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ORGANIC WASTEWATER TREATMENT USING ENZYME IMMOBILIZATION

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Abstract - *Wastewater discharged from restaurants, hotels etc contain high amount of biodegradable organic substances. Low biodegradable oil and grease are also present in this wastewater. If the wastewater is discharged, prior to water treatment, it will produce offensive smell and also that water cannot be reused. The enzyme immobilisation method can reduce oil and grease content to a higher level and odor problems can be completely solved.*

Enzymes such as lipase, protease and amylase were used for enzyme immobilisation. These enzymes are capable of degrading proteins, starch and lipids present in water. Wastewater was collected from a restaurant in Kollam which consumes 500L of water daily. Lipase, Protease and amylase enzymes were extracted in laboratory. Clay beads dried in muffle furnace at 700°C were used for enzyme immobilisation. The immobilised enzymes were taken and tested for treatment of water. Wastewater was given pre treatment in a filter column prior to passing through clay beads. Preliminary filtration and enzyme immobilisation regulates various water quality parameters. Dissolved Oxygen, Biochemical Oxygen Demand, Chemical Oxygen Demand, pH, Chloride, Alkalinity, Oil and Grease, Total Solids, Total Dissolved Solids, Total Suspended Solids, were analysed for raw wastewater, water passed through filtration column and water obtained after immobilised enzyme treatment. The results obtained clearly shows that water was 65% free from pollutants.

Key Words: *Enzyme, Organic wastewater, Water treatment, Immobilisation, Filtration, Water quality analysis*

1. INTRODUCTION

Water scarcity is a very important problem we are facing now. Reuse of water can reduce water scarcity to a great extent. Wastewater can be reused by various treatment methods and there are a lot of methods for treatment of wastewater. Wastewater that is disposed from restaurants and hotels can be also reused after treatment process. An economic and efficient method for treating wastewater discharged from restaurants and food courts were taken into consideration for study.

Wastewater from a restaurant consists of water originating from preparation of food products including meats, food that contain fats, oils, greases, dairy products, etc and intensive use of cleaning agents such as disinfectants, cleaners, floor strippers, and soaps that create higher strength wastewater in restaurants that is more difficult to treat than in a typical residential home. On initial discharge, these wastewaters can contain high levels of inorganic pollutants which can be easily biodegraded, but whose impact load on the ecosystems, either in Total Suspended Solids (TSS), Biochemical Oxygen Demand (BOD), or Chemical Oxygen Demand (COD), may be in the tens of thousands mg/L [13].

Organic toxic waste (oil and grease (O&G)) causes ecology damages for aquatic organisms [7], plant, animal, and equally, mutagenic and carcinogenic for human being [12]. From wastewater source they discharge to form a layer on water surface body. This lead to decreased dissolved oxygen levels in that water. Then oxygen molecules are difficult to be oxidative for microbes on hydrocarbon molecules and cause ecology damages to water bodies [11, 10].

Enzymes are biocatalysts produced by living cells to cause specific biochemical reactions generally forming the various metabolic processes of the cells and are absolute necessary for maintenance and activity of life. [9]

Enzyme-based water treatment has promise to fulfill all the described parameters with efficacy. Furthermore, their immobilization onto a suitable carrier by appropriate immobilization methods has given an additional advantage in the treatment of wastewater. [4]. Physical adsorption is the most useful method for immobilization because it is easy, inexpensive, intoxicant, able to retain the activity and feasible for regeneration [14].

2. MATERIALS AND METHODS

Wastewater sample for analysis is collected from a hotel in Kollam district, Kerala. Hotel Mahalekshmi consumes 500L of water every day. The Hotel serves both non vegetarian and vegetarian food. Wastewater was collected from disposal tank. Enzymes used for treatment of wastewater in this study were Lipase, Amylase and Protease. These enzymes are capable of degrading fats, carbohydrates and proteins present in the wastewater. Organisms that produce enzymes

used for treatment were grown in separate nutrient broth medium and centrifuged at 8000rpm for 15 minutes to extract enzyme. In addition to this a mixed enzyme was prepared by growing all three organisms that produce lipase, amylase and protease enzyme in a single 250mLconical flask.

Clay beads were used as carriers for immobilisation. Clay beads were prepared with 5mm diameter and dried in a muffle furnace at 700°C for 1 hour. Both rough and smooth surface clay beads were prepared. Smooth and rough surface clay beads are given below in figure 1.

As for immobilisation physical adsorption method was adopted. Clay beads along with enzymes were placed in a shaker for 30 minutes at 120rpm. For 5gm beads, 5ml enzyme was used. Rough surface and smooth surface clay beads were shaken separately in all 4 enzymes. Eight combination of immobilised enzyme were prepared to identify the best result.

R1- Rough surface clay beads in lipase enzyme

R2- Rough surface clay beads in amylase enzyme

R3- Rough surface clay beads in protease enzyme

R4- Rough surface clay beads in mixed enzyme

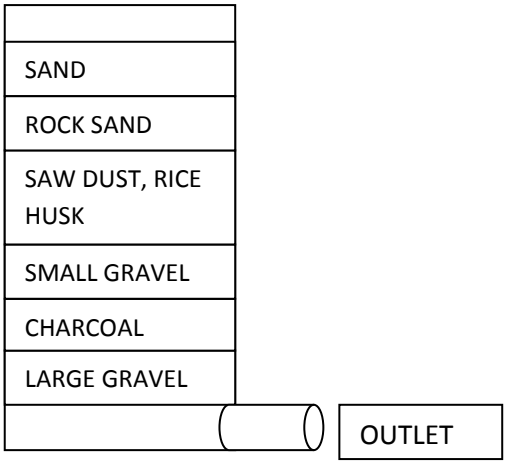
S1- Smooth surface clay beads in lipase enzyme

S2- Smooth surface clay beads in amylase enzyme

S3- Smooth surface clay beads in protease enzyme

S4- Smooth surface clay beads in mixed enzyme

Each immobilised clay beads were placed separately inside a separating funnel, and water to be treated is passed through the separating funnel. For treatment of collected wastewater as a pretreatment method, a filtration column apparatus was used. Wastewater was passed through filtration column prior to flow through separating funnel. The filter column apparatus was 60 X 10 X 10cm in dimension.



Collected sample is labeled as W1. Sample is collected in covered bag to prevent light exposure. Temperature, pH were measured at field. Total Solids (TS), Total Dissolved Solids (TDS), Total Suspended Solids, Chloride, Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Alkalinity, Color, Odor, Oil and Grease were analysed according to APHA protocols for collected wastewater sample W1.

Fig -1: Rough surface and smooth surface clay beads

Wastewater was passed through filtration column and filtered water was collected in a closed container labeled as W2. The water quality parameters pH, color, odor, chloride, TS, TDS, TSS, COD, DO, BOD, Alkalinity, oil and grease were analysed.

Collected wastewater W1 was passed through clay beads immobilized with individual and mixed enzyme separately. Eight separating funnels were used for individual analysis. From this experiment, the best combination of clay bead with enzyme was identified. The best result was obtained for R4- Rough surface clay beads in mixed enzyme.

After preliminary filtration obtained water (W2) was passed through a 250mL separating funnel (SF) containing rough surface clay beads immobilised by mixed enzyme. Water passed through SF was collected in an air tight Glass vessel.

All water quality parameters were analysed for treated water. Treated water was labeled as W3.

3. RESULTS AND DISCUSSIONS

3.1 Wastewater quality analysis

1. Temperature

Temperature of wastewater collected from the field, W1 was 34°C. Temperature of wastewater is a very important parameter. It has a significant effect on chemical reactions, aquatic life and reusability of wastewater. After treatment, temperature obtained was 31°C.

2. Color

Wastewater W1 was reddish yellow initially. After passing through filter column, color changed to yellow. Finally after all treatment color of wastewater was pale yellow which was transparent.

Fig -2: Wastewater sample before treatment and after treatment

3. Odor

Odour is produced in wastewater due to the gases that are produced by the decomposition of organic matter present in the wastewater. Initial wastewater W1 was very offensive. But after treatment very significant change occurred in odor problem. Odour of wastewater W was similar to clay.

4. pH

Determination of pH plays an important role in the waste water treatment process. The term "pH" refers to the measurement of hydrogen ion activity in the solution. The direct measurement of the pH is difficult so specific electrodes are required for quick and accurate pH determination. pH is measured on a scale of 0 to 14, with lower values indicating high H+ (more acidic) and higher values indicating low H+ ion activity (less acidic). A pH of 7 is considered as neutral.

pH of wastewater W1 was obtained as 5.3. For W2 pH obtained was 6.9 and finally for W3, pH was 7.5.

5. Chloride

Chloride exists in all natural waters, the concentrations varies in different sources and reaching a maximum in sea water (up to 35,000 mg/l Cl). Chloride does not pose a health hazard to humans. Initially the chloride content in W1 was obtained as 464.96mg/L. For W2, chloride was 424.96 mg/L and for W3, chloride content raised and result was 549.98mg/L.

The chloride of final water is below permissible limit. Therefore it is safe for discharge and reuse.

6. Dissolved Oxygen

The most negative impact on water is the decrease in dissolved oxygen. The decrease in dissolved oxygen is due o the microorganism present in the wastewater. The dissolved oxygen is used to measure BOD which is an indicator of pollution range of wastewater.

As for the DO values, the DO of W1 was obtained as 3.5mg/L and for W2, DO was 4.1 mg/L and the DO value for W3 was 4.5mg/L.

7. Biochemical Oxygen Demand

Biological oxygen demand is used to express the concentration of organic matter in wastewater. It is a measure of the amount of dissolved oxygen used by microorganisms in the water. If the amount of organic matter in sewage is more, the more oxygen will be utilized by microorganisms to degrade dumping sewage which containing high BOD value.

As for the BOD values, the BOD of W1 was obtained as 34mg/L and for W2, BOD result was 30 mg/L and the BOD value for W3 was 28 mg/L.

The BOD of final water is below 30mg/L which is within the permissible limit. Therefore it is safe for discharge and reuse.

Fu E. Tang and Chung W. Tong conducted a Study of the Garbage Enzyme's Effects in Domestic Wastewater. In their study they observed that garbage enzymes are not suitable for removing BODdue to the high amount of organic material in the garbage enzyme. Compare to that immobilised clay beads reduced BOD levels. [5]

8. Chemical Oxygen Demand

The chemical oxygen demand (COD) is an indicative measure of the amount of oxygen which is utilized for chemical reaction. COD is closely related to BOD, the difference being that BOD is a test of the level of organic matter that can be biologically oxidised while COD is a test of the amount of organic matter that can be chemically oxidised.

The COD obtained for W1 was 416mg/L and COD obtained for W2 was 312mg/L. Finally, for W3, COD value was 208mg/L which is below 250mg/L within the permissible disposal limit. 50% of reduction was obtained.

9. Alkalinity

Alkalinity is a measure of the capacity of water to neutralize acids. Without this acid-neutralizing capacity, any acid added to a flow would cause an immediate change in the pH.

The alkalinity obtained for W1 was 0.25mg/L and result obtained for W2 was 0.4mg/L. Finally, for W3, alkalinity value was 0.5mg/L.

10. Total Solids, Total Dissolved Solids, Total suspended Solids

Total solids are dissolved solids plus suspended and settleable solids in water. The concentration of total dissolved solids affects the water balance in the cells of aquatic organisms. An organism placed in water with a high concentration of solids will shrink somewhat because the water in its cells will tend to move out. Higher concentrations of suspended solids can serve as carriers of toxics, which readily stick to suspended particles. High TSS can cause problems for industrial use, because the solids can clog or scour pipes and machinery. The valuesobtained for TS, TSS and TDS for W1, W2 and W3 were shown in Table 1.

Table -1: Values obtained for TS, TDS, TSS

	Total solids (mg/L)	Total dissolved solids (mg/L)	Total suspended solids (mg/L)
W1	500	500	400
W2	200	400	200
W3	100	200	100

Percentage reduction obtained in Total Solid was 80%. For Total Dissolved Solids, 60% reduction was obtained and 75% reduction was achieved in Total suspended solids.

Nazim and Meera conducted a study on comparison of treatment of Greywater using Garbage and Citrus Enzymes. In the study, the synthetic greywater when treated using (10%) garbage enzyme solution showed more percentage reduction for TDS (23.63%), BOD5 (63.28%) and COD (25.52%) when compared to (10%) citrus enzyme solution [8].

11. Oil and Grease

Problems caused by excessive O&G include a decrease in the cell aqueous phase transfer rates, an obstruction in sedimentation due to the development of filamentous microorganisms, development of sludge with poor activity, clogging and the emergence of unpleasant odors [3].

The oil and grease result obtained for W1 was 3.25mg/L and result obtained for W2 and W3, O&G value was below detectable limit.

Figure 3 shows collected wastewater sample W1, preliminary filtered water W2 and final disposal water is shown in Figure 4.

Fig -3: Wastewater sample before and after filtration

Fig -4: Wastewater after passing through immobilized enzyme system

3.2 Wastewater treatment reactor

A laboratory scale reactor set up was made to treat wastewater from hotels and restaurants. When wastewater passes through inlet of the reactor which consists of a preliminary filtration column, a pump and an immobilised enzyme apparatus, water get cleaned and received through outlet.

Schematic representation of proposed lab scale reactor is shown in Figure 5.

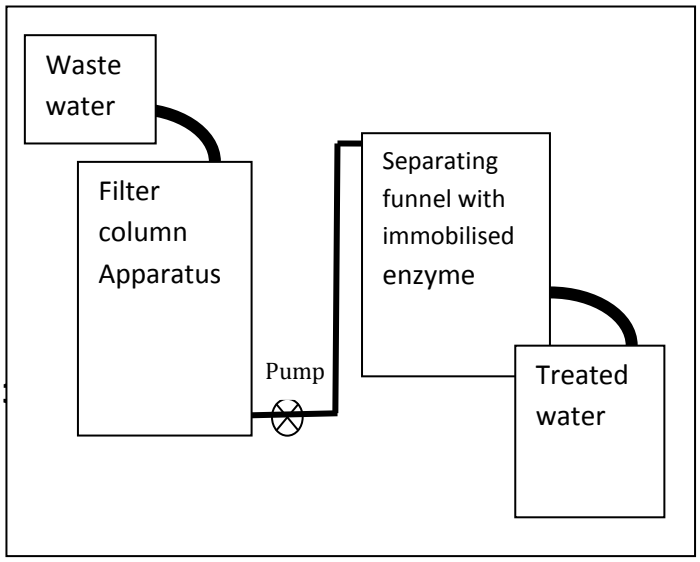


Fig -5: A schematic representation of lab scale reactor

4. CONCLUSION

Hotel wastewater is rich in fats, oil, grease, carbohydrates proteins, etc which are difficult to degrade and costly in treatment process. By using enzymes like lipase, amylase, protease, constituents which are difficult to degrade can be

remediated easily. Enzymes have been employed in numerous fields primarily for their immense catalytic potential. In wastewater treatment, enzymes can be utilized to develop remediation processes that are environmentally less aggressive than conventional techniques. Their versatility and efficiency even in mild reaction conditions gives them an advantage over the conventional physico-chemical treatment methods. Enzymes are immobilised to increase its efficiency. Thus enzymes can be regenerated. Immobilized enzymes achieve greater bioremediation. Clay beads that used as carriers for immobilisation are cheap and effective. Water obtained after this treatment was very much clearer and removed odor problems, TS, TDS, TSS, Oil and Grease to a great extent. Chloride content in water increased but Chloride level of final water was within the permissible limit. This water can be disposed safely to surface bodies and also the water can be reused for gardening purposes.

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