Environmental Chemistry and Microbiology

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Module - 5
Lecture - 22
Nitrogen Chemistry (Part B)

Welcome everyone to our online NPTEL course of Environmental Chemistry and Microbiology. This course will be taught by Professor Sudha Goel and myself, Professor Anjali Pal. We are from the Civil Engineering Department, IIT Kharagpur. We have divided this course into two parts. The first part is Environmental Chemistry. That will be covered by me. The second part is Environmental Microbiology. It will be taught by Professor Sudha Goel. In my first module, I have discussed about the acids, bases and salts. In the second module, I discussed about the chemical equilibrium. In the third module, I have elaborated the chemical kinetics. In the fourth module, I have discussed about the catalysts and catalysis. This is my fifth module and lecture number 22, where I am discussing about the nitrogen chemistry. This is the part B. In my last lecture, I have covered the nitrogen cycle and nitrogen chemistry. That was part A and this is Part B.

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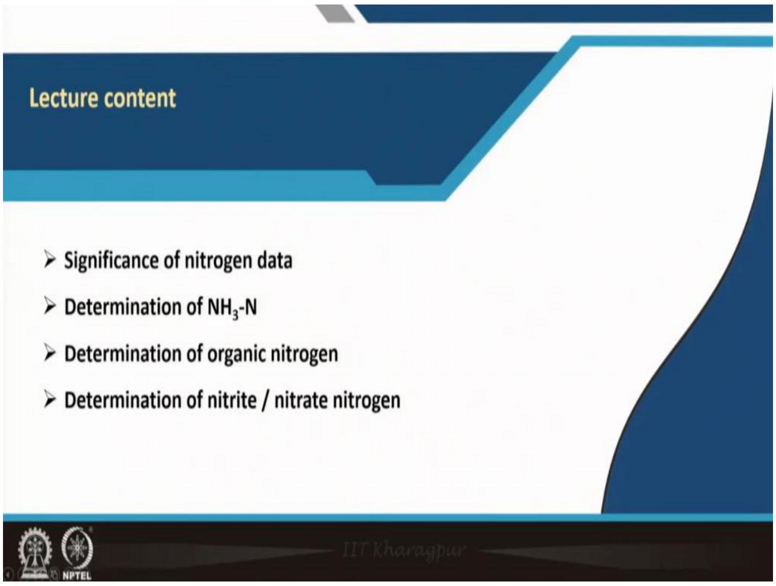

Lecture content

- Significance of nitrogen data
- Determination of NH_3-N
- Determination of organic nitrogen
- Determination of nitrite / nitrate nitrogen

In this lecture content, I will talk about the significance of nitrogen data. I will tell how we can determine the ammonia nitrogen. I will also discuss about the, how to determine the organic nitrogen and the nitrite and nitrate nitrogen.

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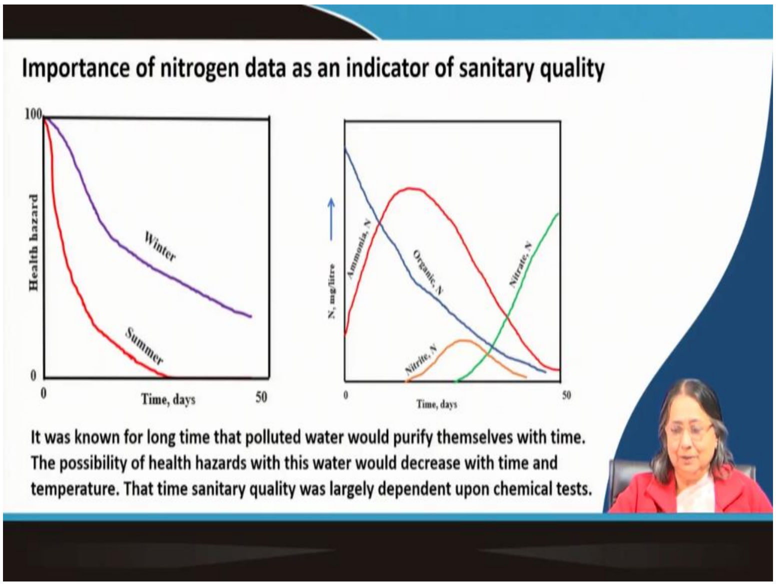

Importance of nitrogen data as an indicator of sanitary quality

It was known for long time that polluted water would purify themselves with time. The possibility of health hazards with this water would decrease with time and temperature. That time sanitary quality was largely dependent upon chemical tests.

First of all, we will see, importance of nitrogen data as an indicator of sanitary quality. It was known for long time that polluted water would purify themselves with time. Say for example, if you put some organic matter which is biodegradable organic matter, in some natural environment then what will happen? Naturally it will be purified with time. The possibility of health hazard with this water would decrease with time and temperature. In comparison to winter the rate of purification is faster in summer. The kinetics have been shown in the last slide. So, time and temperature both has effect. In my previous lecture, I have already told that nitrogen is present as ammonia, organic matter, nitrite or nitrate. So, there are different forms of nitrogen. Now, if you can measure these different types of nitrogen, then you can see with time how the nature of that combined form of nitrogen is changing. On the right-hand side of the last slide, you can see the variations of different form of nitrogen with respect to time. If you measure the organic nitrogen, then you will see that with time organic nitrogen concentration is decreasing. Nitrogen concentration has been expressed in milligram per litre. If you see the of the ammonia nitrogen, then you will find that at first its concentration is increasing. It will go to a peak value and then it will decrease. in between you will see the graph of nitrite nitrogen. It is first increasing and then finally it is decreasing. However, if you see the graph for nitrate nitrogen, you will see it is increasing with time. Now, why it is so? Say for example, you have polluted with some protein molecule. Then what will happen with time? The protein molecule will be first decomposed. It will first form the ammonia. First it will form the ammonia. So, organic nitrogen (protein molecule) will be degraded. So, you will see that organic nitrogen is decreasing. Then, after the degradation, ammonia is produced. So, the concentration of ammonia nitrogen will be increasing and then finally, it is converted to nitrite and finally to nitrate. The highest oxidation state of nitrogen

(+5) is observed in nitrate. So, in aerobic environment ultimately it will be converted to nitrate.

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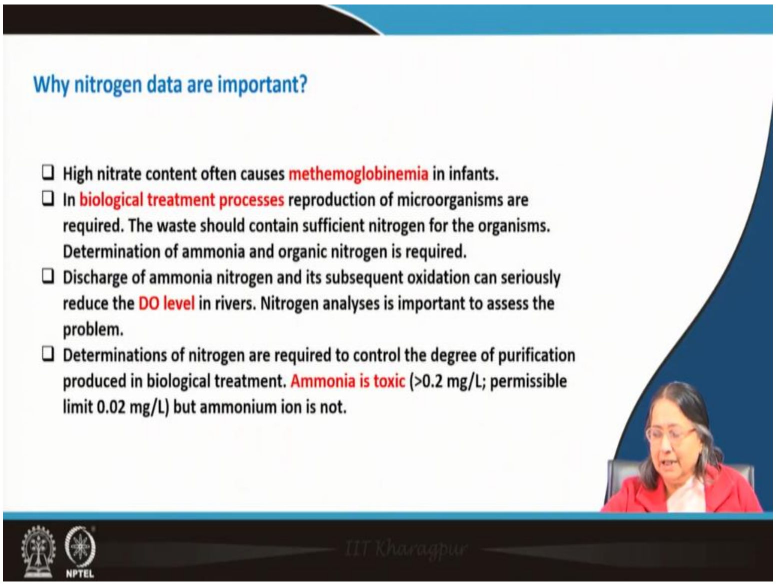

Why nitrogen data are important?

❑ High nitrate content often causes methemoglobinemia in infants.
❑ In biological treatment processes reproduction of microorganisms are required. The waste should contain sufficient nitrogen for the organisms. Determination of ammonia and organic nitrogen is required.
❑ Discharge of ammonia nitrogen and its subsequent oxidation can seriously reduce the DO level in rivers. Nitrogen analyses is important to assess the problem.
❑ Determinations of nitrogen are required to control the degree of purification produced in biological treatment. Ammonia is toxic (>0.2 mg/L; permissible limit 0.02 mg/L) but ammonium ion is not.

why nitrogen data are important? Say for example, high nitrate content often causes methemoglobinemia in infants. This is a disease. So, there is some permissible limit. So, if nitrate concentration is beyond that, then it may cause this type of disease in infants.

Now, in the biological treatment, the reproduction of microorganisms is required. So, if they grow, then the waste should contain sufficient nitrogen for the organism. It is so because for the growth we always need the nitrogen. Say for example, protein molecule contains nitrogen. So, determination of ammonia and organic nitrogen here it is very important.

Now discharge of ammonia nitrogen and its subsequent oxidation can seriously reduce the DO level. I already told you that while discussing about BOD. Nitrogen analysis is important to assess the problem. Oxygen DO level should not go beyond some level. Then anaerobic condition will be generated. Then determination of nitrogen is required to control the degree of purification produced in biological treatment.

Ammonia is very toxic. So, there is some permissible limit (0.2 mg/l). So, it should not go beyond this value. However, ammonium ion is very much needed for plant growth. Ammonium sulphate is used for fertilizer. So, ammonium ion is not toxic, but ammonia is toxic. So, we have to know how much ammonia is present and for that, we need some method. So analytical chemistry is very important for the environmental engineers.

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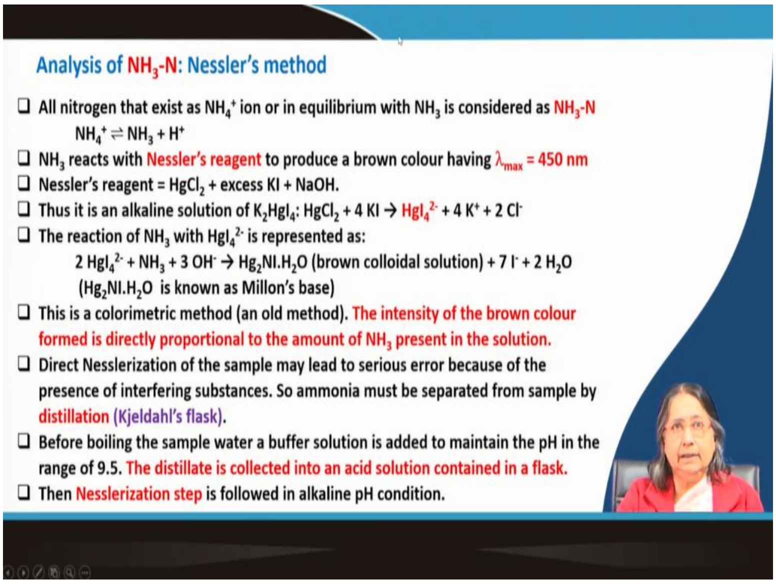

Analysis of NH_3-N: Nessler's method

- ☐ All nitrogen that exist as NH_4^+ ion or in equilibrium with NH_3 is considered as NH_3-N

  $NH_4^+ \rightleftharpoons NH_3 + H^+$
- ☐ NH_3 reacts with Nessler's reagent to produce a brown colour having $\lambda_{max} = 450$ nm
- ☐ Nessler's reagent = $HgCl_2$ + excess KI + NaOH.
- ☐ Thus it is an alkaline solution of K_2HgI_4: $HgCl_2 + 4\,KI \rightarrow HgI_4^{2-} + 4\,K^+ + 2\,Cl^-$
- ☐ The reaction of NH_3 with HgI_4^{2-} is represented as:

  $2\,HgI_4^{2-} + NH_3 + 3\,OH^- \rightarrow Hg_2NI.H_2O$ (brown colloidal solution) $+ 7\,I^- + 2\,H_2O$

  ($Hg_2NI.H_2O$ is known as Millon's base)
- ☐ This is a colorimetric method (an old method). The intensity of the brown colour formed is directly proportional to the amount of NH_3 present in the solution.
- ☐ Direct Nesslerization of the sample may lead to serious error because of the presence of interfering substances. So ammonia must be separated from sample by distillation (Kjeldahl's flask).
- ☐ Before boiling the sample water a buffer solution is added to maintain the pH in the range of 9.5. The distillate is collected into an acid solution contained in a flask.
- ☐ Then Nesslerization step is followed in alkaline pH condition.

I will tell you how to determine different forms of nitrogen. Say for example, how you can determine the ammonia nitrogen? I will explain a very simple and very useful method i.e., Nessler's method. You are very familiar with the equilibrium shown in (1).

$NH_4^+ \leftrightharpoons NH_3 + H^+$……………………….(1)

I told you in my chemical equilibrium lectures that ammonium ion is in equilibrium with ammonia and H^+. So, if you want to shift this equilibrium towards right, then what you have to do? You have to take out this H^+. So, how you can take out? You have to make the solution alkaline. Now, ammonia reacts with Nessler's reagent to produce a brown colour (λ_{max}=450nm). Now, what is Nessler's reagent? Nessler's reagent is nothing but a solution of $HgCl_2$ and excess potassium iodide (KI) in presence of NaOH. So, basically it is an alkaline solution of K_2HgI_4. So, basically $HgCl_2$ and KI in presence of alkali forms K_2HgI_4. Now, the reaction of NH_3 with HgI_4^{2-} is represented as:

$2HgI_4^{2-} + NH_3 + 3OH^- \rightarrow Hg_2NI.H_2O$ (brown colloidal solution) $+7I^- + 2H_2O$…………..(2)

The complex $Hg_2NI.H_2O$ is known as Millon's base. It is a colloidal solution which has the brown colour. You can use this method as a colorimetric method. Intensity of the brown colour is directly proportional to the amount of NH_3 present in the solution. Now, direct Nesslerization of the sample may lead to serious error. Direct Nesslerization means, you take the sample and you put the reagent, i.e., the Nessler's reagent. Then you think that some brown colour will be formed and you can use this as for ammonia nitrogen determination. But it is not like that. It is so because in the sample there may be other things also which may lead to serious error because of the interfering substances. So, the direct method is not possible. Hence, ammonia must be separated from sample by distillation (Kjeldahl's flask).

Ammonia nitrogen is some type of nitrogen which is present either as ammonium ion or as ammonia. Now, you have to consider the equilibrium represented by (1). It is so because if you want to distill out this ammonia as a gas, then you have to make the solution alkaline. If it is ammonium ion, it will be solubilized in water. So, you have to shift the equilibrium completely towards right side. Then you have to H^+. It can be done just making solution alkaline. Then you have to use the Kjeldahl's flask (a type of distillation flask). You can make the solution alkaline by using potassium carbonate. Then you have to heat it. Then ammonia will be produced and it will be steam distilled. So, ammonia will be carried out with the steam. It will be taken out and condensed to another flask. In the other flask you can take some acid (e.g., sulphuric acid) so that ammonia goes there and gets converted to ammonium sulphate. So, ammonia will not go out. As ammonia is volatile, so you have to take proper care. You have to be sure that from the solution, whole ammonia (100%) has come out. Otherwise, your result will be wrong. Before boiling the sample, a buffer solution is added to maintain the pH in the range of 9.5 and the distillate is collected into an acid solution contained in a flask. Whatever interfering ions were there in your sample, they are not coming with the distillate. They are remaining, in that flask, from where you are distilling. So, interfering ions are not present with ammonia. Now, then Nesslerization step is followed in alkaline pH condition. Say for example, you have taken say 200 millilitre water sample. Then you have to distill up to such time that at least 100 millilitre should come. If more comes then it is better. You will be sure that, all ammonia has come there in the sulphuric acid. Now, after it is collected there, then can do two things. The first thing you can do is that, take 100 millilitre sample and put the Nessler's reagent there. Immediately it will be forming brown colour. In another tube, you have to take 100 millilitre of distilled water and then mix it with the same volume of the Nessler's reagent. Then you have to add some source of ammonia nitrogen from outside. It is nothing but ammonium chloride solution. So, you have to prepare a standard ammonium chloride solution (say for example, 1 millilitre of that solution is equivalent to 0.5 milligram of ammonia nitrogen). So, you have to add drop by drop in that blank solution where you have taken the distilled water. Then when you will see the colour of your sample water is matching you can calculate the amount of ammonia nitrogen present. It is so because you know how much ammonium chloride you have added. This is a colorimetric method. Colorimetric means colour matching. You can extend this to spectrophotometry also, because you know that $\lambda_{max} = 450$ nm. You can prepare some standard solutions of ammonium chloride and then you can add the Nessler's reagent. The colour will be developed. They will have different intensities according to the concentration of ammonium chloride. You have some sample solution. There are also you add the same volume of this Nessler's reagent. Then you can match with all the solutions. It is colorimetric. Otherwise, you can make a calibration graph using the spectrophotometer, using the standard solutions. Using the calibration curve, you can determine the ammonia nitrogen in the sample.

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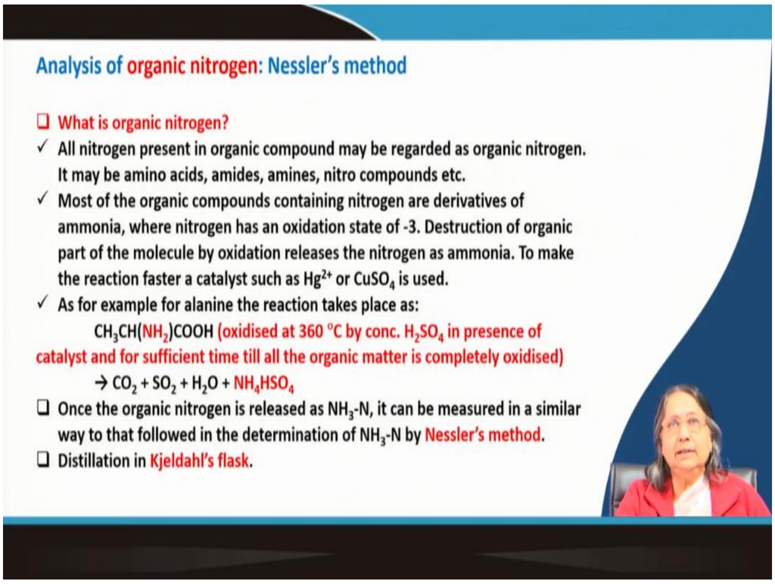

Analysis of organic nitrogen: Nessler's method

☐ What is organic nitrogen?

✓ All nitrogen present in organic compound may be regarded as organic nitrogen. It may be amino acids, amides, amines, nitro compounds etc.

✓ Most of the organic compounds containing nitrogen are derivatives of ammonia, where nitrogen has an oxidation state of -3. Destruction of organic part of the molecule by oxidation releases the nitrogen as ammonia. To make the reaction faster a catalyst such as Hg^{2+} or $CuSO_4$ is used.

✓ As for example for alanine the reaction takes place as:

$CH_3CH(NH_2)COOH$ (oxidised at 360 °C by conc. H_2SO_4 in presence of catalyst and for sufficient time till all the organic matter is completely oxidised)

$\rightarrow CO_2 + SO_2 + H_2O + NH_4HSO_4$

☐ Once the organic nitrogen is released as NH_3-N, it can be measured in a similar way to that followed in the determination of NH_3-N by Nessler's method.

☐ Distillation in Kjeldahl's flask.

Now, what is organic nitrogen? You can apply the same Nessler's method to determine the organic nitrogen also. You have to know what is organic nitrogen. All nitrogen present in organic compound may be regarded as organic nitrogen. It may be amino acid, it may be amides, it may be amines, it may be nitro compounds, it may be proteins. Most of the organic compounds containing nitrogen are derivatives of ammonia. So, if I ask you that in protein, what is the oxidation state of nitrogen? Protein is a very complex molecule. So, what is the oxidation state of nitrogen there? What is the oxidation state of nitrogen in ammonia? It is -3. So, in protein also it is same. When nitrogen has an oxidation state of -3. If you make something to decompose the organic part of the molecule by oxidation, it releases the nitrogen as ammonia. When you decompose the organic molecule, i.e, say for example amines, protein molecules etc., then in the first step it is producing the ammonia. To make the reaction faster, a catalyst such as Hg^{2+} or $CuSO_4$ is used. You have already learnt what is catalyst. It makes a reaction faster. It can be explained by the oxidation of alanine by concentrated sulphuric acid at 360°C in presence of catalyst and for sufficient time till all the organic matter is completely oxidised.

$CH_3CH(NH_2)COOH$ (oxidized at 360°C by conc. H_2SO_4 in presence of catalyst and for sufficient time till all the organic matter is completely oxidized)$\rightarrow CO_2+SO_2+H_2O+NH_4HSO_4$…………………(3)

In (3) you can see that the oxidation state is not changing for nitrogen. But for carbon, you can see that carbon is going to be carbon dioxide. Sulphur dioxide is coming from sulphuric acid. Once the organic nitrogen is released as ammonia nitrogen, it can be measured in a similar way to that followed by the determination of ammonia nitrogen by Nessler's method. You have to add concentrated sulphuric acid to the solution, where you have some organic nitrogen present. You have to heat it at high temperature (360° C), till the organic matter is completely oxidised. How do you know this? You will see lot of fumes are coming out because of this sulphuric acid. In the first stage you will see that it has become black, because of the formation of carbon. Then carbon dioxide will be formed. Ultimately you will see that the black colour has gone and it has become transparent solution. Then you will find that fumes are also gone. So, at that stage you can tell that, the complete decomposition has occurred. So, you can see in the last slide that how from complex molecule ammonia nitrogen is generated. Previously I explained how to determine the ammonia nitrogen. You have to follow the same process by using the Nessler's method.

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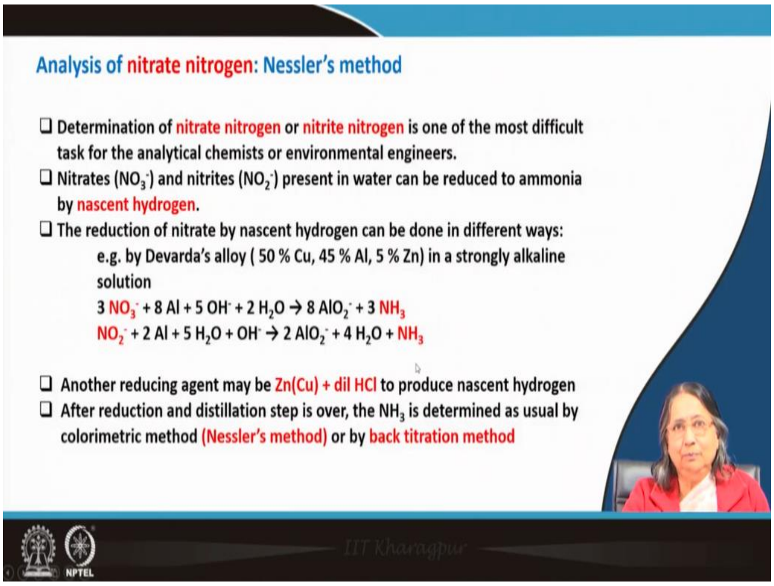

Analysis of nitrate nitrogen: Nessler's method

❑ Determination of nitrate nitrogen or nitrite nitrogen is one of the most difficult task for the analytical chemists or environmental engineers.

❑ Nitrates (NO_3^-) and nitrites (NO_2^-) present in water can be reduced to ammonia by nascent hydrogen.

❑ The reduction of nitrate by nascent hydrogen can be done in different ways:
e.g. by Devarda's alloy (50 % Cu, 45 % Al, 5 % Zn) in a strongly alkaline solution

$3\,NO_3^- + 8\,Al + 5\,OH^- + 2\,H_2O \rightarrow 8\,AlO_2^- + 3\,NH_3$

$NO_2^- + 2\,Al + 5\,H_2O + OH^- \rightarrow 2\,AlO_2^- + 4\,H_2O + NH_3$

❑ Another reducing agent may be Zn(Cu) + dil HCl to produce nascent hydrogen

❑ After reduction and distillation step is over, the NH_3 is determined as usual by colorimetric method (Nessler's method) or by back titration method

Now, how we can determine the nitrate nitrogen? That is also done by Nessler's method. But you have understood till now, that in the Nessler's method we need ammonia or ammonia nitrogen. But nitrate nitrogen is not ammonia nitrogen. It may be present as nitrate or even nitrite. So, how will you convert it to ammonia nitrogen? It can be simply done by reduction. So, how will you reduce it? Determination of nitrate nitrogen or nitrite nitrogen is one of the most difficult tasks. Nitrates and nitrites present in water can be reduced to ammonia by nascent hydrogen. Nascent hydrogen is very active reducing agent. How will you do it? The reduction of nitrate by nascent hydrogen can be done in different ways. I am giving one example. It can be done by Devarda's alloy (50% Cu, 45% Al, 5% Zn) in strong alkaline condition. Devarda's alloy in strongly alkaline medium, will produce the nascent hydrogen which will reduce nitrate to ammonia or nitrite to ammonia. After getting the ammonia nitrogen you can apply the same method. Another reducing agent that may be used is zinc copper. This is a type of alloy. You can prepare it by putting zinc granules to copper sulphate solution. You will see, the blue colour copper is deposited on zinc. After keeping for some time in acid condition, it will produce the nascent hydrogen. Then you have to follow the same method after the reduction i.e., for ammonia nitrogen determination. Now, say for example, you have in your sample all 3 different types of nitrogen. But you want to know how much is ammonia nitrogen, how much is organic nitrogen and how much is nitrate nitrogen. Then you have to do it in stepwise fashion. First you have to take the sample and determine the ammonia nitrogen. So, ammonia nitrogen is gone there. After that, you take the same sample from the Kjeldahl's flask. Then you do the organic nitrogen. And finally, you do the nitrate nitrogen. If you want to do the nitrate nitrogen first, then ammonia nitrogen will also come there. That is why it will not be correct.

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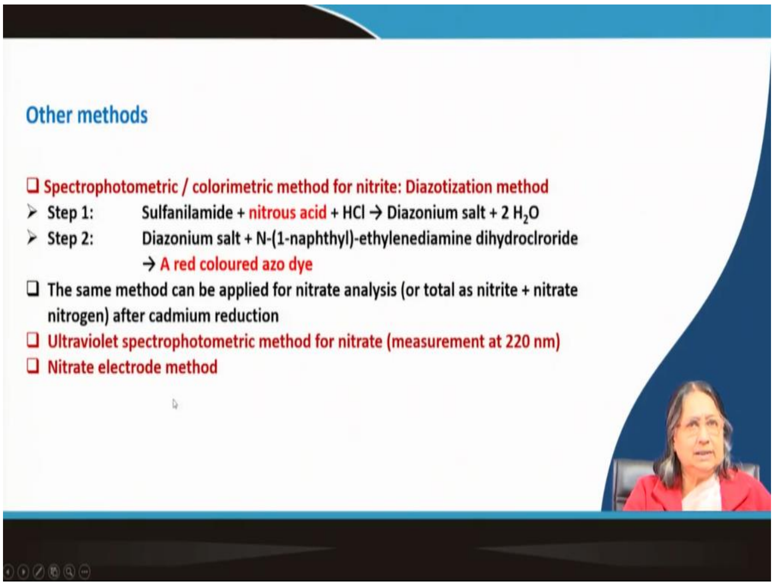

Other methods

❑ Spectrophotometric / colorimetric method for nitrite: Diazotization method
- Step 1: Sulfanilamide + nitrous acid + HCl → Diazonium salt + 2 H_2O
- Step 2: Diazonium salt + N-(1-naphthyl)-ethylenediamine dihydroclroride → A red coloured azo dye

❑ The same method can be applied for nitrate analysis (or total as nitrite + nitrate nitrogen) after cadmium reduction

❑ Ultraviolet spectrophotometric method for nitrate (measurement at 220 nm)

❑ Nitrate electrode method

There are other methods also such as spectrophotometric method by using the diazotization method. What is diazotization? In diazo compound, there is N=N and it has very strong colour. In textile dyes, you will see the 90% dyes are having the diazo group.

There are 2 steps. In the step 1, sulfanilamide, nitrous acid and HCl are allowed to react. They will react to form that diazonium salt. Then this diazonium salt will react with this N-(1-naphthyl)-ethylenediamine dihydrochloride to form a red-coloured azo dye. So, diazo compound is converted to azo compound. Then you can measure the absorbance in the spectrophotometer. So, basically it is a quantitative method. The same method can be applied for nitrite analysis or total as nitrite plus nitrate nitrogen after cadmium reduction. So, this is basically a method for nitrite determination. But if you have nitrate, then you can convert the nitrate to nitrite first, by cadmium reduction. Cadmium amalgam is used for the purpose.

There is another method, i.e., ultraviolet spectrophotometric method for nitrate (λ_{max}=220 nm). This range is not very good, but some people use it because it is the simplest method. But you have to have UV-visible spectrophotometer. It is much costlier than the visible spectrophotometer.

There is also nitrate electrode where you can just put it in the solution and you can get the concentration. But the self-life of this electrode is not much, maybe 1 year or 2 year maximum. Moreover, it is costly also. But it is very user friendly. You do not have to do anything but only calibration and measurement.

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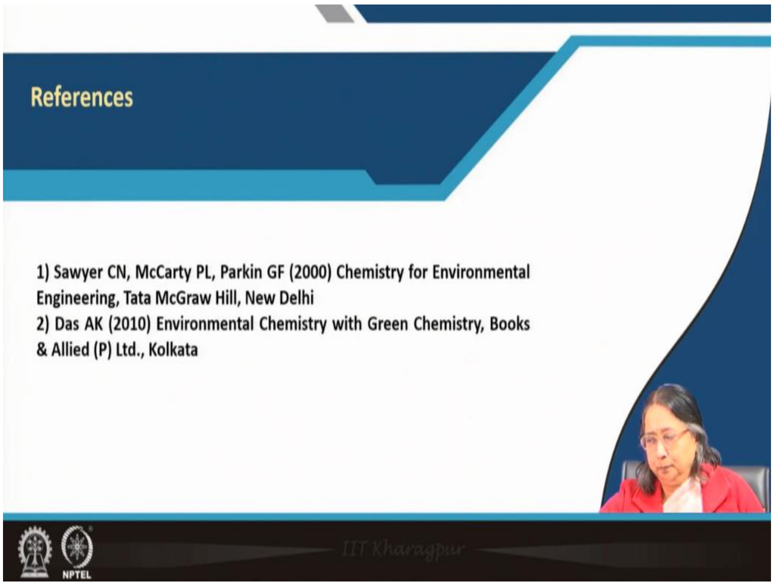

References

1) Sawyer CN, McCarty PL, Parkin GF (2000) Chemistry for Environmental Engineering, Tata McGraw Hill, New Delhi

2) Das AK (2010) Environmental Chemistry with Green Chemistry, Books & Allied (P) Ltd., Kolkata

Now, as a reference, I can tell you about two books, first one is the Sawyer McCarty book and the other one is by Asim K Das.

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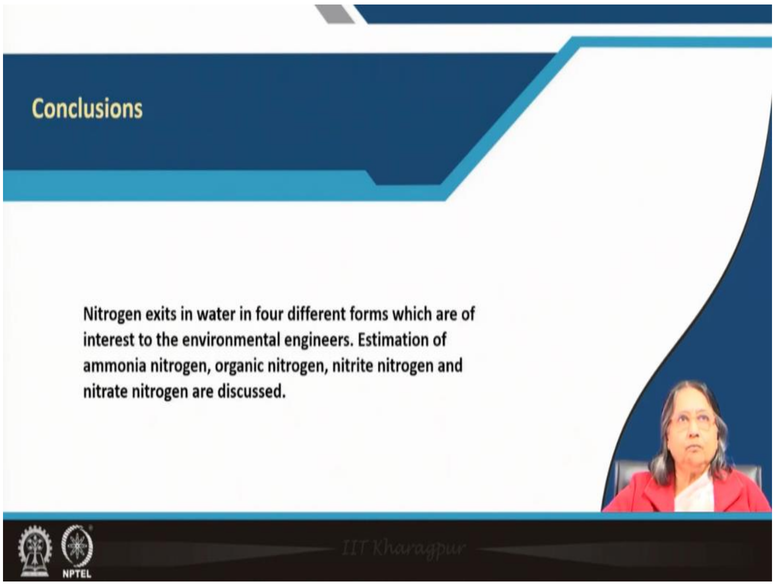

Conclusions

Nitrogen exits in water in four different forms which are of interest to the environmental engineers. Estimation of ammonia nitrogen, organic nitrogen, nitrite nitrogen and nitrate nitrogen are discussed.

In this lecture, I have shown that nitrogen exists in water in 4 different forms which are of interest to the environmental engineers. Estimation of ammonia nitrogen, organic nitrogen, nitrite nitrogen and nitrate nitrogen are discussed. So, this is basically analytical chemistry part. Thank you.