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Assessment of Biodegradability of Organic Acids by a Defined Microbial Mixture

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The environment has become polluted with a variety of pollutants as a result of the industrial development. Such pollutants and their byproducts are adversely affecting our ecosystem. The problem of industrial waste handling and disposal is increasing continuously as more stringent environmental measures are being imposed. During this era of environmental protection, the environmental biotechnologist is faced with the special challenge to become innovative in the development of appropriate low-cost technologies for treatment and/or reuse of waste products prior to discharge into environment.

Untreated waste water usually contains various organic and inorganic pollutants. Major types of organic pollutants found in various wastewaters include phenols, organic acids, pesticides, etc. (Ehrhardt and Rehm 1985; Yarden et al 1989). Higher concentrations of such pollutants will increase the organic content of wastewaters, and, therefore, it becomes absolutely necessary to decrease the pollutional load of such wastewaters down to an acceptable standard limit. This can be achieved by treating such polluted waters by a suitable method, preferably by means of biological unit processes (Mark 1975). The treatment of industrial waste containing both toxic and non- toxic organic compounds are often accomplished by biological processes. Before such treatment, biodegradability of various organic compounds should be known. Specific micro-organisms may be required to biodegrade such compounds. Employment of single micro- organism may not suffice the purpose. A mixture of micro-organisms may have a cumulative effect on increasing the biomass activity, growth efficiency, and enzyme production.

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In addition, mixed cultures in this way serve to overcome feedback regulation and catabolic repression as the products of one micro-organism are substrates for other organisms.

Here, we present systematic studies on the biodegradability of selected organic acids in terms of the Biochemical Oxygen Demand (BOD) by using a defined mixture of micro-organisms. For comparative studies on biodegradability of organic acids, sewage has been used as a source of micro-organisms.

MATERIALS AND METHODS

The defined microbial mixture contained the following seven genera: *Enterobacter, Citrobacter, Pseudomonas, Klebsiella, Yersinia,* and *Serratia.* The cultures were isolated from untreated sewaqe samples collected from a primary treatment plant situated-in the vicinity of Delhi. These were cultured in a medium containing 10g peptone, 5 g NaCl, 3 g beef extract/L, and the pH was adjusted to 6.8 by using biological buffers. These bacteria were grown separately and mixed in equal concentrations (based on optical density) to obtain a defined microbial mixture. The organic acids used for the study were L(+) tartaric acid, oxalic acid, L-glutamic acid, L- ascorbic acid, 1,2 cyclohexane diamine tetra acetic acid (CDTA, trans-1,2 - diaminocyclohexane - N, N, N', N' - tetra acetic acid), ethylene diamine tetra acetic acid(EDTA), citric acid, 4-aminobenzoic acid, sulfosalicylic acid, phthalic acid, nicotinic acid, L- aspartic acid, folic acid, D-gluconic acid and 3,5- dinitrosalicylic acid. Tartaric, oxalic, sulfosalicylic, glutamic and citric acids were procured from E. Merck (India) and were of extra pure grade. The organic acids namely ascorbic, aspartic, nicotinic, folic and 4-aminobenzoic acids were obtained from E. Merck (Germany) in extra pure form. CDTA (98% pure), EDTA (99% pure) and gluconic acid (99% pure) were purchased from Sigma Chemical Company, USA. 3,5 Dinitrosalicylic acid (98% pure) and Phthalic acid (99% pure) were bought from Aldrich Chemical Co. USA.

Chemical Oxygen Demand (COD) of the organic acids was determined using strong chemical oxidant, potassium dichromate, under reflux conditions (APHA 1989). Biochemical Oxygen Demand (BOD) test was carried out in accordance with the method described by Standard Methods for Examination of Water and Wastewater (APHA 1989). BOD values were expressed as mg/L. The test samples were diluted before BOD estimations because of the limited solubility of oxygen in water. Stock solutions of all the organic acids (0.03%) were prepared and for BOD estimation, 2% of stock solution was used.

For BOD test, seeding (Source of micro-organisms) of the test sample is needed to biodegrade the organic matter present. In conventional BOD test settled raw sewage is used as a seeding material. In the present studies, for seeding the test sample, the microbial mixture was used in parallel with settled raw sewage in each set of experiments. A solution containing Glucose and Glutamic Acid (GGA, 150 mg/L each) was employed as a standard solution for calibration of BOD test. Glucose has an exceptionally high and variable oxidation rate but when it is used with glutamic acid, the oxidation rate is stablized and is similar to that obtained with many municipal wastes (APHA 1989).

RESULTS AND DISCUSSION

The present study was conducted in order to determine the biodegradability of selected organic acids by a defined mixture of micro-organisms.

Table 1. BOD and COD (O_2 mg/L) values of certain organic acids (1% w/v) by defined microbial mixture as well as sewage.

Organic acids	COD (mg/L) x 10^3	BOD (O_2 mg/L) Microbial mixture x 10^2	BOD (O_2 mg/L) Sewage x 10^2
Tartaric	6.8	30.4	30.6
Oxalic	1.3	7.0	6.0
Glutamic	8.2	40.1	46.3
Ascorbic	8.9	36.4	36.7
CDTA	10.7	9.7	10.1
EDTA	14.6	3.8	3.5
Citric	7.0	64.1	60.4
4-Aminobenzoic	16.6	8.2	8.6
Sulfosalicylic	8.3	NB*	NB
Phthalic	2.5	NB	NB
Nicotinic	1.5	NB	NB
Aspartic	6.7	37.6	37.3
Folic	6.3	11.5	12.2
Gluconic	7.6	40.2	39.2
3,5 dinitrosalicylic	9.2	7.5	8.3

*NB = Non - biodegradable

Table 1 shows the COD of the acids as determined by reflux method. Table indicates that, out of the selected organic acids, highest COD value (16.6x10^3 mg/L) was obtained in the case of 4- amino-benzoic acid. This indicates that this particular acid is the most chemically degradable as compared with other organic acids studied. EDTA also showed a high COD value which was in the order of 10^4. Other acids such as tartaric acid, citric acid, nicotinic acid, etc. showed COD values in the range of 10^3 to 10^4.

In order to check the feasibility of the technique with respect to the mixture of the defined micro-organisms, the BOD of GGA was determined by using the above mixture. The results were compared with those values obtained by using sewage as seed. The values obtained by the use of defined mixture of micro-organisms are closer to the theoretical value of GGA as compared with sewage. This implies that the micro-organisms in the mixtures are able to biodegrade the organic acids more efficiently than sewage.

The BOD values of the organic acids using the mixture of defined micro-organisms are also presented in Table 1. Highest BOD value was observed in the case of citric acid (6400 mg/L) and glutamic acid (4010 mg/L). Moderate BOD values (3000 - 4000 mg/L) were represented by gluconic acid, aspartic acid, ascorbic acid, and tartaric acid. Oxalic acid, 4- aminobenzoic acid, CDTA, 3,5- dinitrosalicylic acid and folic acid showed BOD values in the lower range, i.e., 600 - 1200 mg/L. Lowest BOD value (380 mg/L) was obtained by EDTA. BOD results in Table 1 demonstrate that the mixture of defined micro-organisms has either better or almost comparable activity with that of sewage. Certain organic acids such as sulphosalicylic, phthalic and nicotinic acids were not degraded either by sewage or by the mixture of defined micro-organisms. It is quite obvious that the micro-organisms in the mixture may not be specific to degrade these particular organic acids.

Figure 1 shows the BOD/COD ratio of the acids by both, mixture of defined micro-organisms as well as sewage. As observed, the BOD/COD ratio of citric acid was 0.76 which is slightly higher than the theoretical value of 0.65. This indicates that citric acid is more biologically degradable with respect to other acids. BOD/COD ratio as observed for tartaric, aspartic, ascorbic, oxalic and gluconic acids was in themoderate range. The lowest BOD/COD ratio of EDTA shows that EDTA is not oxidized biologically as efficiently as obtained by chemical method. Other acids such as CDTA, 4- aminobenzoic, folic and 3,4 -dinitrosalicylic acids showed lower BOD/COD ratio, thereby depicting

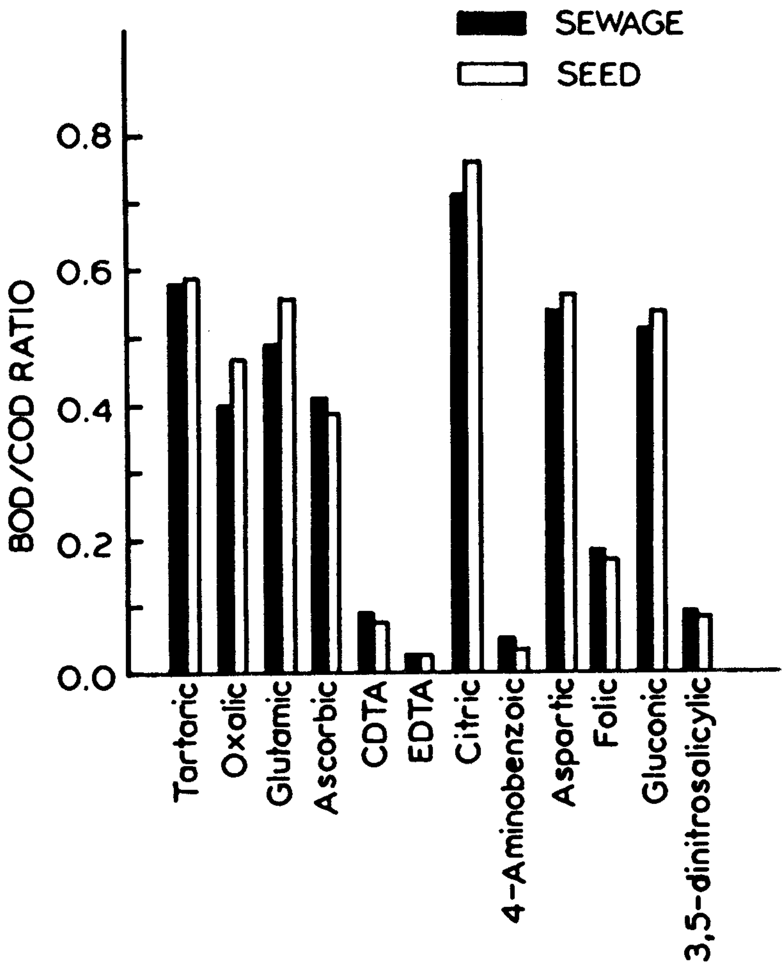

Figure 1 BOD/COD ratio of organic acids as determined by microbial mixture as well as sewage.

that these acids are oxidizable in a better way by chemical means than biological means.

Above study indicates that microbial mixture is able to degrade citric acid more efficiently than other organic acids studied. Since several factors influence the biodegradation of a compound in the environment, the high biodegradation of citric acid may be due to the presence of favourable and necessary growth factors. These factors include essential macro/micro nutrients, sufficient supply of suitable electron acceptors and microbial community present. Among macro-nutrients, some bacteria may utilize both the organic acids as the principal source of carbon and nitrogen for their metabolism. Similarly, other factors, such as pH and temperature of the environment that affect microbial proliferation will influence organic biodegradation (Bollag and Bollag 1992). As microbial enzymes are also responsible for degradation of various organic compounds, there may be certain specific enzymes present in the bacterial cells of this microbial mixture which regulates the degradation of the organic acid.

Certain organic acids showed moderate biodegradation by both microbial mixture as well as sewage. Out of seven micro-organisms present in the mixture, some may have provided favourable conditions to the biodegradation reactions while the others may have inhibited the same. The micro-organisms capable of using these organic acids as nutrients for their growth and metabolism tend to degrade them. The remainder of the micro-organisms of the mixture were unable to utilize these acids, thus, affecting the biodegradation rate. Another major problem is the need to neutralize the organic acids to keep the pH greater than 6 (McKinney 1992). On assumption of the above mentioned factors, organic acids such as oxalic acid, 4-aminobenzoic acid, CDTA, 3,5 dinitrosalicylic acid, folic acid, ascorbic acid, gluconic acid, tartaric acid and aspartic acid were not being degraded completely hence resulted in moderate BOD values.

Being a chelating agent, EDTA is stable against oxidation, thus micro-organisms of the mixture resulted in low biodegradation (Wolf and Culbert 1992). Means et al (1980) also reported that EDTA is susceptible to biodegradation under aerobic conditions. However, the rate of biodegradation is too slow to ensure removal during the biological treatment of sewage.

Sulphosalicylic, phthalic, and nicotinic acids were found to be non-biodegradable. Absence of significant degradation in these organic acids may have been due to several factors which include lack of optimum abiotic conditions as well as the non-existence of specific enzymes in the bacterial cells (Paul 1992). The acids may generate environment which may hinder biodegradation of the concerned acids. Presence of substituents such as nitro (in nicotinic acid), sulfonate etc. tended to diminish biodegradability (Bollag and Bollag, 1992).

The above results on biodegradability studies of organic acids revealed that higher degree of biodegradation occured by a microbial mixture compared with sewage/ activated sludge, used in conventional methods. This mixture of micro-organisms was able to degrade a large number of organic acids with a variable biodegradation rate.

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