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(54) Title: HUMIC ACID-RICH BIOLOGIC SOIL CONDITIONER MADE FROM ALCOHOL WASTE LIQUID AND SLUDGE FROM SUGAR MILL

(57) Abstract: Provided is a humic acid-rich biologic soil conditioner comprising acid & alkali-treated alcohol waste liquid from a sugar mill, peat, and microbe-fermented sludge from a sugar mill. The conditioner is used for the prolongation of fertilizer efficiency and the decrease of heavy metal pollution of plants. Also provided are a microbial starter culture and use thereof.

HUMIC ACID-RICH BIOLOGIC SOIL CONDITIONER MADE FROM ALCOHOL WASTE LIQUID AND SLUDGE FROM SUGAR MILL

FIELD OF THE INVENTION

The invention belongs to the field of fertilizer technology. Specifically the invention relates to a humic acid-rich biologic soil conditioner made from alcohol waste liquid and sludge from a sugar mill and peat.

BACKGROUND OF THE INVENTION

According to prior arts, an organic fertilizer or an organic compound fertilizer may be made from sludge, bagasse, mud, and/or alcohol waste liquid by microbial fermentation.

For example, the patent, CN1171384A, discloses a process for producing an organic compound fertilizer by utilizing molasses alcohol waste liquid, which comprises the steps of mixing bagasse, alcohol waste liquid, sludge, and mud for fermentation by soil microbes.

The patent, CN1327965A, discloses a process for preparing microbial fertilizer by utilizing sludge and waste liquid from a sugar mill, which comprises the steps of culturing nitrogen-fixating bacteria and P/K decomposing bacterium in a culture media added with sludge and/or alcohol waste liquid from sugar mill, and conventional fermentation.

The patent, CN1377958A, discloses a microbial fermentation agent for treating alcohol waste liquid from a sugar mill.

The patent, CN1872803A, discloses a biologic organic fertilizer made from sugar industry waste, breeding industry waste, oil-processing waste, phosphoric fertilizer, trace element fertilizer and silicon fertilizer by using 3 types of microbes including more than 20 species.

The patent, CN101219912A, discloses an aniseed dregs bio-organic fertilizer

made from aniseed dregs, sugar cane sludge, tung bran, organic material for fermentation, and microbes including *Bacillus pumilus*, filamentous fungus, *Actinomyces*, and *Saccharomyces*.

The patent, CN102153413A, discloses an organic and inorganic fertilizer, which comprises green alga, humic acid, bagasse mortar, cork dust, vermiculite, sludge, concentrated solution of organic waste water, bagasse, sugar cane leaves, and microbial liquid.

The patent, CN102826910A, discloses a microbial compound fertilizer, which comprises sugar cane sludge, orange water, tobacco powder, medical stone, *Rehmannia rhizome*, P-K decomposing bacterium, and bentonite.

Although microbes are added in the processes for producing the fertilizers mentioned above, yet both of alcohol waste liquid and sludge from a sugar mill (optionally with other components) must be fermented by additionally adding microbes. Briefly, prior arts teach processes of mixing alcohol waste liquid and sludge for fermentation without selective fermentation of only one of the wastes from a sugar mill.

However, the inventor found that the processes without selective fermentation would decompose some beneficial components (e.g., humic acid), especially those of alcohol waste liquid, and decrease the efficiency of the waste in need of fermentation, resulting in no perfect effects for planting. Therefore, the invention relates to a process comprising a step of specifically fermenting the waste in need by specific microbes, and comprising no step of fermenting the waste unsuitable for fermentation. The process of the invention can retain more beneficial components for promoting the growth of some plants, preferably sugar cane.

Surprisingly, the soil conditioner produced by the process of the invention comprises several specific nutrient components, resulting in not only the supplement of fertilizers, but also the prolongation of fertilizer efficiency and the decrease of heavy metal pollution of plants.

DISCLOSURE OF THE INVENTION

For the solution of the defects of prior arts mentioned above, the invention provides a biologic soil conditioner, wherein the biologic soil conditioner is prepared by acid & alkali-treated alcohol waste liquid from a sugar mill, peat, and microbe-fermented sludge from a sugar mill, and the weight ratio of the acid & alkali-treated alcohol waste liquid from a sugar mill, the peat and the microbe-fermented sludge from a sugar mill is 2000-5000 : 100-300 : 500-800.

In the biologic soil conditioner, the acid & alkali-treated alcohol waste liquid from a sugar mill is the product of alcohol waste liquid from a sugar mill treated by acid and alkali. Preferably the acid & alkali-treated alcohol waste liquid from a sugar mill is prepared by a process consisting of the steps of:

(1) adding 37.5%wt dilute sulfuric acid to alcohol waste liquid from a sugar mill until pH of the waste liquid is less than 1, wherein the waste liquid is reserved in a reservoir;

(2) reserving the waste liquid acquired by step (1) for 5-10 days at normal temperature; and

(3) adding aqueous ammonia to the waste liquid acquired by step (2) until pH of the waste liquid is 6-7, and then acquiring the acid & alkali-treated alcohol waste liquid from a sugar mill.

The term used herein, alcohol waste liquid from a sugar mill, also named as molasses alcohol waste liquid or sugar cane alcohol waste liquid, is acidic organic wastewater from a sugar mill or an alcohol plant of sugar cane. Every year nearly 300 tons of the wastewater are produced and discharged in China. The discharge of the untreated wastewater to a river is generally unallowable due to the pollution from nitrogen, copper and organic matter in the wastewater. Although the wastewater can be used directly for irrigation of sugar cane in China and Brazil, yet the ill-effects of the irrigation include heavy metal pollution of plants, severe plant diseases, and damage of plants due to the viscosity of the wastewater.

In the biologic soil conditioner, the microbe-fermented sludge from a sugar mill is the product of sludge from a sugar mill fermented by microbes. Preferably the microbe-fermented sludge from a sugar mill is prepared by a process consisting of the

steps of:

(I) mixing sludge from a sugar mill and a microbial starter culture, wherein the microbial starter culture comprises *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Aspergillus oryzae* and *Streptomyces microflavus*; and

(II) fermenting the mixture acquired by step (I) for 7-15 days, and then acquiring the microbe-fermented sludge from a sugar mill.

The term used herein, sludge from a sugar mill is the residue produced by clarifying and filtrating sugar cane juice in a process of preparing sugar from sugar cane. The sludge comprises sugar cane fibre, sugar, cerosin, proteins, calcium phosphate for clarifying sugar cane juice and sands from soil of sugar cane. Generally a sugar mill can produce 10000 tons of the sludge per year.

In the microbial starter culture, preferably the weight ratio of *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Aspergillus oryzae* and *Streptomyces microflavus* is 20-40:10-20:15-35:5-15:20-30.

In the microbial starter culture, preferably each of the microbes is provided by the form of microbial powder of each isolated strain, and the number of survival microbes in the powder is more than $100-2 \times 10^9$ /gram. The fermentation of sludge from a sugar mill is based on the common fermentation function of each of the microbes, so commercially-available strains and/or powders of these microbes can be used in the invention.

In the biologic soil conditioner, the peat, also named as turf, peat coal, and/or sooty coal, is the coal with the lowest degree of coalification, and an early component in the formation of humic coal. Peat is an accumulation of partially decayed vegetation, and forms in wetland conditions, where flooding obstructs flows of oxygen from atmosphere. Under proper conditions, peat will turn into lignite coal over geologic periods of time. Peat is soft and easily compressed. Under pressure, water in the peat is forced out. Peat mainly comprises mineral matter and organic matter such as cellulose, hemicellulose, xylogen, humic acid, asphalt and so on.

The invention further provides a process of preparing the biologic soil conditioner mentioned above, wherein the steps of the process are:

A, producing 2000-5000 parts by weight of acid & alkali-treated alcohol waste liquid from a sugar mill;

B, producing 500-800 parts by weight of microbe-fermented sludge from a sugar mill; and

C, mixing the products acquired by steps A and B, and then adding 100-300 parts by weight of peat;

wherein the acid & alkali-treated alcohol waste liquid from a sugar mill is prepared by a process consisting of the steps of:

(1) adding 37.5%wt dilute sulfuric acid to alcohol waste liquid from a sugar mill until pH of the waste liquid is less than 1, wherein the waste liquid is reserved in a reservoir;

(2) reserving the waste liquid acquired by step (1) for 5-10 days at normal temperature; and

(3) adding aqueous ammonia to the waste liquid acquired by step (2) until pH of the waste liquid is 6-7, and then acquiring the acid & alkali-treated alcohol waste liquid from a sugar mill;

and the microbe-fermented sludge from a sugar mill is prepared by a process consisting of the steps of:

(I) mixing sludge from a sugar mill and a microbial starter culture, wherein the microbial starter culture comprises *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Aspergillus oryzae* and *Streptomyces microflavus*; and

(II) fermenting the mixture acquired by step (I) for 7-15 days, and then acquiring the microbe-fermented sludge from a sugar mill.

Preferably the weight ratio of *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Aspergillus oryzae* and *Streptomyces microflavus* is 20-40:10-20:15-35:5-15:20-30.

Preferably each of the microbes in the microbial starter culture is provided by the form of microbial powder of each isolated strain, and the number of survival microbes in the powder is more than $100-2 \times 10^9$ /gram. The strains and/or powders are commercially available. In one embodiment of the invention, 1-2 kg of the

microbial starter culture is added to one ton of the sludge from a sugar mill.

The invention further provides a use of the biologic soil conditioner mentioned above for the prolongation of fertilizer efficiency and the decrease of heavy metal pollution of plants, wherein the dosage of the biologic soil conditioner is 100-1000 kg/667m². Preferably the dosage is 200-800 kg/667m².

The biologic soil conditioner is applied before sowing, during period of growth, or before flowering. In addition, preferably the plant to be applied is sugar cane.

The invention further provides a method of applying the biologic soil conditioner mentioned above, wherein the biologic soil conditioner is applied as basal dressing before sowing, the biologic soil conditioner is applied as side dressing during period of growth, or the biologic soil conditioner is diluted 2-3 times for irrigation.

The invention further provides a microbial starter culture, wherein the microbial starter culture comprises *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Aspergillus oryzae* and *Streptomyces microflavus*.

In the microbial starter culture, preferably the weight ratio of *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Aspergillus oryzae* and *Streptomyces microflavus* is 20-40:10-20:15-35:5-15:20-30.

The invention further provides a use of the microbial starter culture mentioned above for the preparation of a biologic soil conditioner, especially a biologic soil conditioner used for the prolongation of fertilizer efficiency and the decrease of heavy metal pollution of plants.

Sufficiently utilized are nutrient components in the alcohol waste liquid from a sugar mill and sludge from a sugar mill of the biologic soil conditioner of the invention. The alcohol waste liquid from a sugar is treated by acid and alkali without fermentation, resulting in avoiding decomposition of some of nutrient components in the liquid and decreasing toxicity of heavy metal to microbes for fermentation. The sludge from a sugar mill is fermented for the decomposition of components unsuitable for plants and the decrease of the levels of plant-absorbable heavy metals due to the absorption of the heavy metals by microbes.

The biologic soil conditioner of the invention comprises more beneficial nutrient components, resulting in not only the supplement of fertilizers, but also the prolongation of fertilizer efficiency and the decrease of heavy metal pollution of plants.

For a better understanding of the invention, it will now be described in greater detail by reference to specific examples. It should be noted that the examples only exemplify the invention, and should not be construed as limiting the scope of the invention. According to the description of the application, various modifications and alterations of the invention are obvious to a skilled person in the art. The publications cited in the application are used to illustrate the invention, the contents of which are incorporated herein by reference, as if they have been written down herein.

EXAMPLES

The material, microbes and agents used in the examples are commercially available. For example, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Aspergillus oryzae* and *Streptomyces microflavus* were purchased from China General Microbiological Culture Collection Center (CGMCC; cat. No.: AS 1.3376, AS 1.228, AS 3.0276, AS 3.0032, and AS 4.5889), cultured according the recommended guidelines of CGMCC, and lyophilized to form microbial powders, and the alcohol waste liquid and sludge were collected from Guangxi Dongtang Investment Co..

Example 1: Biologic Soil Conditioner of the Invention

10 tons of the alcohol waste liquid were reserved in a reservoir. 37.5%wt sulfuric acid was added into the reservoir until pH of the waste liquid is less than 1. After the pH<1 waste liquid was reserved for 10 days at normal temperature, aqueous ammonia was added to the waste liquid until pH of the waste liquid is 6-7. Hence the acid & alkali-treated alcohol waste liquid was acquired.

4 kg powder of *Bacillus subtilis*, 1 kg powder of *Pseudomonas aeruginosa*, 1.5 kg powder of *Aspergillus niger*, 1.5 kg powder of *Aspergillus oryzae* and 2 kg powder of *Streptomyces microflavus* were mixed thoroughly, and the mixture is the

microbial starter culture for sludge fermentation.

1 ton of the sludge and 2kg of the microbial starter culture were mixed and fermented for 15 days, and the fermented product is the microbe-fermented sludge.

5 tons of the acid & alkali-treated alcohol waste liquid, 0.5 ton of the microbe-fermented sludge and 0.3 ton of peat were mixed thoroughly, and the mixture is the biologic soil conditioner 1.

Example 2: Comparative Biologic Soil Conditioner

5 tons of the (untreated) alcohol waste liquid, 0.5 ton of the (untreated) sludge and 0.3 ton of peat were mixed thoroughly, and 10 kg of the microbial starter culture mentioned in Example 1 were added to the mixture for fermentation for 15 days. Hence the biologic soil conditioner 2 was acquired.

Example 3: Sugar Cane Cultivation by Using Biologic Soil Conditioner

According to the parameters shown in table 1, the biologic soil conditioner 1 mentioned in Example 1 and the biologic soil conditioner 2 mentioned in Example 2 respectively were applied as basal dressing before sowing and as side dressing during the period of growth (also named as the period of elongation; i.e., at the time that 12-13 leaves has grown up in sugar cane). Several indexes including the yield of sugar cane and levels of heavy metals in sugar cane were measured while the sugar cane was harvested. Each of values measured is the mean value calculated from 5 samples, and the area of each of the samples is 667 m².

As shown in table 1, in comparison to the biologic soil conditioner 2, the utilization of the biologic soil conditioner 1 resulted in higher level of yield and bottle green leaves. The results indicate that the fertilizer effect of the biologic soil conditioner of the invention persists until the maturation stage, though it was applied before and/or during the period of growth. More surprisingly, the biologic soil conditioner of the invention can decrease more levels of heavy metals in plants.

Table 1 Effects of Biologic Soil Conditioner

Type of biologic soil conditioner	Dosage of basal dressing (kg/667 m ²)	Dosage of side dressing (kg/667 m ²)	Colour of leaves at the maturation stage	Yield (kg/667 m ²)	Cd (mg/kg)	As (mg/kg)
the biologic soil conditioner 1	300	800	bottle green	4022	0.046	0.017
the biologic soil conditioner 2	300	800	green	3714	0.164	0.152

CLAIMS

1. A biologic soil conditioner, wherein the biologic soil conditioner is prepared by acid & alkali-treated alcohol waste liquid from a sugar mill, peat, and microbe-fermented sludge from a sugar mill, and the weight ratio of the acid & alkali-treated alcohol waste liquid from a sugar mill, the peat and the microbe-fermented sludge from a sugar mill is 2000-5000 : 100-300 : 500-800.

2. The biologic soil conditioner of claim 1, wherein the acid & alkali-treated alcohol waste liquid from a sugar mill is prepared by a process consisting of the steps of:

(1) adding 37.5%wt dilute sulfuric acid to alcohol waste liquid from a sugar mill until pH of the waste liquid is less than 1, wherein the waste liquid is reserved in a reservoir;

(2) reserving the waste liquid acquired by step (1) for 5-10 days at normal temperature; and

(3) adding aqueous ammonia to the waste liquid acquired by step (2) until pH of the waste liquid is 6-7, and then acquiring the acid & alkali-treated alcohol waste liquid from a sugar mill.

3. The biologic soil conditioner of claim 1, wherein the microbe-fermented sludge from a sugar mill is prepared by a process consisting of the steps of:

(I) mixing sludge from a sugar mill and a microbial starter culture, wherein the microbial starter culture comprises *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Aspergillus oryzae* and *Streptomyces microflavus*, preferably the weight ratio of *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Aspergillus oryzae* and *Streptomyces microflavus* is 20-40:10-20:15-35:5-15:20-30; and

(II) fermenting the mixture acquired by step (I) for 7-15 days, and then acquiring the microbe-fermented sludge from a sugar mill.

4. A process of preparing a biologic soil conditioner, wherein the steps of the process are:

A, producing 2000-5000 parts by weight of acid & alkali-treated alcohol waste liquid from a sugar mill;

B, producing 500-800 parts by weight of microbe-fermented sludge from a sugar mill; and

C, mixing the products acquired by steps A and B, and then adding 100-300 parts by weight of peat;

wherein the acid & alkali-treated alcohol waste liquid from a sugar mill is prepared by a process consisting of the steps of:

(1) adding 37.5%wt dilute sulfuric acid to alcohol waste liquid from a sugar mill until pH of the waste liquid is less than 1, wherein the waste liquid is reserved in a reservoir;

(2) reserving the waste liquid acquired by step (1) for 5-10 days at normal temperature; and

(3) adding aqueous ammonia to the waste liquid acquired by step (2) until pH of the waste liquid is 6-7, and then acquiring the acid & alkali-treated alcohol waste liquid from a sugar mill;

and the microbe-fermented sludge from a sugar mill is prepared by a process consisting of the steps of:

(I) mixing sludge from a sugar mill and a microbial starter culture, wherein the microbial starter culture comprises *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Aspergillus oryzae* and *Streptomyces microflavus*, preferably the weight ratio of *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Aspergillus oryzae* and *Streptomyces microflavus* is 20-40:10-20:15-35:5-15:20-30; and

(II) fermenting the mixture acquired by step (I) for 7-15 days, and then acquiring the microbe-fermented sludge from a sugar mill.

5. A use of the biologic soil conditioner of any one of claims 1-3 for the prolongation

of fertilizer efficiency and the decrease of heavy metal pollution of plants, wherein the dosage of the biologic soil conditioner is 100-1000 kg/667m².

6. The use of claim 5, wherein the dosage is 200-800 kg/667m².

7. The use of claim 5 or 6, wherein the plant to be applied is sugar cane.

8. A method of applying the biologic soil conditioner of any one of claims 1-3, wherein the biologic soil conditioner is applied as basal dressing before sowing, the biologic soil conditioner is applied as side dressing during the period of growth, or the biologic soil conditioner is diluted 2-3 times for irrigation.

9. A microbial starter culture, wherein the microbial starter culture comprises *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Aspergillus oryzae* and *Streptomyces microflavus*, preferably the weight ratio of *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Aspergillus oryzae* and *Streptomyces microflavus* is 20-40:10-20:15-35:5-15:20-30.

10. A use of the microbial starter culture of claim 9 for the preparation of a biologic soil conditioner, especially a biologic soil conditioner used for the prolongation of fertilizer efficiency and the decrease of heavy metal pollution of plants.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2014/072654

A. CLASSIFICATION OF SUBJECT MATTER C05G 3/04(2006.01)i According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) C05; C09K17 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) CPRSABS;VEN: waste,sludge,acid,alkali,sugar,cane,ferment+,peat,peats		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	CN 103113170A (YE, CHANGDONG) 22 May 2013 (2013-05-22) see claims 1-9	1-10
PX	CN 103102226A (YE, CHANGDONG) 15 May 2013 (2013-05-15) see claims 1-6	1-10
A	CN 1546437A (UNIV HUAZHONG AGRIC) 17 November 2004 (2004-11-17) see description, embodiments	1-10
A	CN 1327965A (HUANG, MIN ET AL.) 26 December 2001 (2001-12-26) see the whole document	1-10
A	CN 1962561A (WANG, ZHAOLIANG ET AL.) 16 May 2007 (2007-05-16) see the whole document	1-10
A	SU 914614A1 (MOLDAVSKIJ NII POCHVOVEDENIYA) 23 March 1982 (1982-03-23) see the whole document	1-10
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 19 May 2014		Date of mailing of the international search report 28 May 2014
Name and mailing address of the ISA/ STATE INTELLECTUAL PROPERTY OFFICE OF THE P.R.CHINA(ISA/CN) 6,Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088 China Facsimile No. (86-10)62019451		Authorized officer BAL, Youai Telephone No. (86-10)62084399

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

- [1] The subject matter of the present independent claims 1, 4, and 5 is related to a biologic soil conditioner with the same constituents. The subject-matter of claim 9 is a composition of a microbial starter culture. It has no common or corresponding special technical feature between claims 1, 4, 5 and claim 9. Thus, the application does not meet the requirements of unity of invention as defined in Rules 13.1 PCT.

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

- Remark on Protest**
- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/CN2014/072654

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
CN 103113170A	22 May 2013	None	None
CN 103102226A	15 May 2013	None	None
CN 1546437A	17 November 2004	CN 1280239C	18 October 2006
CN 1327965A	26 December 2001	None	
CN 1962561A	16 May 2007	CN 100594203C	17 March 2010
SU 914614A1	23 March 1982	None	None