Research Paper

Biotechnology



Bioremediation of Sewage using Specific Microbial consortium and Biogenic Filter materials B Ravi Kumar **M Lakshmi Prasad ***D Sriniyas

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ABSTRACT

Municipal sewage problems are more complex as the volume of the wastewater is large and it requires huge area. The objective of this work is to evaluate a set of microbial consortium and various kinds of filter materials for domestic wastewater treatment. The present study involves defining an economic solution utilizing various kinds of filter materials. The materials taken into study are selected to work on the basis of adsorption, immobilization and centres for bioconversion. Biofiltration experiments were under taken with various biofilter materials inoculated with a defined microbial community. A lab scale submerged attached growth bioreactors using, natural biogeneic materials as supporting material for the immobilization of specific consortium was applied for the domestic wastewater treatment. The units are tested for their individual capacities and as consortia with an ultimate aim achieving safer environmental standards. However, the key component of the research study is the role of microbial flora.

Keywords : Bioremediation, Sewage treatment, Biogenic Biofilter material, Corn cob as Biofilter material

Introduction

Sewage disposal affects people's immediate environments and leads to water related problems. Domestic wastewater treatment has become a serious aquatic environmental problem for all over the world (Patel and Kanungo 2012). Sewage water often contains pathogenic microorganisms like bacteria, viruses, fungi, algae etc., having the potential to cause diseases that can lead to immense harm to public health. The popular water borne diseases are typhoid, paratyphoid fevers, dysentery, cholera, polio and infectious hepatitis. The responsible organisms occur in the feaces or urine or infected people. Soil contamination and ground water pollution are the major environmental problems of wastewater (Buechler and Gayatri, 2005). Urbanization has given rise to a number of environmental problems such as water supply, wastewater generation and its collection, treatment and disposal in urban areas. In most cases wastewater is let out untreated and it either percolates into the ground and in turn contaminates the groundwater or is discharged into the natural drainage system adding pollution in downstream areas. According to Central Pollution Control Board, India, sewage accounts for more than 75 % of the surface water contamination in India (CPCB, 2009). The United Nation's report on "Water for People, Water for Life" (the first ever UN system wide evaluation on global water resources) has put India at 120th position for water quality among the list of 122 nations covered.

The objective of the sewage treatment is to produce a disposable effluent without causing harm to the surrounding environment and also prevent pollution (Khopkar, 2004). Biofiltration is a pollution control technique using living microorganisms for bioremediation process of pollution. Microorganisms including bacteria and fungi are immobilized in biofilter and degrade the pollutant (Ottengraf, 1983). Adsorption processes are widely applied for separation and purification because of their high reliability, energy efficiency, design flexibility, technological maturity and the ability to regenerate the exhausted adsorbent.

Monteiro *et al.* (2010), reported that biofilm reactor is a popular method for biological treatment of wastewater to combat high organic strengths owing to enhanced mean cell residence time and economical oxygen supply. They also reported that COD removal from wastewater can be achieved by either pure culture organism or mixed culture system.

The biofilter media provide a suitable surface for quick biomass growth, larger surface area for biomass growth and good surface texture to hold biomass against shear and sloughing. It is important to select an appropriate filter backwashing technique for successful operation of a biofilter. The major advantages of biofiltration are, being simple, low construction cost, energy saving, flexible to load variation, inexpensive and easy operation and maintenance. Britt et al. (2011), reported that types of pollutants differ considerably with respect to their chemical structure, filter material with high adsorption capacity and different properties is needed.

Several materials can be considered as attachment media for bio-purification. Natural materials like agar, agarose, collagen, alginates, chitosan and synthetic polymer materials like polyacrylamide, polyurethane, polyethylene glycol and polyvinyl alcohol (Fang *et al.*, 2004), compost, wood fibres and peat (Streese and Stegmann (2005), wood chips (Saliling *et al.* (2007), corn cobs powder (Jignesh *et al.* (2008) offer excellent surface area for microbial attachment. Similarly peat, soil, compost, woodchips, straw or a combination of two or more (Nicolai and Janni, 2001), sisal fiber waste, pumice stone and porous glass beads (Anthony *et al.* 2008), coal, plastic ring, stone chips and wood chips (Raquibul and Delwar, 2009), water hyacinth (Tolu Olufunmilayo Ajayi and Atoke Olaide Ogunbayo, 2012), hydrill and pistia (Patel and Kanungo 2012), also can be utilized for biofiltration.

Various industrial solid wastes, agricultural by-products discards and similar products have adsorption affinity for heavy metals. Coffee grounds (Pandey and Choudhari, 1982), china clay (Macchi *et al.*, 1986), apple wastes (Yadava *et al.*, 1991), peanut skins (Maranon and Sastre, 1991) etc., have also been tested with varying degree of success for removing metals from wastewater.

In the present study, an attempt was made to utilize low cost, economically viable and readily available filter media. A novel and holistic approach was manifested for pollutants removal and efficiency of filter materials were determined in the direction of pollutant removal using a specific consortium of microbes. The aim of the study is to achieve high removal efficiency of pollutants and to maintain an optimal performance in long term run. Novel filter materials were selected to knew the applicability for biofiltration system.

In the present work, two packing materials which were used as support media in biofiltration are analyzed and compared to evaluate their suitability according to physical characteristics. The nature of the packing material in biofilters is an important factor for the success in their construction and operation. The materials studied were chosen in a novel approach, which were not present in previous studies in the field of biofiltration including organic materials.

A set of fifteen different parameters were selected to cope with well established factors, such as a material specific surface area, volume, hydraulic retention time and time period. The economic importance of filter material was highly considered and material selection was done based on purchase cost, availability and durability. One ranking of packing materials was established for each parameter studied in order to define a relative suitability degree. Since biofiltration success generally depends on a combination of the ranked parameters, a procedure was defined to compare packing materials suitability under common situations in biofiltration. The selected phenomena, like biofiltration of intermittent loads of pollutants and biofiltration of waste water were investigated.

Materials and methods

Sewage sample was collected according to standard procedures from APHA (1998). Microorganisms with unique characteristics of degradation, Bio-remediation and transformation were selected and obtained from NCL, Pune and IMTECH, Chandigarh. In the present study, *Bacillus megatherium* (NCIM 2104), *Nitrobacter sps.*, (NCIM 5062), *Nitrosomonas sps.*, (NCIM 5071), *Pseudomonas denitrificans* (NCIM 2038), *Chromatium sps.*, (NCIM 2336), *Bacillus mucilaginosus* (NBDC), *Lactobacillus acidophilus* (NCIM 2285), *Bacillus licheniformis* (MTCC 2450), were used as candidates in the consortium. Preparation of consortium, optimization of inoculum concentration and hydraulic retention time were considered according to previous study, bioremediation of sewage using specific consortium.

Various physico chemical parameters were considered for the present study. They are pH, temperature, electrical conductivity, total solids, total suspended solids, total dissolved solids, alkalinity, hardness, chloride, oil and grease, sludge volume index (SVI), chemical oxygen demand (COD), biological oxygen demand (BOD), Nitrogen – total nitrogen (TN), nitrites (NO₂), nitrates (NO₃) and ammonia (NH₃), phosphorus (as 'P'), sulphides (H₂S). The sewage sample was analyzed for fifteen successive days to standardize the values. Analysis was done according to American Public Health Association methods (APHA, 1998).

Natural and biogenic (or) organic materials like corn cobs and bamboo wood chips were used as filter media. Corn cobs are central stem like structures in which the seeds of corn are attached. Corn cob is made up sclerenchyma and central medulla is made up of parenchyma. Medulla of each cob was removed from each cob and the cob looks like a hollow cylinder. Cavities are present on the cob where the seeds are attached, after removal of seeds each cavity provides good surface area. Wood chips were taken from bamboo plant and each chip results a perfect rectangular cube. In the present study, surface areas of both the filter media were calculated and experimentation was completed with variable volumes & surface areas, hydraulic retention time (HRT), time period for filter media.

Suitable corncobs were selected and medulla present at centre was removed. Hence it looks like a hollow cylinder. Height and outer radius & inner radius were measured carefully and volume was calculated using the formula V= π h (R² - r²). Surface area of the corn cob was calculated using derived formula.

Volume of wood chip was calculated by measuring the length, width and height of the wood chip and substituted in the formula V= I x w x h. Surface area of wood chip (similar to solid rectangular cubes) was calculated using the formula A = 2XY + 2XZ + 2YZ. Length, breadth and height of the cube was measured carefully with millimeter scale and substituted into the formula.

Inoculum rate was kept constant i.e., 0.2% volume of inoculum, work was carried out for variable volumes & surface areas of corn cobs and wood chips, hydraulic retention time (HRT) and time period for the efficiency calculation and physico-chemical parameters.

The effect of various volumes i.e., 10%, 20%, 30% and 40% of hollow cylindrical corn cobs and wood chips as biofilter materials were determined by conducting experiments separately in presence of 0.2% consortium and 12 hours hydraulic retention time. Effect of various HRT's i.e., 8 hours, 9 hours, 10 hours, 11 hours and 12 hours were determined in presence of optimized volume of hollow cylindrical corn cobs and wood chips as biofilter materials and 0.2% inoculum at various HRTs. The effect of various time periods i.e., 10, 20, 30, 40, 50 and 60 days was determined using optimized volume of hollow cylindrical corn cobs and wood chips as biofilter materials along with 0.2% consortium as inoculum & optimized hydraulic retention time for domestic wastewater treatment.

Scanning electron microscopy (SEM)

Sample was prepared using inoculated sewage with specific consortium and filter materials optimized volume and allowed to complete the optimized HRT and time period. Sample was fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 hours at 4°C and post fixed 2% in aqueous osmium tetroixde for 4 hours. Dehydrated in series of graded alcohols and dried to critical point drying with CPD unit. The processed samples were mounted over the stubs with double-sided carbon conductivity tape and a thin layer of gold coat over the samples were done by using an automated sputter coater (Model – JEOL JFC -1600) for three minutes and scanned under scanning electron microscope (SEM Model: JOEL 5600) at required magnifications as per the standard procedures (John and Lonnie, 1998).

Results and discussion

Corn cobs as biofilter materials

Corn cobs of radius of outer radius 1.25 cm radius, inner radius of 0.5 cm and height of various sizes ranging from 5 cm to 10 cm of hollow cylindrical shaped material were used for present study. The mean height of cobs was measured and it was 7.6 cm of height (Fig 5 & 6). Volume of the hollow cylindrical corn cobs was calculated using formula V = π h (R²- r²), and it was observed that each hollow cylindrical corn cob had a volume of 31.32 cm³.

Based on the volume of the reactor, various volumes of hollow cylindrical corn cobs i.e., 160 pieces (10% of working volume), 319 pieces (20% of volume), 479 pieces (30% of volume) and 639 pieces (40% of volume) were used in the present study. Surface area of the corn cob was determined and it was 327.68 cm² for corn cob having the outer diameter of 2.5 cm, inner diameter of 1cm and a mean height of 7.6 cm. The specific surface area of corn cob was determined by dividing the surface area with volume of the corn cob and converted to meter scale and it was 1046.17 m²/m³.

Effect of various volumes of corn cobs as biofilter material along with 0.2% consortium and 12 hours HRT were determined. The results showed the reduction of BOD by 58.08% in presence of 10% volume of corn cobs and 51.98% in presence of 20% volume of filter material. It was observed that the addition of corn cobs at the rate of 10% & 20% volume to the treatment system, the BOD and other parameters were increased when compared to removal efficiency of microorganisms @ 0.2% and 12 hours HRT. Hence, further experimentation was continued by removing the central medullary

portion of the corn cob. It comprises soft parenchymatous tissue. After removal of medulla, the corn cob becomes central hollow cylinder and surface area was calculated and used in the present study.

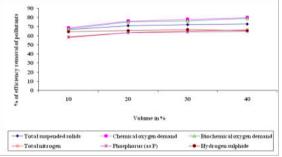
S.	Parameters*	10% volum	ie	20% volur	ne	30% volun	ne	40% volume		
No		corncobs	wood chips	corncobs	wood chips	corncobs	wood chips	corncobs	wood chips	
1	pН	0.1↓	0.1↓	0.03 ↓	0.1 ↑	-	0.1↓	0.1↑	-	
2	Electric conductivity	20.34 ↓	17.97 ↓	20.30 ↓	18.52 ↓	20.66 ↓	20.09 ↓	20.72 ↓	20.04 ↓	
3	Temperature	-	1.0°C ↓	-	-	0.5°C ↓	2.0°C ↓	-	-	
4	Total suspended solids	66.67 ↓	63.83↓	71.02 ↓	65.18 ↓	72.13 ↓	70.99↓	72.8 ↓	71.46 ↓	
5	Volatile suspended solids	60.68 ↓	57.93 ↓	63.99 ↓	58.99 ↓	65.1 ↓	63.57 ↓	65.7 ↓	64.09↓	
6	Chlorides	61.73 ↓	60.13 ↓	67.93↓	62.98 ↓	68.99 ↓	68.07 ↓	69.78↓	68.36 ↓	
7	Hardness	49.19 ↓	50.0 ↓	53.98 ↓	51.5↓	56.96 ↓	53.98 ↓	57.97↓	55.0 ↓	
8	Alkalinity	52.68 ↓	54.06 ↓	57.98 ↓	55.83 ↓	60.02 ↓	58.99↓	60.0 ↓	60.0↓	
9	Chemical oxygen demand	68.62 ↓	66.94 ↓	76.03↓	70.0↓	77.97↓	76.09↓	79.75↓	76.92 ↓	
10	Biochemical oxygen demand	67.34 ↓	67.14 ↓	74.9↓	70.16↓	76.26 ↓	74.66 ↓	78.8 ↓	76.97↓	
11	Total nitrogen Ammonical nitrogen Nitrite-nitrogen (NO ₂ ⁻) Nitrate-nitrogen (NO ₃ ⁻) Kjeldhal nitrogen	57.75↓ 60.15↓ 200.0↑ 51.85↑ 61.18↓	57.02 ↓ 59.08 ↓ 100.0 ↑ 30.3 ↑ 60.58 ↓	63.25 ↓ 65.22 ↓ 100.0 ↑ 33.33 ↑ 69.15 ↓	59.38 ↓ 61.07 ↓ 30.56 63.56 ↓	65.17 ↓ 66.94 ↓ 141.67 51.85 ↑ 69.81 ↓	62.96 ↓ 65.06 ↓ 43.33 ↑ 30.56 ↑ 69.33 ↓	66.37 ↓ 68.13 ↓ 41.67 ↑ 30.56 ↑ 71.26 ↓	64.49 ↓ 66.03 ↓ 1 20.0 69.83 ↓	
12	Phosphorus (as P)	58.51 ↓	58.43 ↓	63.19↓	60.2 ↓	63.99 ↓	63.0 ↓	64.81↓	64.21↓	
13	Oil & grease	4.55 ↓	0.54 ↓	5.98 ↓	3.13 ↓	5.88 ↓	3.57 ↓	9.09 ↓	3.17 ↓	
14	Hydrogen sulphide	64.32 ↓	65.52 ↓	65.52 ↓	66.07 ↓	66.67↓	66.67 ↓	65.48 ↓	67.24 ↓	
15	Sludge volume index	33.33 ↓	25.42 ↓	35.94 ↓	27.42 ↓	38.46 ↓	28.13 ↓	42.03 ↓	29.23 ↓	

Table 1: Effect of various volumes of corn cobs (CC) and wood chips (WC) as biofilter materials in % removal pollutants along with 0.2% consortium as inoculum and 12 hours hydraulic retention time for domestic sewage treatment

*(All parameters are expressed in mg/litre (ppm) except pH, electric conductivity, temperature & SVI)

The results of various volumes of corn cobs (hollow cylindrical) as biofilter material along with 0.2% consortium and 12 hours hydraulic retention time for the treatment of domestic wastewater treatment revealed that a decrease in the BOD by 67.34% in the presence of 10 % volume of hollow cylindrical corn cobs, 74.9% in the presence of 20% volume of filter material, 76.26% in the presence of 30% filter and 78.8% in the presence of 40% filter material (Table 1). After the completion of experiments with various volumes of hollow cylindrical corn cobs as biofilter material, it was noticed that there was a significant variation in the removal efficiency of pollutant. The results were observed to be useful for sewage treatment process (Fig 1). Further experimentation was continued by using 20% of hollow cylindrical corn cobs as optimized volume of biofilter material, 0.2% inoculum at various HRT's like 8 hours, 9 hours, 10 hours, 11 hours and 12 hours to determine the effect of HRT in presence of hollow cylindrical corn cobs as biofilter material.

Fig.1: Effect of % of volume on removal efficiency of pollutants in presence of corn cobs as biofilter material, 0.2% inoculum & 12 hours HRT



Effect of various HRT's in presence of 20 % volume of hollow cylindrical corn cobs as biofilter material, 0.2% inoculum. The

maximum removal efficiency of BOD was obtained after 9 hours of HRT. The results of removal efficiency of BOD demand at different HRTs were tabulated. The treatment of wastewater by the addition of 0.2% inoculum and 20% volume of hollow cylindrical corn cobs as biofilter material for various HRT's revealed the reduction of BOD by 67.03% for 8 hours of HRT, 78.14% for 9 hours HRT 78.1% for 10 hours HRT, 78.63% for 11 hours of HRT and 81.76% for 12 hours of HRT (Table 2).

Table 2: Effect of various HRT's in hours for % removal pollutants along with 20% volume of corn cobs (CC) and 30% wood chips (WC) as biofilter material along with 0.2% consortium as inoculum for domestic sewage treatment

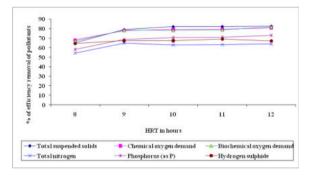
S.No	Parameters*	8 hours		9 hours		10 hours		11 hours		12 hours	
		СС	WC	СС	WC	сс	WC	СС	WC	сс	WC
1	рН	-	0.1	-	-	0.1	-	-	0.1	0.1↓	-
2	Electric conductivity	20.64 ↓	18.93 ↓	20.90 ↓	19.47 ↓	20.46 ↓	22.65 ↓	20.49 ↓	21.30 ↓	20.66 ↓	20.92 ↓
3	Temperature	0.5°C ↓	-	-	-	-	-	0.5°C ↓	1°C ↓	-	-

4	Total suspended solids	64.98 ↓	63.95	78.95↓	70.57 ↓	82.03↓	80.19↓	82.18 ↓	80.94 ↓	82.53 ↓	81.51 ↓
5	Volatile suspended solids	55.86 ↓	54.88 ↓	65.77 ↓	59.02 ↓	66.67↓	64.14 ↓	66.94 ↓	65.17 ↓	67.53↓	66.03↓
6	Chlorides	58.88 ↓	58.33 ↓	68.15↓	62.75↓	68.99↓	67.04 ↓	69.25 ↓	67.53 ↓	70.79↓	67.39 ↓
7	Hardness	42.13 ↓	40.0 ↓	60.0 ↓	50.28 ↓	61.94 ↓	60.59 ↓	62.37 ↓	62.02 ↓	63.04 ↓	63.02 ↓
8	Alkalinity	47.95 ↓	45.48 ↓	61.07 ↓	52.05 ↓	62.96 ↓	60.57 ↓	63.3 ↓	60.95 ↓	64.62 ↓	61.01↓
9	Chemical oxygen demand	68.03↓	67.5↓	77.95↓	72.04 ↓	79.05↓	80.48 ↓	79.49 ↓	80.92 ↓	80.48 ↓	81.76 ↓
10	Biochemical oxygen demand	67.03 ↓	66.84 ↓	78.14 ↓	71.45↓	78.1↓	73.91↓	78.63 ↓	78.38 ↓	81.76↓	80.25 ↓
11	Total nitrogen Ammonical nitrogen Nitrite-nitrogen (NO ₂ ⁻) Nitrate-nitrogen (NO ₃ ⁻) Kjeldhal nitrogen	57.23 ↓ 59.26 ↑ 29.57 ↑	58.03 ↓ 40.0 ↑ 16.67 ↑		62.09 ↓ 500.0 ↑ 46.67 ↑	62.98 ↓ 65.24 ↓ 216.67↑ 21.43 ↑ 67.24 ↓	66.94 ↓ 73.33 ↑ 28.21 ↑	55.56 ↑ 27.78 ↑	67.08↓ 400 ↑ 36.67 ↑	63.91 ↓ 65.9 ↓ 59.26 ↑ 42.42 ↑ 68.52 ↓	65.82 ↓ 68.06 ↓ 400 ↑ 24.24 ↑ 69.52 ↓
12	Phosphorus (as P)	58.04 ↓	55.67 ↓	68.52↓	59.18↓	70.49↓	66.97↓	70.73↓	67.37↓	72.73↓	67.86 ↓
13	Oil & grease	2.94 ↓	1.33↓	2.63 ↓	2.63↓	3.45 ↓	1.19 ↓	3.57 ↓	2.75↓	5.88 ↓	2.63↓
14	Hydrogen sulphide	64.29 ↓	62.07 ↓	67.24 ↓	63.22 ↓	67.24 ↓	67.24 ↓	68.97 ↓	67.86 ↓	67.01↓	67.86 ↓
15	Sludge volume index	40.68 ↓	31.77 ↓	41.67 ↓	32.86 ↓	42.86 ↓	33.33↓	47.69↓	35.59 ↓	49.18 ↓	40.32 ↓

*(All parameters are expressed in mg/litre (ppm) except pH, electric conductivity, temperature & SVI)

After completion of experimentation with 20% volume of hollow cylindrical corn cobs as biofilter material, 0.2 % inoculum at various HRTs, it was noticed that 9 hours HRT shows significant changes during the treatment process. Hence, it was considered 9 hours HRT as optimized HRT (Fig 2). Further experimentation was conducted to determine the viability and increase of performance of filter material. Experiments were conducted for 60 days and samples of wastewater before and after treatment were analyzed for every 10 day interval.

Fig.2: Effect of HRT on removal efficiency of pollutants in presence of 20% volume of corn cobs as biofilter material & 0.2% inoculum



The maximum removal efficiency of BOD was obtained up to 40 days of time period. The results of removal efficiency of BOD at different time periods were tabulated. Effect of time period on 20% volume of corn cobs as biofilter material along with 0.2% consortium as inoculum & 9 hours HRT for domestic wastewater treatment in terms of BOD removal revealed that the reduction of BOD by 83.05% for 10 days time period, 83.15% for 20 days time period, 84.52% for 30 days time period, 84.72% for 40 days time period, 81.38% for 50 days time period and 80.02% for 60 days time period. Results of various other physico-chemical parameters were tabulated (Table 3). After completion of experimentation with 20% volume of hollow cylindrical corn cobs as biofilter material, 0.2 % inoculum and 9 hours HRT at various time periods, it was noticed that the efficiency removal of BOD and other pollutants were increased up to 40 days of operation, later it was observed that a decrease of efficiency removal of BOD and other pollutants for 50 days & 60 days (Fig 3). Hence, 40 days of time period can be consider as optimized time period for the treatment process of domestic wastewater. According to Jignesh et al. (2008), about 10504 MT of maize is produced in India annually and the cobs are thrown as a waste. Hence cobs will be available at free of cost.

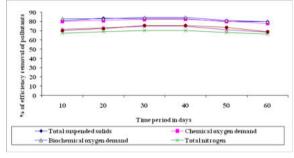
	Table 3: Effect of time period in days for % removal pollutants for 20% volume of corn cobs (CC) for 9 hours HRT and 30% volume of wood chips (WC) as biofilter materials for 10 hours along with 0.2% consortium as inoculum for sewage												
t	treatment												
6	~		aveb 01	20 days	30dave	10 days	50 days	60days					

S.	Parameters*	ers* 10 days		20 days 30		30days		40 days		50days		60days	
No		CC	WC	CC	WC	CC	WC	CC	WC	CC	WC	CC	WC
1	рН	0.1↓	-	-	0.1↓	-	0.1↓	-	0.1↓	-	-	-	0.1↓
2	Electric conductivity	20.94 ↓	19.49 ↓	21.02↓	19.89 ↓	21.09 ↓	21.49 ↓	21.1↓	22.55 ↓	19.96 ↓	22.61 ↓	19.92 ↓	22.18 ↓
3	Temperature	-	-	-	-	0.5°C ↓	-	-	-	-	-	-	-
4	Total suspended solids	81.03 ↓	80.54 ↓	82.8 ↓	81.4 ↓	83.04 ↓	81.88 ↓	82.97 ↓	52.49 ↓	\$0.0 ↓	82.45 ↓	80.1 ↓	80.03 ↓
5	Volatile suspended solids	68.33 ↓	65.91 ↓	69.14 ↓	67.86 ↓	76.05 ↓	67.79 ↓	76.31↓	68.02 ↓	71.43 ↓	68.02 ↓	68.08 ↓	64.14 ↓
6	Chlorides	68.79 ↓	66.29 ↓	71.1↓	68.12 ↓	71.93 ↓	68.8 ↓	71.94 ↓	68.86 ↓	66.14 ↓	68.86 ↓	64.04 ↓	63.73 ↓
7	Hardness	61.48 ↓	61.94 ↓	63.94 ↓	62.94 ↓	65.97 ↓	63.5 ↓	66.09↓	64.13↓	65.0 ↓	64.07↓	64.02 ↓	60.0↓
8	Alkalinity	61.95 ↓	62.06 ↓	65.0 ↓	64.91↓	66.98 ↓	66.09↓	67.0 ↓	66.14 ↓	65.96 ↓	66.14 ↓	64.02 ↓	60.98 ↓
9	Chemical oxygen demand	80.14 ↓	80.98 ↓	81.04 ↓	81.52 ↓	82.13 ↓	82.04 ↓	82.32 ↓	82.63 ↓	80.2 ↓	82.61 ↓	77.96 ↓	78.98 ↓

10	Biochemical oxygen demand	83.05 ↓	80.19 ↓	83.15 ↓	81.01↓	84.52 ↓	82.74 ↓	84.72 ↓	83.52 ↓	81.38 ↓	83.53 ↓	80.02↓	78.99
	Total nitrogen Ammonical nitrogen Nitrite-nitrogen (NO ₂ ⁻) Nitrate-nitrogen (NO ₃ ⁻) Kjeldhal nitrogen	70.28 ↓ 79.17 ↑ 62.96 ↑	69.1 ↓ 50.0 ↑ 27.27 ↑	72.15 ↓ 86.67 ↑ 39.39 ↑	70.14 ↓ 53.33 ↑ 24.24 ↑	73.05 ↓ 59.26 ↑ 30.77 ↑	69.13 ↓ 37.04 ↑ 25.0 ↑	72.99 ↓ 53.33 ↑ 25.0 ↑		70.98 ↓ 25.93 ↑ 43.33 ↑	71.07 ↓ 33.33 ↑ 50.0 ↑	69.23 ↓ 80.0 ↑ 30.3 ↑	39.39 ↑
12	Phosphorus (as P)	71.56 ↓	68.87 ↓	73.08↓	71.58 ↓	74.86 ↓	71.55↓	74.77↓	72.64 ↓	71.3↓	72.95↓	68.63↓	66.13 ↓
13	Oil & grease	3.57 ↓	2.22↓	2.63 ↓	2.04 ↓	2.75↓	3.93 ↓	4.76 ↓	3.03 ↓	5.26 ↓	3.53 ↓	4.63↓	2.82↓
14	Hydrogen sulphide	70.24 ↓	67.74 ↓	70.41↓	70.69 ↓	75.86 ↓	70.69 ↓	75.86 ↓	72.41 ↓	73.81 ↓	72.41 ↓	68.97 ↓	69.05 ↓
15	Sludge volume index	50.00 ↓	35.38 ↓	50.77↓	38.46 ↓	52.86 ↓	42.86 ↓	56.06 ↓	46.88 ↓	56.52 ↓	49.23 ↓	55.65 ↓	51.43

*(All parameters are expressed in mg/litre (ppm) except pH, electric conductivity, temperature & SVI)

Fig.3: Effect of time period on removal efficiency of pollutants in presence of 20% volume of corn cobs as biofilter material, 0.2% inoculum & 9 hours HRT

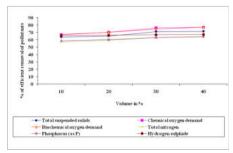


Wood chips as biofilter material

Experiments were conducted using natural biogenic filter media like wood chip was used as filter material. Wood chips of size 8.4 cm X 0.8 cm X 0.7cm, length X breadth X height of equal sized rectangular cube shaped wood pieces were used for present study (Fig 7). Volume of the wood chip was calculated using formula V = I x b x h, and it was observed that each wood chip has a volume of 4.7 cm³. Based on the volume of the reactor, various volumes of wood chips i.e., 1063 pieces (10% of working volume), 2126 pieces (20% of volume), 3189 pieces (30% of volume) and 4252 pieces (40% of volume) were used in the present study. Surface area of the wood chip was determined and it was 26.32 cm² for each wood chip. The specific surface area with volume of the wood chip and converted to meter scale and it was 559.52 m²/m³.

The results of various volumes of wood chips as biofilter material along with 0.2% consortium and 12 hours HRT for the treatment of domestic wastewater treatment revealed that a decrease in the BOD by 67.14% in the presence of 10 % volume of filter material (wood chips), 70.16% in the presence of 20% volume of filter material, 74.66% in the presence of 30% filter and 76.97% in the presence of 40% filter material (Table 1).

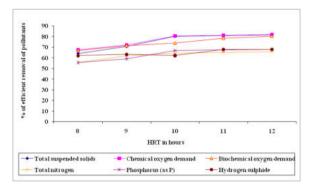
Fig.4: Effect of % of volume on removal efficiency of pollutants in presence of wood chips as biofilter material, 0.2% inoculum & 12 hours HRT



After the completion of experiments with various volumes of wood chips as biofilter material, it was noticed that there was a significant variation in the removal efficiency of pollutant. The results were observed to be useful for sewage treatment process (Fig 4). Further experimentation was continued by using 30% of wood chips as optimized volume of biofilter material, 0.2% inoculum at various HRT's like 8 hours, 9 hours, 10 hours, 11 hours and 12 hours to determine the effect of hydraulic retention time in presence of wood chips as biofilter material.

The maximum removal efficiency of BOD was obtained after 10 hours of hydraulic retention time. The treatment of wastewater by the addition of 0.2% inoculum and 30% volume of wood chips as biofilter material for various HRT's revealed the reduction of BOD by 66.84% for 8 hours of HRT, 71.45% for 9 hours HRT 73.91% for 10 hours HRT, 78.38% for 11 hours of HRT and 80.25% for 12 hours of HRT (Table 2). After completion of experimentation with 30% volume of wood chips as biofilter material, 0.2 % inoculum at various HRTs, it was noticed that 10 hours HRT shows significant changes during the treatment process. Hence, it was considered that 10 hours HRT as optimized HRT (Fig 5). Further experimentation was conducted to determine the viability and increase of performance of filter material. Experiments were conducted for 60 days and samples of wastewater before and after treatment were analyzed for every 10 day interval.

Fig.5: Effect of HRT on removal efficiency of pollutants in presence of 30% volume of wood chips as biofilter material & 0.2% inoculum

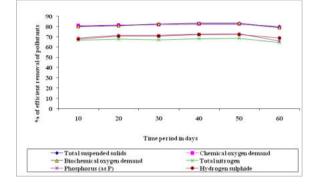


The maximum removal efficiency of BOD was obtained up to 50 days of time period. The results of removal efficiency of BOD at different time periods were tabulated. Effect of time period on 30% volume of wood chips as biofilter material along with 0.2% consortium as inoculum & 10 hours HRT for domestic wastewater treatment in terms of BOD removal revealed that the reduction of BOD by 80.19% for 10 days time period, 81.01% for 20 days time period, 82.74% for 30 days time period, 83.52% for 40 days time period, 83.53% for 50 days time period and 78.99% for 60 days time period. Results of various other physico-chemical parameters were tabulated (Table 3).

After completion of experimentation with 30% volume of wood chips as biofilter material, 0.2 % inoculum and 10 hours HRT at various time periods, it was noticed that the efficiency re-

moval of BOD and other pollutants were increased up to 50 days of operation, later it was observed that a decrease of efficiency removal of BOD and other pollutants for 60 days (Fig 6). Hence, 50 days of time period can be consider as optimized time period for the treatment process of domestic wastewater.

Fig.6: Effect of time period on removal efficiency of pollutants in presence of 30% volume of wood chips as biofilter material, 0.2% inoculum & 10 hours HRT



Miao *et al.* (2005), reported that 97% removal efficiency of rapeseed oil smoke by *pseudomonas* sps., with the help of platane wood chips as biofilter material. Saliling *et al.* (2007), reported that the denitrification rate for wood chips as biofilter media was 1.36kg NO₃-N/m³/d. In conformance with our observation, Wolverton *et al.* (1983), compared various kinds of filter material which are plant free including reeds, cattail, rush and bamboo. They reported that bamboo filter is more efficient than plant free system by reducing 49%, 76% of ammonical nitrogen in 6 hours and 24 hours of hydraulic retention time.

Scanning electron micrographs shows that microbial cells and colonies were adhered to the surface of the cob. The matrix of the filter material corn cob and attachment of bacterial cell on the surface of corn cob was shown in fig 7-8. It indicates that the adsorption capacity of the microbes to the biogenic material is high.

Fig. 7: Scanning electron micrograph of filter material – corn cob matrix

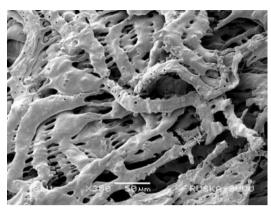
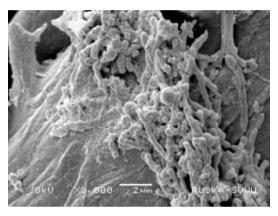


Fig.8: Scanning electron micrograph showing bacteril cells and colonies on the surface of the corn cob



Conclusion

Amongst the materials used for bio-purification, corncobs of specified dimensions are found to be more suitable. Corn cobs with hollow surface produced remarkable results. The time duration was also less for initiation of purification activity. It also produced stabilized results in the repeated experiments. Wood chips also produced promising results and BOD removal efficiencies are slightly lower than corncobs. The utilization of these materials is safe and eco-friendly due to its biogenic nature and they are highly economic for usage because they are available at thrown away costs.

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