Course Name: Industrial Wastewater Treatment

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Week – 11

Lecture 2: Treatment of wastewater produced from pharmaceutical industry

So, welcome back. We are in module 11, lecture 2 and we are discussing about the treatment of the wastewater which is generated from the pharmaceutical industries. So, in this lecture we will be covering the various processes about the production of the antibiotics. So, we will talk about the history of the antibiotics, the manufacturing process of the antibiotics and we will also talk about the wastewater that is generated from the production of the antibiotics as well as we will also talk about what are the wastewater characteristics that is generated from the production of antibiotics. So, antibiotics are the chemicals which are very effective at low concentrations, and they can kill, or they can stop the growth of the disease-causing microbes or the pathogens which are present inside our body.

So, around 90% of the antibiotics so they are isolated from the bacteria, they are isolated from the fungi or the molds and at one time all the antibiotics were being produced from the living organism only and that process was known as the biosynthesis process which is still used in the manufacture of the several antibiotics. So, the antibiotics was first known to the world, and it was used by the Chinese around 2500 years ago and they applied the antibiotics in the form of moldicard of the soybeans so that the infected parts so they can be treated easily by applying such type of moldy curd and they found that it has got a lot of medicinal benefits as well as the therapeutic benefits. Similarly, the Sudanese-Ubian civilization so they also used a type of tetracycline antibiotic as early as 350 AD whereas in Europe near the middle ages so crude plant extracts and the cheese curds were also being used for fighting the infections. So, although these cultures they were using antibiotics, but the general principles of the antibiotic actions were not understood until the 20th century.

So, Louis Pasteur in 1877 it discovered that the growth of the disease-causing anthrax bacteria it could be inhibited by the saprophytic bacteria and he also showed that the large amounts of anthrax bacilli can be given to the animals without having any adverse effect on the animals as long as the saprophytic bacteria or the bacilli were also given to them. So, over the next few years it was basically these observations that the bacteria or basically the fungi or the molds whatever the biological material is being derived from them so it is helping in the prevention of the growth of the disease-causing bacteria as well as the pathogens and that's how the generation or basically we can say the production of the antibiotics it started. So, the production of the antibiotic is a very lengthy as well as the costly process as it includes the living organisms so it must be first of all identified that

what type of antibiotic we are going to manufacture and which organism or the microorganism is responsible for the production of that antibiotics. So, this means that screening process has to be followed so that the identification of that microorganism can be done and there are two types of screening processes for example we are having the primary as well as the secondary screening process. The primary screening process means that the antibiotic that is being produced from the organism so this is basically the performance of that antibiotic is first of all measured and in this process the primary screening process lot of compounds which are produced from the antibiotics so they are rejected and later on when it passes the primary screening process so after that the secondary screening process is done which basically leads to the extensive investigation into the properties of the different compounds so we see that no toxic compounds are produced and whatever the antibiotics are being produced so they do not have an adverse impact on the women who are consuming such type of antibiotics.

So, the toxicity testing is one of the very very important process in the evaluation process and the antibiotics which are having acceptable therapeutic index so that basically are generally taken and they are used for the production of the further antibiotics. So most antibiotics they occur in the nature but they are not available in the quantities which are required for the large scale production of the antibiotics that's why the antibiotic production has to be done so in that case the organism or the microorganism which are producing the antibiotics that has to be grown on a large scale so that we can produce it on a larger scale we can have the high amount of the antibiotics produced so that the further purification of that antibiotics as well as the chemical analysis of the antibiotics can also be done and we can demonstrate that such type of antibiotic which is being produced so that is unique in nature. So the process of the production of the antibiotics it involves the fermentation process so fermentation process means that is first of all we have to isolate the desired microorganism so basically if we want to have the certain antibiotics being produced so that can be produced by a certain microorganism so it means that the isolating identification and the isolation of the microorganism is a very very important is the first and foremost step for the fermentation process. After that the culture is grown and the nutrients as well as the substrate that is required for the growth of such type of microorganism so that basically is added and the culture basically is prepared and after the culturing is done the culture basically is taken to a large-scale production and this leads to the formation of the antibiotics and later on the refining and isolating the final products that is the antibiotic is done and then it can be taken to the end users. So microorganism can be grown either in the solid media or they can be grown in the liquid media so this solid media or the liquid media must have the necessary nutrients which are required for the growth of that microorganism for example when we are considering that the bacteria or basically the fungi or the modes that are producing that antibiotic so we need to know that what nutrients are required what substrate is required for that bacteria to grow in a very large numbers and here the main constituents will always include the carbon nitrogen and

sulfur which is required in very high amounts and similarly it is possible that we may also add certain micronutrients certain inorganic substances which may also be required for the growth of those bacterias and the complex nutrient sources may be also be added so they can be degraded by the enzymes like proteases and diastasis but they are degraded at a very slow and steady rates and similarly the all the optimum conditions for example the pH the temperature and air so these all things need to be controlled so that optimum growth or the maximum growth or rate of the bacteria they can occur in such conditions.

So, when we go for the fermentation process the antibiotic producing microorganism so it must be isolated and the numbers has to increase many folds so that's why it is possible that we have to first of all go for the culturing in a lab so that this culturing sample basically can be done by using a agar containing plate and that initial culture is generally done in the labs and then this initial culture is then taken to the shake flask where the nutrients and the substrate which is required for the growth of those micro organisms so that basically happens in the shake flask and once this suspension which contains isolated bacteria as well as the higher number of the bacteria that basically creates a suspension and this can later on be transferred to a bigger tanks which are known as the seed tanks so where further growth happens and further we have to add certain nutrients or the substrate right so that has to be added so that the large population of the microorganism need to be developed and in this seed tank we we have to have all the conducive conditions for example we can add warm water if you want to control the temperature similarly we have to add the food for example the carbohydrate sources like lactose or glucose sugars which they can easily metabolize and then they can grow in a large numbers in such seed tanks and we have to also add carbon sources for example we can add acetic acid to it we can add alcohols or we can add hydrocarbons to it so that the necessary carbon sources may be available to the bacteria and similarly the nitrogen sources also we had added for example the ammonia salts can be added here so that they can get a high amount of nitrogen for their growth similarly the growth factors for example the vitamins the amino acids the minor nutrients so they all have to be added into the seed tank so that the growth of these microbes can be enhanced and these seed tanks may also be equipped with the mixers so that the contents of the seed tank so they may be moving they may be mixed properly and a pump is also used so that we can deliver the sterilized filtered air and if it is required in such process so after 24 to 28 hours where then the material from the seed tank is transferred to the primary fermentation tanks where the fermentation process takes place and the production of the antibiotics takes place so if we talk about the fermentation process so it is a metabolic process which converts the glucose into acids gases or alcohols so it can be done in absence of the oxygen or any electron transport chain so here no electron transport change is present as we see in case of the respiration so the fermentation pathways always regenerate the coenzyme that is nicotinamide adenine dinucleotide that is NAD plus so that it can release energy in form of the adenosine triphosphates so fermentation yields a net of two ATP molecules per glucose molecules through the process of the glycolysis whereas the aerobic

respiration it can yield up to 32 molecules of ATP per glucose molecules with the aid of the electron transport chain so here basically the coenzyme that is NAD plus is getting converted to NADH and that basically is providing the energy for the reaction so during the fermentation an electron acceptor like pyruvate or acetaldehyde it reacts with the NADH so it forms NAD plus and this basically leads to the generation of the products like carbon dioxide and ethanol so this is known as the ethanol fermentation or basically it can also lead to the formation of lactate so which is known as the lactic acid fermentation so if we talk about the ethanol fermentation so we can see here that the glucose it undergoes glycolysis so here the NAD plus is converted to NADH and here the ADP basically is converted to ATP and we see that the two molecules of pyruvate they are generated during this process and that these two molecules of pyruvate later on they are converted to two molecules of acetaldehyde and two molecules of carbon dioxide and later on this acetaldehyde by the reaction with NADH which basically reduces this acetaldehyde to ethanol so and the NADH basically is converted to NAD plus so here the regeneration of the NAD plus takes place and we get the two molecules of ethanol in this case so we can see here that the glucose is converted to two molecules of ethanol and two molecules of carbon dioxide they are produced in this reaction so such type of fermentation process is known as the ethanol fermentation and here the acetaldehyde is reduced by NADH to ethanol and it basically regenerates the NAD plus which can again be used for the process of the glycolysis and such type of process is generally used in production of the beer or the bread production similarly the another type of fermentation that takes place is the lactic acid fermentation which basically may be used for the flavor and preserving the dairy and the vegetables so here in the lactic acid fermentation the glucose undergoes the glycolysis and again here the NAD plus gets converted to NADH and the two molecules of ADP are converted to two molecules of ATP and we get the two molecules of pyruvate here and this pyruvate can then be reduced to the two lactate molecules where basically NADH converts into NAD plus and this basically leads to the formation of two lactate molecules so you can see here the glucose can be converted to the two molecules of lactate in this lactic acid fermentation process and this process can also be of two type that is it can be homolactic process or it can be heterolactic process in the hemolytic fermentation so NADH it reduces pyruvate directly to form lactate as we see here whereas in the heterolactic process the some lactate is further basically gets converted to ethanol and the carbon dioxide so this process is called heterolactic fermentation so now in the fermentation tank which is basically the larger version of the seed tank so here we are having the same growth media as we have used in case of the seed tank and here the because of the high volume of the tank and high volume of the media these bacteria so they are grown at a very fast rate and they multiply and they are able to grow rapidly in such type of conditions so as they grow so they excrete a large quantities of the desired antibiotics in such cases so in the fermentation tank we get a huge amount of antibiotics that is desired that is that we require to produce and the temperatures are maintained in the fermentation

tank and temperature between 23 to 27.2 degree centigrade so they have been shown to produce a high rate of antibiotics so it will depend upon the type of antibiotics that we are producing so temperature may be then controlled accordingly and similarly the these fermentation tanks are constantly agitated so that whatever the suspension is there so whatever the contents of the fermentation tanks are there so they always remain in suspension so that the required food as well as the required nutrients so they are available to all the microorganisms which basically are multiplying at a faster rate and they are producing the antibiotics similarly if it is required we are having some aerobic bacteria so in that case the aeration may be required so it should be aerated properly as well as the ph needs to be maintained so that the ph that is required for the optimum growth of the bacteria that needs to be controlled so after the three to five days of this fermentation process we can get a very high amount of antibiotic production and after the antibiotics is produced so later on we go for the process of the isolation so in the process of isolation depending upon the various type of antibiotics that are produced depending upon the specific type of antibiotic that is produced so this fermentation broth which is there so it is taken through various purification methods for example if we are having the water soluble antibiotic compound so in that case the ion exchange process may be used for the purification purposes whereas if we are having the oil soluble or hydrophobic antibiotics so in that case we find that for example penicillin so in that case we can use solvent extraction method so we use a solvent in which this type of antibiotic may be soluble and later on the solvent can be recycled or solvent basically can be recovered by the evaporation process so the broth that is prepared so it is treated by a number of organic solvents for example it will again depend upon the type of antibiotics that we are talking about and for example we can use here methyl acetate or methyl isobutyl ketone so it can specifically dissolve that type of antibiotic and the dissolved antibiotic is then recovered by by following the various organic chemical means and once the we get the purified antibiotics so lastly it can be converted into crystalline form or it can be converted into powdered form and later on it can be refined further it can be converted into different type of products or different type of applications can go to the end users so in the refining process the antibiotic can take different type of forms for example here when we have gone through the fermentation process so we have recovered we have purified the antibiotics and that purified form can again be now transferred into different type of applications for example we can produce solutions out of it which can be used for the intravenous bags or for the syringes we can convert the antibiotics into pills or we can convert it into capsule forms we can convert we can have it in the powder form we can have we can convert it into the different type of bindments for example if we talk about the intravenous bag so this crystalline antibiotic that we get so it is dissolved in the solution and then it is put in a bag and that bag is hermetically sealed similarly for the capsules we can fill the half part of the capsule with the powdered antibiotics and later on the top half can be put mechanically in place and then once these products are formed for example the ointments can be formed or it basically

remains in the powder form whatever the form the antibiotics produce so that is ultimately packed and then it is transported to the to the final packaging stations so the quality control process is a very important process during the manufacturing of the antibiotics so here steps must be taken so that we can ensure that no contamination takes place during the production process so it is very very necessary that during the production process we always check that there is no extra contamination that is occurring because of the production processes that we are undergoing so for example here the equipment that we use so they are constantly steam sterilized so they are basically free from the any pathogens that basically can contaminate or any chemical that can contaminate these drugs similarly during the manufacturing process also the quality of all the compounds that we are using for example we use a number of chemicals we use a number of solvents so the quality of these compounds should be checked regularly so that basically we can see that they are having the desired standards so that no toxicity is induced in the final antibiotics similarly there should be a frequent checks on the condition of the microorganism culture for example when we are going for the fermentation process so it is possible that the microorganism culture may be converted into toxic byproducts if the conditions are not conducive so in that case we need to check that the microorganism culture is always having the desired quality and this can be checked by using various chromatographic techniques for example you can use the liquid chromatography or we can use the gas chromatography and then we can check that whatever the antibiotic production is happening or whatever the other compounds or the byproducts that are being created during the culture so they basically are not toxic or they do not induce any contamination to the antibiotics so similarly we have to also check the physical and chemical properties of the finished products also for example the ph the melting point the moisture content so these all needs to be checked before the final finished product is transported so now let us talk about the production of one important type of antibiotic that is the penicillin so this penicillin is produced from the modes of penicillium notatum chrysogenum so this group of the bacteria which is cultured it is under submerged aerobic conditions as the fungi here basically is the aerobic bacteria so it is basically fermented in the aerobic conditions and here the medium that we use is a corn steep liquor a very good nitrogen source similarly we can add here peanuts or the mineral salts and the lactose is added for the carbon source so this culture that basically is there so this culture that is by addition of this medium and the penicillium notatum chrysogenum so this leads the fermentation of the antibiotic that is penicillin is produced and later on once the penicillin is produced from this fermentation process the modes mycelium so they are separated by the process of the filtration later on the filtrate that we get penicillin so then this filtrate is acidified to a suitable ph by using the phosphoric acid and later on the penicillin is removed from this suspension by extracting it with the amyl acetate we can also use butyl acetate also so this penicillin solution is then further extracted by using a buffered solution of salt for example the sodium chloride can be used here so that the penicillin which is there it is it can be precipitated out it can be removed from the

from the solution then the isolated penicillin is finally purified by by extracting it with an organic solvent and from that organic solvent we finally get the purified penicillin form so we can see here this process of the penicillin production so we are having the penicillium we are having the penicillium chrysogenum and this basically is taken into the lab where basically we use it we use a number of shaker flask so that we can grow this penicillium we can isolate and we can grow penicillium chrysogenum in the under controlled conditions and later on it can be transferred to a seed tank where the growth of this fungi is further enhanced and later on from the seed tank we can take some solution into the fermentation tank where basically the fermentation of this fungi is done on a large scale and the seed tank constantly replenish the fungi into the fermentation tank and this fermentation tank leads the formation of a high amount of antibiotics into it and later on the suspension basically may be filtered and then from the filtrate extraction may be done from the penciling by using the first extract like for example we have used the ethyl acetate or the butyl acetate so that can be used similarly then the second extraction is done where we use salts and later on we can go for the third extraction where we can again use certain solvents so that we can get the purified penicillin from it and after this we pass it through the vacuum crystallization so that the crystals basically may be formed and later on the slurry that is that contains crystals so that can be filtered through a ceramic filtration process and we can get the crystals here and the crystal wash can also be again filtered so that we can get the further crystals that basically are there in the wash process and then it can be taken to a vacuum dryer and from the vacuum dryer we get the penicillin in the crystalline form so then we can have another type of antibiotic also that is known as streptomycin so the the production of the streptomycin also uses the similar processes as we have seen in the case of the production of the penicillin so here the species known as the streptomyces griseus so it is used for the culturing of the streptomycin so the culture media may contains lot of glucose it may contain corn steep liquor and it may contain other substances for example can have the micronutrients and the other growth factors and other growth requirements so they should be added into the culture so the fermentation broth which is then formed which contains the streptomycin anti-biting so this has to be filtered and the filtrate is later on it adsorbed onto the charcoal or a resin so here the streptomycin that is formed so that gets adsorbed onto the charcoal or that gets exchanged into the resin and later on from the resin or from the charcoal the streptomycin can be eluted by using the dilute acid so we pass dilute acids through the column where basically the streptomycin has been absorbed so this column is of charcoal or resin so from here whatever the dilute acid eluent we get so it contains the streptomycin antibiotic so this eluent that we get so it is further neutralized and it is further concentrated so that we can purify the streptomycin and then the crude streptomycin is further then precipitated and we add acetone to it so that the precipitation may happen and then it is further purified so the yield of the streptomycin may be small in comparison to the raw material that we use and it is reported that the fermentation process more than 90 percent of the raw material that we use so it

becomes a waste so the waste mycelium that we get so this can be either used as a manure or it can also be used as a livestock feed or it can also be disposed of by using the safe methods of disposal so during the process of the production of the antibiotics lot of waste water can be generated for example this liquid waste that is being generated can be divided into two following groups so we can have the spent liquor that is being generated from the fermentation process we can have the wash waters which is being generated from the cleaning of the floors from the equipments from the steaming process etc the waste may contain a lot of acids bases and solvents which we are using during the extraction and the purification process so this may also find its way into the waste water the filter aid which is used in the filtration so it is known as the diatomaceous earth so this generally forms the solid waste so it does not form the part of the liquid waste however some suspended solids may come into the wastewater and similarly the condensates which are coming out from the condensers which are used in the evaporation and drying processes may also basically become the wastewater and it basically enters the wastewater stream so if we talk about the wastewater characteristics so a lot of unconsumed raw materials for example the nutrient broth that we are using the metal salts that we use for the precipitation purposes the starch we use nitrates phosphates and so they all basically becomes the characteristics of the wastewater that is generated from the antibiotics plants similarly this wastewater may have the values of high cod high bod suspended solids will also be high and the pH basically may range from 4 to 8 and because we are using lot of steam for the disinfection purpose for the sterilization purposes so that's why we find that the steam may also contain the chemicals like phenols the detergents the disinfectants which are used for the maintaining the sterility of the antibiotic plant similarly we also find that lot of metals and halogen impurities so they also basically may become the part of the wastewater and these metals or halogens so they may be used for the precipitation of the mother liquor in which the we are fermenting the antibiotics and then we also find that lot of solvents so they also come into the wastewater because it is being used for the purification and the extraction processes similarly the recycling of the solvents also leads to the formation of a mixable organic solvents and they find their way into the wastewater so the antibiotic waste characteristics if we talk about the pencil plant so it may be colorless and it has got a fruity smell and the bod basically may be quite high for example when we talk of bod 5 so it is maybe between 650 milligrams per liter to 5500 milligrams per liter similarly the free ammonia nitrogen may be there for example it may range from 0 to 5.6 milligrams per liter we can also have nitrate nitrogen which may happen because of the nitrification process of the ammonical nitrogen so it may be nearly 0.1 to 0.5 milligrams per liter similarly we also find phosphates into the process because of addition of certain phosphate as nutrients as well as we may also use phosphoric acid somewhere so in that case also the phosphate may be coming out into the effluents so the phosphates concentration may be between 18 to 700 milligrams per liter if we talk about the streptomycin plant so we find that the color may be pale yellow and the order may be there which may be septic order similarly the bod 5 concentrations

are also high that is it may range from 500 to 2810 milligrams per liter we can have free ammonia which may be ranging from 0.3 to 18.2 milligrams per liter nitrate nitrogen is also there which is nearly 0.8 milligrams per liter and the phosphates are also found in the wastewater of the antibiotics produced from the streptomycin plant so that may vary from 9 to 700 milligrams per liter so here the total solids are also introduced for example you can see that very high amount of total solids nearly 480 to 26200 milligrams per liter may be entering into the base water so this may be because of the molds that we use so we are filtering that molds also but again it is possible that some amount of these molds may come as a solids into the base water similarly there can be a very high amount of dissolved solids also that we use as we are using a number of acids we are using a number of salts so that basically increases the concentration of the solids and in the penicillin plant we find that nearly 480 to 26200 milligram per liter of total solids may present whereas in the streptomycin plant we find that nearly 960 to 4950 milligrams per liter of total solids are present and the suspended solids may range from 70 to 1080 millions per liter in the penicillin plant whereas it may range from 80 to 1800 milligrams per liter in case of the streptomycin plant so we are having a higher fraction of the dissolved solids in comparison to the suspended solids and we find that the dissolved solids basically may be volatile in nature because we are using a number of organic compounds like solvents other chemicals which are organic in nature so find that the total volatile solids may be as high as 200 to 12180 similarly we are also basically having a lot of microbes being growing into the solutions into suspension because of fermentation process so that's why the volatile solids basically are very high similarly in case of streptomycin plant also we find that the volatile solids may vary from 480 milligrams per liter to 3070 milligrams per liter and the pH of the penicillin plant base water may be ranging between 3.9 to 7.8 whereas the pH of the streptomycin plant may vary from 2.9 to 8.7 so the pH may vary from acidic to the basic conditions in this case so we end here our discussion on the antibiotic production process and in the next turn we'll be talking about the production of the synthetic drugs and these are the references that we've used for the preparation of this lecture.

Thank you