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DETERMINATION OF THE AMOUNT OF NITROGEN IN CYANOBACTERIA BY KJELDAHL METHOD

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THESIS ABSTRACT

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Determination of the amount of nitrogen in Cyanobacteria by Kjeldahl Method

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This thesis is going to determinate the amount of nitrogen from the cyanobacteria sample by Kjeldahl method. The cyanobacteria sample was collected by Saloy method from Kuralanjärvi with Helsinki Engineer Office in the summer at 2008. Kuralajärvi is a lake that has a terrible problem with cyanobacteria near Naantali, in Finland.

Also some other methods for controlling and removing cyanobacteria will be discussed. The damage cyanobacteria brought to the environment and human beings, the reason of the harmful algae blooms, and the way to control its blooming by technology management will be researched.

Key Words

Cyanobacteria, Nitrogen, Kjeldahl method, Saloy method, control, water blooms, algae blooms, Red-tide, Technology Management

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ABSTRACT

1 INTRODUCTION

With the development of industrialization, cyanobacteria is a problem for lakes and rivers all round the world. The phenomenon of water blooms and red-tide is frequently identified. Many times, it influences human life and it is hazardous to health. The control of cyanobacteria is an international problem. On a worldwide basis, cyanobacteria water blooms appeare in the developed countries such like European countries, such like in Finland, the United States of America and Japan. In the Early Stages of Industrialization, most of them resorted to the combined method by technological management and environment monitoring.

In Japan, the Prevention Ordinance of Lake Eutrophication was carried out, and the control strategies of the comprehensive nutrients were set out at the beginning of 2000. In Denmark, the factories are forcibly to use technology of biological removal of phosphorus and nitrogen to the waste waters the factories produced to the city during the producing by the government. The USA, Environment protection Agency even used biological controlling methods, which include aquatic vegetations' restoration, to recommend indigene fishes all over again, to remove the invasive categories, to revision the structures, and administrative management, the results are highly visible, but it takes a long time period, the cooperation of different departments are required, and the cost was prodigious.

From the 1980th, the controlling and removing work of cyanobacteria were started in China. The controlling and removing methods used nowadays are machinery salvage, chemical fixation of phosphorus, chemical inhibition, US-ultrasonic sound, microbial treatment, electromagnetic processing, and photochemical catalysis. But there are many problems of these methods. Some of them lack consistency and systematic. Some of them are too expensive. In addition, some of methods are not 100 percent completed, and some of them even gave another extra pollutions. Especially, chemical inhibition helps at the current moment, but after a while, it brings out bigger problems.

Large area blue green algae bloomed in the city of Wuxi in the Jiangxi Province, China in 2007. The direct influence was that people could not get drinking water, because the underground water and the lake water were too heavily polluted, and all kind of waters from supermarkets were sold out.

Dian Chi, it is a lake in City of Kun Ming that all year round spring city of Yunnan Province in the south of China. It was named as the Pearl of the Plateau. It offered drinking and using water for all the people from the spring city – Kun Ming. But now, because of the lack knowledge for environment protecting, every spring and summer time, water blooms and algae blooms were occurred, the startlight of the Pearl of the Plateau disappeared, it is a really pitiful thing. Nowadays, people realized it is serious problems, and they also tried to make it better, but the method what they used didn't help so much, and water blooms and algae blooms occur every year still.

Kurala Lake was one of the most heavily cyanobacteria infested lakes in Finland. It is located in Naantali, the southwest of Finland. From 2005 to 2008, Saloy Company which is a Finnish engineering office of Helsinki, started to control and remove the cyanobacteria from Kurala Lake, it acquired remarkable results. Before the removing of cyanobacteria by Saloy method, the summer cottages people from the lakeside cloud not go swimming, and many fishes were dead, but now, both the water and the living environment for fish turned better.

In this thesis, the Saloy method will be discussed. The sample which was used was collected by Saloy method from Kuralanjärvi in July, 2008. Until now, it is the best way to remove the cyanobacteria, Saloy method was first carried out to clean small lakes like Kurala Lake, but now, the Saloy method to control the cyanobacteria is also working in big areas, such like the Gulf of Finland and the Baltic Sea area. It is believed to remove the cyanobacteria away from lakes is more technical, efficient and better for environment than just kill the cyanobacteria.

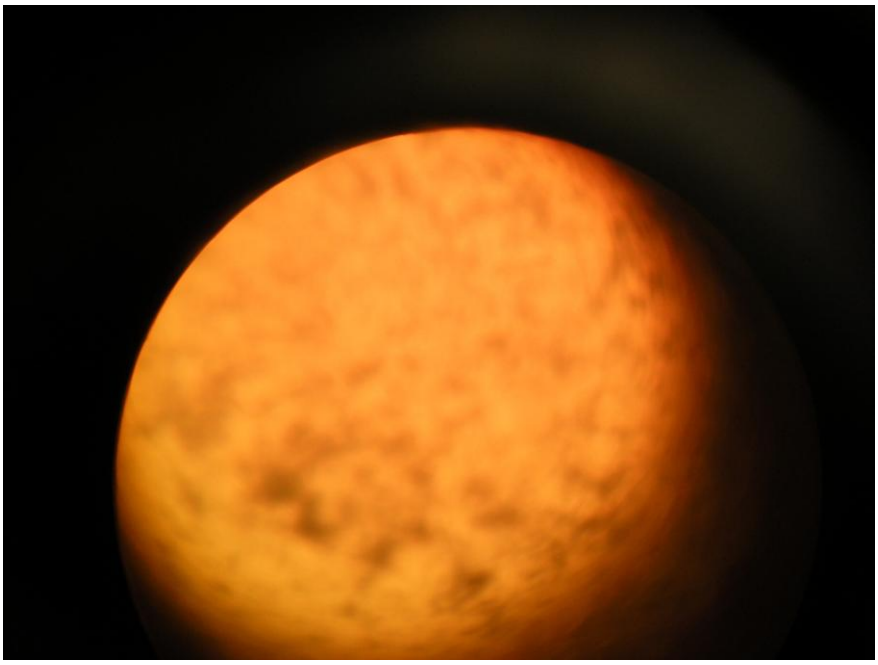
In this thesis, the following questions will be answered: How to control the cyanobacteria and how to resort the samples? Why the simple cyanobacteria became a big problem for whole world since it was a good algae to keep the environmental balance? What is causing the algae blooming?

2 THEORY OF CYANOBACTERIA

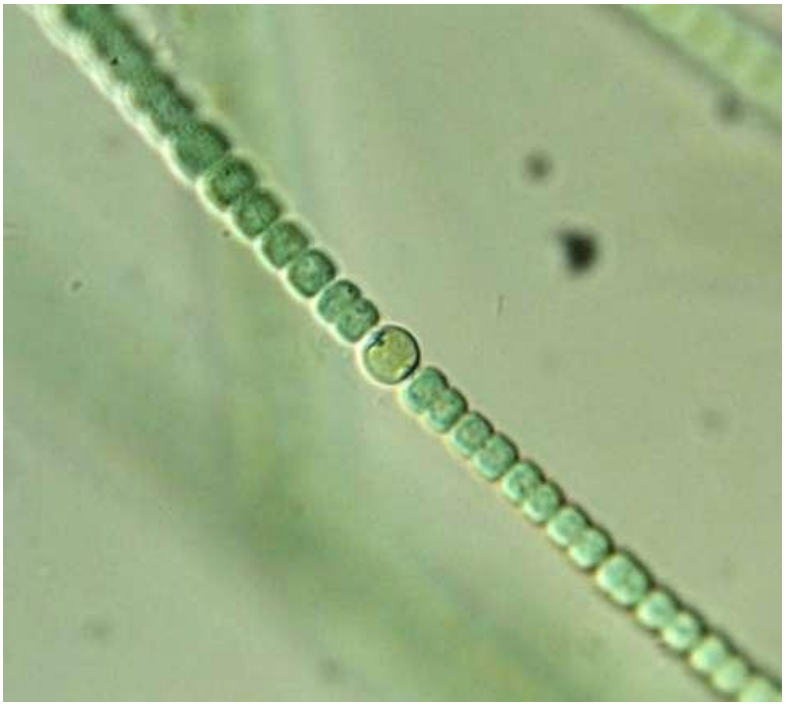
Cyanobacteria which are the scientific names of blue algae, blue-green algae, or green algae. Blue-green algae is named from the color (from Greek), because every cyanobacteria contains one kind of special blue pigment. But not every cyanobacterium is blue color, because different cyanobacteria contain different color pigments, such as some of them contain chlorophyll, some contains xanthin, some contains renieratene, some contains cyanobacteria lencocyan, and some contains cyanobacteria rhodophyll, ect. Such as the Red Sea which divides Africa from Asia, called by the color, because there is cyanobacteria rhodophyll showed in the water. (Cyanobacteria. 2010)

Nutrients are the reason for the algae growing, in the algae cell; it contains over 20 kinds of elements. There are some elements were contained over 0.01% in the algae, so we call them macro-elements or major elements, such like carbon (C), oxygen (O), hydrogen (H), nitrogen (N), phosphorus (P), sulfur (S), potassium (K), magnesium (Mg), Calcium (Ca), Sodium (Na), chlorine (Cl). The left which were contained less than 0.01%, they were named as trace elements, such like Iron (Fe), Manganese (Mn), Copper (Cu), Zinc (Zn), Boron (B), Silicon (Si), Molybdenum (Mo), Cobalt (Co) and so on. For the most of water body, the elements to limit the algae' growing is nitrogen and phosphorus, sometimes CO_2 can be a reason, too. (Cyanobacteria. 2010)

There are about 2000 different kinds of cyanobacteria, in China the recorded types of cyanobacteria are over 900. Because the cell surface of most of the cyanobacteria is covered by kind of pectin, so it is known as myxophyceae, too. Among all kinds of algae organisms, cyanobacteria is the most simple and original one. Cyanobacteria are prokaryotic organism, and photosynthetic. There are two pictures (Graph 1 and 2) from the dry cyanobacteria sample. We can easily find out that, cyanobacteria do have a simple structure.



GRAPH 1. Cyanobacteria via microscope (photo by author)



GRAPH 2. Structure of cyanobacteria (copyright from farm3 2010.)

2.1 Cyanobacteria now and earlier

Cyanobacteria were very important for the health and growth of many organisms, and also environment. Cyanobacteria are the earliest photosynthesis oxygen-evolving organism, and they are acting an important part in changing the scale free heating to aerobic of the atmosphere environment from the surface of the earth. And also, they are one of the very few groups of organisms that can convert inert atmosphere nitrogen to an organic form, such as nitrate or ammonia.

There are many cyanobacteria can directly regular the nitrogen in the atmosphere, because there is azotase in cyanobacteria, it can process biological nitrogen fixation directly, to improve the soil, and let plants grow better.

And also, part of the algae can used as food or medicine, such as, the very famous long thread moss, the common nostoc (it is called also edible tree fungus), spirulina and so on. But nowadays, in many lakes, rivers and small pools because of too over nutrients, in summer time, cyanobacteria grow very fast, and in the surface of lakes or pools, there are so much green color floating bubbles with terrible odor, we call it water bloom.

It happens in big zone also, we call it Green-Tide, and it is opposites with the Red-Tide which appear in the ocean or seaside. The Green-Tide makes the water worse, sometimes when it is very bad, all of the oxygen in the water will be used, and it will cause the death of the fish. Even worse is that in about 50% of cyanobacteria, there is cyanobacteria toxin, cyanobacteria toxin is not only leading the death of fishes and farm animals, but also the big reason for human being’s liver cancer.

There are many reasons caused the cyanobacteria blooms, such as chemical fertilizer loss; wastewaters from house using - include the wastewater from family using and the cleaning liquid contains phosphorus; farm animals - the faeces from the farm animals contain a lot of nutrients wastes, such like nitrogen and phosphorus, and it leads to eutrophication; industrialization pollutions - include chemical fertilizer plant and wastewater pollutions. Combustion mineral fuel - in Baltic Sea, 30% of nitrogen and in Mississippi River, about 13% nitrogen all comes from it.

The influences of cyanobacteria to waters include the following: Algae changes the taste of the water to sweet, bitter and sour. In summer time, because of the hot weather, cyanobacteria grow fast, the odor influence the human being and also the environment. Change of the color is the secondary impact by cyanobacteria. Large amount of the cyanobacteria, turn water to green. About 50% of the cyanobacteria are toxins. They are bad for the organisms, the water and also a major risk for the health of human beings. Dermatitis of the skin is caused by the toxin from cyanobacteria. The other name of the cyanobacteria is myxphoyceae because of the pectin in the surface of the algae cell.

2.2 Controlling and removal cyanobacteria with Saloy method

Saloy method is named from the company name of Saloy Ltd. Saloy Ltd is a Finnish engineering company that founded in 1978. They have a true pioneer in finding a solution for cyanobacteria. (Saloy Accessed 2009)

Until now, Saloy method is the best method to remove the cyanobacteria. It was first carried out to cleaning small lakes like Kuralanjärvi, but now, it is also working for big areas, such like the Gulf of Finland and the Baltic Sea area. It is a Finnish solution for blue-green algae. (Saloy Accessed 2009)

Saloy Ltd has developed a method through which the internal load cycle can be interrupted by removing blue-green algae from the lake or water pools. The harmful algae would be removed from the water by using a filter made of a special filtering fabric that collects the algae while allowing water to run through.

In the following graph (Graph 3) we can see the pump switch on the left side. It can control the internal running water. There is a long and big special fabric as a filter, and the water goes through this filter. The cyanobacteria will stay on the filter and they can be taken off by a vacuum-cleaner. The over running water is now clean.

GRAPH 3. Taking cyanobacteria from Kurala Lake by Saloy method (photo by author 2008)

2.3 The advantage in Soloy method

Removing the algae is crucial in stopping the cycle of internal load. When you remove the algae, the oxygen levels on the bottom will be improved at the same time while collecting the algae and phosphorus, the source of eutrophication. Reduction of internal load of bodies of water through reduction of blue-green algae makes even more sense, when you can stop the phosphorus flow into the water at the same time. Saloy offers a solution to this problem as well. (Saloy 2010)

According to Saloy's experience, cyanobacteria presence is at highest where there are high amounts of nutrients. The presence of algae in different parts of the water can be charted precisely though the use of a special instrument, which reacts to the color pigments of the cyanobacteria. When high amounts of cyanobacteria are present, the phosphorus concentration is charted upstream until the phosphorus source can be found. (Saloy Acessed 2009)

The phosphorus is removed with a precipitation device using ferric sulfate. The precipitated phosphorus can then be removed from the bottom of the stream. Utilizing the precipitated phosphorus as a fertilizer is being studied. A similar method for removing phosphorus from wastewater has been used for years. (Saloy Accessed 2009)

Biological method is a method to use the adsorption and flocculation of the cyanobacteria to the biomembrane, to break down the cyanobacteria from the water, part of them to be precipitate, the other part to be oxidized by the micro organism after adsorption. After the biological reactor running steadily, this method can take 60% to 70% cyanobacteria from the water. But while taking the caynobacteria from the water, it also retro gradation many other organic organisms, so the water quality will be reduced.

Until now, the common method for controlling the cyanobacteria is believed as oxidation pretreatment. This method is used in 50% of the cleaning. During the process of presaturation with oxygen, the chlorine will react with the organic in the water. Halogenated organic compounds will be formed and there are dangerous to human beings. For example, haloform (THM) can cause cancer.

3 DETERMINATION OF THE AMOUNT OF NITROGEN IN CYANOBACTERIA BY KJELDAHL METHOD

3.1 Principle of the Kjeldahl method

Johan Kjeldahl, a Danish chemist, who found out a way to test the amount of protein by exterminating the amount of nitrogen in 1883. Because the nitrogen amount of protein in food is very steady. Average amount is about 16%. This method is used to determinate the amount of nitrogen to find out the amount of protein in the food. The Kjeldahl method is widely used in analytical chemistry to determinate the amount of nitrogen. (Methods 2007)

In this method samples are heated with acid and catalyst, to dissolve proteins, and let the dissolved ammonia combine with the acid to produce ammonium sulfate. Then with series of processes like digestion process, distillation process and titration process nitrogen amount can be determinated. In this thesis, Kjeldahl method was used to determinate the nitrogen amount in cyanobacteria in both the liquid samples and the solid samples. (ISO 2010)

Samples are organic substances containing nitrogen. In the experiment, ammonia combines with acid to produce ammonium sulfate. An alkaline solution was used to distillate. Firstly, boric acid was used to titrate the solutions, and then use the standard sulphric acid (H_2SO_4) solution for back titration.

a. The ammonia from the samples will react as the following reaction
   Organic N + H_2SO_4 → $(NH_4)_2SO_4$ + H_2O + CO_2 + other

b. Though Kjeldahl equipment to combine with alkaline
   $(NH_4)_2SO_4$ + 2 NaOH → 2 NH_3↑ + Na_2SO_4 + 2 H_2O

c. A carefully measured standardized acid solution is used to titrate, and then based on the amount of used acid to determinate the amount of nitrogen.

$2\ NH_3 + 2\ H_2SO_4 \rightarrow (NH_4)_2SO_4 + H_2SO_4$ (no color change)

$(NH_4)_2SO_4 + H_2SO_4 + 2\ NaOH \rightarrow Na_2SO_4 + (NH_4)_2SO_4 + 2\ H_2O$

3.2 Procedure in handling the liquid part

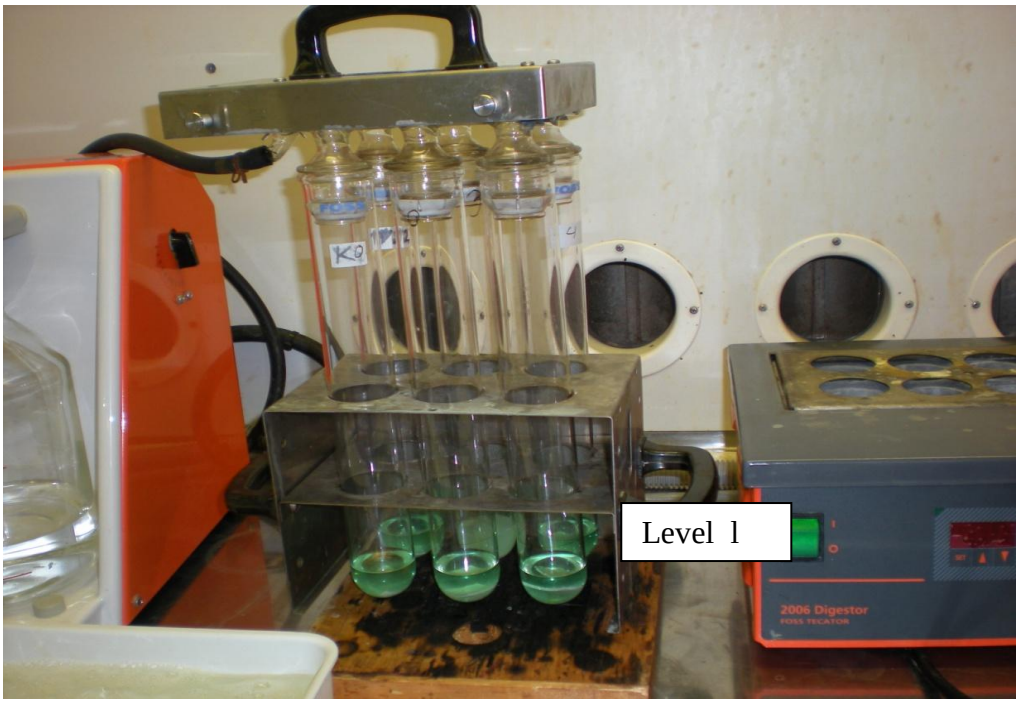
3.2.1 Digestion part

Six tubes were prepared for all the solutions in this experiment, and they were marked as O_1 (distillated water), O_2 (distillated water), 1 (cyanobacteria sample water) (graph 4), 2 (cyanobacteria sample water), 3 (stock solution), and 4 (stock solution).

GRAPH 4. Sample water

5 ml concentrated H_2SO_4, 35 mg Devarda's Alloy and 2 g catalyst $CuSO_4 \cdot 5H_2O$ were added to each tube. A piece of ceramic was added to each tube, for preventing the over cooking. The tubes were left for about 20 minutes at room temperature.

The tubes were heated and the temperature was set to 100 °C at the beginning. The temperature were rised 20 °C to the final temperature 370 °C. When the bubbles showed over level 1 (graph 5), the temperature was set not to rise for a while. When the bubbles went down, under level 1, the temperature could start to rise again. These actions were taken so the samples wouldn't burn. When the bubbles disappeared, then the temperature could rise faster and higher, for example: from 300 °C to 350 °C.



GRAPH 5. Digestion part of the experiment

Table 1. The amount of Alloy and catalyst used in the experiment

	O_1	O_2	1	2	3	4
Alloy (g)	0.0364	0.0351	0.0352	0.0367	0.0357	0.0355
Catalyst (g)	2.0014	2.0064	2.0053	1.9990	2.0039	2.0043

Table 2. The observations during the heating

Time	T (°C)	O_1	O_2	1	2	3	4
12:20	32	normal	normal	normal	normal	normal	normal
13:00	220	normal	normal	too much bubbles	too much bubbles	normal	normal
13:30	240	normal	normal	too much bubbles	too much bubbles	normal	normal
		It stayed at 241°C for 30 minutes.					
14:00	371	All the tubes were full with fog or steam					

3.2.2 Distillation part

300 g NaOH was dissolve into 1000 ml distillated water to conical flask, to make the alkali solution for Kjeldahl equipment (Graph 6).

GRAPH 6. Distillation with Kjeldahl equipment

Set the distillation of alkali NaOH 3 X 10 ml for 6 minutes for each distillation. During the distillation, we have to make sure that:

i. The pipes are deeply in the water samples which should be distillated
ii. Make sure all the solution on the pipe goes to the flask.
iii. While changing tubes, wear gloves on, because they might be hot.

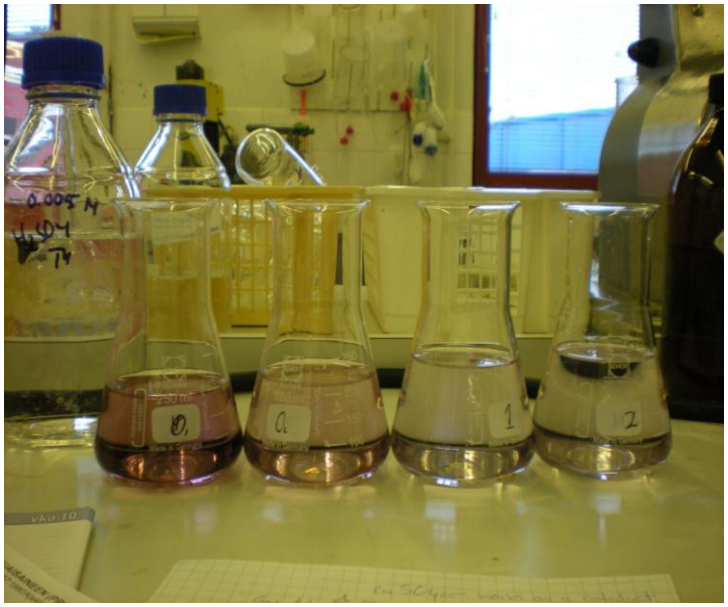
Measure 10 g (9.9999 g was used) boric acid and dissolve to 500 ml distillated water. Because H_3BO_4 is not easy to dissolve, a stirrer was used.

20 ml of H_3BO_4 solution was measured to each flask. A few drops of methyl red indicator was added to each flask.

3.2.3 Titration part

0.005 M H_2SO_4 solution was prepared for titration of the distillated solutions O_1, O_2, 1, 2, 3 and 4. (Graph 7) The sample was titrated. The required volumes are listed in Table 3.

GRAPH 7. The titration equipment



GRAPH 8. The solutions after titration

Table 3. The amount used of H_2SO_4 solution

Flask No.	Titration 1 (ml)	Titration 2 (ml)
O_1	0.58	0.67
O_2	0.76	0.79
1	25.93	26.04
2	26.00	26.09
3	3.70	3.80
4	3.64	3.94
Notice: the result in flask No. O_2, it went over, so 0.67 ml was used for calculations.		

3.2.4 Calculation of the liquid part

The formula used for the liquid part's calculation is as following.

$$X = [(V_3 – V_4) * c * 14 *2 * 1000] / V$$

Where,

X = the amount of nitrogen in the sample, mg/l

V_3 = the volume of H_2SO_4 used for titration

V_4 = the volume of H_2SO_4 used for titration of the zero sample

c = the concentration of H_2SO_4, 0.005 M

V = the volume of the sample amount, 50 ml

14 = the molar mass of nitrogen

1000 = change ml to l

2 = acid mass molarity change of nitrogen

Then we can get:

V_4 = (0.58 ml + 0.67 ml)/2 = 0.625 ml, in the following calculation, it is a constant amount

V = 50 ml, it is a constant amount

The amount of nitrogen in the algae sample is

X_1 = [(26.04 – 0.625) * 0.005 * 14 * 2 * 1000] / 50 = 71.05 mg/l

X_2 = [(26.09 – 0.625) * 0.005 * 14 * 2 * 1000] / 50 = 71.302 mg/l

The average amount of nitrogen in the algae sample is:

X_{ave} = (71.05 + 71.302) mg/l / 2 = 71.176 mg/l

The amount of the nitrogen in standard sample is

X_3 = [(3.70 – 0.625) * 0.005 * 14 * 2 * 1000] / 50 = 8.61 mg/l

X_4 = [(3.64 – 0.625) * 0.005 * 14 * 2 * 1000] / 50 = 8.442 mg/l

The average amount of the nitrogen in standard sample is:

X_{ave} = (8.61 + 8.442) mg/l / 2 = 8.526 mg/l

3.3 Procedure in handling the solid part

The theory of procedure in this part is the same as for the liquid part. The difference is that only a small amount of the solid sample was mixed with the distillated water.

3.3.1 Digestion part

Six tubes were prepared for all the solutions in this experiment, and are marked as O_1 (distillated water), O_2 (distillated water), 1 (dry cyanobacteria sample) (graph 9), 2 (dry cyanobacteria sample), 3 (dry cyanobacteria sample), and 4 (dry cyanobacteria sample).

GRAPH 9. The dry cyanobacteria sample

5 ml concentrated H_2SO_4, 35 mg Devarda's Alloy and 2 g catalyst $CuSO_4 \cdot 5H_2O$ were added to each tube. The exact masses are found in table 4.

A piece of ceramic was added to each tube, for preventing the over cooking. The tubes were left for about 20 minutes at room temperature. The tubes were heated and the temperature was set to 100 °C at the beginning. The temperature rised 20 °C to the final temperature 370 °C. When the bubbles showed over the level 1 (Graph 4), the temperature was set not to rise. When the bubbles went down, under level 1, the temperature could start to rise again. These actions were taken so the samples wouldn't burn. When the bubbles disappeared, then the temperature could rise faster and higher, for example: from 300 °C to 350 °C.

Table 4. The used amount of Alloy and Catalyst

	O_1	O_2	1	2	3	4
Alloy (g)	0.0350	0.0363	0.0353	0.0357	0.0349	0.0359
Catalyst (g)	2.0099	2.0014	2.0065	1.9987	2.0002	2.0200

Table 5. The observation from 6 tubes during the digestion

T (°C)	O_1	O_2	1	2	3	4
20	normal	normal	normal	normal	normal	normal
220	normal	normal	too much bubbles	too much bubbles	normal	normal
240	normal	normal	too much bubbles	too much bubbles	normal	normal
	It stayed at 241°C for 30 minutes.					
350	All the tubes were full with fog or steam.					

3.3.2 Distillation part

Measure 300 g NaOH to dissolve to 1000 ml distillated water to conical flask, to make the solution as alkali for Kjeldahl equipment.

Set the distillation of alkali NaOH to 9 X 10 ml for 6 minutes for each distillation. During the distillation, we have to make sure that:

iv. The pipes are deeply in the water samples which should be distillated
v. Make sure all the solution on the pipe goes to the flask.
vi. While changing the tubes, wear gloves on, because they might be hot.

10 g (10.0033 g was used) of boric acid was dissolved to 500 ml distillated water. Because H_3BO_4 is not easy to dissolve, so use a stirrer to help to mix up the solution. 20 ml of H_3BO_4 solution was measured to each flask. A few drops of methyl red indicator was added to each flask.

3.3.3 Titration part

0.005 M H_2SO_4 solutions was prepared for titration of the distillated solutions O_1, O_2, 1, 2, 3 and 4. The samples were titrated. The required volumes are listed in Table 6.

Table 6. The amount of 0.005 M H_2SO_4 solution used

Flask No.	Titration 3 (ml)	Titration 4 (ml)
O_1		
O_2	0.85	
1	50.03	50.85
2	43.44	
3	54.84	57.88
4	55.49	
Notice: the result in flask No. O_2, it went over, so 0,85 ml was used for calculations. In the first tube, nothing happened.		

3.3.4 Calculation of the solid part

The formula used in the solid part's calculation is as following:

$$X = [\ 10 * (\ V_3 - V_4) * c * 14 * 2 * 1000\ ] / m$$

Here we know

$V_4 = 0.85$ ml

m = 2 g

The amount of nitrogen in the algae sample is

$X_1 = [\ 10 * (50.03 - 0.85) * 0{,}005 * 14 * 2 * 1000\ ] / 2 = 34.426$ mg/l

$X_2 = [\ 10 * (43.44 - 0.85) * 0{,}005 * 14 * 2 * 1000\ ] / 2 = 29.813$ mg/l

$X_3 = [\ 10 * (54.84 - 0.85) * 0{,}005 * 14 * 2 * 1000\ ] / 2 = 37.793$ mg/l

$X_4 = [\ 10 * (55.49 - 0.85) * 0{,}005 * 14 * 2 * 1000\ ] / 2 = 38.248$ mg/l

4 CONCLUSION

Nitrogen is everywhere in our living environment, in the air (there are 78% nitrogen), in the food, in the water and in the ground. Nitrogen is one of the most important element to keep the balance for the atmospheric pressure of our earth. If there isn't enough nitrogen gas, the atmospheric pressure will be too weak for human beings. Nitrogen is not reacting with water, but it is dissolved in the water. There is a very emblematical example, in the Qinghai-Tibet Plateau, the low atmospheric pressure cause by the limited nitrogen, leads to that people have difficulties to live there. Both human beings, animals and plants need nitrogen. Especially plants need plenty of nitrogen as a nutrient for growing.

Nowadays, because of many reasons, people use too much products that consists of nitrogen nutrients. According to this research, it is not hard to find out that the large amount of nitrogen causes eutrophication, and the eutrophication causes the cyanobacteria blooms and the Red-Tide. Due to an official investigation of 623 lakes from east America made by EPA – Environment Protecting Agency of the United States of American, reported that nitrogen stand for 60% of the water quality.

From the experiment, we can find out that the nitrogen amount in the Kurala Lake is 72 mg/l in liquid phase and 37 mg/l in dry solid phase. Because same of the samples wasting during the experiment, for example there would be some samples left on the tubes or glass, the real amount of nitrogen in the cyanobacteria is higher than these results show. During the experiment of the dry sample, many algae were left on the tube, which affect the results.

The nitrogen amount in normal water is 0.029 – 1.508 mg/l, and in the small lakes in cities the amount is 0.262 – 20.82 mg/l. Based on these numbers, we can say that the most important action to control the cyanobacteria of lakes is to control the nitrogen amount in the lakes.

In Baltic Sea, 50% of the nitrogen comes from the fertilizer loss. Around Kurula Lake, there are many apple tree gardens and potato fields, so it is believed that over 70% of the eutrophication is caused of the large amount of chemical fertilizer used on them. To control the cyanobacteria one need to control the use of chemical fertilizers around Kurala Lake.

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