sample	1 Mi n.	3 Mi n.	5 Mi n.	10 Min.	30 Mi n.	60 Mi n.
Mud A	0.32	0.32	0.32	0.32		
Mud A + 3% biomass B	0.27	0.24	0.25	0.26	0.25	
Mud A + 3% biomass B	0.25	0.26	0.28	0.23	0.21	0.22
Mud A + 3% biomass A1	0.24	0.24	0.24	0.24	0.23	
Mud A + 3% biomass A2	0.24	0.22	0.22	0.20	0.14	0.13
Mud A + 3% biomass A3	0.18	0.15	0.15	0.16	0.19	
Mud A + biomass B	0.08	0.06	0.06	0.05	0.04	0.04
Mud A + oil from Strain A	0.04	0.04	0.04	0.04	0.05	0.05
Mud A + 3% biomass A4	0.23	0.21	0.21	0.21	0.21	
Mud A + 3% biomass A1	0.17	0.16	0.15	0.15	0.14	0.13

**Table 12. Metal to metal lubricity with water based drilling mud
(measurements taken immediately after hot rolling)**

5

Sample	1 Min.	3 Mi n.	5 Min.	10 Mi n.	30 Mi n.	60 Mi n.	90 Min. .
Mud A	0.32	0.32	0.32	0.32			
Mud A + 6% biomass A5	0.17	0.16	0.16	0.16	0.15	0.14	0.12
Mud A + 3% biomass A5	0.23	0.16	0.13	0.11	0.09	0.07	

Sample	1 Min.	3 Min.	5 Min.	10 Min.	30 Min.	60 Min.	90 Min.
Mud A + 3% biomass A1	0.20	0.15	0.15	0.13	0.11	0.09	
Mud A + 3% biomass A2	0.10	0.10	0.10	0.10	0.10	0.10	
Mud A + 3% biomass A3	0.11	0.11	0.11	0.11	0.10	0.10	
Mud A + 3% MIL-LUBE	0.05	0.05	0.05	0.05	0.05		
Mud A + 3% Eco Global Solutions DFL	0.03	0.03	0.04	0.09	0.05		

Table 13. Metal to metal lubricity with water based drilling mud

Sample	1 Min.	3 Min.	5 Min.	10 Min.	30 Min.	60 Min.	90 Min.
Mud D	0.24	0.26	0.27	0.27	0.27	0.26	
Mud D + 3% biomass B	0.23	0.23	0.23	0.23	0.21	0.19	
Mud D + 3% biomass A2	0.20	0.20	0.21	0.20	0.21	0.20	
Mud D + 3% biomass A2	0.13	0.13	0.13	0.13	0.13	0.14	0.14

Table 14. Metal to metal lubricity with synthetic based drilling mud

Sample	1 Min.	3 Min.	5 Min.	10 Min.	30 Min.	60 Min.
Mud B	0.13	0.13	0.13			
Mud B + 3% biomass A1	0.19	0.14	0.13	0.11	0.11	0.11
Mud B + 3% biomass A2	0.10	0.09	0.10	0.10	0.10	0.10
Mud B + 3% biomass A5	0.12	0.11	0.10	0.08	0.08	0.12
Mud B + oil from Strain A	0.11	0.10	0.10	0.09	0.10	0.09

5

Table 15. Metal to metal lubricity with salty water based drilling mud (measurements taken immediately after hot rolling)

Sample	1 Min.	3 Min.	5 Min.	10 Min.	30 Min.	60 Min.
Mud C	0.24	0.23	0.22	0.20	0.19	
Mud C + oil from Strain A	0.22	0.20	0.18	0.16	0.15	
Mud C + 3% biomass A1	0.25	0.23	0.22	0.21	0.19	0.16
Mud C + 3% biomass A2	0.19	0.25	0.10	0.07	0.06	0.05
Mud C + 3% biomass B	0.24	0.22	0.21	0.21	0.19	

The changes in the lubricity of the drilling fluid when the biomass or oils are added can be expressed in Table 16 as a percent reduction in torque (ratio of difference in lubricity to lubricity of mud without microalgal cells/microalgal oil).

Table 16. Percent Torque Reduction at 60 minutes

Sample	Water-based mud	Synthetic oil -based mud	Salty water-based mud
3% whole cells (Strain A)	57%	15%	13%
3% lysed cells (Strain A)	58%	23%	67%
3% whole cells (Strain B)	77%	8%	2%

5

As shown in Figure 2, the water-based mud formulated with whole or lysed cells demonstrated reduction in coefficient of lubricity as a function of time. Based on the reductions in the coefficient of lubricity, the torque reduction resulting from the use of whole or lysed cells is estimated to be 57-77%. Synthetic based muds containing whole cells were found to demonstrate a trend of decreasing coefficients of lubricity as shown in Figure 3, corresponding to approximately 8-15% torque reduction. Synthetic based muds containing lysed cells were found to have a lower coefficient of lubricity (0.1), corresponding to a reduction in torque of about 23%. In salty water based muds, formulations with lysed cells showed the greatest decrease in coefficient of lubricity over time, corresponding to a torque reduction of approximately 67% as shown in Figure 4.

The coefficients of lubricity mud containing the oils from Strains A and B were compared to mud containing commercially available extreme pressure lubricants DFL EcoGlobal or Baker Hughes Mil-Lube, a vegetable oil lube. As seen in Figure 1 and Table 17, the reductions in coefficient of lubricity and associated torque reduction due to addition of oils from Strain A and B in the mud were found to be comparable to the commercial lubricants.

Table 17. Percent Torque Reduction

Sample	Water-based mud	Synthetic oil -based mud	Salty water-based mud
Free oil (Strain A)	87%		
Free oil (Strain B)	84%	31%	19%
Mil-lube	84%		
EcoGlobal DFL	84%		

Example 2

Extreme pressure lubricity tests were performed using Fann EP/Lubricity tester model 21200 with results given in Table 18. Significant increases were seen in film strength upon addition of 3% biomass from strain B.

5 **Table 18. Extreme pressure tests**

Sample	Sample Preparation	Torque (inch pounds)	Scar Width (hundredths of an inch)	Film Strength (PSI)
Mud A	Hot rolled 16 hrs at 120°C	150	17.50	4571
Mud A + 3% biomass B	Hot rolled 16 hrs at 120°C	150	9.50	8421
Mud A + 3% oil from Strain A	Hot rolled 16 hrs at 120°C	150	15.00	5333
Mud B + 3% oil from Strain A	Hot rolled 16 hrs at 120°C	150	11.00	7273
Mud A + 3% oil from Strain A	Hot rolled 16 hrs at 120°C and blended 10 minutes prior to testing	150	11.50	6957
Mud A + 3% MIL-LUBE	Hot rolled 16 hrs at 120°C and blended 10 minutes prior to testing	150	7.50	10667
Mud A + 3% EcoGlobal DFL	Hot rolled 16 hrs at 120°C and blended 10 minutes prior to testing	150	8.50	9412

Example 3

Cells from strain B isolated from the culture broth or drum dried were lysed using a homogenizer at 500 bar pressure (7,252 psi) to determine effect of pressure on cell breakage. As seen Table 19 and Figure 5, about 45% of the cells were lysed at this pressure, with greater lysis seen in the drum dried biomass.

Table 19. Percent lysis at 500 bar

Strain	Broth	Drum dried
B	28	45

Example 4

A field trial using water based muds containing microalgal cells from Strain A was conducted to assess efficacy in increasing the rate of penetration and reducing drill bit drag. The water based muds were prepared using a formulation provided in Table 20.

Table 20. Water-based drilling mud formulation

Products	Unit Sizes	Concentrations	Estimated Usages
Xanthan gum	25 lbs sack	1.5 ppb*	35 sacks
Soda ash	50 lbs sack	0.25 ppb	3 sacks
White Starch	50 lbs sack	4.0 ppb	46 stacks
Polyanionic cellulose (PAC) LV	50 lbs sack	0.5 ppb	6 sacks
Caustic Soda	50 lbs sack	0.15 ppb	3 sacks
Glutaraldehyde	44.6 lbs x 5 gal pail	0.5 ppb	7 pails
Strain A whole microalgal cell	1 lb	17 ppb	8200 lbs

*ppb = pounds per barrel

Xanthan gum was used as for rheology control in this trial. Starch is a quality fluid loss additive and was used in the trial to provide excellent low end rheology enhancement in conjunction with xanthan gum. Glutaraldehyde was employed as a biocide. Polyanionic cellulose (PAC) was added for viscosity and filtration control. Caustic soda was added to control alkalinity, while soda ash was used to precipitate hardness to allow calcium-sensitive materials such as PAC to function efficiently. The calcium was controlled between 100-200 ppm with soda ash, and the p_f (i.e., a measurement of alkalinity) was controlled between 0.5-1.0 with caustic soda.

Wells having the primary system parameters provided in Tables 21-22 were drilled at a Catoosa Testing Facility in Hallet, Oklahoma, where soil formation at 1300 feet total vertical distance (TVD) was composed of a shale layer.

Table 21. System properties

Properties	Parameters	Units
Surface Density	8.6–8.8	Ppg
Low shear rate viscosity (LSRV)	4,000–8,000	cPs
Yield Point (YP)	8–14	Lbs/100 ft ²
6 θ (contingency hole)	8-10	Rpm
3 θ (contingency hole)	8-10	Rpm
10 Sec Gel	6-10	Lbs/100 ft ²
10 Min Gel	8-14	Lbs/100 ft ²
API Fluid Loss (30 min)	> 10.0	cc

Table 22. Drilling parameters

Hole Size	8.5 inches
Starting Depth: (MD)	500 feet
Interval TD: (MD)	1,900 feet
Interval Length	1,400 feet
Estimated Washout Generated:	1.0% by volume
Last Casing I.D.:	9.625 inches
Last Casing Shoe: (MD)	500 feet
New Surface Volume:	350 barrels
Volume Carried Forward:	0 barrels
Open Hole Volume:	99 barrels
Solids Control Efficiency:	90.0%
Maximum Drill Solids at Suction:	5.0%
Flow Rate:	10 BPM
Maximum Drilled Solids in Annulus:	8.0% by volume
Volume of Dilution Fluid Used:	188 barrels
Maximum Uniform Drilling Rate Allowed:	120 feet per hour
Casing and Open Hole Volume:	136 barrels
Total Interval Volume:	575 barrels

- 5 To measure the effect of using whole microalgal cells on the drill bit's rate of penetration (ROP), wells were created by drilling a vertical 8.5 inch diameter hole to the kick off point (KOP) at 750 feet measured depth (MD) and then drilling a curve at 10° per 100 feet, achieving 90° at +/- 1650 feet MD, as shown in Figure 6 (drilled using a 1.5 degree bent-housing motor operated and a GX-30CDX tricone bit (Baker Hughes).
- 10 After reaching the landing point, 180 feet was drilled laterally by rotating. Whole microalgal cells were then added to the water based mud for the drill bit and allowed to incubate for 1 or 2 hours, before proceeding with drilling along a lateral

section. Data was collected on an NOV Totco system connected to a top drive on an oil rig.

The use of the whole cells appeared to increase the rate of penetration by 20% after 2 hour incubation, as shown in Table 23. There was no change in the rate of penetration with the 1 hour incubation period, and this confirmed that circulation time and shearing were necessary to activate lubricity (e.g., weaken cells to enable rupture). This field trial showed that the use of whole cells either reduced drilling time or increased drilling distance.

Table 23. Percent Increase in Average Rate of Penetration (ROP)

Sample	Average ROP (ft/hr)	Standard deviation	% increase in ROP relative to No MEOCs
Mud	56	4	N/A
Mud + whole microalgal cells from Strain A + 1hr incubation	56	5	0
Mud + whole microalgal cells from Strain A + 2hr incubation	68	5	20%

10

To measure the effect of using whole microalgal cells on the drag encountered by the drill bit, the bottom hole assemblies (BHAs) were pulled out of the aforementioned dug wells and dragged with no rotation along the 45 degree and 60 degree portion of the curve (Figures 7 and 8). These drills were either treated with the water based mud alone or with water based mud in combination with the whole microalgal cells. Data was collected on an NOV Totco system connected to a top drive on an oil rig. On average, hook weight was reduced by 27%, with a maximum reduction of 50% in the presence of encapsulated oil.

The changes in drag resulting from the addition of whole microalgal cells to the water based mud are expressed in Table 24. The drag change was computed by taking the difference between the drag when mud alone was used and the drag when mud with whole microalgal cells were used, then dividing that difference by the drag

20

when mud alone was used. These ratios were averaged to arrive at the percent drag reduction at both the 45- and 60- degree portions of the curve.

Table 24. Percent Drag Reduction

		Mud	Mud + whole cells		
	Depth	Drag (lb)	Drag (lb)	Drag change	% Drag reduction
60°	1330	54,000	39,000	0.277777778	32%
	1325	58,000	41,000	0.293103448	
	1320	59,000	43,500	0.262711864	
	1315	58,000	44,800	0.227586207	
	1310	57,000	61,400	-0.077192982	
	1305	79,000	44,400	0.437974684	
	1300	88,000	43,100	0.510227273	
	1295	68,000	34,100	0.498529412	
	1290	66,000	37,000	0.439393939	
	1285	57,000	36,700	0.356140351	
45°	1171	41,000	32,300	0.212195122	24%
	1166	45,000	33,800	0.248888889	
	1161	47,000	35,500	0.244680851	
	1156	43,000	36,600	0.148837209	
	1151	43,000	33,000	0.23255814	
	1146	43,000	32,600	0.241860465	
	1141	42,000	33,000	0.214285714	
	1136	56,000	34,000	0.392857143	
	1131	43,000	34,600	0.195348837	
	1126	48,000	34,200	0.2875	
	1121	40,000	34,400	0.14	
	1116	46,000	34,400	0.252173913	
	1111	50,000	34,800	0.304	

- 5 As illustrated in Fig. 7 and 8, the addition of the whole microalgal cells to the water-based mud demonstrated a reduction in hook weight (lb) as a function of bit height. Based on the reduction of hook weight, the use of the mud system with whole microalgal cells led to: (1) a 24% reduction in drag in the 45-degree section of the curve; and (2) a 32% reduction in drag in the 60-degree section of the curve.

- 10 Rotational torque for the top drive was measured by analyzing average torque while rotating off-bottom prior to rotationally drilling in the absence of product. Following encapsulated oil addition, rotational torque was measured at the same points while tripping out. As the pumps were off for the measurements tripping out, a

correction factor was applied based on three separate readings done while the pumps were on. On average, rotational torque required to rotate the drill string and bottom hole assembly (BHA) was ~250 ft*lbs lower when the pumps were on vs. when they were off at the same measured distance (MD), presumably because rotation of the drill bit cones when the pumps were on enabled easier rotation of the entire BHA or because of increased removal of cuttings due to circulation. In the presence of encapsulated oil, rotational torque was reduced by as much as 45% (Figure 9).

Lateral drilling was performed in the presence and absence of encapsulated oil, rotating at 40-45 RPM and with a weight on bit of 15,000 lbs. Following addition of the encapsulated oil and incubation for 2 hours, rate of penetration (ROP) increased by ~20% (Figure 10).

Example 5

The strains and lubricant in Table 25 below were prepared or obtained and subjected to testing described in Examples 6 and 7.

Table 25. Biomass / lubricant

Biomass Name	Source	Description
Strain C - oil	Prototheca moriformis	Solvent extracted oil
Strain C	Prototheca moriformis	Dried whole cells
Strain D	Prototheca moriformis	Dried whole cells
Strain E	Auxenochlorella protothecoides	Dried whole cells
Strain F	Saccharomyces cerevisiae	Dried whole cells
Strain G	Rhodoturula glutinis	Dried whole cells
Stabil Lube	Ptarmigan Energy	Drilling fluid lubricant

Strains C was derived from UTEX 1435 classical mutagenized for higher oil production and further transformed with the following plasmid pSZ2533 (SEQ ID NO: 1) for production of triacylglycerides with high oleic acid and low linoleic acid profile. The construct disrupts a single copy of the FATA1 allele while simultaneously expressing a *Saccharomyces cerevisiae* sucrose invertase and overexpressing a *P. moriformis* KASII gene (PmKASII). Relevant restriction sites in the construct pSZ2533 FATA13':::CrTUB2:ScSUC2:CvNR::PmUAPA1:PmKASII-CvNR::FATA1 5' are indicated in lowercase, bold and underlining and are 5'-3' *BspQ* I, *Kpn* I, *Asc* I, *Mfe* I, *EcoRV*, *Spe*I, *Asc*I, *Cla*I, *Sac* I, *BspQ* I, respectively.

BspQI sites delimit the 5' and 3' ends of the transforming DNA. Bold, lowercase sequences represent FATA1 3' genomic DNA that permit targeted integration at FATA1 locus via homologous recombination. The *C. reinhardtii* β -tubulin promoter driving the expression of the yeast sucrose invertase gene is indicated by boxed text.

- 5 The initiator ATG and terminator TGA for invertase are indicated by uppercase, bold italics while the coding region is indicated in lowercase italics The *Chlorella vulgaris* nitrate reductase 3' UTR is indicated by lowercase underlined text followed by the *P. moriformis* UAPA1 promoter, indicated by boxed italics text. The Initiator ATG and terminator TGA codons of the PmKASII are indicated by uppercase, bold italics,
- 10 while the remainder of the coding region is indicated by bold italics. The *Chlorella protothecoides* S106 stearyl-ACP desaturase transit peptide is located between initiator ATG and the *Asc* I site. The *C. vulgaris* nitrate reductase 3' UTR is again indicated by lowercase underlined text followed by the FATA1 5' genomic region indicated by bold, lowercase text.

- 15 Nucleotide sequence of transforming DNA contained in pSZ2533:

gctcttcacccaactcagataataccaatacccctcttctctctcatccattcagtacccccccttct
cttcccaaagcagcaagecggtggttacagaagaacaatcggttcgcgcaagtcgcccagcactgcccgcag
gcgccgcgcccagcagcccgttggccacacaggcaacgaatacattcaatagggggcctcgagaatggaagg
agcggtaaagggtacaggagcactgcgcacaaggggctgtgcaggagtgactgactggcgggcagacggcg
20 **caccgcgggcgaggcaagcaggggaagattgaagcgccaggaggaggatgctgattgaggggggcatcgag**
tctctcttggacccgggataaggaagcaaatattcgccgggttgggtgtgtgtgtgcacgttttcttctcagagtcgt
gggtgtgcttcaggaggatataagcagcaggatcgaaatcccgcgaccagcggttccccatccagccaaccacc
tgtcggttacctttcttgcgctatgacacttcagcaaaaggtagggcggtgctgcgagacggcttccggcgctgcatgca
acaccgatgatgcttcgacccccgaagctccttggggctgcatggcgctccgatgccgctccagggcgagcgctgtt
25 taaatagccagggccccgattgcaaagacattatagcgagctacaaagccatattcaaacacctagatcactaccactct
acacaggccactcgagcttgtgatcgactccgctaagggggcgcccttctctcttctcgtttcagtcacaacccgcaaac**gg**
cgcgccATGctgctgcaggccttctgttctgctggccggcttcgccccaagatcagcgctccatgacgaacga
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agaaggacgccaagtggcactgtacttcagtagaaccgaacgacacccgttgggggacgcccttgttctggggc
30 **cacgccacgtccgacgacctgaccaactgggaggaccagcccatcgccatgccccgaagcgcaacgactccggc**
gccttctccggctccatggtggtgactacaacaacacctccggcttcttaacgacaccatcgaccgcgccagcgct
gcgtggccatctggacctacaacaccccgagtcggaggagcagtagatctctacagcctggacggcggtacacc
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ccctcccagaagtggatcatgaccgcggccaagtcccaggactacaagatcgagatctactcctccgacgacctgaa
 gtcttggaaagtggagtcgcgttcgccaacgagggcttctcggtaccagtagagtgccccggcctgatcgaggt
 cccaccgagcaggaccccagcaagtctactgggtgatgttcattccatcaacccccggcgccccggcgggcggt
 cctcaaccagtacttcgtcggcagctcaacggcacccacttcgaggecttcgacaaccagtccegcgtggtggacttc
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 caccgagtaccaggccaacccggagacggagctgatcaacctgaaggccgagccgactcgaacatcagcaacgc
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 accggcacccctggagttcgagctggtgtacgccgtcaacaccaccagacgatctccaagtccgtgttcgaggacctct
 10 ccctctggttcaagggcctggaggaccccaggagtagctccgcatgggcttcgaggtgtccgctcctccttctctctg
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 cgaaacgttcacagcctaggg gatatc atagcgactgtctacccccgacctgtgcgaggcagaaattatatacaaga
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 gcagggtcaagctgctccaaaactcttgggcggttccggacggacggctaccgcggtgcgccctgacgc
 cactgttcggaagcagcgcgctgcatgggcagcgccgctgcggtgcgccacggaccgcatgatccaccggaaa
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**ggtgccatggcgcgctactaccctcgctatgactcgggtcctcgccggctggcggtgctgacaattcgtttagtgga
gcagcgactccattcagctaccagtcgaactcagtgggcacagtgactccgctcttc** (SEQ ID NO:1)

Strain D was derived from UTEX 1435 mutagenized for higher oil production and further transformed with a plasmid to disrupt a stearyl-ACP desaturase site
5 followed by further mutagenesis. The plasmid was constructed in accordance with methods and sequences described in WO2008/151149, WO2010/063031, WO2010/063032, WO2011/150411, and WO2013/158938 and comprises a *C. reinhardtii* β -tubulin promoter driving the expression *Saccharomyces cerevisiae* sucrose invertase gene with a *Chlorella protothecoides* Ef1 3' UTR and a *Prototheca*
10 *moriformis* endogenous *AMT3* promoter driving expression of an exogenous acyl-ACP thioesterase from *Cuphea. Wrightii* fused to a transit peptide from *Prototheca moriformis* fatty acid desaturase with a *Chlorella vulgaris* nitrate reductase 3' UTR.

Strain E is a *Chlorella protothecoides* (UTEX 250) strain obtained from the Culture Collection of Alga at the University of Texas (Austin, TX, USA).

15 A strain of oleaginous yeast *R. glutini* (Strain G) and a strain of non-oleaginous yeast *S. cerevisiae* (Strain F) were cultivated in a nutrient rich complex seed medium (Table 26) at 30 °C and 200 rpm. Primary 250-mL flasks containing 50-60 mL seed medium were inoculated with 1.0-1.5 mL cryopreserved cells (cell bank). At an OD (A_{600}) >3, primary flask cultures were used to inoculate secondary
20 flasks containing 60-300 mL seed medium to an initial OD of 0.1-0.2. Strains of yeast were propagated as required by sub-culturing a well-grown inoculum culture (OD >3) into seed medium at OD 0.1-0.2. For production fermentations, the seed culture was cultivated to OD >3 and the seed inoculum volume was typically 10% of the fermentation starting volume (also referred to as the after inoculation volume).
25 The *S. cerevisiae* strain was propagated through two seed flask stages (primary -> secondary -> production fermentation-AIV) to prepare the inoculum for the production fermenter. *R. glutinis* strain was propagated through four seed culture stages (primary -> secondary -> 3rd stage -> 4th stage -> production fermentation) to prepare the inoculum for the production fermenter.

30 The *R. glutinis* and *S. cerevisiae* cultures were cultivated in 15-L lab scale fermenters in a nutrient rich defined medium (Table 26 and Table 27). These fermentations were controlled at a temperature of 30 °C, a pH of 5 and dissolved

oxygen (DO) >30% of air saturation. The fermentations were aerated at 1.4 volume air/volume medium with automatic control of agitation at 400 – 1000 rpm as required to control DO. A 13% (w/w) potassium hydroxide solution was used to control pH. The cultures were fed a 71% (w/w dry solids) corn syrup solution on demand in order to maintain residual glucose concentrations in the broth between 0 and 20 g/L. The *S. cerevisiae* cultures were harvested after cultivation for ~ 4 days and 320 – 460 grams of glucose were consumed per liter after inoculation volume (g/L – AIV). The *R. glutinis* cultures containing 33% oil were harvested after cultivation for ~ 3 days and 230 – 260 grams of glucose were consumed per liter after inoculation volume (g/L – AIV). The *R. glutinis* cultures containing 44% oil were harvested after cultivation for 6 - 7 days and 420 – 450 grams of glucose were consumed used per liter after inoculation volume (g/L – AIV).

Table 26. Composition of seed medium for cultivation of yeast strains.

Medium was prepared by sterilizing in an autoclave at >121°C for >20 minutes or passing through a sterile 0.2 micron membrane filter.

Medium Components	Concentration (starting fermentation volume basis)
Peptone	20 g/L
Yeast Extract	10 g/L
Thiamine-HCl*	1.005 mg/L
d-Biotin*	0.015 mg/L
Cyanocobalimin*	0.012 mg/L
Calcium Pantothenate*	0.030 mg/L
p-aminobenzoic acid*	0.060 mg/L
Glucose*	20 g/L
Potassium Hydrogen Phthalate*	5.1 g/L

*Sterilized separately and combined aseptically to achieve the final concentration

Table 27. Composition of production fermentation medium for cultivation of yeast strains.

Medium was prepared by sterilizing in an autoclave at >121°C for >20 minutes or passing through a sterile 0.2 micron membrane filter.

Medium Components	Concentration (starting fermentation volume basis)
KH ₂ PO ₄	10.00 g/L
NaCl	0.50 g/L
MgSO ₄ *7H ₂ O	3.00 g/L
CaCl ₂ *2H ₂ O	0.50 g/L
(NH ₄) ₂ SO ₄	10.00 g/L
Antifoam 204 (Sigma Chemicals)	0.26 mL/L
Biotin*	0.30 mg/L
Calcium Pantothenate*	3.60 mg/L
Thiamine HCl*	3.60 mg/L
CuSO ₄ *5H ₂ O*	1.60 mg/L
COCl ₂ *6H ₂ O*	4.76 mg/L
ZnSO ₄ *7H ₂ O*	52.83 mg/L
MnSO ₄ *H ₂ O*	43.38 mg/L
Na ₂ MoO ₄ *2H ₂ O*	4.84 mg/L
FeSO ₄ *7H ₂ O*	55.56 mg/L
97DE Corn Syrup (71% dry solids)*	40-60 g/L

5 *Sterilized separately and combined aseptically to achieve the final concentration

Example 6

Burst strengths of the biomass in the previous examples were determined by comparing the amount of free oil released for cells of increasing oil content as a function of pressure. Dried biomass was suspended in de-ionized water to 10% total solids, as measured on a Mettler Toledo moisture analyzer by adding 1g of liquid to a tared glass filter paper and drying at 100 °C. The suspension is processed through a Niro Panda lab scale homogenizer unit at the indicated pressures (0, 500, and 750 bar) and collected for free oil analysis. Free oil is extracted from the lysed broth by diluting 0.5g of sample into 3mL de-ionized H₂O followed by gentle mixing with a 1:2 hexane and isopropyl alcohol solution for 30 seconds and centrifuged at 12,000

rpm for 5 minutes. The hexane layer containing the oil is transferred with a pipet to a pre-weighed aluminum tray and allowed to evaporate for 60 minutes in a fume hood. The dry oil in the pan is weighed and the % lysis for each sample is determined by dividing the free oil by the total oil available as determined by acid hydrolysis and gas chromatography. Results are summarized in Figure 12.

Example 7

The amounts of additives in water were normalized to strain A containing 55% lipid content. The additives (Figure 13) were mixed in water to a final concentration of 3% by weight for solid samples (which is 2% total oil by volume for strain A) and 2% by volume for liquid samples. The suspensions were mixed for 3 minutes at low shear using a Hamilton Beach Mixer and then transferred into the sample cup of an OFI Lubricity Meter (model #112-00). For the lubricity coefficient test, 150 in-pounds of force (the equivalent of 5,000 to 10,000 PSI pressure on the intermediate fluid) is applied between two hardened steel surfaces, a block, and a ring rotating at 60 RPM. The % torque reduction is then calculated against the base fluid from the meter reading as described in the equipment manual. Results are shown in Figure 13.

Example 8

Drilling fluid containing encapsulated oil was used in a project involving the excavation of two 21 foot diameter, 3.4 mile long light rail tunnels with Earth Pressure Balance Machines (EPBM, Robbins and Hitachi). The project encountered hard, dry sand conditions that slowed the machine from an average advance rate of greater than 70mm/min to 20mm/min. Muck collected and compacted within cutter head openings, causing maintenance and operational delays and increasing down time. Attempts to clear the muck and increase the advance rate using a drilling detergent and wetting agent were unsuccessful, while use of an anti-clay polymer led only to a slight improvement. Use of the encapsulated oil however, led to immediate and substantial improvement.

The drilling fluid containing encapsulated oil was used in 4 rings (5 foot drives) and compared to the previous 98 rings in the same soil conditions. The encapsulated oil was found to have a 72% improvement in advance rate in just 20 feet, improved consistency of movement, and improved productivity from 45 feet/day to 90 feet/day. A comparison of the advance rate of encapsulated oil with bentonite as

compared to bentonite only and bentonite with detergent is shown in Figure 14, with details of increased rate of penetration by ring section shown in Figure 15. Figure 16 illustrates the time savings achieved with use of encapsulated oil, as the installation rate doubled from 9 rings/day to 18 rings/day.

5 It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in
10 their entirety for all purposes.

Informal Sequence Listing

SEQ ID NO:1

Nucleotide sequence of transforming DNA contained in pSZ2533:

5 **gctcttcacccaactcagataataccaataacccctcttctctctctatccattcagtaaccccccttct**
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caccgccccgcaggcaagcaggggaagattgaagcggcagggaggaggatgctgattgaggggggcatcgacg
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Claims:

1. A fluid for use in a wellbore operation, the fluid comprising water, an oleaginous microbial cell, and a solvent, and optionally one or more of a surfactant, alcohol, demulsifier, or combinations thereof.
- 5 2. The fluid of claim 1, comprising a surfactant and an alcohol.
3. The fluid of any one of the preceding claims, wherein the solvent is an ester or a terpene.
4. The fluid of any one of the preceding claims, wherein the solvent is ethyl lactate.
- 10 5. The fluid of any one of the preceding claims, wherein the solvent is d-limonene.
6. The fluid of any one of the preceding claims, wherein the surfactant is a fatty acid soap.
7. The fluid of any one of the preceding claims, wherein the surfactant is
15 ethoxylated castor oil, polyoxyethylene sorbitan monopalmitate, or polyethylene glycol or combinations thereof.
8. The fluid of any one of the preceding claims, wherein the alcohol is isopropanol.
9. The fluid of any one of the preceding claims, wherein the surfactant and
20 solvent together form a microemulsion.
10. The drilling fluid of any one of the preceding claims, wherein the microbial cell is an oleaginous bacteria, yeast, or microalgae.
11. The drilling fluid of any one of the preceding claims, wherein the microbial cell is obtained from a heterotrophic oleaginous microalgae.
- 25 12. The drilling fluid of any one of the preceding claims, wherein the microbial cell is obtained from *Parachlorella*, *Prototheca*, or *Chlorella*.

13. The drilling fluid of any one of the preceding claims, wherein the microbial cell is obtained from *Prototheca moriformis*.
14. A method of conducting a wellbore operation, the method comprising introducing into the wellbore a fluid of any one of the preceding claims.
- 5 15. The method of claim 14, wherein the wellbore operation is a drilling, completion, stimulation, hydraulic fracturing, acidification, sand control, or workover operation.

Figure 1

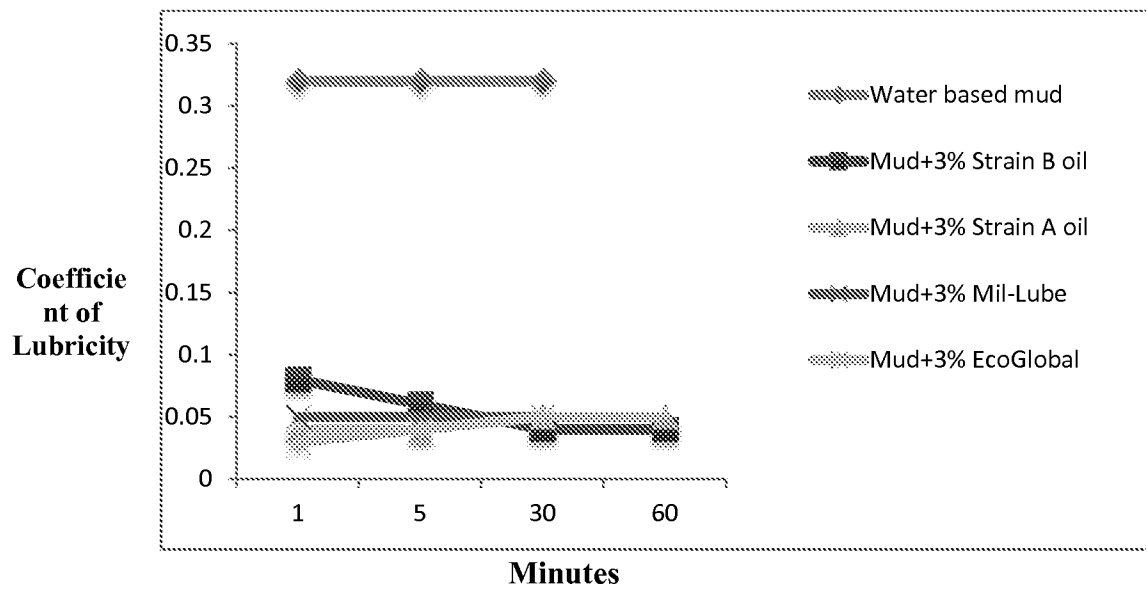
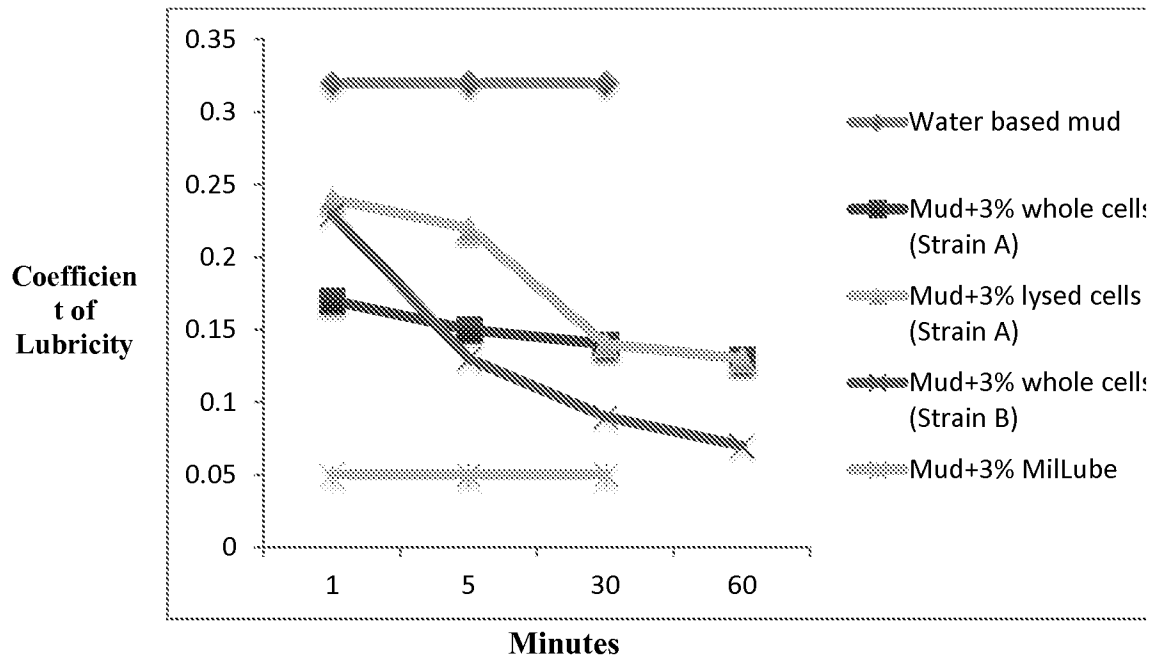
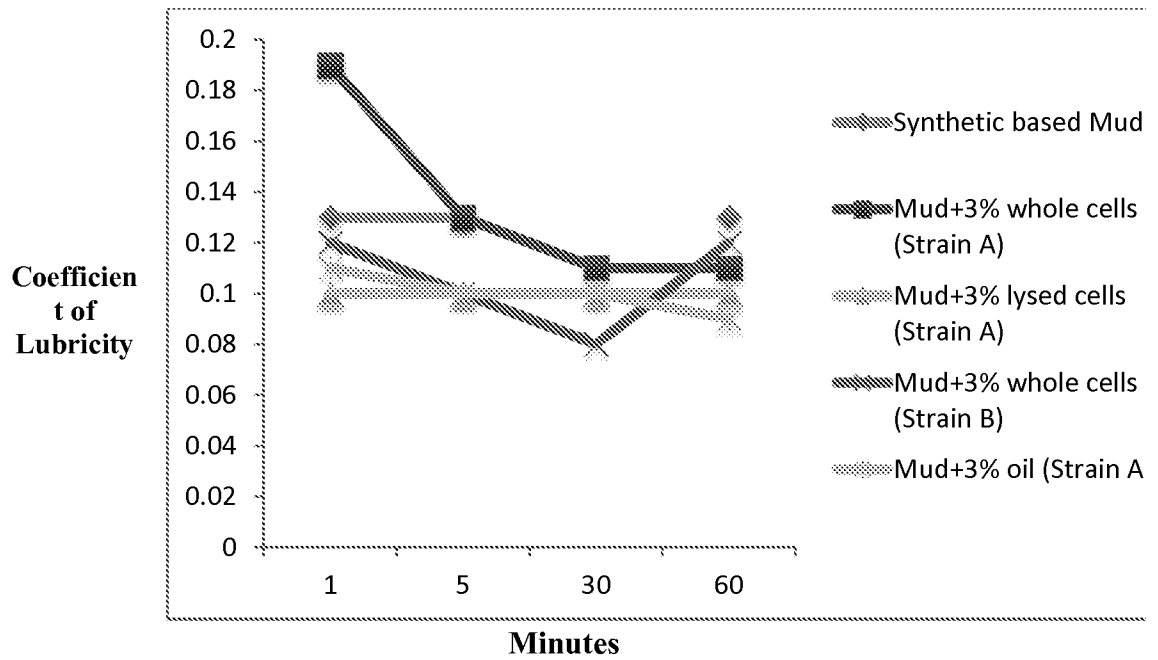
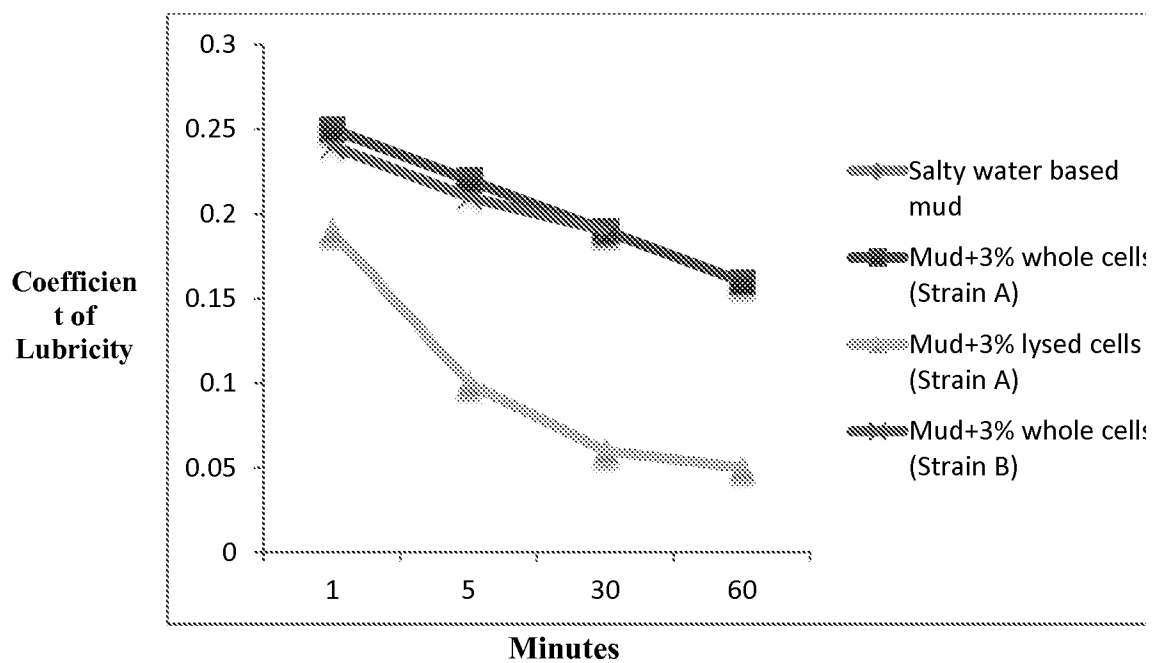
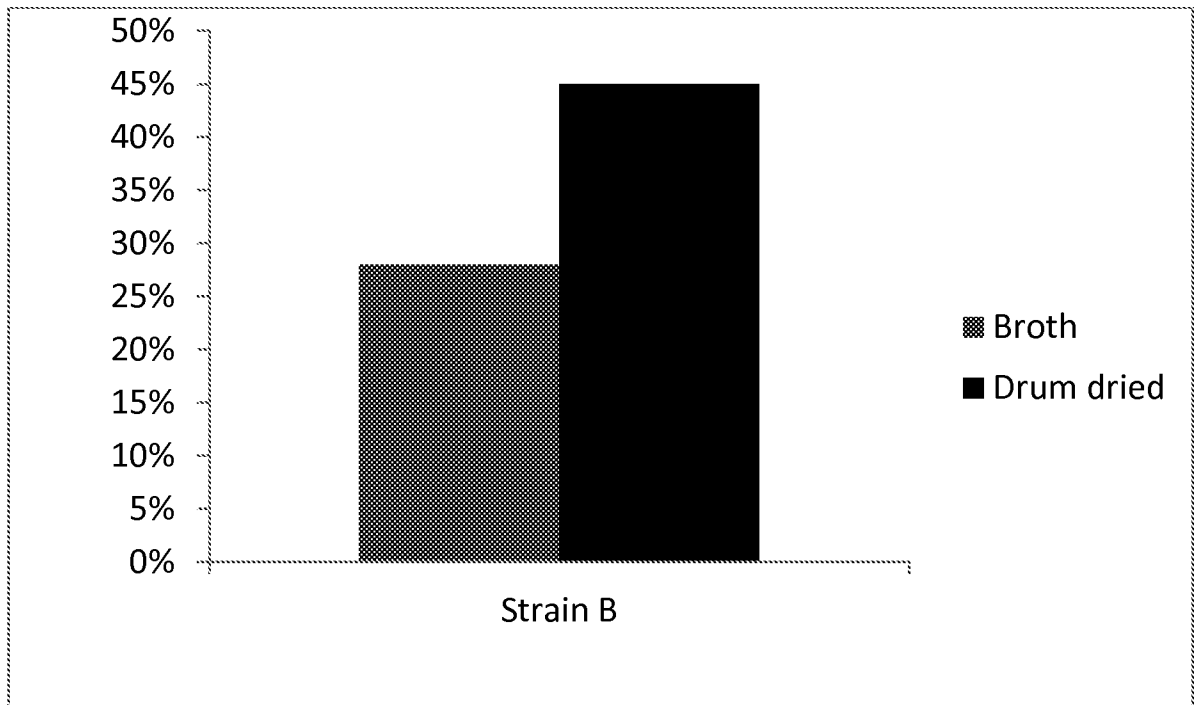


Figure 2



3/16**Figure 3**

4/16**Figure 4**

5/16**Figure 5**

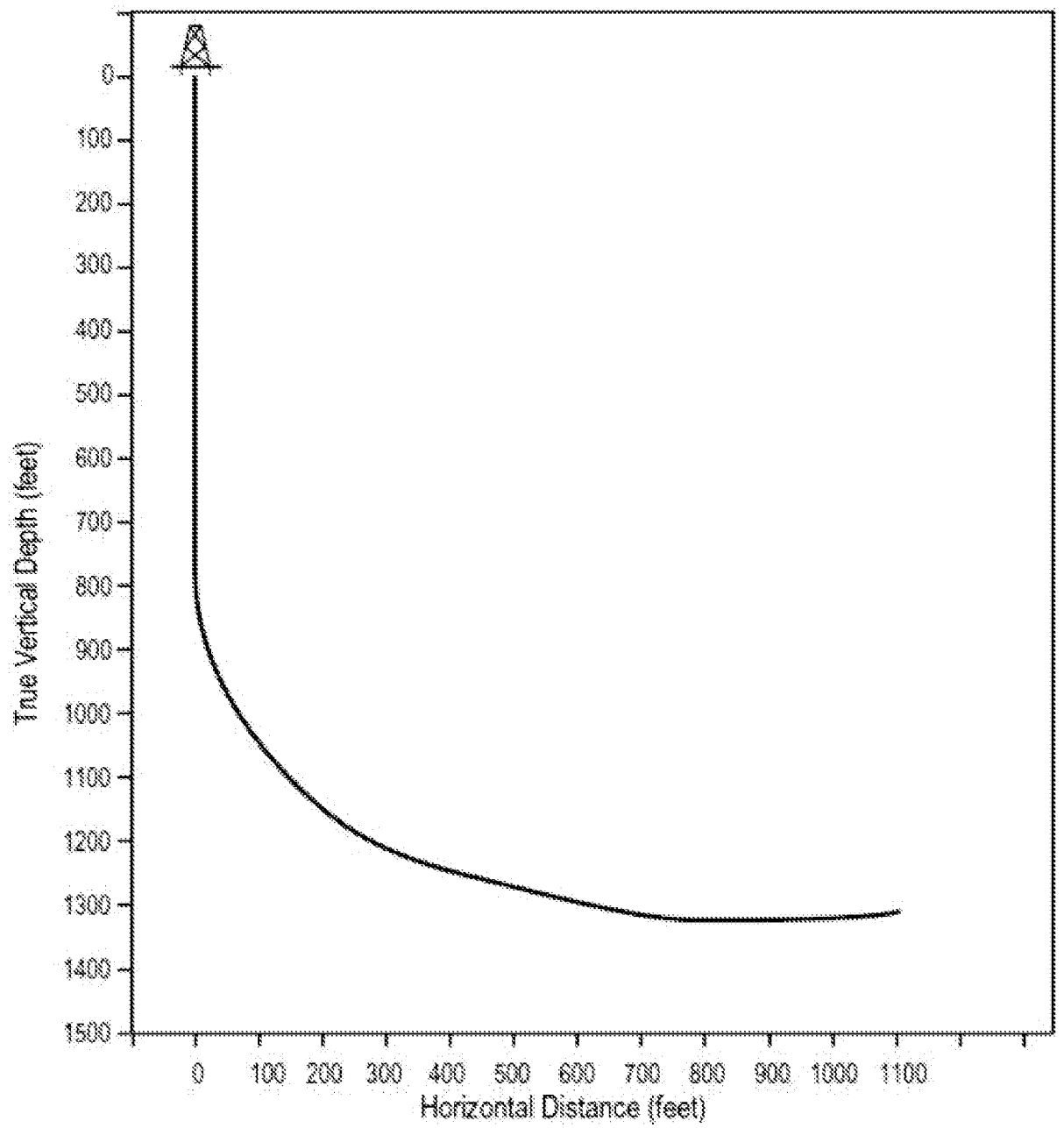
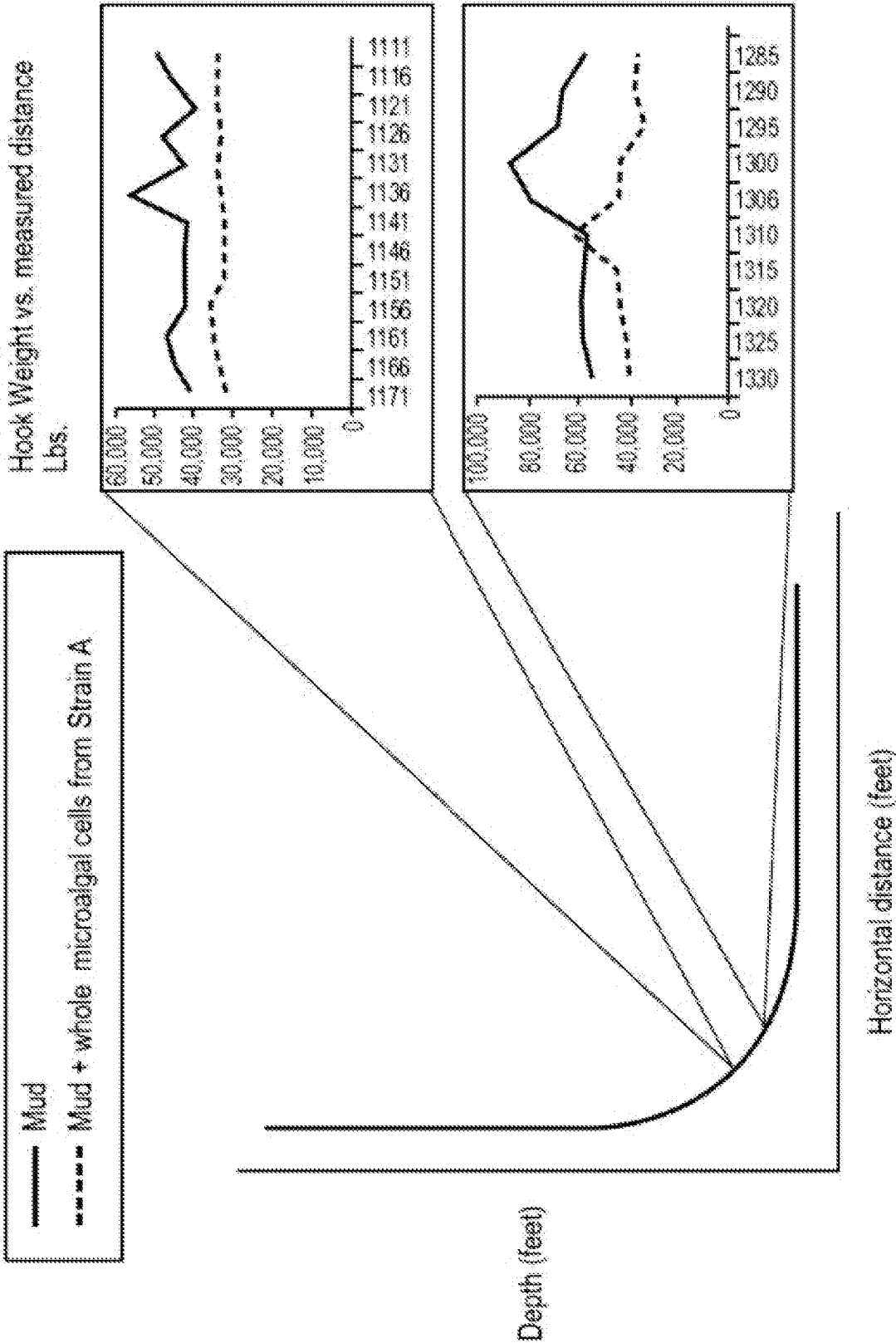
6/16**Figure 6**

Figure 7



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Figure 8

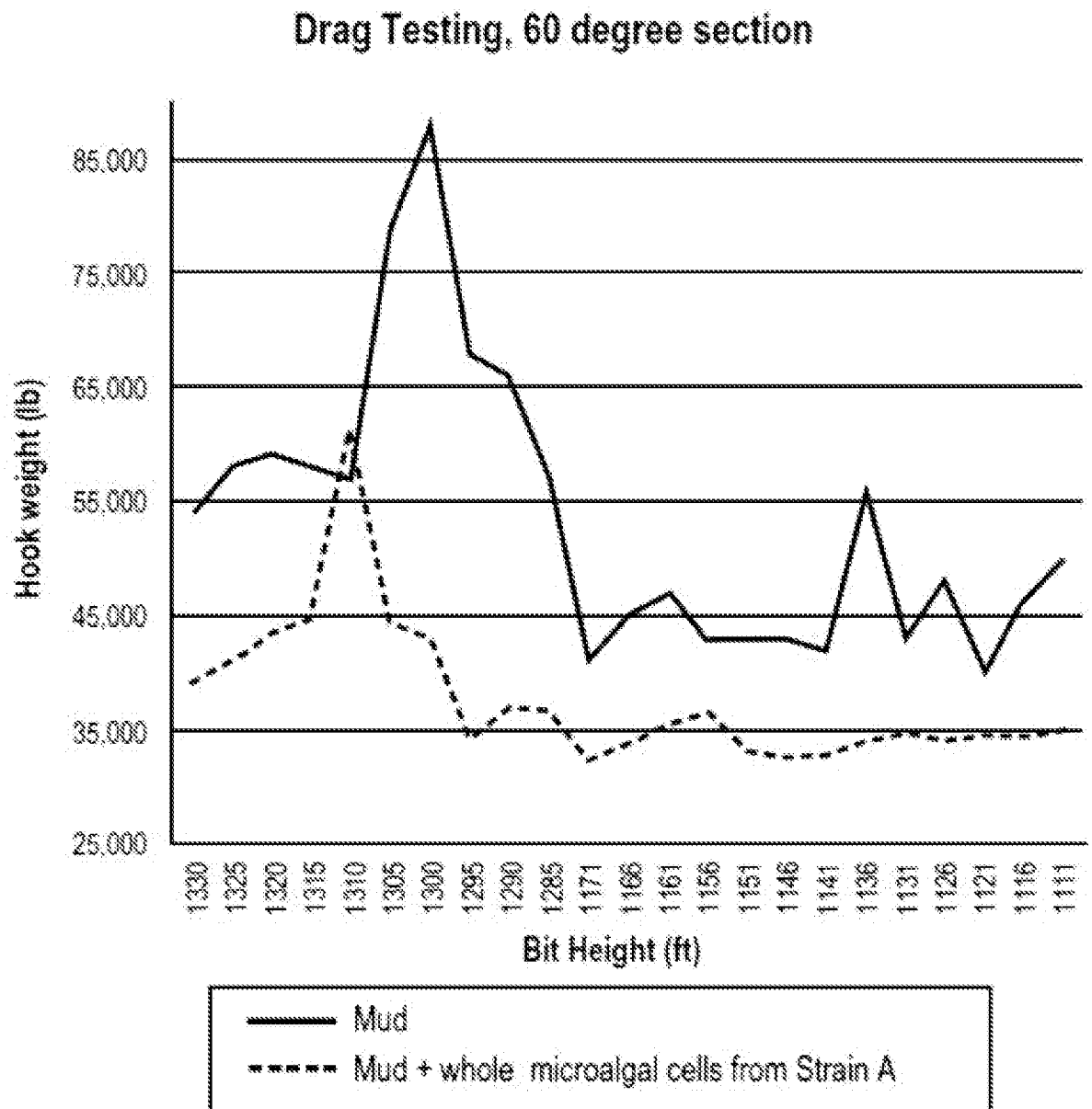
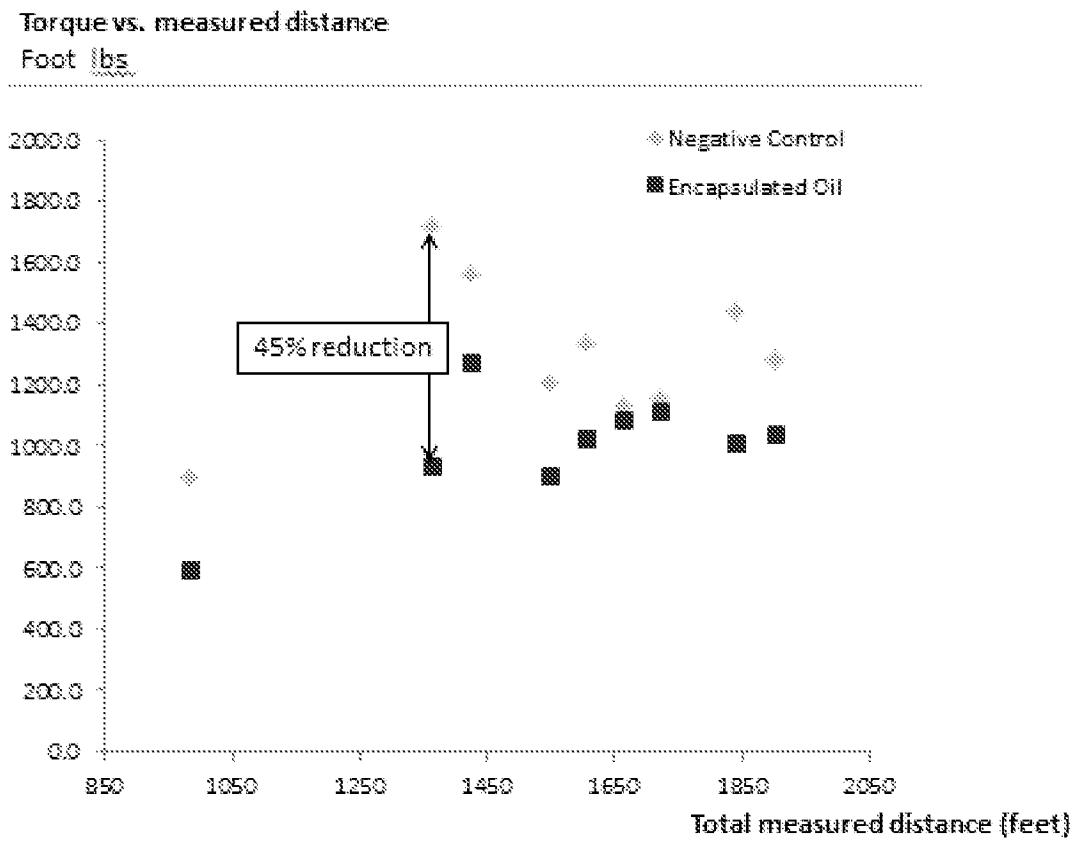


Figure 9



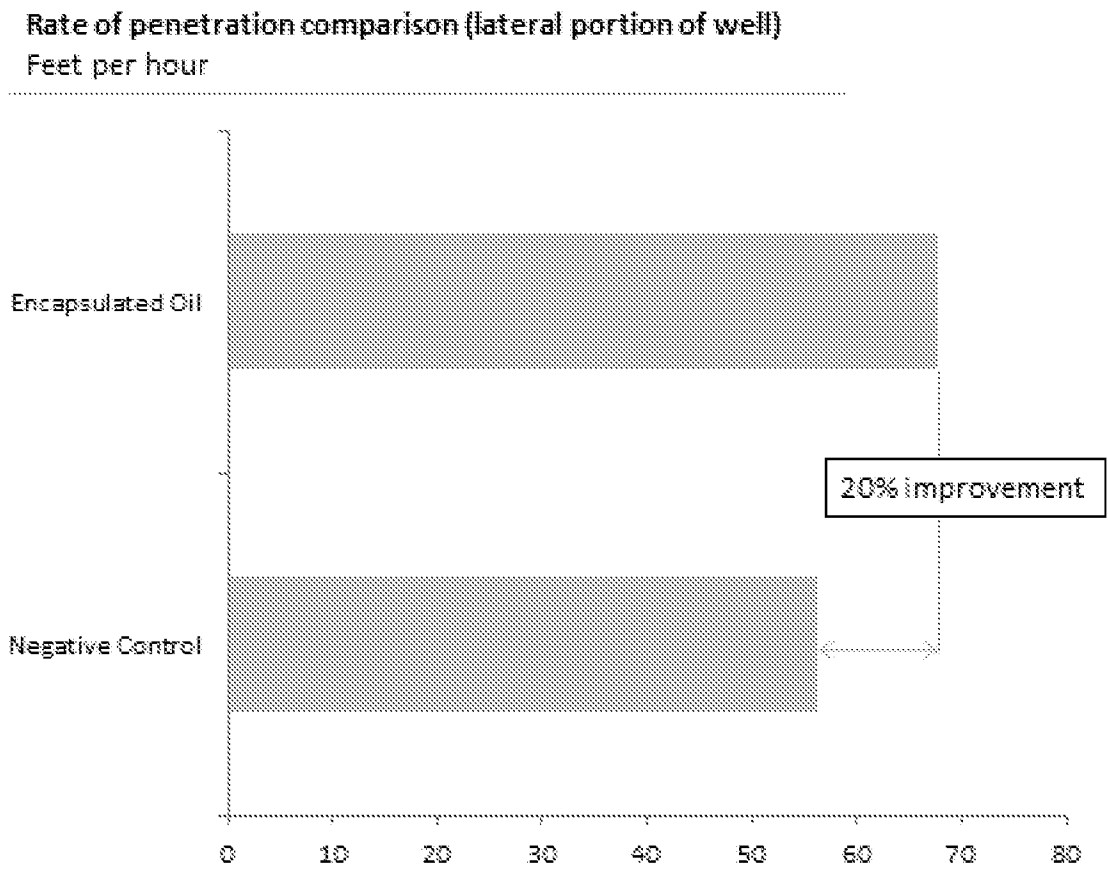
10/16**Figure 10**

Figure 11

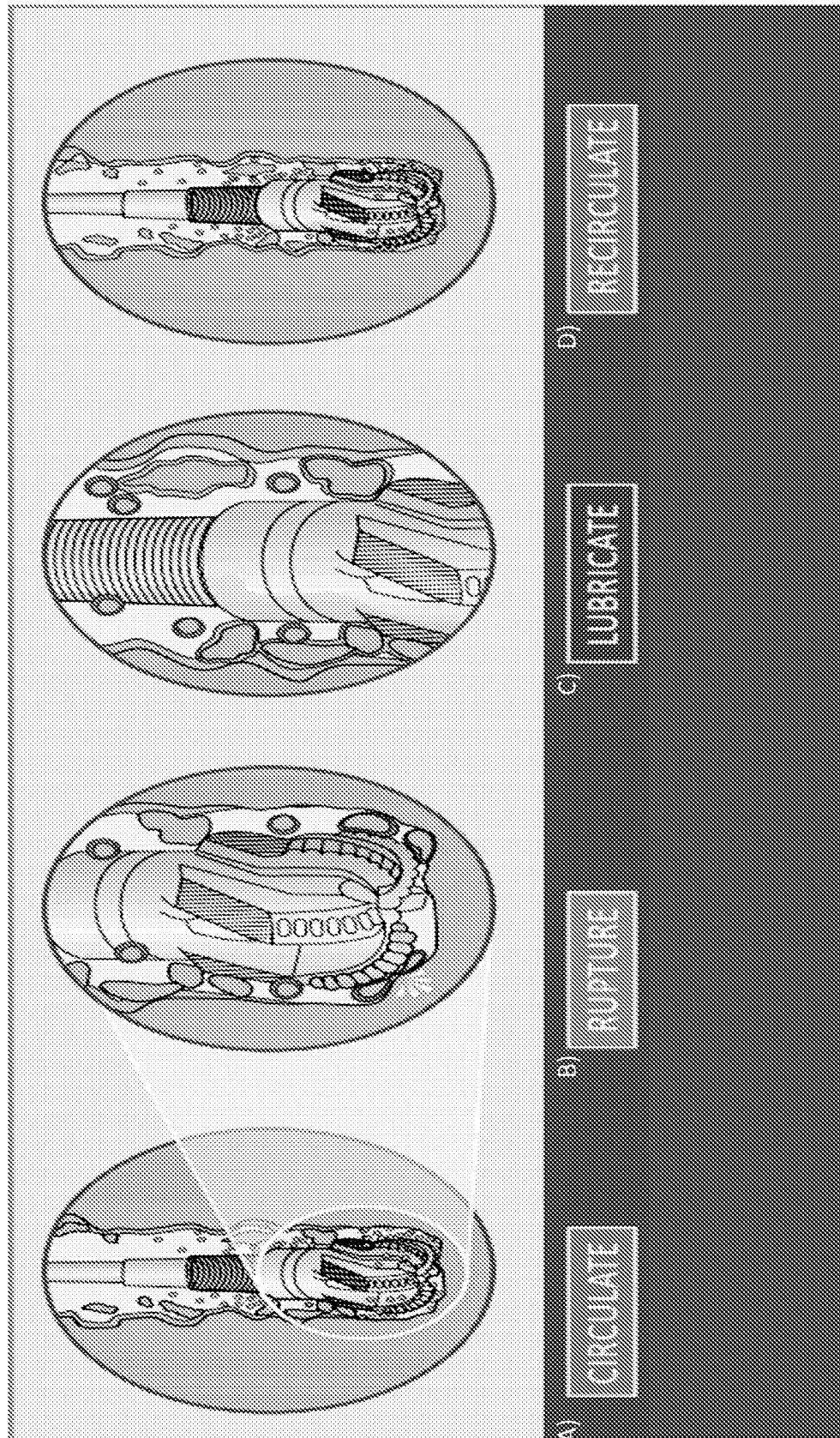


Figure 12

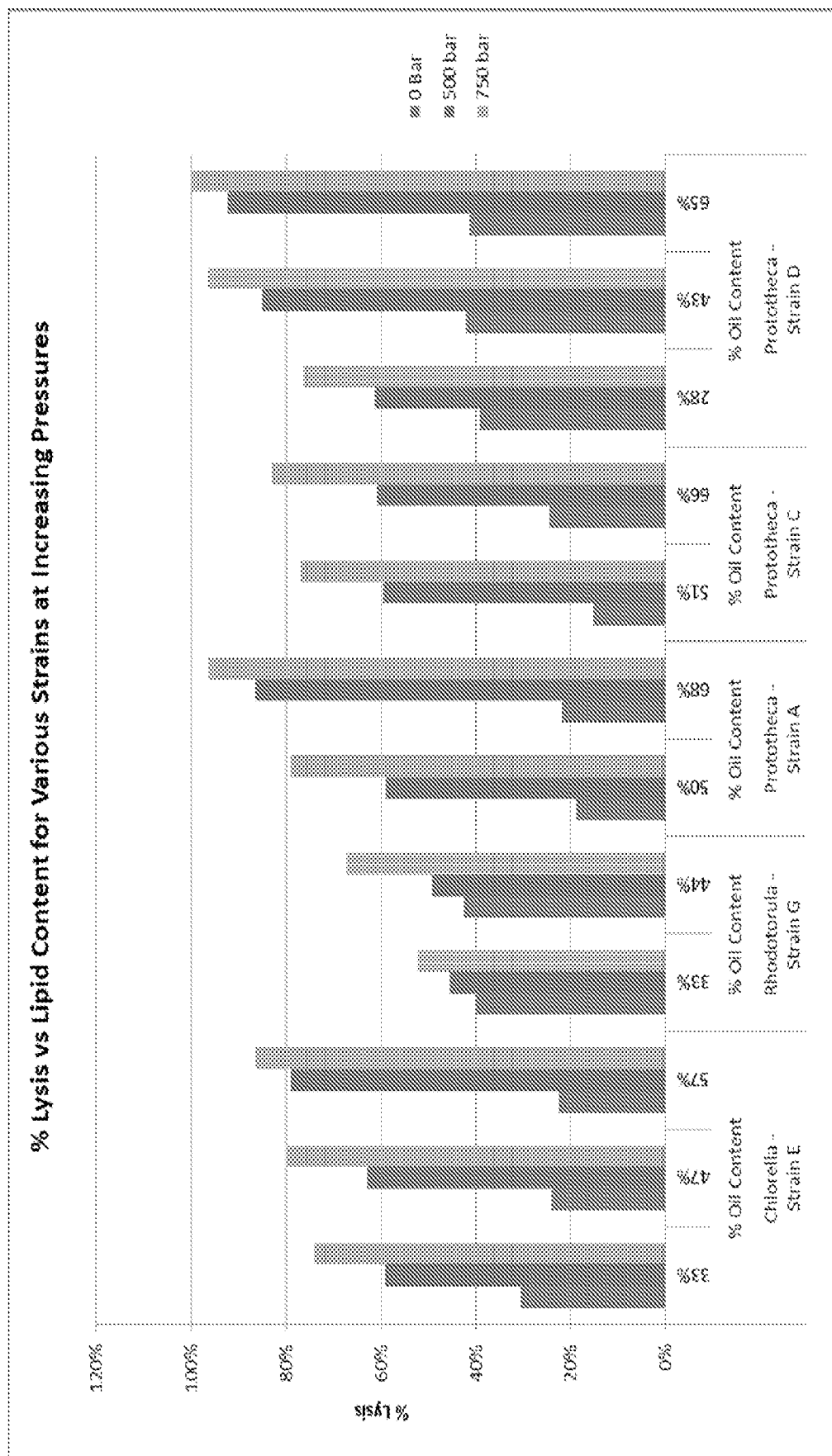


Figure 13

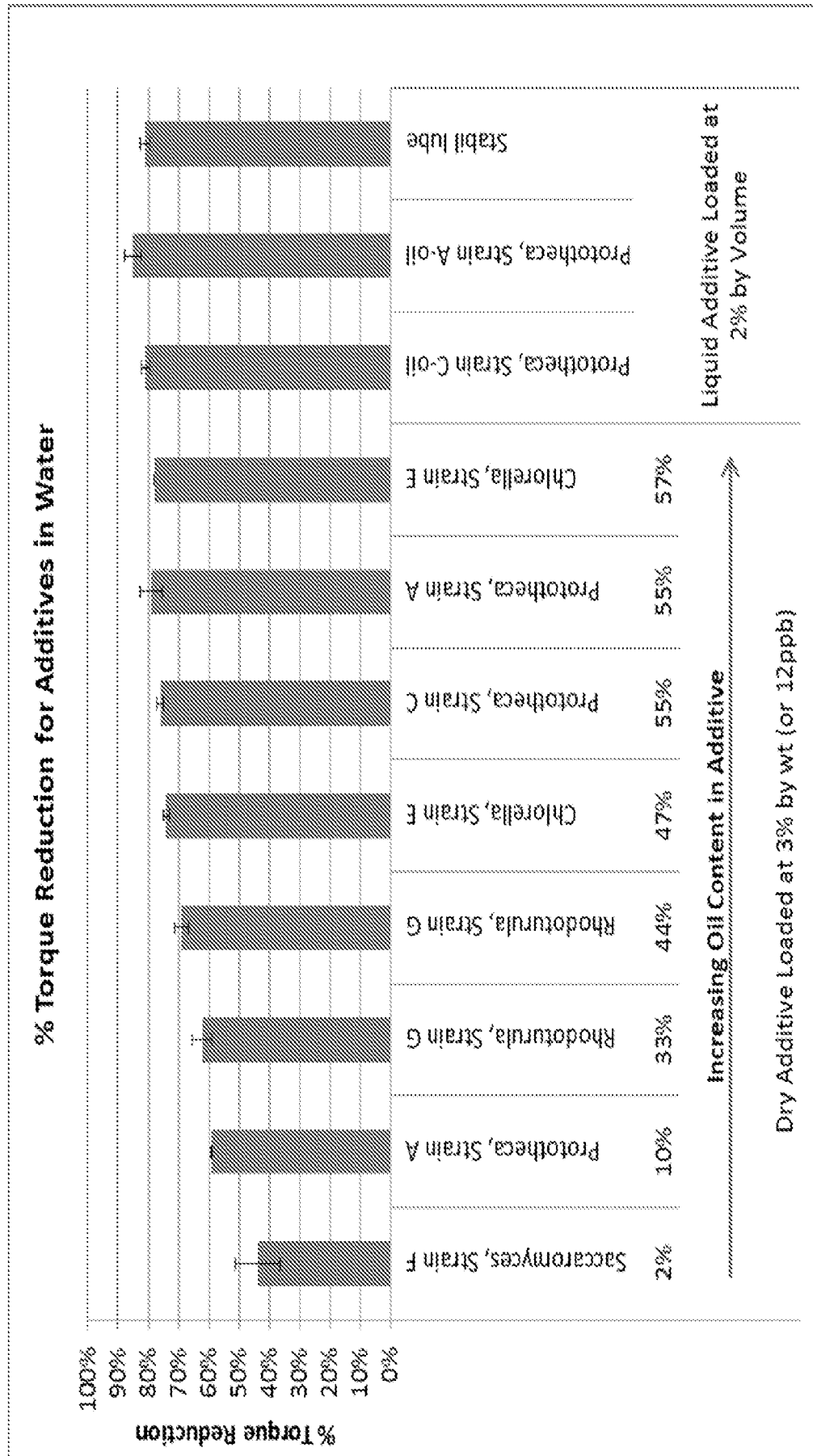
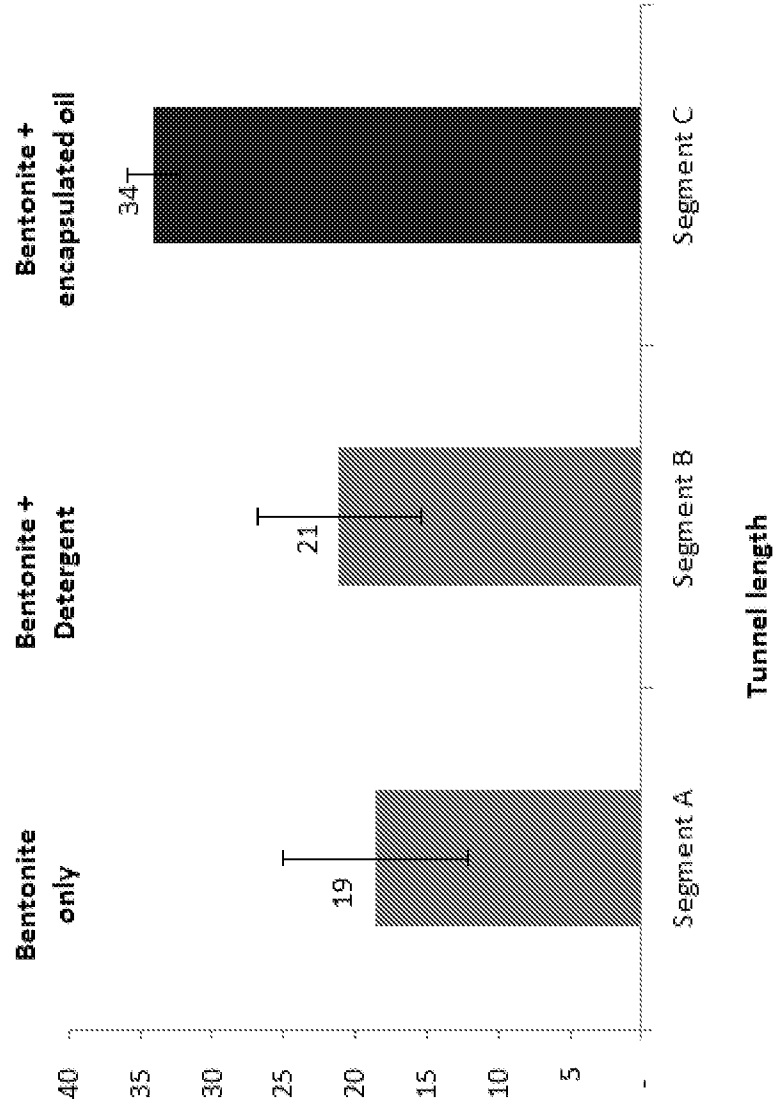
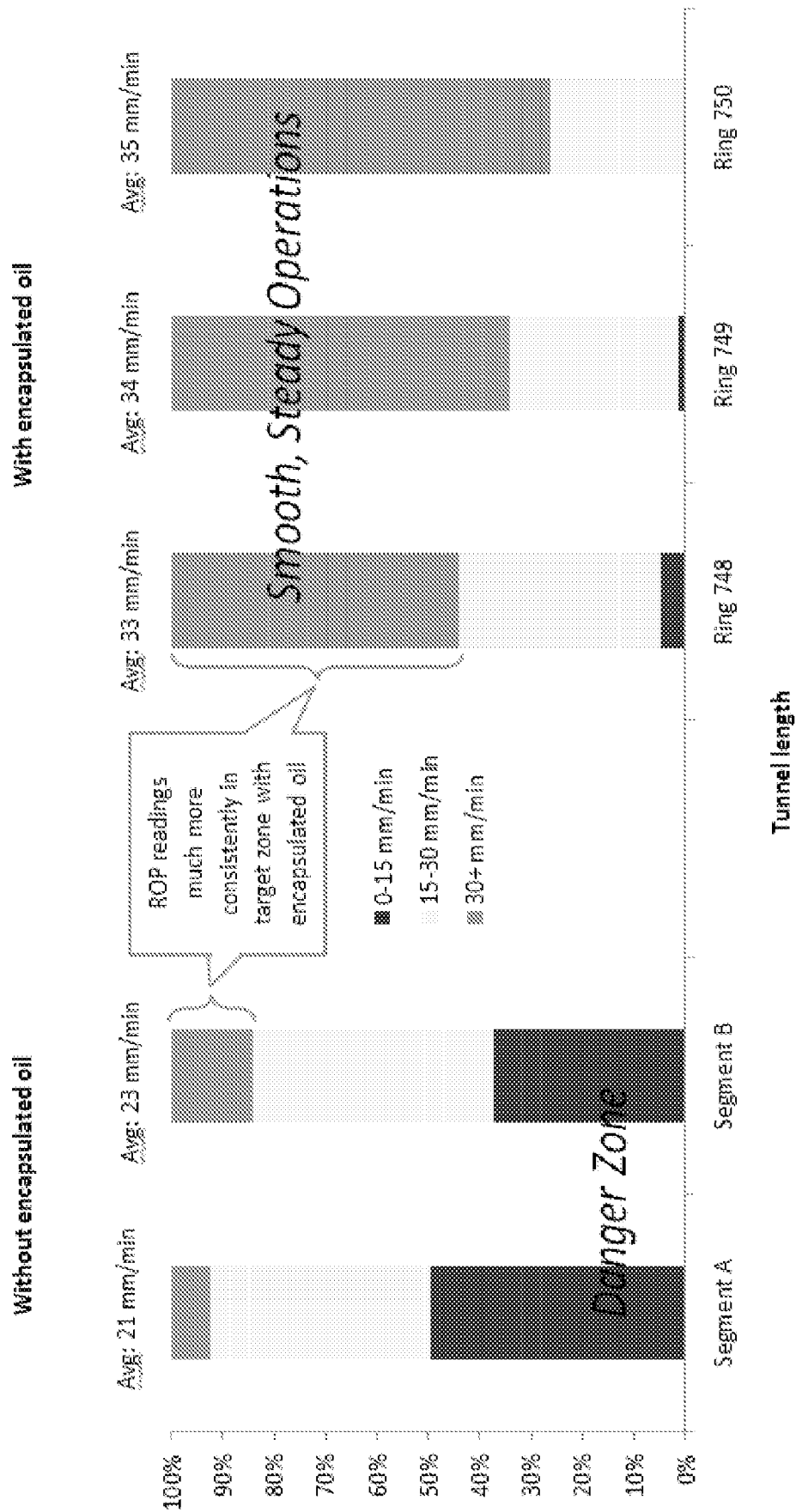


Figure 14



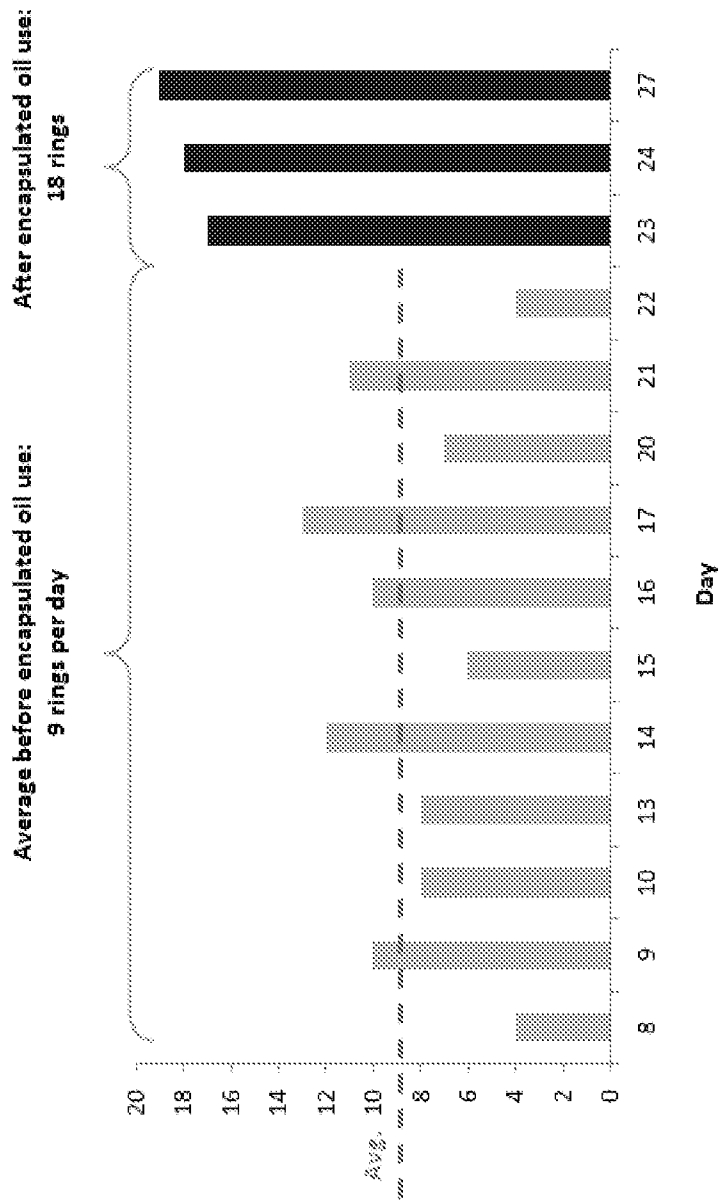
Segment A: Rings 650-699 (250 feet)
Segment B: Rings 700-747 (240 feet)
Segment C: Rings 748-751 (20 feet)

Figure 15



Segment A: Rings 650-699 (250 feet)
Segment B: Rings 700-747 (240 feet)

Figure 16



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/039130

A. CLASSIFICATION OF SUBJECT MATTER

INV. C09K8/08 C09K8/575 C09K8/68 C09K8/74 C09K8/86
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C09K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, COMPENDEX, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012/135756 A2 (SOLAZYME INC [US]; RAKITSKY WALTER [US]; SOUSA SONIA [US]; DAY ANTHONY) 4 October 2012 (2012-10-04) page 3 - page 6 page 14 - page 18 page 45 - page 50; claims; examples -----	1-15
X	US 3 598 181 A (WEGNER EUGENE H ET AL) 10 August 1971 (1971-08-10) column 2 - column 3; examples -----	1,2, 9-11,14, 15
X	US 2010/233761 A1 (CZARTOSKI THOMAS J [US] ET AL) 16 September 2010 (2010-09-16) page 4 - page 5; claims; example 8 -----	1,10-13
X	US 6 180 376 B1 (LIDDELL JOHN MACDONALD [GB]) 30 January 2001 (2001-01-30) examples -----	1,10,11



Further documents are listed in the continuation of Box C.



See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search

8 September 2015

Date of mailing of the international search report

24/09/2015

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Authorized officer

Doslik, Natasa

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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