

Isolation and identification of bacterial flora from bat guano and its study on bioremediation of industrial waste

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Manuscript details:

Available online on
<http://www.ijlsci.in>

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:

Khadse TA and Gadhikar YA (2017) Isolation and identification of bacterial flora from bat guano and its study on bioremediation of industrial waste, *Int. J. of Life Sciences, Special Issue, A8*: 41-46.

Acknowledgements:

Authors express their thanks to U.G.C, India for providing financial support to this research. We also grateful to Ms. Smita Pandya, Director and Environment Consultant, Global Scientific Incorporation and MTCC, Chandigarh

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ABSTRACT

Preliminary investigation were carried to identify the bacteria from bat guano. Morphological and biochemical test were performed and the identification of bacteria was made. Guano is rich in existing organic fertilizers with a better balance of essential N-P-K, wealth of microorganism and much higher level of organic matter. Various bacteria present in guano have proved to be efficient for breakdown of industrial effluents i.e. by bioremediation process of microbes which clean up toxic substances from the industrial waste. Recent research indicates that different bacteria confined in guano may actually provide important medicinal materials. The present work is an attempt to study the effect of bat guano on bioremediation of industrial waste effluents from Mahananda dairy and Coca cola bottling plants. The results showed that with a period of 15 days there was a remarkable reduction in the Chemical Oxygen Demand (COD) values up to 50%-70%, thus stabilizing the industrial effluents. Besides this, values of various physico-chemical parameters were notably found to reduce suggesting that industrial effluents can be effectively treated by bat guano.

Keywords: Bat guano, Bacteria, Bioremediation, Industrial effluents.

INTRODUCTION

The word guano originated from the Quichua language of the Inca civilization and means "the dropping of sea birds". It describes both bat and sea bird manure. Bat guano provides a considerable supply of richest fertilizer. Recent research indicates that different bacteria confined in guano may actually provide important medicinal material. A marvelous symbiosis exists between the microorganisms and bats. Countless microbes are regularly excreted along with waste products and together with soil organisms they constitute the microbial population of a bat guano deposit. Their main function is to accelerate the process of breaking down of organic matter in guano.

Bioremediation is a spontaneous or managed process in which biological (especially micro biological) catalyst act on pollutants and thereby

remove environmental contamination Bioremediation technology have become famous in early 1980's for site cleanup. Various bacteria present in guano have proved to be efficient for breakdown of industrial waste i.e by bioremediation process of microbes which clean up toxic substances from the industrial waste. By considering this aspect present study was carried out to assess the effect of bat guano as a bioremediator. Bat guano is a safe and environment friendly alternative to harmful chemicals. Kelehar (1998) suggested that bat guano is 100% organic and natural. According to Alper (1983), bioremediation is at least six times cheaper than confinement.

In the present investigation, an attempt is made to isolate the bacterial flora from the bat guano, by morphological, biochemical studies and its effect as a bioremediator was observed on industrial waste water effluents namely Local dairy industry and soft drink (Coca cola) bottling plant at Nagpur.

MATERIALS AND METHODS

Bat guano samples were collected from the roosting sites of bats from the Urban area of Amravati City. The dropping was collected from a clean site with negligible contamination of soil manually by using gloves and placed in sterile polythene bags. Bat guano samples were brought to the laboratory, weighted and immediately process for serial dilution and culturing of the bacteria. Isolation of bacteria was performed by making serial dilution.

Morphological and biochemical test were also performed. Morphological characteristics such as shape and size were determined under light microscope and

using gram staining. The bacterial isolates were biochemically characterized by sugar fermentation test and IMVIC test. Identification of bacteria was made by MTTCC Chandigarh.

The sterile sampling bottles were used to collect the industrial effluents sample from Mahananda dairy and Coca cola bottling plants. Dissolve Oxygen was fixed in situ immediately. The samples were brought to the laboratory and various parameters like pH, Dissolved Oxygen (DO), Total suspended solids (TDS), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), sulphate, chlorine and oil and grease were analyzed. Statistical analysis of the COD reduction results was carried out using two-way ANOVA test by utilizing SPSS utility package.

RESULTS AND DISCUSSION

In the present investigation, moist bat guano samples green and yellow were collected from roosting sites of the bat colonies and were brought the laboratory on cultured on nutrient agar plates at 37°C. After 24 hrs a luxuriant growth as obtained from the guano samples. Seven morphologically different colonies three each from bat guano were obtained (Table 1).

All colonies obtained were transfer to nutrient agar slants in order to get pure isolates of each colonies and heavy growth was obtained in pure nutrient agar slants (Fig.1). The isolated colonies were streaked on selective and differential media to enhance the growth of desired organism. The media used are Bismuth sulphite agar (BSA)(Fig.2), Deoxycholate agar (DCA) Mac Conkey (MaC) and Eosin methylene blue agar (EMB).

Table.1. Colony morphology of bacteria observed on nutrient agar plated from serial dilution 10⁴

Medium	Colony	Growth	Colony Morphology				
			Size	Shape	Colour	Edges	Opacity
Yellow moist guano	C-I	Present	Medium	Circular	Red	Entire	Opaque
	C-II	Present	Medium	Circular	White	Entire	Opaque
	C-III	Present	Small	Circular	Yellow	Entire	Opaque
Green moist guano	C-I	Present	Small	Circular	Red	Entire	Opaque
	C-II	Present	Large	Circular	White	Entire	Transparent
	C-III	Present	Medium	Circular	Yellow	Erose	Opaque



Fig.1.



Fig.2.

Fig.1. Nutrient agar slant showing colonies of bat guano

Fig.2. Isolated colonies on BSA

Table 4 : Physicochemical parameters analysed before and after treatment with bat guano of locally dairy waste sample.

Parameters	Raw effluents without treatment(mg/L)	After treatment(mg/L)
pH	8.5	7.8
Dissolved Oxygen(DO)	Nil	5.0
Oil Grease	18.3	9.8
Total Suspended Solids(TSS)	280	65
Biological Oxygen Demand(BOD)	1400	500
Chemical Oxygen Demand (COD)	257	590
Chlorides	2650	590
Sulphates	39.0	19.9
Total Dissolved Solids (TDS)	34.8	42.2

Table 5 : Physicochemical parameters analysed before and after treatment with bat guano of soft drink bottling plant sample

Parameters	Raw effluents without treatment(mg/L)	After treatment(mg/L)
pH	9.8	7.4
Dissolved Oxygen(DO)	Nil	4.2
Oil Grease	21.6	8.2
Total Suspended Solids(TSS)	210	42
Biological Oxygen Demand(BOD)	280	32
Chemical Oxygen Demand (COD)	1200	390
Chlorides	29.8	19.24
Sulphates	31.2	26.5
Total Dissolved Solids (TDS)	1567	380

The isolated colonies from nutrient agar slants were used to study gram reaction. Isolates from yellow moist guano showed that presence of organism which were short rods gram -ve and few were short and long rods gram +ve .In addition green moist guano showed presence of gram -ve short rod and short rod coccobacilli, cocci in bunches which were gram +ve. Identification of bacteria was made by MTCC, Chandigarh. *Serratia marcescens* and *Bacillus pantothenicus* are the two bacteria which were identified. *Serratia marcescens* are rod shaped gram

-ve bacteria and *Bacillus pantothenicus* are rod shaped gram +ve bacteria. The result obtained from the present study indicate that bat guano contain several different bacterial group i.e gram positive and negative . Literature reveals that not much work has been carried out on the present topic. There have been several made to examine the type of bacteria that can be isolated from faecal material of different animal species and human (Gilliland *et al.*,1975); (Mitsuoka, 1974) and (Zammi,1974). Faecal bacteria are isolated and identified from Swine by Salantro *et al.* (1977).

Gram positive *Bifidobacterium suis* and *Lactobacillus acidophilus* have been identified from swine faeces by Gavani *et al.* (2006).

From chicken faeces five strains of *Lactobacillus thermotolerant* lactic acid bacteria were isolated. These strains were characterized taxonomically by Niamsup *et al.* (2003). They were heterofermentive lactobacilli that produce DL-lactic acid. *Paenibacillus favisporus* sp.nov.a xylon degrading microorganism, a sporulated bacterium was isolated from recent and old cow dung and rectal samples by Veazquez *et al.* (2004).

In the present study, analysis of bat guano for its main constituents were performed by the standard methods. The values were compared with the known values of the constituents of the bat guano. It was found that the Nitrogen and Phosphorus were

comparatively very high whereas Potassium values analyzed were extremely low. The organic matter was found to be substantial in amount.

Certain physico-chemical parameters of two industrial effluents were analyzed in the present investigation and the results are given in Table.4 and 5.

After treating the industrial effluents with guano sample at different concentration, the COD reduction values obtained are given in Table.6 and 7. Table 6.1 shows the percentage data calculated from the values of Table 6. Table 7.1 shows the percentage data calculated from the values of Table 7.

The result indicates that the minimum dosage of sample inoculum for reducing the COD value for the dairy waste found to be 10mL which reduced the COD level from 2570mg/L to 590mg/L.

Table 6 : Results of COD reduction values of the dairy waste industrial effluents by treating with different concentration concentrations of bat guano samples

Conc.of bat guano	COD reduction values mg/L				Total
	1 st day	7 th	15 th	21 th	
2mL	2650	2253	1850	1800	8553
4mL	2700	2295	1755	1720	8470
6mL	2700	2095	690	690	6175
8mL	2600	2210	610	610	6120
10mL	2570	2185	590	590	5963

Table 6.1 : Percentage data of COD reduction values (dairy waste)

Conc of bat guano	Percentage data			
	1 st day	7 th	15 th	21 th
2mL	30.98	26.34	21.63	21.05
4mL	31.88	27.10	20.72	20.31
6mL	43.72	33.93	11.17	11.17
8mL	42.48	36.11	11.44	9.97
10 mL	43.10	36.64	10.36	9.89

Table 7 : Results of COD reduction values of the soft drink industrial effluents by treating with different concentration concentrations of bat guano samples

Conc.of bat guano	COD reduction values mg/L				Total
	1 st day	7 th	15 th	21 th	
2mL	1200	1020	395	390	3005
4mL	1250	1063	350	350	3013
6mL	1250	1013	373	365	3001
8mL	1250	1089	386	380	3105
10mL	1250	1056	352	350	3008

Table 7.1:Percentage data of COD reduction values (Soft drink waste)

Conc of bat guano	Percentage data			
	1 st day	7 th	15 th	21 th
2mL	39.93	33.94	13.14	12.98
4mL	41.49	35.28	11.62	11.62
6mL	41.65	33.76	12.43	12.16
8mL	40.26	35.07	12.43	12.24
10 mL	41.56	35.11	11.70	11.64

Table 8:Significance by 2-way ANOVA test for the effect of bat guano on the reduction of COD values of the local dairy waste effluent.

Source of Variation	SS	Df	MSS	F tab	F cal	Result
Concentration of Sample in ml	1771828	4	442956.9	3.922105	3.25916	Significant
Days	9238495	3	3079498	27.26702	3.4903	Significant
Error	1355263	12	112938.6			
Total	12365585	19				

Table 9:Significance by 2-way ANOVA test for the effect of bat guano on the reduction of COD values of the soft drink waste effluent

Source of Variation	SS	Df	MSS	F tab	F cal	Result
Concentration of Sample in ml	1949.8	4	487.45	1.845802	3.25916	Significant
Days	3095137	3	1031712	1790.183	3.4903	Significant
Error	6915.8	12	576.3167			
Total	3104003	19				

Where SS:Sum of squares ,df: Degree of freedom, MSS: Mean sum of square, F tab: F tabulated , Fcal :F calculated.

The minimum dosage for reducing the COD value for soft drink was found to be 2ml, where it reduced the COD level from 1200mg/L to 390 mg/L. Though the optimization of COD values was obtained on 15th day of the experiment, the values remained more or less stabilized till 21st day. The similar study was performed to assess the effect of bat guano as a soil cleanser against fungi for bioremediation of lake soil (Pawar and Deshmukh,2004). Biodegradation of lignocelulosic waste by *Aspergillus terreus* was reported first time by Emitiazi *et al.* (2001). Literature reveals a reduction in the COD value of lignocellulosic waste by 40 -80 after treating with the fungus *Aspergillus terreus*. The presence of bat guano brings about biodegradation of toxic compounds in favourable environmental condition (Coyane,1999).

Two-way analysis of variance (ANOVA) test performed for COD values in dairy waste water effluent sample showed that there is a significant difference between

concentration of samples and between the days on which the effluents was treated with bat guano sample, since $F_{cal} > F_{tab}$ at 5% level of significance (Table 8). Whereas, for soft drink the test was found to be insignificant as there was not significant difference between the treatment values (Table 9).

CONCLUSION

High values of Nitrogen, Phosphorus, Potassium and organic matter in the bat guano analysis reveals that, bat guano can become a good manure and can be used as a fertilizer. An attempt should be made to identify further the bacterial flora of bat guano so that it can be used as soil cleaner. From the optimization experiment, it can be concluded that less concentrations of bat guano seed can reduce the chemical oxygen demand (COD) levels significantly and stabilize the industrial waste water. The reduced

values of the physiochemical parameters of dairy waste and Coca cola bottling plant by using bat guano suggest that industrial waste can be effectively treated by bat guano.

Conflicts of interest: The authors stated that no conflicts of interest.

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