Water Quality Management Practices

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Role of enzymes and algae in biological wastewater treatment

Hello everyone, welcome to this NPTEL online certification course on Water Quality Management Practices. My name is Gourav, Professor Gourav Bhowmick from the Department of Agriculture and Food Engineering of Indian Institute of Technology Kharagpur. So, in this particular lecture we will be discussing about the role of enzymes and the algae in biological wastewater treatment which is a part of the module 6 Fundamentals and Principles of Biological wastewater treatment. So, in this particular lecture video lecture material I would say like in we will be covering the concepts like the enzymes and their mechanisms of enzymatic reactions. The selection of enzymes for treatment of wastewater the different enzyme models, the role of microbial enzyme for wastewater treatment, enhancing the wastewater treatment efficiency using enzyme, algae and their use in wastewater remediation, types of algae used in wastewater treatment, application of algae in wastewater treatment etcetera. To start with we all know that what is a what is an enzyme.

It is a it is a actually nothing, but a versatile high molecular weighted proteins ranging from 1000 to almost 20 lakh Dalton used by living cells that act as efficient biocatalyst for cellular reactions and all. So, they processes highly rated catalytic properties compared to other chemical catalyst because this is considered as one of the finest biocatalyst possible and it actually degrade the pollutant present in the wastewater and produces the biological macromolecules from this from the complex molecules and all. It generally it consists of at least one polypeptide myotene and it is normally made up of different amino acids group if you can see this picture in this primary structure of an enzyme. So, which normally associated with some amide or the peptide bond in a linear chain ok.

So, what are the advantages of this bacterial enzymatic enzymes for the treatment of wastewater? Because first of all whenever we use the a bacterial enzyme and use this kind of enzyme for the treatment of wastewater, it normally produces the pollutant load like anything. It require very low energy because it is a biological process. It is cost effective and it they cannot complete with they cannot normally compete with other microbial forms. Hence higher performance is there and they have higher reaction kinetics and it work under the mild reaction conditions like you know like ambient temperature or the pH. Different macro and microorganisms like bacteria, fungi, nutrients produces enzymes which is a wide scope of uses across the industries whether it be food, textile etcetera.

And also they are easily adaptable to the different harsh conditions of the environment and all ok. So, that is the reason why we need to go ahead with the enzymatic processes in the treatment for the treatment of wastewater and which is very widespread in nowadays in different advanced wastewater treatment systems and all. So, how we need to understand that how this enzymatic action takes place, how enzymes actually works on treating the wastewater pollutant, the pollutant present in the wastewater and how it actually performs. First one first of all it depends on the nature of an enzyme and the substrate interaction. The catalytic reaction that occurs it can be it is normally it normally occurs on the active or the catalytic site.

We normally call the active site of enzymes ok, which is normally constituted of some amino acid joined through several peptide chains and all. In this catalytic site or this active sites, the side chains of the amino acid residue provide the enzymatic group to bind with a specific group of substrate ok. The enzyme normally create a transition state complex during this catalytic reaction and it returns to its original state after the disassociation of the products in the reactions. So, so there are two different models which are mostly very famous when which actually very nicely describe the mechanism of enzymatic reactions. First one is the Fisher template model or also known as the lock and key mechanism lock and key model and the second one is the induced feed model and all ok.

As you can see in this picture, so I will be discussing about it more in the coming in the coming slide. So, we will be able to understand that how actually this Fisher template model and the induced feed model actually works. From the picture you can see the enzyme in the picture a enzyme and substrate. So, enzyme has a particular active sites which is exactly designed to feed the substrate like a lock and key mechanism and that is why the enzymatic substrate complex actually performs it generates. And this way they actually consume the they actually reduces the pollutant load in the system.

But induced feed model says that the active site is not fixed, it is like lightly you know fixable they actually fix it according to the availability according to the size of the substrate and the based on that they literally change their active site design a bit. So, that it will or it will particularly preferably like you know fix into the into this into the their active site properly and this is how the product is developed. So, in case of lock and key mechanism as I was discussing that it is proposed by the German chemist Emil Fisher in 1894. So, it normally it elucidates that the elucidates that the interaction between the substrate used in the enzymatic reaction and enzyme in terms of the lock and key analogy ok. In this according to this theory both the enzyme and the substrate have complementary geometric shapes and orientations which each other in which they feed easily just as a key feeds into the lock.

So, here the enzyme the active sites present in the enzyme represents as a lock and have the ability to accept the specific substrate and this is exemplified as a keys ok. The shape of both the enzyme and substrate do not normally affect each other and thus the substrate remains stabilized for long. Then there come the induced feed model. In case of induced feed model the scientist Koshland he like in 1963 postulated that an induced feed model can overcome the drawback of lock and key model because of the flexibility of the catalytic site in of an enzyme. Because in some cases Fisher template model it fails to you know kind of give any particular search any particular you know ideal idea to this concept that when in case the active site of the enzyme is not fitting to the substrate what happens in that case.

So, how still they can consume it and how they still they can perform their operations. That particular drawbacks can be easily like you know easily showcased by this induced feed model where they have seen they have it is mentioned that the substrate induced the conformational changes in its catalytic site of the enzyme. So, that the substrate can perfectly fit on its active site in a convenient manner and it catalyzes the chemical reaction successfully, understood. So, this model also proposes the competitive inhibitions allosteric variations and the denaturation of the enzymes during the inactivation as well. What type of enzyme we should actually select for the treatment of wastewater? There are majorly 6 functional classes of enzymes based on their catalytic reactions hydrolysis, the oxido reductase, isomerases like transferase, lyases and the ligases.

So, out of all these things the hydro hydrolysis and the oxido reductases are the widely used enzyme in the wastewater treatment scenario or the in this particular subjects due to their wide range of biocatalytic properties with most pollutant present in the wastewater ok. Other than the lacase and the peroxidases are also common mostly employed for

eliminating the organic micro contaminant from the wastewater due to their wide ranging substrate specific as a specificities and all ok. So, however, you just try to remember this oxido reductases and hydrolysis are one of the most commonly used enzymes in wastewater treatments in a in this particular subject. Now, selection of enzyme. When we treat the wastewater what do we mean by the treating? Treating means we have to somehow get rid of the pollutant load from the system.

So, when we try to get rid of the pollutant load from the systems by using a specific treatment procedure. So, whenever we use the enzyme we have to make sure that that enzyme is target specific that the pollutant that is present in your wastewater is actually specific to that kind of pollutant only you introduce the enzyme which can target on that pollutant. Normally there are different type of pollutants like we can say like carbohydrate same as like you know the nutrient available in the in our food it is majorly like that only we have carbohydrate fat a protein based pollutants and all right. So, and sometimes heavy metals sometimes the temperature base any other and so on so forth we already discussed about these things. So, for degrading the animal fat oil and grease we can target the enzyme called the lipase.

For targeting the protease protein component we can we can incorporate the enzymes called the protease. For tackling the phosphate organophosphates chlorinated compounds and other organic compounds we can target the carboxyl esterases and all. Like this organophosphates compounds can also hydrolyzed via the phosphotry esterases enzymes as well ok. And the carbon and halogen bonds of halogenated aliphatic compounds can cleave by haloalkine di halogens and all. So, based on the type of the pollutant we can modulate we can actually choose the type of enzyme that we need to provide into the systems.

Like for the horst so this is the this is from some different literatures which is not I mean like not very specific, but you can definitely get some idea about the requirement of a specific enzyme for specific type of pollutants ok. Like different kind of lacase collected from the this choriolopsis gallica has a higher and the speedier oxidation rate compared to other fungus lacases. Different types of lipase enzyme hydrolysis hydrolyze the animal fat vegetable oil and floating grease present in the food waste. Three types of lipases are there lipase 1, 2, 3 from the aspergillus species, candida species or portion species degrade the animal fat vegetable oil and the floating greases respectively and respectively and all. So, this is like very specific to this kind to this to the pollutant.

So, that is why we have to select the enzyme based on the type of pollutant that we are targeting. In general what is the role of microbial enzyme for wastewater treatment? The mixture of this carbohydrates and the protease and the lipase enzymes it can remove up to

90 percent of the COD and the 90 percent of the BOD from the BRIORI wastewater according to the study given study done by the Eisen et al in 2009. So, you can easily understand that just by providing this biological I mean like this compounds like you know this enzymes this carbohydrates and protease and lipase they effectively remove this 90 percent of COD. That means, if suppose you have a suppose you have a 1000 milligram per liter of COD load in your system. So, it can remove up to 900 milligram per liter from the systems.

So, which is a huge and this much of removal if it can take place in any enzymatic biological treatment processes it drastically actually I would say like outperform all the existing wastewater treatment systems like you know which does not actually employ the enzymatic treatment processes and all. Because most of the aerobic treatment processes or the aerobic treatment processes it somehow stuck up to 80-85 percentage of COD removal and all. However, in case of enzymatic year because we are normally providing this kind of enzymes with a targeted pollutant. So, our efficiency is much higher the chances of getting much higher removal efficiency is more prominent in this kind of reactors. So, that is why this enzymatic specific enzyme based biological treatment systems are actually work much nice much better way than the existing treatment systems which only randomly when we put the aerobic or anaerobic treatment systems you understand.

So, this is the reason why the role of microbial enzyme come into the picture. There are other studies with the enzymatic treatment duration of 96 hour the combined enzymes reduces 50 percent and 100 percent is of total suspended solid and total hydrocarbon respectively it is quite huge. The two major nutrients such as the ammonium nitrogen and the phosphate also eliminated with the removal efficiency of 100 percentage and 91.6 percentage respectively from brewery wastewater. That means, those enzymes are capable of removing 100 percent ammonium nitrogen from the system ok, which is one of the best I mean like I would say like you know the system that is possible and you need we can employ this kind of system this kind of it needs some certain expertise certain you know the experience I would also say to be the to work on this kind of systems, but however, once it is developed and it is started running properly your treatment since treatment plant can achieve maximum treatment efficiency.

Maximum COD removal efficiency of 54.3 percent is achieved with the pH of 4.8 from distill wastewater after 72 hour in the aerobic oxidation. So, you can see the difference ok. So, this is to just to give you one example this is not actually true all the cases like it is not that low COD removal in some cases the aerobic oxidation can go as high as 80 percent 85 percent is removal of COD as well ok.

However, the comparatively the enzymatic reactions are much more efficient. The protease, amylase and the lipase enzyme extracted from the organic kitchen waste is effective for the removal of 93.5 percentage total suspended solid, 90.8 percentage total dissolved solid, 94.3 percentage of total oil and grease, 75 percentage of the biochemical oxygen demand, 97.4 percentage of the chemical oxygen demand and 99.3 percentage of the chloride from the domestic wastewater as per the research done by Joseph et al 2020. If you want to read this paper the reference in the reference section it is given the paper details you just go through it and try to understand the functionality how this kind of enzymes are nowadays employed in the wastewater treatment system the advanced design of wastewater treatment systems and it is it performed much better than the existing ones and can be the future of wastewater treatment system design I would say. Forget about future even nowadays also lot of people started employing in the real life real field scenarios and all. In general, the if you see the role of microbial enzyme the detoxification treatment of toxic organic and inorganic pollutant using different type of microbial enzymes are eco friendly and sustainable approach for the treatment of wastewater.

Second is the micro enzymes it extract the energy from the organic matter present in the wastewater through this energy yielding biological reactions which facilitated by the enzymes when pollutants are oxidized by the microbiota. Biological treatment through this microbial enzyme is definitely advantageous due to its action due to the action of enzyme on pollutants even when they are present in a very dilute solution. This is a very important thing because sometimes what happened of some type of genobiotic compound it stays in the wastewater treatment systems in a very low concentration. Suppose the pharmaceutical product there are type different type of pharmaceutical pollutant which stays in the wastewater in the hospital wastewater and all in a very minute amount ok. So, in this because of this minute amount in say like in nanogram per liter level.

So, once it is in nanogram per liter level so, what happens it is very hard for any specific type of treatment to work on it on any generic treatment to work on this kind of pollutant which is in a very minor amount. However, it is still detrimental it can be carcinogenic it can be very dangerous for human consumption or any other activities. So, still the those pollutants should be removed. However, it is hard to remove hard to be removed from the existing systems. So, that is why we we introduce this kind of enzymatic reactions enzymatic treatment systems because enzyme can work on it particularly targeting that particular type of enzyme particular type of pollutant and remove it from the system.

And this is where the this kind of enzymatic microbial enzymatic biological treatment systems come into the picture more and more with time to come. Then oxidoreductase enzymes which can contribute to the hemification of different phenolic compounds that

are produced from the decomposition of lignin in soil or aqueous environment. Oxidereductase also can remove different toxic genobiotic compounds as I just mentioned like phenolic or aniline compounds present in the wastewater via polymerization of or the copolymerization with the hemix substances. These oxidoreductases are actually quite commonly used very I mean say famous in wastewater treatment systems they people use it a lot and in there are different studies done in the microbial fuel cell like studies also. In the microbial fuel cell means it is where we use some fuel cell technology where we have the anodic and the cathodic compartment and all.

In this anodic compartment we use a specific type of enzyme and it reacts it actually acts on the type of pollutant we are actually supplying in a targeted manner. And because of that it not only reduces the pollutant load, but also it generates the electricity out of it ok. In coming lecture in the end of this lecture series I will be discussing about those systems as well this advanced water treatment system that is coming into the picture. Nowadays all over world people are working on it that needs to be discussed you need to understand those advancement in the water treatment technologies as well wastewater treatment technologies as well. There are species like you know candida tropicalis or the dibyromasis polymorphos and this filamentous fungi like penicillium gastivorus and also ambelopsis isabellina yeast which decolorize the 100 million per liter of synthetic dye using the manganese dependent oxalo reductase enzyme within 16 to 48 hours.

This removal of dye is a major problem in various industries and all like whether it be paint industry, whether it be other industries and all like you know where they uses some synthetic dye and all like in the textile industries and all. So, in order to get rid of this additional synthetic dye which is very hard to degrade and also which is very dangerous for the human consumptions and has a very strict law by most of the governmental body all over the world and it needs to be removed before it reaches to the surface water bodies. So, this can be done by oxalo reductase enzyme as I was discussing. So, and specific and you can see the removal it can be removed completely within 16 to 48 hour by different studies. It also could remove the chlorinated phenolic compound found in the effluent of the paper and pulp industries.

This white row fungi releases the various extracellular oxalo reductase enzymes from their mycelium into the environment that assist to the degrade the industrial wastewater content in different genobiotic and phenolic compounds. Lacases can catalyze the oxidation reduction reaction of phenolic compounds and methoxy phenolic acids with simultaneous reduction of the subatomic oxygen to water. So, this you just try just these are the examples of how beneficiary how how the wastewater treatment system the treatment plant designers and the researcher actually benefited by introducing the enzyme

to the system the to the unit. Different microbiota produces intra and extracellular a lackages which can catalyze the oxidation of different phenolic compounds. The three different types of fungal a lackages collected from a trimetes species, pycnoporous species and the plurota species, de-lignifies the high quality paper pulp and almost 90 percent of the lignin was removed after the lacase mediator treatment via H_2O_2 bleaching.

So, this is done in IIT, KGB itself. So, IIT Kharagpur itself this research and it is actually the it showcases the advancement of the treatment system, advancement of the wastewater treatment system using a different type of enzymes and all. We can further enhance the treatment capacity of efficiency of wastewater treatment systems by this immobilized enzymes like for recyclability require the low maintenance, easy metal and nutrient recovery for ecological and the cost effective approach. How heavy metal and mercury is recovered? See immobilized papain enzyme on sodium alginate matrix can remove the mercury too and removal efficiency can be as high as 98.

88 percent within 8 minutes. Immobilized U.S. remove the heavy metal ions such as mercury, copper, silver, zinc, cadmium from the wastewater. Genetically engineered microorganism like the genomic component through the recombinant deoxyribonucleic acid technology it create the strain for the treatment of wastewater that can survive in extreme environmental conditions. So, now, we are not only introducing those enzymes, but we also genetically engineered them so to make it more effective for treating the wastewater targeted wastewater and all. So, we use some recombinant DNA technologies and also that it target that particular type of waste, a particular type of pollutant in our wastewater treatment system and we can remove those pollutant from the systems in a much efficient and highly effective way. There are transgenic plants development like genes from the animals, microbites and other plants degrade different genobiotic compounds.

This cytochrome P450 genes enhances the metabolism of the genobiotic compound like herbicides and the volatile halogenated hydrocarbons from the contaminated waste groundwater. There are studies where genetically modified microbes can also be utilized as a microbial biosensors for the rapid and precise detection of pollutant present in the wastewater. So, how it works? This microbial biosensors it is nothing, but how it reacts I mean like suppose it is once you final once you have a particular type of load pollutant load say your BOD is 100 milligram per liter in your wastewater. So, once you introduce a particular type of enzyme after say 2 hour you check you identify that the final wastewater or final wastewater like you know it comes down to say 50. So, 50 percent removal is possible within 2 hour then in 5 hour it is a say 100 percent removal is possible.

So, this way you make the calibration curve and all and then at different COD you try to

check the what is the change that it happens when the when it is instead of 100 milligram per liter when we introduce 200 milligram per liter how the changes are happening place changes are taking place in with timely manner. Based on that we go we prepare a calibration curve and from there when we introduce that system to a unknown pollutant load sample that from unknown pollutant load also you can easily from the from the removal curve that you will get after within a couple of hour you will be able to tell that what was the in what was the actual pollutant load of the system. So, this is just to give you one example how it can work ok. This microbial biosensors are so, they will assist you to understanding the pollutant load they can assist you to understand the actual target pollutant load from your wastewater and all. So, what is the potential of algae in the wastewater systems? So, we already discussed about the enzymes.

So, now, let us discuss about the algae how why how algae is more important and how it is performing and its revolutionizing the wastewater treatment system as we can see today. So, it can remove the pollutants, nutrients and the toxic metals and the pathogens from the wastewater. The photosynthetic algae has been used for tertiary wastewater treatment due to its easy adaptable nature in the aquatic environment along with the high biomass productivity compared to other photosynthetic microorganisms. And also algal biomass after the treatment is done it is harvested from the wastewater and can be used for the feedstock for the biofuel production and all. So, that is also something of additional economic benefit.

And the primary and secondary treatment in normally required to reduce the concentration of organic matter and other pollutant concentration if present in the wastewater. But further this kind of algae based tertiary treatment it is symbiotic to algae and bacterial amyloids, the amyloids the treatment efficiency as microalgae remove the impurities via their cellular metabolism. So, our target is to increase the efficiency and algae can work as a very nice polishing treatment technology for removing further impurities from the wastewater. In general this algae assisted tertiary treatments it provide an economic approach by reducing the energy consumption and carbon footprint of the conventional wastewater treatment processes. It also eliminate has a capacity to eliminate almost 90 percent of the nitrogen and phosphorus present in the wastewater.

There are hundreds of study available in the in the in the Google in the you can easily search for it where they have found that this efficiency can be further increased with time with the with the more efficient design of algal bioreactor coming into the picture right now. Harvested algal biomass can be transformed into valuable carbon neutral biofuels such as bioethanol, biodiesel, other other valuable byproduct like biogas, bio fertilizer, cosmetics, medicines and animal feed or the cattle feed etcetera. There are like the

hundreds of application of this harvested algal biomass once it is utilized in the once it is utilized for consuming the wastewater from the consuming the pollutant from the wastewater. This microalgae assisted wastewater normally it provides newer revenues to the biological treatment system which possess a significant advantages over the conventional ones. So, also it normally utilize the solar light and atmospheric carbon dioxide as energy and carbon source and also it can be classified as eukaryotic or prokaryotic cyanobacteria based on its utilization of energy and the carbon source.

Different type of unicellular green microalgae species like chlorella and the cenodesmus species and all they are widely used in the wastewater treatment system. Diverse group of algal species like the chlorophyc like you know chlorophyc like you know green algae, the cyanophyc like you know the blue green algae or the chrysophyc like you know golden algae and the baserophyc like the diatoms are found in different wastewater treatment systems. This diatom I am telling you just go through it and try to understand some more details read a little bit more about this diatom. Diatom is will be the future of wastewater treatment systems has a capacity to be the future of wastewater treatment systems for its huge benefit the way it in the increases its biomass it is a huge reproduction rate and it is the way it consumes the amount of the consumes the amount of pollutant from the wastewater it is enormous and there are hundreds of literature there are like lot of people all over the world now focusing on diatom application in the wastewater treatment systems. Algal groups which are classified based upon the different types of intra and extracellular structure lifespan and the pigmentation and the storage yields.

So, in general how we where we apply the algae in the wastewater treatment systems? First to remove the nitrogen and phosphorus this kind of inorganic substances which still stays in the system even after employing couple of treatment units. So, primary and secondary treatment units. So, we can remove those nitrogen and phosphorus using the microalgae. It can be we can be used for removal of chemical and the biochemical oxygen demons COD, BOD removal as you have there are literature where they found out that mixed concentration of this filamentous cyanobacteria can remove 98.2 and 84 percent of the COD and the total organic carbon respectively from the domestic wastewater treatment system.

Reduction of polyform and the other pathogen microalgae can also remove the 99 percent of the total polyform successfully from the municipal wastewater via high rate algal photobioreactor with 22 gram per meter square of meter square per day meter square per day of biomass production. So, it is not only helping get rid of the pollutant from the wastewater, but also it produces some biomass that can be utilized for further byproduct recovery. Other than that it also can remove the heavy metal by the process of biosurptions.

There are there are different kind of literatures which showcases that it can remove 100 percent of the heavy metal from the systems from the synthetic wastewater within a certain period of time. There are literatures which is available to which can that this algae can remove the pesticide and other genomic compound from the system as well.

As you can see from this example this study where this Chlamydomonas rain hardity. So, they effectively degraded 100 percent of the pesticide trichloroform or TCF commonly known as a TCF from the synthetic wastewater with a maximum concentration of 100 milligram per liter of TCF which is quite huge, but still that algae is successful in removing this genomic compounds from the systems. So, if you can see this five example that is given it is the one of the major nuisance in wastewater treatment system which still can present even after the primary and the secondary treatment is done. So, you can easily identify easily understand how effective how important it is to employ the system employ procedures like you know this algae in the wastewater treatment scenarios and all ok. So, in general we come to the conclusion we discussed about the enzymes which comes as a very cost effective and environment environmental friendly solution for wastewater treatment and its crucial role in a diverse and because of its diverse catalytic properties it can act as a crucial it can play a crucial role in wastewater treatment scenario.

The use majorly in the wastewater treatment scenario it proves to be much more advantageous for removing the contaminants in the wastewater with a focus on BOD and COD removal. Whereas the algae are found to be the one of the play can play a major role in removing different pollutants nutrient and the pathogens and very emerging contaminants that is that is coming into the picture day to day basis then this algae can act a very good role in removing all this pollutants and all. These are the different references I would like you to go through it and actually read those paper it will give you a very good understanding about the discussions that we had today ok. So, thank you so much I hope you understand the role of enzyme and the algae in details in the and the how they can play a very futuristic role in the in the wastewater treatment scenario and the research is still going on to be to make it more effective in future ok. Thank you so much I hope you got to know some very good understanding about the algae and the application of enzymes in wastewater treatment systems we will see you in the next video. Thank you.