

## Notice

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## DESCRIPTION CN118894603A

Methods to enhance cadmium accumulation in duckweed using dopamine

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利用多巴胺增强浮萍镉积累的方法

[0001]

Technical Field

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技术领域

**[n0001]**

This invention belongs to the field of phytoremediation of water bodies containing heavy metal cadmium, specifically relating to a method for enhancing cadmium accumulation in duckweed using dopamine.

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本发明属于植物修复水体重金属镉技术领域，具体来说涉及一种利用多巴胺增强浮萍镉积累的方法。

**[0003]**

Background Technology

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背景技术

**[n0002]**

Cadmium pollution in water bodies poses a serious threat to human health and the ecological environment. However, remediating cadmium pollution is difficult and lacks effective methods.

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水体镉污染严重威胁人体健康和生态环境，然而，修复镉污染难度大且缺少有效方法。

Duckweed is characterized by its wide distribution, ease of acquisition, and strong growth and reproduction capabilities, making it a potential cadmium hyperaccumulator.

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浮萍具有分布广，易获取和生长繁殖能力强等特点，是潜在的镉超富集植物。

Duckweed can remove cadmium ions from water through a bioaccumulation process. In 25L of untreated industrial wastewater, 200g (fresh weight) of duckweed (*Lemna gibba* L.) can be removed.

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浮萍可以通过生物蓄积过程去除水体中的镉离子，在25L未经处理的工业废水中，生长的200g(鲜重)浮萍(*Lemna gibba* L.)

Under greenhouse conditions and natural light for 21 days, the Cd concentration was reduced from 0.74 mg/L to 0.071 mg/L, demonstrating a highly efficient cadmium wastewater purification capability. However, the treatment cycle is relatively long, and the cadmium adsorption capacity of duckweed needs to be improved. Therefore, finding methods to promote the adsorption of cadmium ions by duckweed is of great significance.

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在温室条件下接受自然光周期21天，将Cd浓度从0.74mg/L降低至0.071mg/L，具备高效镉污水净化能力，然而该处理周期较长，浮萍吸附镉能力有待提升。因此，寻找促进浮萍吸附镉离子的方法具有重要意义。

**[0005]**

Summary of the Invention

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发明内容

**[n0003]**

To address the shortcomings of existing technologies, the present invention aims to provide a method for enhancing cadmium accumulation in duckweed using dopamine.

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针对现有技术的不足，本发明的目的在于提供一种利用多巴胺增强浮萍镉积累的方法。

**[n0004]**

The objective of this invention is achieved through the following technical solution.

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本发明的目的是通过下述技术方案予以实现的。

**[n0005]**

A method for enhancing cadmium accumulation in duckweed using dopamine includes the following steps:

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一种利用多巴胺增强浮萍镉积累的方法，包括以下步骤：

[n0006]

Step 1: Add dopamine to the culture medium to obtain a culture solution. Cultivate duckweed in the culture solution for at least 23 hours to obtain pretreated duckweed. Add dopamine to the liquid to be treated to be adsorbed with  $\text{Cd}^{2+}$ . Add the pretreated duckweed to the liquid to be treated and cultivate for at least 23 hours. The dopamine concentration in both the culture solution and the liquid to be treated after adding dopamine is  $C_i$ . Make  $C_i$  take different values. Based on the phenotype of the pretreated duckweed after cultivation, determine the  $C_i$  corresponding to the optimal phenotype after cultivation when  $C_i$  takes different values.  $C_{\max}$  is the optimal phenotype after cultivation.

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步骤1，向培养基中加入多巴胺，得到培养液，将浮萍在培养液中培养至少23h，得到预处理浮萍；向待被吸附 $\text{Cd}^{2+}$ 的待处理液体中加入多巴胺，将预处理浮萍加入待处理液体中培养至少23h，其中，培养液和加入多巴胺后待处理液体中多巴胺浓度均为 $C_i$ ，使 $C_i$ 取不同值，根据预处理浮萍的培养后表型确定 $C_i$ 取不同值时最优培养后表型所对应的 $C_i$ 为 $C_{\max}$ ；

#### [n0007]

In step 1, the amount of duckweed added to each 1L of culture medium is 3-5g, and the amount of pretreated duckweed added to each 1L of liquid to be treated is 3-5g.

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在所述步骤1中，每1L所述培养液中加入浮萍的量为3~5g，每1L所述待处理液体中加入预处理浮萍的量为3~5g。

#### [n0008]

In step 1,  $0 < C_{\text{sub}i}$ , preferably  $0 < C_{\text{sub}i} < 500 \mu\text{M}$ .

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在所述步骤1中， $0 < C_{\text{sub}i}$ ，优选为 $0 < C_{\text{sub}i} < 500 \mu\text{M}$ 。

#### [n0009]

In step 1, the phenotype of the pretreated duckweed after cultivation is determined based on the root breakage rate, and the optimal phenotype after cultivation is 1 to 3 with the lowest root breakage rate.

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在所述步骤1中，根据断根率确定预处理浮萍的培养后表型，最优培养后表型为断根率最小的1~3个。

## [n0010]

Step 2: Place the pretreated duckweed in a culture medium containing dopamine at a concentration of  $C_{\text{max}}$  and culture for at least 23 hours to obtain duckweed ready for use;

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步骤2，将预处理浮萍放入含有多巴胺浓度为 $C_{\text{max}}$ 的培养基中培养至少23h，得到待使用浮萍；

## [n0011]

In step 2, the amount of pretreated duckweed added to each 1L of the culture medium is 3-5g.

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在所述步骤2中，每1L所述培养基中加入预处理浮萍的量为3~5g。

## [n0012]

Step 3, Formal Treatment: Add dopamine to the liquid to be treated ( $C_{\text{d}^{2+}}$  to be adsorbed) so that the concentration of dopamine in the liquid to be treated ( $C_{\text{d}^{2+}}$  to be adsorbed) is  $C_{\text{max}}$ . Then add duckweed to be used and incubate for at least 23 hours.

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步骤3，正式处理：向待被吸附 $\text{Cd}^{2+}$ 的待处理液体中加入多巴胺，以使待被吸附 $\text{Cd}^{2+}$ 的待处理液体中多巴胺的浓度为 $C_{\text{max}}$ ，再加入待使用浮萍，培养至少23h。

### [n0013]

In step 3, the amount of duckweed to be used added to each 1L of the liquid to be treated is 3-5g.

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在所述步骤3中，每1L所述待处理液体中加入待使用浮萍的量为3~5g。

### [n0014]

In the above technical solution, the culture medium is Datko medium.

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在上述技术方案中，所述培养基为Datko培养基。

### [n0015]

In the above technical solution, the culture temperature is 20-25°C.

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在上述技术方案中，所述培养的温度为20~25°C。



**[n0016]**

The use of dopamine in increasing cadmium accumulation in duckweed.

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多巴胺在提高浮萍镉积累中的用途。

**[n0017]**

This invention discovers that dopamine enhances the cadmium resistance and cadmium accumulation capacity of duckweed, increasing its cadmium accumulation capacity by 30%, thus enabling it to be used in the remediation of cadmium pollution in water bodies.

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本发明发现多巴胺增强浮萍镉抗性和镉富集能力，使浮萍镉富集能力增强30%，使其具备应用于修复水体镉污染的实际能力。

**[0021]**

Attached Figure Description

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附图说明

**[n0018]**

Figure 1 shows (a) growth status (scale bar 1cm), (b) root breakage rate statistics and (c) photographs of Examples 1-5, Comparative Example 1 and CK group (CK in Figure 1);

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图1为实施例1~5、对比例1和CK组(图1中CK)的(a)生长状况(标尺1cm)、(b)断根率统计和(c)照片；

[n0019]

Figure 2 shows the cadmium content in duckweed obtained in Example 3 and Comparative Example 1.

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图2为实施例3和对比例1得到浮萍中镉含量。

[0024]

Detailed Implementation

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具体实施方式

[n0020]

The technical solution of the present invention will be further described below with reference to specific embodiments.

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下面结合具体实施例进一步说明本发明的技术方案。

#### [n0021]

The duckweed (*Lemna turionifera* 5511) was harvested from a freshwater lake in Xiqing District, Tianjin.

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浮萍(*Lemna turionifera* 5511)采摘自天津市西青区淡水湖。

#### [n0022]

After harvesting, the duckweed was washed with deionized water, soaked in 15wt% NaClO aqueous solution for 10 min, and then washed 4 times with sterile water. Before use, it was cultured in Datko medium at a temperature of  $23\pm 2^{\circ}\text{C}$  under long-day conditions (16h light /8h dark) with a light intensity of  $45\mu\text{mol m}^{-2}\text{s}^{-1}$ .

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浮萍在采摘后经去离子水清洗，用15wt%NaClO水溶液浸泡10min，再用无菌水清洗4次，使用前培养于Datko培养基中，培养温度 $23\pm 2^{\circ}\text{C}$ ，长日照光照(16h光照/8h黑暗)，光照强度为 $45\mu\text{mol m}^{-2}\text{s}^{-1}$ 。

#### [n0023]

All the culture media described below are Datko media, and the Datko media formulation is shown in Table 1.

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下述培养基均为Datko培养基，Datko培养基配方如表1所示。

[n0024]

Table 1. Datko medium (pH 5.6-5.8) formulation

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表1Datko培养基(PH 5.6-5.8)配方

[n0027]

The specific steps for ICP testing of cadmium content in duckweed are as follows: After harvesting the duckweed, dry it at 60°C for 6 hours and nitrify it overnight.

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ICP检测浮萍中镉含量的具体步骤如下：收获浮萍后，在60°C下干燥6h，硝化过夜。

[n0028]

In the following examples, an aqueous solution of CdCl<sub>2</sub> was used to simulate the liquid to be treated by adsorbing Cd<sup>2+</sup>, and the concentration of CdCl<sub>2</sub> in the aqueous solution was 50 μM.

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下述实施例中采用CdCl<sub>2</sub>水溶液模拟待被吸附Cd<sup>2+</sup>的待处理液体，CdCl<sub>2</sub>水溶液中CdCl<sub>2</sub>的浓度为50μM。

### [n0029]

In the examples described below, the culture temperature was 23±2°C, with long day illumination (16h light/8h darkness) and a light intensity of 45μmol m<sup>-2</sup>s<sup>-1</sup>.

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下述实施例中培养的温度为23±2°C，长日照光照(16h光照/8h黑暗)，光照强度为45μmol m<sup>-2</sup>s<sup>-1</sup>。

### [n0030]

#### Comparative Example 1

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#### 对比例1

[n0031]

Duckweed was added to the liquid to be treated to adsorb Cd<sup>2+</sup> and cultured for 48 h. The amount of duckweed added to each 1 L of liquid to be treated was 4 g.

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向待被吸附Cd<sup>2+</sup>的待处理液体中加入浮萍，培养48h，其中，每1L待处理液体中加入浮萍的量为4g。

The growth status of duckweed at 24 and 48 hours of cultivation is shown in Figure 1a, the root breakage rate at 24 hours of cultivation is shown in Figure 1b, and the photograph is shown in Figure 1c.

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在培养的第24和48小时，浮萍生长状况如图1的a所示，在培养的第24小时的断根率如图1的b所示、照片如图1的c所示。

The cadmium content in duckweed was determined by ICP (Inductively Coupled Plasma Emission Spectrometry, Agilent ICP-OES 725ES) after 48 hours of cultivation.

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利用ICP(电感耦合等离子体发射光谱仪，ICP,Agilent ICP-OES 725ES)检测培养48h后得到浮萍中镉含量。

[n0032]

CK group

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CK组

**[n0033]**

Duckweed was cultured in Datko medium for 48 hours. The growth status of duckweed at the 24th and 48th hours of culture is shown in Figure 1a. The root breakage rate at the 24th hour of culture is shown in Figure 1b. The photograph is shown in Figure 1c.

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将浮萍在Datko培养基中培养48h，在培养的第24和48小时浮萍的生长状况如图1的a所示，在培养的第24小时的断根率如图1的b所示、照片如图1的c所示。

**[n0034]**

Examples 1-5

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实施例1～5

**[n0035]**

A method for enhancing cadmium accumulation in duckweed using dopamine includes the following steps:

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一种利用多巴胺增强浮萍镉积累的方法，包括以下步骤：

[n0036]

Step 1: Add dopamine to the culture medium to obtain a culture solution. Cultivate duckweed in the culture solution for 24 hours to obtain pretreated duckweed. Add dopamine to the liquid to be treated (containing  $Cd^{2+}$  to be adsorbed), then add the pretreated duckweed and cultivate for 48 hours. The dopamine concentration in both the culture solution and the liquid to be treated after adding dopamine is  $C_i$ , allowing  $C_i$  to take different values. The phenotype of the pretreated duckweed after cultivation is determined based on the root breakage rate. The phenotypes and growth status at 24 and 48 hours are shown in Figure 1a, and the root breakage rate at 24 hours is shown in Figure 1b. The optimal phenotypes after pretreatment of duckweed with different  $C_{NER25}$  values were determined to be  $C_{NER26}$ , which corresponds to  $C_{NER27}$ . The optimal phenotypes after pretreatment were the two with the lowest root breakage rates. 4g of duckweed was added to each 1L of culture medium, and 4g of pretreated duckweed was added to each 1L of the liquid to be treated. The values of  $C_{NER28}$  are shown in Table 2.

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步骤1，向培养基中加入多巴胺，得到培养液，将浮萍在培养液中培养24h，得到预处理浮萍；向待被吸附Cd<sup>2+</sup>的待处理液体中加入多巴胺，再加入预处理浮萍，培养48h，其中，培养液和加入多巴胺后待处理液体中多巴胺浓度均为C<sub>i</sub>，使C<sub>i</sub>取不同值，根据预处理浮萍的培养后表型(根据断根率确定预处理浮萍的培养后表型，在第24和48小时的生长状况如图1的a所示，在第24小时的断根率如图1的b所示)确定C<sub>i</sub>取不同值时预处理浮萍最优培养后表型所对应的C<sub>i</sub>为C<sub>max</sub>，最优培养后表型为断根率最小的2个，每1L培养液中加入浮萍的量为4g，每1L待处理液体中加入预处理浮萍的量为4g；C<sub>i</sub>的取值如表2所示。

[n0037]

Table 2 shows the values of C<sub>i</sub>.

表2C<sub>i</sub>的取值

[n0038]

Example <!

实施例 <!

[CDATA[C<sub>i</sub>(unit: μM)]]> Example 1 10 Example 2 20 Example 3 50 Example 4 100  
Example 5 200

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[CDATA[C<sub>i</sub>(单位： μM)]]> 实施例1 10 实施例2 20 实施例3 50 实施例4 100 实施例5 200

[n0039]

As shown in Figure 1, by comparing Examples 1-5 and Comparative Example 1, it can be seen that the addition of exogenous dopamine significantly improved the cadmium resistance of duckweed.

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如图1所示，通过对比实施例1～5以及对比例1可知，外源多巴胺的添加显著提升了浮萍的镉抗性。

As can be seen in Figure 1a, the yellowing of the leaves was alleviated by exogenous dopamine.

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可以在图1的a中看到，叶片的黄化情况被外源多巴胺缓解。

As shown in Figure 1b, the root breakage rate of duckweed was 0% without the addition of cadmium (CK group), while the root breakage rates of duckweed were 21.1%, 20.7%, 10.4%, 6.6%, 6.2% and 7.9% after applying cadmium stress (Comparative Example 1) and adding 10, 20, 50, 100 and 200 μM dopamine, respectively.

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同时如图1的b所示，在不添加镉时浮萍的断根率为0%(CK组)，而在施加镉胁迫(对比例1)和分别添加10、20、50、100和200 $\mu$ M多巴胺后，浮萍的断根率分别为21.1%,20.7%,10.4%,6.6%,6.2%和7.9%。

It can be seen that the root breakage rate of duckweed was significantly reduced with the addition of exogenous dopamine. The two lowest root breakage rates were observed when 50  $\mu$ M and 100  $\mu$ M dopamine were added. Considering the cost,  $C_{i} = 50 \mu\text{M}$  with a lower dopamine concentration was selected as  $C_{\text{max}}$ .

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可以看出浮萍的断根率在外源多巴胺的添加下被显著降低，最小的2个断根率在外源添加50 $\mu$ M和100 $\mu$ M多巴胺时被观察到，考虑到成本，选取多巴胺浓度更低的 $C_{i} = 50\mu\text{M}$ 作为 $C_{\text{max}}$ 。

Furthermore, as can be seen in Figure 1c, the addition of  $C_{i} = 50\mu\text{M}$  (Example 3) dopamine significantly reduced the root breakage rate of duckweed.

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另外在图1的c中也可以看出， $C_{i} = 50\mu\text{M}$ (实施例3)多巴胺的添加显著降低了浮萍的断根率。

Therefore,  $C_{\text{max}} = 50\mu\text{M}$  was selected.

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因此，选择 $C_{\text{max}} = 50\mu\text{M}$ 。

#### [n0040]

Step 2: Place the pretreated duckweed in a culture medium containing dopamine at a concentration of  $C_{\text{max}}$  and culture for 24 hours to obtain duckweed ready for use; wherein, the amount of pretreated duckweed added to each 1L of culture medium is 4g.

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步骤2，将预处理浮萍放入含有多巴胺浓度为 $C_{\text{max}}$ 的培养基中培养24h，得到待使用浮萍；其中，每1L培养基中加入预处理浮萍的量为4g。

#### [n0041]

Step 3, Formal Treatment: Add dopamine to the liquid to be treated ( $\text{Cd}^{2+}$  to be adsorbed) to make the dopamine concentration in the liquid to be treated ( $\text{Cd}^{2+}$  to be adsorbed) equal to that of  $C_{\text{max}}$ . Then add duckweed to be used and incubate for 48 hours. The amount of duckweed to be used added to each 1L of liquid to be treated is 4g. The cadmium content in the duckweed after 48 hours of incubation is detected by ICP (Inductively Coupled Plasma Emission Spectrometry, ICP, Agilent ICP-OES 725ES).

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步骤3，正式处理：向待被吸附 $\text{Cd}^{2+}$ 的待处理液体中加入多巴胺，以使待被吸附 $\text{Cd}^{2+}$ 的待处理液体中多巴胺的浓度为 $C_{\text{max}}$ ，再加入待使用浮萍，培养48h，其中，每1L待处理液体中加入待使用浮萍的量为4g，利用ICP(电感耦合等离子体发射光谱仪，ICP,Agilent ICP-OES 725ES)检测培养48h后得到浮萍中镉含量。

[n0042]

As shown in Figure 2, the cadmium content in the duckweed obtained in Comparative Example 1 was 341.37 mg/kg, while the cadmium content in the duckweed obtained in Example 3 was 442.41 mg/kg. It can be seen that the dopamine treatment enhanced the cadmium accumulation capacity of duckweed by 30%, making it capable of being used to remediate cadmium pollution in water bodies.

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通过实施例3和对比例1的ICP测试可知，如图2所示，对比例1所得浮萍内镉含量为341.37mg/kg，实施例3得到浮萍中镉含量为442.41mg/kg，可见多巴胺处理下，将浮萍镉富集能力增强30%，使其具备应用于修复水体镉污染的实际能力。

[n0043]

The present invention has been described above by way of example. It should be noted that any simple modifications, alterations or other equivalent substitutions that can be made by those skilled in the art without creative effort without departing from the core of the present invention fall within the protection scope of the present invention.

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以上对本发明做了示例性的描述，应该说明的是，在不脱离本发明的核心的情况下，任何简单的变形、修改或者其他本领域技术人员能够不花费创造性劳动的等同替换均落入本发明的保护范围。