Application of an alkaliphilic bacterium, *Kurthia sp.* for treating wastewaters

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Introduction

The isolation and characterization of microorganisms that are able to thrive in extreme environments have received a great deal of attention because of their potential biotechnological applications. The alkaliphiles are unique microorganisms, with great potential for microbiology and biotechnological exploitation for the environmental applications. The aspects that have received the most attention in recent years include (i) extra cellular enzymes and their genetic analysis, (ii) mechanisms of membrane transport and pH regulation, and (iii) the taxonomy of alkaliphilic microorganisms. Alkaline enzymes should find additional uses in various fields of industry, such as chiral-molecule synthesis, biological wood pulping, and more production of sophisticated enzyme detergents. Furthermore, alkaliphiles may be very good general genetic resources for such applications as production of signal peptides for secretion and promoters for hyper production of enzymes.

Several microorganisms exhibit more than one pH optimum for growth depending on the growth conditions, particularly nutrients, metal ions, and temperature. The term “alkaliphile” is used for microorganisms that grow optimally or very well at pH values above 9.0. The first paper concerning an alkaline enzyme of alkaliphilic microorganisms was published in 1971 (Horikoshi 1971). Over the past two decades, our studies have focused on the enzymology, physiology, ecology, taxonomy, molecular biology and genetics of alkaliphilic microorganisms. Industrial applications of these microorganisms have also been investigated extensively and some enzymes, such as alkaline proteases, alkaline amylases and alkaline cellulases, have been put to use on an industrial scale (Fujiwara and Yamamoto 1987; Ishikawa et al. 1993). Subsequently, many microbiologists have published numerous papers on alkaliphilic microorganisms in various fields. Cell surface of alkaliphiles can maintain the intracellular pH values neutral in alkaline environments of pH 10-13. In 1995, new host vector systems were developed by using alkaliphilic *Bacillus* C-125 mutants that are alkaline sensitive, and genes responsible for alkaliphily have been investigated (Kudo et al. 1990; Takagi et al. 1996). Although alkaliphiles have been used for a number of industrial applications but there is no research publication regarding the neutralization of textile industrial wastewater using them? Some work on biological neutralization by a mixture of bacteria in the presence of sugars has been considered for patenting (Indian Patent No. IN191632 2004).

The pH of industrial wastewater directly affects the physiological functions and nutrient utilization by microbes and in turn it affects the aerobic biological treatment. Extremes in pH can reduce the ETP (effluent treatment plant) to a lifeless, smelly bog. The pH of inlet or outlet of wastewater is important in maintaining a proper ecological balance. pH measurements are used as a guide to the proper neutralization of wastewater, as well as to monitor the final effluent quality. Although a number of chemical treatments are available to neutralize the alkaline wastewater but these methods are conventional, expensive, hazardous and further pollute the environment. These chemical based methods also hinder the biological treatment of wastewater.

Today’s more stringent laws and more frequent checks by the authorities reflect the environmental concerns now held by much of society. Thus, for instance, now the pH of wastewater of industries such as textiles may only deviate minimally from the neutral point when discharged into a receiving watercourse or sewerage system.

In present study, a biological technology for neutralization was developed to treat the alkaline industrial waste water. This is a striking example of applied research wherein the use of existing knowledge of extremophilic bacteria was manipulated. This technology offers a cutting-edge biotechnological tool to treat the alkaline waste water in an efficient, advanced and economic way. This technology excludes the use of chemicals as used in the conventional treatment of waste water.

Material and methods

The bacterial strain in the present study has been isolated from the six months old activated sludge from the ETP of a local textile industry. To isolate a potential bacterial isolate, 10 g activated sludge from the said site was added in the 500 ml autoclaved flask containing 100 ml activated sludge extract, 100 ml alkaline bacillus medium and 50 ul Candid B (anti-fungal). Alkaline bacillus medium contained 1gm peptone, 0.5 gm yeast extract, 1g glucose, 0.1g K_2HPo_4 and 1g Na_2CO_3. Peptone and yeast extract were autoclaved at 15psi while glucose, K_2HPo_4 and Na_2CO_3 were autoclaved at 10 psi. After autoclaving the different ingredients at different psi, all the ingredients are mixed together aseptically. The enrichment flask was kept at 100 rpm for 48 hours at 35^0C.

On the basis of colony morphology and color, two bacterial isolates were selected for further study and checking their capability for neutralizing the alkaline wastewater. The single isolated colonies were picked and streaked on fresh plates containing the same medium. The above step was repeated till pure colonies were obtained.

In neutralization experiment, 200 ml textile industrial wastewater of high pH (12.00) was taken in 500 ml glass flask at two places and each bacterial growth was added individually. Bacterial pellets as prepared above were added. The flasks were kept under shaking conditions (100-120 rpm). pH was monitored by a pH meter.

Effect of glucose was also studied during neutralization experiments. Biological neutralization experiments were conducted as mentioned above with an addition of 0.10% glucose to the waste water.

The selected alkaliphilic bacterium, capable of neutralizing alkaline waste water was identified by IMTECH Chandigarrh, India. For starch degradation experiment, a smear of *Kurthia sp.* was made in the centre of the experimental starch agar media plates using a sterile inoculation loop. In control, no smear was made on the media. The plates were then incubated for 48 hours at 37^oC in an inverted position. A lawn of growth was visible after 48 hours and the plates were flooded with Lugol's Iodine. Lugol's Iodine was allowed to react for one minute and was poured off. Destaining was performed using distilled water.

Results and Discussion

In order to explore the potential alkaliphilic bacteria for neutralization of alkaline textile industrial wastewater, total two bacteria were isolated from the pipe through which textile industrial activated sludge has been passed over a period of years. Theses bacterial isolates were selected to check their capability for neutralizing the alkaline waste water.

Out of two, only one isolate was found capable to grow on high pH (12.00) and bring down the pH of wastewater within a short period of 2 hour (Table 1). This bacterium was identified as *Kurthia sp.* and the main characteristic features are: gram positive, motile, catalase positive, capable to grow in a high pH environment (pH 12.00) and capable to hydrolyze the starch.

It was observed that ABM medium of high pH is a suitable medium to grow the *Kurthia sp.* Neutralization of alkaline textile wastewater was also done with lyophilized powder of *Kurthia sp.* Bacterial pellet of 40 ml culture (O.D. = 2.00) was lyophilized and added to 500ml flask containing 200 ml alkaline textile wastewater. Inoculated flask was kept at 35^0C for two hours. Neutralization of alkaline waste water could be achieved in just two hours.

Bacterial Isolates	Reduction in pH of waste water during course of time				
	0 hr	0.5hr	1.0hr	1.5hr	2.0hr
Isolate 1	12.1	12.1	12.0	11.8	**11.6**
Kurthia sp.	12.1	10.5	9.3	8.4	**7.1**

Table 1 : pH reduction of waste water by alkaliphilic bacteria

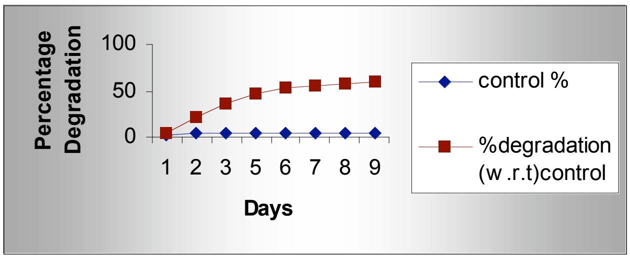

Figure 1: Degradation of starch by *Kurthia sp.*

Experiments were also performed to study the effect of nutrient application (0.1% glucose) on neutralization of effluent. As glucose is provided as a substrate to the bacteria pH was brought down to the neutral within 0.5 hour. This result implies that glucose is easy consumable substrate for bacteria than any other substrate present in the effluent and also assumptions could made that for utilizing or consuming glucose, bacteria definitely has to release some enzymes or acids.

Kurthia sp. was found positive for starch hydrolysis (Figure 1). Amylase (∝-1, 4-glucan 4-glucanohydrolase) is an enzyme that degrades starch to oligosaccharides and in turn to maltose and glucose by hydrolyzing ∝1-4, glycosidic bonds. In digestion, the role of ∝ - amylase is primarily the first reaction of this process, generating oligosaccharides that are then hydrolyzed by other enzymes. On the basis of these results, one could infer that the bacteria might have secreted extra cellular enzyme. More degradation of starch can be observed at pH 10.50 while at pH 7.5 degradation is less.

Conclusions

The neutralization of alkaline wastewater using *Kurhtia sp.* is an economical and effective process. In conventional acid-neutralization process, tonnes of acid are used for the neutralization while the developed biological process decreases the cost drastically. This process of neutralization (Indian Patent Application No. 0816DEL2005) by biological means is quite safe process as the utilization of acid in large quantities for the neutralization of wastewaters is not safe for the industry and strong acid has dangerous effect on the health of workers as well as on the industrial processes. Use of large quantity of acid also increases the volume of industrial wastewaters to be drained out in the main stream. Moreover, the use of acid for neutralization results in generation of dissolved solids while the developed biological process does not generate solids. For effective and fast treatment of waste waters, this kind of bacteria can be immobilized on suitable support.

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