Notice

DESCRIPTION CN113273483A

A method for optimizing culture conditions suitable for the rapid propagation of duckweed and its application

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一种适用于浮萍快速繁殖的培养条件优化方法及应用

[0001]

Technical Field

技术领域

[n0001]

This invention relates to the field of rapid propagation of aquatic plants, specifically to a method and application for optimizing culture conditions suitable for the rapid propagation of duckweed.

本发明涉及水生植物快繁领域，具体是涉及一种适用于浮萍快速繁殖的培养条件优化方法及应用。

[0003]

Background Technology

背景技术

[n0002]

Duckweed is a common aquatic plant that, under suitable environmental conditions, has outstanding characteristics such as rapid growth and reproduction, strong nitrogen and phosphorus absorption capacity, rich plant protein content, and easy recycling. It has been widely used in paddy field weed control, high-protein feed, new biofuel preparation, and sewage treatment.

浮萍是一类常见的水生植物，在适宜的环境条件下具有生长繁殖迅速、氮磷吸收能力强、植物蛋白含量丰富、易于回收利用等突出特点，已被广泛应用于稻田控草、高蛋白饲料、新型生物燃料制备、污水处理。

Therefore, obtaining a large quantity of duckweed in a short period of time is of great significance for the promotion and application of duckweed.

因此在短时间内获得大量浮萍对于浮萍的推广应用具有重要意义。

[n0003]

Because environmental factors interact, it is difficult to establish the optimal plant cultivation scheme through single-factor experiments.

由于环境因子有交互作用，通过单因素试验很难建立最优的植物培养方案。

Chinese patent application CN105638484A discloses a method for indoor cultivation and propagation of duckweed. By reasonably controlling the light cycle and culture medium, the number of leaves is used as a response index to determine the growth of duckweed. However, this method does not consider the interaction effect of multiple factors, and there are also shortcomings in the selection of response index.

中国专利申请CN105638484A公布了浮萍室内培养与繁殖的方法，通过合理控制光照周期，培养基，利用叶片数作为响应指标作为判定浮萍生长的指标，但该方法没有考虑多因素的互作效应，在响应指标上的选取也存在有不足。

Chinese patent application CN103333068A discloses a method for optimizing the extraction of chlorogenic acid from Jerusalem artichoke straw using response surface methodology.

中国专利申请CN103333068A公开了利用响应面法优化菊芋秸秆绿原酸的提取方法。

Response surface methodology is a statistical method used to fit the functional relationship between factors and response values using a multiple quadratic regression equation. By analyzing the regression equation, it seeks the optimal process parameters and solves multivariate problems. However, current applications are limited to optimizing extraction conditions and process parameters.

响应面是一种用于采用多元二次回归方程来拟合因素与响应值之间的函数关系，通过对回归方程的分析来寻求最优工艺参数，解决多变量问题的一种统计方法。 但是目前利用还局限在提取条件的优化和工艺参数的优化。

[n0004]

Current research indicates that duckweed grows best when the pH is controlled between 6.5 and 7.5, and its relative growth rate is higher under high light intensity, with the optimal light intensity being 6000 lx. Duckweed can still grow at temperatures of 5-7°C, but its growth is severely inhibited, and if the temperature continues to drop, it will sink to the bottom in a dormant state.

目前研究表明，控制6.5-7.5的pH时，浮萍生长条件最好，并且浮萍在高光强下的相对生长率更高，最适光强在6000lx；浮萍在温度为5-7°C时仍旧能够生长，但是生长受到了严重的抑制，温度继续降低则会以休眠体的形式沉入水底。

Duckweed grows well at 25°C, but its growth rate decreases at 35°C. Duckweed's tolerance to low pH is limited to 5-6, and it can grow normally between 6-9. Under suitable conditions, the bioaccumulation of *Lemna minor* is greater than that of *Lemna minor* and *Lemna minor*. Under medium and high nitrogen concentrations, an ammonium-nitrate ratio of 1/4 or 0/5 and a phosphorus concentration of 0.1–0.3 mmol/L are more suitable for the growth of *Lemna minor* and *Lemna minor*. In addition, the ratio of ammonium nitrogen to nitrate nitrogen also affects duckweed biomass. However, these studies are limited to single-factor cultivation of duckweed, and the multi-factor interaction effects during the cultivation process are often overlooked.

25°C下浮萍生长状况好，35°C生长率下降。 浮萍对pH的低耐受限为5-6，在6-9之间能够正常生长。适宜环境下的青萍生物积累量大于少根紫萍和多根紫萍，中、高氮浓度下1/4、0/5的铵硝比，磷浓度0.1～0.3mmol/L更适合少根紫萍和青萍的生长。 此外铵态氮与硝态氮的比值也对浮萍生物量存在影响。 但这些研究都仅仅局限于对浮萍的单因素培养，培养过程中的多因素互作效应往往被忽视。

[n0005]

In summary, current duckweed propagation technology still has shortcomings such as ignoring the interaction effects of multiple factors, inaccurate calculation of duckweed growth rate due to simple cultivation equipment, and using simple indicators to measure duckweed growth.

综上所述，现阶段浮萍扩繁技术中还存在如忽视多因素互作效应、培养设备简单导致浮萍增长率计算不准确、以简单指标来衡量浮萍增长等不足。

[0008]

Summary of the Invention

发明内容

[n0006]

The purpose of this invention is to overcome the shortcomings of the existing technology and provide a method and application for optimizing culture conditions suitable for the rapid propagation of duckweed that can reduce the number of experiments.

本发明的目的就是为了克服上述现有技术存在的缺陷而提供一种可减少试验数量的适用于浮萍快速繁殖的培养条件优化方法及应用。

[n0007]

The objective of this invention can be achieved through the following technical solutions:

本发明的目的可以通过以下技术方案来实现：

[n0008]

A method for optimizing culture conditions suitable for the rapid propagation of duckweed includes the following steps:

一种适用于浮萍快速繁殖的培养条件优化方法，包括以下步骤：

[n0009]

The main factors affecting duckweed growth were selected, and the optimal growth conditions of duckweed under each main factor were obtained by single-factor experiments.

选取影响浮萍生长的主效因素，以单因素试验获得浮萍在每种主效因素下的最适生长条件；

[n0010]

Using the optimal growth conditions under each major factor as the median, a fractional factorial experiment was conducted to screen the major factors affecting duckweed growth.

以每种主效因素下的最适生长条件为中间值进行分式析因试验，筛选影响浮萍生长的主效因子；

[n0011]

Using the main effect factor as the independent variable and the duckweed ratio growth rate as the response value Y, a response surface optimization experiment was conducted to obtain optimized nutrient content data of the culture medium.

以所述主效因子为自变量，以浮萍比增长率为响应值Y，进行响应面优化试验，获得优化的培养液养分含量数据；

[n0012]

A quadratic multiple regression model was established based on the optimized nutrient content data of the culture medium.

基于所述优化的培养液养分含量数据建立二次多元回归模型；

[n0013]

The response surface plot of the nutrient solution optimal conditions for duckweed growth was obtained based on the aforementioned quadratic multiple regression model.

基于所述二次多元回归模型获得指示浮萍生长的营养液最优条件的响应面图。

[n0014]

Furthermore, the main factors include culture medium pH, culture medium concentration, nitrogen-to-phosphorus ratio, and ammonium nitrogen ratio.

进一步地，所述主效因素包括培养液pH、培养液浓度、氮磷浓度比和铵态氮比例。

[n0015]

Furthermore, the fractional factorial experiments and response surface optimization experiments were designed based on Minitab19 software.

进一步地，所述分式析因试验和响应面优化试验基于Minitab19软件设计。

[n0016]

Furthermore, the single-factor experiment, fractional factorial experiment, and response surface optimization experiment are based on experimental data obtained from biological hydroponics. During biological hydroponics, duckweed is washed to remove algae and insects, sprayed with copper sulfate solution, and the light status of duckweed is adjusted regularly.

进一步地，所述单因素试验、分式析因试验和响应面优化试验基于生物水培获得试验数据，生物水培时，将浮萍洗净除去藻类与昆虫，喷洒硫酸铜溶液，定时调整浮萍的光照状态。

[n0017]

Furthermore, the biological hydroponics is implemented based on a cultivation device, which includes a nutrient solution holding unit and a duckweed growth weighing unit, wherein the duckweed growth weighing unit includes a nylon mesh bottom.

进一步地，所述生物水培基于培养装置实现，所述培养装置包括营养液盛放单元和浮萍生长称重单元，所述浮萍生长称重单元包括尼龙网底部。

[n0018]

Furthermore, both the nutrient solution holding unit and the duckweed growth weighing unit are made of transparent polypropylene material.

进一步地，所述营养液盛放单元和浮萍生长称重单元均由透明聚丙烯材料制成。

[n0019]

Furthermore, the duckweed includes duckweed, duckweed with few roots, or duckweed with many roots.

进一步地，所述浮萍包括青萍、少根紫萍或多根浮萍。

[n0020]

Furthermore, in each single-factor experiment, the remaining factors were kept at their original condition levels.

进一步地，在进行每个单因素试验时，其余因素保持原始条件水平。

[n0021]

Furthermore, in the single-factor experiment, fractional factorial experiment, and response surface optimization experiment, the duckweed ratio growth rate was calculated using a logarithmic model.

进一步地，所述单因素试验、分式析因试验和响应面优化试验中，浮萍比增长率采用对数模型计算获得。

[n0022]

The present invention also provides an application of the cultivation condition optimization method described above, which enables rapid propagation of duckweed based on the obtained optimal nutrient solution conditions.

本发明还提供一种如上所述的培养条件优化方法的应用，基于获得的所述营养液最优条件进行浮萍快速扩繁。

[n0023]

Compared with existing technologies, this invention uses fractional factorial design and response surface methodology to optimize duckweed culture conditions, providing a foundation for the broad application prospects of duckweed and offering the following beneficial effects:

与现有技术相比，本发明采用分式析因设计和响应面法优化浮萍培养条件，为浮萍广阔的应用前景提供了基础，具有以下有益效果：

[n0024]

1. This invention applies response surface methodology to the cultivation of duckweed. While improving the growth rate of duckweed, it overcomes the shortcomings of single-factor experiments, such as the large number of experiments, long cycle, and heavy workload, as well as the lack of interaction between factors. Only a small number of experiments are needed to obtain optimization results, thus obtaining suitable conditions for duckweed growth and the maximum growth rate.

1、本发明将响应面优化方法应用于浮萍的培养，在提高浮萍的生长速率的同时，克服了单因素试验次数多，周期较长和工作量大，而且缺少各因素之间的交互作用等缺点，仅需少量试验即可得到优化结果，得到浮萍生长的适宜的条件和最大的生长速率。

[n0025]

2. This invention measures the fresh weight of duckweed using a self-made strainer device, and combines manual counting with computer counting to measure the number of duckweed leaves and plants. It has high accuracy in measuring response variables and is simple and easy to implement.

2、本发明通过自制漏网装置测量浮萍的鲜重，利用人工计数和电脑计数结合测量浮萍的叶片数和株数，对响应变量的测量具有很高的准确度并且简便易行。

[n0026]

3. The growth of duckweed was fitted using an exponential growth curve. The growth status of duckweed was reflected by the final constructed response variable—the ratio growth rate. This method integrates data from various growth stages of duckweed, has time attributes, and is highly accurate.

3、利用指数增长曲线对浮萍的生长进行了拟合，通过最后构建的响应变量—比增长率来反应浮萍的生长状况，综合了浮萍各个生长时期的数据，具备时间属性，准确度高。

[n0027]

4. This invention reduces accidental experimental errors by cleaning the original duckweed seed before cultivation, spraying it with 2 mg/L copper sulfate, and rotating the culture cups.

4、本发明通过培养前对浮萍原种进行清理，喷洒2mg/L硫酸铜，进行培养杯的轮换减少偶然的实验误差。

[0031]

Attached Figure Description

附图说明

[n0028]

Figure 1 is a schematic diagram of nutrient solution container unit A;

图1为营养液盛放单元A示意图；

[n0029]

Figure 2 is a schematic diagram of the duckweed growth weighing unit B, where (2a) is a side view and (2b) is a top view;

图2为浮萍生长称重单元B示意图，其中，(2a)为侧视图，(2b)为俯视图；

[n0030]

Figure 3 shows the effect of different single factors of the present invention on Aquatic Plantago asiatica.

图3为本发明不同单因素对青萍的影响图；

[n0031]

Figure 4 is a three-dimensional response surface plot of the growth rate of the ratio of nitrogen to phosphorus concentration to culture medium concentration, ammonium nitrogen ratio, and base 10.

图4为培养液浓度、铵态氮比例、10为底的氮磷浓度比对数对青萍比增长率的响应面三维图；

[n0032]

Figure 5 shows the effect of different single factors of the present invention on duckweed;

图5为本发明不同单因素对紫萍的影响图；

[n0033]

Figure 6 is a three-dimensional response surface plot of the growth rate of the ratio of nitrogen to phosphorus concentration to the growth rate of the ratio of Azolla to culture medium concentration, ammonium nitrogen ratio, and nitrogen-to-phosphorus concentration (base 10).

图6为本发明培养液浓度、铵态氮比例、10为底的氮磷浓度比对数对紫萍比增长率的响应面三维图；

[n0034]

Figure 7 is a schematic diagram of the process of the present invention.

图7为本发明的流程示意图。

[0039]

Detailed Implementation

具体实施方式

[n0035]

The present invention will now be described in detail with reference to the accompanying drawings and specific embodiments.

下面结合附图和具体实施例对本发明进行详细说明。

This embodiment is implemented based on the technical solution of the present invention, and provides detailed implementation methods and specific operation processes. However, the scope of protection of the present invention is not limited to the following embodiment.

本实施例以本发明技术方案为前提进行实施，给出了详细的实施方式和具体的操作过程，但本发明的保护范围不限于下述的实施例。

[n0036]

Referring to Figure 7, the present invention provides a method for optimizing culture conditions suitable for the rapid propagation of duckweed, comprising the following steps:

参考图7所示，本发明提供一种适用于浮萍快速繁殖的培养条件优化方法，包括以下步骤：

[n0037]

S101. Select the main factors affecting duckweed growth and obtain the optimal growth conditions of duckweed under each main factor through single-factor experiments.

S101、选取影响浮萍生长的主效因素，以单因素试验获得浮萍在每种主效因素下的最适生长条件；

[n0038]

S102. Using the optimal growth conditions under each major factor as the median value, conduct fractional factorial experiments to screen the major factors affecting duckweed growth.

S102、以每种主效因素下的最适生长条件为中间值进行分式析因试验，筛选影响浮萍生长的主效因子；

[n0039]

S103. Using the main effect factor as the independent variable and the duckweed ratio growth rate as the response value Y, a response surface optimization experiment was conducted to obtain optimized nutrient content data of the culture medium.

S103、以所述主效因子为自变量，以浮萍比增长率为响应值Y，进行响应面优化试验，获得优化的培养液养分含量数据；

[n0040]

S104. Establish a quadratic multiple regression model based on the optimized nutrient content data of the culture medium;

S104、基于所述优化的培养液养分含量数据建立二次多元回归模型；

[n0041]

S105. Based on the aforementioned quadratic multiple regression model, obtain the response surface plot of the nutrient solution optimal conditions for duckweed growth.

S105、基于所述二次多元回归模型获得指示浮萍生长的营养液最优条件的响应面图。

[n0042]

The above method screens and optimizes the main factors of conditions such as pH, concentration, nitrogen-phosphorus ratio, and ammonium nitrogen ratio in the culture medium to obtain the optimal reproductive conditions for duckweed, thereby controlling the rapid reproduction of duckweed and laying the foundation for the broad application prospects of duckweed.

上述方法对培养液pH、浓度、氮磷浓度比、铵态氮比例等条件进行筛选主效因子和优化，获得浮萍最优繁殖条件，以控制浮萍快速繁殖，为浮萍广阔的应用前景提供了基础。

[n0043]

In this method, single-factor experiments, fractional factorial experiments, and response surface optimization experiments are based on experimental data obtained from biological hydroponics.

本方法中，单因素试验、分式析因试验和响应面优化试验基于生物水培获得试验数据。

When using biological hydroponics, wash the duckweed to remove algae and insects, spray it with copper sulfate solution (such as 2 mg/L copper sulfate), and adjust the light conditions of the duckweed regularly.

生物水培时，将浮萍洗净除去藻类与昆虫，喷洒硫酸铜溶液(如2mg/L硫酸铜)，定时调整浮萍的光照状态

[n0044]

Biological hydroponics is achieved based on a cultivation device, which includes a nutrient solution holding unit A and a duckweed growth weighing unit B, as shown in Figures 1 and 2, both of which are made of transparent polypropylene material.

生物水培基于培养装置实现，培养装置，包括营养液盛放单元A和和浮萍生长称重单元B，如图1和图2所示，均由透明聚丙烯材料制成。

[n0045]

In a specific embodiment, the nutrient solution holding unit A is 10cm high and 7cm in diameter, and can be wrapped with tin foil; the duckweed growth weighing unit B is 3.5cm high and 7cm in diameter, with a bottom diameter of 6cm. The duckweed growth weighing unit B has a 0.5cm outer edge for easy handling, and the bottom is a nylon mesh with a 1mm aperture.

在某一具体实施例中，营养液盛放单元A高10cm，直径7cm，外可包锡纸；浮萍生长称重单元B高3.5cm，直径7cm，下端直径6cm，浮萍生长称重单元B有0.5cm外缘以方便取放，底端为孔径为1mm的尼龙网。

The duckweed nutrient solution is placed in the nutrient solution holding unit A. Every three days, deionized water is used to replenish the lost water to ensure the normal water requirements of the duckweed. The duckweed growth weighing unit B uses nylon netting to trap the duckweed and harvests it regularly.

浮萍营养液置于营养液盛放单元A中，每三天利用去离子水补充损失的水分以保证浮萍的正常水分需求，浮萍生长称重单元B通过尼龙网截留浮萍，定期对浮萍进行收获。

After harvesting, absorb the moisture with absorbent paper and then weigh the product using an electronic balance.

收获后利用吸水纸吸干水分，随后利用电子天平进行称重。

[n0046]

In the single-factor experiment, fractional factorial experiment, and response surface optimization experiment of this method, the growth rate of duckweed ratio was calculated using a logarithmic model.

本方法的单因素试验、分式析因试验和响应面优化试验中，浮萍比增长率采用对数模型计算获得。

[n0047]

In a specific embodiment, the logarithmic model can be expressed as: lny = kx + b, where y is the number of duckweed plants, leaves, or fresh weight (g), k is the duckweed growth rate, x is the culture period (d), and b is a set parameter.

在某一具体实施例中，对数模型可表示为：lny＝kx+b，其中y为浮萍的株数、叶片数或鲜重(g)，k为浮萍比增长率，x为培养周期(d)，b为设定参数。

The methods for recording the number of leaves, the number of plants, and the fresh weight include the following:

记录叶片数、株数、鲜重的方法包括如下：

[n0048]

Number of plants and leaves: When the number of leaves is small, they are counted manually. When the number of leaves is large, photos are taken with a mobile phone and uploaded to a computer for accurate counting using drawing software. At the same time, the number of mouse clicks is recorded using keyboard and mouse wizard software.

株数、叶片数：在叶片数少时通过人工计数，当叶片数数量大时，通过手机拍照后上传电脑，通过画图软件进行精确计数，同时借助键鼠精灵软件记录鼠标点击数。

[n0049]

Fresh weight: Weighing using an electronic balance.

鲜重：利用电子天平进行称重。

[n0050]

In the single-factor experiment, the required duckweed variety was selected, and attached algae and insects were removed. After washing, it was pre-cultured, and 2 mg/L copper sulfate was sprayed on its surface to inhibit algae growth and help algae become the dominant group in the culture system.

在单因素试验中，取试验所需浮萍品种，去除附着藻类与昆虫，洗净后进行预培养，在其表面喷洒2mg/L硫酸铜抑制藻类发生，协助藻类成为培养体系中优势群体。

Subsequently, hydroponics was carried out using Hogland culture medium.

随后利用hogland培养液进行水培。

During the cultivation process, the position of the culture cup should be changed daily to ensure uniform light exposure.

在培养过程中每日更换培养杯位置，使光照均匀。

Key factors such as culture medium pH, concentration, nitrogen-phosphorus ratio, and ammonium nitrogen ratio were selected, and different levels were set based on the values of each factor in the Hogland culture medium.

选用如培养液pH、浓度、氮磷浓度比、铵态氮比例等主效因素，基于hogland培养液中各因素值，分别设置不同的水平。

A fixed amount of duckweed (approximately 0.4 g of duckweed per L of culture medium) was added to different levels of culture medium for each treatment.

将定量浮萍(大约0.4g每L培养液的浮萍)投入各个处理的不同水平培养液中。

The number of leaves, number of plants, and fresh weight of duckweed were recorded regularly. After batch processing of the data using pivot tables in EXCEL software, a logarithmic model was used to construct response variables describing duckweed growth. Finally, the optimal conditions for duckweed under each factor were determined.

定期记录浮萍的叶片数、株数、鲜重，利用EXCEL软件的数据透视表对数据进行批量处理之后，通过对数模型构建描述浮萍生长的响应变量，最后确定每种因素下浮萍最适的条件。

Other environmental conditions for duckweed growth were set according to the study as follows: light intensity 2500 lx, light cycle 16 h/d, temperature 25°C, and 1x Hoagland culture medium.

浮萍生长的其他环境条件根据研究，设定为光照强度2500lx，光照周期16h/d、温度25°C、1倍Hoagland培养液。

[n0051]

In the fractional factorial experiment, based on factors such as pH, concentration, nitrogen-phosphorus ratio, and ammonium nitrogen ratio of the culture medium, the optimal growth conditions of duckweed under each single factor obtained from the single factor experiment were used as the median value. The experiment was designed according to the factorial design of Minitab19 software to screen out the main factors affecting duckweed growth with a smaller number of experiments.

分式析因试验中，基于培养液pH、浓度、氮磷浓度比、铵态氮比例等因素，以单因素试验得到的浮萍在每种单因素下的最适生长条件为中间值，根据Minitab19软件因子设计进行试验设计，以较小的实验次数筛选到影响浮萍生长的主效因子。

[n0052]

In the response surface methodology experiment, based on the results of the fractional factorial experiment, the major effect factor with significant influence was used as the independent variable, and the specific growth rate of duckweed was used as the response value Y. The experiment was designed using Minitab19 software according to the Box-Behnken Design principle to determine the optimal nutrient content of the culture medium.

响应面优化方法试验中，根据分式析因实验的结果，以影响显著的主效因子为自变量，浮萍的比增长率为响应值Y，利用Minitab19软件根据Box-Behnken Design设计原则进行试验设计，确定优化的培养液养分含量。

[n0053]

The above method is applicable to common duckweed species including the common green duckweed (Lemna minor L.) and the less-rooted purple duckweed (Spirodelaoligorrhiza (Kurz) Hegelm.).

上述方法可适用的浮萍包括常见的青萍(Lemna minor L.)、少根紫萍(Spirodelaoligorrhiza(Kurz) Hegelm.

) and multi-rooted duckweed (Spirodela polyrhiza (Linn.)

)和多根浮萍(Spirodela polyrhiza(Linn.

Schleid.

)Schleid.)。

[n0054]

Example 1

实施例1

[n0055]

The main materials in this embodiment are: KNO₃, NH₄H₂PO₄, Hoagland culture medium (one concentration), deionized water, HCl, NaOH, CuSO₄, and duckweed.

本实施例的主要材料：KNO₃，NH₄H₂PO₄，Hoagland培养液(一倍浓度)，去离子水，HCl，NaOH，CuSO₄，青萍。

[n0056]

(1) Single-factor experiments were conducted: The original culture conditions were set as pH 6.0, light intensity 2500 lx, photoperiod 16 h/d, temperature 25°C, and 1 times Hoagland culture medium for culturing *Pediatrica spp.*. Single-factor experiments were designed, and the following treatments were made for each factor: pH: 3, 4, 5, 6, 7, 8, 9, 10, 11 (nine levels); phosphorus concentration: 0 mmol/L, 0.25 mmol/L, 0.5 mmol/L, 1 mmol/L, 2 mmol/L, 4 mmol /L (six levels); culture medium concentration: 0.25×, 0.5×, 1×, 1.5×, 2×, 4× (six levels); ammonium nitrogen ratio: 0%, 20%, 40%, 60%, 80%, 100% (six levels).

---

(1)进行单因素试验：设置原始培养条件为pH 6.0、光照强度2500lx，光照周期16h/d、温度25°C、1倍Hoagland培养液培养青萍，设计单因素试验，对每个因素做出如下处理：pH：3、4、5、6、7、8、9、10、11九个水平；磷浓度为0mmol/L、0.25mmol/L、0.5mmol/L、1mmol/L、2mmol/L、4mmol/L六个水平；培养液浓度为0.25×、0.5×、1×、1.5×、2×、4×六个水平；铵态氮比例为0%、20%、40%、60%、80%、100%六个水平。

When conducting each single-factor experiment, the other factors are kept at the original conditions.

在进行每个单因素试验时，其余因素保持原始条件的水平。

Four replicates were set up at each level. The culture was carried out in disposable plastic cups with a volume of 300ml and 250ml of culture medium. The fresh weight, number of plants and number of leaves of the duckweed were recorded every 3 days. At the time of inoculation, algae and insects attached to the duckweed were removed. The duckweed was sprayed with 2mg/L copper sulfate and cultured for 15 days. During the culture period, the order of the culture cups was rotated, but the culture medium was not changed. Deionized water was used to replenish the water.

各水平设置4个重复，用容积为300ml的塑料一次性水杯培养，培养液为250ml，每3天记录青萍的鲜重、株数和叶片数，接种时去除青萍附着藻类与昆虫，喷洒2mg/L硫酸铜，培养15天，培养期间轮换培养杯顺序，不更换培养液，用去离子水补充水分。

The experimental results were analyzed using an Excel pivot table, and the results are shown in Figure 3.

试验结果用EXCEL数据透视表进行分析，结果见图3。

It can be seen that the optimal single-factor culture conditions for *Peperomia stenoptera* are pH=8, phosphorus concentration=0.5mmol/L, culture medium concentration=0.5 times, and ammonium nitrogen ratio=20%.

可以看出，青萍最适的单因素培养条件为pH＝8、磷浓度＝0.5mmol/L、培养液浓度为0.5倍、铵态氮比例为20%。

[n0057]

(2) Conduct factorial experiments: The high and low levels of each single factor are obtained from the conclusions drawn from the single factor experiments. The factorial design table is obtained using Minitab19 software. The corresponding treatments are set according to the table and the experiment is carried out.

---

(2)进行分式析因试验：通过单因素试验得出的结论得到各单因素的高水平和低水平，运用Minitab19软件得到分式析因设计表，根据表格设置相应处理进行试验。

Before cultivation, wash the duckweed with 2 mg/L copper sulfate solution to inhibit algal growth. Record the number of duckweed leaves every 2 days. Cultivate for 10 days without changing the culture medium, and replenish water with deionized water.

培养前用2mg/L硫酸铜溶液清洗青萍抑制藻类生长，每2天记录青萍的叶片数，培养10天，培养期间不更换培养液，用去离子水补充水分。

The experimental results were processed using Minitab19 for factor analysis.

实验结果用Minitab19处理进行因子分析。

The fractional factorial design and results are shown in Table 1.

分式析因设计及结果见表1。

[n0058]

Table 1. Fractional Factorial Design (Main Factor Screening Design)

表1分式析因设计(主效因子筛选设计)

[n0060]

As shown in Figure 5, concentration, NH4NER6NER7, and the logarithm of the nitrogen-phosphorus ratio are the main factors affecting the growth of *Lysimachia christinae*. The optimal conditions obtained from the single-factor experiments are the zero level of the Box-Behnken experimental design.

由图5可知，浓度、NH₄⁺、氮磷比对数为影响青萍生长的主效因子，以单因素试验所得最适条件为Box-Behnken试验设计的零水平。

[n0061]

(3) Conduct response surface optimization experiments: Box-Behnken experimental design was carried out on the three main factors obtained by the fractional factorial design. The

corresponding treatments obtained from the design table were tested. Before cultivation, duckweed was washed with 2 mg/L copper sulfate solution to inhibit algae growth. The number of duckweed leaves was recorded every 2 days. The culture was carried out for 10 days. The culture medium was not changed during the cultivation period. After algae appeared, they were removed manually. Deionized water was used to replenish the water.

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(3)进行响应面优化试验：根据分式析因设计得到的三个主效因子对其进行Box-Behnken试验设计，根据设计表格得到的相应处理进行试验，培养前用2mg/L硫酸铜溶液清洗青萍以抑制藻类生长，每2天记录青萍的叶片数，培养10天，培养期间不更换培养液，出现藻类后人工去除，补充水分用去离子水。

The experimental results were processed using Minitab19 for response surface optimization analysis.

实验结果用Minitab19处理进行响应面优化分析。

The response surface design and results are shown in Table 2.

响应面设计及结果见表2。

[n0062]

Table 2 Response Surface Optimization Design

表2响应面优化设计

[n0065]

(4) Perform multiple regression analysis based on the data from step (3) and establish a quadratic multiple regression model equation: Y = 0.139 + 0.1693X1 + 0.00093X2 + 0.202X3 - 0.0662X1*X1 - 0.000020X2*X2 - 0.0600X3*X3 + 0.000690X1*X2 - 0.0268X1*X3 - 0.000281X2*X3.

---

(4)根据步骤(3)的数据进行多元回归分析，建立二次多元回归模型方程：Y＝0.139+0.1693X1+0.00093X2+0.202X3-0.0662X1*X1-0.000020X2*X2-0.0600X3*X3+0.000690X1*X2-0.0268X1*X3-0.000281X2*X3。

Where the response value Y is the growth rate of the Aquatic Plantago asiatica ratio, X1 is the concentration of the culture medium, X2 is the proportion of ammonium nitrogen, and X3 is the logarithm of the nitrogen-phosphorus concentration ratio (10).

其中响应值Y为青萍比增长率，X1为培养液浓度、X2为铵态氮比例、X3为氮磷浓度比的10为底对数。

[n0066]

(5) Using Minitab19 software, the relationship between the independent variable and the response value Y is plotted and analyzed based on the quadratic multiple regression model to obtain the response surface plot of the regression equation, which is used to indicate the optimal nutrient solution conditions for duckweed growth, thereby obtaining the optimal conditions for duckweed growth.

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(5)利用Minitab19软件根据二次多元回归模型进行绘图分析自变量和响应值Y的关系，得到回归方程的响应面图，用于指示浮萍生长的营养液最优条件，从而得到浮萍生长的最适条件。

[n0067]

Figure 4 is a three-dimensional response surface plot of the logarithm of culture medium concentration, ammonium nitrogen ratio, and nitrogen-phosphorus concentration ratio to the growth rate of *Leptochloa crus-galli* in this embodiment. In the figure, a is a three-dimensional response surface plot of concentration and ammonium nitrogen ratio to the growth rate of *Leptochloa crus-galli*, b is a three-dimensional response surface plot of concentration and nitrogen-phosphorus concentration ratio to the growth rate of *Leptochloa crus-galli*, and c is a three-dimensional response surface plot of the logarithm of ammonium nitrogen ratio and nitrogen-phosphorus concentration ratio to the growth rate of *Leptochloa crus-galli*.

图4为本实施例培养液浓度、铵态氮比例、氮磷浓度比的10为底对数对青萍比增长率的响应面三维图，其中，a为浓度和铵态氮比例对青萍比增长率的响应面三维图，b为浓度和氮磷浓度比的10为底对数对青萍比增长率的响应面三维图，c为铵态氮比例和氮磷浓度比的10为底对数对青萍比增长率的响应面三维图。

It can be seen that the three effects interact with each other.

可以看出，三种效应存在交互作用。

[n0068]

According to the model, the optimal nutrient concentration for cultivating *Peperomia stenoptera* is 1.187 times the normal concentration, the ammonium nitrogen ratio is 0.523, and the lg(N/P) ratio is 1.340.

根据模型可知，青萍培养的最适培养液养分为1.187倍浓度，铵态氮比例为0.523，lg(N/P)为1.340。

Under these conditions, the maximum specific growth rate of duckweed is 0.390.

在此条件下青萍最大比增长率为0.390。

[n0069]

Example 2

实施例2

[n0070]

The main materials in this embodiment are: KNO₃, NH₄H₂PO₄, Hoagland culture medium (one concentration), deionized water, HCl, NaOH, CuSO₄, and duckweed.

本实施例的主要材料：KNO₃，NH₄H₂PO₄，Hoagland培养液(一倍浓度)，去离子水，HCl，NaOH，CuSO₄，紫萍。

[n0071]

(1) Single-factor experiments were conducted: The original culture conditions were set as pH 6.0, light intensity 2500 lx, photoperiod 16 h/d, temperature 25°C, and 1 times Hoagland culture medium for culturing *Pediatrica spp.*. Single-factor experiments were designed, and the following treatments were made for each factor: pH: 3, 4, 5, 6, 7, 8, 9, 10, 11 (nine levels); phosphorus concentration: 0 mmol/L, 0.25 mmol/L, 0.5 mmol/L, 1 mmol/L, 2 mmol/L, 4 mmol

/L (six levels); culture medium concentration: 0.25×, 0.5×, 1×, 1.5×, 2×, 4× (six levels); ammonium nitrogen ratio: 0%, 20%, 40%, 60%, 80%, 100% (six levels).

---

(1)进行单因素试验：设置原始培养条件为pH 6.0、光照强度2500lx，光照周期16h/d、温度25°C、1倍Hoagland培养液培养青萍，设计单因素试验，对每个因素做出如下处理：pH：3、4、5、6、7、8、9、10、11九个水平；磷浓度为0mmol/L、0.25mmol/L、0.5mmol/L、1mmol/L、2mmol/L、4mmol/L六个水平；培养液浓度为0.25×、0.5×、1×、1.5×、2×、4×六个水平；铵态氮比例为0%、20%、40%、60%、80%、100%六个水平。

When conducting each single-factor experiment, the other factors are kept at the original conditions.

在进行每个单因素试验时，其余因素保持原始条件的水平。

Four replicates were set up at each level. The culture was carried out in disposable plastic cups with a volume of 300ml and 250ml of culture medium. The fresh weight, number of plants and number of leaves of the duckweed were recorded every 3 days. Algae and insects attached to the duckweed were removed before inoculation. Copper sulfate of 2mg/L was sprayed. The culture was carried out for 15 days. During the culture period, the order of the culture cups was rotated. The culture medium was not changed. Deionized water was used to replenish the water.

各水平设置4个重复，用容积为300ml的塑料一次性水杯培养，培养液为250ml，每3天记录紫萍的鲜重、株数和叶片数，接种时去除紫萍附着藻类与昆虫，喷洒2mg/L硫酸铜，培养15天，培养期间轮换培养杯顺序，不更换培养液，用去离子水补充水分。

The experimental results were analyzed using an Excel pivot table.

试验结果用EXCEL数据透视表进行分析。

The results are shown in Figure 5.

结果见图5。

It can be seen that the optimal single-factor culture conditions for *Lemna minor* are pH=6, phosphorus concentration=1mmol/L, culture medium concentration=1, and ammonium nitrogen ratio=40%.

可以看出，紫萍最适的单因素培养条件为pH＝6、磷浓度＝1mmol/L、培养液浓度为1倍、铵态氮比例为40%。

[n0072]

(2) Conduct factorial experiments: The high and low levels of each single factor are obtained from the conclusions drawn from the single factor experiments. The factorial design table is obtained using Minitab19 software. The corresponding treatments are set according to the table and the experiment is carried out.

---

(2)进行分式析因试验：通过单因素试验得出的结论得到各单因素的高水平和低水平，运用Minitab19软件得到分式析因设计表，根据表格设置相应处理进行试验。

Before cultivation, wash the duckweed with 2 mg/L copper sulfate solution to inhibit algal growth. Record the number of duckweed leaves every 2 days. Cultivate for 10 days without changing the culture medium, and replenish water with deionized water.

培养前用2mg/L硫酸铜溶液清洗紫萍抑制藻类生长，每2天记录紫萍的叶片数，培养10天，培养期间不更换培养液，用去离子水补充水分。

The experimental results were processed using Minitab19 for factor analysis.

实验结果用Minitab19处理进行因子分析。

The fractional factorial design and results are shown in Table 3.

分式析因设计及结果见表3。

[n0073]

Table 3. Fractional Factorial Design (Main Factor Screening Design)

表3分式析因设计(主效因子筛选设计)

[n0075]

As shown in Figure 5, the concentrations of NH4NER13 and NER14, and the logarithm of the nitrogen-phosphorus ratio are the main factors affecting the growth of *Lemna minor*. The optimal conditions obtained from the single-factor experiments are the zero level of the Box-Behnken experimental design.

由图5可知，浓度，NH₄⁺，氮磷比对数为影响紫萍生长的主效因子，以单因素试验所得最适条件为Box-Behnken试验设计的零水平。

[n0076]

(3) Conduct response surface optimization experiments: Box-Behnken experimental design was carried out on the three main factors obtained by the fractional factorial design. The corresponding treatments obtained by the design table were tested. Before cultivation,

duckweed was washed with 2 mg/L copper sulfate solution to inhibit algae growth. The number of duckweed leaves was recorded every 2 days. The culture was carried out for 10 days. The culture medium was not changed during the cultivation period. Algae were removed manually after they appeared. Deionized water was used to replenish the water.

---

(3)进行响应面优化试验：根据分式析因设计得到的三个主要因素对其进行Box-Behnken试验设计，根据设计表格得到的相应处理进行试验，培养前用2mg/L硫酸铜溶液清洗紫萍以抑制藻类生长，每2天记录紫萍的叶片数，培养10天，培养期间不更换培养液，出现藻类后人工去除，补充水分用去离子水。

The experimental results were processed using Minitab19 for response surface optimization analysis.

实验结果用Minitab19处理进行响应面优化分析。

The response surface design and results are shown in Table 4.

响应面设计及结果见表4。

[n0077]

Table 4 Response Surface Optimization Design

表4响应面优化设计

[n0080]

(4) Perform multiple regression analysis based on the data from step (3) and establish a quadratic multiple regression model equation: Y = 0.104 + 0.0853X1 - 0.00031X2 + 0.158X3 - 0.0263X1*X1 - 0.000003X2*X2 - 0.0410X3*X3 + 0.000843X1*X2 - 0.0420X1*X3 - 0.000792X2*X3.

---

(4)根据步骤(3)的数据进行多元回归分析，建立二次多元回归模型方程：Y＝0.104+0.0853X1-0.00031X2+0.158X3-0.0263X1*X1-0.000003X2*X2-0.0410X3*X3+0.000843X1*X2-0.0420X1*X3-0.000792X2*X3。

Where the response value Y is the growth rate of the aquatic plant, X1 is the concentration of the culture medium, X2 is the proportion of ammonium nitrogen, and X3 is the logarithm of the nitrogen-phosphorus concentration ratio (10).

其中响应值Y为紫萍比增长率，X1为培养液浓度、X2为铵态氮比例、X3为氮磷浓度比的10为底对数。

[n0081]

(5) Using Minitab19 software, the relationship between the independent variable and the response value Y is plotted and analyzed based on the quadratic multiple regression model to obtain the response surface plot of the regression equation and the optimal conditions for duckweed growth are obtained.

---

(5)利用Minitab19软件根据二次多元回归模型进行绘图分析自变量和响应值Y的关系，得到回归方程的响应面图，得到浮萍生长的最适条件。

[n0082]

Figure 6 shows the three-dimensional response surface plots of the 10-base logarithm of culture medium concentration, ammonium nitrogen ratio, and nitrogen-phosphorus ratio to the growth rate of *Leptochloa crus-galli*. In the figure, a is the three-dimensional response surface plot of the 10-base logarithm of concentration and nitrogen-phosphorus ratio to the growth rate of *Leptochloa crus-galli*, b is the three-dimensional response surface plot of the concentration and ammonium nitrogen ratio to the growth rate of *Leptochloa crus-galli*, and c is the three-dimensional response surface plot of the 10-base logarithm of ammonium nitrogen ratio and nitrogen-phosphorus ratio to the growth rate of *Leptochloa crus-galli*.

图6为培养液浓度、铵态氮比例、氮磷浓度比的10为底对数对紫萍比增长率的响应面三维图，其中，a为浓度和氮磷浓度比的10为底对数对紫萍比增长率的响应面三维图，b为浓度和铵态氮比例对紫萍

比增长率的响应面三维图，c为铵态氮比例和氮磷浓度比的10为底对数对紫萍比增长率的响应面三维图。

It can be seen that the three effects interact with each other.

可以看出，三种效应存在交互作用。

[n0083]

According to the model, the optimal nutrient concentration for culturing *Lysimachia christinae* is 0.936 times the concentration of nutrients, with an ammonium nitrogen ratio of 20% and a lg(N/P) ratio of 1.25.

根据模型可知，紫萍培养的最适培养液养分为0.936倍浓度，铵态氮比例为20%，lg(N/P)为1.25。

Under these conditions, the maximum growth rate of *Lysimachia christinae* is 0.231.

在此条件下紫萍最大的生长速率为0.231。

[n0084]

The preferred embodiments of the present invention have been described in detail above.

以上详细描述了本发明的较佳具体实施例。

It should be understood that those skilled in the art can make numerous modifications and variations based on the concept of this invention without creative effort.

应当理解，本领域的普通技术人员无需创造性劳动就可以根据本发明的构思作出诸多修改和变化。

Therefore, any technical solution that can be obtained by those skilled in the art based on the concept of this invention and through logical analysis, reasoning, or limited experimentation on the basis of the prior art should be within the scope of protection defined by the claims.

因此，凡本技术领域中技术人员依本发明的构思在现有技术的基础上通过逻辑分析、推理或者有限的实验可以得到的技术方案，皆应在由权利要求书所确定的保护范围内。