Water Quality Management Practices

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Week – 02

Lecture – 10

Determination of Nitrogen, Phosphorus & Microbial Counts

Hello everyone, welcome to this NPTEL online certification course on Water Quality Management Practices. My name is Gaurav, Professor Gaurav Dharbomic from the Department of Agricultural and Food Engineering of Indian Institute of Technology Kharagpur, India. So, continuing to my discussion with the quantity estimation of major pollutants in water in the waste water, today we will be giving you the last lecture of this module and we will discuss about the how determination procedure for nitrogen, phosphorus and the microbial counts. The concept that I will be covering here majorly the determination of nitrogenous compound, the ammoniacal nitrogen, nitrate nitrogen, nitrate nitrogen and the total Kjeldahl nitrogen and determination of phosphorus concentration and the determination of most probable number for coliform microorganisms quantification. Determination of nitrogen in general the nitrogen content in sanitary sewage is about 15 to 50 milligram per liter. In waste water it may present in organic or inorganic form under reduced or oxidized form.

In general they present either in nitrite, nitrate, ammoniacal or Kjeldahl nitrogen form. What is this Kjeldahl nitrogen? Kjeldahl nitrogen is kind of a giving us combined understanding about the the ammoniacal as well as the organic nitrogen present in your waste water. So, we have nitrite and nitrate and then we have ammonia and organic nitrogen. So, ammonia and organic nitrogen gives us the understanding about the total Kjeldahl nitrogen and nitrate and nitrate is also separate.

So, which is also considered as inorganic component of nitrogen waste present in the waste water. So, this ammoniacal nitrogen in order to determine this ammoniacal nitrogen the sample pH has to be adjusted to 9.5 using 6 normal sodium hydroxide solution to reduce the hydrolysis of organic nitrogenous compounds. To determine the ammonia by titration method normally we select the sample volume of 250, 100, 50, 25 milliliter for 2 to 10, 10 to 20, 20 to 50 and 50 to 100 milligram per liter of ammoniacal nitrogen concentration present in a sample respectively. We need to collect approximately 1 gram of dry weight of sludge or the sediment sample in crucible and then wash and dilute it to 250 ml in a general flux.

So, this procedure is only this procedure is for the sludge the solids . In order to we then we carry out the distillation process in a boric acid solution and collect only 100 ml in of the distillate. This then in terms of waste water because our concern is majorly waste water in case of waste water what we do we add 25 ml of borate buffer to 500 ml of waste water sample and adjust the pH to 9.5 using 6 normal sodium hydroxide solution. Then we carry the distillation at 6 to 10 milliliter per minute with the delivery tube tip being immersed in a recovering receiving boric acid solution.

Then we collect at least 200 ml of distillate from the top and then we dilute it to 500 ml with

the standard 0.02 normal sulfuric acid titrant in presence of mixed indicators containing methyl red and the methylene blue. When the color turns from the to the till the color turns to the pale lavenderish in nature. The same process has to be carried out for a blank solution as well like not the waste water, but the actual distilled water and all. And then what we will do then ammonical nitrogen present in the sample can be easily estimated with this equation for liquid sample.

Ammonical nitrogen (mg/L) = $\frac{(A-B)\times 280}{Volume\ of\ Sample\ (mL)}$. This A is nothing, but the volume of sulfuric acid titrated for sample and in ml and B is the volume of sulfuric acid titrated for blank in ml as well. And for the sludge also is the same, but the unit will be milligram per kg. For nitrite nitrogen NO 2 negative is determined by associating the this diatodite sulfur and amide with NAD dehydrochloride through the intermediate formation of a reddish purple azo dye at pH 2 to 2.5. Presence of nitrogen trichloride gives the false red color after the addition of this color regions and any suspended solid in the sample must be filtered before only as the presence of colored ions may actually alter the color. Fresh samples should be used for nitrates determination as bacteria present may start converting the nitrate into nitrate very fast or to ammonia. The sample can only be also be stored between 4 degree to minus 20 degree Celsius for the next 1 or 2 days. And this nitrate nitrogen then we filter 50 ml of this wastewater sample through a 0.45 micron pore size of membrane.

Grass filter paper normally we can take and then we bring the pH down to bit bit around 5 to 9 with 1 normal HCl or ammonium hydroxide followed by addition of 2 ml of color reagent. We use the spectrophotometer between 10 minutes to 200 2 hours to measure the absorbance at 543 nanometer. Using this light path as 1 5 and 10 centimeter for 2 to for 25 2 to 6 and the less than 2 micro liter microgram per liter of nitrite concentration respectively to plot the absorbance and formulate a standard curve from which we can easily find out the concentration of the sample the estimate the concentration of the sample. So, how we can do it for the nitrate nitrogen? We determine the nitrate nitrogen using the spectrophotometer at only 1 wavelength as only the 1 wavelength and sometimes is difficult going to a it like you know it is being able to absorb the UV light as well like other dissolved organic matter and solute. So, the UV spectrophore nitrite and nitrite nitrate and nitrate are similar.

However, nitrate concentration is usually much higher in polluted water than the nitrate because it is it can be easily converted into nitrate. This UV absorbance spectrum reaches to a peak of 203 nanometer when nitrate nitrogen concentration is less than 3 milligram per liter and the slit width of slit width of spectrophotometer may also affect this profile of this UV that also we need to be concerned we need to be concerned about. In general what we do we prepare the filtered sample water at the wastewater and take 50 ml of it and we add the 1 ml of 1 normal HCL solution and we mixed it well. Then we measure the absorbance and 220 nanometer against the distilled water sample for which the absorbance is set to 0 to obtain the actual nitrate absorbance rating . Now, this absorbance rating is determined at 275 nanometer to determine the interference due to the presence of dissolved organic matter and from the absorbance reading at 220 nanometer subtract twice the absorbance reading at 275 nanometer to get the absorbance due to the nitrate ions.

We can also do the construct the standard curve for absorbance of nitrate versus the known nitrate nitrogen concentration and thus using this collected sample absorbance the nitrate concentration present can be easily estimated from this curve. From this graph if you see the sea surface nitrate concentration in milli mole of milli mole normal it in like you know in in

in in per meter cube. If you see its nitrogen per meter cube if you see the purple ones are actually considering like you know giving us a very low concentration whereas, the red and the the the yellowish ones it is giving us the high concentration which is normally is the in the polar region. Total Zelda nitrogen another very important thing as I was discussing you that the total Zelda nitrogen it is represented by the ammoniacal nitrogen plus what the organic nitrogen remember. So, it it includes the proteins peptides urea and many other synthetic organic compounds as well .

So, fresh samples you should always be used and you should analyze it with the fresh sample only then you have to acidify it with the concentrated sulphuric acid say at 1.5 to 2 pH and then store it at 4 degree Celsius. Then you can select the different sample size and you based on that you add 800 ml of Kjeldahl flux added in the Kjeldahl flux and you can see the Kjeldahl flux how it looks like and then you adjust the sample pH to 7.0. Now you can add 25 ml of borate buffer followed by 6 normal of NaOH till the pH of 9.5 is achieved thenboil the solution after adding the glass beads . Now cool the Kjeldahl flux and you carefully add 20 ml of digestion reagent consisting of 6.7 gram of K_2SO_4 and 0.365 gram of $CuSO_4$ and 6.7 milliliter of concentrated H_2SO_4 in the distillation flask.

Now we boil the arrangement till profuse white fumes where you start observing and the volume comes down to 25 to 50 ml. Now you continue the digestion for 30 minute till the sample turns into pale green and cool it and dilute it with a 300 ml of distilled water. Now what you need to do once you added with this distilled water you connect the flux in a steamed out distillation apparatus and the pH of the solution should not should obviously, exit the 11.0 I mean like the pH and now you collect a 200 ml of distillate from this in this 50 in a 50 ml boric acid solution with the tip of the condenser below the level of boric acid absorbent solution and maintain the temperature in the condenser below 29 degree Celsius. You there are apparatus nowadays which are available which can do this kind of operations by itself.

Now you have to titrate it using 0.02 normal H_2SO_4. You titrate it until the color turns into pale lavender. Now this organic nitrogen can be easily estimated by this equation $\frac{(A-B)\times 280}{Volume\ of\ Sample\ (mL)}$ that you have. A is nothing, but the volume of sulphuric acid that is needed for titrating the sample.

B is the volume of sulphuric acid that is needed for titrating the blank . The summation of ammoniacal nitrogen and organic nitrogen gives us the TKN the total geldal nitrogen present in your sample understood. This is the standard methods given by the American Public Health Associations there are other methods as well, but this is one of the simplest one and that is been widely followed. Determination of phosphate phosphorus in general in wastewater majorly the phosphorus is coming from the detrituses and the body of aquatic organisms only. Other than that nowadays people you know nowadays agricultural runoff is becoming one of the major source of this phosphorus present in the present in the wastewater.

It normally can be classified in it normally stays in the form of orthophosphate or the condensed phosphate or organically bound phosphates is not it. So, what are the major sources that I was saying it is pollution from the sewage, industrial waste, application of the fertilizers and the from the natural contact with the minerals that can also leach some amount of phosphorus to the groundwater or the surface water. We normally calculate this phosphorus in two steps . The first one we convert the phosphorus into dissolved orthophosphate and the second we this dissolved orthophosphate it determined by following

the colorimetric method. So, once we know the presence of dissolved orthophosphate in the colorimetric method we based on the equations that we developed for this conversion process we can easily get the value of the phosphate present in your in a sample.

The process is very simple we take the 50 ml of water in a sample of gilder flask and add 1 ml of concentrated H2SO4 and followed by 5 ml of concentrated HNO3. Then we digested to a volume of 10 ml and continue until the solution becomes colorless. We then cool down the sample and add 20 ml of distilled water, add a drop of phenolphthalein indicator and start adding one normal NaOH solution slowly until the faint pink color appears again. Then we transfer it to 100 ml of volumetric flask to dilute it up to 100 ml with the distilled water and take 20 ml of the sample we add 4 ml of ammonium molybdenum molybdate reagent followed by 10 drops of stannous chloride reagent. After 10 minute we measure the absorbance in a spectrophotometer at 690 nanometer of for its absorbance value and compare it with the calibration curve using the distilled water as a blank.

So, from there we can easily calculate the phosphate using this following formula. The weight of the phosphorus in milligram and in approximately 104.5 milliliter of milliliter of in final volume divided by the volume of the sample multiplied by the 1000. From here we can get the value of phosphorus in milligram per liter. So, this nitrogen and phosphorus as I was saying it is one of the major kind of nuisance sometime for our wastewater treatment plant.

So, that we have to worry about we normally also make sure that our nutrients are at ease we try to reduce the concentration of it by we reduce try to employ some amount of treatment plant and all. So, specifically for it. So, . So, separate like other than that we another important pollutant that we also need to understand is the microbial count. The majorly the colony forms which are used to detect the bacterial contamination.

So, the um multi tube multiple tube fermentation technique is generally used to identify the presence of coliforms which would act as an indicator organisms in general. The coliforms being the the enterobacteriaceae family and are facultative and anaerobes and non spore forming and they are gram negative in nature . The most probable number MPN it actually represents the number of this multiple tube fermentations that is the probability of the number of poly forms present in a sample per 100 ml that is one of the most famous way of representing the MPNs. How we can determine the this microbial count? We can simply suspend you know 35.6 gram of laurel tryptos broth or L T L T B in 1 liter of distilled water and dissolve the medium completely.

We use 3 groups of 5 test tubes and add 9 ml of broth to each test tube containing inverted derham tubes to detect the fermentation grass. Now sterilize before doing a we have to sterilize and then we have to sterilize all the tubes and in in an autoclave at 120 degree 21 degree Celsius for 15 minutes and we cool it down in a laminar flow chamber under UV light. So, to reduce any kind of you know contamination to reduce the chances of any kind of contamination. Then we transfer 1 ml of sample to each 5 tubes of 1 group of broth and we add 0.1 ml of sample to 5 tubes of next group of broth and we add 0.

01 ml of say sample of 5 tubes of remaining last group of broth tubes. Then we incubate the tubes at 35 degree Celsius for 24 hour if no gas or acid reaction evident by the shades of yellow color is evident after 24 hour continue we continue it until 48 hour and reexamine. After incubation we observe the fermentation gas in the derham tubes and we record the number of positive result from each set and compare it with the standard chart that is given for to give

the presumptive polyform count per 100 ml and for the combination of tubes not appearing in the standard chart MPN can be determined using the following formula majorly if the number of positive tubes in 200 divided by root over of ml of sample in negative tubes multiplied by ml of sample in all tubes. So, in general we in this particular lecture video it was quite fast because I do not want to go in much details about it you can easily get all this information in the in any regular you know later video I mean regular textbo also that how to determine the TKN how to determine the nitrite nitrate phosphate phosphorus or the MPN.

So, that is why I make it very short. So, we know that, but however, it is very important for us to at least discuss during this lecture series because we need to understand that how this how this nitrogen and the phosphorus is impacting our treatment in it. Specially the nitrogen when we talk about it is very important because unless until we know the negative effect of nitrogen in our waste water in our treatment I mean like in the follow up systems it is very hard for us to understand and also we need to understand the basic testing test procedures and all right. So, normally whenever you will be designing we will be discussing in coming modules when you will be designing any say like aerobic or aerobic treatment unit we need to be sure about it that our treatment system is designed in such a way that it is it can biologically remove the nitrogen or the phosphorus present in our systems specifically in nitrogen. How we can do? We can do it aerobically as you know the nitrification process it requires the oxygen it is oxygen scavenging process. However, it requires oxygen and it that because of the process of nitrification the ammonia will be converted into nitrate and nitrate finally.

This nitrate if you want to remove it further remove it from the system further that also we can do. How we can do? You can remove that additional amount of nitrate by the process called denitrification . There are by denitrification process this nitrate will be converted into dinitrogen gas. You can do further some more stuffs you can do this there are concepts like you know an amox process which is coming by which actually we can actually directly remove the ammonia into dinitrogen gas is also possible. There are processes by which we can actually do the the we call the air stripping processes by which also we can actually get rid of the ammonia from the systems.

Anyway so, there are there are different procedures by which actually we can get rid of the nitrogen from the waste water and in this is one of the foremost necessity for whenever we will be designing in future that different type of treatment plant for our city for say municipality or say like for our industry that how much is the nitrogen component and it whether it is it can remove the nitrogen or not because that is one of the major criteria for any regulatory bodies. We discussed about the how we can treat this how we can actually determine this different type of nitrogen components. We discuss about the ammoniacal nitrogen, we discuss about the nitrate nitrite, we discuss how phosphate phosphorus can also be determined, we also discussed how the MPN the most probable number in number of like microorganisms per 100 ml of say waste water is present that is also kind of a one of the best quantifying criteria that whether your final effluent is easily dischargeable into the surface water or not. Even that is one of the major criteria for you to understand whether it is a drinkable or not. If it is it is 0, if that MPN value is 0 almost 0 then it can be almost considered as a in the portable water or drinkable water considering all the other factors are .

So, these are the some of the things in this particular lecture module I hope you understood different major pollutants that is present in the waste water and you also

understand that the what are the significance of all these pollutants and from next module onwards we will be going into more in details about the process factors about the process chemistry and also the we will do some reactor engineering and then we will continue with the actual plant design and how actually we need to go designing the different plant which can actually treat those waste water or the water which is very important for us. I was as I was saying it is very important for all of us to understand this water engineering and where the water is coming into your glass or what is the fate of the water that is coming into your household isn't it. So, this is very important for all of us to know and we will be discussing more in details about all these things in the coming lecture. Please follow these references where they from where actually this materials are like materials materials are actually been taken and this is the papers and the bos are very important very nice and lot of information can be gathered through while you read these bos and all . So, thank you so much I hope you understand and actually enjoy this particular module and we will see you in the next module. Thank you so much.