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Recycling of Liquid Waste from Hospital using Selected Microbial Cultures

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ABSTRACT

Application of biological mechanisms or employment of microorganisms is in practise for many years in the treatment of sewage. In the present study selected microorganisms (*Pseudomonas* sp., *Klebsiella* sp., *E.coli*, *Lactobacillus* sp., and *Candida* sp., were employed to treat the sewage of a local hospital. This sewage is found to contain considerable quantity of alcohol and phenol. The efficiency of the above cultures either separately or in different combinations was studied. The original levels of BOD (285 mg O_2/l) and COD (680 mg O_2/l) of the sewage were reduced to 16.4 O_2/l and 260 O_2/l respectively in 72 h when sterile sewage was incorporated with *Pseudomonas* sp. at a level of 26 X 10^5 CFU/ml. The corresponding BOD and COD in the control was 79 mg O_2/l and 655 mg O_2/l. The *Candida* sp., at an incorporation level of 35 X 10^5 CFU/ml reduced the BOD and COD of the sewage to 18.3 mg O_2/l and 240 mg O_2/l respectively. *E.coli*, *Lactobacillus* sp., *Klebsiella* sp., at an incorporation level of 28 X 10^5, 27 X 10^5 CFU/ml reduced BOD to 23.6, 19.9, 26.5 mg O_2/l respectively. From the original value of 160 mg/l, nitrate reduced to 8.9, 46.8, 4.3, 49.2 and 6.8 mg/l respectively when *Pseudomonas* sp., *Klebsiella* sp., *E.coli*, *Lactobacillus* sp., and *Candida* sp., were incorporated separately in the sewage. The value in control was 135 mg/l mg/l at 72 /h of incubation. The phosphate was reduced to 4.7, 3.9, 2.8, 5.3 and 2.9 mg/l respectively. The value in the control was 10.8 mg/l. The above said cultures (*Pseudomonas* sp., *Klebsiella* sp., *E.coli*, *Lactobacillus* sp., and *Candida* sp.,) were mixed at a ratio of 26:23:28:27:35 and incorporated in the sewage at a mixed population of 120 X 10^5 CFU/ml which reduced the BOD to 14.2 mg O_2/l and COD to 138 mg O_2/l in 48 h of incorporation. Thus it was found that the efficiency of mixed cultures found to be higher than the individual cultures.

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Introduction

Explosive growth in human population, reckless use of natural, rapid industrialisation and urbanisation has resulted into generation of huge amount of wastewater. Among the various types of waste generated from different sectors like industries, restaurants, factories, public sectors, municipal sewage etc., hospital waste is the most dangerous and hazardous to the environment in general and to the human community in particular [1].

Medical or hospital waste have witnessed an increase in the past ten years due to the increased number in size of the health care facilities, medical services and the used of medical disposal products [2]. Hospital or biomedical waste can pose a grave hazard to patients in health care centres, to workers and to community if proper waste management is not exercised. Hospital liquid waste also forms an important source of hazardous and infections waste which has been covered under biomedical waste regulations [3].

The liquid waste from hospital include sewage and wash water from testing laboratory and operations theatres, microbiological laboratory, chemical therapy, radioactive therapy, X-ray labs and other infectious waste generating sections. This liquid waste is hard to recycles as it contains materials like phenol, alcohol [4,5], reagents, antibiotics [6,7,8], detergents, disinfectants [9] [4], other mixed pharmaceuticals [10,5] and also pathogenic micro flora [11,12,13]. The rate of generation (quantity of liquid waste) of such waste varies from hospital to hospital, country to country and also the type of hospital [14,3,15]. The quantity of liquid waste generated in hospitals depends on the number of beds, outpatients, by - standers, visitors, canteen and laundry facilities available etc. The liquid waste being discharged from hospital and the sludge formed during the effluent treatment both have a potential risk of transmitting infectious diseases besides the involvement of chemical hazards [16,17,13]. In developing countries like India, hospital wastewater treatment plants are not common and therefore liquid waste from most of the hospitals let out untreated and are directly linked to the public sewer or discharged in open land [18]. Such improper disposal of waste leads to environmental pollution leading to the active spread of communicable diseases [16,19,20,21]. It is to be noted that a sizeable portion of the wastewater in a municipal area is contributed by the hospitals. Domestic liquid wastes of a city are currently being treated as bulk by corporate sectors by waste stabilisation system in which minimum power is

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required, but it is handicapped with limited success in eliminating the pathogens. Nevertheless waste stabilisation process is in vogue [22, 23]. Since other advanced treatment strategies are observed to be the cost prohibitive in Indian cities. The problems with reference to the pathogens in normal waste stabilisation are high if hospital drainages are linked to the public sewer. Therefore it is felt that the wastewater from hospitals require specialised and isolated treatments in view of the specialised constituents of hospital liquid waste. Moreover many hospitals are handicapped with limited land facilities. Therefore it is required to identify cost effective, less land consuming methodologies to treat hospital effluent to an acceptable quality. In this context, it is felt that a detailed investigation concerned with the isolated treatment of hospital wastes is necessary. Hence a field work was carried out in a local hospital which has bed strength of 500 and out patient of round 2000 and has a canteen annexed to it. Presently the liquid wastes are passed through an extended aeration system. But the quality of tested effluent does not satisfy the norms. Hence in the present study an attempt was made to treat the hospital liquid waste using selected microorganisms either separately or in different combinations.

Materials and Methods

Study area (Waste water treatment System)

Meenakshi Mission Hospital Research Centre is an existing unit and the unit is involved in the process of hospital activities treating diseased inpatients and out patient by means of surgery etc. This hospital is situated in the lake area of Madurai - Melur – Chennai Highway in the outskirts of the Madurai city. It is having a wastewater treatment system for treating the discharged wastewater from the hospital. The unit is designed to treat 100 KLD of effluent from various sources like septic tank overflows, canteen waste, floor waste, wastewater from operation theatres and wash water etc.

Source of the Effluent

Sewage is generated from the following point sources

a) Wastewater from main and annexure buildings of the hospital
b) Wash water from wards, operation theatres and laboratories.
c) Working women's hostel
d) Canteen and mess
e) Rejected effluents from water treatment plants.

The wastewater generated in the hospital is from various departments which includes routine clinical, cytotoxic, infection, pathological, pharmaceutical, testing laboratories, radiological wastes and operations. This wastewater along with the domestic waste from staff quarters, hostel, canteen and doctor's quarters are brought into common septic tanks.

Quantity of sewage generation

This hospital disposes around 2.5 Lakhs of litres of wastewater per day. The present treatment system consists of Septic tanks, Equalisation tank, Aeration tank, Settling tank, Chlorination tank, Polishing tank and Sludge drying beds.

Collection of samples

The samples were collected in a clean sterilised polyethylene can of 5 liters capacity according to the methods prescribed in [24] APHA (1998). They were immediately transported to the laboratory and subjected to bioremediation studies in the laboratory. Wastewater was collected from the treatment system of MMHRC at the entry point of the equalisation tank. It was sterilised in an autoclave at 121º C at 15 lbs for 15 minutes to kill all the microbes in it.

Isolation of microbes

Microbial colonies were isolated from the wastewater samples by adopting pour plate techniques. The samples were serially diluted up to 10^9 dilutions and plated in nutrient agar medium and incubated for 24 h at 37º C. Pure isolated, morphologically different colonies were marked and picked up using the inoculation loop. The colonies were sub cultured in slants. The slant cultures were transferred to nutrient broth and then to nutrient agar for the identification of individual cultures. Isolation and identification were done in the laboratory according to colony characteristics, biochemical test, characterisation of bacteria etc [25, 26, 27]. Five promising cultures were selected and identified up to genus level as *Pseudomonas* sp., *Klebsiella* sp., *E.coli*, *Lactobacillus* sp., and *Candida* sp.

Development of Microbial isolated and mixed culture

Isolated microbes i.e selected individual microbial cultures and mixed culture was dissolved in 0.85 % saline in room temperature before it is utilised for biodegradation studies. Before subjecting the wastes to aerobic degradation using developed microbial cultures, the optimum inoculation and optimum dose and optimum HRT were found by running the experiment continuously aerated for 10 days at room temperature with 2 mg/l of dissolved oxygen by different doses of 1, 2, 4, 6, 8 ml respectively of microbial cultures. The chemical content and maximum reduction in the organic content was fixed as the optimum inoculums dose for the degradation of the wastewater.

Optimization of hydraulic retention time (HRT)

The reactors were run with different HRT namely 2, 4, 6, 8, 10 days by replacing the required quantities from each reactor with fresh material i.e. 500, 250, 167, 125 and 100 ml in the respective reactors. The experiment was run for a period of 10 days and the chemical content were analysed daily.

Optimization of inoculums dose of individual selected microbial seed culture

One liter (1-L) aliquots of hospital liquid waste samples were taken in 2- L conical flask. Since the D.O level in the wastewater samples was zero initially, the aliquots were aerated continuously using aerators. The aerators were run with full capacity of aerator (i.e) 2.5 l / minute. When the DO levels in the aerators attained 2 mg/l they were inoculated with individual selected culture and mixed seed culture into the respective flasks. They were aerated continuously for 10 days at room temperature. From the previous experiment optimum inoculums doses were fixed as 8 ml of cultures with rate of 120 X 10^5 CFU/ml and 125 ml of HRT in the reactor (i.e. 125 ml of fermenting material was removed daily and replaced with fresh raw sterile liquid hospital waste). The experiment was run for a period of 10 days maintaining the DO level of 2 mg/l. The samples were examined daily for the chemical content as shown in Table.1.

Table 1. Parameters and methods for the examination of wastewater

S. No	Parameters of wastewater analysis	Method
1.	COD	Dichromate reflux
2.	BOD	Azide modification
3	Phosphate	Colorimetric
4.	Nitrate	Colorimetric
5.	Nitrite	Cd column reduction
6.	Nitrogen	Microkjedahl method

Results

The results are presented in Table 2 and Fig 1-6. The experiment was initially conducted in order to find out the optimum inoculums dose and HRT. It is noted that the results showed a rise in percentage reduction in all the parameters such as BOD, COD, nitrate, nitrate, nitrogen and phosphate with increase in the inoculums dose from 2 ml to 8 ml/l. Similarly the chemical parameters got reduced with the rise in the HRT from 2nd to 6th day. The percentage reduction obtained in BOD when inoculated with *Pseudomonas* sp. was 83 % at 2 days HRT, 88 % at 4 day HRT, 93 % at 6 day HRT.

The original levels of BOD (285 mg O_2/l) and COD (680 mg O_2/l) of the sewage were reduced to 16.4 O_2/l and 260 O_2/l respectively in 72 h when sterile sewage was incorporated with *Pseudomonas* sp. at a level of 26 X 10^5 CFU/ml. The corresponding BOD and COD in the control was 79 mg O_2/l and 655 mg O_2/l. The *Candida* sp., at an incorporation level of 35 X 10^5 CFU/ml reduced the BOD and COD of the sewage to 18.3 mg O_2/l and 240 mg O_2/l respectively. *E.coli*, *Lactobacillus* sp., *Klebsiella* sp., at an incorporation level of 28 X 10^5, 27 X 10^5 CFU/ml reduced BOD to 23.6, 19.9, 26.5 mg O_2/l respectively.

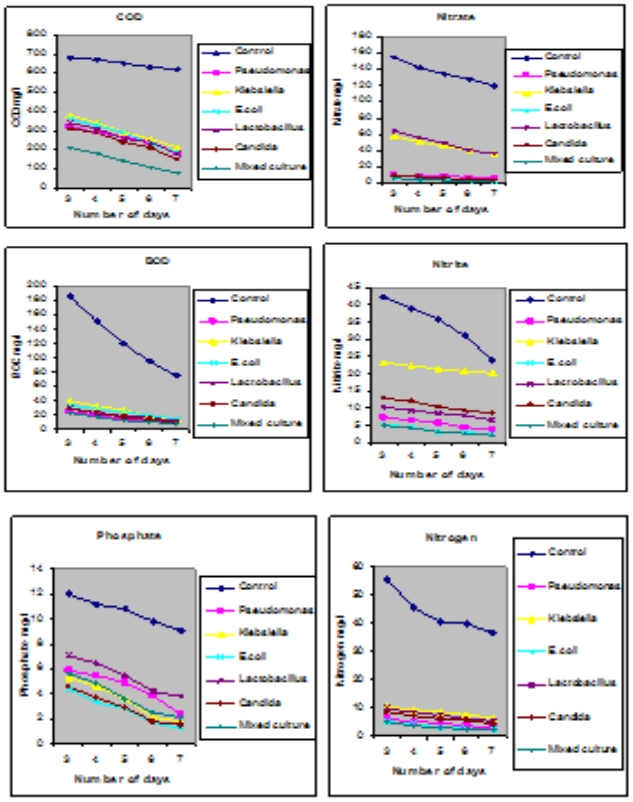

Fig.1- 6. Chemical parameters of different microbial sp. employed for the treatment of hospital liquid waste.

From the original value of 160 mg/l, nitrate reduced to 8.9, 46.8, 4.3, 49.2 and 6.8 mg/l respectively when *Pseudomonas* sp., *Klebsiella* sp., *E.coli*, *Lactobacillus* sp., and *Candida* sp., were incorporated separately in the sewage. The value in control was 135 mg/l at 72 /h of incubation. The phosphate was reduced to 4.7, 3.9, 2.8, 5.3 and 2.9 mg/l respectively. The value in the control was 10.8 mg/l.

The cultures (*Pseudomonas* sp., *Klebsiella* sp., *E.coli*, *Lactobacillus* sp., and *Candida* sp.) were mixed at a ratio of 26:23:28:27:35 and incorporated in the sewage at a mixed population of 120 X 10^5 CFU/ml which reduced the BOD to 14.2 mg O_2/l and COD to 138 mg O_2/l in 48 h of incorporation.

Discussion

It is noted that from the Table 2 that there is a gradual rise in the percentage reduction in the COD, BOD in all the (5 + 1) mixed + individual cultures during the course of experiment which indicates that the organisms inoculated were capable of degradation of the organic matter present in the waste. It is also noted there was a gradual reduction in all the chemical parameters (COD, BOD, Phosphate, nitrate, nitrite and nitrogen) when the inoculums dose was from 2 ml to 8 ml. The data showed that 8 days HRT was optimum for the degradation of raw hospital effluent. Among the various cultures inoculated into wastewater for treatment *Pseudomonas* sp. and *Candida* sp. were able to reduce the BOD to 95 and COD to 80 percent respectively with the inoculums dose of 8 ml/l at the rate of 26 X 10^5 CFU/ml and 35 X 10^5 CFU/ml. *Pseudomonas* sp. and *Candida* sp. could able to survive in high COD during treatment and bring about significant reduction in COD during growth. These microorganisms posses a greater resistance to high shear forces nozzle of the jet loop reactors [28]. COD removal up to 90 percent by *Pseudomonas* sp. isolated from activated sludge has been reported [29,30].

It may be also due to environmental factors such as pH, temperature and other substrates in the environment that affect the growth of microorganisms and their degradation ability. *Pseudomonas* sp. could degrade the organic content at a wide rang of pH, from 5.5 to 8 with an optimum pH of 6.3 [31, 32]. According to [33] 60 to 65 percent of the organic matter present in the sewage is utilised by microbial biomass for their growth and energy and thus purifies the wastewater. Our results are in agreement with the reports mentioned above and are correlated with the fact that there would be an increase in biosorption and biodegradation in the specific rate of organic content at pH of 6 - 6.28 in *Pseudomonas* sp. when compared to other species [34]. The phosphate level decreased to a permissible level (10 mg/l) in 5 days of inoculation of *Pseudomonas* sp., *Candida* sp. and mixed microbial cultures.

Table 2. Performance of 2 L reactor in the aerobic treatment of hospital liquid waste by employing different microbial cultures.

S.No	Parameters	Pseudomonas	Klebsiella	E.coli	Lactobacillus	Candida	Mixed culture	Control
1.	COD	260	296	280	270	240	144	650
2.	BOD	16.54	26.4	23.84	19.84	18.2	14.16	189
3.	Phosphate	4.5	3.48	2.68	5.4	2.9	3.72	10.58
4.	Nitrate	8.6	46.5	4.4	49.4	6.5	4.1	135
5.	Nitrite	5.5	21.58	3.6	8.5	10.6	3.4	34.5
6.	Nitrogen	4.48	8.4	3.4	7.4	6.4	3.2	43.7

Note:

- Values are designated as mg/l
- Values are the average of 5 days analysis
- HRT is 8 days
- DO - 3 mg/l
- Dose - 8 ml/l
- Loading volume ml/d - 125 ml

The results obtained in the case of the above microbial species as indicated earlier are in agreement with the findings of [35, 36, 37].

The polyphosphate accumulation took place in wastewater only when cells were not actively multiplying during lag and /or stationary growth phase [38, 39].

In order to analyse the better performance of these cultures, comparison study was made between the individual cells and mixed microbial cultures. The mixed culture was able to do the job well. All the parameters such as BOD, COD, Phosphate, Nitrate, Nitrite and Nitrogen were reduced to an acceptable limit of wastewater in 5 days of inoculation with mixed microbial cultures. The results of the present study well coincided with the studies of [40] Asthana *et al*. (2001). With reference to BOD and COD removal higher ability of removal of the organic content of the wastewater was observed in mixed microbial culture which may be due to the fact the adaptability of the mixed bacteria existing in the wastewater was stronger than in dispersive status themselves [41, 42]. The over all performance of the reactor runs was very good for optimum inoculums 8 ml/l and an optimum HRT (8 days) over a period of 10 days.

Conclusion

Among various cultures individual inoculated for degradation studies, the efficiency of Pseudomonas sp. was better (93 % of BOD removal) when compared to all other organisms in degrading the organic waste of hospital wastewater. The over all performance of the reactor runs was very good for optimum inoculums 8 ml/l and an optimum HRT (8 days) over a period of 10 days. The mixed culture was able to reduce the BOD to 95 percent in 48 h. Thus it was found that the efficiency of mixed culture is higher than individual cultures.

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