International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:5, No:4, 2011

Neutralization of Alkaline Waste-Waters using a Blend of Microorganisms

Rita Kumar, Alka Sharma, Purnima Dhall, Niha M. Kulshreshtha, Anil Kumar

***Abstract*—**The efficient operation of any biological treatment process requires pre-treatment of incompatible pollutants such as acids, bases, oil, toxic substances, etc. which hamper the treatment of other major components which are otherwise degradable. The pre-treatment of alkaline waste-waters, generated from various industries like textile, paper & pulp, potato-processing industries, etc., having a pH of 10 or higher, is essential. The pre-treatment, i.e., neutralization of such alkaline waste-waters can be achieved by chemical as well as biological means. However, the biological pre-treatment offers better package over the chemical means by being safe and economical. The biological pre-treatment can be accomplished by using a blend of microorganisms able to withstand such harsh alkaline conditions. In the present study, for the proper pre-treatment of alkaline waste-waters, a package of alkalophilic bacteria is formulated to neutralise the alkaline pH of the industrial waste-waters. The developed microbial package is cost-effective as well as environmental friendly.

***Keywords*—**alkaline, alkalophilic bacteria, biological, pollutants, textile.

I. Introduction

THE future of mankind depends on water availability in both quantitative and qualitative terms. In the developing countries, rapid urbanization and industrialization is making the satisfactory collection, treatment and disposal of liquid effluents a formidable problem with serious implications for public health [9]. India is facing a serious environmental management crisis, particularly with respect to pollution of air and water [1]. Water is polluted mainly by traditional organic wastes, wastes generated through industrial processes, chemical agents from fertilizers and pesticides and silt from degraded catchments. The contaminants in waste-wasters can be removed by physical, chemical and biological unit processes. Out of these processes, biological treatment process is the most suitable and is used primarily to remove biodegradable organic substances in waste-waters [10].

Rita Kumar is with the Institute of Genomics and Integrative Biology, Mall Road, Delhi-110007, India (phone: 91-11-27666156 etx. 154; fax: 91-11-27667471; email: rita@igib.res.in)

Alka Sharma was with the Institute of Genomics and Integrative Biology, Mall Road, Delhi-110007, India (email: alka@dbt.nic.in)

Purnima Dhall is with the Institute of Genomics and Integrative Biology, Mall Road, Delhi-110007, India (email: purnimadh@igib.in)

Niha M. Kulshreshtha is with the Institute of Genomics and Integrative Biology, Mall Road, Delhi-110007, India (email: nihamk24@gmail.com)

Anil Kumar was with the Institute of Genomics and Integrative Biology, Mall Road, Delhi-110007, India (email: anilcbt@yahoo.com)

Some of the effluents from industrial processes pose a major problem for treatment as they are alkaline in natureIndustries producing alkaline waste-waters include textile industries, paper and pulp industries and many others. These alkaline waste-waters have a high pH ranging from 8.0-12.0. Since the pH of alkaline waste-waters is very high, generally more than 9, so it is not permissible to drain out such waste-waters as such, in natural water bodies [6]. Moreover, their treatment also becomes a challenging task. Therefore, the alkaline effluents need to be first neutralized and then sent for further treatment process before disposal.

At present, for neutralization of such alkaline waste-waters, acid is used in large quantities which are often economically not feasible [8]. An alternative solution to the problem can be neutralizing such alkaline waste-waters by biological means using microorganisms. Literature reveals that microorganisms, which grow at high pH, have been utilized as a source of large number of enzymes [3] used in industries such as detergent, hide- dehairing, pulp milling and food processing, for gelatine decomposition from spent X-ray films [5] and degradation of rayon waste [4]. However, the potential of microorganisms for the neutralization of alkaline waste-waters has not yet been explored.

An attempt has been made to isolate a number of alkalophilic bacteria from the source habitat and to formulate a package of the same useful for the neutralization of alkaline waste-waters.

II. Materials And Methods

A. Microorganisms and Culture Conditions

A number of strains of alkalophilic bacteria were isolated from source habitat i.e., the effluent from textile industries. All the isolated bacterial strains were grown in medium A having pH ranging from 8.0 to 11.0, to select the alkalophilic bacteria. Medium A contained 1.5g beef extract, 2.0g yeast extract, 5.0g peptone and 5.0g sodium chloride per litre of distilled water. Tris-HCl buffer was used for adjusting pH values up to 9.0, whereas, $NaHCO_3$-Na_2CO_3 buffer was used to adjust pH values from 9.0-11.0. The media was sterilized at 121^oC for 15 minutes at 15lbs pressure. All the cultures were inoculated in the medium having different pH values and incubated at 37^oC, 100 rpm for 16-18 hours. The growth of all the isolated cultures was observed in terms of optical density at 650nm.

B. Selection of Microorganisms

The microorganisms were selected on the basis of their growth at high pH in medium A. The selected bacteria were identified. The growth curve of the selected bacteria was studied. On the basis of growth curve, the generation time and growth rate were calculated using the following formulae [11]:

$$k = \frac{\log N_t - \log N_o}{0.301\ t}$$

where, 'N_t' is the no. of cells at time't', 'N_o' is the number of cells initially and 't' is the time interval.

$$h = \frac{0.693}{k}$$

where, 'h' is the generation time and 'k' is the growth rate constant

The selected microorganisms were propagated in two different media i.e., medium A and Medium B for obtaining better growth at different pH values. Medium B comprised of 5.6 g pancreatic digest of casein 1.6g sodium chloride, 0.83g dibasic potassium phosphate, 0.83g dextrose per litre. Desired pH values were obtained by using NaOH and $NaHCO_3$ under sterile conditions. The media were sterilized at 121°C for 15 minutes at 15lbs pressure. Inoculated cultures were incubated at 37°C, 100 rpm for 16-18 hours. The growth of the selected microorganisms was observed in both the medium in terms of optical density at 650nm.

C. Acclimatization of Selected Microorganisms in Medium B

Acclimatization of selected, identified microorganisms was carried out by growing the bacterial cultures in medium B having values of pH ranging from 8.0 – 11.0. Acclimatization of the selected bacterial strains to different pH values in medium B was accomplished by sub-culturing the bacterial cultures a number of times at different pH values in the increasing order.

D. Formulation of Microbial Consortium

For the formulation of microbial consortium, the acclimatized bacterial cultures were grown individually at pH 9.5. After obtaining optimum growth, the cultures of selected microorganisms were mixed in equal proportions on the basis of their optical density values. The resultant bacterial suspension was centrifuged at 8000 rpm for 20-30 minutes at 4°C. The pellet was collected, washed with 50mM phosphate buffer, pH 6.8 and re-centrifuged at 8000 rpm for 20-30 minutes at 4°C. The resultant pellet was collected and used for the experiment.

E. Acid production by Microbial Consortium

Production of acid by alkalophilic bacteria was observed in medium B as well as in medium C. Medium C contained 1.5 g peptone, 5.3 g NaCl, 0.12 g yeast extract, 0.016 g beef extract, 0.3 g K_2HPO_4 per litre. The required pH of the media was adjusted by using NaOH under sterile conditions. The acid production by microbial consortium was observed by monitoring the pH of the media with the help of pH electrode at different time intervals. Phenol red indicator was added to Change in colour of phenol red from red to orange and orange to yellow was also an indication of acid production by the bacteria.

F. Acid Production by Microbial Consortium in Medium C in the Presence of Carbohydrates

For enhancing the activity of microorganisms to produce acids, stock solutions of 10.0% glucose and 10.0% sucrose as sources of carbohydrates were prepared and appropriate aliquots were added individually in medium C to achieve a final concentration of 1.0%. Phenol red was added to the medium containing carbohydrates, to act as an indicator of acid production.

G. Acid Production by Microbial Consortium in Textile-Industry Effluent in the Presence of Carbohydrates

Alkaline waste-water from textile industry was selected for neutralization study using the formulated microbial consortium. For this, the initial pH of the waste-water was measured. Microbial consortium was added in 500 ml effluent and incubated at 37°C , 100 rpm for a period of 2-7 days. Similar experiment was set up with the effluent containing carbohydrates. The change in pH of the effluent was observed at regular time intervals with the help of a pH electrode.

III. Results

Out of a number of microorganisms isolated from textile industry effluent, only two showed vibrant growth at pH 9.0 in medium A (Table 1). Both the microorganisms were selected for further study and were identified as *Bacillus alkalophilus* or *B. alkalophilius* (corresponding to ATCC No. 27647) and *Bacillus sp.* or *B. sp.* (corresponding to ATCC No. 27337). To study which media supported best the growth of these two bacteria, two media, medium A and medium B, with pH ranging from 8 to 11.0, were tested. It was observed that both microorganisms showed better growth in medium B as compared to medium A (Table 2). So, medium B was selected for further studies.

TABLE I
GROWTH OF ISOLATED MICROORGANISMS AT DIFFERENT PH VALUES IN MEDIUM A

	Microorganisms						
pH	1	2	3	4	5	6	7
8.0	+	+	+	+	+	+	+
8.5	+	+	+	-	+	-	+
9.0	-	-	++	-	++	-	-
9.5	-	-	-	-	-	-	-
10.0	-	-	-	-	-	-	-
10.5	-	-	-	-	-	-	-
11.0	-	-	-	-	-	-	-

(-) No growth, (+) Poor growth, (++) Good growth

TABLE II
COMPARISON OF GROWTH OF SELECTED BACTERIAL CULTURES ON AGAR PLATES IN MEDIUM A AND MEDIUM B

pH	Medium A		Medium B	
	B. alkalophilus	*B. sp.*	*B. alkalophilus*	*B. sp.*
8.0	+	+	++	++
8.5	+	+	++	++
9.0	++	++	+++	+++
9.5	-	-	+++	+++
10.0	-	-	++	+
10.5	-	-	+	+
11.0	-	-	+	+

(-) No growth, (+) Poor growth, (++) Good growth

The acclimatization of selected microorganisms at different pH values in medium B has been carried out. It was observed that both microorganisms showed optimum growth at pH 9.5. As compared to pH 9.5, the growth of both microorganisms decreased gradually at pH 10.0, 10.5 and 11.0. The bacteria were acclimatized to higher pH values i.e., pH 11.0, till good growth was observed.

Acid production by the microbial consortium was measured as a change in pH, in medium C. Production of acid was observed by observing the change in pH values over different incubation periods and simultaneously visualizing the change in colour of the medium from red to orange to yellow.

In an appropriate medium lacking carbohydrates, the overall change in pH was observed from 0.5 to 2.0 pH units, after an incubation period of 2-5 days, as shown in Table 3.

Carbohydrates i.e., glucose and sucrose were used for enhancing the activity of acid production by the bacteria. Presence of carbohydrates in the medium supported the growth of bacteria and enhanced the acid production. In the presence of sucrose, after 2-5 days of incubation period, acid production by both the bacteria was indicated by a shift in pH values towards the acidic range by 1.5 to 4.0 pH units. Table 3 also represents the acid production by bacteria in the presence of glucose.

The pH of the medium significantly changed by 1.5 to 4.0 pH units towards the acidic range. The decrease in pH of the medium was also indicated by the colour change of phenol red from red to orange to orangish-yellow to yellow. The results of the change in pH of the medium in the presence of glucose were comparable with the results of change in pH in the presence of sucrose.

Fig 1 depicts the neutralization of textile waste-waters both in the presence as well as absence of carbohydrates i.e., glucose and sucrose, by the formulated microbial consortium.

TABLE III
ACID PRODUCTION BY MICROBIAL CONSORTIUM IN MEDIUM C

Experimental conditions	Initial pH of the medium	pH of the medium			
		after 2 days incubation		after 5 days incubation	
		pH	Δ pH	pH	ΔpH
Without carbohydrates	9.0	8.0	1.0	7.32	1.68
	9.5	8.6	0.9	7.96	1.54
	10.0	9.12.0	0.88	8.96	1.04
	10.5	9.95	0.55	9.63	0.87
With 1% sucrose	9.0	6.24	2.76	5.26	3.74
	9.5	7.78	1.72	6.68	2.82
	10.0	8.22	1.78	7.13	2.87
	10.5	8.90	1.60	7.85	2.65
With 1% glucose	9.0	6.50	2.50	5.23	3.77
	9.5	7.94	1.56	6.44	3.06
	10.0	8.44	1.56	7.47	2.53
	10.5	9.02	1.48	8.26	2.24

The package bacteria chosen for the study were able to produce more acid in the presence of carbohydrates than without carbohydrates. The shift in pH unit was observed to be from 2.02-2.61 units towards the acidic range. It is evident from the above data that the presence of carbohydrates in the medium supported the growth of bacteria.

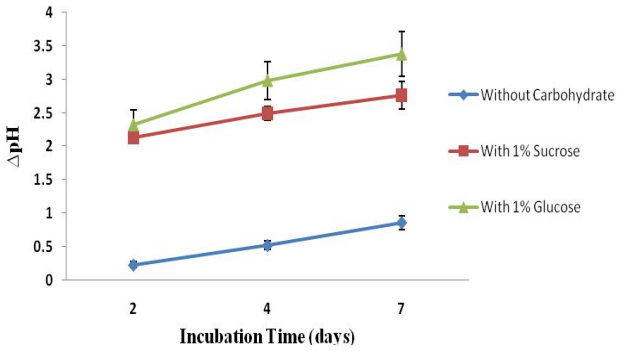

Fig. 1 represents the neutralization of alkaline waste-water from textile industry by microbial consortium

IV. DISCUSSION

The screening of different bacterial isolates led to the selection of only two microorganisms which grew well at pH 9.0 in medium A. The selected bacteria were isolated from the source habitat i.e., alkaline waste-water of textile industry with the expectation that these organisms will be able to tolerate and grow at higher pH values, performing all the metabolic activities essential for survival.

The growth of the selected microorganisms was compared in two media, medium A and medium B and it was observed that the growth of both the microorganisms was better in medium B. This may have been due to the better buffering capacity and presence of suitable ingredients in medium B required for the growth of both the bacteria.

Both the organisms showed optimum growth till pH 9.5, whereas, at pH 10.0, 10.5 and 11.0 there was a decline in the

growth capacity, representing the facultative alkalophilic nature of the microorganisms used. However, when the microorganisms were sub-cultured a number of times at a particular pH, they were acclimatized slowly at higher pH values. In this way both the strains were acclimatized at higher pH values i.e., 11.0.

Acid production was observed both with *Bacillus alkalophilus and Bacillus sp.* in medium C, in the presence as well as in the absence of carbohydrates. Decrease in pH with concomitant change in colour of phenol red of the medium indicated acid production by both the bacteria in synergism. The colour change was in the order of red (alkaline pH) to orange (neutral pH) followed by yellow (acidic pH). In the absence of carbohydrates, the quantity of acid produced was lower, than in the presence of carbohydrates as evident from the change in pH units depicted in Fig 1. This change in pH of the medium may possibly be attributed to the production of organic acids in the medium by the alkaline amylase. This is in agreement with the metabolic products of some alkaliphilic members of genus Bacillus [7]. With glucose as substrate, lactate, acetate and formate are possibly the major end products, the proportions of which depend on the cultural conditions [2].

Bacteria usually utilize carbohydrates by one of the two metabolic processes i.e., fermentation and respiration. Acid production by the microbial consortium may have been due to the carbohydrate metabolism. Among carbohydrates, glucose is metabolised oxidatively to gluconic acid and a local decrease in pH may have been due to the H^+ ions given off by the gluconic acid. The gluconic acid can be further converted to other organic acids. Bacteria can also utilize gluconate as a sole carbon source and thus enhance their growth. Although the bacteria are highly diverse in their metabolism, they do not contain separate metabolic pathways for each of the substances they use. In most cases, they channel a diversity of materials into a relatively few number of central pathways such as Embden-Meyerhoff-Parnas pathway. Those of them, which have an aerobic mode of respiration, undergo glycolysis and Kreb's cycle for the complete oxidation of organic compounds, thereby producing an array of organic acids.

Since textile industries are emanating effluents of a very high pH i.e. up to 12.0, so alkaline waste-water from textile industry was selected for neutralization studies by using alkalophilic bacteria. Neutralization of textile waste-water in the absence of carbohydrates and presence of carbohydrates by microbial consortium was observed (Fig 1). The microbial consortium was able to neutralize waste-water from textile industry. The package bacteria chosen for the study produced more acid in the presence of carbohydrates than without carbohydrates. Higher acid production in the presence of carbohydrates may have been due to the utilization of carbohydrates by bacteria as a source of carbon. Some industrial waste-waters may be rich in high carbohydrate content (eg. starch) and may help in producing acid by using the available carbohydrates in the waste-waters. By using the microbial consortium, the addition of industrial effluents (rich in carbohydrate contents) in the alkaline waste-water (lacking carbohydrate content) may be advantageous for neutralization of these combined effluents.

ACKNOWLEDGEMENT

The authors acknowledge the textile mill authorities for extending their cooperation for providing wastewater samples, whenever required and the generous hospitality offered to us upon each visit. We are also thankful to CSIR for providing the necessary infrastructure.

REFERENCES

[1] D. Chakraborti, B. Das, M.T. Murrill, "Examining India's Groundwater Quality Management," Environmental Science and Technology., vol 45 , pp. 27–33, 2011.
[2] J.M. Gee, B.M. Lund, G. Metcalf, J.L. Peel, "Properties of a new group of alkalophilic bacteria," Journal of General Microbiology., vol 117, pp. 9-17, 1980.
[3] K. Horikoshi, "Alkaliphiles: some applications of their products for biotechnology," Microbiology and Molecular Biology Reviews., vol 63, pp. 735-750, 1999.
[4] Y. Ikura, K. Horikoshi, "Isolation and some properties of alkalophilic bacteria utilizing rayon waste," Agricultural and Biological Chemistry., vol 41, pp. 1373-1377, 1977.
[5] A. Masui, M. Yasuda, N. Fujiwara, H. Ishikawa, "Enzymatic Hydrolysis of Gelatin Layers on Used Lith Film Using Thermostable Alkaline Protease for Recovery of Silver and PET Film,". Biotechnology Progress., vol 20, pp. 1267–1269, 2004.
[6] W. Mayes, P. Younger, J. "Aumoönier, Buffering of Alkaline Steel Slag Leachate across a Natural Wetland," Environmental Science and Technology., vol 40, pp. 1237-1243, 2006.
[7] S. Paavilainen, P. Helisto, T. Korpela, "Conversion of carbohydrates to organic acids by alkaliphilic Bacilli," Journal of Fermentation and Bioengineering., vol 78, 217-222, 1994.
[8] M. Prisciandaro, G.M. Celso, F. Vegliò, "Development of a reliable alkaline wastewater treatment process: optimization of the pre-treatment step," Water Research., vol 39, pp. 5055-5063, 2005.
[9] L. Ritter, K. Solomon, P. Sibley, K. Hall, P. Keen, G. Mattu, B. Linton, "Sources, pathways, and relative risks of contaminants in surface water and groundwater: a perspective prepared for the Walkerton inquiry," Journal of Toxicology and Environmental Health A., vol 65, pp. 1-142, 2002.
[10] C. R. Soccol, L.P. Vandenberghe, A.L. Woiciechowski, V. Thomaz-Soccol, C. T. Correia, A. Pandey, "Bioremediation: an important alternative for soil and industrial wastes clean-up," Indian Journal of Experimental Biology., vol 41, pp. 1030-1045, 2003.
[11] R.Y. Stanier, J.L. Ingraham, M.L. Wheelis, P.R Painter,. General Microbiology. MacMillan Education Ltd. Hongkong. pp 689, 1992.