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MICROBIAL CONSORTIUM: A NEW APPROACH IN EFFECTIVE BIOREMEDIATION OF SEWAGE WATER

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ABSTRACT
Present study was taken up to prepare effective microbial consortia with concomitant enzymatic activity for the effective bioremediation of sewage water. Three different consortia were prepared and the compatibility of the microbial strains within the consortia was checked by various microbial and biochemical activities. The microbial consortia were prepared by using molasses as medium and were used on sewage water for the bioremediation of various elements like BOD, COD, N, P, K, C, macro elements like Zn, Ca and heavy metals at different temperatures. Effective microbial consortia 3 was found best among the three as it showed the maximum affectivity required for bioremediation of sewage water and it was monitored for 30 days showing gradual decrease in the N, P, K, C, macro elements like Zn, Ca and heavy metals of the sewage water. Nitrogen content showed reduction from 32.6 mg/l in untreated sewage water to 25, 24, 22, 18 and finally 16.3 mg/l in treated sewage water which was within the permissible limit as per WHO. Phosphorus and potassium also showed the reduction. Total phosphorus showed reduction from 6.2 mg/l in untreated sewage to 3, 2, 1, 0.7, 0.5 mg/l in treated sewage water. Total potassium also showed reduction from 68 to 39 mg/l in EM treated sewage water. Similar reducing of BOD and COD was also observed from untreated sewage water to treated one. Reduced offensive smell and Foul odours the bioremediation of sewage water by the microbial consortia is highly significant. It reduces the time span of biodegradation.

KETWORDS:- Bioremediation, Sewage water, Microbial consortia, BOD, COD.

INTRODUCTION

Wastewater is a complex mixture of natural, inorganic and organic material mixed with man-made substances. It contains everything discharged to the sewer, including materials washed from roads and roofs. It is this complex mixture that ends up at the wastewater treatment plant for purification. Wastewater may be categorized into domestic (sanitary) wastewater also known as sewage, industrial (trade) wastewaters and municipal wastewater which is a mixture of the two. Sewage is correctly the subset of wastewater that is contaminated with faecal matter and urine. The strength and composition of sewage changes on an hourly, daily and seasonal basis, with the average strength dependent on per capita water usage, infiltration, surface run-off as well as local habits and diet. Sewage is 99.9% water with the material that require to be removed amounting to just 0.1% by volume. This solid material is a mixture of faeces, food particles, grease, oils, soaps, salts, metals, detergent, plastics, sand and grit. The organic fraction is composed of proteins, carbohydrates and fats which reflect the diet of the community served by the treatment system (Metcalf and Eddy, 1991). Waste characteristics vary according to the extent of urbanization, the income level of the area and the degree of its industrialization and commercialization. The composition of municipal waste depends to a large extent on the affluence of the population contributing to the waste stream. It is essential to know the composition of waste, both at the source and at discharge, to assess the most suitable option for disposal and recovery. For example, the feasibility of composting is determined by a combination of the quantities of waste generated and the proportion of organic waste, amongst other factors. Waste usually can be processed for disposal or recycling by one or more steps. The first step is the preliminary and primary treatment which is a physico-chemical treatment. Because of the objectionable properties of the effluent, the secondary treatment, which is biological treatment, is employed. The operation involves the biological degradation of organics, both dissolved and suspended materials by microorganisms under controlled conditions. Biological treatment can be accomplished in a number of ways, but the basic characteristic of the system is the use of mixed microbial culture: bacteria, fungi and/or algae, for the conversion of pollutants. In most cases, organic materials are converted to oxidized products, mostly carbon dioxide and new microbial cells (the sludge). The organic materials serve as an energy and carbon sources for cell growth (Chui *et al.*, 2006). A major problem facing municipalities throughout the world is the treatment, disposal and/or recycling of sewage sludge. Generally, sludge from municipal waste mainly consists of biodegradable organic materials with a significant amount of inorganic matter. However, sludge exhibits wide variations in the physical, chemical and biological properties (Javaid, 2006). A new technology has been introduced in which a consortium of naturally occurring microorganisms is developed according to the principles of EM-technology (Effective Microorganisms) and it is mainly used for foul odour elimination and organic waste composting. The concept behind beneficial and effective microorganisms (EM) technology was developed by

Professor Teruo Higa, at the University of Ryukyus, Okinawa, Japan (Sangakkara, 2002). EM contains various aerobic and anaerobic microbial species of photosynthetic bacteria, lactic acid bacteria, yeasts, actinomycetes and fermenting fungi and their mode of action improves biodegradation of organic wastes and deodorization of foul odour. EM is a natural, probiotic technology operational for over 25years around the world. It is based on beneficial and effective microorganisms. The microbes in EM are non-harmful, non-pathogenic, not-genetically-engineered or modified and not-chemically-synthesized (Higa, 2002). Jochen *et al.* (2008) have also described EM as a multi-culture of coexisting anaerobic and aerobic beneficial microorganisms. The main species involved in EM include: Lactic acid bacteria: *Lactobacillus plantarum, Lactobacillus casei, Streptoccus lactis*; Photosynthetic bacteria: *Rhodopseudomonas palustrus, Rhodobacter spaeroides*; Yeasts: *Saccharomyces cerevisiae*, *Candida utilis*, Actinomycetes: *Streptomyces albus*, *Streptomyces griseus*, fermenting fungi *Aspergillus niger,Fusarium oxysporum.*

Isolation of most effective microorganisms

Following groups of microorganisms which constituted the effective microbial consortium were isolated:

Lactic acid bacteria were isolated from curd samples which were obtained from different sources like Khyber, Zum Zum, Milkman, Snow Cap and Amul *etc*. The samples were collected and serial dilution was done on de Man Rogosa and Sharpe agar medium (MRS).

Isolation of *Pseudomonas* species Soil samples were collected from several areas of garages, containing spilled oil soils, randomly 15-20cm beneath the surface using sterile spatula and were placed in sterile screw capped vials and were purified and isolated by repeated sub culturing into basal medium (King's B) until purified strains were obtained.

Isolation of Yeasts Dry yeast granules were used for isolation of yeasts. One gram of yeast granules was suspended in 9 ml of distilled water and 0.1ml of culture suspension was withdrawn and spread on Potato Dextrose Agar medium and the culture plates were incubated at 27^{0}C for 48 hrs.

Isolation of Actinomycetes one gram from the thoroughly mixed soil sample taken from decomposing pile of garbage was suspended in 100 ml sterile distilled water and incubated in an orbital shaking incubator at 28^{0}C with shaking at 140 rpm for 30 min and grown on Starch Casein Agar medium and incubated at 28^{0}C for 10 days.

Isolation of Fermenting fungi The samples of onion, garlic, banana peel, saffron corms and rice were taken and suspension was made in distilled water and 0.1ml of suspension was taken and inoculated on czapek's dox medium at 27^{0}C for 48 hrs.The fungal species were identified by morphological and physiological tests like spore type, LCB staining and amylase production.

Characterization of the microbial isolates

Morphological characterization

All the Effective microorganisms were examined for the colony morphology, cell shape, gram reaction and spore forming ability as per the standard procedures given by Anonymous (1957) and Barthalomew and Mittewer (1950). Colony morphology was studied with the help of magnifying lens and cell shape of the isolates under microscope.

Biochemical characterization

The Biochemical characterization of the isolates was carried out as per the procedures given by Cappuccino and Sherman (2009). The tests which were carried out are outlined as under: iMviC, Catalase tes ,Urease test, Oxidase test ,Starch hydrolysis .Casein hydrolysis and Hydrogen sulphide production

Qualitative screening of fungal isolates for their relative amylolytic activity

All the collected fungal isolates were primarily screened for their amylolytic activity on Yeast Peptone Soluble Starch medium (YPSS) by observing zone of clearance.

Phosphate solubilisation: All the isolated cultures of *Pseudomonas* were grown in Tryptic soya Agar (TSA) broth. Log phase growing cells of each culture (15 µl) were spotted on Pikovskaya's medium plates. These plates were incubated at 28^{0}C for 3-4days. Zone of solubilisation and colony size were measured using a measuring scale and these values were used to calculate solubilisation index

Chitinase assay: The chitinase activity of *Pseudomonas* isolates was estimated as per Reissig *et al.* (1995) and for preparation of colloidal chitin the method of Berger and Reynolds (1958) was adopted. Petri plates amended with 0.3% colloidal chitin were incubated at 30 ^{0}C for 7 days, then iodine was added to these plates. Development of halo zone around the colony after addition of iodine was considered as positive for chitinase enzyme production.

Antimicrobial activity

In vitro antimicrobial activity of the isolates against *Fusarium oxysporum, Fusarium moniliforme, Bacillus subtilis* and *E. coli* on potato dextrose agar (PDA) plates and nutrient agar plates was examined by dual inoculation technique (Sakthivel *et al.*, 1986). The fungal pathogen was inoculated on the plates containing potato dextrose agar medium and bacterial pathogen on nutrient agar medium and incubated at 28 $\pm 2^{0}$C for 72 hours. With the help of sterile cork borer, the disc of fungal growth from this plate was taken out and placed at the centre of the fresh potato dextrose agar medium containing plate. 24 hours old growth of each bacterial isolate was then streaked on either side of the disc and kept for incubation at 28 $\pm 2^{0}$C for 72 hours. After the incubation for 72 hours, the plates were visually observed for the inhibition of fungal pathogen by comparing with the control PDA plate inoculated with only fungal pathogen.

Compatibility: To study the antagonistic properties, a single bacterial strain was streaked as a straight line in the centre of nutrient agar and fungal strains on Potato Dextrose Agar plates. Cultures to be tested were streaked perpendicularly across the initial culture and incubated at 28 ^{0}C for 48 to 96 hours. Lack of microbial growth (zone of inhibition) at the intersections was indicative of the antagonism of the cultures (Oskay, 2009) but the cultures growing in the close proximity were compatible to each other. Then these compatible cultures were used in formulation of three different consortia.

Formulation of Effective microbial consortium: Microbial consortium was prepared by mixing 3 g of molasses in 100 ml of distilled water. The pH of the broth was adjusted to 7 with 0.1 N HCl. 1 ml of *Pseudomonas*

bacterial culture was added to 100 ml of the molasses and incubated at 30 ^{0}C at 120 rpm for two days. In the second step, 1 ml of Actinomycetes culture was added to the broth containing *Pseudomonas* bacteria. This mixture was continuously shaken at 120 rpm at 30^{0}C for another two days. The final step was to add 1 ml each of the cultures of lactic acid bacteria, yeast and fermenting fungi to broth containing other two species and shaken at 120 rpm at 30^{0}C for another two days. The microbial consortia designated as Effective Microorganisms (EM) were stored for further studies.

Sewage water Management using Effective microbial consortium: Sewage water sample from STP Hazratbal was collected in sterile bottles and 250ml of sewage sample was treated with 10 ml of Effective microbial consortium and different parameters like N, P, K, C, BOD, COD and Heavy metals were assessed at different intervals of time, at different pH, temperature and molasses concentration.

***In vitro* biodegradation:** Treatment of sewage water with: EM1**,** EM2**,** EM3 and Sewage water without any treatment (Control) Replications: 3, Design :CRD

Effect of temperature on growth of effective microorganisms

Growth of various constituent microorganisms in various effective microbial consortia was checked by growing these microbial consortia at different temperatures

Effect of Temperature on total N, P, K, C, BOD, COD and heavy metals using various consortia:

All the three different effective microbial consortia were grown at three different temperatures (20, 25 and 30^{0}C) and various elements in sewage water with effective microbial consortia were estimated like N, P, K, C, BOD, COD and heavy metals and was observed for steep decrease in values from original to permissible values so that sewage water can be reused in agricultural activities.

Analysis of treated sewage

Estimation of organic carbon, Estimation of total nitrogen, Estimation of total phosphorus and Estimation of total potassium

Estimation of heavy metals

Procedure: Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detecting metals and metalloids in environmental samples (Baron, 1990; Dauvaltermic, 1998). 5ml of Sewage water sample was diluted to 50 ml with double distilled water (DDW) and Heavy metals like Cd, Ni, Pb were detected using (AAS) Atomic Absorption Spectrometre (GBS Scientific Equipment Pvt. Ltd, Australia).The standard solution (MERCK manufacturers, Mumbai, India) for Cd, Ni, Pb was used for calibration. The heavy metal contents were directly calculated by the computer software AVANTA-2.0 version preinstalled in computer connected to AAS.

Estimation of BOD (Biological oxygen demand) and Estimation of COD (Chemical oxygen demand)

Statistical analysis

The data recorded in triplicate for various parameters was subjected to ANOVA (analysis of variance) in accordance with completely randomized block design using MINITAB statistical package to quantify and evaluate the sources of variation (Gomez and Gomez, 1984).

RESULTS

TABLE 1: Morphological characteristics of various isolates

Isolate	Colony Morphology	Cell shape	Gram reaction
SL1	Creamy, raised, entire medium size	Cocci	Positive
SL4	Creamy, raised, irregular medium sized	Short rods	Positive
SL9	Creamy, minute, lightly raised, irregular	Short rods	Positive
SP1	Small, raised entire, Orange, circular, Opaque	Short rods	Negative
SP6	Yellowish pale, entire, flat, undulate, clear	Short rods	Negative
SP6	Pinkish, raised, circular, entire, opaque	Long rods	Negative
SS1	Creamy to white colour, fluffy and smooth margin	Oval	Positive
SS4	Mucoid, creamish irregular with filamentous margin	Filamentous	Positive
SS7	Colonies were either white or off white	Oval	Positive
SA1	Mycelial, filamentous creamish white	Short filamentous	Negative
SA3	Mycelial spiral, creamish white	Long rods	Negative
SA5	Mycelia spiral, creamish white	Long rods	Negative
SF1	Garlic	Carbon black or very dark brown spores, colourless conidiophores and spores Colorless to light yellow	Negative
SF6	Rice	A hyphae that is hyaline and septate, Pale to yellow	Negative
SF16	Saffron corm	Carbon black or very dark brown spores colourless conidiophores and spores Colorless to light yellow	Negative

TABLE 2: Compatibility between various organisms

Lactobacillus	*Pseudomonas*	*Sacchromyces*	Actinomycetes	Fermenting fungi
SL1	SP1	SS1	SA1	SF6
SL4	SP5	SS4	SA3	SF1
SL9	SP6	SS7	SA5	SF16

TABLE 3: Formulation of Effective Microbial Consortia

Type of consortia	Isolates
EM1	SL1
	SP1
	SS1
	SA1
	SF6
EM2	SL4
	SP5
	SS4
	SA3
	SF1
EM3	SL9
	SP6
	SS7
	SA5
	SF16

TABLE 4: Chitinase activity exhibited by isolates of *Pseudomonas*

Isolate	Solubilization zone(mm)	Solubilization Index	P-released (mg/ml)
SP1	7.057	1.837	101.510
SP2	5.117	2.303	96.503
SP3	6.057	2.403	92.333
SP4	4.090	2.103	88.543
SP5	7.110	2.203	105.833
SP6	8.037	1.960	107.443
SP7	4.220	1.807	65.320
SP8	3.630	1.187	47.307
SP9	2.147	1.500	55.823
SP10	3.570	1.277	62.203
C.D ($p \leq 0.05$)	0.079	0.119	0.204

TABLE 5: Phosphate solubilisation by bacterial isolates

Isolate	Chitinase activity *(units/ml)	** CS:CZ (Ratio)
SP1	22.18	3.67
SP2	11.05	1.63
SP3	15.33	2.85
SP4	9.41	2.09
SP5	33.34	4.08
SP6	25.12	3.06
SP7	13.82	3.11
SP8	12.73	3.05
SP9	11.21	1.63
SP10	14.69	3.45
C.D($p \leq 0.05$)	0.07	0.17

** C:Z depicts ratio of colony size to the zone of clearance

* One unit of enzyme activity (IU) is the amount of enzyme required for the formation of one micromole of product (N-acetyl glucosamine per minute) under the assay condition

TABLE 6: Effect of temperature on Total N, P, K, C, BOD, COD, heavy metals using various consortia

Type of consortium	Parameters	Before treatment values(mg/l)	Temperature (^oC)			Mean	C.D ($p \leq 0.5$)	WHO Permissible Limits(mg/l)
			20	25	30			
	N	32.6	20.3	16.3	18.3	18.3	0.119	37.0
	P	6.2	0.4	0.3	0.4	0.3	0.11	8.6
	K	95.1	68.0	45.0	61.0	59.6	9.67	75.0
	C	1.85	0.8	0.6	0.7	0.7	0.06	0.87
	BOD	57.6	51.2	26.3	39.5	39.0	0.61	80.0
EM1	COD	71.4	47.0	29.0	31.3	35.8	0.39	150
	Mg	50	35.1	30.5	41.7	35.7	0.106	100
	Pb, Cd, Ni	0.00	0.0	0.0	0.0	0.0	-	840,85,420ppm
	Fe	7.0	2.1	1.0	1.0	1.4	0.09	5.0
	Zn	7.0	1.9	0.9	1.2	1.3	0.09	5.0
	Ca	240	235	160	175	190	10.22	230
	N	32.6	20.3	18.3	22.3	20.3	0.11	37.0
	P	6.2	0.5	0.3	0.6	0.4	0.07	8.6
	K	95.1	50.2	48.2	54.1	50.8	0.09	75,0
	C	1.85	0.6	0.8	0.7	0.7	0.07	50.0
	BOD	57.6	39.1	47.2	41.2	42.5	0.10	80.0
EM2	COD	71.4	51.1	66.3	57.2	58.2	0.39	150
	Mg	50	47.1	39.6	42.2	42.9	0.38	100
	Pb, Cd, Ni	0.00	0.0	0.0	0.0	0.0	-	840,85,420ppm
	Fe	7.0	1.2	1.0	1.0	1.1	0.08	5.0
	Zn	7.0	1.4	0.8	1.5	1.2	0.49	5.0
	Ca	240	135	120	161	499	10.90	230
	N	32.6	26.5	24.3	28.5	26.5	0.53	37.0
	P	6.2	1.0	0.7	0.7	0.8	0.07	8.6
	K	95.1	67.6	53.0	62.3	60.9	10.69	75,0
	C	1.85	30.5	25.2	32.5	19.8	0.55	50.0
	BOD	57.6	42.3	33.5	39.3	38.4	0.62	80.0
EM3	COD	71.4	60.4	51.5	57.6	56.5	0.66	150
	Mg	50	45.4	36.8	40.3	40.8	0.50	100
	Pb, Cd, Ni	0.00	0.0	0.0	0.0	0.0	-	840,85,420ppm
	Fe	7.0	8.3	1.2	1.2	3.6	0.46	5.0
	Zn	7.0	91.6	1.5	1.1	31.4	6.70	5.0
	Ca	240	176	135	151	154	25.96	230

Mean: temperature: 44.59 31.67 38.35
Consortia : EM1:35.82, EM2:35.10 , EM3:43.68
Parameters:N:21.5,P:0.38,K:50.63,C:10.07.BOD:39.73,COD:49.42,Mg:39.65,Fe:1.69,Zn:11.09,Ca:157.85
C.D($p \leq 0.05$)Temperature: 0.3438 (Significant)
Consortia 0.3438 (Significant)
Parameters: 0.6276 (Significant)

SUMMARY AND CONCLUSION

Among the three effective microbial consortia (EM1, EM2 and EM3) used during *in vitro* biodegradation, EM3 was the most promising one for *in vitro* biodegradation on the basis of reduction of macro nutrients like N, P, K and micro-nutrients like Ca, Zn, BOD, COD and heavy metals like Pb, Ni in sewage water treated with effective microbial consortia and the effect of various parameters like temperature, pH, molasses concentration and incubation period on reduction of nutrients was assessed. Effective microbial consortia contained constituent microbes like *Lactobacillus, Pseudomonas, Saccharomyces, Streptomyces* and *Aspergillus.*Chemical characteristics of effective microbial treated sewage water like BOD and COD was found better as compared to untreated sewage water.

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