Notice

DESCRIPTION CN120092689A

A cultivation method to increase starch yield from duckweed thallus

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一种提高浮萍叶状体淀粉产量的培养方法

[0001]

Technical Field

技术领域

[n0001]

This invention belongs to the field of duckweed biomass energy technology, and in particular refers to a cultivation method for increasing the starch yield of duckweed thallus.

本发明属于浮萍生物质能源技术领域，尤其是指一种提高浮萍叶状体淀粉产量的培养方法。

[0003]

Background Technology

背景技术

[n0002]

Duckweed has become a starch resource with development potential due to its advantages such as fast growth rate, high nutrient utilization efficiency, and strong adaptability to various environments.

浮萍因其生长速度快、营养利用效率高、在各种环境下都可生长适应能力强等优势，成为具有开发潜力的淀粉资源来源。

Paradoxically, during the growth of duckweed, the utilization rate of its nutrients, i.e., biomass accumulation, is negatively correlated with starch accumulation (i.e., fast growth results in low starch content; high starch content results in relatively slow growth). In other words, it is currently difficult to achieve a balance between energy supply primarily for duckweed growth and starch accumulation.

矛盾的是，在浮萍生长过程中其营养的利用率即生物量积累与淀粉积累呈负相关性(即生长快，淀粉含量低；淀粉含量高，生长相对缓慢)，也就是说在能量供应方面主要用于浮萍生长还是淀粉积累，目前来说很难达到一个平衡。

How to simultaneously increase the biomass and starch accumulation of duckweed to achieve effective output of final starch has always been a major challenge in the duckweed cultivation process and is also the key to the industrialization of duckweed starch.

如何同时提升浮萍的生物量及淀粉积累在最终淀粉产量达到有效输出一直是浮萍培养过程中的一大难题，也是浮萍淀粉实现产业化的关键。

[n0003]

Currently, duckweed cultivation mainly involves several modes, including autotrophic, heterotrophic, and co-culture.

目前，浮萍培养主要涉及自养、异养和联合培养等几种模式。

Generally, under optimal growth conditions, the starch content of duckweed remains at a relatively low level. However, under stress conditions such as nutrient limitation, salinity, strong light, and plant hormone treatment, or after switching to a heterotrophic growth mode, the starch content of duckweed will increase.

一般来说，在最佳生长条件下，浮萍淀粉含量维持在相对较低的水平，但在营养限制、盐度、强光、植物激素处理等胁迫条件下，或在转换为异养生长模式后，浮萍淀粉含量会增加。

Nitrogen (N), phosphorus (P), and sulfur (S) are essential macronutrients for plant growth and development, participating in the formation of cell structures and high-energy molecules (nucleic acids, proteins, chlorophyll, adenosine triphosphate, and phospholipids). Duckweed can survive and even reproduce in the absence of these macronutrients for a long time. Currently, the two most studied starch induction methods are N-restriction (NL) and P-restriction (PL). However, both strategies come at the cost of sacrificing biomass production, leading to the accumulation of starch in duckweed. Therefore, despite the high starch content, starch yield may be significantly affected due to the trade-off between starch induction and plant growth. Therefore, restricting a single element does not significantly increase the final starch yield. While heterotrophic and co-culture methods can effectively increase biomass, their effect on starch yield is not significant. Furthermore, since glucose is

often used as the carbon source during the culture process, the cost of the culture process increases, making it unsuitable for subsequent industrial production. Therefore, seeking low-cost and high-efficiency training methods is particularly important.

氮(N)、磷(P)和硫(S)是植物生长发育所必需的常量营养素，参与细胞结构和高能分子(核酸、蛋白质、叶绿素、三磷酸腺苷和磷脂)的形成。 在长时间缺乏这些常量营养素的情况下，浮萍可以存活甚至繁殖。 目前，研究最多的是N限制(NL)和P限制(PL)两种淀粉诱导方法。 然而，这两种策略以牺牲生物量生产为代价，引发了浮萍中淀粉的积累。 因此，尽管淀粉含量较高，但由于淀粉诱导和植物生长之间的权衡，淀粉产量可能会受到很大影响。 所以关于单一限制某种元素来说，对最终淀粉产量并没有很大的提升。 而异养和联合培养方式能有效的提升生物量但关于淀粉产量的提升效果并不明显，此外由于培养过程中所用的碳源常为葡萄糖使得培养过程中的成本上升，并不适合后续的工业化生产。 所以寻求成本低、效率高的培养方式显得尤为重要。

[n0004]

Since duckweed has a rapid growth period and a starch accumulation period during its growth process, nutrient solution can be added according to the corresponding growth stage to ensure that both biomass can be increased and starch can be accumulated in large quantities.

由于浮萍在生长过程中存在一个快速生长期和淀粉积累期，可根据生长时期去对应的添加营养液，保证既能提高生物量也可大量积累淀粉。

[0007]

Summary of the Invention

发明内容

[n0005]

This addresses the technical problems in existing technologies, such as the inability to achieve a balance between duckweed biomass and starch content, thus failing to achieve a satisfactory final starch yield.

针对现有技术中存在无法使浮萍生物量和淀粉含量达到平衡，使最终淀粉产量达到较好程度等技术问题。

This invention provides a cultivation method to increase starch yield in duckweed thallus, which involves dynamically controlling the concentration of added nutrient solution.

本发明提供一种提高浮萍叶状体淀粉产量的培养方法，该方法是通过动态调控加入营养液的浓度。

During the rapid growth period of duckweed, a higher concentration of nutrient solution can promote rapid growth and accumulate sufficient biomass. After entering the starch accumulation period, reducing the concentration of nutrient solution can induce duckweed to use more energy for starch synthesis, thereby achieving a better starch yield and realizing a dual increase in biomass and starch accumulation. Since it is a complex system regulated by nutrient solution, there is no limitation to certain elements that sacrifice biomass to achieve high starch yield, nor are there problems such as high cost of adding exogenous carbon sources. The method is simple and easy to operate.

在浮萍生长的快速生长期，较高浓度的营养液能够促进浮萍快速增长，积累足够的生物量；进入淀粉积累期后，降低营养液浓度能够诱导浮萍将更多的能量用于淀粉合成，从而达到较好的淀粉产量，实现生物量与淀粉积累的双重提升，由于是营养液调控的复杂体系不存在限制某种元素牺牲生物量以达到高淀粉量，也不存在外源添加碳源成本高等问题，方法简单易操作。

[n0006]

This invention is achieved through the following technical solution:

本发明通过以下技术方案实现：

[n0007]

The first objective of this invention is to provide a cultivation method for increasing starch yield in duckweed thallus, comprising the following steps:

本发明第一个目的是提供一种提高浮萍叶状体淀粉产量的培养方法，包括以下步骤：

[n0008]

(1) The activated duckweed was cultured under light in the first nutrient solution;

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(1)将活化后的浮萍在第一营养液中光照培养；

[n0009]

(2) Rapid growth period: The duckweed obtained in step (1) is introduced into the second nutrient solution for light cultivation; the second nutrient solution is obtained by diluting the first nutrient solution to a concentration of 40-50% by volume ratio, and the concentration is kept stable during the period and the second nutrient solution is added subsequently.

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(2)快速生长期：将步骤(1)所得浮萍接入第二营养液中光照培养；所述第二营养液通过将第一营养液按体积比稀释至浓度为40～50%得到，期间保持浓度稳定后续补充第二营养液；

[n0010]

(3) Starch accumulation period: Reduce the volume concentration of the second nutrient solution in step (2) to 30% to 40% of the concentration of the first nutrient solution, continue to cultivate under light, and then add distilled water.

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(3)淀粉积累期：将步骤(2)中所述第二营养液的体积浓度降低至第一营养液浓度的30%～40%，继续光照培养，后续补充蒸馏水。

[n0011]

In one embodiment of the present invention, in step (1), the duckweed is selected from one or more of the genera *Lemna minor*, *Lemna minor*, *Lemna spp.*, *Lemna spp.*, and *Lemna spp.*.

在本发明的一个实施例中，步骤(1)中，所述浮萍选自多根紫萍属、少根紫萍属、青萍属、芜萍属和扁无根萍属中的一种或多种。

[n0012]

In one embodiment of the present invention, in step (1), the first nutrient solution comprises the following elemental components by weight percentage: calcium 0.2 g/L to 1 g/L, iron 0.001 g/L to 0.0005 g/L, magnesium 0.01 g/L to 0.05 g/L, boron 0.0001 g/L to 0.0005 g/L, zinc 0.00001 g/L to 0.00005 g/L, copper 0.000004 g/L～0.00002 g/L, molybdenum 0.00001 g/L～0.

00005 g/L, sodium 0.000004 g/L～0.00002 g/L, manganese 0.0002 g/L～0.001 g/L, nitrogen 0.06 g/L～0.3 g/L, phosphorus 0.006 g/L～0.03 g/L, and potassium 0.12 g/L～0.6 g/L.

在本发明的一个实施例中，步骤(1)中，按照重量百分比计，所述第一营养液包括以下元素组分：钙0.2g/L～1g/L、铁0.001g/L～0.0005g/L、镁0.01g/L～0.05g/L、硼0.0001g/L～0.0005g/L、锌0.00001g/L～0.00005g/L、铜0.000004g/L～0.00002g/L、钼0.00001g/L～0.00005g/L、钠0.000004g/L～0.00002g/L、锰0.0002g/L～0.001g/L、氮0.06g/L～0.3g/L、磷0.006g/L～0.03g/L和钾0.12g/L～0.6g/L。

[n0013]

In one embodiment of the present invention, the first nutrient solution comprises the following components: Ca(NO3)2・4H3O 12g/L～60g/L, KNO4 20g/L～25g/L, KH5PO6 1g/L～1.5g/L, tartaric acid 0.5g/L～1g/L, FeCl7・6H8O 1g/L～2g/L, EDTA 1.5g/L～2g/L, MgSO9・7H10O 5g/L～10g/L, H11BO12 0.5g/L～1g/L, ZnSO13・7H14O 0.05g/L～0.1g/L, Na₂MoO₄・2H₂O 0.02g/L～0.025g/L, CuSO₄・5H₂O 0.015g/L～0.02g/L, MnCl₂・4H₂O 0.5g/L～1g/L.

在本发明的一个实施例中，所述第一营养液包括以下组分：Ca(NO₃)₂・4H₂O 12g/L～60g/L、KNO₃20 g/L～25g/L、KH₂

/sub>PO₄1 g/L～1.5g/L、酒石酸0.5g/L～1g/L、FeCl₃・6H<sub>2<
/sub>O1g/L～2g/L、EDTA 1.5g/L～2g/L、MgSO₄・7H₂O 5g/L～10g
/L、H₃BO₃0.5 g/L～1g/L、ZnSO₄・7H₂O
0.05g/L～0.1g/L、Na₂MoO₄・2H₂O 0.02g/L～0.025g
/L、CuSO₄・5H₂O 0.015g/L～0.02g/L、MnCl<sub>2<
/sub>・4H₂O 0.5g/L～1g/L。

[n0014]

In one embodiment of the present invention, the pH value of the first nutrient solution is 5 to 5.5;

在本发明的一个实施例中，所述第一营养液的pH值为5～5.5；

[n0015]

And/or, the pH value of the second nutrient solution is 5 to 5.5; the pH value of the second nutrient solution is obtained by adjustment.

和/或，所述第二营养液的pH值为5～5.5；第二营养液的pH值通过调控得到。

[n0016]

In one embodiment of the present invention, in step (2), the volume of duckweed introduced is 1% to 10% of the nutrient solution.

在本发明的一个实施例中，步骤(2)中，所述浮萍的接入体积为营养液的1%～10%。

[n0017]

In one embodiment of the present invention, in step (2), the diluent used for dilution is distilled water.

在本发明的一个实施例中，步骤(2)中，所述稀释采用的稀释剂为蒸馏水。

[n0018]

In one embodiment of the present invention, in step (2), the light cultivation time is 1 to 3 days.

在本发明的一个实施例中，步骤(2)中，所述光照培养的时间为1～3天。

[n0019]

In one embodiment of the present invention, in step (3), the light cultivation time is 4 to 7 days.

在本发明的一个实施例中，步骤(3)中，所述光照培养的时间为4～7天。

[n0020]

In one embodiment of the present invention, the light intensity of the light culture is 85 μmol /m²/s to 100 μmol/m²/s.

在本发明的一个实施例中，所述光照培养的光照强度为85μmol/m²/s～100μmol /m²/s。

[n0021]

A second objective of this invention is to provide a duckweed thallus obtained by the aforementioned cultivation method, wherein the biomass of the duckweed thallus is ≥140. 57±0.85 g/m² and the starch yield is ≥58.87±0.47 g/m².

本发明第二个目的是提供所述培养方法得到的浮萍叶状体，所述浮萍叶状体的生物量≥140.57±0. 85g/m²；淀粉产量≥58.87±0.47g/m²。

[n0022]

The technical solution of the present invention has the following advantages over the prior art:

本发明的上述技术方案相比现有技术具有以下优点：

[n0023]

(1) The cultivation method provided by the present invention has low cost, good effect and short cycle.

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(1)本发明提供的培养方式成本低，效果好，周期短。

[n0024]

(2) Compared with conventional cultivation, the present invention can simultaneously increase the biomass and starch content of duckweed by changing the nutrient concentration according to different growth stages of duckweed. That is, the biomass reaches more than 140.57±0.85g/m² and the starch yield reaches more than 58.87±0.47g /m².

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(2)较常规培养，本发明根据浮萍不同生长时期，改变营养浓度方法可同时提升浮萍的生物量及淀粉含量，即生物量达到140.57±0.85g/m²以上，淀粉产量达到58.87±0.47g/m²以上。

[n0025]

(3) This invention provides a new approach to increasing the yield of duckweed starch and lays the foundation for the development of duckweed starch resources.

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(3)本发明为提升浮萍淀粉产量提供一种新思路，同时为开发浮萍淀粉资源奠定基础。

[0029]

Attached Figure Description

附图说明

[n0026]

To make the content of this invention easier to understand, the invention will be further described in detail below with reference to specific embodiments and accompanying drawings, wherein...

为了使本发明的内容更容易被清楚的理解，下面根据本发明的具体实施例并结合附图，对本发明作进一步详细的说明，其中，

[n0027]

Figure 1 shows the growth status of duckweed cultured in Examples 1-2 and Comparative Examples 1-2 of the present invention;

图1为本发明实施例1～2和对比例1～2培养的浮萍生长状态图；

[n0028]

Figure 2 shows the starch yield of duckweed cultivated in Examples 1-2 and Comparative Examples 1-2 at different stages of the present invention.

图2为本发明实施例1～2和对比例1～2培养的浮萍不同时期淀粉产量图。

[0033]

Detailed Implementation

具体实施方式

[n0029]

The present invention will be further described below with reference to the accompanying drawings and specific embodiments, so that those skilled in the art can better understand and implement the present invention. However, the embodiments described are not intended to limit the present invention.

下面结合附图和具体实施例对本发明作进一步说明，以使本领域的技术人员可以更好地理解本发明并能予以实施，但所举实施例不作为对本发明的限定。

[n0030]

This invention provides a cultivation method for increasing starch yield in duckweed thallus, as detailed below:

本发明提供了一种提高浮萍叶状体淀粉产量的培养方法，具体如下：

[n0031]

Germplasm was picked from a plate and added to a culture flask containing the first nutrient solution. The flask was then exposed to light to obtain activated germplasm.

平板挑取种质加入装有第一营养液的培养瓶中，光照，获得活化的种质；

[n0032]

The activated duckweed was cultured under light in the first nutrient solution;

将活化后的浮萍在第一营养液中光照培养；

[n0033]

A second nutrient solution is added to the cultivation device. The volume concentration of the second nutrient solution is 40%–50% of that of the first nutrient solution. 1–10% of the working volume of the second nutrient solution is added to the duckweed. During the duckweed's growth, the nutrient solution concentration is dynamically adjusted according to the growth stage: 1) Rapid growth period (days 1–3): Maintain the nutrient solution concentration at 40%–50% of the original concentration (first nutrient solution concentration). At this time, the chlorophyll content of the duckweed is 0.15 mg/g–0.30 mg/g. The evaporation volume is replenished with the corresponding concentration of nutrient solution. 2) Starch accumulation period (days 4–7): Gradually reduce the nutrient solution concentration to 30%–40% of the original concentration (first nutrient solution concentration). At this time, the chlorophyll content of the duckweed is 0.10 mg/g–0.20 mg/g. The evaporation volume of the nutrient solution is replenished with the corresponding

amount of distilled water. This is done 2–3 times, approximately every 1–2 days, while maintaining a stable nutrient solution volume. Ultimately, duckweed thallus with high starch yield is obtained.

在培养装置中加入第二营养液，第二营养液的体积浓度为第一营养液的40%～50%，按工作体积的1～10%接入浮萍，在浮萍生长过程中根据生长阶段动态调整营养液浓度：1)快速生长期(第1～3天)：维持营养液浓度为原浓度(第一营养液浓度)的40%～50%，此时浮萍叶绿素含量为0.15mg/g～0.30mg/g，蒸发体积补充相应浓度的营养液；2)淀粉积累期(第4～7天)：逐步降低营养液浓度至原浓度(第一营养液浓度)的30%～40%，此时浮萍叶绿素含量为0.10mg/g～0.20mg/g，营养液蒸发体积补充相应的蒸馏水，补充次数为2～3次，约每1～2天补充蒸馏水，期间保持营养液体积稳定，最终获得淀粉产量较高的浮萍叶状体。

[n0034]

The preparation method of the second nutrient solution is as follows: at room temperature, prepare the first nutrient solution according to the normal duckweed expansion culture, and then add the corresponding pure water at the volume ratio and mix well to obtain a nutrient solution with a concentration of 40% to 50%.

其中，第二营养液的制备方法是：常温下，根据正常浮萍扩培配制第一营养液，之后再以体积比加入相应纯水混匀，得到40%～50%浓度的营养液。

[n0035]

The nutrient solution used in this invention comprises the following components: calcium, iron, magnesium, boron, zinc, copper, molybdenum, sodium, manganese, nitrogen, phosphorus, and potassium. By weight percentage, the first nutrient solution comprises the following elemental components: calcium 0.2 g/L to 1 g/L, iron 0.001 g/L to 0.0005 g/L, magnesium 0.01 g/L to 0.05 g/L, boron 0.0001 g/L to 0.0005 g/L, and zinc 0.00001 g/L to 0.00001 g/L. 0.00005 g/L, copper 0.000004 g/L～0.00002 g/L, molybdenum 0.00001 g/L～0.00005 g/L, sodium 0.000004 g/L～0.00002 g/L, manganese 0.0002 g/L～0.001 g/L, nitrogen 0.06 g/L～0.3 g/L, phosphorus 0.006 g/L～0.03 g/L, and potassium 0.12 g/L～0.6 g/L.

本发明使用的营养液的组成成分包括：钙、铁、镁、硼、锌、铜、钼、钠、锰、氮、磷、钾；按照重量百分比计，所述第一营养液包括以下元素组分：钙0.2g/L～1g/L、铁0.001g/L～0.0005g/L、镁0.01g/L～0.05g/L、硼0.0001g/L～0.0005g/L、锌0.00001g/L～0.00005g/L、铜0.000004g/L～0.00002g/L、钼0.00001g/L～0.00005g/L、钠0.000004g/L～0.00002g/L、锰0.0002g/L～0.001g/L、氮0.06g/L～0.3g/L、磷0.006g/L～0.03g/L和钾0.12g/L～0.6g/L。

[n0036]

The cultivation conditions of this invention are: a photoperiodic environment with alternating light and dark conditions and a light intensity of 85 μmol/m²/s to 100 μmol /m²/s, and cultivation at room temperature.

本发明的培养条件为：光暗交替条件为24h光量的光周期环境，光照强度为85μmol/m²/s～100μmol/m²/s、室温条件下培养。

[n0037]

The cultivation device of the present invention is a commercially available plastic basket with dimensions of length * width * height (166mm × 114mm × 58mm), but is not limited to this model; it can be enlarged or reduced proportionally.

本发明的培养装置为市面上可购买到的长*宽*高(166mm×114mm×58mm)的塑料筐，但不限于该型号，同比例扩大或缩小均可。

[n0038]

The nutrient solution used in this invention consists of commercially available components, including elements such as calcium, iron, magnesium, boron, zinc, copper, molybdenum, sodium, manganese, nitrogen, phosphorus, and potassium.

本发明使用的营养液组成成分均来自于商业途径，含有钙、铁、镁、硼、锌、铜、钼、钠、锰、氮、磷、钾等元素均可。

[n0039]

The duckweed used in this invention is a dominant duckweed plant with a high starch content in the genus *Lemna*, including but not limited to *Lemna minor* ZH0196.

本发明使用的浮萍为浮萍属中淀粉含量较高的优势浮萍植株；包括但不限于多根紫萍ZH0196。。

[n0040]

Furthermore, the described duckweed morphology refers to duckweed leaf-like structures.

进一步地，所述的浮萍形态均为浮萍叶状体。

[n0041]

Furthermore, the species of duckweed mentioned include, but are not limited to, the genera *Lemna minor*, *Lemna spicata*, *Lemna spp.*, *Lemna spp.*, and *Lemna spp.*.

进一步地，所述的浮萍品种归属包括但不限于多根紫萍属、少根紫萍属、青萍属、芜萍属、扁无根萍属。

[n0042]

Furthermore, the lighting conditions can be any fluorescent lamp that can achieve the required light intensity.

进一步地，所述的光照条件为能达到光照强度的日光灯均可。

[n0043]

The present invention also provides a duckweed with high biomass and high starch content obtained by the above method, which ultimately achieves high starch yield.

本发明还提供了一种由上述方法培养得到的高生物量、高淀粉含量的浮萍，最终达到高淀粉产量。

[n0044]

The high-starch-content duckweed thallus of the present invention can be applied in the fields of biomass energy and agricultural biotechnology.

本发明的高淀粉含量浮萍叶状体可应用在生物质能源及农业生物技术领域。

[n0045]

Unless otherwise specified, the experimental methods used in the following examples are conventional methods, and the materials and reagents used are commercially available.

下述实施例中所使用的实验方法如无特殊说明，均为常规方法，所用的材料、试剂等，如无特殊说明，均可从商业途径得到。

[n0046]

The testing methods involved in the embodiments of this invention are as follows:

本发明实施例中涉及的测试方法：

[n0047]

1.

1.

Determination of duckweed biomass

浮萍生物量的测定

[n0048]

Collect duckweed at different stages, transfer it to a filter bag, centrifuge for 5 minutes to remove surface free water, and then weigh it using a balance.

收集不同时期浮萍，将其转移到滤袋中，离心5min，以去除表面自由水，然后使用天平称重。

Then, the sample was processed in a freeze dryer for 48 hours, the dry weight was measured, and the biomass was calculated based on the culture area per unit area.

然后，样品在冷冻干燥机中处理48h，测定干重，再根据培养面积进行单位面积换算即为生物量。

[n0049]

2.

2.

Starch content determination

淀粉含量测定

[n0050]

The freeze-dried sample was crushed and ground into granules in a mortar.

将冻干后的样品在研钵中进行粉碎，研磨成颗粒状。

Disperse 0.1g of sample in 0.2mL of 80% ethanol, then add 2mL of 2M KOH and stir in an ice bath for 20min.

取0.1g样品在0.2mL80%乙醇中分散后，加入2mL2M的KOH冰浴搅拌20min。

Then add 8 mL of 1.2 M sodium acetate buffer (pH 3.8), 0.1 mL of α-amylase and amyloglucosidase, mix well and incubate at 50 °C for 30 min.

之后加入8mL1.2M醋酸钠缓冲液(pH3.8)、0.1mLα-淀粉酶和淀粉葡萄糖苷酶，混匀50°C水浴30min。

Transfer the sample to a final volume of 100 mL, centrifuge a unit volume of the supernatant, and then perform the determination.

转移样品定容至100mL，取单位体积离心后上清进行测定。

The total starch content was determined using the Megazyme Total Starch Detection Kit.

使用Megazyme淀粉总量检测试剂盒进行测定。

[n0051]

3.

3.

Determination of starch yield

淀粉产量的测定

[n0052]

The product of the measured starch content and biomass is the starch yield, which can be expressed as:

将测得的淀粉含量与生物量的乘积即为淀粉产量，可表达为：

[n0053]

Starch yield (g/m²) = Biomass (g/m²) × Starch content (%).

淀粉产量(g/m²)＝生物量(g/m²)×淀粉含量(%)。

[n0054]

The composition (g/L) of the nutrient solution involved in the embodiments of the present invention:

本发明实施例中涉及的营养液的组成(g/L)：

[n0055]

Ca(NO₃)₂・4H₂O 12g, KNO₃25 g, KH₂PO₄1.4 g, tartaric acid 0.6g, FeCl₃・6H₂O1g, EDTA1.8g, MgSO₄・7H₂O 10g, H₃BO₃0.6 g, ZnSO₄・7H₂O 0.044g, Na₂MoO₄・2H₂O 0.024g, CuSO₄・5H₂O 0.016g, MnCl₂・4H₂O 0.8g, pH 5-5.5.

Ca(NO₃)₂・4H₂O 12g、KNO₃25 g、KH₂PO₄1.4 g、酒石酸0.6g、FeCl₃・6H₂O1g、EDTA1.8g、MgSO₄・7H₂O 10g、H₃BO₃0.6 g、ZnSO₄・7H₂O 0.044g、Na₂MoO₄・2H₂O 0.024g、CuSO₄・5H₂O 0.016g、MnCl₂・4H₂O 0.8g，pH5～5.5。

[n0056]

Example 1:

实施例1：

[n0057]

This embodiment provides a method for cultivating high-density, high-starch-yield duckweed thallus, including the following steps:

本实施例提供了一种高密度、高淀粉产量浮萍叶状体的培养方法，包括如下步骤：

[n0058]

(1) Activation of multi-rooted Azolla ZH0196 germplasm in conical flask: Germplasm was picked from a plate and added to a 250mL culture flask containing 150mL nutrient solution. After 24 hours of light exposure and 15 days of light exposure, the germplasm was activated.

---

(1)锥形瓶活化多根紫萍ZH0196种质：平板挑取种质加入装有150mL营养液的250mL培养瓶中，24h光照15d，获得活化的种质。

[n0059]

(2) The activated germplasm was inoculated into a culture device containing nutrient solution at an inoculation rate of 80% and cultured under light for 24 hours and 4 days.

---

(2)将活化种质按80%接种量接入含有营养液的培养装置中进行24h光照扩培4d。

[n0060]

(3) Add 45% nutrient solution with an initial pH of 5 to 5.5 to the culture device, and add 5% of the expanded duckweed into the culture device according to the working volume, and maintain a nutrient concentration of 45% for the first 3 days.

---

(3)在培养装置中加入初始pH为5～5.5的45%浓度的营养液，按工作体积的5%将扩培后的浮萍接入培养装置中，前3天维持45%的营养浓度。

As the duckweed grows, starting from the 4th day, gradually reduce the nutrient concentration to 35%. The reduced amount of nutrient solution is replenished with distilled water in the later stages to maintain the volume of the culture solution. Replenish once every 1-2 days, for a total of 3 times. The entire process is carried out under full light at a temperature of 25°C. After 5 days of growth, high-density duckweed with high starch content can be obtained after harvesting.

随着浮萍的生长，从第4天开始，逐步降低营养浓度至35%，营养液的减少量后期以蒸馏水补充，维持培养液的体积，每1～2d补充一次，共补充3次，整个过程全光照培养，温度为25°C，待生长至5d，收获后可得到高密度、高淀粉含量的浮萍。

[n0061]

Example 2:

实施例2：

[n0062]

This embodiment provides a method for cultivating high-density, high-starch-yield duckweed thallus, including the following steps:

本实施例提供了一种高密度、高淀粉产量浮萍叶状体的培养方法，包括如下步骤：

[n0063]

(1) Activation of multi-rooted Azolla ZH0196 germplasm in conical flask: Germplasm was picked from a plate and added to a 250mL culture flask containing 150mL nutrient solution. After 24 hours of light exposure and 15 days of light exposure, the germplasm was activated.

---

(1)锥形瓶活化多根紫萍ZH0196种质：平板挑取种质加入装有150mL营养液的250mL培养瓶中，24h光照15d，获得活化的种质。

[n0064]

(2) The activated germplasm was inoculated into a culture device containing nutrient solution at an inoculation rate of 80% and cultured under light for 24 hours and 4 days.

---

(2)将活化种质按80%接种量接入含有营养液的培养装置中进行24h光照扩培4d。

[n0065]

(3) Add 50% nutrient solution with an initial pH of 5 to 5.5 to the culture device, and add 5% of the expanded duckweed into the culture device according to the working volume. Maintain the nutrient solution concentration at 50% for the first 3 days.

---

(3)在培养装置中加入初始pH为5～5.5的50%浓度的营养液，按工作体积的5%将扩培后的浮萍接入培养装置中，前3天维持营养液浓度为50%。

As the duckweed grows, starting from the 4th day, gradually reduce the nutrient solution concentration to 40%. The reduced amount of nutrient solution is replenished with distilled water in the later stages to maintain the volume of the culture solution. Replenish once every 1-2 days, for a total of 3 times. The entire process is carried out under full light at a temperature of 25°C. After 5 days of growth, high-density duckweed with high starch content can be obtained after harvesting.

随着浮萍的生长，从第4天开始逐步降低营养液浓度至40%，营养液的减少量后期以蒸馏水补充，维持培养液的体积，每1～2d补充一次，共补充3次，整个过程全光照培养，温度为25°C，待生长至5d，收获后可得到高密度、高淀粉含量的浮萍。

[n0066]

Comparative Example 1:

对比例1：

[n0067]

This comparative example provides a method for cultivating high-density, high-starch-yield duckweed thallus, including the following steps:

本对比例提供了一种高密度、高淀粉产量浮萍叶状体的培养方法，包括如下步骤：

[n0068]

(1) Activation of multi-rooted Azolla ZH0196 germplasm in conical flask: Germplasm was picked from a plate and added to a 250mL culture flask containing 150mL nutrient solution. After 24 hours of light exposure and 15 days of light exposure, the germplasm was activated.

---

(1)锥形瓶活化多根紫萍ZH0196种质：平板挑取种质加入装有150mL营养液的250mL培养瓶中，24h光照15d，获得活化的种质。

[n0069]

(2) The activated germplasm was inoculated into a culture device containing nutrient solution at an inoculation rate of 80% and cultured under light for 24 hours and 4 days.

---

(2)将活化种质按80%接种量接入含有营养液的培养装置中进行24h光照扩培4d。

[n0070]

(3) Add 20% nutrient solution with an initial pH of 5 to 5.5 to the culture device. Add 5% of the expanded duckweed into the culture device according to the working volume. As the duckweed grows, the amount of nutrient solution is reduced and then replenished with distilled water to maintain the initial culture solution volume. Replenish once every 1 to 2 days for a total of 3 times. The entire process is carried out under full light and at a temperature of 25°C. After 4 days of growth, duckweed with high density and high starch content can be obtained after harvest.

---

(3)在培养装置中加入初始pH为5～5.5的20%浓度的营养液，按工作体积的5%将扩培后的浮萍接入培养装置中，随着浮萍的生长，营养液的减少量后期以蒸馏水补充，维持初始培养液的体积，每1～2d补充一次，共补充3次，整个过程全光照培养，温度为25°C，待生长至4d，收获后可得到高密度、高淀粉含量的浮萍。

[n0071]

Comparative Example 2:

对比例2：

[n0072]

This comparative example provides a method for cultivating high-density, high-starch-yield duckweed thallus, including the following steps:

本对比例提供了一种高密度、高淀粉产量浮萍叶状体的培养方法，包括如下步骤：

[n0073]

(1) Activation of multi-rooted Azolla ZH0196 germplasm in conical flask: Germplasm was picked from a plate and added to a 250mL culture flask containing 150mL nutrient solution. After 24 hours of light exposure and 15 days of light exposure, the germplasm was activated.

---

(1)锥形瓶活化多根紫萍ZH0196种质：平板挑取种质加入装有150mL营养液的250mL培养瓶中，24h光照15d，获得活化的种质。

[n0074]

(2) The activated germplasm was inoculated into a culture device containing nutrient solution at an inoculation rate of 80% and cultured under light for 24 hours and 4 days.

---

(2)将活化种质按80%接种量接入含有营养液的培养装置中进行24h光照扩培4d。

[n0075]

(3) Add 60% nutrient solution with an initial pH of 5 to 5.5 to the culture device. Add 5% of the expanded duckweed into the culture device according to the working volume. As the duckweed grows, the amount of nutrient solution is reduced and then replenished with distilled water to maintain the initial culture solution volume. Replenish once every 1 to 2 days, for a total of 3 times. The entire process is carried out under full light and at a temperature of 25°C. After 6 days of growth, duckweed with high density and high starch content can be obtained after harvest.

---

(3)在培养装置中加入初始pH为5～5.5的60%浓度的营养液，按工作体积的5%将扩培后的浮萍接入培养装置中，随着浮萍的生长，营养液的减少量后期以蒸馏水补充，维持初始培养液的体积，每1～2d补充一次，共补充3次，整个过程全光照培养，温度为25℃，待生长至6d，收获后可得到高密度、高淀粉含量的浮萍。

[n0076]

Comparative Example 3:

对比例3：

[n0077]

This comparative example provides a method for cultivating high-density, high-starch-yield duckweed thallus, including the following steps:

本对比例提供了一种高密度、高淀粉产量浮萍叶状体的培养方法，包括如下步骤：

[n0078]

(1) Activation of multi-rooted Azolla ZH0196 germplasm in conical flask: Germplasm was picked from a plate and added to a 250mL culture flask containing 150mL nutrient solution. After 24 hours of light exposure and 15 days of light exposure, the germplasm was activated.

---

(1)锥形瓶活化多根紫萍ZH0196种质：平板挑取种质加入装有150mL营养液的250mL培养瓶中，24h光照15d，获得活化的种质。

[n0079]

(2) The activated germplasm was inoculated into a culture device containing nutrient solution at an inoculation rate of 80% and cultured under light for 24 hours and 4 days.

---

(2)将活化种质按80%接种量接入含有营养液的培养装置中进行24h光照扩培4d。

[n0080]

(3) Add 40% nutrient solution with an initial pH of 5 to 5.5 to the culture device. Add 5% of the expanded duckweed into the culture device according to the working volume. As the duckweed grows, the amount of nutrient solution is reduced and then replenished with distilled water to maintain the initial culture solution volume. Replenish once every 1 to 2 days, for a total of 3 times. The entire process is carried out under full light and at a temperature of 25°C. After 5 days of growth, duckweed with high density and high starch content can be obtained after harvest.

---

(3)在培养装置中加入初始pH为5～5.5的40%浓度的营养液，按工作体积的5%将扩培后的浮萍接入培养装置中，随着浮萍的生长，营养液的减少量后期以蒸馏水补充，维持初始培养液的体积，每1～2d补充一次，共补充3次，整个过程全光照培养，温度为25°C，待生长至5d，收获后可得到高密度、高淀粉含量的浮萍。

[n0081]

Comparative Example 4:

对比例4：

[n0082]

This comparative example provides a method for cultivating high-density, high-starch-yield duckweed thallus, including the following steps:

本对比例提供了一种高密度、高淀粉产量浮萍叶状体的培养方法，包括如下步骤：

[n0083]

(1) Activation of multi-rooted Azolla ZH0196 germplasm in conical flask: Germplasm was picked from a plate and added to a 250mL culture flask containing 150mL nutrient solution. After 24 hours of light exposure and 15 days of light exposure, the germplasm was activated.

---

(1)锥形瓶活化多根紫萍ZH0196种质：平板挑取种质加入装有150mL营养液的250mL培养瓶中，24h光照15d，获得活化的种质。

[n0084]

(2) The activated germplasm was inoculated into a culture device containing nutrient solution at an inoculation rate of 80% and cultured under light for 24 hours and 4 days.

---

(2)将活化种质按80%接种量接入含有营养液的培养装置中进行24h光照扩培4d。

[n0085]

(3) Add 50% nutrient solution with an initial pH of 5 to 5.5 to the culture device. Add 5% of the expanded duckweed into the culture device according to the working volume. As the duckweed grows, the amount of nutrient solution is reduced and then replenished with distilled water to maintain the initial culture solution volume. Replenish once every 1 to 2 days, for a total of 3 times. The entire process is carried out under full light and at a temperature of 25°C. After 5 days of growth, duckweed with high density and high starch content can be obtained after harvest.

---

(3)在培养装置中加入初始pH为5～5.5的50%浓度的营养液，按工作体积的5%将扩培后的浮萍接入培养装置中，随着浮萍的生长，营养液的减少量后期以蒸馏水补充，维持初始培养液的体积，每1～2d补充一次，共补充3次，整个过程全光照培养，温度为25℃，待生长至5d，收获后可得到高密度、高淀粉含量的浮萍。

[n0086]

Figure 1 shows the growth status of duckweed cultured in Examples 1-2 and Comparative Examples 1-2 of the present invention. As can be seen from Figure 1, the higher the nutrient concentration, the better it is for the growth of thallus, and the less starch accumulates.

本发明实施例1～2和对比例1～2培养的浮萍生长状态图如图1所示；通过图1可以看出，营养浓度越高越用于叶状体的生长，淀粉积累量少。

[n0087]

Figure 2 shows the starch yield of duckweed cultivated in Examples 1-2 and Comparative Examples 1-2 at different stages. As can be seen from Figure 2, different nutrient concentrations lead to different thallus growth cycles.

本发明实施例1～2和对比例1～2培养的浮萍不同时期淀粉产量图如图2所示；由图2可以看出，不同营养浓度培养会导致叶状体生长周期不同。

[n0088]

Table 1 shows the optimal biomass, starch content, and yield of duckweed thallus cultured in Examples 1-2 and Comparative Examples 1-2, as detailed below:

表1为实施例1～2及对比例1～2培养的浮萍叶状体最佳生物量、淀粉含量及产量情况，具体如下：

[n0089]

Table 1

表1

[n0090]

<![CDATA[Biomass/gm²]]> Starch Content % <![CDATA[Starch Yield/gm²]]> Example 1 145.68±0.53 42.60±0.46 61.77±0.44 Example 2 140.57±0.85 41.88±0.53 58.87±0.47 Comparative Example 1 127.98±4.80 42.06±0.76 53.80±1.06 Comparative Example 2 171.05±3.60 31.13±0.22 53.24±0.74 Comparative Example 3 131.44±0.93 37.82±0.33 49.71±0.56 Comparative Example 4 138.21±1.08 35.11±0.28 48.37±0.77

<![CDATA[生物量/gm²]]> 淀粉含量% <![CDATA[淀粉产量/gm²]]> 实施例1 145.68±0.53 42.60±0.46 61.77±0.44 实施例2 140.57±0.85 41.88±0.53 58.87±0.47 对比例1 127.98±4.80 42.06±0.76 53.80±1.06 对比例2 171.05±3.60 31.13±0.22 53.24±0.74 对比例3 131.44±0.93 37.82±0.33 49.71±0.56 对比例4 138.21±1.08 35.11±0.28 48.37±0.77

[n0091]

As shown in Table 1, by changing the nutrient concentration according to different growth stages of duckweed, the biomass of duckweed reached a maximum of 145.68±0.53 g /m² and the starch yield reached a maximum of 61.77±0.44 g/m², which is much higher than the comparative method using a single nutrient solution concentration.

通过表1可以看出，根据浮萍不同生长时期，改变营养浓度方法使得浮萍的生物量最高达到145.68±0.53g/m²，淀粉产量最高达到61.77±0.44g/m²，远高于采用单一营养液浓度的对比例。

This is mainly because duckweed in Examples 1 and 2 requires a higher concentration of nutrient solution in the early stages of growth to support rapid cell division and growth.

这主要是因为实施例1、2浮萍在生长初期需要较高的营养液浓度来支持快速的细胞分裂和生长。

At this stage, sufficient nutrition can promote the development of duckweed's leaves and roots, laying a good foundation for subsequent growth; while in the later stages of growth, the growth rate of duckweed gradually slows down, but starch accumulation continues.

此时，充足的营养可以促进浮萍的叶片和根系的发育，为后续的生长打下良好的基础；而在生长后期，浮萍的生长速度逐渐减缓，但淀粉的积累仍在继续。

At this time, appropriately reducing the concentration of nutrient solution can prevent excessive nutrient supply from causing excessive plant growth, while maintaining a certain nutrient level to maintain starch synthesis and accumulation, ultimately increasing starch yield.

此时，适当降低营养液浓度可以避免过度的营养供应导致植株徒长，同时保持一定的营养水平以维持淀粉的合成和积累，最终提升淀粉产量。

Comparative Examples 1 and 3 clearly show that when the nutrient concentration is high, it is mainly used for biomass accumulation, and the starch accumulation is not high. In Comparative Examples 3 and 4, when a single nutrient concentration is used for cultivation, as the duckweed grows, the nutrients can no longer supply the duckweed for growth in the later stages. Although starch is being accumulated, the starch yield at this time is not as high as in Examples 1 and 2.

对比例1、3能明显看出营养浓度高时主要用于生物量的积累，淀粉积累程度不高；对比例3、4使用单一营养浓度培养随着浮萍的生长，后期营养已供应不上浮萍的生长，虽在积累淀粉但此时的淀粉产量不如实施例1、2.。

[n0092]

Obviously, the above embodiments are merely examples for clear illustration and are not intended to limit the implementation.

显然，上述实施例仅仅是为清楚地说明所作的举例，并非对实施方式的限定。

For those skilled in the art, other variations or modifications can be made based on the above description.

对于所属领域的普通技术人员来说，在上述说明的基础上还可以做出其它不同形式变化或变动。

It is neither necessary nor possible to exhaustively list all possible implementation methods here.

这里无需也无法对所有的实施方式予以穷举。

However, any obvious changes or modifications derived therefrom are still within the scope of protection of this invention.

而由此所引申出的显而易见的变化或变动仍处于本发明创造的保护范围之中。