0273-2289/91/2829—0827$02.80

Novel Biotreatment Process for Glycol Waters

L. M. V. RAJA, G. ELAMVALUTHY, R. PALANIAPPAN, AND R. M. KRISHNAN*

Centre for Research and Development, Southern Petrochemical Industries Corporation, Ltd., Tuticorin 628005, India

ABSTRACT

Propylene oxide (PO), propylene glycol (PG), and polyols are produced from propylene via propylene chlorohydrin. Effluents from these plants contain biological oxygen demand/chemical oxygen demand (BOD/COD) loads besides high chloride concentrations. The high salinity poses severe problem to adopt conventional methods like activated sludge processes. Presently, a simple, economically viable and versatile microbiological process has been developed to get more than 90% biodegradation in terms of BOD/COD, utilizing specially developed *Pseudomonas* and *Aerobacter*. The process can tolerate high salinity up to 10 wt% NaCl or 5 wt% $CaCl_2$ and can withstand wide variations in pH (5.5–11.0) and temperature (15–45°C). The biodegradation of glycols involves two steps. The enzymatic conversion of glycols to carboxylic and hydroxycarboxylic acids is aided by *Pseudomonas*. Further degradation to CO_2 and H_2O by carboxylic acid utilizing *Aerobacter*, and possible metabolic degradative pathway of glycols are discussed. Various process parameters obtained in the lab scale (50 L bioreactor) and pilot scale (20 m^3 bioreactor), and unique features of our process are also discussed.

Index Entries: Glycols; epoxides; glycerol; biodegradation; *Pseudomonas; Aerobacter.*

*Author to whom all correspondence and reprint requests should be addressed.

INTRODUCTION

Fragile balance between different ecosystems and the impact of industrial activity on tilting this balance is realized to be an important issue nowadays. Every society, whether developing or developed, has become aware of the cost of growth and the inevitable production of effluents, along with useful products. Disposal of untreated effluents leading to the alienation of the environment cannot continue forever. For the present generation, "preserve or perish" is not just a choice but, in fact, an admonition. As a result, great demands are made on the treatment of effluents from chemical plants resulting from the presence of pollutants with highly variable physicochemical properties and ecological impacts. An example is the treatment of effluents from propylene glycol (PG), propylene oxide (PO), and polyol plants. Even though the emission of glycols and polyols into the atmosphere during processing does not pose any hazard as such to the environment (*1*), there is a direct effect when these chemicals are disposed of into the soil or water as a result of high oxygen demand they generate. For example, 1 ppm of PG will exert 1.68 ppm of chemical oxygen demand (COD), and 1 ppm of PO will exert 2.21 ppm of COD.

The treatment of these effluents by conventional techniques, like activated sludge processes and activated carbon adsorption, is possible; but there is a price for achieving low COD levels through these processes by way of substantial energy and capital requirements. The very nature of the chlorohydrin process employed for the manufacture of propylene oxide makes the effluent highly saline, either by the presence of calcium chloride or sodium chloride.

As is well known, the high salinity induces a large osmotic pressure inside the organisms, leading to dehydration. Only a few organisms have developed protective systems to survive this salinity and yet degrade the glycols. The salinity problem is also accentuated by the calcium ion and high alkalinity, causing destabilization of the cell walls and denaturation of the cell wall proteins, exoenzymes, and so on. Further, it is well known that compounds with asymmetric carbon atom are difficult to degrade by microbial means.

Given these difficulties, one can appreciate why the biological treatment of glycol waters has so far been done only through activated sludge processes, notwithstanding their inherent drawbacks, like long retention times, the need for reduced inlet salinity, their high aeration requirements, and so on. The basic deficiency of the activated sludge processes is that the actual degrading organisms are only a minuscule fraction, compared to the mass of population handled simultaneously. In other words, instead of achieving an efficient growth of the target organisms and consequent degradation of the pollutant compounds, the effort is spread out and wasted, to a large extent. Consequently, a huge biological sludge has to be handled, separated, and disposed of. Also, the entire process flowsheet becomes unwieldy.

As an alternative to activated sludge processes, a simple, novel, and economically viable SPIC glycol biotreatment process (SGBP) has now been developed, utilizing a mixed culture of the specially developed bacteria from the genera *Pseudomonas* and *Aerobacter*. In developing this process, we have been guided by the general philosophy of our earlier SPIC urea biohydrolysis process (SUBP), namely, the exploitation of the special features of the developed organisms, and achieving their dominance over the invaders.

In this report, unique features and various advantages of our glycol biotreatment process compared to the activated sludge processes are discussed in detail. This novel bioprocess is being brought to a commercial scale to treat 120 m^3/h of PO/PG–Polyol effluents. The treatment plant is now under construction at Manali Petrochemicals Ltd., Madras, India, and is expected to be commissioned in June 1990. The same process has also been tested to treat ethylene oxide/ethylene glycol and glycerol effluents.

SELECTION AND DEVELOPMENT OF ORGANISMS

Since glycol waste waters are saline, any chosen bacterium must be able to metabolize glycol, even under saline stress. This requirement limits the choice of organisms. Out of many bacterial species screened for glycol degradation in 4% $CaCl_2$ solution, only eight species utilized ethylene glycol, propylene glycol, and epoxides. Further, only two organisms tolerated and degraded polyether polyols and polyester polyols to some extent.

Isolation and Enrichment of the Organisms

These bacteria capable of utilizing glycols and epoxides as sole carbon source, even in saline solution, and also tolerate polyols were originally isolated from soil, and their halotolerance and glycol degradation ability at high concentrations were enhanced by open enrichment technique and in vivo chemical mutagenesis. After a thorough evaluation under an extremely varying composition changes (maximum glycol concentration: 40,000 ppm and maximum salt concentration of either 50,000 ppm of $CaCl_2$ or 100,000 ppm of NaCl), including the ability to withstand traces of chlorohydrocarbons, like dichloropropane (DCP), up to 15 ppm, two bacterial species were finally chosen and were identified as special strains of *Pseudomonas* and *Aerobacter*. The microbiological features of the chosen strains were tested by the methods described in Bergey's manual of systematic bacteriology (2). These engineered bacterial species were found to utilize a variety of organic and inorganic nitrogen sources. Whereas the *Pseudomonas* was able to utilize PG and also other degradation intermediates of PG, there was an accumulation of volatile acids (VA) whose degradation proceeded only slowly. On the other hand, the *Aerobacter* was a little ineffective in degrading the original substrate PG, but was effective in degrading the subsequent metabolites like VA quickly. Glycol utilization by both

Pseudomonas and *Aerobacter* was found inhibited by Zinc (10–50 ppm), EDTA (5–20 ppm), and also the easily available carbohydrates, like sugar. The final degradation products of both the species were observed to be just CO_2 and H_2O.

MATERIALS AND METHODS

Preparatory Studies

These organisms were initially maintained on a bovillion agar slant, and were then grown in shaker flasks. At the first stage, the cultures were grown in a 100 mL synthetic seed medium containing nitrogen sources, like yeast extract and peptone, and mineral sources, like ammonium sulfate and potassium chloride. Subsequent cultivations were done in 1 L glycol process effluent itself. Required organic nutrients were added to the effluent containing 4% $CaCl_2$, 1% $CaCO_3$, 0.25% $Ca(OH)_2$, with traces of NaCl and Na_2CO_3. Prior to inoculation, the pH of the medium was adjusted to around 8. The bacteria were allowed to grow in open air for 36 h in a rotary shaker at room temperature.

Subsequent to the cultivation in 1 L open flasks, the biological oxidation of saline glycol waters (with 4% $CaCl_2$), containing either PO/PG or ethylene glycol, was carried out in 100 L vessels under batch conditions. Both *Pseudomonas* and *Aerobacter* were grown separately, and also, as a mixed culture. The bacterial growth was followed by monitoring optical absorbancy at 620 nm. Studies are in progress to relate this optical density to the actual bacterial cell concentration/mL of the culture medium. For every run, the biodegradation of glycols and epoxides were followed by monitoring the COD, BOD, pH, and also, volatile acids.

Analytical Aspects

Standard analytical methods were used for the analysis of $CaCl_2$ and total dissolved solids (TDS). The chemical oxygen demand (COD), dissolved oxygen (DO), and 5-d biological oxygen demand (BOD_5) were measured by the standard procedures of the US Environmental Protection Agency (EPA). The determination of COD was further cross-checked by three different methods to assure reliability, since it is very difficult to estimate a reliable COD in highly saline solutions like glycol waste waters (*3*).

Chloride Interference

Interference of chloride ion in COD determination had earlier been reported by several authors (*4–7*), and the usual technique to correct the error caused by the presence of chloride is to add mercuric sulfate at a ratio of 1:10. However, Bauman (*4*) reported that some portion of the chloride is uncomplexed even after a 1:20 addition of $HgSO_4$ in highly

saline waters. Hence, he developed a "chlorine recovery method" to get reproducible COD values. His method is now recommended by USEPA and American Water Works Association (AWWA) for COD determination in highly saline solutions.

The second method is the one developed by Burns and Marshall (*5*). They derived a special chloride-correction factor for COD determination through graphical means by estimating COD with various NaCl concentrations. They used only mercuric sulfate for chloride complexation. Their method with the special chloride-correction obtained through graphical means is said to be applicable even for highly saline solutions. For our samples, we determined the chloride correction factor in a 4% $CaCl_2$ solution. It is to be mentioned here that the chloride interference caused by $CaCl_2$ is much higher than the interference caused by NaCl. Also, the chloride oxidation is higher in solutions containing low organic content.

A third method of determining COD in highly saline waters is by the use of permanganate instead of dichromate as oxidizing agent.

All the three methods gave near equivalent results. For quick estimation, the second and third methods are preferred.

BOD Determination

In the BOD_5 determinations, chloride interference was totally removed by treating with sodium thiosulfate. Graphical method for BOD determination (*8*) was also followed in this study and was compared with the normal dilution method. In general, the BOD values of the glycol waters were about 35–45% of the corresponding COD values.

Volatile Acids Estimation

For the volatile acids estimation, the bacteria from the bioreactor sample were first removed by centrifugation or microfiltration. The volatile acids were then separated (*3*) and concentrated by electrodialysis. The separated acids were subsequently analyzed by HPLC, using the column (Microbak-C-10). The column was calibrated with standard organic acids like lactic acid, pyruvic acid, and formic acid, using phosophate buffer with flow rate of 1 mL/min.

GROWTH AND KINETICS

Effect of Initial Substrate Concentration

The batch growth and degradation kinetics of the bacteria *Pseudomonas* and *Aerobacter* were followed individually, and also in combination in nonsaline waters at different initial concentrations of PO. The profiles are shown in Figs. 1, 2, and 3. It can be observed that only *Pseudomonas* was able to achieve 90% COD reduction in 24 h for all the four concentrations

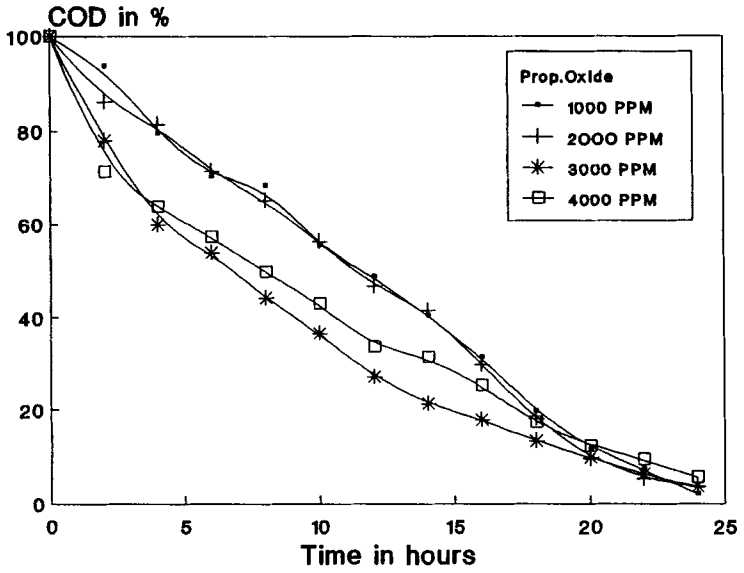


Fig. 1. Biodegradation profile by *Pseudomonas.*

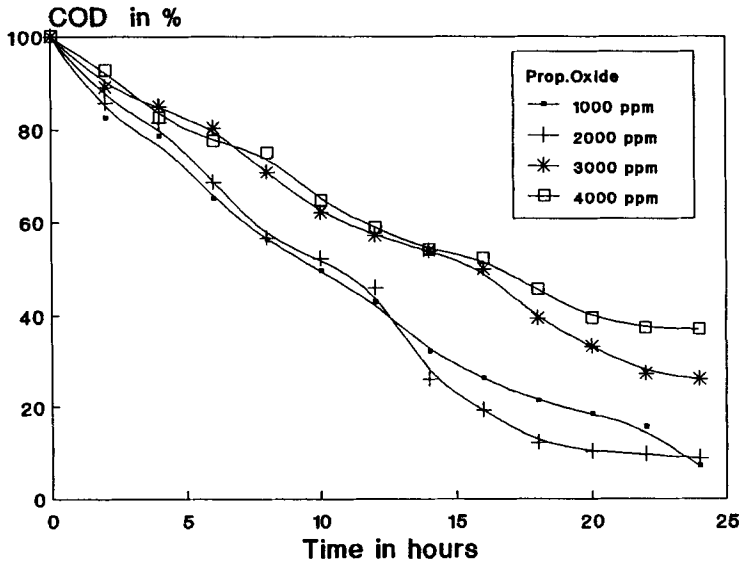


Fig. 2. Biodegradation profile by *Aerobacter.*

1000, 2000, 3000, and 4000 ppm of PO. The bacterium *Aerobacter* was found to be ineffective at concentrations higher than 2000 ppm of PO. Even in terms of the rate of degradation in the exponential phase, *Pseudomonas* exhibited a faster rate at 3000 ppm of PO (and lesser rates at other concentrations), whereas *Aerobacter* did so only at much lower concentration (i.e., less than 2000 ppm of PO). It is probable that the bacterium *Aerobacter* is more susceptible to initial substrate inhibition. Further, for both organisms, the biodegradation of PO appears to be growth associated.

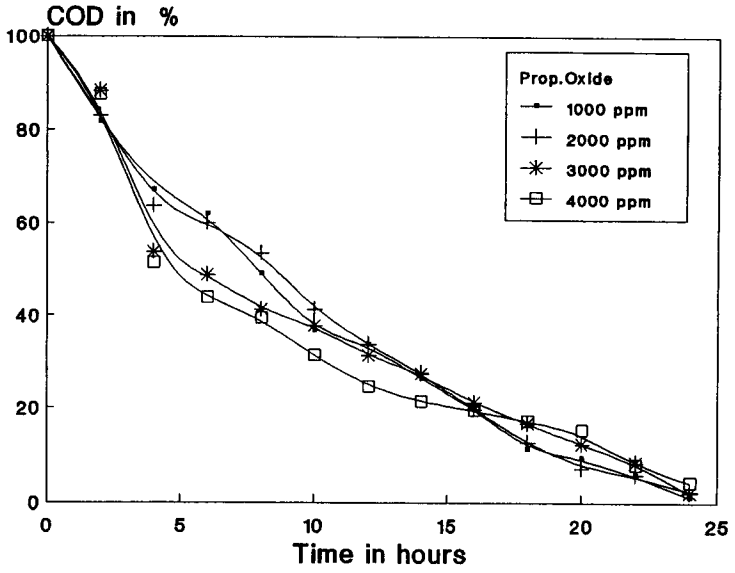


Fig. 3. Biodegradation profile by *Aerobacter* and *Pseudomonas*.

Synergistic Effect

However, when the two bacteria were grown in a mixed culture, a higher rate of degradation was achieved in the exponential phase at a concentration of 4000 ppm of PO. In other words, there appeared to be a synergistic effect in the mixed culture. Probably the two bacteria grew and exhibited the phenomenon of mutualism. This conclusion was supported, when one compared the buildup trend of VA, as shown in Figs. 4, 5, and 6. In the case of *Pseudomonas* (Fig. 4), VA built up to a sharper peak in about 8–12 h at a faster rate and degraded further slowly. In the case of *Aerobacter* (Fig. 5), the buildup of VA was slow, whereas the degradation of VA was fast and nearly complete. Even the peak values of VA for *Aerobacter* were only 50 to 60% of that of *Pseudomonas*. A synergistic behavior was also seen in the VA trend of the mixed culture (Fig. 6). Additional confirmation through the relative assaying of *Pseudomonas* and *Aerobacter* is presently being attempted.

Two Stages of the Biodegradation

This sort of behavior observed through VA and COD reduction suggested a broad two-stage breakup of the PO/PG degradation process.

$$\text{PO/POG} \longrightarrow \text{VA} \longrightarrow \text{CO}_2 + \text{H}_2\text{O}.$$

Whereas *Pseudomonas* was effective in carrying out the first stage (i.e., the conversion of PO/PG to carboxylic and hydroxycarboxylic acids), *Aerobacter* was effective in the second stage (i.e., the conversion of acids to CO_2 and H_2O). However, the stages were not sharp, but diffusive. In

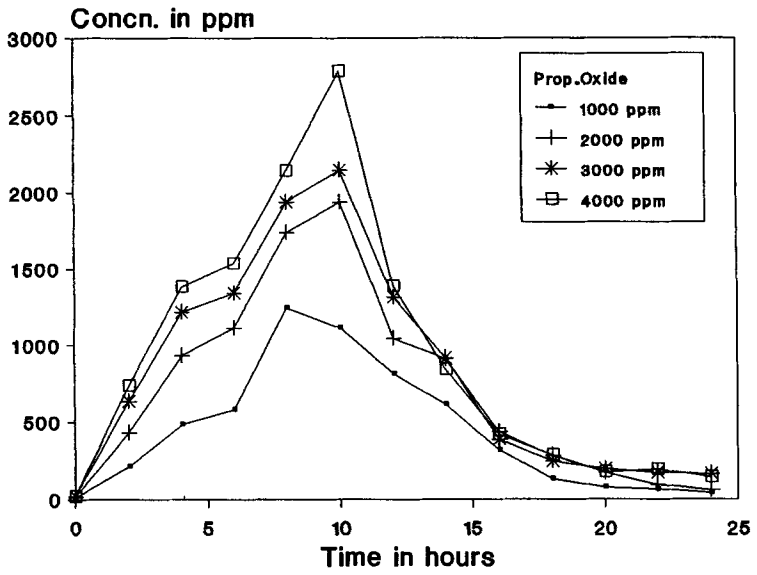


Fig. 4. Volatile acid profile by *Pseudomonas*.

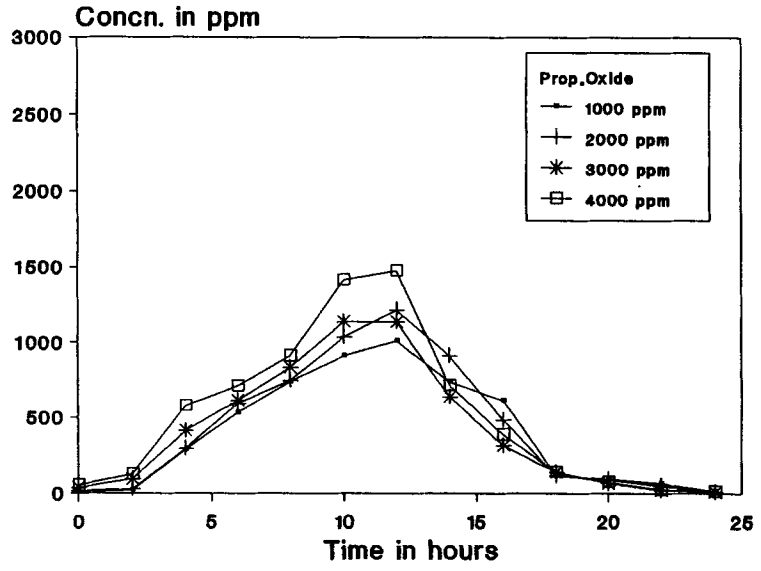


Fig. 5. Volatile acid profile by *Aerobacter*.

reality, there may be a series of successive acids formed before the final decarboxylation to CO_2 and H_2O. In fact, the pH dropped sharply and picked up only gradually to a neutral value, and CO_2 was expelled from the medium by agitation.

General Trend of Degradation

Now, when the two bacteria were grown in a saline medium, all the above observations were seen (though not clearly) at a delayed time. Even

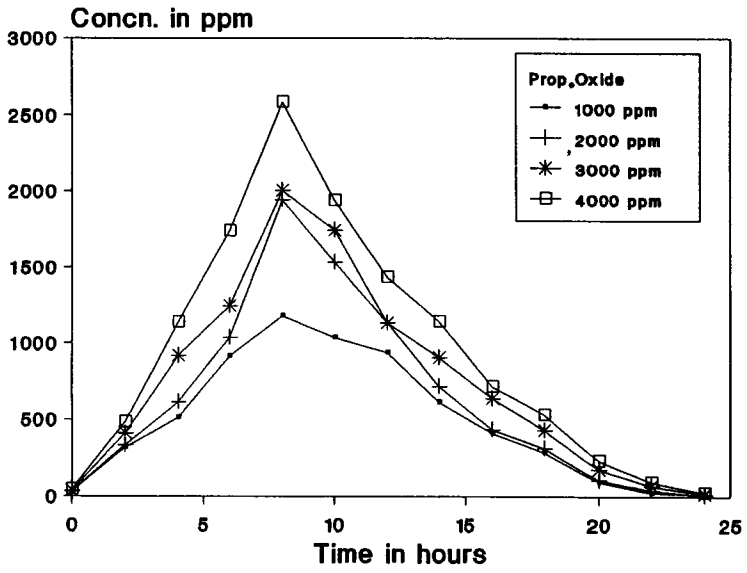


Fig. 6. Volatile acid profile by *Pseudomonas* and *Aerobacter*.

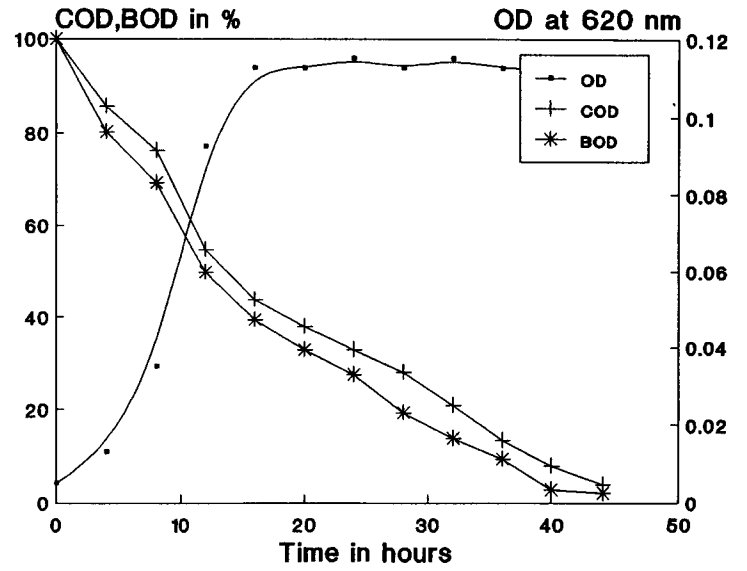


Fig. 7. Biodegradation of PG by glycol degrading bacteria.

the 90% COD reduction was observed only beyond 40 h in a 4% $CaCl_2$ solution. Studies are in progress to assay the relative concentration of *Pseudomonas* and *Aerobacter* during biodegradation process. A general trend of the COD/BOD reduction, microbial growth, volatile acids buildup, and pH reduction is shown in Figs. 7 and 8. Here, the BOD reduction followed the COD reduction in a parallel fashion, thus emphasizing the complete biodegradability of the PO/PG effuents. Further, the exponential growth phase of the mixed culture was complete in about 15–18 h.

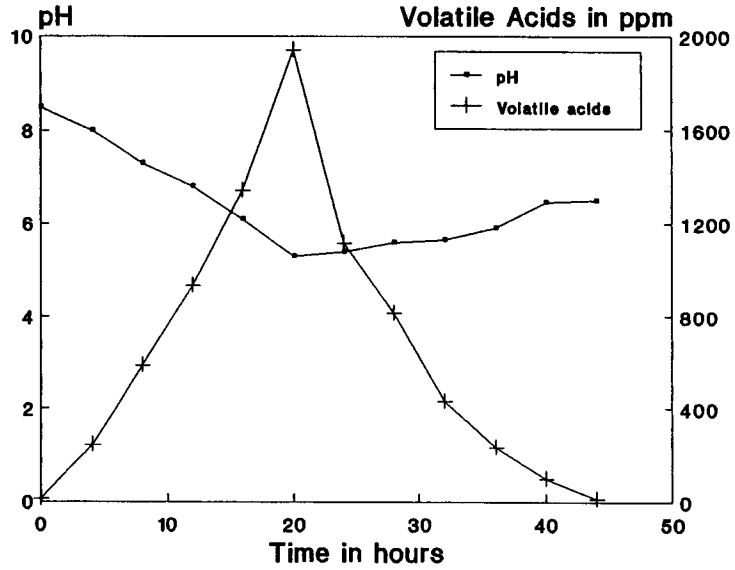


Fig. 8. Biodegradation of PG by glycol degrading bacteria.

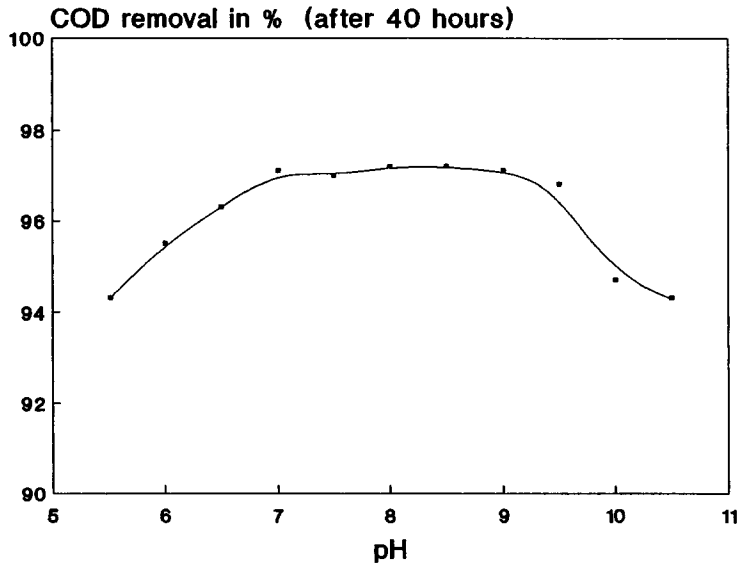


Fig. 9. Effect of pH on biodegradation of PG.

Effect of pH

Regarding the effect of initial pH on batch biodegradation of propylene glycol, it was clear from Fig. 9 that the COD removal after 40 h was nearly the same in the pH range of 6.5–9.5. Even in a wider range of 5.5–11.0, the COD reduction was nearly 94%.

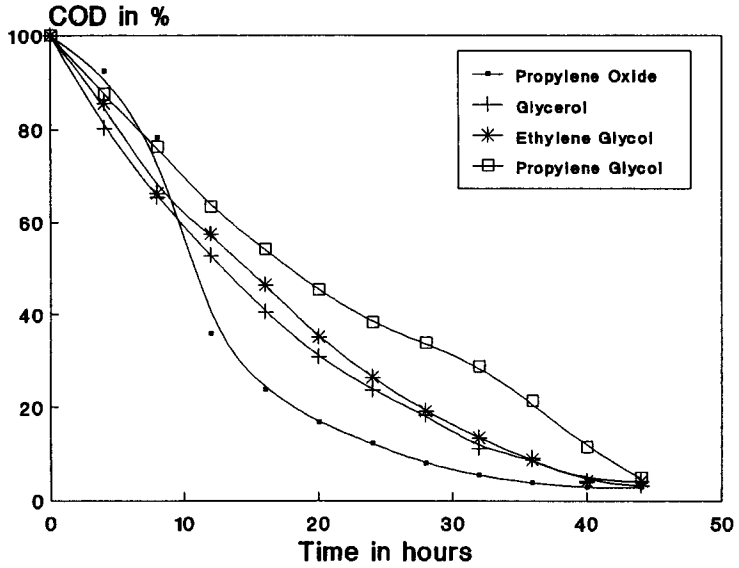


Fig. 10. Biodegradation profile with different substrates.

Effect of Temperature

Since the process has to operate at varying ambient temperatures, the batch biodegradation was followed at various temperatures in the range of 15–45°C. The optimum temperature for biodegradation was observed to be around 38°C. At lower temperatures, the degradation time got extended by about 10% of the one at optimum temperature.

Versatility of the Culture

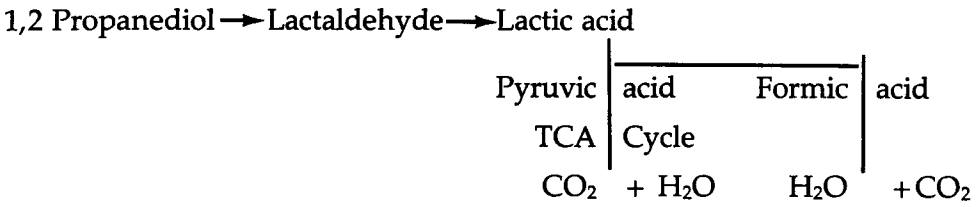
The versatility of the culture was checked by testing the biodegradation of different substrates, like propylene oxide, glycerol, ethylene glycol, and propylene glycol. The biodegradation profiles of these substrates are shown in Fig. 10. The biodegradation of propylene oxide appeared to be faster, followed by glycerol, ethylene glycol, and propylene glycol.

BIODEGRADATION PATHWAYS

Even though we had phenomenological reasons to believe that the biodegradation occurred through two broad stages, namely, buildup of carboxylic and hydroxycarboxylic acids, and subsequent decarboxylation, we wanted to confirm and ascertain, if possible, the identity of the acids accumulating at the end of the first stage.

The bacterium *Pseudomonas* was allowed to grow in 4% $CaCl_2$ effluent containing 5000 ppm of PG at room temperature, with mild agitation, for

12–15 h. The VA formation and the pH reduction were monitored continuously. The liquid chromatogram clearly showed the presence of lactic acid, pyruvic acid, and formic acid. Based on the above results, a possible metabolic pathway for propylene glycol degradation was considered, as below.



First, PG is converted to lactic acid via lactaldehyde. This lactic acid is further converted to pyruvic acid and formic acid. Since the cells require a lot of energy for decarboxylation and dehydrogenation, the pyruvic acid formed from lactic acid enters the TCA cycle, and ultimately, CO_2 and H_2O are released. Similar observations were also made by Yoshitake et al. (*9*) for soil bacterium, and by Huff and Rudhey (*10*) for rumen bacteria.

PROCESS REALIZATION

Subsequent to the batch studies, the biodegradation of glycol waters was carried out in continuously stirred tank reactors (CSTR) at two scaleup levels of 3.2 and 20 m^3. The residence time inside the reactor was maintained at 40 h, based on the batch biodegradation process. The determination of the biological pathways and our empirical reasoning gradually led us to a modified plug flow reactor in the form of multiple CSTRs in series. A study of reaction rate vs conversion on the lines of Levenspiel (*11*) and Atkinson (*12*) convinced us about the need for a minimum of five reactors in series. The methodology by which this configuration was arrived at, and the process modeling attempts, will be published separately. Subsequently, one more reactor was added to take care of the additional design load suggested by the client. A six CSTR setup with a residence time of 8 h/bioreactor was found economical to degrade 120 m^3/h of PO/PG effluent, containing an initial 2300 ppm of COD. A scaled down version of the final combination having a total reactor vol of 50 L has been continuously running for the past one and one-half years, and the characteristics of the effluent before and after the biotreatment are given in Table 1. The final process parameters, like the COD levels in each stage, the pH reduction in each stage, the residence time, and the nutrient loads, were established by extensive pilot plant runs.

Unique Features of the Process

The commercial plant to treat 120 m^3/h of PO/PG–Polyol effluent is now under construction at Manali Petrochemicals Ltd., Madras, India. The chosen process scheme consisting of removal of suspended solids,

Table 1
Physico-Chemical Properties of Glycol Effluent

	Untreated	Treated
1. Temperature	95°C	35-40°C
2. pH	11.2	5.8-7.0
3. Total dissolved solids	40000	40000
Calcium chloride %	3.87	3.87
Calcium carbonate %	1.2	< 100 ppm
Sodium carbonate %	0.08	0.08
Sodium chloride %	0.03	0.03
4. Total suspended solids (ppm)	7500	< 100
5. B O D (ppm)	1130	< 100
6. C O D (ppm)	2300	< 250
7. Dissolved Oxygen(ppm)	NIL	2-3

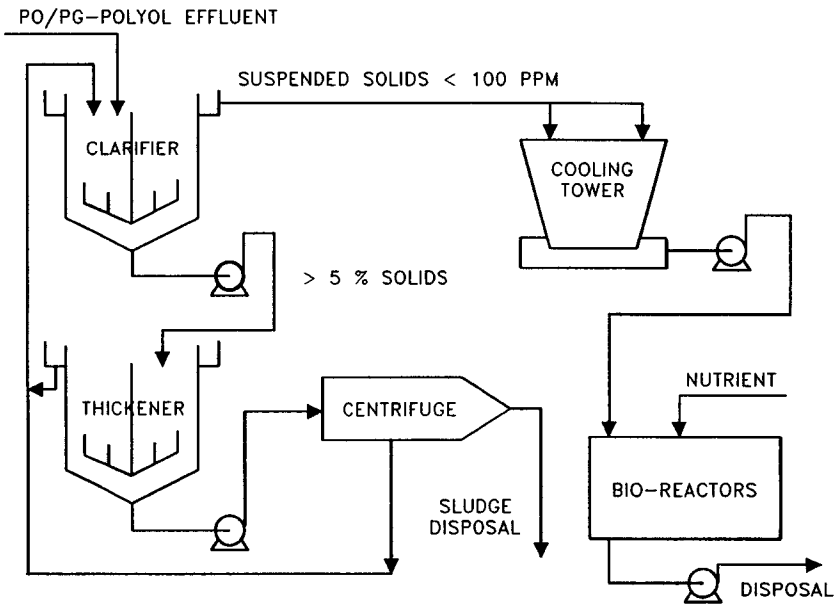


Fig. 11. SPIC glycol biotreatment process.

cooling of the effluent, biological treatment, treated effluent disposal, and mineral sludge removal, is shown in Fig. 11. The scheme as proposed is much simpler than the activated sludge process. The number of equipments are very few, and the floor area occupied is also small. The unique features of the process are

1. The process can withstand wide variations in pH.
2. There is no need for aeration and, hence, no sludge production.

3. The process can withstand chlorohydrocarons, like 1,2 dichloropropane, up to the level of 15 ppm.
4. The energy and chemical demands of this process are less, compared to activated sludge processes.
5. The process is quite amenable to easy and faster startups and shutdowns.
6. The capital cost of the process is only about 70% of that of the activated sludge process.
7. The operating cost of the commercial plant is expected to be about 50% of the activated sludge process.

CONCLUSION

With so much of development occurring in biotechnology, it is no longer necessary to continue with inherently inefficient (though reliable) activated sludge processes for saline glycol effluents. It is well possible to identify target organisms, enhance their biodegradation ability through modern techniques, and achieve pollution targets cost-effectively. We earnestly hope that our methodology of employing mixed cultures of specially developed target organisms to accomplish specific objects would be a welcome development in the effluent treatment field.

ACKNOWLEDGMENTS

The authors wish to thank P. Mohan, N. Shanmugam, and other colleagues for their continued assistance throughout the project period.

REFERENCES

1. Abele, L., Brochhagen, F. K., and Walber, U. (1984), Polyurethane Handbook, Gunter, Oertel, ed., Hanser Publishers, Munich, Vienna, New York, 599–603.
2. Noel, R. Krieg and Holt, J. G. (1984), Bergey's Manual of Systematic Bacteriology, Williams and Wilkins, Baltimore, MD.
3. Greenberg, A. E., Trussell, R. R., and Cleseeri, L. S., eds. (1985), *Standard Methods for the Examination of Water and Waste Water.* Amer. Pub. Health Assn., Washington, DC.
4. Baumann, F. J. (1974), *Anal. Chem.* **46,** 1337.
5. Burns, E. R. and Marshall, C. (1965), *Water Pollut. Control Fed.* **37,** 1716.
6. Cripps, J. M. and Jekins, D. A. (1964), *Anal. Chem.* **36,** 1240.
7. Dobbs, R. A. and Williams, R. J. (1963), *Anal. Chem.* **35,** 1064.

8. Woodring, S. L. and Clifford, D. A. (1980), *J. Water. Pollut. Control Fed.* **60,** 537.
9. Yoshitake, Tanaka, Fujii, K., Tanaka, A., and Fukui, S. (1975), *J. Ferment. Technol.* **53,** 354–362.
10. Huff, E. and Rudhey, H. (1959), *J. Biol. Chem.* **234,** 1060.
11. Levenspiel, O. (1969), *Chemcial Reaction Engineering,* Wiley Eastern, New Delhi, 150–152.
12. Atkinson, B. and Mavituna, F. (1983), Biochemical Engineering and Biotechnology Handbook, The Nature Press, USA, 620–624.