

DESCRIPTION

Title of the Invention: ALGACULTURE SYSTEM AND ALGACULTURE METHOD
USING SAME

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Technical Field

[0001] The present invention relates to an algaculture system and algaculture method using same.

10 Background Art

[0002] In order to solve the issues of global warming, the importance of aiming for “carbon neutrality”, that is, reducing greenhouse gas emissions to zero, has been raised both domestically and internationally. Possible strategies to achieve carbon neutrality can be roughly divided into two categories: (i) technology development and behavior
15 change to reduce anthropogenic CO₂ emissions; and (ii) development of negative emission technologies to collect and absorb CO₂ emitted into the environment.

[0003] However, compared with the strategy (i), the strategy (ii) of technology development has not progressed sufficiently, and radical research and development are
20 needed.

[0004] Meanwhile, natural processes (CO₂ fixation) carried out by macroalgae for low concentrations of CO₂ present in the atmosphere are attracting attention. It is known that as shown in Table 1, algal (3G) biomass, to which macroalgae and microalgae
25 belong, has higher productivity per year than starch-carbohydrate (1G) and woody (2G) biomass, and macroalgae in particular have significantly higher CO₂ absorption · fixation ratios and relatively simpler biomass energy production processes and production conditions compared with other biomass (Non-Patent Document 1).

30 [0005]
[Table 1]

	Starch-carbohydrate (1G)	Woody (2G)	Algal (3G)	
Raw materials	Agricultural products (Corn, etc.)	Forest (Cedar, etc.)	Microalgae (Spirulina, etc.)	Macroalgae (<i>Ecklonia cava</i> ssp. <i>kurome</i> ^{*1})/(<i>Sargassum muticum</i>)
Biomass productivity (t/ha/year)	11	9	10~20	30/210
CO ₂ absorption and fixation amount per unit area (kg-CO ₂ /m ² /year)	1.6	0.84	1.5~2.9	3.3/8.8
CO ₂ fixation ratio	2.3	1	7.6	13/240
Production process of biomass energy	Simple	Complex (Lignin removal)	Simple	Simple
Problems	Competition with food	Land is needed	Land is needed, Risk of contamination, High cost	Expansion of seaweed beds
Production conditions	Sunlight, CO ₂ , freshwater, land, fertilizer, pesticides	Sunlight, CO ₂ , freshwater, land, fertilizer, pesticides	Sunlight, CO ₂ , freshwater/brackish water, terrestrial	Sunlight, CO ₂ , seawater

^{*1} A subspecies of *Ecklonia cava*

[0006] Japan is an island nation surrounded by the sea and has an exclusive economic zone that is approximately 12 times the size of the country (6th largest in the world).
5 For this reason, there are many algal beds suitable for cultivating macroalgae.

[0007] However, in order to achieve the above-mentioned carbon neutrality, it is important to expand algal beds, and it is desired to also improve the production

efficiency of macroalgae in each algal bed. In addition, the maturation period of macroalgae is limited to a part of the year. It has to be said that this is far from achieving stable carbon neutrality throughout the year.

5 Related Art Document

Non-Patent Document

[0008] Non-Patent Document 1: Keiei Sensa, "Industrial Applications of Microalgae", Toray Corporate Business Research, Inc., December 2021, p. 5-9

10 Summary of Invention

Problem to be Solved by the Invention

[0009] The present invention is to solve the above-described issues, and it is an object thereof to provide an algae cultivation system and a method for cultivating algae using the same, which are capable of cultivating various species of algae such as macroalgae
15 more efficiently and being adapted to various production scales.

Means for Solving the Problem

[0010] The present invention provides an algae cultivation system, comprising:
an algae cultivation means to which algae are secured and that is immersed in
20 water; and
an epiphytic substrate that is disposed below the algae cultivation means with a space between the epiphytic substrate and the algae cultivation means,
wherein a shortest distance between the algae cultivation means and the epiphytic substrate is 3.0 m to 14.5 m.

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[0011] In one embodiment, the water is natural seawater or artificial seawater.

[0012] In a further embodiment, the algae are at least one species of macroalgae selected from the group consisting of brown algae of the family *Sargassaceae* and brown
30 algae of the family *Laminariaceae*.

[0013] In a further embodiment, the algae cultivation means is secured in a state of being suspended in the seawater.

[0014] The present invention also provides a method for cultivating algae, comprising:
5 securing algae to the algae cultivation means in the algae cultivation system in water;

 conjugatively cultivating the algae on the epiphytic substrate in the algae cultivation system, by means of zoospores or fertilized eggs released from the algae; and
 collecting the algae from both the algae cultivation means and the epiphytic
10 substrate.

[0015] In one embodiment, the water is natural seawater or artificial seawater.

[0016] In one embodiment, the algae are at least one species of macroalgae selected
15 from the group consisting of *Sargassum horneri*, *Sargassum muticum*, *Sargassum micracanthum*, *Sargassum thunbergii*, *Sargassum siliquastrum*, *Sargassum piluliferum*, *Sargassum autumnale*, *Sargassum macrocarpum*, *Sargassum ringgoldianum ssp. coreanum*, *Sargassum yendoii*, *Sargassum patens*, *Saccharina japonica*, *Undaria pinnatifida*, *Eckloniopsis radicata*, *Eisenia bicyclis*, *Ecklonia cava ssp. kurome*, *Ecklonia cava*, and *Eisenia nipponica*.
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[0017] In a further embodiment, the algae are continuously cultivated throughout the year while changing the species thereof according to a maturation period thereof.

25 [0018] The present invention also provides a method for producing a raw material for bioethanol, comprising:

 securing algae to the algae cultivation means in the algae cultivation system in water;

 conjugatively cultivating the algae on the epiphytic substrate in the algae
30 cultivation system, by means of zoospores or fertilized eggs released from the algae; and
 a step of collecting the algae as the raw material for bioethanol from both the algae

cultivation means and the epiphytic substrate.

[0019] In one embodiment, the water is natural seawater or artificial seawater.

5 [0020] In one embodiment, the algae are at least one species of macroalgae selected from the group consisting of *Sargassum horneri*, *Sargassum muticum*, *Sargassum micracanthum*, *Sargassum thunbergii*, *Sargassum siliquastrum*, *Sargassum piluliferum*, *Sargassum autumnale*, *Sargassum macrocarpum*, *Sargassum ringgoldianum ssp. coreanum*, *Sargassum yendoii*, *Sargassum patens*, *Saccharina japonica*, *Undaria pinnatifida*, *Eckloniopsis radicata*, *Eisenia bicyclis*, *Ecklonia cava ssp. kurome*, *Ecklonia cava*, and *Eisenia nipponica*.

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[0021] In a further embodiment, the algae are continuously cultivated throughout the year while changing the species thereof according to a maturation period thereof.

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Effects of the Invention

[0022] According to the present invention, algae such as macroalgae can be efficiently cultivated by increasing the productivity per unit plot of algal bed. Also, no special machinery or the like is required for this cultivation. In addition, it is possible to

20 cultivate algae almost continuously throughout the year, and the resulting algae can be provided more stably as a raw material for, for example, autumn spike ethanol.

Brief Description of Drawings

[0023] FIG. 1 is a schematic view showing an example of an algae cultivation system

25 of the present invention.

FIG. 2 is a schematic view showing another example of the algae cultivation system of the present invention.

FIG. 3 is a schematic view showing another example of the algae cultivation system of the present invention.

30 FIG. 4 is a schematic view showing another example of the algae cultivation system of the present invention.

FIG. 5 is a diagram showing a relationship between the species of macroalgae that can be cultivated using the algae cultivation system of the present invention and their maturation period (the time of maximum present abundance) in Japan.

5 Description of Embodiments

[0024] Algae Cultivation System

The algae cultivation system of the present invention will be described with reference to the accompanying drawings. The constituent elements denoted by the same reference numerals in all the following drawings are the same as those shown in
10 the other drawings.

[0025] FIG. 1 is a schematic view showing an example of an algae cultivation system of the present invention. An algae cultivation system (hereafter also simply referred to as a “system”) 100 includes an algae cultivation means 110 and an epiphytic substrate
15 120.

[0026] The algae cultivation means 110 to which algae are secured in advance is immersed in water. The algae cultivation means 110 is, for example, in the form of a net, rope, or wire, and is made of materials such as synthetic fibers (e.g., nylon or
20 polyester, or a combination thereof), natural fibers (e.g., hemp, cotton, silk, or straw, or a combination thereof), metals (e.g., stainless steel, tungsten, aluminum, or copper, or a combination thereof), or the like. Preferred examples of the algae cultivation means 110 include a fishing net. If the algae cultivation means 110 is a fishing net, none of the thickness (*gou*), the mesh size (*sunme*), the number of meshes in the vertical
25 direction (*kake*), and the size in the horizontal direction (*ken*) of the fishing net are limited, and the number and the size of fishing nets may be selected as appropriate by those skilled in the art.

[0027] Algae 106 to be cultivated are secured to the algae cultivation means 110. The
30 algae 106 that are secured may be of a single species or two or more species in a combination. For example, the algae 106 are secured by attaching them beforehand to

a thread known to those skilled in the art (e.g., Cremona (vinylon) thread) and winding the thread around the algae cultivation means 110.

[0028] The algae 106 may be any algae that can grow in freshwater, brackish water, or seawater. The species of algae 106 to be cultivated may be selected according to water 142 under the cultivation environment. In the present invention, it is preferred that the algae 106 be algae that can grow under seawater, because the marine environment can be used for mass cultivation. It is also preferable that the algae 106 be macroalgae for the reason that they have a high CO₂ fixation ratio as described above.

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[0029] If the environment (i.e., surrounding water) in which the algae 106 grow is seawater, the seawater may be either natural seawater or artificial seawater. For example, if the environment in which the algae 106 grow is natural seawater, the algae cultivation system of the present invention is disposed in the sea. Alternatively, if the environment in which the algae 106 grow is artificial seawater, the algae cultivation system of the present invention does not have to be disposed in the sea. For example, the system can be used for land-based cultivation in a factory or other land-based environment.

[0030] There is no particular limitation on the algae 106 belonging to macroalgae, but examples thereof include those belonging to brown algae of the family Sargassaceae and brown algae of the family Laminariaceae.

[0031] There is no particular limitation on the algae 106 belonging to macroalgae, but examples thereof include *Sargassum horneri*, *Sargassum muticum*, *Sargassum micracanthum*, *Sargassum thunbergii*, *Sargassum siliquastrum*, *Sargassum piluliferum*, *Sargassum autumnale*, *Sargassum macrocarpum*, *Sargassum ringgoldianum ssp. coreanum*, *Sargassum yendoii*, *Sargassum patens*, *Saccharina japonica*, *Undaria pinnatifida*, *Eckloniopsis radicata*, *Eisenia bicyclis*, *Ecklonia cava*, *Ecklonia cava ssp. kurome* (a subspecies of *Ecklonia cava*), *Eisenia nipponica*, *Heribaudiella fluviatilis*, *Sargassum thunbergii*, *Batrachospermum gelatinosum*,

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Prasiola japonica, and combinations thereof.

[0032] As the algae 106 belonging to macroalgae that can be secured to the algae cultivation means 110, those listed above may be used alone or in a combination of two
5 or more according to their maturation period.

[0033] For example, from January to May in Japan, *Sargassum horneri* and *Sargassum muticum* may be used alone or in a combination as the algae 106.

10 [0034] From March to May in Japan, *Sargassum horneri*, *Sargassum muticum*, and *Sargassum micracanthum* may be used alone or in a combination as the algae 106.

[0035] From June to August in Japan, *Sargassum thunbergii*, *Sargassum siliquastrum*, *Sargassum piluliferum*, *Sargassum yendoi*, *Sargassum patens*,
15 *Sargassum macrocarpum*, *Sargassum ringgoldianum ssp. coreanum*, and *Eckloniopsis radicata* may be used alone or in a combination as the algae 106.

[0036] From July to September in Japan, *Sargassum autumnale*, *Sargassum macrocarpum*, *Sargassum ringgoldianum ssp. coreanum*, *Eckloniopsis radicata*,
20 *Eisenia bicyclis*, *Ecklonia cava ssp. kurome*, *Ecklonia cava*, and *Eisenia nipponica* may be used alone or in a combination as the algae 106.

[0037] From September to November in Japan, *Sargassum autumnale*, *Sargassum macrocarpum*, *Sargassum ringgoldianum ssp. coreanum*, *Eckloniopsis radicata*,
25 *Eisenia bicyclis*, *Ecklonia cava ssp. kurome*, *Ecklonia cava*, and *Eisenia nipponica* may be used alone or in a combination as the algae 106.

[0038] From September to December in Japan, *Sargassum autumnale* may be used as the algae 106.

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[0039] In FIG. 1, ends 112 and 114 of the algae cultivation means 110 are secured to a

float 130 floating on a water surface 140, and the portion between the ends 112 and 114 is secured to the float 130 at any intervals and disposed in a suspended state in the water 142, for example, so that it is warped in a gentle U-shape as a whole. The float 130 may be secured using, for example, wires 132 and anchors 134 to prevent the float
5 130 from moving in any direction on the water surface 140.

[0040] The algae cultivation means 110 may also have a cylindrical (e.g., circular cylindrical or angular cylindrical) net 145 (e.g., fishing net) that extends in the vertical direction in water and is disposed around the outer periphery of the algae cultivation
10 means 110. The cylindrical net 145 can protect the algae 106 against water movement such as tidal currents, and can set up a calm cultivation environment within the net 145. A lower edge 146 of the net 145 may not be in contact with a water bottom 147 so that the net 145 floats in the water 142, or may be in contact with the water bottom 147.

[0041] The epiphytic substrate 120 is a man-made material that is disposed on a water bottom surface 150 in the water 142, and is made of a relatively thin material having a predetermined flat surface, such as a fabric, sheet, or plate having a predetermined thickness. Specifically, the epiphytic substrate 120 may be a woven, knitted, or non-
15 woven fabric made of: synthetic fibers made of nylon or polyester; natural fibers such as
20 hemp, cotton, silk, or straw; or metal filaments made of stainless steel, tungsten, aluminum, or copper; or a combination thereof, may be a sheet made of thermoplastic resins (e.g., nylon or polyester, or a combination thereof), elastomer, or rubber, or a combination thereof; or a plate (e.g., encompassing a hollow plate) made of thermoplastic resins (e.g., nylon or polyester, or a combination thereof), elastomer, rubber, stainless
25 steel, tungsten, aluminum, copper, wood, concrete, mortar, or glass, or a combination thereof.

[0042] There is no particular limitation on the thickness of the epiphytic substrate 120, but it is preferably 30 cm to 100 cm, and more preferably 50 cm to 70 cm. If the
30 thickness of the epiphytic substrate 120 is less than 30 cm, disadvantages may arise such as the epiphytic substrate 120 not being strong enough or being easily swept away

by water currents such as tidal currents. If the thickness of the epiphytic substrate 120 is more than 100 cm, the epiphytic substrate 120 may become heavier as a whole, which may increase the difficulty in placing it on the water bottom surface 150 and/or in retrieving and collecting it from the water 142.

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[0043] In the present invention, as the epiphytic substrate 120, a plurality of the above-mentioned fabrics, sheets, and/or plates that have been cut as appropriate may be combined and disposed on the water bottom surface 150.

10 [0044] There is no particular limitation on the size of the epiphytic substrate 120, but it is designed to be larger than the above-described algae cultivation means 110 suspended thereabove, or equal to or larger than the horizontal cross section of the above-described cylindrical net 145 that extends in the vertical direction.

15 [0045] In the cultivation system 100 of the present invention, algae 106' are secured (attached) to the epiphytic substrate 120 via temporary roots (attachments). The algae 106' are not artificially arranged, but are self-grown on the epiphytic substrate 120 from zoospores or fertilized eggs 160 released from the algae 106 suspended from the algae cultivation means 110. The algae 106' may be of the same species as the algae 106
20 suspended from the algae cultivation means 110, but may be those self-grown from other zoospores or fertilized eggs that have drifted in the water 142 and been provided from the outside.

[0046] FIG. 2 is a schematic view showing another example of the algae cultivation
25 system of the present invention.

[0047] In an algae cultivation system 200 shown in FIG. 2, a plurality of folds 122' are provided in an epiphytic substrate 120'. If the epiphytic substrate 120' has such a form, those skilled in the art can use the folds 122' of the epiphytic substrate 110 to pull up the
30 epiphytic substrate to the water surface 140, thereby easily collecting the algae 106' self-grown on the epiphytic substrate 110.

[0048] Referring again to FIG. 1, the algae 106 secured to the algae cultivation means 110 release germ cells or fertilized eggs 160 into the water 142 as they grow.

5 [0049] For example, if the algae 106 are brown algae of the family Laminariaceae, their sporophytes release zoospores, which are germ cells, and gametophytes. If the algae 106 are brown algae of the family Sargassaceae, they release fertilized eggs when matured.

10 [0050] In the present invention, such zoospores (germ cells) and fertilized eggs released from the algae 106 can be caused to reach the epiphytic substrate 110. Subsequently, by means of the germ cells or fertilized eggs 160 that have reached the epiphytic substrate 110, the algae 106' are grown "conjugatively" with the above-described algae 106, as a result of which the algae 106 and 106' can be cultivated on both the algae
15 cultivation means 110 and the epiphytic substrate 120 that is disposed therebelow.

[0051] There is no particular limitation on the shortest distance between the algae cultivation means 110 and the epiphytic substrate 120, because it varies depending on the species of seaweed to be cultivated, the degree of water movement such as tidal
20 currents at the cultivation site, and the like, but it is 3.0 m to 14.5 m, and preferably 4.0 m to 10 m. If the shortest distance between the algae cultivation means 110 and the epiphytic substrate 120 is less than 3.0 m, the influence of tidal currents is likely to be exerted, and thus, for example, zoospores (germ cells) or fertilized eggs 160 released from the algae 106 may be excessively swept away by water currents such as tidal currents
25 before they reach the epiphytic substrate 120 that is disposed therebelow, as a result of which it may be difficult to conjugatively cultivate the algae 106' on the epiphytic substrate 120. If the shortest distance between the algae cultivation means 110 and the epiphytic substrate 120 is more than 14.5 m, the resulting depth may make it difficult for the epiphytic substrate 120 to receive the amount of sunlight necessary for
30 the cultivation of the algae 106'.

[0052] FIG. 3 is a schematic view showing another example of the algae cultivation system of the present invention.

[0053] In an algae cultivation system 300 shown in FIG. 3, the algae cultivation means
5 110 is secured to a plurality of buoys 330 instead of the float 130 shown in FIG. 1. If the algae cultivation means 110 is secured not to a float but to such buoys 330, the resulting algae cultivation system 300 can have a simpler or more compact configuration.

[0054] In the algae cultivation system 300 shown in FIG. 3 as well, germ cells or
10 fertilized eggs 160 released from the algae 106 suspended from the algae cultivation means 110 move to the epiphytic substrate 120, thereby making it possible to cultivate the algae 106' on the epiphytic substrate 120 in conjugation with the algae 106.

[0055] FIG. 4 is a schematic view showing another example of the algae cultivation
15 system of the present invention.

[0056] In an algae cultivation system 400 shown in FIG. 4, the algae cultivation means
110 is secured to a plurality of buoys 330 instead of the float 130 shown in FIG. 1. Furthermore, ends 412 and 414 of the algae cultivation means 110 are secured to a
20 frame 430 floating on the water surface 140, and the frame 430 is secured via a plurality of rigid posts 432 to the anchors 134 that are arranged in the water 142.

[0057] With this configuration, the movement of the frame 430 on the water surface
140 is limited. This reduces the possibility of the algae cultivation system 400 being
25 swept away to other locations due to, for example, strong winds.

[0058] In the algae cultivation system 4 shown in FIG. 4 as well, germ cells or fertilized
eggs 160 released from the algae 106 suspended from the algae cultivation means 110
move to the epiphytic substrate 120, thereby making it possible to cultivate the algae
30 106' on the epiphytic substrate 120 in conjugation with the algae 106.

[0059] The algae cultivation system of the present invention can cultivate a larger quantity of algae in a limited area of the plot as seen from the water surface. In particular, the algae 106' on the epiphytic substrate 120 can grow in conjugation with the algae 106 suspended from the algae cultivation means 110, and both the algae 106
5 and 106' can be easily collected.

[0060] Method for Cultivating Algae

Next, a method for cultivating algae of the present invention will be described.

10 [0061] In the method of the present invention, algae are first secured to an algae cultivation means in the above-described algae cultivation system in water (e.g., freshwater, brackish water, or seawater).

[0062] The algae secured to the algae cultivation means are preferably germlings that
15 have been pre-grown in a manner known to those skilled in the art. In the present invention, it is preferable to conduct cultivation at an appropriately increased cultivation density in order to avoid the algae becoming food for fish and shellfish, which can cause rocky-shore denudation. Algae such as macroalgae secrete bromophenols such as 2,4-dibromophenol and 2,4,6-tribromophenol outside the algae when the cultivation density
20 is controlled. Such bromophenols function as chemical defense substances (repellents) against fish, shellfish, and other marine algae-eating animals, resulting in the avoidance of the above-mentioned rocky-shore denudation.

[0063] Subsequently, by means of germ cells or fertilized eggs released from the algae,
25 the algae are conjugatively cultivated on the epiphytic substrate in the algae cultivation system.

[0064] In the case of cultivating algae (germlings) secured to the algae cultivation means in the ocean, the release of germ cells or fertilized eggs from the algae is caused
30 to occur by taking out them to the sea (placing them in the sea) and waiting for the algae themselves to spontaneously initiate the release after a predetermined period of time.

This predetermined period of time is not necessarily limited because it varies depending on the species of algae to be cultivated, the time of year, and the cultivation environment (e.g., water temperature, cultivation density), but it is, for example, preferably 150 days to 360 days after the algae are secured to the algae cultivation means (or taken out to the sea). Furthermore, the period of time until the released germ cells or fertilized eggs are accepted by the epiphytic substrate and conjugative cultivation occurs is not necessarily limited because it also varies depending on the species of algae to be cultivated, the time of year, and the cultivation environment (e.g., water temperature, cultivation density), but it is, for example, preferably 30 days to 60 days after the germ cells or fertilized eggs are released from the algae.

[0065] Subsequently, algae are collected from both the algae cultivation means and the epiphytic substrate.

[0066] That is to say, in the method of the present invention, target algae can be collected not only from the algae cultivation means but also from the epiphytic substrate, and thus the amount of resulting algae collected is definitely higher than when the algae cultivation means is used alone.

[0067] In this manner, algae cultivation can be more efficiently performed.

[0068] The algae cultivated by the above-described method can be used as, for example, a raw material for bioethanol, which can be obtained using the method described below. If such algae are used as a raw material for bioethanol, it is desirable to be able to constantly obtain a certain amount of the raw material regardless of the season. In the present invention, different species of algae to be cultivated are preferably selected according to the season in order to enable the constant availability of such a raw material for bioethanol.

[0069] FIG. 5 is a diagram showing a relationship between the species of macroalgae that can be cultivated using the algae cultivation system of the present invention and

their maturation period (the time of maximum present abundance) in Tohoku and regions of south thereof in Japan.

[0070] The macroalgae that can be cultivated in the seas around Japan have different
5 maturation periods depending on their species, and as a whole, any of the algae can be obtained between January and December.

[0071] As shown in FIG. 5, for example, the months from January to May in Japan correspond to the maturation periods of *Sargassum horneri* and *Sargassum muticum*.

10 The months from March to May in Japan correspond to the maturation periods of *Sargassum horneri*, *Sargassum muticum*, and *Sargassum micracanthum*. The months from June to August in Japan correspond to the maturation periods of *Sargassum thunbergii*, *Sargassum siliquastrum*, *Sargassum piluliferum*, *Sargassum yendoii*, and *Sargassum patens*. The months from July to September in Japan
15 correspond to the maturation periods of *Sargassum macrocarpum*, *Sargassum ringgoldianum ssp. coreanum*, and *Eckloniopsis radicata*. The months from September to November in Japan correspond to the maturation periods of *Sargassum autumnale*, *Eisenia bicyclis*, *Ecklonia cava ssp. kurome*, *Ecklonia cava*, and *Eisenia nipponica*. The months from September to December in Japan correspond to the
20 maturation periods of *Sargassum autumnale*.

[0072] Therefore, it is possible to constantly produce the algae serving as a raw material for bioethanol throughout the year from January to December, by appropriately selecting the algae to be cultivated.

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[0073] The algae (e.g., macroalgae) that can be obtained by the above-described process are rich in sugars such as cellulose, hemicellulose, alginic acid, mannitol, and laminaran. Ethanol (i.e., bioethanol) can be produced from the sugars that are thus contained in the algae.

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[0074] Method for Producing Bioethanol

Methods for producing bioethanol using cultivated algae as a raw material for bioethanol are known, and the production can be performed, for example, using arming yeast with predetermined enzymes displayed on the cell surface layer.

5 [0075] Examples of the enzymes displayed on the cell surface of arming yeast include endoglucanase, β -glucosidase, cellobiohydrolase, xylanase, β -xylosidase, xylose isomerase, exo-/endo-alginate lyase, and laminaran degrading enzyme (Gly5M).

[0076] Specific examples of the arming yeast include cellulolytic arming yeast (Fujita
10 et al., Appl. Environ. Microbiol., 68, 5136-5141 (2002); Bae et al., Appl. Environ. Microbiol., 81, 59-66 (2015)), hemicellulolytic arming yeast (Fujita et al., J. Mol. Catalys. 17, 189-195 (2002); Ota, et al., Biotechnol. Progress, 29, 346-351 (2013); Sasaki, et al., Biotechnol. Progress, 33, 1068-1076 (2017)), alginate/mannitol degrading arming yeast (Takagi et al., Appl. Microbiol., Biotechnol., 101, 6627-6636 (2017), and laminaran
15 degrading arming yeast (Motone et al., J. Biotechnol., 231, 129-135 (2016)).

[0077] Bioethanol obtained by such a method can be used, for example, as an alternative automobile fuel to gasoline. In addition, since the bioethanol is produced through algae cultivation as described above, there is no issue of competition with food
20 production.

[0078] As mentioned above, the present invention makes it possible to constantly produce algae, which also enables a stable supply of the resulting bioethanol to the market. Furthermore, since macroalgae with high CO₂ absorption/fixation ratios can
25 be cultivated, the present invention is also useful as a negative emission technology to collect and absorb CO₂ emitted into the environment. As a result, the present invention can also contribute to the realization of carbon neutrality.

Examples

30 [0079] Hereinafter, the present invention will be described in detail by way of examples. Note that the present invention is not limited to these examples.

[0080] Example 1: Fabrication of Algae Cultivation System (R1)

An algae cultivation system (R1) shown in FIG. 1 was fabricated as follows.

5 [0081] Specifically, brown algae of the family *Laminariaceae*, which were macroalgae produced in Example 2 below, were seeded in December 2021 as algae 106 onto Cremona threads (36 threads, left-hand 3-strand twist, 5 m long), and the threads were wound around a 12 mm diameter polyrope with a left twist to fabricate an algae cultivation means 110, and ten of which were prepared and suspended from the float
10 130. The prepared algae cultivation means 110 were surrounded by a fishing net (the net 145) and secured by means of the anchors 134 at a location (cultivation site) approximately 100 m offshore from the port of Kohama, Toba City, Mie Prefecture, Japan. The epiphytic substrate 120 (with a thickness of 50 cm) made of fabric constituted by a combination of synthetic and natural fibers was laid on the seafloor at
15 the cultivation site, and the ends of the attachment substrate 120 were secured to a seabed surface 150 in order to prevent the substrate from being moved by tidal currents. The shortest distance between the algae cultivation means 110 and the epiphytic substrate 120 at this cultivation site was approximately 8 m. The titled algae cultivation system (R1) was thus fabricated.

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[0082] Example 2: Cultivation of Macroalgae

The algae cultivation system (R1) fabricated in Example 1 was used to cultivate brown algae of the family *Laminariaceae* as follows.

25 [0083] First, free-living gametophytes subcultured in the laboratory were pulverized with a mixer and then sprayed onto Cremona threads. The Cremona threads were submerged in a container filled with seawater and cultured at 18°C to 20°C. This culturing process was continued until the germlings (sporophytes) reached a size at which the germlings could be seen by the naked eye. The Cremona threads were then
30 wound around a polyrope as described above and taken out to the sea. After about six months of cultivation near the sea surface, it was confirmed that *Ecklonia cava ssp.*

kurome (a subspecies of *Ecklonia cava*) had grown significantly as brown algae of the family *Laminariaceae* seeded onto the algae cultivation means 110 and that similar brown algae of the family *Laminariaceae* were growing conjugatively on the epiphytic substrate 120 that was disposed below the algae cultivation means 110. Subsequently, approximately 20 kg (in a dry mass) of *Ecklonia cava ssp. kurome* was obtained from the algae cultivation means 110 and the epiphytic substrate 120.

[0084] Reference Examples 1 to 4: Ethanol Production from Macroalgae

1 kg of *Ecklonia cava ssp. kurome* obtained in Example 2 was cut into 5 to 50 mm pieces and added to a normal yeast culture solution. Then, each arming yeast with predetermined enzymes displayed on the cell surface layer (Murai et. al., Appl. Environ. Microbiol., 63, 1362-1366 (1997)) as shown in Table 2 was added in equal proportions (OD 20 to 30) to this culture solution and cultured anaerobically at 30°C for 24 to 48 hours to obtain 150 g to 200 g of ethanol from each culture solution.

[0085]

[Table 2]

	Arming yeast used
Reference Example 1	Cellulolytic arming yeast (Fujita et al., Appl. Environ. Microbiol., 68, 5136-5141 (2002); Bae et al., Appl. Environ. Microbiol., 81, 59-66 (2015))
Reference Example 2	Hemicellulose degrading arming yeast (Fujita et al., J. Mol. Catalys. 17, 189-195 (2002); Ota, et al., Biotechnol. Progress, 29, 346-351 (2013); Sasaki, et al., Biotechnol. Progress, 33, 1068-1076 (2017))
Reference Example 3	Alginate and mannitol degrading arming yeast (Takagi et al., Appl. Microbiol., Biotechnol., 101, 6627-6636 (2017))
Reference Example 4	Laminaran degrading arming yeast (Motone et al., J. Biotechnol., 231, 129-135 (2016))

[0086] Macroalgae such as *Ecklonia cava ssp. kurome* (a subspecies of *Ecklonia cava*) are rich in sugars such as cellulose, hemicellulose, alginic acid, mannitol, and laminaran, and the *Ecklonia cava ssp. Kurome* obtained from the cultivation in Example 2 was also

rich in such sugars. Therefore, desired ethanol was efficiently produced when the culture process was performed using each arming yeast as shown in Table 2.

Industrial Applicability

- 5 [0087] The present invention is useful, for example, for obtaining a raw material for bioethanol production.

Reference Signs List

[0088]

10	100, 200, 300, 400	Algae cultivation system
	106, 106'	Algae
	110	Algae cultivation means
	112, 114, 412, 414	End
	120, 120'	Epiphytic substrate
15	122'	Fold
	130	Float
	132	Wire
	134	Anchor
	140	Water surface
20	142	Water
	145	Net
	146	Lower edge
	147	Water bottom
	150	Water bottom surface
25	160	Zoospore or fertilized egg
	330	Buoy
	430	Frame
	432	Post

CLAIMS

1. An algae cultivation system, comprising:
an algae cultivation means to which algae are secured and that is immersed in
5 water; and
an epiphytic substrate that is disposed below the algae cultivation means with
a space between the epiphytic substrate and the algae cultivation means,
wherein a shortest distance between the algae cultivation means and the
epiphytic substrate is 3.0 m to 14.5 m.
10
2. The algae cultivation system according to claim 1, wherein the water is natural
seawater or artificial seawater.
3. The algae cultivation system according to claim 2, wherein the algae are at least
15 one species of macroalgae selected from the group consisting of brown algae of the family
Sargassaceae and brown algae of the family *Laminariaceae*.
4. The algae cultivation system according to claim 2, wherein the algae cultivation
means is secured in a state of being suspended in the seawater.
20
5. A method for cultivating algae, comprising:
securing algae to the algae cultivation means in the algae cultivation system
according to any one of claims 1 to 4 in water;
conjugatively cultivating the algae on the epiphytic substrate in the algae
25 cultivation system, by means of zoospores or fertilized eggs released from the algae; and
collecting the algae from both the algae cultivation means and the epiphytic
substrate.
6. The method according to claim 5, wherein the water is natural seawater or
30 artificial seawater.

7. The method according to claim 6, wherein the algae are at least one species of macroalgae selected from the group consisting of *Sargassum horneri*, *Sargassum muticum*, *Sargassum micracanthum*, *Sargassum thunbergii*, *Sargassum siliquastrum*, *Sargassum piluliferum*, *Sargassum autumnale*, *Sargassum macrocarpum*, *Sargassum ringgoldianum ssp. coreanum*, *Sargassum yendoi*, *Sargassum patens*, *Saccharina japonica*, *Undaria pinnatifida*, *Eckloniopsis radicata*, *Eisenia bicyclis*, *Ecklonia cava ssp. kurome*, *Ecklonia cava*, and *Eisenia nipponica*.
8. The method according to claim 7, wherein the algae are continuously cultivated throughout the year while changing the species thereof according to a maturation period thereof.
9. A method for producing a raw material for bioethanol, comprising:
securing algae to the algae cultivation means in the algae cultivation system according to any one of claims 1 to 4 in water;
conjugatively cultivating the algae on the epiphytic substrate in the algae cultivation system, by means of zoospores or fertilized eggs released from the algae; and
a step of collecting the algae as the raw material for bioethanol from both the algae cultivation means and the epiphytic substrate.
10. The method according to claim 9, wherein the water is natural seawater or artificial seawater.
11. The method according to claim 10, wherein the algae are at least one species of macroalgae selected from the group consisting of *Sargassum horneri*, *Sargassum muticum*, *Sargassum micracanthum*, *Sargassum thunbergii*, *Sargassum siliquastrum*, *Sargassum piluliferum*, *Sargassum autumnale*, *Sargassum macrocarpum*, *Sargassum ringgoldianum ssp. coreanum*, *Sargassum yendoi*, *Sargassum patens*, *Saccharina japonica*, *Undaria pinnatifida*, *Eckloniopsis radicata*, *Eisenia bicyclis*, *Ecklonia cava ssp. kurome*, *Ecklonia cava*, and *Eisenia nipponica*.

12. The method according to claim 11, wherein the algae are continuously cultivated throughout the year while changing the species thereof according to a maturation period thereof.