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Evaluation of Performance of Cowdung as Microbial Inoculum in Industrial Wastewater Treatment and its Environmental Implications

Tiham Quraishi, Anuja Kenekar, Prafull Ranadive and Ganesh Kamath*

Organica Biotech Private Limited, 36, Ujagar Industrial Estate, WT Patil Marg, Govandi, Mumbai – 400088, Maharashtra, India; charu@organicabiotech.com, anuja@organicabiotech.com, prafull@organicabiotech.com, kamath@organicabiotech.com

Abstract

Objectives: To assess the efficacy of cowdung as a microbial inoculum for the bioremediation of wastewater from industrial effluents. **Methods/Statistical Analysis**: Microbiological analysis of multiple cowdung samples was carried out to assess the population of Gram positive and Gram-negative population. The variation in microbial population of the cowdung in relation to its moisture content was assessed. Further, the performance of cowdung in comparison to commercially available microbial formulation CleanMaxx was studied. **Findings:** The microbiological analysis of cowdung revealed that the population and stability of the Gram-negative bacteria is higher than Gram positive ones. Almost 85% were lactose fermenters which in turn could be considered as indicators of pathogenic load of the cowdung. Effluent treatment simulation study using cowdung revealed 70% COD reduction as compared to CleanMaxx which could effectively reduce up to 90% COD. **Application/Improvements:** The study demonstrates the use of cowdung for industrial wastewaters to be irrational and non-sustainable. Conclusively, the study emphasises the use of scientifically designed microbial products for effluent treatment over the unmitigated use of cowdung.

Keywords: Cow dung, Microbial Inoculum, Waste Water Treatment

1. Introduction

Industrial wastewater is one of the major sources in the pollution of water environment. During the last century, huge amount of industrial wastewater was discharged into rivers, lakes and coastal areas. This has resulted in serious consequences to aquatic environment as well as human life. With an increasing demand of natural water resources and unabated pollution caused by industrial discharges into the environment, it had become necessary to take some immediate corrective measures to solve the grave issue of water pollution[1].

More than 13,468 mega liters of wastewater is generated per day by industries in India; of which only about 60% is treated while the rest is left untreated and directly discharged in nearby water bodies or land[2]. To restrict industries from continuing this unethical disposal of effluents, Central Pollution Control Board (CPCB) initially introduced a policy wherein, industries had to pay a penalty for the amount of pollutant that they discharged into the environment. Instead of holding down the hazardous levels of discharged effluent, as an effect of this policy, industries ended up paying huge amounts of penalties. Owing to this, CPCB had to impose stringent rules for industrial waste water treatment plants. Zero Liquid Discharge (ZLD) is one such effort. The ZLD policy was firstly introduced for four industrial sectors (textile, distilleries, pulp and paper and tanneries) in India. Since March 2015, CPCB has issued notifications to 9 State Pollution Control Boards of states along the Ganga basin. Other than these, stringent cut off limits have been imposed on the quality parameters of the wastewater from the industries that do not fall under ZLD scheme[3]. With being obligated to reduce the toxic load of the

**Author for correspondence*

effluents, industries are in immediate need of approaches that will remediate the effluent quickly and efficiently, so that it can be safely released in the environment. There exist many chemical and physical methods for wastewater remediation. However, they are not only costly but also do not offer environmental sustainability. In such a scenario, microbial remediation of the industrial effluents is the most suitable solution to alleviate this problem to a large extent.

Cowdung has been identified as source of microbes since ancient times and there is no dearth of examples to support this[4-6]. Cowdung is extensively used for bio-remediation and has been found to be best suited for remediation of heavy metals from the environment due to its bio sorption potential. However, limited amount of research has been carried out on the microbiological content cowdung and its possible implications when used for wastewater remediation; especially in aerated systems. Also, the mode of action of cowdung in effluent treatment such as reduction in Chemical Oxygen Demand (COD), Total Dissolved Solids (TDS) and Total Suspended Solids (TSS) appears to be non-specific and is unexplored till date. Moreover, its availability as well as purity is always an issue of concern.

The present study helps to bring focus to the microbiological content and environmental implications of using cowdung in an effluent treatment plant. It also points out the problems associated with use of cowdung in an effluent treatment plant; including its availability and cost. Additionally, it also provides a new solution to the problem associated with use of cowdung in effluent treatment process.

2. Materials and Methods

Fresh cowdung samples were collected from different locations. Moisture content was determined using moisture analyzer (Contech CBB50). To determine the moisture content of the samples, 5 gm of each was loaded into the weighing pan and subjected to rapid evaporation of water content. Minimum 50 gm of each cowdung sample was subjected to air drying for at least 21 days. Both fresh samples and air-dried samples were subjected to estimation of total aerobic count and total count of lactose fermenting bacteria.

For estimation of total aerobic count in the samples, 1 gm of fresh homogeneous sample was dispensed in 100 ml of sterile phosphate buffer saline (pH: 7.3 ± 0.2) containing 8 gm/lit NaCl, 0.2 gm/lit KCl, 0.2 gm/lit KH_2PO_4. The suspension was vigorously shaken manually and kept on rotary shaker with 120 rpm for 15 minutes. The (10^{-1}) suspension so prepared was subjected to serial dilution till 10^{-7} dilutions. For each of the last four dilutions, 0.1 ml was spread onto trypticase soya agar plates containing 15gm/lit casein enzymatic hydrolysate, 5 gm/lit sodium chloride, and 5 gm/lit soya peptones with 2% agar at pH 7.2 $\pm$0.2. All the plates were incubated at 35^0C for 48 hours.

To roughly estimate the count of gram negative bacteria, above mentioned dilutions used for total aerobic count were plated onto Mac-Conkey agar plates to estimate the number of lactose fermenting microbes. CFU per gram for all the methods was calculated using the following formula

CFU/ml = (no. of colonies x dilution factor) / volume of culture spread onto the plate

Residual samples were subjected to air drying until there is no further decrease in moisture content. These air-dried samples were subjected to aerobic as well as anaerobic count as per the procedure described above. Quantitative evaluation of aerobic and lactose fermenting microbial count was carried out periodically after 7 days up to 21 days.

The efficiency of dried cowdung sample for effluent treatment was studied by subjecting suspension of 20% solution of cowdung for treatment of three different types of effluent belonging to dairy and chemical synthesis industry. One part of 20% solution of cowdung was mixed 9 parts of effluent sample in an aerated reactor of 5 Lit capacities and the system was subjected to aerobic treatment. Constant supply of air was provided by using laboratory scale aerators fixed at bottom of the reactors. Effluent sample without addition of cowdung suspension were also kept for aerobic treatment as a negative control. A commercial product "CleanMaxx" from Organica Biotech Pvt Ltd was used as positive control of the experiment. Effluent samples under treatment were sampled after interval of 5 days up to 15 days and tested for Chemical COD. All samples were digested in COD digestor by Hanna reactor HI839800 and readings were recorded on Hanna photometer HI83099.

3. Results and Discussion

3.1 Microbiological Analysis of Cowdung

Moisture content of any samples plays a crucial role in its total viable population of microbes present therein. Therefore, before getting any insight of the quantum and type of microbial population, moisture content of the samples was determined at different time-points of the study. Average moisture content of fresh cowdung sample was found out to be 78.68 %. However, it is found to decrease with increase in duration of drying of the samples. **Figure 1** shows the trend in reduction of moisture content of the cowdung on drying over the period of 21 days. It is interpretative that there is significant decrease in moisture content of all the samples during initial 7 days of drying. Up to maximum of 14 days, most moisture is removed from the sample and the value reaches to approximately 6%. After this there is no significant reduction in moisture content irrespective of the duration of drying. It is a general observation made by many researchers that, more the moisture content, more the viable microbial population in any sample[7,8] and similar theory applies to cowdung as well.

Average count of all fresh cowdung samples were noted to be approximately 10^7 Colony Forming Unit (CFU)/gm. It is found much in accordance with previous studies[5,9] where count of fresh cow dung samples was found to range between 10^6 and 10^8 CFU/gm. Total viable count of each sample after 0, 7, 14, and 21 days from collection of samples are noted in **Table 1**. As evident from the table, there is variation in count owing to different sources of dung samples. Diversity also varied from sample to sample. As the moisture content of the sample decreased, a significant decrease in count was observed. At moisture content of approximately 6%, average count observed was ~10^4 CFU/ml. Another noteworthy observation was, most of the population that could be cultivated comprised of Gram negative bacteria. To authenticate this observation, dilutions of cowdung samples were also plated onto Mac Conkey agar plates. The count of lactose fermenting bacteria at different time-points is also noted in **Table 1**. Morphological identification under microscope also indicated that almost all Gram negative belonged to coccobacillus family; of which majority have been identified to be either potent or opportunistic pathogens[10]. Very few colonies of Gram positive bacteria could be isolated. Moreover, it was observed in our study that the presence of Gram positive bacteria majorly depended on the source of cowdung sample.

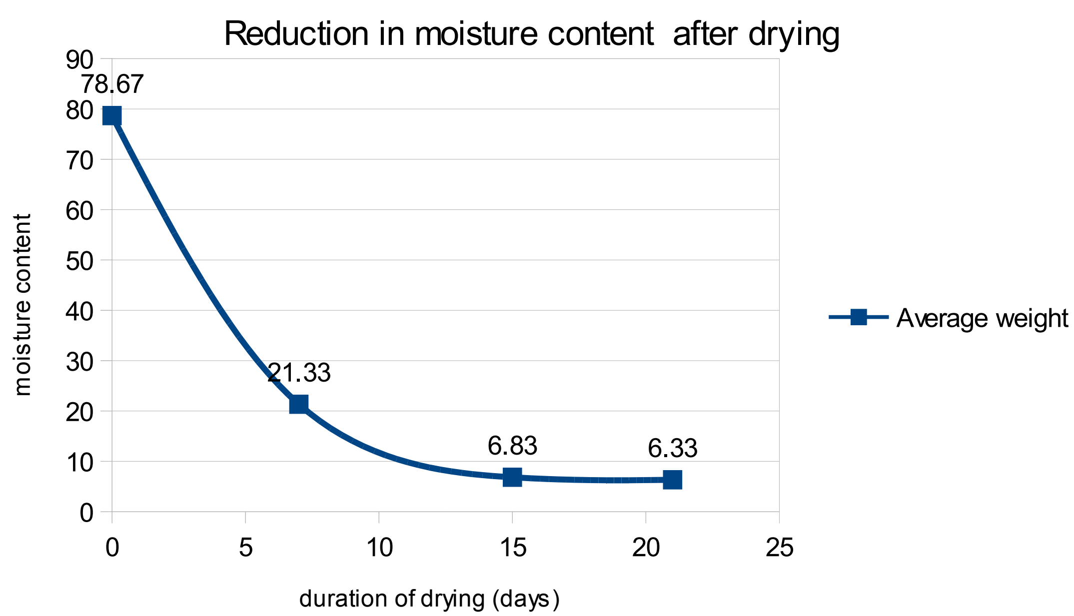

Figure 1. Trend in decrease in moisture content of cow dung samples over the period of 21 days.

Table 1. Total viable and lactose fermenting count of cow dung samples at up to 21 days

Sample Name	Count in samples at different time points (CFU/gm)							
	Fresh samples (0 days)		7 days old samples		14 days old samples		21 days old samples	
	CBC	LFC	CBC	LFC	CBC	LFC	CBC	LFC
CD 1	3.7 x 10^9	~10^4	2 x 10^5	~10^3	3 x 10^4	~10^3	~10^4	~10^3
CD 2	7.9 x 10^8	~10^4	1.7 x 10^5	~10^5	1.2 x 10^4	~10^4	~10^3	~10^3
CD 3	1.1 x 10^7	~10^4	9.8 x10^6	~10^4	7.5 x 10^4	~10^3	~10^3	~10^3
CD 4	1.4 x 10^5	~10^4	3.7 x 10^4	~10^2	3 x 10^3	~10^2	~10^3	~10^2
CD 5	9.6 x 10^6	~10^4	2.5 x 10^5	~10^3	4.9 x 10^3	~10^3	~10^3	~10^2
CD 6	6.2 x 10^7	~10^4	2.1 x 10^8	~10^4	5.6 x 10^4	~10^3	~10^3	~10^3
CD 7	5.3 x10^7	~10^4	6 x10^6	~10^3	5.7 x 10^4	<10^2	~10^3	<10^2
CD 8	1.8 x 10^8	~10^4	8 x 10^6	~10^4	1.7 x10^4	~10^3	~10^3	~10^3
CD 9	4.9 x 10^6	~10^4	2.1 x10^5	~10^3	2.6 x 10^4	~10^3	~10^4	~10^3
CD 10	8.2 x 10^5	~10^4	4. x10^5	~10^2	8.8 x 10^3	<10^2	~10^3	<10^2

(CBC: Total cultivable bacterial count; LFC: Total lactose fermenting count)

Another significant interpretation that can be drawn from **Table 1** is regarding relative decrease in counts of total viable bacteria and total lactose fermenting bacteria. It is remarkable that though there is a sharp decrease in total aerobic counts after reduction in moisture content, the count of lactose fermenting bacteria however, doesn't show proportionate decrease in count. While log difference in total viable count was 4, it was maximum 2 in case of lactose fermenters. Approximately 10^3 CFU/gm of lactose fermenting bacteria were found to be present in cowdung irrespective of the moisture content or duration of drying. Relation between lactose fermenters and Gram negative enteric pathogens has long been established and in this regard, the lactose fermenting population observed in cowdung can also be linked with pathogenic load of the same. In other words, the pathogenic population is more stable than the non-pathogenic one. Our findings support the previously quoted findings stating about abundance of pathogenic bacteria in cowdung samples. Indeed, in actual scenario, it is not feasible to have fresh cowdung for application in an effluent treatment plant and most of the times it is in dried forms. Alternatively, it can be said that the microbial population that is being used in an effluent treatment plant is that of potentially pathogenic cultures. Such pathogens commonly found in bovine manure are with the greatest risk of infection for human. The different type of pathogens reported to be present in bovine feces are bacteria like *E. coli, Listeria spp, Listeria monocytogenes, E. coli* O157, *Salmonella spp. Mycobacterium paratuberculosis;* protozoans like *Cryptosporidia parvum, Giardia spp,* and viruses Bovine Virus, Diarrhea Virus, Coronavirus Foot and Mouth Disease Virus[11,12]. ***E. coli*** **O157:H7** found in cattle manure has been reported as the most notorious pathogen which produces a potent toxin that can cause serious infection in humans. The amount of *E. coli* O157 shed in the manure is estimated to be between 3 – 50,000 CFU/gram of feces. It should be noted that the *E. coli* O157 infective dose for humans is about 10[13,14]. Having been known of the fact that treated effluents are ultimately discharged in the environment, option of using cowdung for wastewater treatment seems questionable pertaining to environmental sustainability and the associated health hazards.

Though, our study has been restricted to cultivable bacteria in the total population, similar observation has been made pertaining non-cultivable count as well. Metagenomic analysis which can analyze both cultivable and non-cultivable bacteria have been carried out by[6] It was observed in their study that the largest genotypic population (almost 85 %) was non-cultivable and more than 90% was strict anaerobes; thus making availability of aerobic bacteria which considered as beneficial such as next to impossible. Moreover, most of the cultivable count was contributed by pathogens residing the gut flora belonging to Enterobacteriaceae family.

There are many literature reports where bacteria have been used for wastewater treatment[14-18] and most of them mention the use of Bacilli for remediate of wastewater.

These microbes may not inherently be present in the wastewater; neither can they be abundantly found in cowdung. Hence, they need to be added from outside and use of consortia of microbes specially designed to remediate the industrial wastewaters seems to be best option ahead.

3.2 Practical Problems Associated with Cowdung

Other than microbiological facts stated above, use of cowdung has other problems as well when it comes to using it in an effluent treatment plant. The primary problem is with its availability. In recent years, the growth in livestock production has reduced considerably due to increasing costs of animal rearing. Farmer and animal breeders are hence turning towards more profitable outputs sources such as meat. As a consequence, the value shares of cow dung in livestock production to only 9% of the total output[19]. This has made cow dung increasingly difficult to get and expensive over the past few decades. Considering the fact that proportion of useful bacteria is very less in the cowdung, huge volumes needs to be added in an effluent treatment plant. Larger the volume needed in an effluent treatment plant, larger is the cost of transportation. While most supply of cowdung is made from rural parts of India, industries are mostly based near urban part. With this fact, there is an addition to transportation cost owing to larger distances to be travelled.

Cowdung is primarily used in agricultural sector either as a starter for composting or directly as manure. Due to rampant use of chemical fertilizer, the soil quality is being decreasing day by day and to compensate the loss, soil conditioning is being carried out by layering the entire field by cow dung containing manure[20]. Alternatively, cowdung is been traditionally used for Biogas production; mostly because of presence of strict anaerobes which otherwise cannot be cultivated easily. With more waste being generated, the demand of cowdung is also increasing and is being given high priority owing to better revenue generation potential[21]. Looking at such huge demand in agricultural and energy sector, industrial demands are insignificant and most supply is diverted to the former.

Irrespective of the availability, purity of the cowdung remains the second major concern. Due to limited availability and high supply of cowdung is inadvertently being mixed with other dung sample to meet the demand. But, this won't suffice the purpose of wastewater remediation, as the flora in dung of other animals is different than that of cowdung and is insignificant for the purpose of remediation[22]. Moreover, the composition of cowdung also contains lot of insoluble matter which may lead to sludge bulking or choking in an aerated system.

3.3 Effluent Treatment Efficiency of Cowdung over CleanMaxx

To determine the efficacy of cowdung in industrial wastewater treatment, another study was conducted wherein definite concentration of cow dung were used for remediation of wastewater; keeping commercially available product CleanMaxx by Organica Biotech Pvt Ltd as a control of the study. The important factors that are kept in check during the effluent treatment process i. e. COD, TDS and TSS were determined at different time points. The values of COD are noted in **Table 2**. The values show that there is gradual reduction in COD using cowdung as well as CleanMaxx. However, maximum COD reduction was achieved using CleanMaxx. Like CleanMaxx, there are many products available which comprise microbes in concentrated form and are specially designed to serve the purpose of remediation of industrial wastewaters. Along with being efficient in wastewater remediation, these products require less space, thus offering ease of store

Table 2. COD reduction in industrial wastewaters using cow dung and CleanMaxx

Duration of Treatment in Days	Values of COD in ppm					
	Effluent 1		Effluent 2		Effluent 3	
	CleanMaxx	Cow dung	CleanMaxx	Cow dung	CleanMaxx	Cow dung
0	1650		3646		8743	
5	934	897	2950	2848	6552	7836
10	564	622	1184	1674	3978	5120
15	174	263	913	1260	2195	4332

and transportation and have a longer shelf life. The above mentioned finding justifies the use of such formulation over non-specific sources of microbes such as cowdung. Another observation made during the study was addition of cowdung leads to excessive foaming in the treatment systems which is not a good indication for smooth functioning of the plant. This may be due to reduction in dissolved oxygen which impedes the growth of aerobic bacteria.

The above stated facts and observation made in this study collectively questions the safety as well of specificity of the bacteria used in cowdung in effluent treatment plants. While there are definite scientific reports on use and applicability of cowdung in composting, as an agricultural manure, as a bio-sorbent and even antimicrobial agent and even in anaerobic digestion of waste[23,24], available literature reports fail to justify the use of cowdung in effluent treatment plants.

4. Conclusion

Cowdung has been inadvertently used as an additive in an effluent treatment plant as a source of microbes without getting an insight into its microbiological content. The present study has been carried out to scientifically evaluate the performance of cowdung as a source of microbial inoculum in industrial wastewater treatment. The microbiological analysis concludes that majority of the cultivable microbes present in the cowdung are of pathogenic nature. Effluent treatment efficiency of cowdung has also been studied by simulating the condition in a typical effluent treatment plant. The studies concluded that CleanMaxx, a commercially available microbial formulation is far better than using cowdung as a source of microbes in terms of efficiency of waste water treatment, environmental safety and ease of application as well. Availability issue and economics of using cowdung in an effluent treatment plant has also been explained. Collectively use of commercially available microbial formulation especially designed to be used for remediation of industrial effluents seems to be a better option than using cowdung.

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