



(51) International Patent Classification:

D21H 17/00 (2006.01) D21H 21/14 (2006.01)
D21H 17/02 (2006.01) D21H 21/28 (2006.01)
D21H 17/65 (2006.01) C12R 1/89 (2006.01)
D06P 1/34 (2006.01)

(21) International Application Number:

PCT/IL20 18/050320

(22) International Filing Date:

20 March 2018 (20.03.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/473,549 20 March 2017 (20.03.2017) U S

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ,

CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available):

ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: COMPOSITION COMPRISING CULTIVATED MICROALGAE FOR USE IN COLORING PROCESSES



Figure 1

(57) Abstract: The invention is directed to composition for use in the process of coloring surfaces, such as fabrics, comprising cultivated microalgae, processes for its preparation and use thereof.



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COMPOSITION COMPRISING CULTIVATED MICROALGAE FOR USE IN COLORING PROCESSES

TECHNOLOGICAL FIELD

The present invention is directed to composition for use in the process of coloring surfaces, such as fabrics, comprising cultivated microalgae.

BACKGROUND

Microalgae are microscopic unicellular organisms capable to convert solar energy to chemical energy via photosynthesis. They contain numerous bioactive compounds that can be harnessed for commercial use. The potential of microalgal photosynthesis for the production of valuable compounds or for energetic use is widely recognized due to their more efficient utilization of sunlight energy as compared with higher plants. Microalgae can be used to produce a wide range of metabolites such as proteins, lipids, carbohydrates, carotenoids or vitamins for health, food and feed additives, cosmetics and for energy production.

Nowadays, there are numerous commercial applications of microalgae such as microalgae can be used to enhance the nutritional value of food and animal feed owing to their chemical composition; they play a crucial role in aquaculture and they can be incorporated into cosmetics. Moreover, they are cultivated as a source of highly valuable molecules. For example, polyunsaturated fatty acid oils are added to infant formulas and nutritional supplements and pigments are important as natural dyes.

Microalgae have three fundamental attributes that can be converted into technical and commercial advantages. They are genetically a very diverse group of organisms with a wide range of physiological and biochemical characteristics; thus, they naturally produce many different and unusual fats, sugars, bioactive compounds, etc. They can cost-effectively incorporate the stable isotopes ^{13}C , ^{15}N and ^2H into their biomass and thus into various compounds they produce. They comprise a large, unexplored group of organisms, and thus provide a virtually untapped source of products.

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In recent years, microalgae apart from being used as single-cell proteins, they are projected as living-cell factories for the production of bio-fuels and various beneficial biochemicals used in food, aquaculture, poultry and pharmaceutical industries due to presence of different useful compounds (*J. Algal Biomass Utiln.* 2012, 3 (4): 89-100).

The pigment content in microalgae is a specific feature of each species. Its evaluation is essential as an indirect measure of cell growth, as well as a parameter to check the trophic level of waters. Components of algae are frequently used in cosmetics as thickening agents, water-binding agents, and antioxidants. Some microalgal species are established in the skin care market, the main ones being *Arthrospira* and *Chlorella*. Microalgae extracts can be mainly found in face and skin care products (e.g., anti-aging cream, refreshing or regenerant care products, emollient and as an anti-irritant in peelers). Microalgae are also represented in sun protection and hair care products. Typical species that are used for cosmetics are *Chondrus crispus*, *Mastocarpus stellatus*, *Ascophyllum nodosum*, *Ahria esculenta*, *Spirulina platensis*, *Nannochloropsis oculata*, *Chlorella vulgaris* and *Dunaliella salina*. Microalgae are a polyphyletic and biochemically diverse assemblage of chlorophyll-*a* containing microorganisms capable of oxygenic photosynthesis that are predominantly found in aquatic environments with observed high levels of ultraviolet (UV) radiation.

Microalgal pigment has commercial uses as a natural food coloring and cosmetic ingredient. Some microalgae contain substantial amounts of Carotene (besides beta carotene). Other types of coloring appear in microalgae as well. Beta carotene is used as a food coloring (with a major application in providing the yellow color to margarine), as a food additive to enhance the color of the flesh of fish and the yolk of eggs, and to improve the health and fertility of grain-fed cattle. Natural Beta Carotene has physical properties that make it superior to synthetic. In particular, natural Beta Carotene is fat soluble.

Marine microalgae are organisms that live in complex habitats submitted to extreme conditions (for example, changes of salinity, temperature, nutrients, UV-Vis irradiation, etc.), therefore, they must adapt rapidly to the new environmental conditions

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to survive, producing a great variety of secondary (biologically active) metabolites, which cannot be found in other organisms.

Since the properties of microalgae is greatly influenced by their environment and culture conditions, for the purpose of preparing commercially standardized bulk reagents suitable for the industry there was a need for a method of growing microalgae in a cultivated manner which uses the outdoor sunlight as a source of energy. US5534417 discloses an apparatus for confined growth of microalgae in an assembly of vertical, transparent tubes through which nutrient and air is carried with carbon dioxide. The microalgae is periodically harvested from the tubes. US5643585 discloses a non-soluble particulate coloring material comprises dry ground red microalgae-derived material as the base coloring matter for use in the coloring of food products and cosmetics.

GENERAL DESCRIPTION

The inventors of the present application have found that cultivated microalgae may be used for coloring surfaces, such as for example paper, a fiber, a fabric, a cloth, a garment, wool, silk, cotton, leather, polymeric material and any combination thereof.

Thus, the present invention provides a composition comprising at least one cultivated microalgae and at least one mordant.

The invention further provides a composition comprising at least one cultivated microalgae and at least one mordant, for use in a process of coloring a surface.

In a further aspect the invention provides a composition comprising at least one cultivated microalgae and at least one mordant, for use in coloring at least one of a fiber, a fabric.

The invention provides a process for the preparation of a composition according to any one of the preceding claims, comprising the steps of: mixing at least one cultivated microalgae with at least one of at least one mordant, and optionally at least one of at least one enzyme, at least one wetting agent, at least one dye binder, at least one thickening agent and any combinations thereof.

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In another one of its aspects, the invention provides a fiber comprising a composition of the invention. In another aspect, the invention provides a fabric comprising a composition of the invention.

In another aspect, the invention provides a process of coloring a surface comprising the steps of providing a composition of the invention, mixing said composition with a fabric. In some embodiments, said mixture of composition and fabric are heated to a temperature in the range of 30 - 100C. In another embodiments, said fabric is pretreated. In some embodiments, said pretreatment is cationization of said fabric (i.e. making said fabric impregnated with a cationic solution). In other embodiments, said composition of the invention is used to print a pattern on said fabric using a printer or a pattern mold. In some embodiments the printed fabric is baked in an oven for fixing said pattern on said fabric.

BRIEF DESCRIPTION OF THE DRAWINGS

The subject matter regarded as the invention is particularly pointed out and distinctly claimed in the concluding portion of the specification. The invention, however, both as to organization and method of operation, together with objects, features, and advantages thereof, may best be understood by reference to the following detailed description when read with the accompanying drawings in which:

Figure 1 shows the results of the coloring process using a composition of the invention as described in the example herein.

DETAILED DESCRIPTION OF EMBODIMENTS

The invention provides a coloring and/or dyeing composition comprising at least one cultivated microalgae and at least one mordant.

The term "*composition*" as used in the context of the present invention should be understood to encompass any composition that is suitable for coloring and/or dyeing, i.e. transferring a color onto a surface, such as for example a fiber, a fabric and so forth.

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The term "*microalgae*" should be understood to encompass are unicellular photosynthesis forming species which exist individually, or in chains or groups, having a size that can range from a few micrometers (μm) to a few hundreds of micrometers. Microalgae do not have roots, stems, or leaves. Microalgae biomass is typically measured with chlorophyll concentrations.

In some embodiments, said cultivated microalgae is selected from red algae, brown algae, green algae, red algae, blue algae and mixtures thereof. In other embodiments, said cultivated microalgae is a red microalgae. In yet further embodiments, said cultivated red microalgae is selected from the genus *Porphyridium*, *Rhodella*, *Hematococcus (pluvialis)*, *Dunaliella (bradawil)* and any mixture thereof.

The natural types of microalgae are usually found in freshwater and marine systems living in both the water column and sediment, however, the microalgae used in the present invention are "*cultivated microalgae*" thus, they are grown and cultured outside their natural marine environment, in regulated and controlled environments. In some embodiments, said cultivated microalgae are from a source that is other than a marine source. In further embodiments, said cultivated microalgae are none naturally grown microalgae. In further embodiments, said cultivated microalgae are none marine grown microalgae. In some other embodiments, said cultivated microalgae is grown/cultivated outside a marine environment (the cultivated microalgae used in the present invention are not harvested from a marine or natural water source). For example, in some embodiments, said cultivated microalgae is grown/cultivated in a microorganism growth apparatus. An example of a microorganism growth apparatus, including a process for growing said microalgae in the apparatus can be found in US5534417, which is herein incorporated in its entirety.

In some embodiments, a composition of the invention comprises at least 0.5% by weight of said at least one cultivated microalgae. In other embodiments, a composition of the invention comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20% by weight of said at least one cultivated microalgae. In further embodiments, a composition of the invention comprises between about 5% to about 40% by weight of said at least one cultivated microalgae. In further embodiments, a composition of the

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invention comprises between about 1% to about 20% by weight of said at least one cultivated microalgae. In further embodiments, a composition of the invention comprises between about 5% to about 25% by weight of said at least one cultivated microalgae.

The term "*mordant*" as used herein should be understood to encompass any type of dye fixative, a substance used to set (i.e. bind) the coloring composition of the invention on a fiber and/or a fabric.

In some embodiments, said at least one mordant is from a natural source. In some embodiments, said at least one mordant is selected from Oak galls, Pomegranate rind Juniper needles and any combinations thereof. In other embodiments, said at least one mordant is from a synthetic source, selected from Tannic acid, Tartaric acid, Iron (II) sulfate heptahydrate, Aluminum potassium sulfate dodecahydrate, FeSO₄ and any combinations thereof. In other embodiments, said at least one mordant is tannic acid.

In some embodiments, a composition of the invention further comprises at least one of the additive list consisting of: at least one enzyme (such as for example cellulose degrading enzyme), at least one dye binder (an agent that allows for the color composition to bind to the fiber of the fabric, such as AVCO BINDER D), at least one thickening agent (an agent that prevents the color composition from spreading by capillary attraction on the fabric or fibers) and any combinations thereof. In some embodiments, said at least one enzyme is a cellulose degradation enzyme.

Typical thickening agents are starch derivatives, flour, gum arabic, guar gumderivatives, tamarind, sodium alginate, sodium polyacrylate, gum Senegal and gum tragacanth, British gum or dextrine and albumen. Other thickeners include, alginate-based thickeners (such as AVCO-PRINT ESO) and cellulose based thickeners (such as THICKENER CM-7).

The present invention provides a composition comprising cultivated microalgae, for use in a process of coloring a surface. When referring to a process of coloring a surface, it should be understood to encompass any type of coloring process including,

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dyeing and printing any type of color or pattern on a surface, thus endowing said surface with a color or a pattern with color.

In some embodiments, said process of coloring is dyeing (i.e. a process wherein the fabric, part of it or in its entirety, is immersed in the coloring composition of the invention and thus being endowed with a uniform color). In other embodiments, said process of coloring is printing (i.e. a process of applying color to fabric in definite patterns or designs. In printing, wooden blocks, stencils, engraved plates, rollers, or silkscreens or printers can be used to place the composition on the fabric).

In some embodiments said surface is a paper, a fiber, a yarn, a fabric, a cloth, a garment, wool, silk, cotton, leather, polymeric material and any combination thereof.

In a further aspect the invention provides a composition comprising cultivated microalgae, for use in a process of coloring a fiber.

The term "*fiber*" should be understood to encompass a thread that can be used to make a yarn, fabric, knitted, woven, nonwoven fabric, cloth, paper, having a length longer than it is width.

In some embodiments, said fiber is a natural fiber. Such fibers include cellulose based fibers, protein-based fibers, mineral based fibers. Such natural fibers include but are not limited to: Seed fiber (fibers collected from seeds or seed cases, e.g. cotton and kapok, Leaf fiber (fibers collected from leaves, e.g., sansevieria, fique, sisal, banana and agave), Bast fiber (fibers are collected from the skin or bast surrounding the stem of their respective plant. These fibers have higher tensile strength than other fibers. Therefore, these fibers are used for durable yarn, fabric, packaging, and paper. Some examples are flax, jute, kenaf, industrial hemp, ramie, rattan, and vine fibers), Skin fiber, Fruit fiber (fibers are collected from the fruit of the plant, e.g. coconut (coir) fiber), Stalk fiber (fibers that are actually the stalks of the plant, e.g. straws of wheat, rice, barley, and other crops including bamboo and grass, tree wood), Animal fibers (generally comprise proteins such as collagen, keratin and fibroin; examples include silk, sinew, wool, catgut, angora, mohair and alpaca, animal hair (wool or hairs): fiber or wool taken from animals or hairy

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mammals. e.g. sheep's wool, goat hair (cashmere, mohair), alpaca hair, horse hair, etc.
silk fiber: fiber secreted by glands (often located near the mouth) of insects during the preparation of cocoons, avian fiber: fibers from birds, e.g. feathers and feather fiber).

In other embodiments, said fiber is a manufactured fiber. Such fibers include, but are not limited to natural sugar based manufactured fibers - PLA, chitosan, protein based - rubber, azlon, cellulose based - rayon, lyocell, acetate, triacetate, synthetic polymers such as - acrylic, anidex, aramid, elastoester, fluoropolymer, lastrile, melamine, modacrylic, novoloid, nylon, nitril, olefin, PBI, polyester, rubber (synthetic), saran, spandex, sulfar, vinal, vinyon.

In other embodiments said fiber is a synthetic or artificial fiber (such as for example Nylon, Olefin, Acrylic, Polyester, Rayon, artificial silk, Vinyon, Saran, Spandex, Vinalon, Aramids, Nomex, Kevlar, Twaron, Modal, Dyneema/Spectra, PBI (Polybenzimidazole fiber), Sulfar, Lyocell, PLA, M-5 (PIPD fiber), Orion, Zylon (PBO fiber), Vectran (TLCP fiber) made from Vectra LCP polymer, Derclon, Acrylonitrile rubber, Glass fiber, metallic fiber, expanded polystyrene flakes, urea-formaldehyde foam resin, polyurethane foam, phenolic resin foam and any combinations thereof.

The invention further provides a composition comprising at least one cultivated microalgae, for use in a process for coloring a fabric. In some embodiments, said fabric is selected from a woven fabric, a nonwoven fabric, a knitted fabric or any combinations thereof.

In some other embodiments, a composition of the invention further comprises at least one further agent selected from an anti-irritating agent, an anti-microbial agent, vitamins, anti-septic, anti-inflammatory, anti-oxidants, an anti-sceptic agent, a softening agent.

In some embodiments, said fabric is used in the preparation of a garment. In other embodiments, said fabric is used in the preparation of a medical fabric (such as for example a bandage, a wound dressing, a casting form, a pressure wound dressing, an

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hygienic napkin, a sanitary napkin, burn dressing and so forth). In some embodiments, said fabric is used in the preparation of a wearable medical device.

The invention further provides a process for the preparation of a composition according to any one of the preceding claims, comprising the steps of: mixing at least one cultivated microalgae with at least one at least one mordant (fixating agent), and optionally at least one of at least one enzyme, at least one wetting agent, at least one binding agent, a thickening agent and any combinations thereof.

In a further aspect the invention provides a fiber comprising a composition of the invention. In a further aspect the invention provides a fabric comprising a composition of the invention.

EXPERIMENTAL SECTION

Cultivated Microalgae: Red microalgae of the type *Porphyridium sp.* The microalgae were cultivated under controlled conditions as disclosed in US5534417 and provided as biomass (either in a liquid form, a frozen biomass form or a powder).

Preparing the dyeing composition for cellulose based fabrics

400 ml of cultivated microalgae biomass was mixed with 200 ml of distilled water. 12g of tannic acid (mordant) was added to the mixture.

Preparing the cellulose based fabric for dyeing

Cationization solution: a mixture of 200 ml water, 1.2g AVCOPRET Ultra Cat.

Cationization process: The solution was added with a fabric sample (20g of cellulose based fabrics such as modal, bamboo or cotton) to a container which was heated in an oven for 20 minutes at 50C. After additional 50 minutes the fabrics were taken out of the container.

Dyeing process for cellulose based fabrics

The process was performed in container of 1 liter. 600 ml of the dyeing composition was added to the container together with 20g of fabric that has been prepared by the cationization process.

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The container was introduced into an oven for 30 minutes at 80C. After 30 minutes the oven was stopped. The container was opened, and small piece of the fabric were taken. The color of sample was pink. Then the container was entered into the over again for another 20 minutes at 98 C. The oven was stopped, and another sample of the fabric was taken, having beige/brown color. After taking the fabric out of the container it was dried.

Preparing the dyeing composition for synthetic fabrics

Fabrics: Nylon - Semi - Dull

Materials: Enzyme - Avco - Zim und 3548, Mordant - Tannic acid, Biomass - Red micro algae, Biomass - Blue / Green micro algae. Microalgae were cultivated under controlled conditions as disclosed in US5534417.

Eight types of compositions were prepared:

Composition 1: Red Biomass (50 ml), Enzyme (50 ml), water (100 ml)

Composition 3: Red Biomass (50 ml), No Enzyme, water (100 ml)

Composition 5: Blue/Green Biomass (50 ml), Enzyme (50 ml), Water (100 ml)

Composition 7: Blue/Green Biomass (50 ml), No Enzyme, Water (100 ml)

Composition 2: Red Biomass (50 ml), Enzyme (50 ml), Mordant (50 ml), water (100 ml)

Composition 4: Red Biomass (50 ml), No Enzyme, Mordant (50 ml), Water (100 ml)

Composition 6: Blue/Green Biomass (50 ml), Enzyme (50 ml), Mordant (50 ml), Water (100 ml).

Composition 8: Blue/Green Biomass (50 ml), No Enzyme, Mordant (50 ml), Water (100 ml).

Each composition was added to a container with fabric sample and entered into an oven for 120 minutes in 98C. At the end of the oven treatment the fabric samples were taken out of the container and rinsed twice (10 minutes with warm water at 40C and another 10 minutes with tap cold water). The fabrics were then spinned and washed in a washing machine. Figure 1 shows the coloring results. Table 1 below describes the results.

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Table 1 - Results of coloring compositions 1 - 8 above

Sample number	Pigment and other materials	Color recieved
(1)	Red with enzyme	Beige
(3)	Red with no enzyme	Light Beige
(2)	Red with enzyme and with mordant	Green-Brown
(4)	Red with no enzyme with mordant	Green-Blue
(5)	Green/Blue with enzyme	Lemon green
(7)	Green/Blue with no enzyme	Lemon green
(6)	Green/Blue with enzyme and with mordant	Green-Brown
(8)	Green/Blue with no enzyme with mordant	Dark Green-Brown

As can be understood from the table above, the compositions wherein mordant was added provided a fabric that was colored with stable color that did not wash off.

Preparing the coloring composition for printing on fabrics

Materials - Biomass of red micro algae (frozen) (cultivated under controlled conditions as disclosed in US5534417), Enzyme: Avco - Zimund 3548, Water: distilled water, Binder: Avcobinder D, Fixator: UP. Fabrics - Modal after washing in washing machine, Cotton - P.G. 76/68 677327

30g of frozen biomass was melted, and mixed with 125 ml water, 15 ml enzyme. The mixture was stirred for 5 hours at 40C. To the mixture 48 ml binder and 3 ml fixator were added.

The composition was applied to the fabric by hand printing and drying the printed fabrics with warm air. Each printed fabric was baked in an oven heated to 120C for 2 minutes.

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CLAIMS:

1. A composition comprising at least one cultivated microalgae and at least one mordant.
2. A composition according to claim 1, wherein said at least one cultivated microalgae is selected from red algae, brown algae, green algae, red algae, blue algae and any combinations thereof.
3. A composition according to claims 1 or 2, wherein said at least one cultivated microalgae is a red microalgae.
4. A composition according to claim 3, wherein said cultivated red microalgae is selected from the genus *Porphyridium*, *Rhodella*, *Hematococcus (pluvialis)*, *Dunaliella (bradawil)* and any mixture thereof.
5. A composition according any one of the preceding claims, wherein said cultivated microalgae comprises at least 0.5% of said cultivated microalgae.
6. A composition according to any one of the preceding claims, wherein said at least one mordant is selected from Tannic acid, Tartaric acid, Iron (II) sulfate heptahydrate, Aluminum potassium sulfate dodecahydrate, FeSO_4 and any combinations thereof.
7. A composition according to any one of the preceding claims, wherein said at least one mordant is tannic acid.
8. A composition according to any one of the preceding claims, further comprising at least one of the additive list consisting of: at least one enzyme, at least one dye binder, at least one thickening agent and any combinations thereof.
9. A composition according to claim 8, wherein said at least one enzyme is a cellulose degradation enzyme.
10. A composition according to any one of the preceding claims, for use in a process of coloring a surface.
11. A composition according to claim 10, wherein said surface is paper, fiber, yarn, fabric, cloth, garment, silk, wool, cotton, leather, polymeric material and any combination thereof.
12. A composition according to any one of claims 1 to 9, for use in coloring at least one of a fiber, a fabric.
13. A composition according to claim 12, wherein said fiber is selected from a natural fiber, a synthetic fiber or any combination thereof.

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14. A composition according to claim 12, wherein said fabric is a woven or nonwoven fabric.
15. A composition according to claims 10 to 13, wherein said coloring is dyeing.
16. A composition according to claims 10 to 13, wherein said coloring is printing.
17. A process for the preparation of a composition according to any one of the preceding claims, comprising the steps of: mixing at least one cultivated microalgae with at least one mordant and optionally at least one of at least one enzyme, at least one wetting agent, at least one dye binder, at least one thickening agent and any combinations thereof.
18. A fiber comprising a composition according to any one of claims 1 to 17.
19. A fabric comprising a composition according to any one of claims 1 to 17.
20. A process of coloring a fabric comprising the steps of providing a composition of the invention, mixing said composition with a fabric.
21. A process of claim 20, wherein said mixture of composition and fabric are heated to a temperature in the range of 30 - 100C.
22. A process of claim 20 or 21, wherein said fabric is pretreated.



Figure 1

INTERNATIONAL SEARCH REPORT

International application No PCT/IL2018/050320

A. CLASSIFICATION OF SUBJECT MATTER
 INV. D21H17/00 D21H17/02 D21H17/65 D06P1/34 D21H21/14
 D21H21/28 C12R1/89

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)
 D21H D06P C12R D06M

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 , WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X A	KR 101 413 874 BI (RESTORE LABS CO LTD [KR]) 1 July 2014 (2014-07-01) paragraphs [0013] , [0021] , [0043] - [0048] ; claims -----	1, 6, 10-17 2-5 , 7-9
X A	CN 103 045 478 B (YANTAI INST COASTAL ZONE RES CAS) 8 February 2017 (2017-02-08) examples -----	1, 6, 10-17 2-5 , 7-9
A	US 5 643 585 A (ARAD SHOSHANA [IL] ET AL) 1 July 1997 (1997-07-01) cited in the application the whole document -----	18-22

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents :

<p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p>
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Date of the actual completion of the international search 8 June 2018	Date of mailing of the international search report 15/06/2018
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Koegl er-Hoffmann , S
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No PCT/IL2018/050320
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