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Anaerobic biodegradation of trichloroethylene with the addition of sugar using activated carbon-fluidized beds

Huang, Suxuan, D.Eng.Sc.

New Jersey Institute of Technology, 1989

ANAEROBIC BIODEGRADATION OF TRICHLOROETHYLENE WITH THE

ADDITION OF SUGAR USING ACTIVATED CARBON FLUIDIZED BEDS

by

Suxuan Huang

Dissertation submitted to the Faculty of Graduate School of

the New Jersey Institute Of Technology

in partial fulfillment of the requirements for the degree

of Doctor of Engineering Science - Environmental Engineering

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APPROVAL SHEET

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Activated Carbon Fluidized Beds

Name of Candidate: Suxuan Huang
Doctor of Engineering Science, 1989

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ABSTRACT

Title of Thesis :   Anaerobic Biodegradation of Trichloroet-
                    hylene With the Addition of Sugar Using
                    Activated Carbon Fluidized Beds

Suxuan Huang, Doctor of Engineering Science, 1989

Thesis Directed by : Dr. Yeun C. Wu
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Anaerobic biodegradation of trichloroethylene (TCE) was carried out in a two-stage granular activated carbon fluidized bed bioreactor. The intermediate products were identified as: dichloroethylene (DCE), vinyl chloride (VC), 1,2-dichloroethane (DCA) and chloroethane (CA). Of the three geometric isomers of DCE, the trans-1,2 dichloroethylene (TDCE) was found to be the most predominant species. The production of DCA suggested a diverted reaction sequence from the conventional sequential reductive dechlorination pathway postulated in the past literature. CA was believed to be a product of VC and/or DCA. The co-substrate glucose was implicated for this reaction specificity. Based on our data and on other's work, a modified degradation pathway for TCE in anaerobic environment is postulated. The quantitative production of CA strongly implied a potential for complete mineralization of TCE under reductive conditions.

The rate constant, k, of TCE biodegradation in this

process was found to be 8.7 min^{-1}. The reaction kinetics resembled that of Michaelis-Menten model with the maximum rate, V_m and Michaelis-Menten constant, K_m, determined as 1.63 mg/L-min and 0.11 mg/L, respectively. The kinetic constants for TDCE conversion were: $k = 0.3$ min^{-1}, $V_m = 0.06$ mg/L-min and $K_m = 0.10$ mg/L.

The material balance performed on one stage of the system revealed that, in the range of influent TCE concentration of 0.02 - 4.64 mg/L, about 1% of the TCE introduced into the system volatilized to the reactor headspace, a maximum of 2% remained unaltered on the granular activated carbon (GAC) particles, and a maximum of 35% escaped biotransformation and eluted with the effluent of the first stage. Over 62% of the TCE was biotransformed to the DCEs, about 75% of which was further transformed to CA. The percent reduction of TCE from the aqueous phase ranged from 81 to 98% for the first stage and 98 to 100% for the entire system.

The optimum recycle rate was found to range from 750 to 850 ml/min. With a target effluent TCE concentration of below 20 ug/L and allowing a contact time of one minute, the nutrient feed rate must be < 4 ml/min. A glucose/TCE ratio of greater than 4 seemed to result in the early cleavage of double bond in TDCE and thus lead to the production of CA via DCA rather than VC.

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To my beloved mother, Yu-Lan Chuang

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CHAPTER I

INTRODUCTION

Groundwater is one of the most important sources of potable water in the United States. Although much smaller in volume compared to the oceans, groundwater represents the second largest reservoir of water in this country since it has a much larger volume than the polar ice caps, surface water and atmospheric water. In the United States, groundwater constitutes about 95% of all available fresh water. More than half of the population depends on groundwater as its primary source of drinking water and some 97% of rural domestic water supplies are obtained solely from groundwater [1]. As a result of the rapid economic and industrial growth, reliance on groundwater as source of water for drinking, for municipal, agricultural and industrial uses has intensified. In the period of 1950 through 1980, U. S. reliance on groundwater grew 2.35 times faster than the population [2].

Unfortunately, analyses of groundwater samples have shown that large portions of the subsurface aquifers in this country have been contaminated by organic compounds. A more recent survey conducted by the Environmental Protection Agency (EPA) has revealed that 22% of approximately 466 randomly sampled utilities that use groundwater have produced drinking water that has volatile organic compounds

(VOCs) at detectable levels [3]. Major concerns are associated with the haloaliphatic hydrocarbons containing from 1 to 3 carbon atoms because of their suspected carcinogenic or mutagenic nature [4, 5, 6, 7, 8]. These compounds are exclusively products of human activities and have found their ways to the groundwater as a result of accidental spills, leaks in distribution lines and aquifers, and inadequate waste disposal practices. They are highly resistant to biodegradation in aerobic environment and are therefore persistent in contaminated water [9, 10, 11]. Among the VOCs found in the groundwater, trichloroethylene (TCE) is the most frequently detected and at the highest level [12, 13]. In the groundwater near some industrial sites, concentration of TCE has been found to be as high as a thousand mg/L [14].

TCE is a major industrial solvent having a worldwide annual production of as high as 234,000 metric tons. It is used for degreasing and cleaning metal parts and electronic components. It has found wide application in industries such as metal finishing, battery manufacture, paint and ink formulator, etc. Therefore, it has been found to exist in the wastewater of 21 out of 32 industrial categories and subcategories analyzed for organic priority pollutants [15].

TCE is a colorless, nonflammable liquid. It has a molecular weight of 131, 81% of which being chlorine. Its

boiling point is $87^{\circ}C$ and its melting point is $-73^{\circ}C$. TCE is a volatile compound having a vapor pressure of 77 millimeters of mercury at room temperature. It has a low solubility of 1,100 micrograms per liter in water at $25^{\circ}C$. The molecular formula of TCE is $CHCl:CCl_2$ [16].

TCE has been tested positive in causing cancer in laboratory animals [17, 18, 19, 20]. It has also exhibited severe effects on the human nervous system, the lungs, the liver and the kidneys, leading to headache, dizziness, vertigo, nausea and vomitting, irregular heart beat, sleepiness, fatigue, blurred vision, and intoxication similar to that caused by alcohols. Most data concerning human absorption of TCE has been obtained with inhalation as the route of exposure. Central nervous system (CNS) depression has been observed for both short- and long-term exposure to TCE vapor. Progressive decline in human psychomotor function was recorded after an exposure to concentrations of TCE of 1 mg/L and higher for a period of less than three hours. Some epidemiological studies also revealed evidences of increased nervous system disorders in occupational exposures to TCE at levels below 0.5 mg/L for 5 to 15 years duration. Because TCE is suspected to be a human carcinogen, the recommended concentration of TCE in water for maximum protection of human health is 0 [21].

In view of the hazardous threat posed by TCE to human

health and the environment, various technologies have been developed in an effort to remove TCE from aqueous solutions. These technologies are based primarily on the physical and chemical properties of TCE. For example, the air stripping technique is endorsed based upon the high volatility of TCE. However, since subsurface aquifers are rarely in contact with the atmosphere, removal of TCE via volatilization or photolysis is unlikely. The current practice involves pumping the groundwater to the surface and stripping out the TCE in aeration towers or passing the groundwater through a column containing sorbent upon which TCE is adsorbed and separated from the water. Unfortunately, both these processes effect only a mere transfer of TCE from one environmental medium to another and thus convert a water pollution problem into air or soil pollution problem if no post treatment procedure is implemented. Chemical transformation has been attempted but is almost always prohibitively expensive.

Biodegradation is drawing considerable attention due to its potential of converting TCE into harmless compounds such as Cl^-, CO_2 and/or methane. The process can be carried out either aerobically or anaerobically. Anaerobic treatment, as opposed to aerobic treatment, has been found to have the following advantages: (1) A high degree of waste stabilization is possible, (2) Only a small portion of the waste is converted to new cells, which facilitates disposal proce-

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dure, (3) Low nutrient requirement, which is very important when one deals with industrial wastes which normally lack theses materials, (4) No oxygen required and, '(5) The end product methane is a useful source of fuel. On the other hand, the disadvantages of anaerobic processes include: (1) The need for relatively high temperature, (2) Dilute wastes may not produce methane in quantity sufficient to be of any significant value as a fuel, (3) Slow rate of growth of the methanogenic bacteria leads to a long acclimation period prior to start-up [22]. Nevertheless, for a properly designed and engineered process, maximum utilization of its advantages is possible and these advantages normally far outweigh the disadvantages. Moreover, many organohalogen compounds partition to and residue in anaerobic sediments. Therefore any process proven to be successful in treating aqueous solutions contaminated with organohalogen compounds anaerobically will likely be implementable in the in-situ remediation of groundwater.

Earlier survey on the biodegradation of TCE in groundwater showed that halogenated hydrocarbons such as TCE is non-biodegradable within the concentration range of 10 to 100 ug/L in unsaturated (aerobic) condition but may be degraded anaerobically [23, 24]. A study conducted under both aerobic and anaerobic conditions also indicated that at TCE levels of 10 to 200 ug/L, biodegradation occured to some extent anaerobically but not aerobically [25, 26]. TCE was

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found to be degraded aerobically only under special condition such as in the presence of methane gas or some aromatic compounds, e.g., phenol [27, 28]. Hence, unless 'methanogenic bacteria are also present in situ, which is unlikely because methanogenic bacteria are anaerobes, provision of methane to subsurface aquifers will constitute additional operating costs. In the second case, since aromatic compounds such as phenol are also toxic, their use as induction agents for TCE biodegradation is not considered recommendable.

On the other hand, past analyses of the composition of contaminated groundwater revealed that anaerobic biodegradation of TCE in subsurface was rarely complete. The proposed pathways all pointed to a sequential reductive dechlorination of TECE or TCE, which resulted in the production of VC: TECE --- TCE --- DCEs --- VC [29, 30]. VC was considered equally or even more dangerous to human health and the environment, in view of its tumor-causing and recalcitrant nature [31]. This phenomenon has precluded the endorsement of anaerobic biodegradation as an alternative treatment process for TCE. However, recent developments in this area have demonstrated that TECE, the precursor of TCE, is partially mineralized to CO_2 in a continuous-flow fixed film methanogenic column [32]. Separate studies have also shown that VC can be further degraded anaerobically to chloroethane, which is readily transformed to nonvolatile compounds

[33, 34]. Our preliminary investigation on the anaerobic biodegradation of TCE carried out in the same fluidized-bed bioreactor system as the present study revealed that mechanism other than, but paralled, the sequential dechlorination of TCE had taken place, which resulted in the production of the more readily degradable DCA rather than VC [35]. To the author's knowledge, this is the first report implicating DCA as an integral component in the anaerobic biodegradation pathway of TCE with supporting experimental evidences. This strongly suggests the possibility of attaining complete mineralization of TCE under reductive conditions.

Until now, opinion among workers as to the ultimate fate of TCE in these completely different environments is still very much divided. Nonetheless, potential exists for the complete mineralization of TCE under anaerobic conditions. This must not be undermined because it constitutes a great leap forward in human endeavour to develop a feasible alternate process for contaminated groundwater remediation. The key word is "anaerobic", a condition most likely to prevail in field sites because subsurface aquifers are rarely aerobic. Furthermore, anaerobic processes need no supply of molecular oxygen, which constitutes a great savings on both capital and operating investments.

Due to the hydrophobocity and low solubility of TCE in water, it is very difficult to render TCE available to the

microbial population for biodegradation. One way of solving this problem is to find a medium where both the microorganisms and TCE come in contact with each other and carry out the desired biological transformation reaction. Activated carbon, being porous, possesses a tremendous surface area suitable for concentrating large amount of microbial population [36, 37]. The high affinity between activated carbon and many priority pollutants such as TCE, VC, DCEs, etc. and the long retention time activated carbon can afford for gradual biodegradation of refractory compounds has been well documented [38, 39, 40, 41]. Fluidization of activated carbon is expected to bring about marked enhancement in the mass transfer characteristics of the process. Fluidized-bed reactors have found wide application including wastewater treatment processes because its numerous interesting features such as the mobility of the particles, reduced risk of blockage, and small pressure drop [42]. Fluidized bed bioreactors (FBBR) have been successfully applied in many water and wastewater treatment processes [43, 44, 45, 46, 47]. Advantages of the FBBR include: (1) Steady reactor biomass hold-up, which paves the way to steady performance characteristics, (2) High support surface area per unit reactor volume, (3) The existence of a uniform shear field for adequate control over the biomass associated with individual particle, (4) Operation with either steady or unsteady particle biomass hold-up is possible, (5) Continuous

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removal and return of particles without disturbance of the attached biomass is possibles, and (6) Minimum hardware and operator involvement in attaining all the above conditions [48]. While use of activated carbon may sound expensive because of its relatively short service life and regeneration process is needed to compensate for it, growth of microorganisms on its surface has a potential of eliminating this step through the process of bioregeneration [49].

Many organic compounds that are very resistant to biodegradation have been found to be metabolized eventually by microorganisms when another readily utilizable substrate is present in the medium [50]. This phenomenon is termed "co-metabolism". Glucose, being one of the simplest carbohydrates [51], has been shown to enhance the biodegradation of many recalcitrant compounds, including TCE [14, 52]. In this study, glucose was employed as the co-substrate in the full-scale activated carbon fluidized bed bioreactor system for the anaerobic biodegradation of TCE. The proposed process is a recent innovation intended to combine the attractive features rendered by FBBR, GAC, co-metabolism with glucose and anaerobic biotransformation for the ultimate mineralization of TCE. The achievement of this goal is dictated by the knowledge associated with the fundamental aspects of the process. The objectives of this study, therefore, include: (1) To determine the effects of operating variables, viz., TCE influent concentration, nutrient pumping rate, recircu-

lation rate, loading and glucose/TCE ratio on the process performance; (2) To evaluate the relative distribution of TCE and the other chlorinated ethenes in the various phases of the system; (3) To assess the possibility of attaining complete mineralization of TCE in an anaerobic fluidized bed bioreactor using glucose as the co-substrate; (4) To be able to appreciate the roles played by glucose and activated carbon in the process; and (5) To derive the pertinent kinetic parameters and a generalized correlation that will be useful for future design purposes.

CHAPTER II

BACKGROUND INFORMATION

Literature Review

Several technologies have been demonstrated to be capable of removing TCE from contaminated surface and/or groundwaters. They include air stripping, activated carbon adsorption, reverse osmosis, incineration, and biodegradation. Developments in these technologies are summarized below.

1. Air Stripping

The Air stripping technique has been conventionally used in water and wastewater treatment plant for the removal of contaminants such as ammonia, carbon dioxide, and hydrogen sulfide. Application of air stripping for the removal of TCE from drinking water is a rather recent innovation. The influent water is introduced at the top of a cylindrical tower filled with plastic or ceramic packing and cascades down through the void spaces of the packing. Air is blown countercurrently from the bottom of the tower and contacted with the thin film of contaminated water on the surface of the packing. This contact transfers the TCE from the aqueous solution to the air. This process relies on the high volatility and hydrophobic characteristics of TCE in bringing about the physical separation of TCE from the contaminated water [53].

The major drawback of this process is the possibility of polluting the atmosphere if the volume and concentration of TCE in the air discharge are large enough to constitute a potential hazard to the environment. In this case, post treatment of the air must be carried out prior to discharge. Two methods are currently applicable: One is to pass the contaminated air through a granular activated carbon (GAC) unit to effect the transfer of TCE from the air to the GAC particles through adsorption. With this combined aeration and GAC process, the TCE-contaminated groundwater of Rockaway, New Jersey has been rendered safe for residential consumption [54]. The second method is to use steam instead of air as the stripping medium. The steam removes the TCE from the water in exactly the same manner as the air in the air stripping column, but is subsequently cooled down prior to discharge. Thus TCE is removed from the air discharge via condensation with the steam.

Cost analysis of the process, without consideration of any post treatment steps, has indicated that the treatment cost increases almost linearly as the temperature decreases and the overall treatment cost is relatively insensitive to changes in the operating variables in the vicinity of the optimum region. In other words, there is not much room left for manipulation of operating variables in order to enhance process performance [55]. Therefore, air stripping as an alternative process for the removal of TCE from contaminated

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waters is technically possible but treatment cost is quite high.

2. Activated Carbon Adsorption

In the past, attention had been focused on demonstrating the effectiveness of GAC in treating surface water [56]. More recently, research projects have turned to aspects such as the feasibility of implementing GAC for the removal of VOCs and SOCs (synthetic organic chemicals) from groundwater.

TCE has been found to be readily adsorbed unto activated carbon due to its low solubility in water and its high affinity towards activated carbon [51, 57, 58, 59]. Disadvantages associated with this process include: (1) the requirement of a pretreatment step such as aeration and/or solid separation of the influent water, (2) an online monitoring system for the control of the operating paremeters is necessary to prevent breakthrough incidences, (3) thermal regeneration of carbon is not only expensive but also presents potential hazard such as the emission of toxic substances like dioxins [60]. Therefore, the GAC adsorption process cannot be considered cost-effective unless it is used in conjunction with biological treatment so that the GAC can be regenerated by biolgical rather than thermal means.

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3. Reverse Osmosis (RO)

This technology uses a thin film composite membrane for the separation of TCE from water. TCE is initially sorbed onto the membrane and then escapes from the membrane as the contact time increases. Experimental results have indicated that this technology is capable of rejecting over 99% of some of the volatile organic compounds in distilled water. For contaminated groundwater, the water is filtered prior to passage through the RO units. It has been found that the unit removes 99, 97, and 78% of the TCE in the tested water at contact times of one, five, and thirteen hours, respectively. Further work has to be done to properly assess the feasibility of implementing this technology as an alternative process for the elimination of TCE [61].

4. Chemical Degradation

Ozone oxidation is by far the most popular among the chemical means used in the destruction of VOCs. It is currently being studied extensively in DWRD's (Drinking Water Research Division of EPA) in-house pilot-plant facilities. The removal efficiency for alkenes was found to improve with increased ozone dosage [56].

5. Radiation Treatment

Ultraviolet light also shows some promise in removing some organic contaminants, particularly when used in conjunction with ozone or hydrogen peroxide. A research project

is now underway at Los Angeles Department of Water and Power, which has been designed to study the water-phase oxidation of VOCs and SOCs to CO_2 and water. Another research project funded by the American Water Works Association Research Foundation intends to evaluate the gas-phase oxidation of VOCs using the same technique [56]. Gehringer et. al. [62] used a -radiation to induce degradation of trace amounts of TCE (70 - 440 ug/L) in drinking water had been investigated. The cost has been estimated as about US $0.20 per cubic meter of water treated.

6.   Catalytic Photolysis

TCE was reduced to methane and/or ethane in a water photolysis system where solar energy was used to excite the electron-transfer reaction resulting in the cleavage of water to H_2 and OH^-. This reaction was catalyzed by superfine colloidal platinum [63].

7.   Biodegradation

Aerobic Biodegradation: Experimental results obtained for aerobic degradation of halogenated ethenes have been rather conflicting. While several research groups found no evidence of aerobic biodegradation of these compounds [11, 23, 61], Tabak et. al. [65] claimed the removal of over 50% of di-, tri-, and tetrachloroethene at an initial concentration of 10 mg/L in 1 week by a sewage inoculum which utilized yeast extract as the primary substrate.   In 1985,

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Wilson and Wilson [27] reported that TCE was aerobically biodegraded to CO_2 by methanotrophs, the growth of which had been promoted by exposing unsaturated soil microflora to natural gas. The results showed that TCE at an average concentration of 150 ug/L was lowered by about one order of magnitude within a two-day residence time. This is in great contrast with the results they obtained in their earlier work where no biodegradation occurred in the same soil but without prior exposure to natural gas.

A separate work done by Fogel [66] also postulated the involvement of methanotrophs in the aerobic biodegradation of TCE [66]. The inoculum, designated CL-M, was a methane-utilizing culture isolated from a lake sediment. After two days of incubation, about 23% of the labelled TCE had appeared as $^{14}CO_2$, 34% was associated with the bacteria, and the rest remained in solution as nonvolatile or nonchlorinated species. The rate of TCE degradation was determined as 2×10^{-6} umol/hr-ug protein. Moreover, of the six chloroethenes tested, VC was found to be the most readily degraded. Thus, in the order of decending ease of degradation: VC, 1,2-DCE, Vinylidene chloride, TCE, and TECE. In fact, TECE was not degraded at all under the experimental conditions employed.

A pure culture capable of metabolizing TCE under aerobic conditions was first isolated by Nelson et. al. [67] and

was designated strain G4. The physiochemical and morphological features of this strain G4 suggested that the isolate belong to the genus Acinetobacter. It was also observed that although the isolate could grow on substrates such as glucose, succinate, acetate and ethanol, TCE degradation was largely impaired when these were used in place of the water obtained from an industrial waste treatment facility in the contaminated site. This implies that a certain constituent present in the water may act as an inducer for the synthesis of the enzyme capable of degrading TCE. Furthermore, no growth occurred with methane or methanol, indicating that the microorganism involved is not a methanotroph.

In their subsequent study, Nelson et. al. [68] identified phenol as the constituent in the water from which strain G4 was isolated that was responsible for the complete biodegradation of TCE . It was then suggested that the degradation of TCE by strain G4 followed an aromatic degradative pathway involving a meta fission inducible by aromatic compounds such as phenol and toluene. In their experiments, strain G4 degraded almost all detectable TCE when phenol was included in the incubation mixture. However, when a protein inhibitor such as chloramphenacol was added, TCE degradation was inhibited. On the other hand, cells that were preinduced with phenol degraded TCE as well even in the presence of chloramphenacol and the omission of phenol from the mixture. Moreover, stoichiometric amounts

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of chloride ions (2.7 - 2.9 Cl$^-$/TCE molecule) were produced, implicating dechlorination as the key mechanism in the bio-degradation of TCE.

The involvement of an aromatic degradative pathway in the aerobic cometabolism of TCE was further demonstrated by carrying out phenol induction on natural microflora of three different environmental water samples, namely, estuarine, river and groundwater [28]. It was noted that TCE degradation was greatest when groundwater samples were enriched for phenol degraders prior to exposure to TCE. In addition to this, laboratory strains capable of degrading aromatic compounds such as naphthalein, biphenyl, phenol and toluene were also tested for TCE degradative activity. It was then postulated that during the induction period, the microorganisms were stimulated to produce three active enzymes: toluene dioxygenase, cis-dihydrodiol dehydrogenase and catechol-2,3-dioxygenase. The results showed that microorganisms which were deficient in toluene dioxygenase could not degrade TCE. This strongly implicates toluene dioxygenase as the active enzyme which plays the vital role in the aerobic degradation of TCE.

Recently, Wackett and Gibson [69] explicitly demonstrated the involvement of toluene dioxygenase as the common enzyme responsible for the aerobic degradation of TCE. With toluene-induced cells of Pseudomonas putida F1, TCE was

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removed from the growth media at a significantly higher initial rate than the methanotroph and other mixed cultures. In order to gain an insight as to the degradative potential of P. putida F1 and the range of chlorinated olefins that could be oxidized by toluene dioxygenase, ethylene and some chlorinated ethenes were employed as growth substrates. Cis-1,2-DCE was degraded at a rate comparable to that of TCE. The other two isomeric DCEs were degraded in considerably lower rates. TECE and VC were not degraded at all. The negative result for VC degradation is in great contrast with the observation of other investigators concerning aerobic biodegradation of VC, i.e., having the least number of chlorine, VC is oxidized at the highest rate among the chlorinated ethenes.

Contrary to some earlier investigations, Fliermans et. al. [14] were able to show that over 99% degradation of TCE could occur in unsaturated (aerobic) subsurface sediments. The microbial consortia were heterotrophs which utilized a variety of energy sources including tryptone-yeast extract, methanol, methane, and propane. Of the energy sources tested, methane was found to impart slow but effective stimulation of TCE degraders. Glucose enrichment cultures grew rapidly but lost their TCE-degrading activity within a couple of transfers. The optimum temperature for growth of the enrichments and subsequent TCE consumption ranged from 22 to

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37°C and the optimum pH ranged from 7.0 to 8.1. The major end products of the degradation process were hydrochloric acid and carbon dioxide. The minor products were chlorinated organic compounds such as DCE, VC and chloroform, which constituted less than 10% of the TCE loss. An incubation period of at least two weeks was required for observable TCE utilization. The upper limit of TCE concentration degradable by the enrichments was found to be approximately 300 mg/L. Experimental evidences also suggested that the TCE degradation observed in the study was associated with cometabolism rather than microbial growth or energy production. Furthermore, it was found that substrates for promotion of other microbial growth were essential for appreciable TCE degradation, implying possible symbiosis between different species.

Another pure culture capable of utilizing TCE as carbon source under aerobic conditions was isolated from soil and water collected from a landfill sites with historical contamination of industrial 1,2-DCA and 1,2-dichloropropane by Vandenbergh and Kunka [52]. The isolate was a gram-negative, oxidase positive, motile, rod-shaped bacterium. It was identified as Pseudomonas fluorescens and designated PFL12. Biodegradation of TCE by PFL12 was not significant; for with an initial concentration of 100 mg/L, only 2 mg/L was degraded after 24 hours of incubation.

Anaerobic Biodegradation:

In 1980, field data obtained from the Palo Alto groundwater recharge project provided evidences of long-term biodegradation of some halogenated organics. In an attempt to confirm these findings, Bouwer el. al. [25] carried out both aerobic and anaerobic biodegradability studies of several chlorinated aliphatics at low concentration (10 - 200 ug/L), including TCE. It was found that under aerobic conditions none of the compounds investigated was biodegraded. However, under anaerobic conditions, TCE was found to be slightly degraded after an incubation period of 16 weeks. In their subsequent study, Bouwer and McCarty claimed that anaerobic biodegradation of TECE and TCE occurred only during vigorous methanogenesis supported by growth of acetate-utilizing microorganisms [26]. This strongly suggested a co-metabolism process.

A report by Parsons et. al. [29] about the appearance of compounds such as cis- and trans-1,2 dichloroethylene, and vinyl chloride in well water initially contaminated by tetrachloroethylene (TECE) spills but not by any of the aforementioned compounds has alarmed reaserchers in this area on the possibility of producing vinyl chloride as the result of incomplete reductive dehalogenation of TECE. Although not explicitly stated in their report, it was implied that the TCE originally present as an impurity in the TECE used in their study was biotransformed in a similar way as

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the TECE, thus yielding VC as the end product of degrada-
tion. In order to prove this, Kleopfer et. al. [70] used
TCE with single atom ^{13}C labelling technique to monitor the
degradation process on soils collected from a TCE spill site
in Des Moines. Their results showed that DCE, which was not
present initially, increased in amount gradually with time.
This confirmed that TCE indeed underwent biotransformation
to DCE under anaerobic conditions. Under this context, they
predicted the inevitable formation of VC as the product of
the reductive dehalogenation process.

The kinetics of the depletion of TCE under different
kinds of environmental conditions found in aquifers was
first investigated by Barrio-Lage et. al. [71]. It was
noted that the depletion followed non-linear forms of the
Michaelis-Menten kinetics in highly organic sediments but
followed a linear form in calcareous sedimentary rocks. The
K_m values obtained for the depletion of TCE, as well as
those for 1,1-DCE, cis- and trans-DCE [72] showed a strong
correlation between the values of K_m and the percent total
organic carbon content of the sediments. The smallest first
order rate constant found for the rock sediment was attri-
buted to the fact that it contained the least microbial
biomass and organic carbon.

The question still remains whether VC is the final end
product of the anaerobic biotransformation of TCE or, fur-

ther degradation of VC can take place and thus leads to the complete mineralization of TCE. More recent work by Vogel and McCarty [32] on the biotransformation of TECE under methanogenic environment has clearly demonstrated that TCE and VC are the major intermediates in TECE biotransformation and 24% of the TECE has been mineralized to CO_2 in a continuous-flow fixed-film column.

Wilson et. al. [74] also examined the anaerobic biotransformations of selected halogenated aliphatic hydrocarbons in microcosms constructed with authentic aquifer material which received municipal landfill leachate under methanogenic conditions. It was found out that long lag time was required before significant degradation occurred for TCE, 1,1-DCE and TDCE. At least 16 weeks of incubation was needed before complete disappearance of these compounds was noted. VC was identified as the daughter product of 1,1-DCE, TDCE and TCE. However, it was also observed that the parent compounds did not transform quantitatively into their daughter products; only trace amounts of VC were detected at the end of the experiment. This was in great contrast to the accumulation of VC reported by Vogel et. al. in their acetate-driven reactors [73]. Therefore, in their conclusion, Wilson et. al. indicated that although anaerobic biodegradation of chloroalkenes had indeed led to the production of more hazardous, more mobile, and more recalcitrant

compounds, sequential reductive dechlorination of chloroalkenes need not result in the accumulation of the products and reduction could be rapid and extensive once activity began. Moreover, the lag times could be long from the experimental point of view but might be short when compared to the residence time of the pollutants in the subsurface [74].

Fathepure et. al. [75] tested several species of anaerobes in an attempt to identify the specific bacteria that were responsible for the reductive dehalogenation of TECE. The anaerobes included four types of acetate-utilizing methanogens, two types of Clostridium, a dechlorinating bacterium (designated DCB-1), a benzoate oxidizer (BZ-2), and a Desulfovibrio desulfuricans. The highest rate of dechlorination of TECE was attained using DCB-1, followed by Methanosarcina sp. and M. mazei. No significant dechlorination of TECE was observed with the other cultures tested. In their subsequent experiments, they used several dechlorinating methanogenic enrichments, which were obtained from an anaerobic sewage sludge and grown on various types of chlorinated compounds. The chlorophenol and chlorobenzoate enrichments showed the maximum dechlorination of TECE to TCE. Furthermore, a much faster rate of TECE reduction was associated with the consortium having mixed cultures of DCB-1, benzoate degrader (BZ-2) and Methanospirillium sp. strain PM-1. Because the rates of transformation decreased as

chlorine was removed in anaerobic condition but increased in aerobic environment, they suggested a two-stage process consisting of an anaerobic reductive dechlorination followed by aerobic oxidation for decontaminating water containing complex mixtures of C_1 and C_2 chlorinated ethenes such as TECE and TCE.

The partial mineralization of TCE to CO_2 under anaerobic conditions [32] and the biotransformation of VC to CA [72, 33] aroused the speculation that the process involved not only methanogenic bacteria but also other microbial species capable of reductive dechlorination. Baek and Jaffe [33] conducted a series of experiments desinged to unveil the roles played by the methanogens, as well as the non-methanogenic bacteria in effecting the anaerobic biotransformation of TCE. Their results showed that in the media containing only non-methanogenic fermenters or methanogens, depletion of TCE was not significantly different from autoclaved samples. The production of chlorinated intermediates such as DCE, VC and CA, as well as methane, was minimal in both cultures. However, when the two cultures were mixed together, degradation of TCE was markedly enhanced and dramatic increase in the production of CA and methane was registered. This phenomenon was attributed to the possible symbiosis between the fermenters and methanogens: In the fermentation process, the fermenters oxidized a reduced

electron carrier such as $NADH_2$ to NAD and H_2. These H_2 were utilized by the methanogens as eletron donors. The process was termed "interspecies hydrogen transfer". Moreover, the production of considerable amounts of VC and CA in samples containing the mixed culture led them to hypothesize that the fermenters made use of the carbon-carbon double bond of VC in inserting the H_2 from the $NADH_2$ during the fermentative metabolism, thus reducing VC to CA. The important roles of the non-methanogenic fermenters were thus seen to be of two facets: (1) supplying hydrogens for methanogenesis and (2) saturating the carbon-carbon double bond of VC. Since CA had been found to be relatively easier to mineralize [76], the results of this investigation further proved the existence of the possible complete mineralization of TCE.

Principle and Theory

In a fluidized bed reactors, the hydrodynamic behavior of the solid particles is very much dependent on the velocity of the fluid through the column. At very low velocities the particles are not disturbed so that the fixed bed behavior prevails. At the other extreme, however, the velocity is so high that particle entrainment occurs. In this condition the system operates essentially as a transport reactor. Most fluidized bed catalytic reactors operate in the bubbling regime, where the fluid flows with intermediate velo-

city. [77]

Due to the mixing in the bubbling regime and to the high specific transfer area associated with the small particles, fluidized beds can be operated nearly isothermally. Therefore, the temperature and concentration differences between the fluid and the particle surface are usually small. As a consequence, external temperature and concentration gradients can be neglected in the design of fluidized-bed reactors, i. e., the global reaction rate is the same as the intrinsic rate, evaluated at bulk values of the temperature and concentration. Chu et. al. [77] has summarized the heat and mass transfer characteristics in such a reactor with the following correlation for the range $30 < d_p G/u(1 - e_B) < 5,000$:

$$j_D \text{ or } j_H = 1.77 \ [d_p \ G/(u)(1 - e_B)]^{-0.44}$$

where e_B = void fraction of the bed, G = superficial mass velocity, d_p = average particle diameter. j_D and j_H are defined as follows:

$$j_D = [k_m \ d/G] \ [a_m/a_t] \ [u/d \ D]^{2/3}$$

where k_m = mass transfer coefficient based on a unit of transfer surface, i. e., a unit of external area of the catalyst particle; a_m = external area per unit mass; d = density of fluid; a_t = total surface area; u = absolute viscosity of fluid; D = molecular diffusivity of component

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being transfered.

An FBBR operates in much the same way as a heterogenous catalytic fluidized bed reactor: The immmobilized biomass (attached microorganisms) provides the enzyme that catalyzes the biochemical reaction in a way analogous to what a chemical catalyst does in ordinary heterogenous catalytic reactions. Therefore the compendium of principles and correlations employed in the analysis and modelling of a heterogenous catalytic reactor is equally applicable in the simulation of an FBBR.

The formulation of a rate equation for the conversion of the reactants into products in a heterogenous process must include the following sequence of steps: (1) Transfer of reactants from the bulk fluid to the fluid-solid interface (external surface of the solid particle). This step is characterized by mass transfer through a liquid film governed by Fick's law of diffusion, (2) Intraparticle transport of reactants within the solid particle, if the particle is porous, (3) Adsorption of reactants at the active sites, (4) Conversion of adsorbed reactants to products (surface reaction, i. e., the intrinsic chemical step), (5) Desorption of adsorbed products, (6) Transport of products from the interior sites to the outer surface of the solid particle, and (7) Transport of products from the fluid-solid interface into the bulk fluid stream. At steady state the rates of

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all the individual steps will be the same.

It is obvious from the foregoing discussion that, unless simplifying assumptions are made, a complete simulation of an FBBR requires evaluation of all the rate constants involved in the entire transport and reaction process. In view of this complexity and the limitation of the present knowledge about FBBR, Atkinson et. al. [48] derived a pseudo-analytical equation based on the theory of chemostat, relating performance with the properties of the microbe-substrate system, the extent of the surface films, and the flow rates. According to the theory, the conversion efficiency of a completely mixed FBBR with steady particle biomass hold-up depends upon the following dimensionless parameters: (1) A dimensionless detention time parameter, $F/V \, G_{max}$; (2) A dimensionless bed biomass hold-up parameter, $d_b \, L \, A_s/Y \, K_m$; (3) A dimensionless inlet concentration, C_I/K_m ; and (4) A dimensionless parameter, $d_b \, G_{max}/Y \, K_m \, D_e$, which characterizes the diffusion limitation within the biomass film, where F = volumetric flow rate, V = reactor volume, L = biofilm thickness, A_s = support surface area per unit reactor volume, C_I = inlet concentration, G_{max} = maximum specific growth rate, d_b = biomass density, Y = yield coefficient, K_m = Monod coefficient, D_e = effective diffusion coefficient within the biomass. Thus for a given reaction, i.e., when G_{max}, K_m, Y and D_e are known, the process effi-

ciency,

$$C_o/C_I = f (F/V, d_b LA_s, C_I, L) \quad ,$$

and the corresponding volumetric rate of reaction, R_v is given by:

$$R_v/G_{max} K_m = (F/V \; G_{max}) \; (C_I/K_m) \; (1 - C_o/C_I)$$

CHAPTER III

EXPERIMENTAL

Experimental Set-Up And Operating Conditions

There were two identical anaerobic biological systems in operation simultaneously: System 1 being fed at a rate of 4 to about 18 ml/min while System 2 at half these rates. Each system, as depicted in Figure 1, consisted of two columns, designated Stage 1 and Stage 2, mounted with a difference in elevation of about two feet. Each column was five feet tall with an inside diameter of two inches and was maintained at about 35°C by a heating tape. Because of the difference in the elevation of the two columns, the effluent of the first stage flowed freely to the bottom of the second stage. The effluent from the second stage was pumped back to the bottom of the first stage at a rate of approximately 0.81 liters per minute to maintain activated carbon fluidization in each column. This had resulted in a hydraulic retention time of about 3.8 minutes per column per pass. These value were selected based on the hydrodynamic tests perfomed prior to actual operation. See subsequent section on hydrodynamic characteristics.

The activated carbon media were Filtrasorb 400 from Calgon Carbon Corporation (about 325 grams/column) supported on a 1-1/2 inch layer of 6 mm glass beads followed by an

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1 - Recycle
2 - Sampling Port
3 - Gas Line
4 - Activated Carbon
5 - 6-mm Glass Beads
6 - 18-mm Glass Marbles
7 - Pump
8 - Nutrient Feed
9 - Overflow
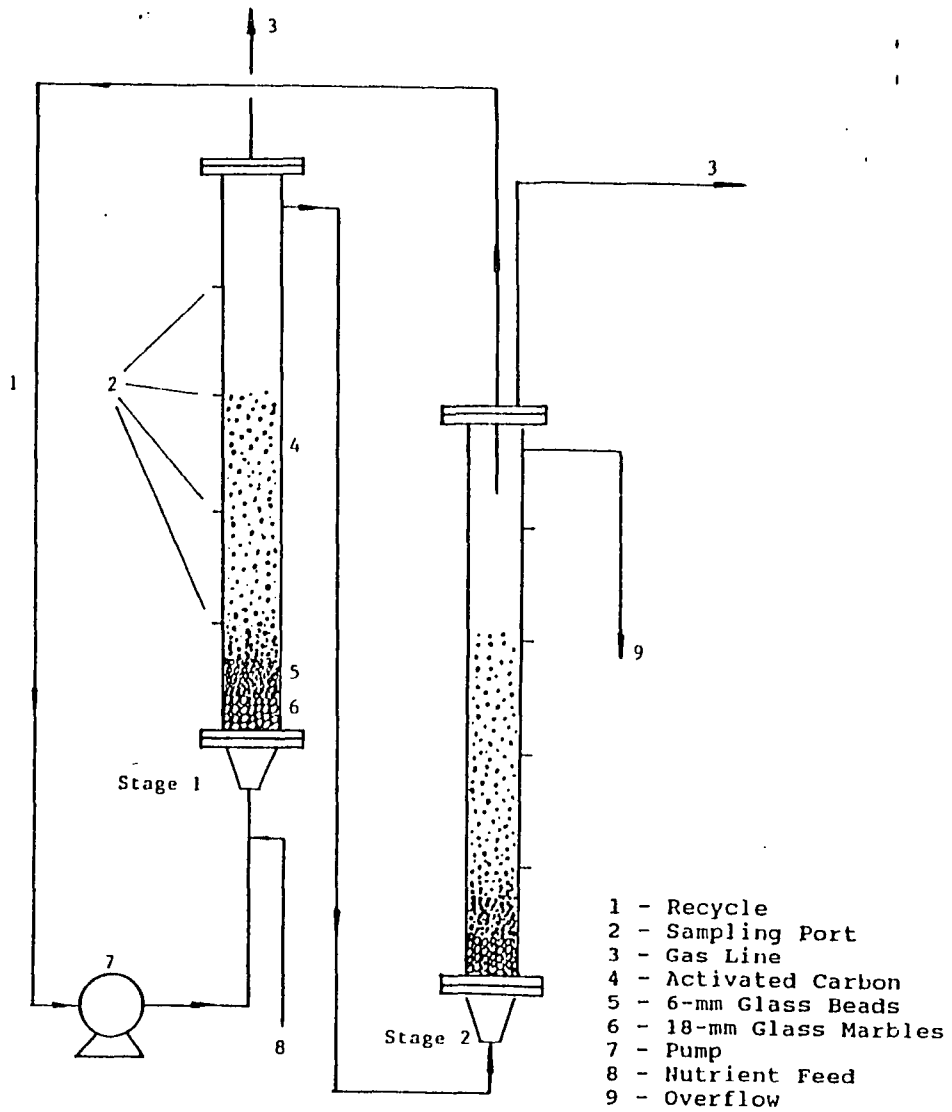
Figure 1:   Schematic   Flow Diagram of the   Two-Stage
        Anaerobic Fluidized Bed Biofilm Reactor

inch layer of 18 mm glass marbles. The initial bed height
was maintained at about 0.33 m. Since the expanded bed
height was approximately 28% [78] of the total column
height, the effective contact time in the column was only 1
minute. The systems were inoculated with a mixed microbial
culture taken from an anaerobic digester and acclimated to a
defined synthetic wastewater as the influent feed. During
the period of two-month acclimation, a layer of immobilized
biomass was established on the surface of the activated
carbon particle. This caused a change of color of the GAC
particles from black to gray, as observed visually. The
variables investigated in the present study included the
following:

Table 1: Scope of Study --- Variables Tested

Variable	Value
Initial TCE Concentration (Feed Tank)	0.3 - 480 mg/L
Infulent TCE Concentration (Stage 1)	0.02 - 2.3 mg/L
Nutrient Feed Rate	1.8 - 30.6 ml/min
Recycle Rate	666 - 921 ml/min
Glucose/TCE Ratio	0.63 - 1000

At least a 2-week period was allocated for each change of
concentration.

The composition of the stock solution used for the preparation of the defined media is listed in Table 2. Trace amounts of $MnCl_2 \cdot 4H_2O$, $CoCl_2 \cdot 6H_2O$, H_3BO_3, $CuCl_2 \cdot 2H_2O$, $Na_2MoO_4 \cdot H_2O$, and $ZnCl_2$ were also added. The stock solution of TCE was prepared by dissolving reagent grade (99.99% pure) TCE in reagent grade methanol. In all experiments, the glucose concentration was fixed at 300 mg/L.

Table 2:   The Composition of the Stock Solution for
the Defined Medium

Solution	Constituents	Concentration, [g/L]
1	$(NH_4)_2HPO_4$	26.7
2	$CaCl_2 \cdot 2H_2O$	16.7
	NH_4Cl	26.6
	$MgCl_2 \cdot 6H_2O$	120.0
	KCl	86.7
3	Biotin	0.002
	Folic Acid	0.002
4	$FeCl_2 \cdot 4H_2O$	370.

The concentration of each constituent in the feed tank, as well as the corresponding concentration in the influent stream to the first stage, after taking into account the dilution caused by the recycle stream from the second stage, are listed in Table 3. The calculations were based on a recycle rate of 793 ml/min and a feeding rate of 4 ml/min, which were the prevailent conditions for system 1 in most parts of the study. Since System 2 was fed at half the rate

but at the same recycle rate, the composition of the influent stream to System 2 is expected to be about half that of system 1.

Table 3: The Composition of Feed Solution Before And After Dilution

Constituents	Concentration, [mg/L]	
	Before Dilution	After Dilution
$(NH_4)_2HPO_4$	37.1	0.18
$CaCl_2 \cdot 2H_2O$	9.3	0.04
NH_4Cl	14.8	0.07
$MgCl_2 \cdot 6H_2O$	66.7	0.33
KCl	48.2	0.24
Biotin	0.003	1.5×10^{-5}
Folic Acid	0.003	1.5×10^{-5}
$FeCl_2 \cdot 4H_2O$	103	0.52

The dissolved oxygen (D. O.) of the feed solution was below 1.0 most of the time. The deoxygenation was achieved by purging nitrogen gas (99.998% pure, purchased from Spectra Gas) with air stone through the measured volume of tap water. This usually lowered the D. O. level to about 1.5. Further deoxygenation was effected by adding small amounts of Na_2 S. The pH of the solution was adjusted with sodium phosphate buffer (dibasic) to a value of 8.0 or above so as to maintain a pH of about 7.4 in the column after dilution by the recycle stream.

Immediately after the feed solution was prepared, the feed tanks were covered tightly to minimize volatilization of TCE. Furthermore, the concentration of TCE in the feed

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tanks was monitored and was found to decrease exponentially with time, as shown in Figure 2. However, for the first three hours following preparation, the concentration of TCE remained essentially constant. Based on the pumping rate and the length of the tubing leading to the column, it was estimated that a maximum of about 50 minutes and 100 minutes was required, respectively, for the feed solution to reach Systems 1 and 2. Therefore, collection of effluent samples were carried out about one and two hours after the commencement of feed for Systems 1 and 2, respectively.

Sampling And Analysis

Effluent samples, as well as gas samples from the head spaces, of both the first and the second stages, were collected and analyzed for TCE, the intermediate and the end products. In sampling, sufficient amount of copper sulfate was added to the sampling bottles to quench the biological reaction so that no further biotransformation of the concerned substances could occur prior to analysis. A mass balance was then performed based on the loading input to the system. All samples were analyzed using a gas chromatograph (Hewlett Packard 5890) equipped with a flame ionization detector, which was kept at 250°C and a 8' x 1/8" o.d. stainless steel column packed with 60/80 Carbopack B/1% SP-1000 (Supelco, Inc., Bellefont, P. A.). The nitrogen carrier gas flowrate was 40 ml/min, the flow rates of hydrogen and
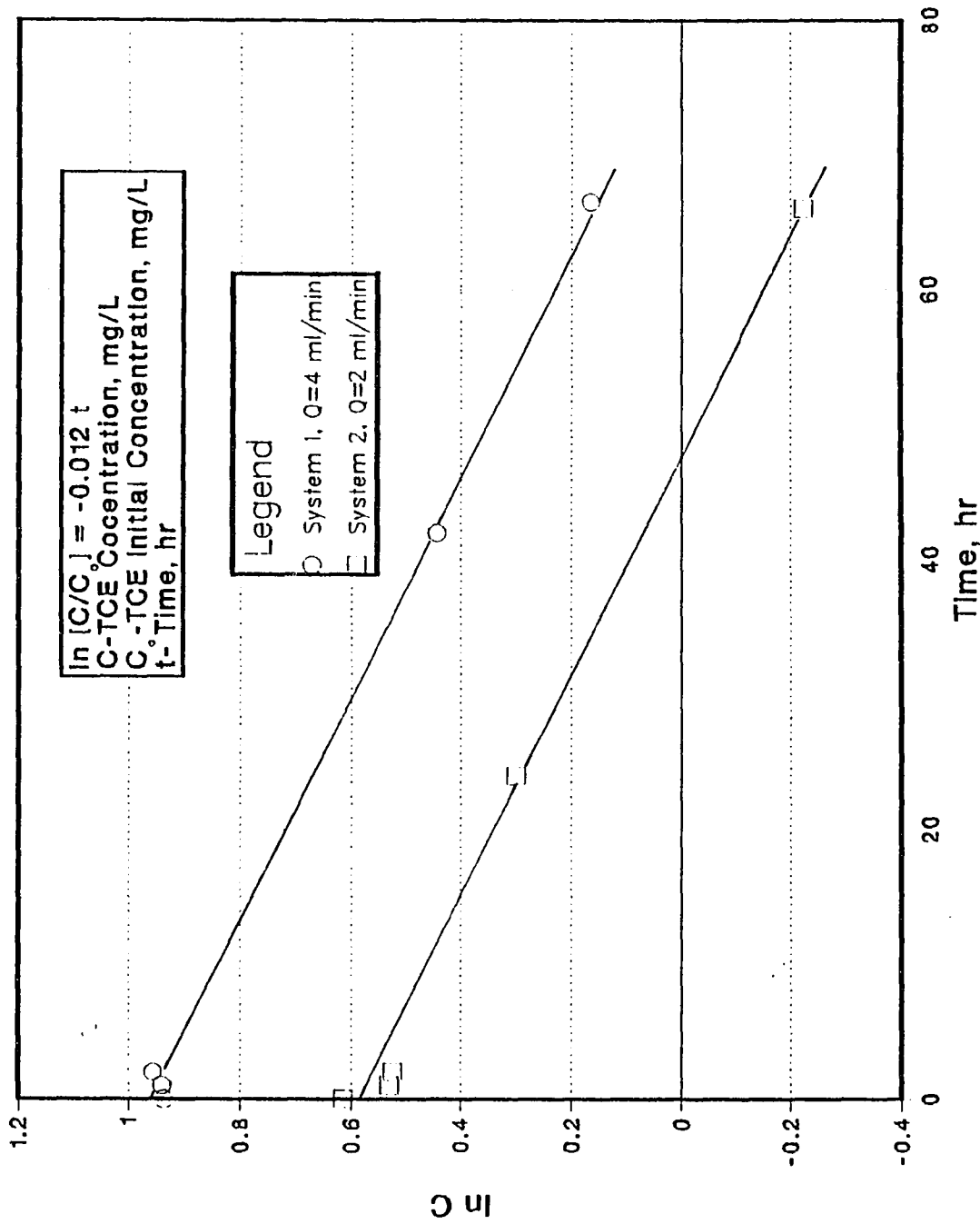
FIGURE 2 RATE OF VOLATILIZATION OF TCE IN FEED TANK

air were 30 and 70 ml/min, respectively. The column was initially 45°C, followed by a linear temperature gradient of 12°C/min to 200°C. Peak integrations were obtained with a Hewlett-Packard Integrator. The GC was coupled with a purge and trap system, Tekmar Model 4000 (Tekmar Co., Cincinnati, Ohio) and the analyses were carried out in accordance with EPA Method 601. The conditions employed in this procedure were as follows: Purge gas nitrogen flow rate = 40 ml/min; purge time = 11 minutes; desorb time = 4 minutes; bake time = 10 minutes.

Collection of gas samples was carried out as follows. First, the volume of the column headspace was accurately measured as 128.7 ml. The gas sample was then collected in a Tenax trap, which had its one end connected to the reactor headspace and the other to a well-sealed container having a high vacuum. The rate of flow of gas through the trap was controlled by installing a precalibrated orifice (Hamilton 33 Gauge Orifice) between the trap and the evacuated contai-ner, which served as a suction in drawing the gas sample through the trap once its valve was opened. Knowing the flowrate through the orifice (10 ml/min in our case), the amount of sample trapped was thus controlled by setting the sampling time. Due to the limited size of the headspace, the period for the gas collection was restricted to no longer than 10 minutes. The interval between sampling was at least 2 hours to prevent extraneous suction of TCE from

the liquid phase. A total volume of 50 milliliters had been found to be sufficient for a reliable GC analysis. After collection, the Tenax trap was then removed from the system, mounted on the purge and trap system and analyzed for VOCs.

A calibration curve for methane was obtained by the following procedure: (1) Predetermined volumes of 1% CH_4 standard were withdrawn from a disposable cylinder equipped with septum with a gas tight syringe, (2) The standard was injected slowly into a tenax trap plugged at both ends with a thin layer of glass wool and baked for 10 minutes at $250^{o}C$ prior to use, (3) The trap was assembled on the purge and trap unit and analysis was carried out in the same way as in the analyses of the gas samples collected from the headspace, (4) Since there was a doubt whether or not tenax traps were appropriate for CH_4 analysis, the % recovery was determined by directly injecting a known amount of CH_4 standard into the GC and the resulting area compared with that obtained by using the trap, (5) The recovey was found to be only 36%. Therefore the values obtained in the analyses of the headspace samples were corrected for the discrepancy.

In addition to volatilization into the headspace on top of the column, the TCE can also be adsorbed onto the activated carbon particles and/or subsequently be transformed to other compounds. Since the present study aimed at investigating the possibility of the ultimate transformation of TCE

into non-toxic substance and not the mere physical transfer of TCE from one phase to another, it is necessary to be able to quantify the amount that is transformed and that which is left unchanged on the surface of the carbon. Quantification of transformed TCE was done by measuring the amount of the possible products of the reaction, e. g., VC, DCE, DCA, etc. with GC. The residual TCE on the activated carbon was quantified as follows. Samples of activated carbon (about 0.5 to 2 g) were withdrawn from the column by opening the valve on the sampling port and allowing the liquid to drain through a piece of filtering cloth. The filtered carbon particles were quickly placed in a vial containing 20 ml of CS_2. Ten ml of distilled water was then added, which stayed on top of the CS_2 layer and prevented the excessive escape of CS_2 and TCE to the air. The vial was sealed and placed on a shaker operating at 240 rpm for 48 hours. It was then stored at $10^{\circ}C$ for at least overnight for complete separation of the layers, which were subsequently analyzed with the purge and trap and GC.

The parameters measured and the corresponding analytical techniques employed are presented in Table 4.

Tests On The Hydrodynamic Characteristics Of The FBBR

Since an FBBR represents a reactor configuration that makes use of fluid mechanics behavior to attain process intensification [48], a careful examination of its hydrody-

Table 4: The Analytical Methods Used In The Analyses Of The Pertinent Parameters

Parameter	Analytical Method
TCE	EPA Method 601
Glucose	Anthrone test
TDCE	EPA Method 601
1,1-DCE	EPA Method 601
VC	EPA Method 601
1,2-DCA	EPA Method 601
NH_4^+-N	Orion Model 601A
Alkalinity as $CaCO_3$	Standard Methods
pH	pH meter
PO_4^{-3}-P	Standard Methods
Gas measurement	Volumetric Method
Flow rate	Volumetric Method

Note: TDCE = Trans-1,2-Dichloroethylene
        1,1-DCE = 1,1-Dichloroethylene
        1,2-DCA = 1,2-Dichloroethane

namic characteristics prior to actual operation of the process is needed to gain an insight of what to anticipate and, so that operation in the optimal region can be assured. In this context, a series of tests was performed using a column containing only GAC particles. The axial pressure drop was measured with a U-tube manometer. Because the pressure drop was small, carbon tetrachloride (CCl_4, density = 1.594[20] g/ml) was used in place of mercury to minimize reading error. Although CCl_4 is not miscible with water, it is also colorless. Therefore to make the water-CCl_4 inter-face distinctively visible, a dye was added, coloring the CCl_4 column orange. The velocity of the upflowing water was determined by dividing the measured volumetric flow rate by

the column cross-sectional area. The bed porosity was obtained by measuring the bed height before and after the bed expansion.

CHAPTER IV

RESULTS AND DISCUSSIONS

Hydrodynamic Characteristics of the FBBR

Figures 3 through 5 illustrate the effect of the fluid velocity on the hydrodynamic behavior of the system. All three figures point to a velocity of 2×10^{-3} m/s as the critical velocity for incipient fluidization, the breakpoint on the curves of axial pressure drop (P) vs. the fluid superficial velocity (v), bed porosity (e) vs. v, and fluidized bed height (H) vs. v. This critical velocity is seen to be independent of the initial bed height. Below this value, Figure 3 showed that the reactor assumed a packed bed configuration, where P varied linearly, but markedly, with v. In this region, e was zero (Figure 4) and there was essentially no change in H (Figure 5).

Between $v = 2 \times 10^{-3}$ to 6×10^{-3} m/s, there was a dramatic increase in e with v. P continued to vary linearly with v but with a significantly lower slope. This region is thus designated as the transition region from a packed bed to a fully fluidized-bed configuration for beyond this region the GAC particles are fully fluidized and P is independent of v. There is also a corresponding enhancement in the bed porosity, i. e., e is greater than 0.4.

Based on the results obtained in this test, for an

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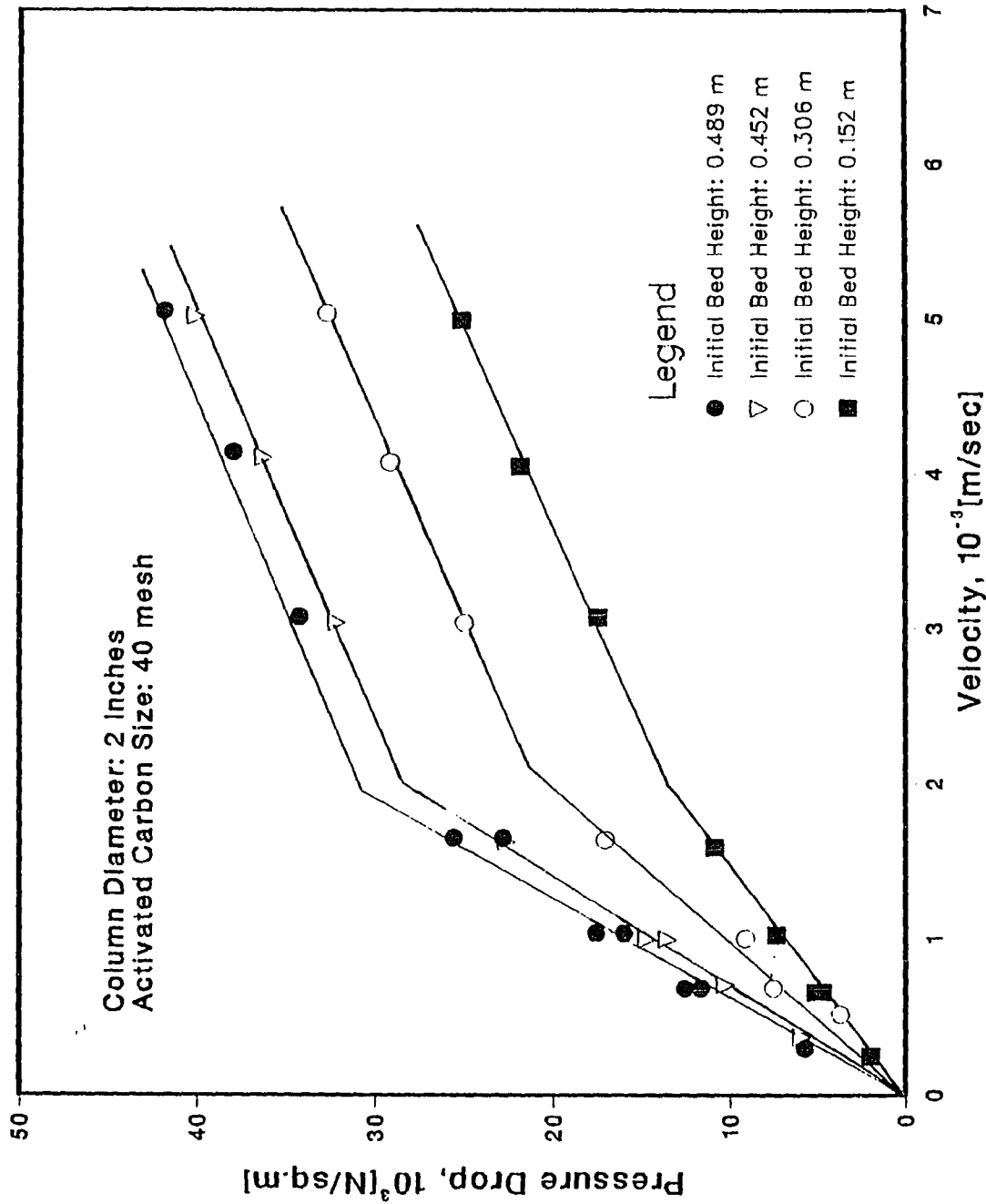
FIGURE 3   EFFECT OF VELOCITY ON PRESSURE DROP OF THE BED

Column Diameter: 2 Inches
Activated Carbon Size: 40 mesh

Legend
● Initial Bed Height: 0.489 m
▽ Initial Bed Height: 0.452 m
○ Initial Bed Height: 0.306 m
■ Initial Bed Height: 0.152 m

Porosity, %

Velocity, 10^{-3} [m/sec]
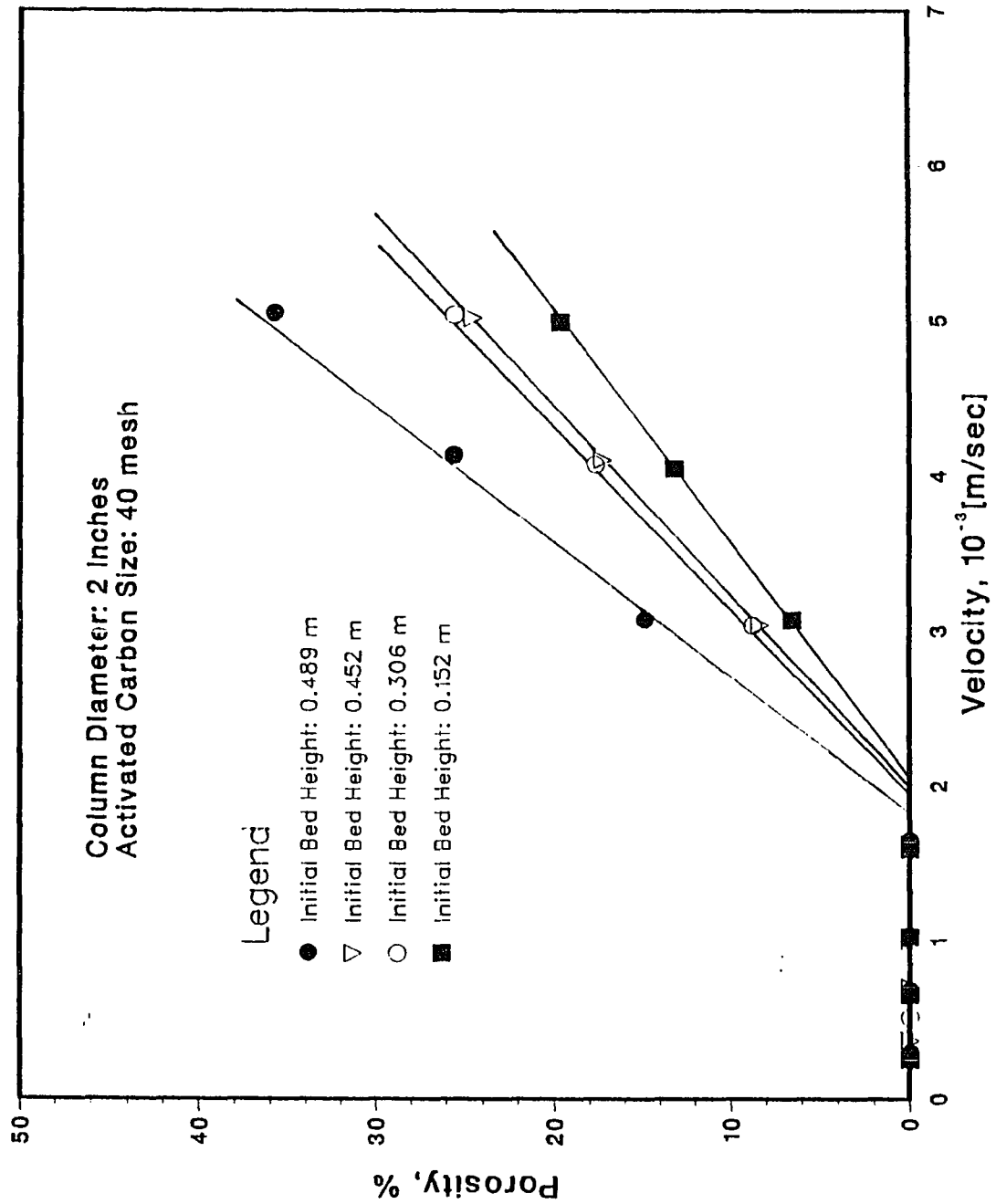
FIGURE 4   EFFECT OF VELOCITY ON POROSITY OF FLUIDIZED BED

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Column Diameter: 2 Inches
Activated Carbon Size: 40 mesh

Legend

● Initial Bed Height: 0.489 m
▽ Initial Bed Height: 0.452 m
○ Initial Bed Height: 0.306 m
■ Initial Bed Height: 0.152 m

Velocity, 10^{-3}[m/sec]

Fluidized Bed Height, [m]
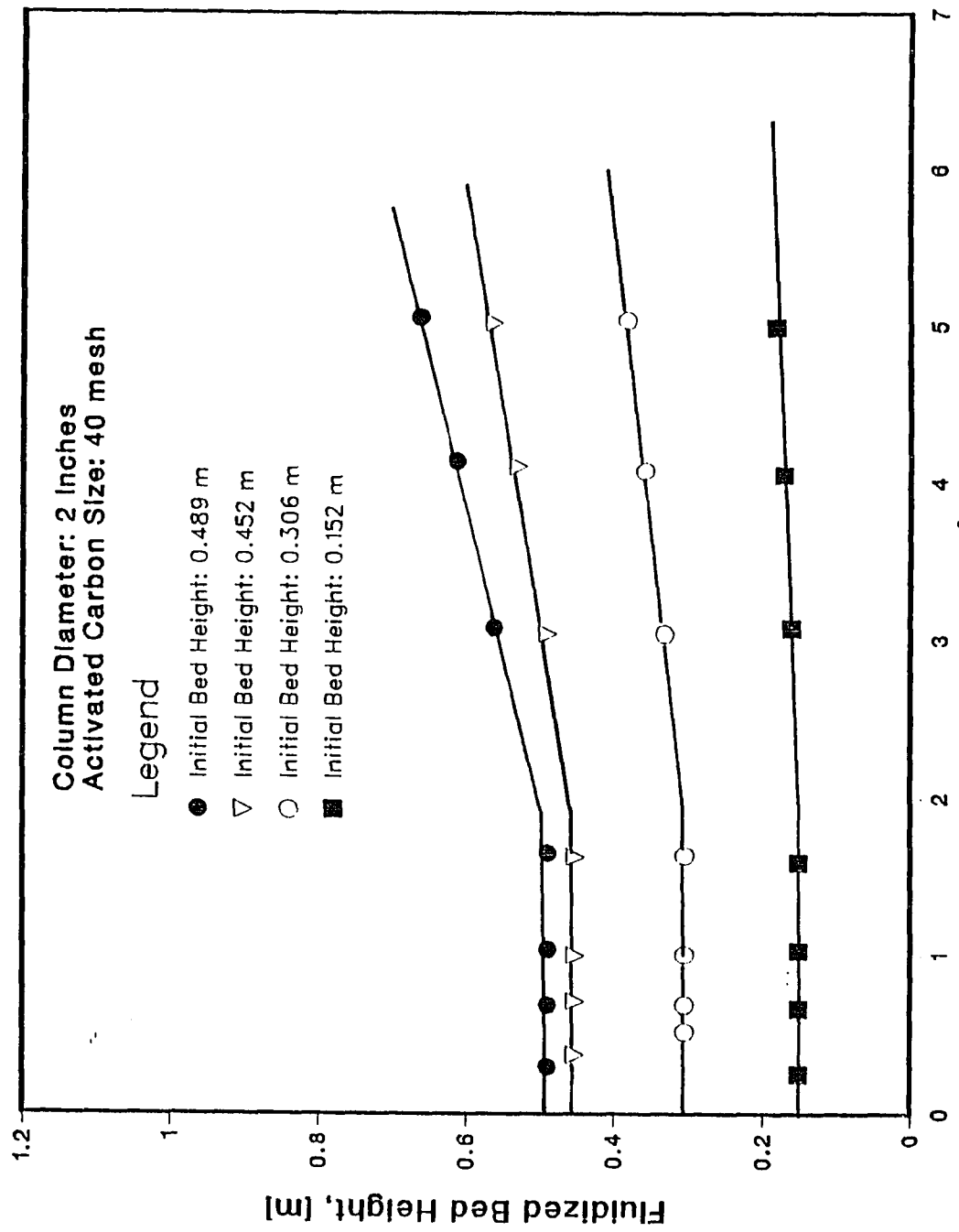
FIGURE 5   EFFECT OF VELOCITY ON FLUIDIZED BED HEIGHT

Table 5: Hydrodynamic Characteristics of the Fluidized Bed

Column Diameter : 2"   Activated Carbon Size: 40 mesh

Column Volume V = 3088.9 ml

Fixed Bed Ho, m	Feed Rate [cub.m/sec] x10^6	Velocity [m/sec] x10^3	Fluidized Bed Height, H, m	(H-Ho) m	e %	P-Po N/sq.m
0.489	0.968	0.295	0.489	0.00	0	575.7
0.489	2.250	0.685	0.489	0.00	0	1255.3
0.489	2.250	0.685	0.489	0.00	0	1167.0
0.489	3.410	1.038	0.489	0.00	0	1755.3
0.489	3.410	1.038	0.489	0.00	0	1598.5
0.489	5.420	1.650	0.489	0.00	0	2559.6
0.489	5.420	1.650	0.489	0.00	0	2285.0
0.489	10.100	3.074	0.562	0.07	14.9	3422.5
0.489	13.600	4.140	0.614	0.13	25.6	3795.3
0.489	16.600	5.050	0.664	0.18	35.8	4187.6
0.452	1.230	0.374	0.452	0.00	0	595.3
0.452	2.340	0.712	0.452	0.00	0	1029.7
0.452	3.300	1.004	0.452	0.00	0	1471.0
0.452	5.370	1.630	0.452	0.00	0	2265.4
0.452	10.000	3.040	0.490	0.04	8.4	3216.7
0.452	13.500	4.110	0.530	0.08	17.3	3628.6
0.452	16.500	5.020	0.563	0.11	24.6	4011.1
0.306	1.700	0.520	0.306	0.00	0	377.6
0.306	2.270	0.690	0.306	0.00	0'	754.2
0.306	3.370	1.010	0.306	0.00	0	913.0
0.306	5.390	1.640	0.306	0.00	0	1706.4
0.306	10.000	3.040	0.333	0.03	8.8	2500.8
0.306	13.420	4.080	0.360	0.05	17.7	2922.5
0.306	16.550	5.040	0.384	0.08	25.6	3275.5
0.152	0.808	0.250	0.152	0.00	0	203.0
0.152	2.170	0.66	0.152	0.00	0	515.8
0.152	3.370	1.03	0.152	0.00	0	734.5
0.152	5.220	1.59	0.152	0.00	0	1088.6
0.152	10.100	3.07	0.162	0.01	6.6	1745.6
0.152	13.300	4.05	0.172	0.02	13.2	2187.0
0.152	16.400	4.99	0.182	0.03	19.7	2520.4

Note :   e = [(H-Ho)/Ho]x100%, porosity of the Fluidized Bed

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initial bed height of 0.33 m used in this study, a superficial velocity of 6.5 x 10^{-3} m/s, which is equivalent to a Q_r of 800 ml/min was adopted in evaluating the effects of operating variables other than Q_r on the process performance.

Effect Of Recirculation Rate, Q_r

As shown in Figures 6 through 9, the effect of Q_r on the composition of the effluent can be divided into three regions. In Region 1, where Q_r is below 750 ml/min (superficial velocity = 0.02 ft/s), the effluent TCE and TDCE levels decrease as Q_r increases in both systems. This is attributed to the enhanced substrate utilization as a result of more adequate supply of nutrient for the promotion of greater growth. Sanders and Characklis [80] investigated the oxygen utilization by attached slime organisms in a continuous culture and observed maximum growth of slime at the upper limit of the velocity range of 0.1 to 1 ft/s. Experiments by the Ministry of Technology of Great Britian on attached microbial growths also demonstrated a maximum slime development between 0.5 to 0.9 ft/s [81]. When the superficial velocity of the fluid in the column is low, complete fluidization of the medium can not be attained. This causes a decline in the bed porosity (Figure 4) and may lead to severe attrition between particles, which in turn may be detrimental to microbial growth. As seen from Figure
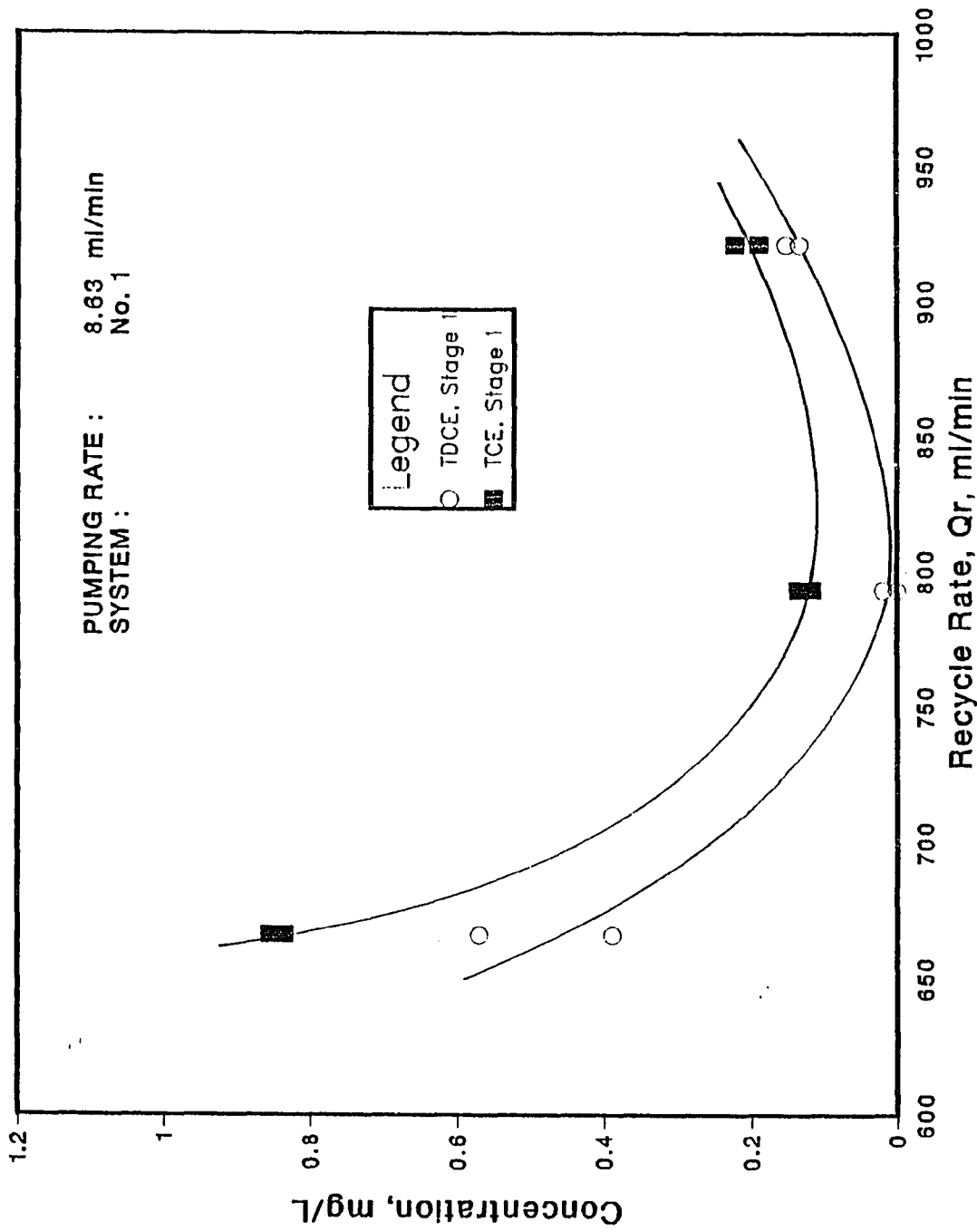
FIGURE 6   EFFECT OF RECYCLE RATE ON EFFLUENT COMPOSITION (1)

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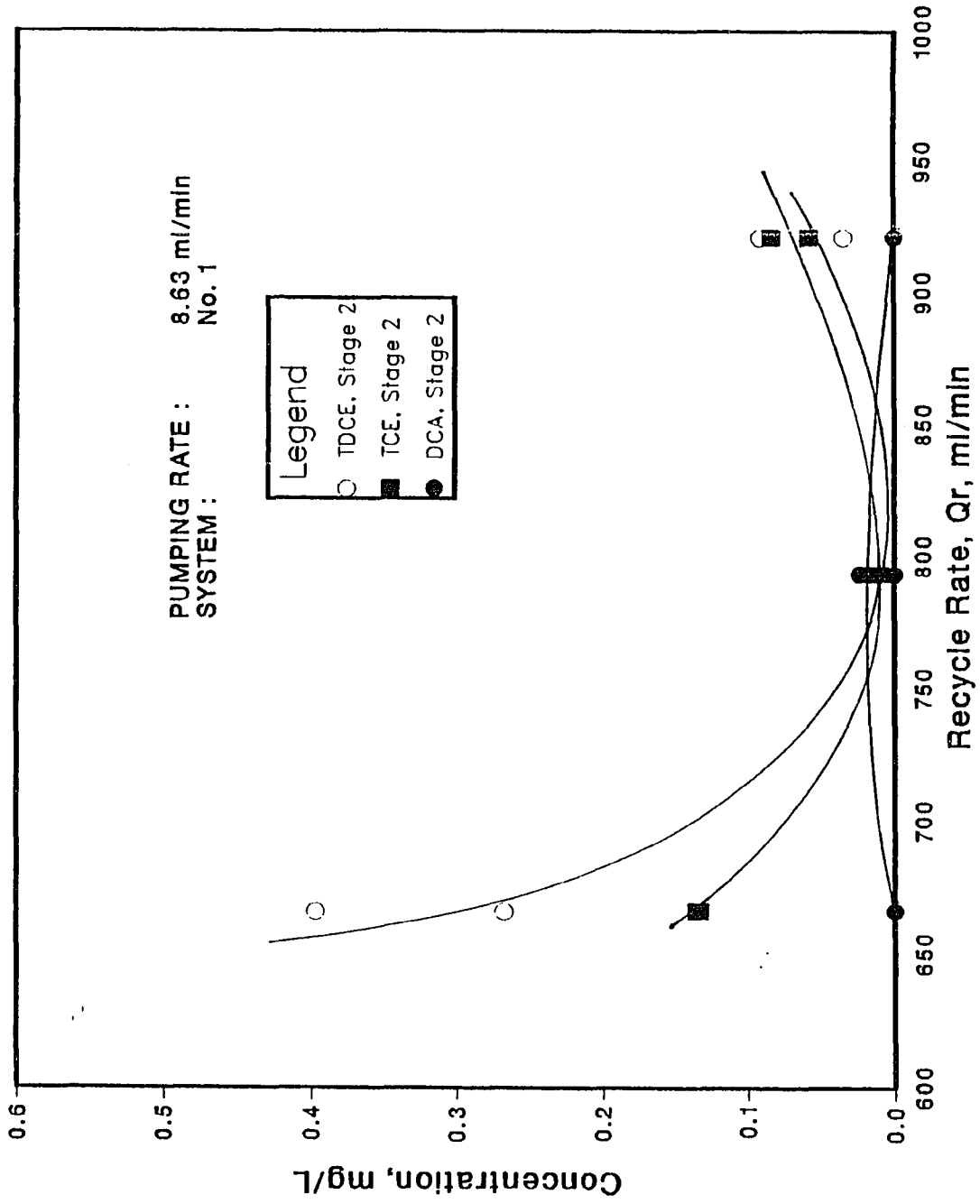
FIGURE 7   EFFECT OF RECYCLE RATE ON EFFLUENT COMPOSITION (2)
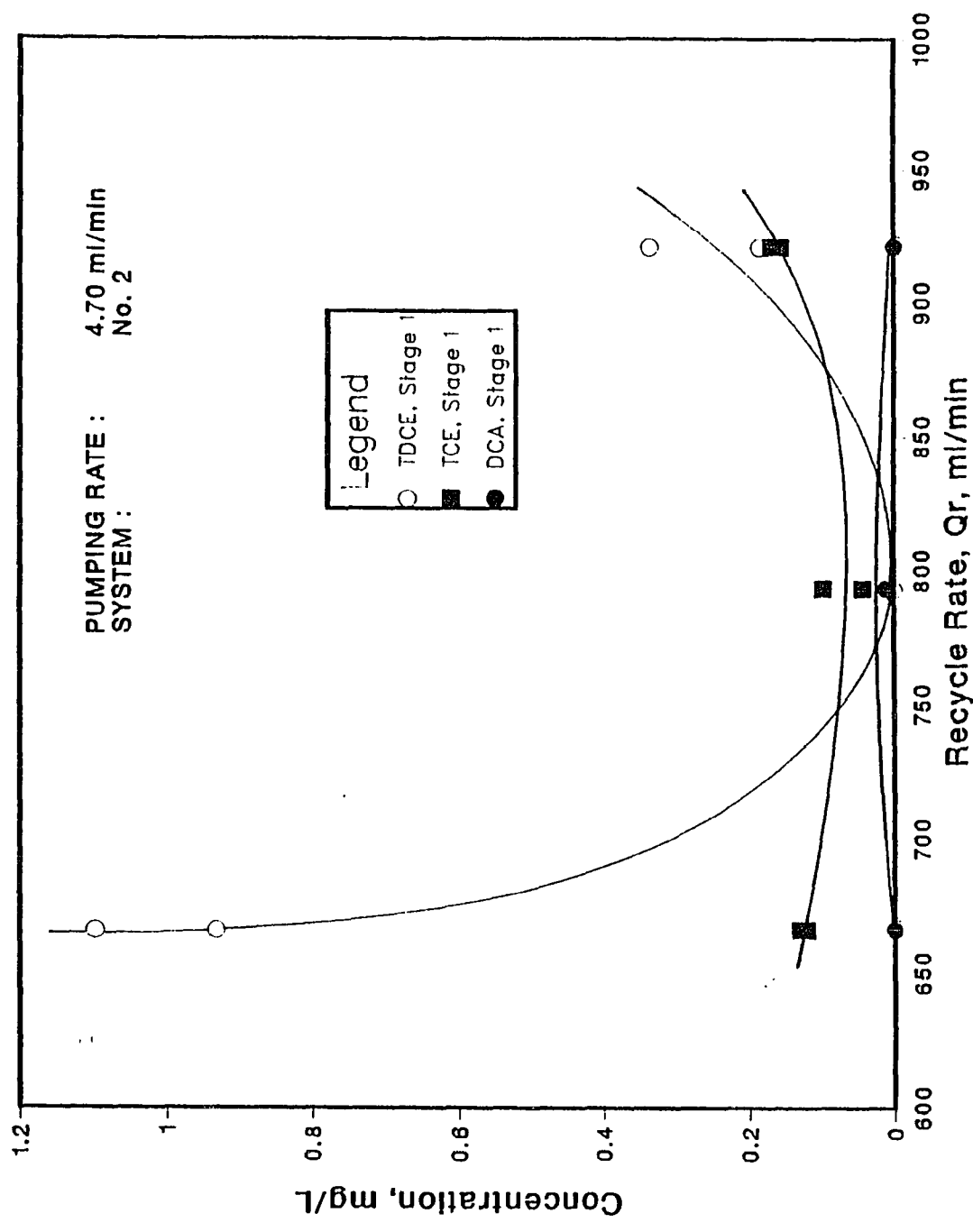
FIGURE 8   EFFECT OF RECYCLE RATE ON EFFLUENT COMPOSITION (3)
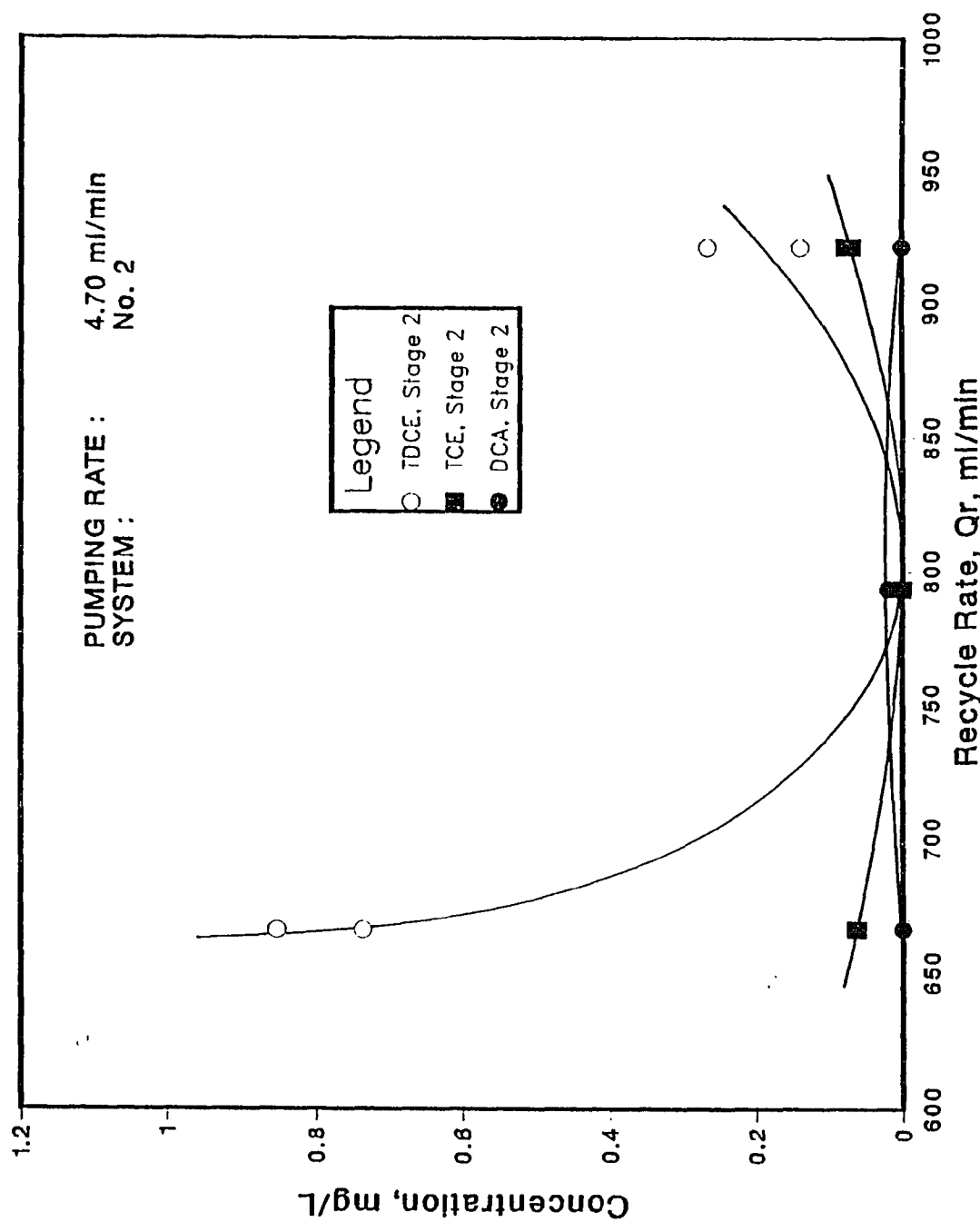
FIGURE 9   EFFECT OF RECYCLE RATE ON EFFLUENT COMPOSITION (4)

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Table 6: Effect of Recycle Rate on Effluent Composition

System	Q	Qr	Stage 1 TDCE	DCA	TCE	Stage 2 TDCE	DCA	TCE
	[ml/min]		[mg/L]			[mg/L]		
	8.63	666.0	0.389	nd	0.855	0.268	nd	0.136
	8.63	666.0	0.571	nd	0.836	0.397	nd	0.134
	8.63	793.0	0.022	nd	0.136	0.015	0.024	0.010
1	8.63	793.0	0.018	nd	0.118	0.014	0.03	0.015
	8.63	921.0	0.150	nd	0.189	0.035	nd	0.058
	8.63	921.0	0.135	nd	0.222	0.092	nd	0.084
	4.70	666.0	0.932	nd	0.123	0.738	nd	0.062
	4.70	666.0	1.098	nd	0.130	0.854	nd	0.064
	4.70	793.0	nd	0.012	0.099	0.008	0.019	nd
2	4.70	793.0	nd	0.010	0.044	0.004	0.020	nd
	4.70	921.0	0.187	nd	0.169	0.139	nd	0.078
	4.70	921.0	0.338	nd	0.158	0.268	nd	0.069

4, with an initial bed height of about 0.3 m, operation below a superficial velocity of 6 x 10^{-3} m/s, i.e., Q_r < 750 ml/min will result in porosity values smaller than 0.4, which is far below the optimum value found in the literature [78]. Furthermore, inadequate fluidization may result in sluggish transport of the substrate to the microbial film on the activated carbon as a result of thicker liquid film surrounding the GAC particles. Under this condition, transport of the substrate to the carbon particles will likely be governed by Fick's diffusion law. Kinetics of adsorption of substrate onto activated carbon particles has been found to be greatly affected by the intensity of agitation imparted on the particles [82].

In the second region where Q_r ranges from 750 to 850 ml/min, a minimum amount of TCE was detected in the effluent of both Stages 1 and 2 for the two systems. The optimum Q_r is estimated as 820 ml/min. It must be noted that the production of DCA in both stages of System 2 is maximum in this region. When Q_r was greater than 850 ml/min, abrupt increase in the effluent concentrations of TCE and TDCE were observed. This is probably due to the insufficient hydraulic retention time (HRT) provided for the microorganisms to act on the substrates. Reaction could not go to completion. The reduction in HRT significantly offsets the improvement in mass transfer characteristics imparted by enhanced fluidization and increased reactant concentration per unit time.

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Moreover, at extremely high superficial velocity, scouring of biomass may take place as a result of high shear stress [81]. Consequently, much of TCE and TDCE escaped degradation and eluted from the system.

The effect of HRT on the process performance can also be delineated by comparing the effluent composition of the two stages of System1. It must be noted that while DCA was detected at the optimum Q_r in the second stage, no DCA was found in the first stage. This strongly indicates that installation of the second stage has prolonged the HRT so that further transformation can take place. On the other hand, DCA was detected in the effluents of both stages of System 2, which was fed at about half the rate for System 1. This pointed to the fact that the process performance is also dependent on the feed rate of TCE.

Effect Of Nutrient Feed Rate, Q

For a given value of glucose/TCE ratio and recirculation rate, Q_r, a change in the nutrient feed rate, Q, has a profound influence on the composition of the effluents from both stages of the system. This is due to the fact that the sequential dehalogenation of TCE is a series reaction. An increase in the feed rate of TCE connotates an increase in the concentration of the reactant in the column, a condition that will favor the forward reaction, i.e., the production

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of the intermediate and end products. Since the transformation of TCE to TDCE is considerably faster than the subsequent reactions (See discussion on "Reaction Kinetics"), the concentration of TDCE in the effluents was expotential for both stages, as illustrated in Figures 10 and 11.

There was also a slight increase in the concentration of VC in the headspace of Stage 1 initially followed by an abrupt decline when Q was increased beyond 5 ml/min (See Figure 12). In the second stage, VC also diminished with increasing Q, but the change was gradual rather than abrupt. This is attributed to the inhibition exerted by TCE on the microorganisms in the first stage, which must have experienced greater toxicity than those in the second stage because TCE was introduced to the first stage. As indicated by our data on the percent reduction of TCE, as well as the effluent TDCE concentration for Stage 1, most of the TCE introduced into the system was removed in the first stage and converted to TDCE. The TCE concentration had been reduced to a considerably more tolerable level. Thus the microorganisms in the second stage could retain most of their degradative activity. This phenomenon has been substantiated in part by the results obtained for the production of methane in the two stages (Figures 12 and 13). The concentration of methane decreased with increasing Q in Stage 1 but, the reversed trend was observed in Stage 2. Indeed, the biofilm thickness on the GAC particles in the
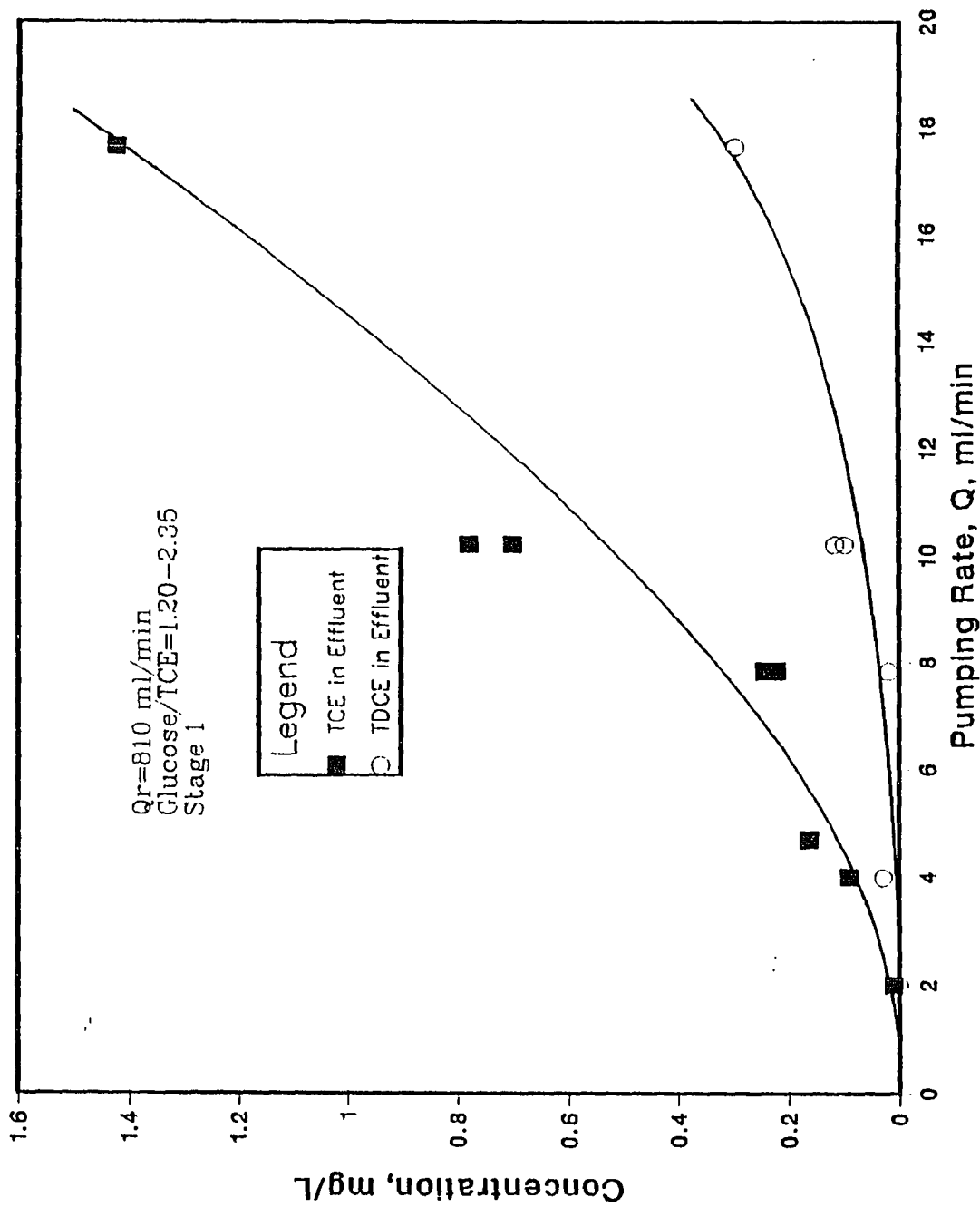
FIGURE 10   EFFECT OF PUMPING RATE ON EFFLUENT COMPOSITION
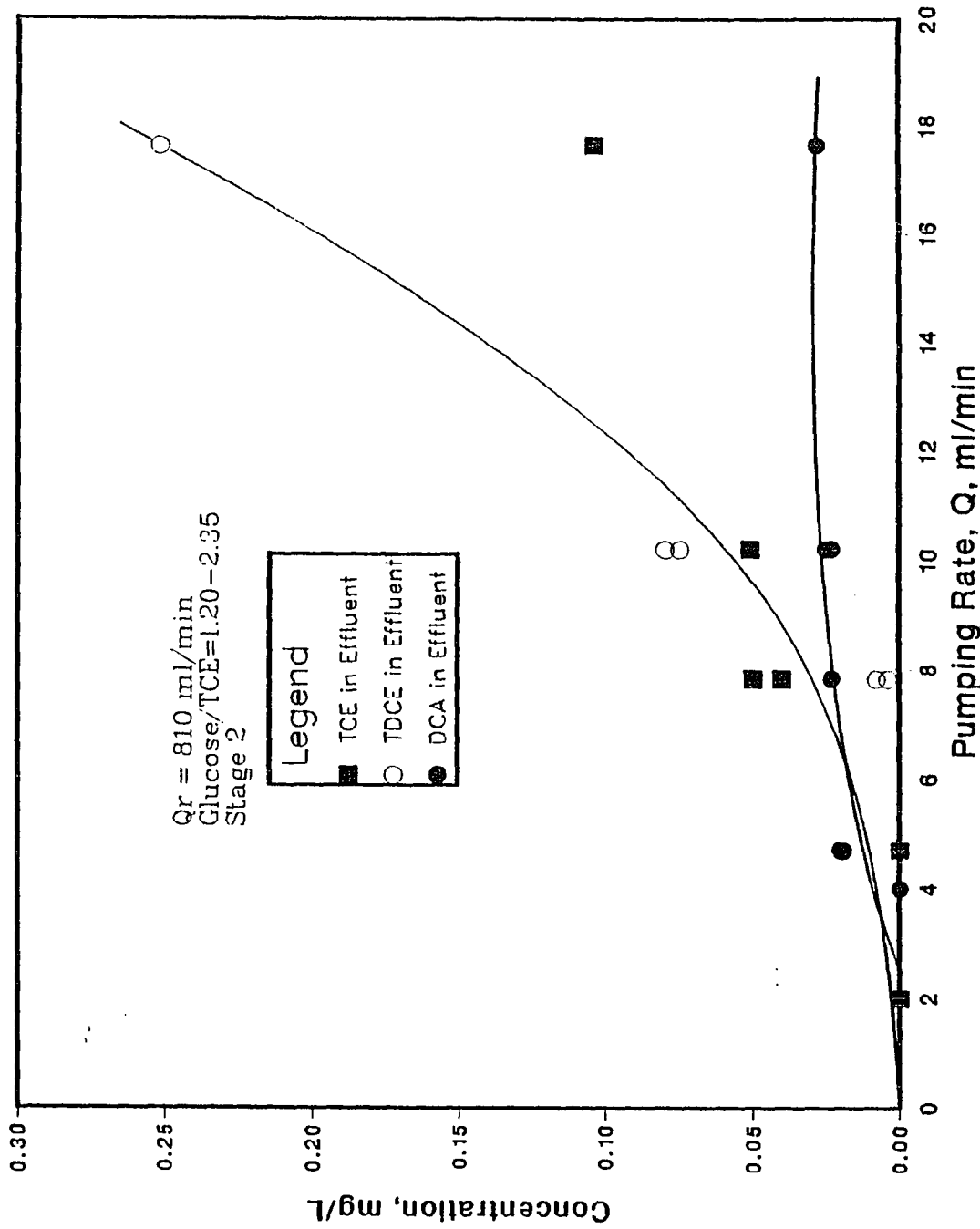
FIGURE 11 EFFECT OF PUMPING RATE ON EFFLUENT COMPOSITION

Table 7: Effect of Pumping Rate on Effluent Composition

Recycle Rate Qr = 810 ml/min

Pumping Rate	Stage 1			Stage 2		
	TCE	TDCE	DCA	TCE	TDCE	DCA
ml/min		mg/L			mg/L	
2.00	0.010	nd	nd	nd	nd	nd
4.00	0.091	0.03	nd	nd	nd	nd
4.70	0.164	nd	0.012	nd	nd	0.020
7.83	0.222	0.02	nd	0.04	0.008	0.021
7.83	0.244	0.03	nd	0.05	0.004	0.023
10.20	0.789	0.10	nd	0.05	0.080	0.025
10.20	0.707	0.12	nd	0.05	0.075	0.023
17.64	1.425	0.30	nd	0.10	0.252	0.026

GAS COMPOSITION IN HEADSPACE 1
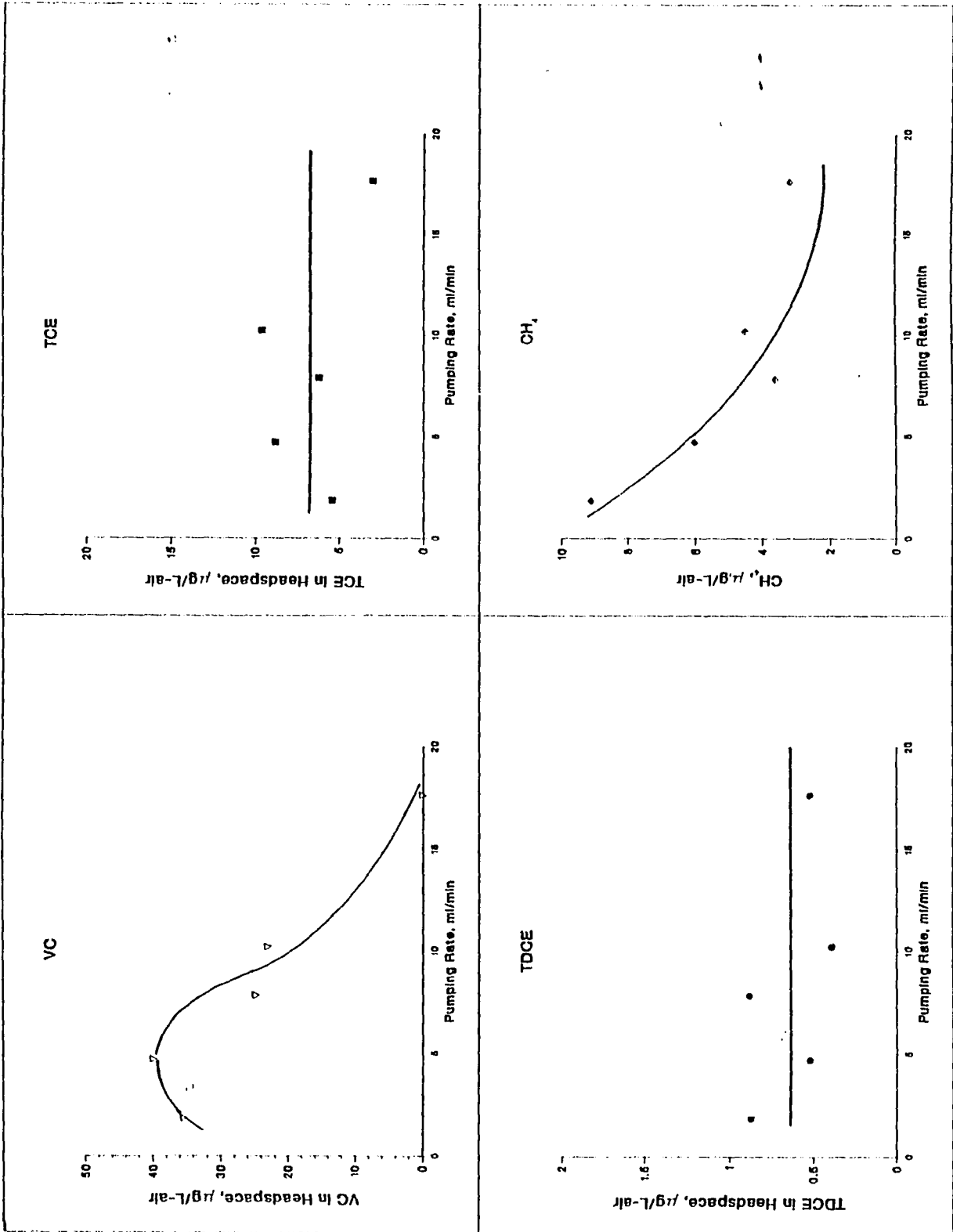
FIGURE 12   EFFECT OF PUMPING RATE ON GAS COMPOSITION

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GAS COMPOSITION IN HEADSPACE 2
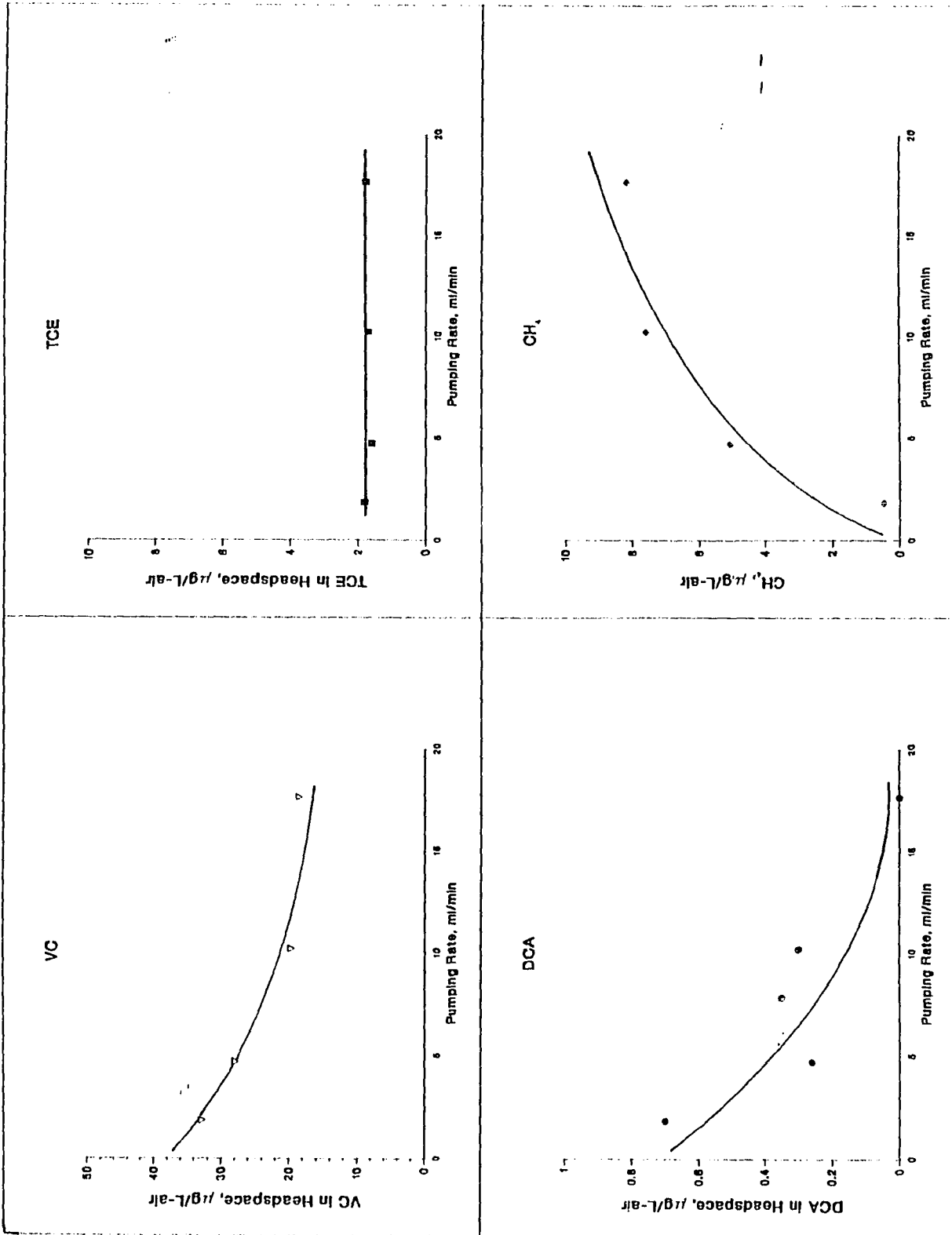
FIGURE 13   EFFECT OF PUMPING RATE ON GAS COMPOSITION

Table 8: Effect of Pumping Rate on Gas Composition

Recycle Rate Q_r = 810 ml/min

Stage	Pumping Rate [ml/min]	VC	TCE	TDCE	CH$_4$
				[ug/L-Air]	
	1.83	35.40	5.40	0.87	9.13
1	4.70	40.00	8.80	0.52	6.02
	7.83	24.80	6.20	0.88	3.61
	10.20	23.02	9.60	0.39	4.51
	17.64	nd	3.00	0.52	3.19
		VC	TCE	DCA	CH$_4$
	1.83	32.75	1.80	0.70	0.48
2	4.70	27.90	1.60	0.26	5.07
	7.83	–	–	0.35	–
	10.20	19.62	1.70	0.30	7.61
	17.64	18.50	1.80	0.00	8.20

second stage was observed to be orders of magnitude greater than that in the first stage.

Both the concentrations of TCE and TDCE 'in the headspace remained essentially constant over the range of Q investigated, indicating that the amount of these compounds that volatilized were independent of Q under steady state operation. Furthermore, the concentration of TCE in the headspace of Stage 2 was considerably lower than that in Stage 1, as expected.

While the effluent concentration of TCE in the first stage varied exponentially with Q as in the case of TDCE, it changed linearly with Q in the second stage, i.e., the effect of Q was comparatively less. This is because the second stage provides an extention of the HRT so that more TCE conversion can take place. In this case the reaction is more likely to approach completion. Indeed DCA was detected in Stage 2 but not in Stage 1 over the range of Q investigated. In summary, a slower feed rate seemed to favor the reaction towards the production of DCA rather than VC in the early stage of the process. Moreover, although the two systems appear to be identical in terms of physical configuration, viz., exactly the same geometric size and shape, supplied with exactly the same substrate, they can be completely different in the characteristics of the biota that developed due to the difference in the feed rate.

Effect Of Influent TCE Concentration

Our studies on TCE removal by the continuous upflow anaerobic activated carbon fluidized bed system have shown that this proposed process is capable of removing TCE from the aqueous phase for a concentration range of 3.2 to 476 mg/L (corresponding to a concentration range of 0.02 to 2.3 mg/L for the first stage). As expected, the TCE level in the effluents of both systems increases with increasing influent TCE concentration, as shown in Tables 9 and 10. For the range of initial TCE concentration investigated, the percent removal in the first stage varied from 81 to 98, while the residual TCE was almost completely removed in the second stage, as listed in tables.

Using Stage 1 data and one minute as the effective contact time, the removal rates of TCE were calculated and plotted against the influent concentration in Figure 14. A straight line was obtained, which could well be represented by the following expression with a near perfect correlation:

$$R = -0.0034 + 0.9479 \ (Si)_d$$

where R is the overall removal rate in mg/L-min, $(Si)_d$ is the influent TCE concentration into the first stage in mg/L.

Table 9:   Effect Of Influent TCE Concentration On TCE Reduction

System 1   Pumping Rate, $Q = 4.0$ ml/min
                                 Recycle Rate, $Q_r = 793$ ml/min

Si	Si_d	TCE(1)	Reduction (1)	TCE(2)	Reduction (2)
mg/L	mg/L	mg/L	%	mg/L	%
7.77	0.038	0.007	81.58	nd	100.0
16.00	0.079	0.013	83.54	nd	100.0
21.53	0.106	0.016	84.91	nd	100.0
50.74	0.249	0.018	83.13	nd	100.0
53.13	0.261	0.041	84.29	nd	100.0
78.34	0.385	0.064	83.38	nd	100.0
81.06	0.398	0.049	87.70	nd	100.0
84.97	0.418	0.050	88.70	nd	100.0
148.65	0.727	0.122	83.22	nd	100.0
150.49	0.736	0.139	81.10	nd	100.0
182.16	0.895	0.111	90.00	nd	100.0
181.42	0.892	0.107	88.00	nd	100.0
216.35	1.063	0.096	90.97	nd	100.0
222.67	1.089	0.181	90.80	0.003	98.3
223.66	1.099	0.071	93.54	nd	100.0
412.64	2.028	0.157	92.26	nd	100.0
476.41	2.341	0.162	93.08	0.003	98.1

Note : (1) means first stage;
        (2) means second stage.

Table 10:   Effect Of Influent TCE Concentration On TCE Reduction

System 2

Pumping Rate, Q = 2.0 ml/min
Recycle Rate, Q_r = 793 ml/min

Si	Si_d	TCE(1)	Reduction (1)	TCE(2)	Reduction (2)
mg/L	mg/L	mg/L	%	mg/L	%
8.54	0.021	0.002	90.48	nd	100.0
10.72	0.026	0.003	88.46	nd	100.0
19.42	0.048	0.005	89.50	nd	100.0
89.47	0.220	0.024	89.10	nd	100.0
55.77	0.137	0.008	94.16	nd	100.0
78.86	0.194	0.010	94.84	nd	100.0
81.76	0.201	0.012	94.03	nd	100.0
124.83	0.307	0.030	90.22	nd	100.0
127.51	0.313	0.020	93.61	nd	100.0
126.07	0.310	0.015	95.16	nd	100.0
130.13	0.320	0.040	87.50	nd	100.0
163.48	0.402	0.045	88.80	nd	100.0
168.36	0.414	0.035	91.55	nd	100.0
154.53	0.380	0.040	89.47	nd	100.0
294.08	0.723	0.043	94.05	nd	100.0
410.00	1.007	0.056	94.44	nd	100.0
457.52	1.124	0.028	97.51	nd	100.0

Note: (1) means Stage 1;
         (2) means Stage 2.
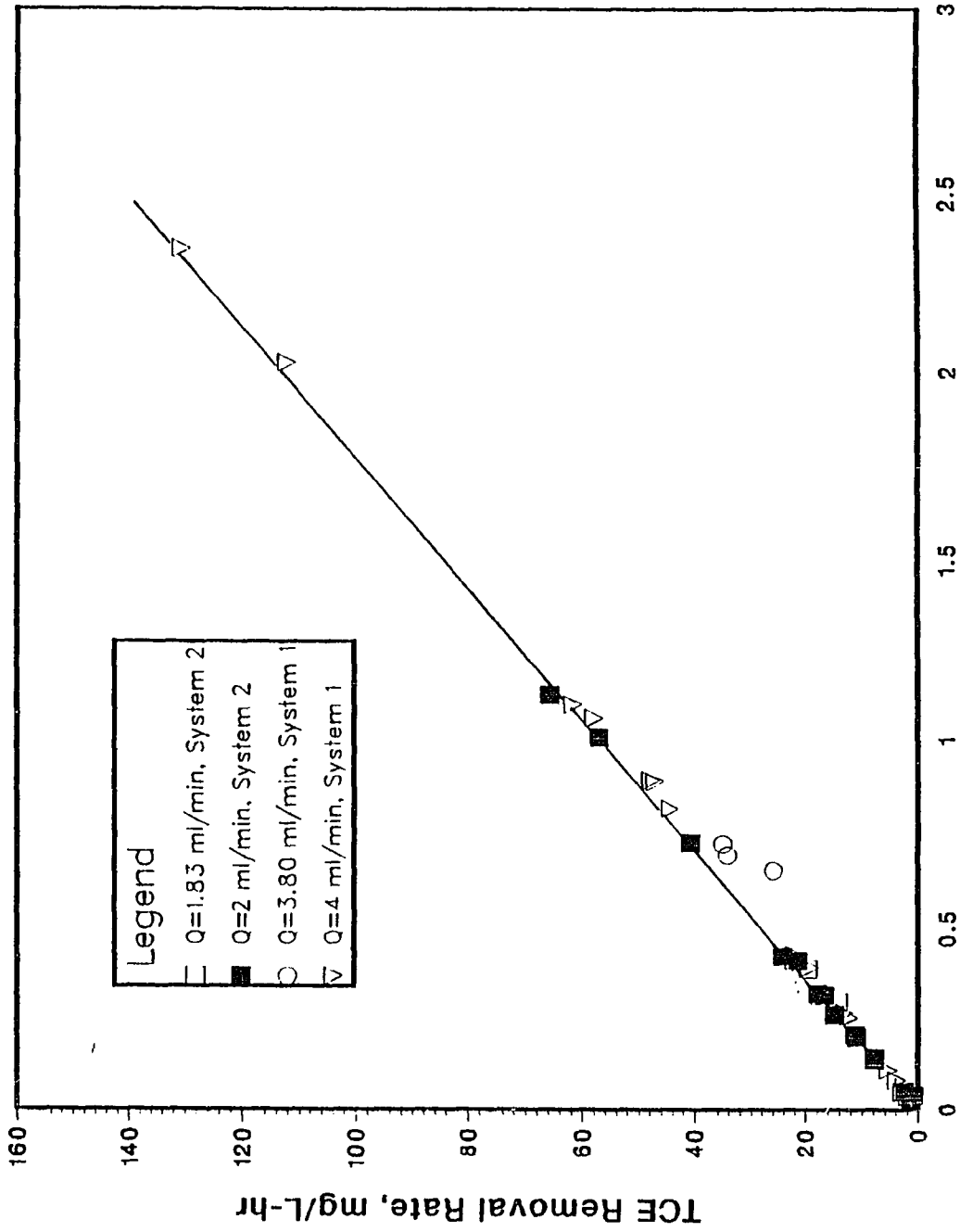
FIGURE 14   EFFECT OF TCE CONCENTRATION ON TCE REMOVAL RATE

Effect Of TCE Loading

In an FBBR, loading is one of the most important para-
meters used in describing the process perfomance for it
incorporates the effects of HRT and influent concentration.
In our case, it combines the effects of Q_r, Q and $(Si)_d$. As
expected, an increase in the TCE loading results in an
increase in both the effluent TCE and TDCE concentrations.
As shown in Figure 15, while the effluent TCE concentration
continued to increase with the loading, the effluent TDCE
concentration seemed to level off beyond a loading of 10
mg/L-hr. The same trend was observed with the VC and DCA in
the headspace (Figure 16). This had been accompanied by a
reversed trend in the solid phase: Both VC and CA decreased
with increasing TCE loading, as illustrated in Figure 17.
Methane was seen to increase initially with the TCE loading
(Figure 16). This is attributed to an increase in the
glucose loading. The decline in the concentration of met-
hane beyond a loading of about 12 mg/L-hr is attributable to
the inhibition of microbial activity at high TCE loading.
Figure 18 depicts the effect of TCE loading on the removal
rate of TCE. It is interesting to note that, in the range
of loading investigated, the removal rate increases linearly
with the loading and does not level off. This lack of
equilibrium is probably due to the continual renewal, and
thus exposure of the active sites on the GAC particles as a
result of microbial action [39].

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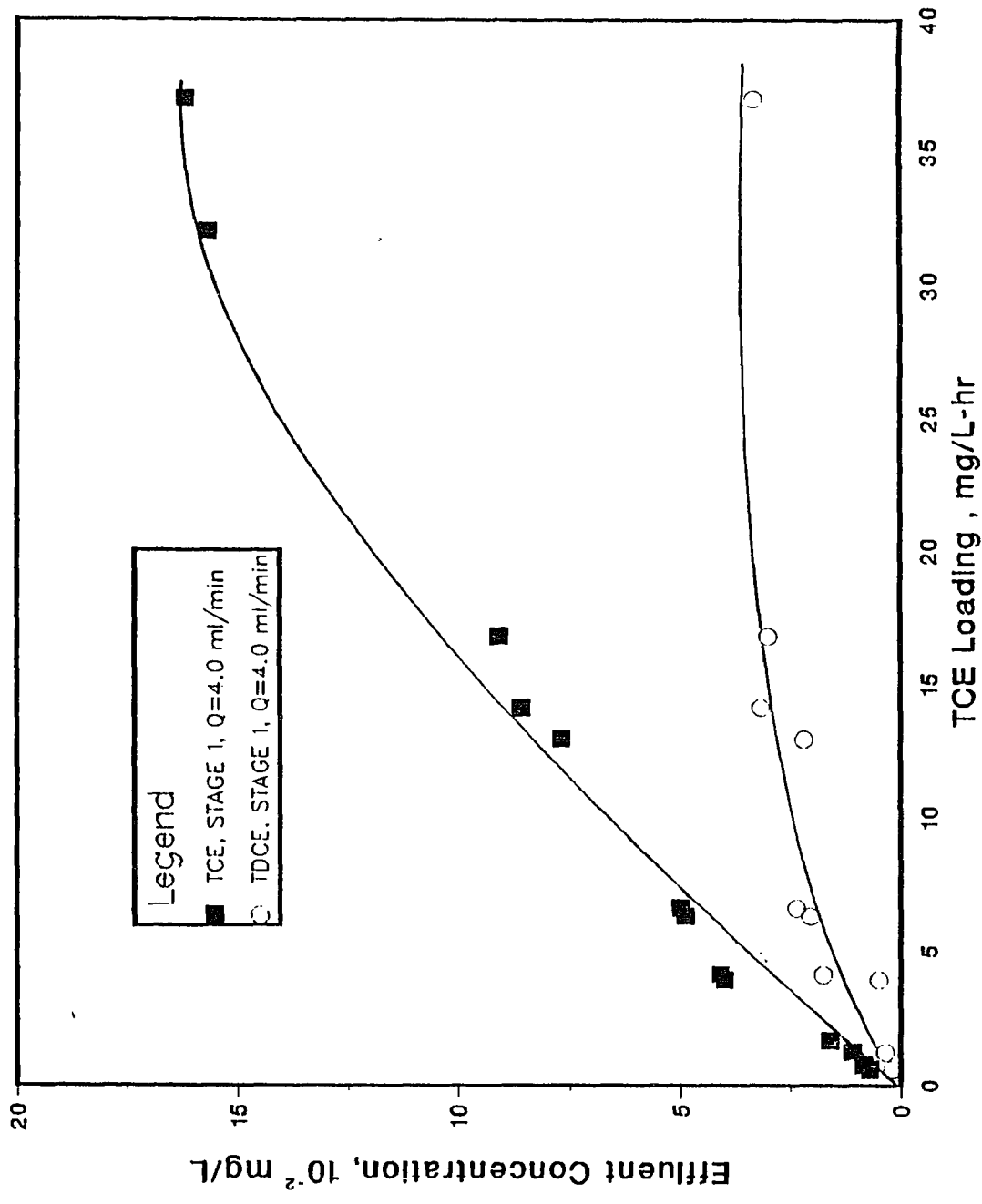
FIGURE 15   EFFECT OF TCE LOADING ON EFFLUENT CONCENTRATION (SYSTEM 1)
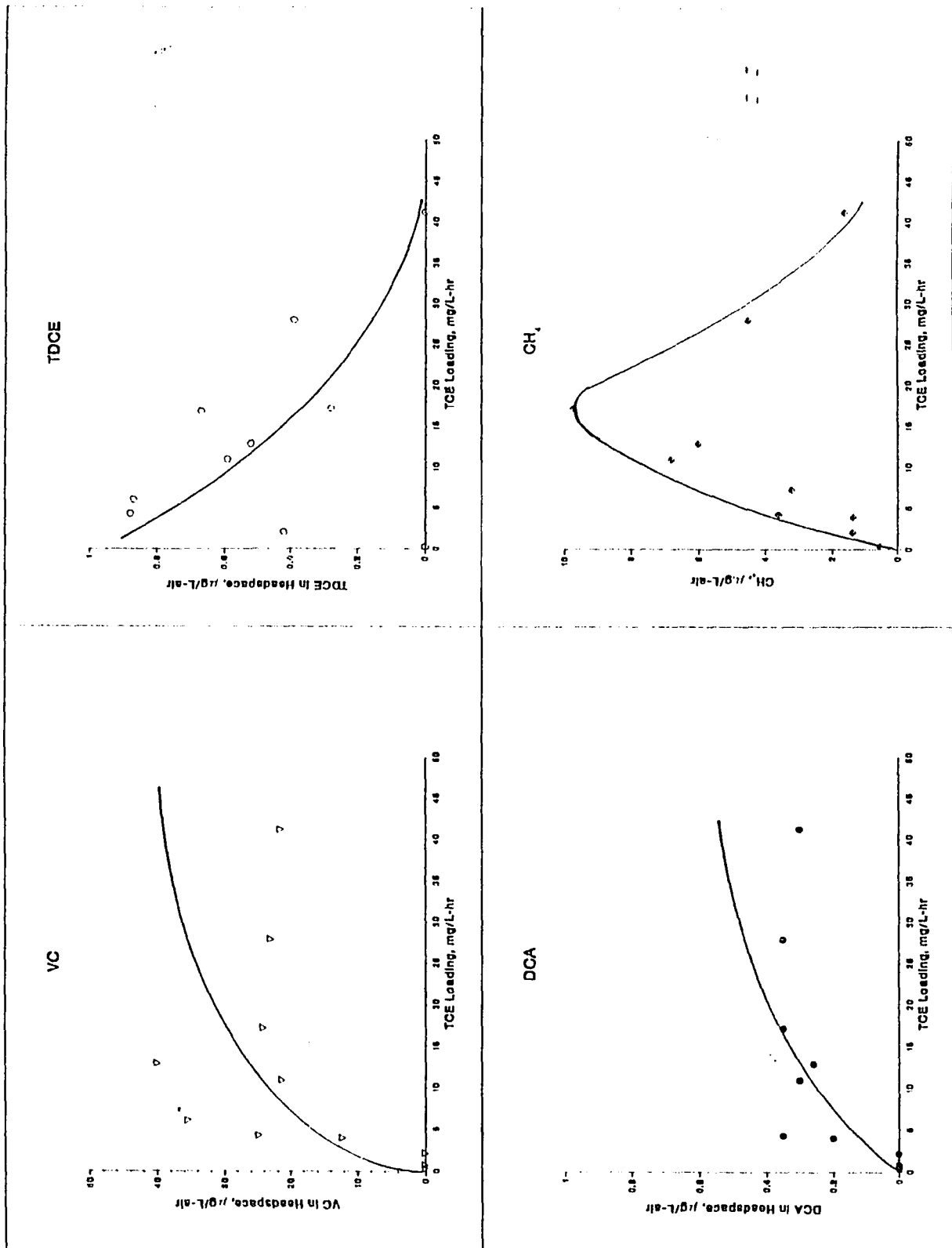
FIGURE 16   EFFECT OF TCE LOADING ON GAS COMPOSITION

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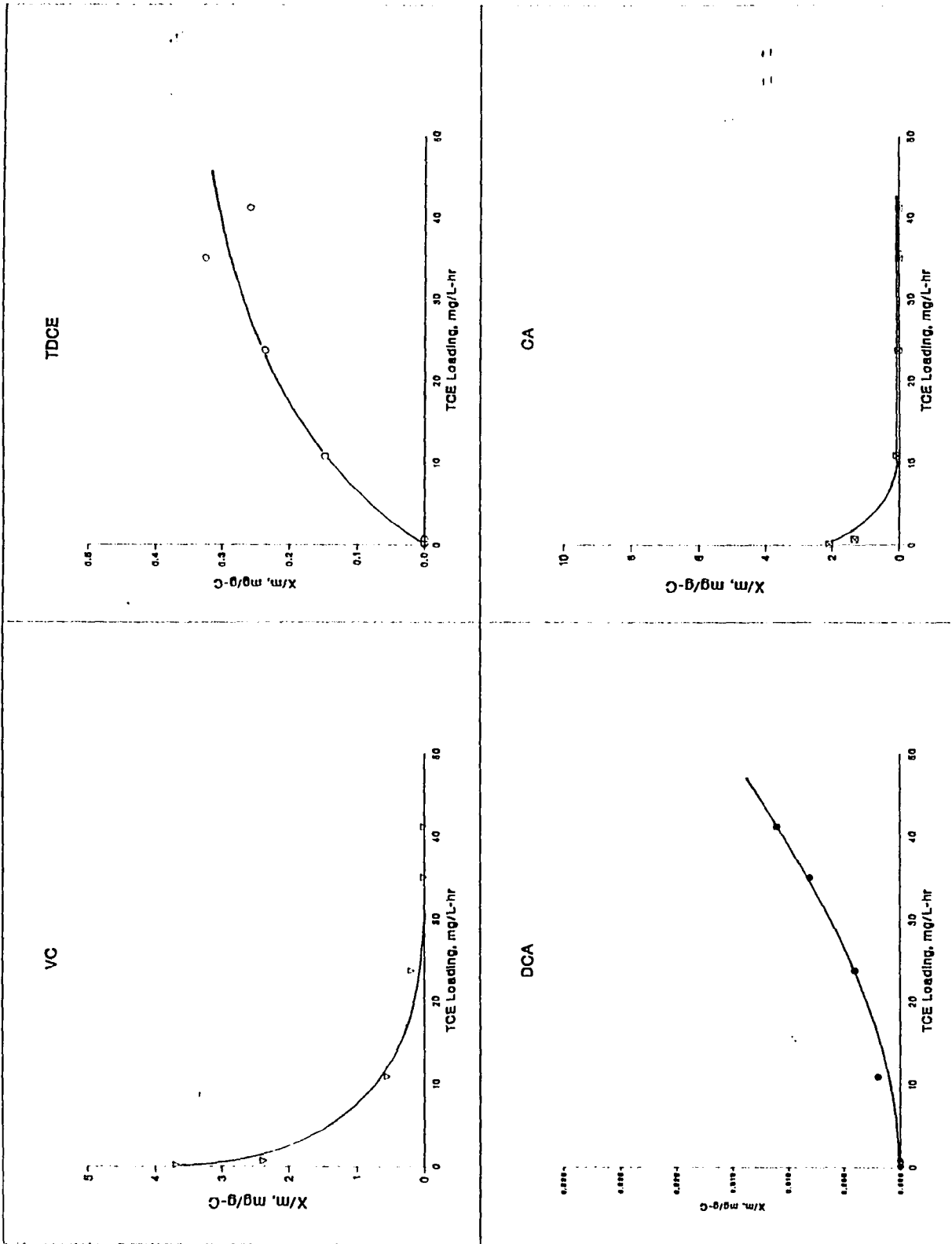
FIGURE 17 COMPOSITION OF VOCs ADSORBED ON GAC PARTICLES

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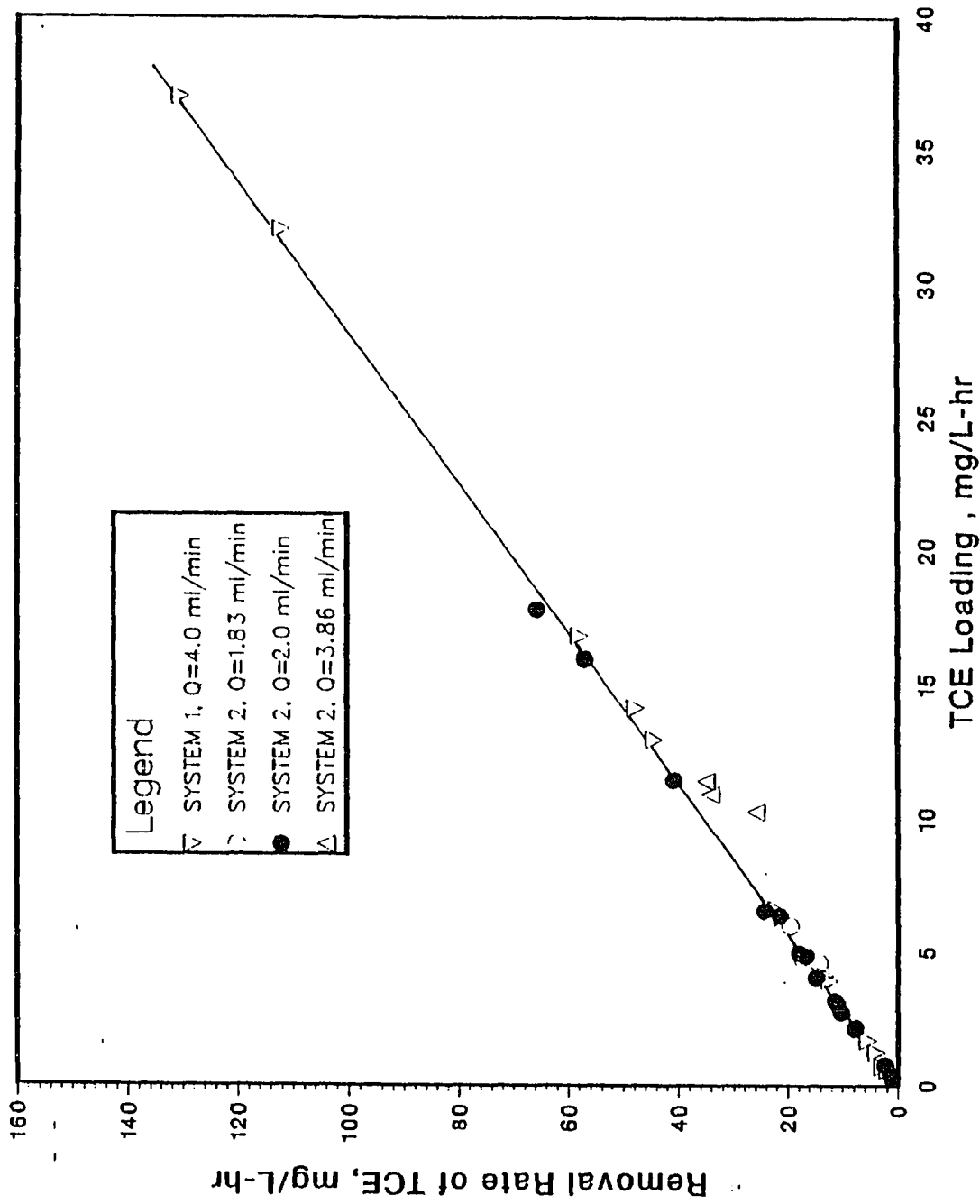
FIGURE 18   EFFECT OF TCE LOADING ON REMOVAL RATE OF TCE

Table 11: Effect of TCE Loading on the TCE Removal Results (1)

System 1 : Stage 1

Pumping Rate Q = 3.86 - 4.0 ml/min

Recycle Rate Qr = 793 ml/min

Inlet		Outlet			TCE Removal Rate	
Loading [mg/L-hr]	TCE [mg/L]	TDCE	DCA 0.01[mg/L]	TCE	[mg/L-min]	[mg/L-hr]
0.61	0.038	0.20	nd	0.70	0.031	1.86
0.71	0.048	-	-	0.85	0.048	2.88
1.24	0.079	0.35	nd	1.30	0.066	3.96
1.67	0.106	-	nd	1.60	0.090	5.40
3.94	0.249	0.51	nd	4.20	0.207	12.42
4.13	0.261	1.77	nd	4.10	0.220	13.20
6.30	0.398	2.05	nd	4.90	0.349	20.94
6.60	0.418	2.36	nd	5.00	0.368	22.08
10.27	0.650	5.00	nd	10.80	0.432	25.92
10.92	0.691	3.10	nd	12.10	0.570	34.20
11.41	0.722	3.80	nd	13.80	0.584	35.04
12.94	0.818	2.19	nd	7.70	0.741	44.46
14.13	0.894	3.16	nd	8.60	0.797	47.82
16.80	1.063	3.00	nd	9.60	0.967	58.02
32.04	2.028	-	nd	15.70	1.871	112.26
37.02	2.341	3.33	nd	16.20	2.179	130.74

Table 12:   Effect of TCE Loading on TCE Removal Results (2)

System 2   :   Stage 1

Pumping Rate Q = 1.83 - 2.0 ml/min

Recycle Rate Qr = 793 ml/min

Inlet		Outlet			TCE Removal Rate	
Loading [mg/L-hr]	TCE [mg/L]	TDCE	DCA 0.01[mg/L]	TCE	[mg/L-min]	[mg/L-hr]
4.61	0.292	nd	nd	1.40	0.238	14.28
4.80	0.305	nd	3.90	1.50	0.290	17.40
6.00	0.382	nd	nd	5.50	0.327	19.62
0.33	0.021	nd	nd	0.20	0.019	1.14
0.42	0.026	nd	0.20	0.30	0.023	1.38
0.76	0.048	nd	0.50	0.80	0.040	2.40
2.10	0.133	nd	-	0.81	0.129	7.74
2.17	0.137	nd	2.00	0.83	0.129	7.74
2.73	0.173	nd	-	0.20	0.171	10.26
3.07	0.194	nd	2.50	1.00	0.184	11.04
3.17	0.201	nd	-	1.20	0.189	11.34
4.04	0.255	nd	-	0.70	0.248	14.88
4.85	0.307	nd	-	3.00	0.277	16.62
4.96	0.313	nd	3.70	1.60	0.297	17.82
6.36	0.402	nd	2.70	3.50	0.357	21.42
6.55	0.414	nd	2.50	1.00	0.404	24.24
11.42	0.723	nd	4.80	4.70	0.680	40.80
15.93	1.007	nd	-	5.60	0.951	57.06
17.77	1.124	nd	3.10	2.80	1.096	65.76

Effect Of Methanol On Gas Composition

Since the solvent methanol (MeOH) added to the system also underwent anaerobic degradation, its presence in the system could have either positive or negative effect on the biotransformation of TCE. While Wackett and Gibson [69] found no evidence of enhanced TCE degradation by the aerobic pure culture P. putida F1 with the addition of MeOH, Fathepure et. al. [75] found maximum TCE degradation under reductive environment using methanol in place of acetate as the growth substrate. This was attributed to the higher reducing equivalents (6) gained during the metabolism of methanol than that of acetate (2 reducing equivalents). Baek and Jaffe [33] also reported a maximum production of VC with medium containing 1% methanol.

In our study, the effect of MeOH on the process performance was studied under constant glucose loading. As shown in Figures 19 and 20, with an increase in the MeOH/TCE ratio, there was a continuous decline in the production of VC accompanied by an increase in the levels of both CA and DCA in the aqueous phase. This seems to imply that MeOH enhanced biodegradation process, leading to a more complete dechlorination of TCE to CA. However, beyond a ratio of about 8, the concentrations of CA and DCA decreased with increasing ratio. This is due to a decrease in the TCE concentration. The abrupt increase in the CH_4 concentration
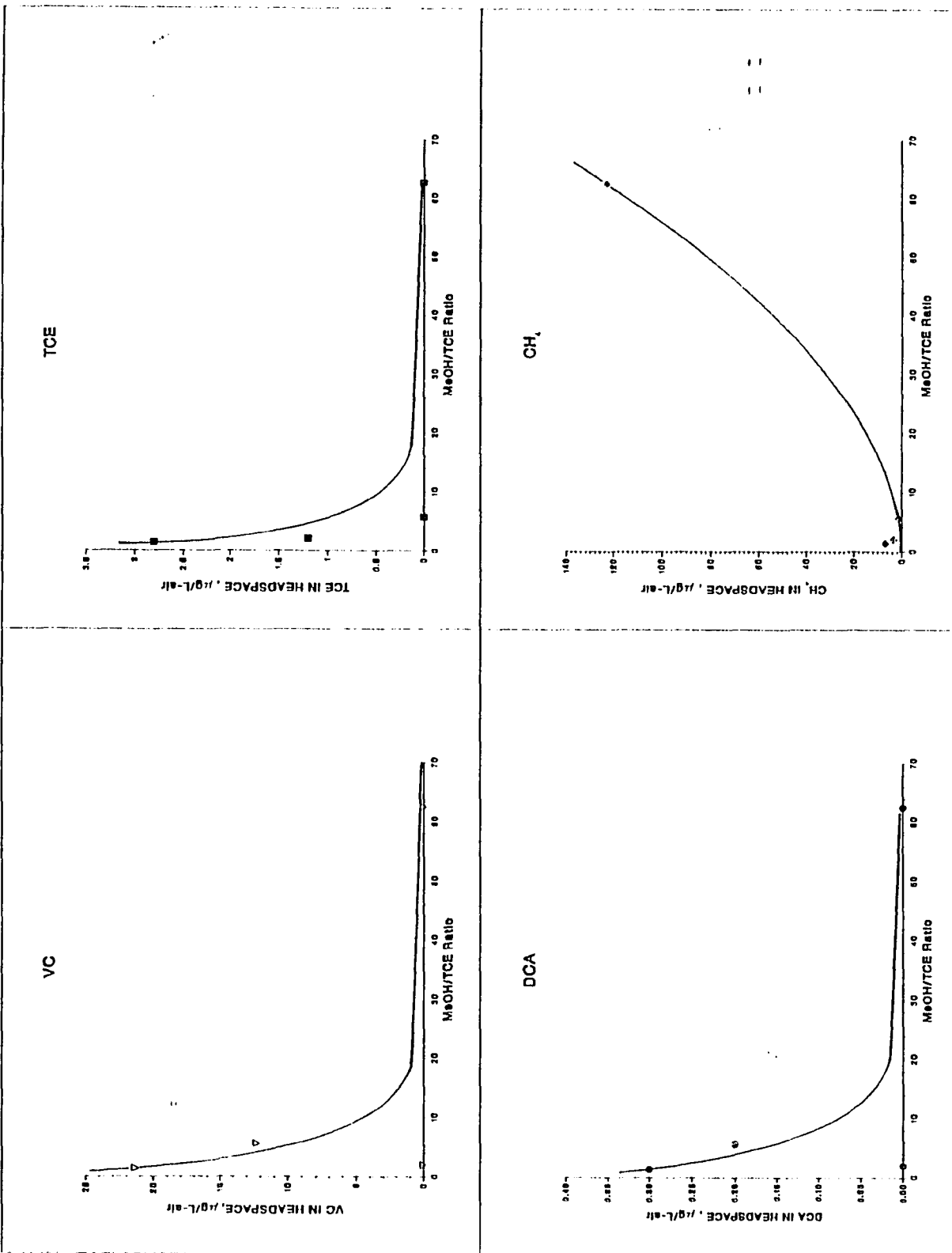
FIGURE 19 EFFECT OF MeOH/TCE RATIO ON GAS COMPOSITION

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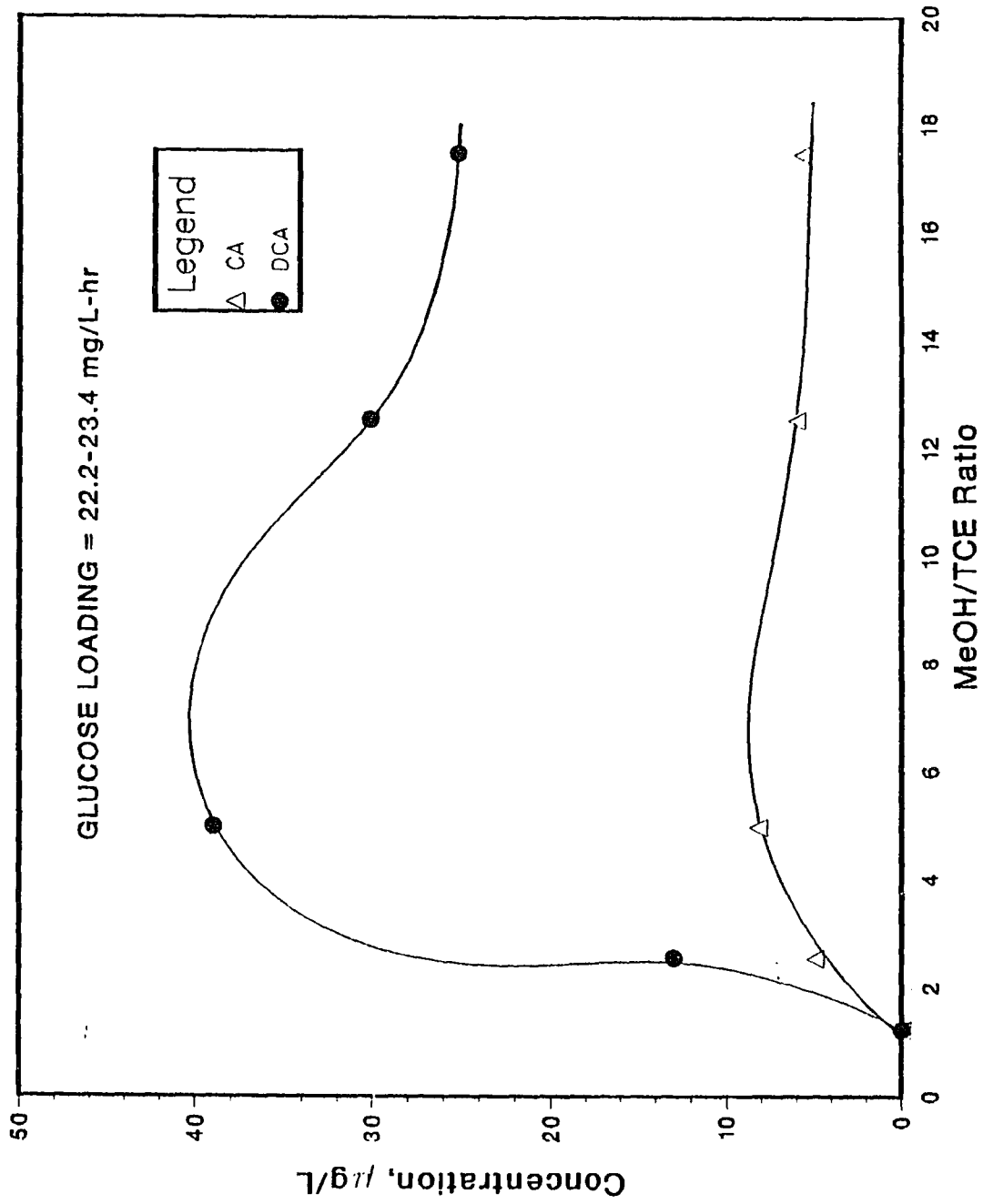
FIGURE 20 EFFECT OF MeOH/TCE RATIO ON EFFLUENT COMPOSITION

in the headspace is attributed to the high concentration of MeOH in the feed.

Evidence Of Biologically-Mediated Transformation

The fact that the degradation attained in this study was biologically mediated had been confirmed by carrying out tests on a blank column (designated System 0) containing only activated carbon and no microorganisms. It can be seen from Table 13 that under similar conditions, no methane was produced in System 0, while methane was detected for all gas samples collected from System 1. The TCE concentration in the gas sample from both systems did not change significantly, indicating that the fraction of TCE that volatilized to the headspace of the column would be the same in both seeded and unseeded systems provided the operating conditions were kept the same.

In the liquid samples, TDCE was not detected in the blank until visual observation indicated apparent growth of microorganisms on the activated carbon particles. See Figure 21. The TDCE level at this time, however, was always orders of magnitude lower than that in System 1, indicating further that transformation of TCE to TDCE in the seeded columns was indeed biologically mediated. Moreover, the TDCE in the effluent decreased to undetectable value when high dosage of sodium azide was introduced to System 0 in an attempt to kill the microorganisms. On the other hand, the

Table 13: Comparison Between Seeded And Unseeded Systems

TCE Loading	Unseeded		Seeded	
mg/L-hr	CH_4	TDCE	CH_4	TDCE
10.72	nd	nd	–	0.050
10.79	nd	nd	–	–
10.92	–	–	6.83	0.031
11.41	–	–	–	0.038
13.67	nd	nd	–	–
18.67	–	–	5.91	–
23.61	nd	0.02	–	–
27.90	–	–	4.52	0.100
39.21	nd	nd	–	0.203
43.72	–	nd	3.19	0.297
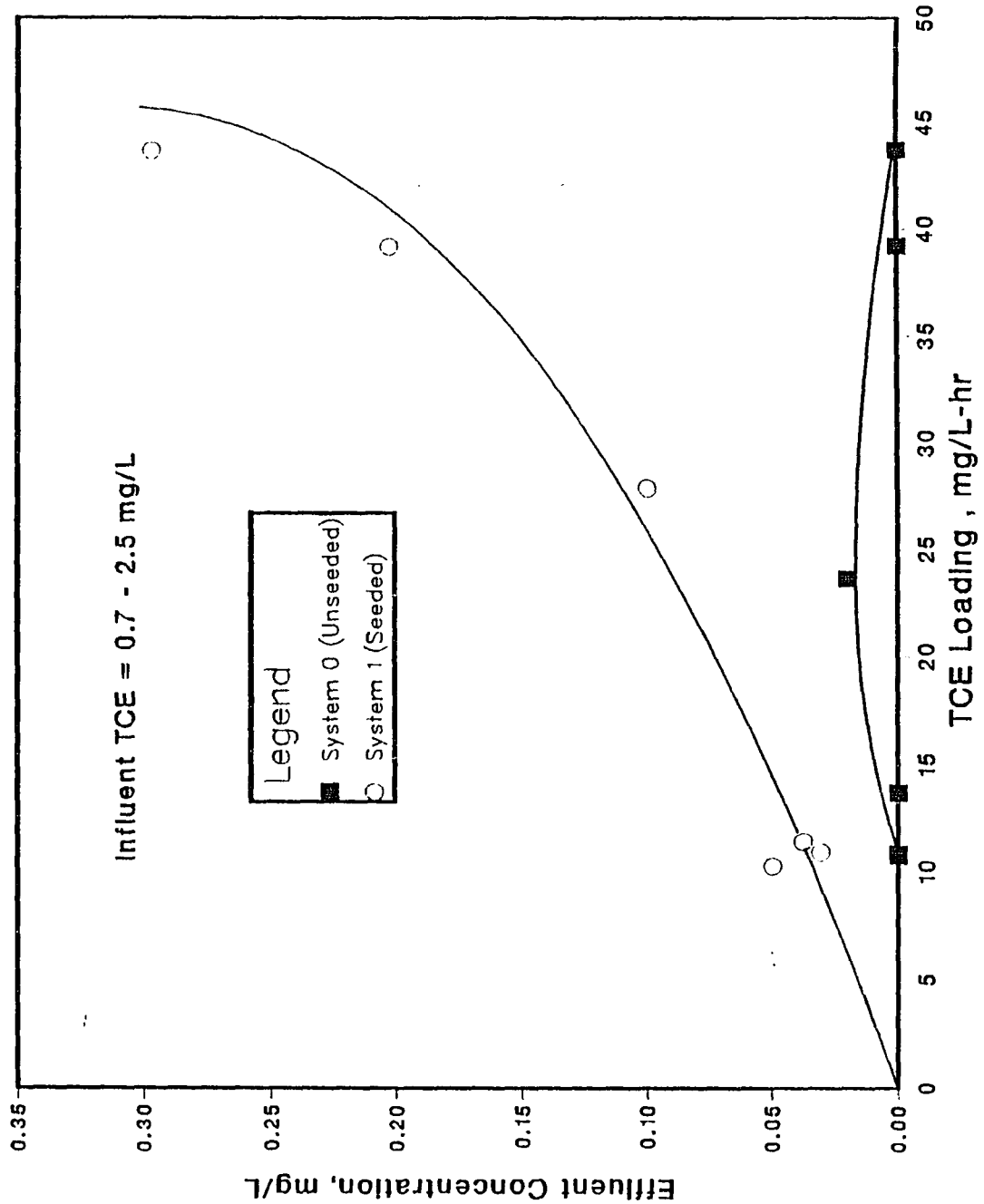
FIGURE 21 COMPARISON OF EFFLUENT TDCE IN SEEDED AND UNSEEDED SYSTEMS

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effluent TCE concentration in System 0 was always less than that in System 1, as shown in Figure 22, because System 0 was started months later than System 1 so that most of the TCE was adsorbed on the fresher activated carbon particles, which had not yet attained saturation at the time of the experiment.

Figure 23 is a comparison of the amount of TCE detected on the GAC particles in the seeded and unseeded systems. It must be noted that at low TCE loadings the residual TCE that remained unaltered on the GAC of the seeded system is small compared to the unseeded system where the TCE removal is merely through adsorption. Most of the TCE initially adsorbed in the seeded system were biotransformed into other products. (See section on "Effect Of Loading"). At high TCE loadings the rate of transformation reaction has become controlling, so accumulation of residual TCE is inevitable. If the trend continues to follow the path as illustrated in the figure and the loading is raised to an excessively high level so that the curve for the seeded systems intersects that for the unseeded system, then the GAC surface is said to be devoid of active biomass and the mechanism of TCE removal will likely be that of adsorption on the GAC alone. There are two possible reasons for this: First, impairment of microbial activity due to extreme toxicity of TCE at high loading. Second is that the microbial film is extremely thin, e. g., 1 micron, which corresponds to a monolayer
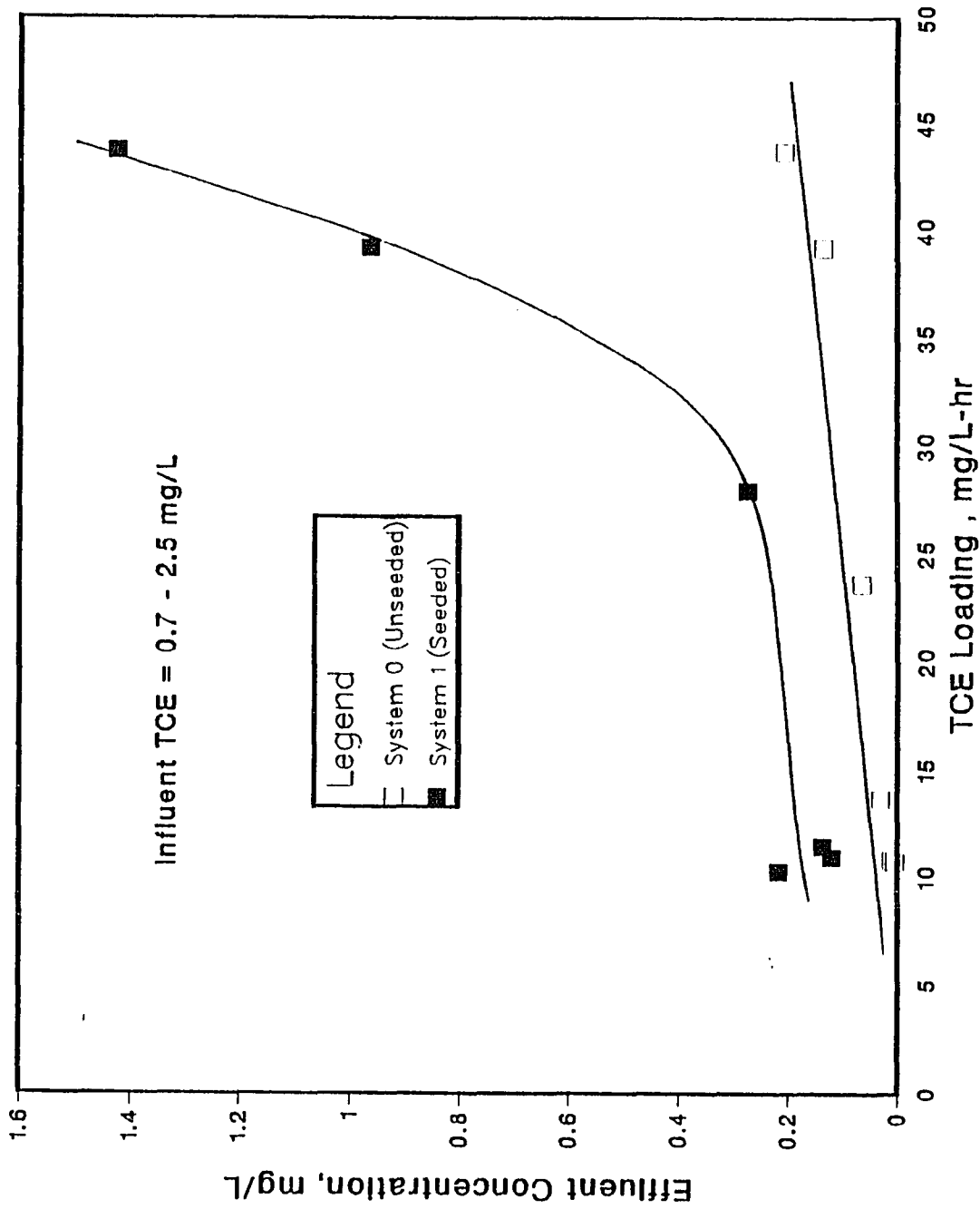
FIGURE 22   COMPARISON OF EFFLUENT TCE IN SEEDED AND UNSEEDED SYSTEMS

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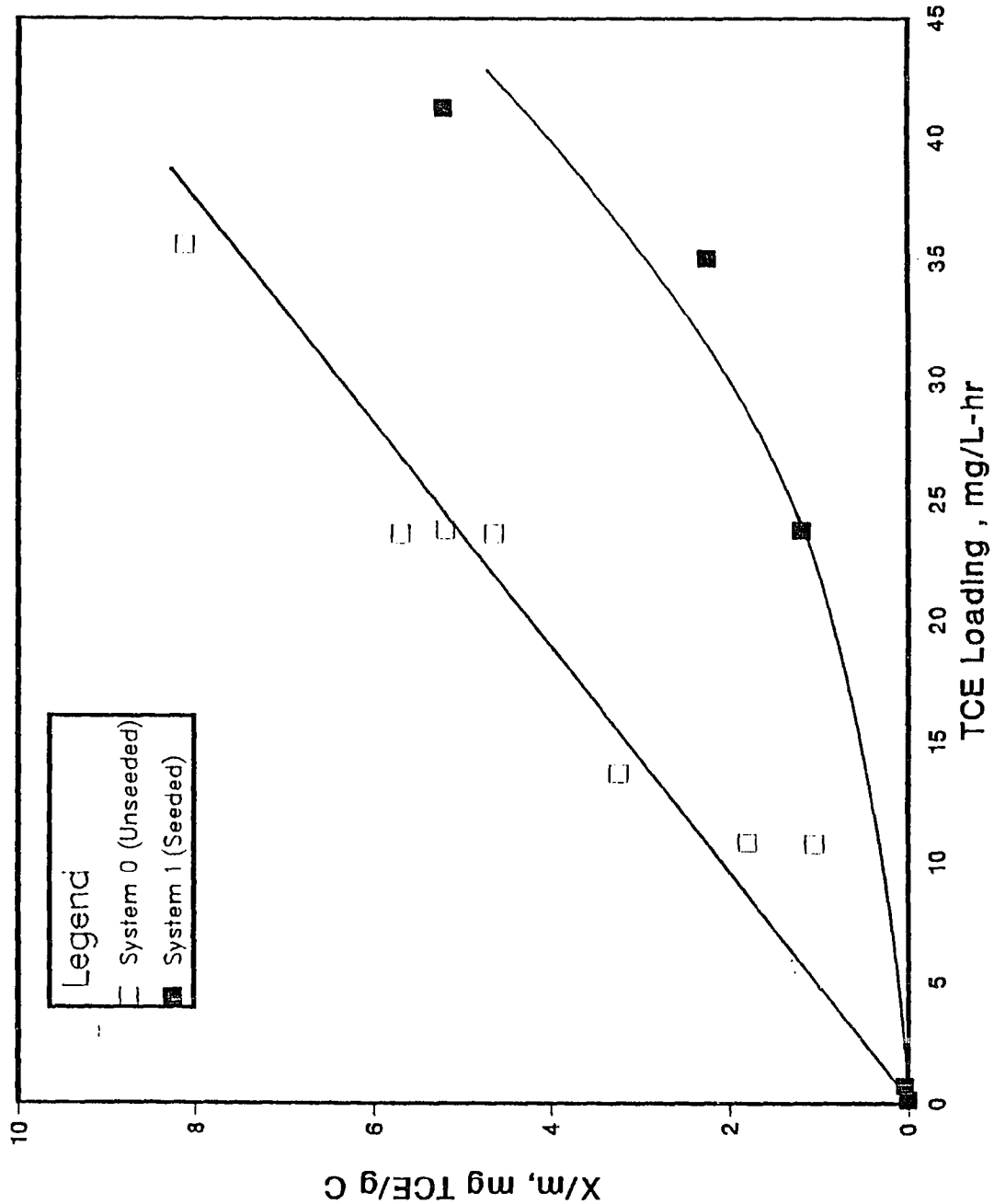
FIGURE 23 COMPARISON OF ADSORBED AND RESIDUAL TCE ON GAC PARTICLES

X/m, mg TCE/g C

TCE Loading , mg/L-hr

Legend
System 0 (Unseeded)
System 1 (Seeded)

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coverage of the surface. In their development of a predic-
tive model for a continuous stirred tank fermenter (CSTF),
Atkinson and Davies [83] stated that in the case of thin
microbial films, as indicated by the value of the dimension-
less parameter

$$B = (A/V) \ (d_b \ k_3/Y) \ L$$

where A = area of the film, V = liquid volume in fermenter,
k_3 = biological rate equation coefficient, Y = yield coeffi-
cient, L = film thickness, the larger the influent concen-
tration the closer the approximation to the simple CSTF
theory, that is, ignoring the contribution of the microbial
film to the fermenter performance.

However, in a completely mixed microbial film fermenter
(CMMFF) such as the FBBR used in the present study, due to
the large surface area, B can never be zero unless the
surface is sterile. In fact, the value of B in our case can
be estimated as follows: A = 325 g x 1000 m^2/g [84], V = 3
L (neglecting the volume occupied by GAC particles), d_b =
1.03 g/ml, k_3 = 1.7 x 10^5 ml/g, Y = 0.5 [85]. Therefore, B
= 379 x 10^5. This indicates that the contribution of the
microorganisms in the present work can be neglected only
when inhibition is incurred at extremely high TCE loading.

Effect Of Glucose/TCE Ratio

As stated before, many recalcitrant organic compounds

were found to be biodegradable when sugar was added into the growth medium [50, 86]. The same effect was found in case of the biodegradation of TCE [14,33]. However, it is not clear until now whether glucose has acted as a co-substrate in effecting co-metabolism of TCE or as a pregrowth substrate for the induction of the pertinent enzyme needed in the decomposition of TCE. Most anaerobic degradative pathways for TCE point to the reductive dechlorination reaction whereby the chlorine in the TCE is replaced sequentially by hydrogen. Hydrogen is a strong electron donor, and TCE can be the electron acceptor. Hydrogen has also been implicated as being the source of energy in attaining dehalogenation of many haloaromatic and haloaliphatic compounds [87, 72]. In the most recent work on the anaerobic biodegradation of TCE, hydrogen was shown to be responsible in the cleavage of the double bond in VC, leading to the production of the single-bonded CA [33]. The source of hydrogen, however, is not exactly known. Nonetheless, the production of hydrogen from the anaerobic fermentation of a wide spectrum of hydrocarbons, of which glucose is one of the most readily degradable, is well documented [88]. Therefore, if glucose is selected as the source of hydrogen in effecting the reductive dechlorination of TCE, the concentration of glucose in the system is expected to have a significant influence on the composition of the finished water and thus the process performance.

When all the other factors in the system are kept constant, the ratio of glucose loading to that of TCE is the parameter used to describe the above effect. For the present study, the influent glucose concentration has not been altered in the course of the experiments. Therefore, the variation in the glucose/TCE ratio (Hereafter referred to as 'ratio') is in fact a measure of the change in TCE concentration relative to that of glucose in the feed solution.

Figures 24 and 25 showed that there was a decrease in the concentration of effluent TCE as well as TDCE with an increase in the ratio for System 1. Figures 26 and 27 are similar plots for System 2, in which the same conditions have been maintained except for the feed rate: System 2 was fed at half the rate of System 1. It is noted that although the general trend is the same as in System 1, i.e., there is a decrease in the effluent concentrations of TCE and intermediate product with an increase in the ratio, TDCE has not been detected in this system. Instead, DCA has been indentified as the major intermediate in the liquid phase. The production of CA in both systems followed the same trend, i. e., CA decreased initially with the ratio; it started increasing with the ratio at the ratio of 4 and then levelled off, as depicted in Figure 28. The effluent TCE concentration for System 1 was almost always three times more than that of System 2, as shown in Figures 24 and 26.
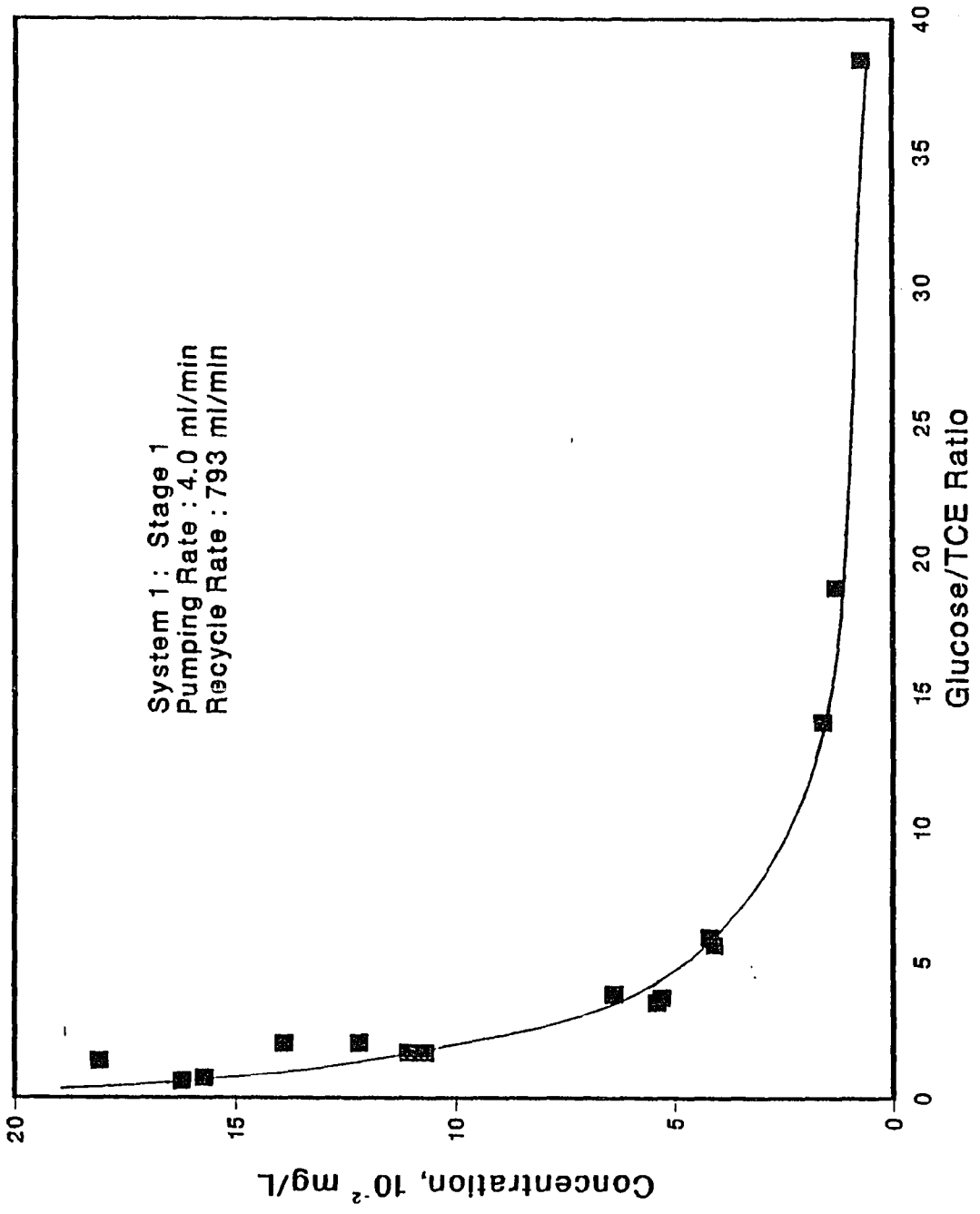
FIGURE 24   EFFECT OF GLUCOSE/TCE RATIO ON EFFLUENT TCE CONCENTRATION (1)
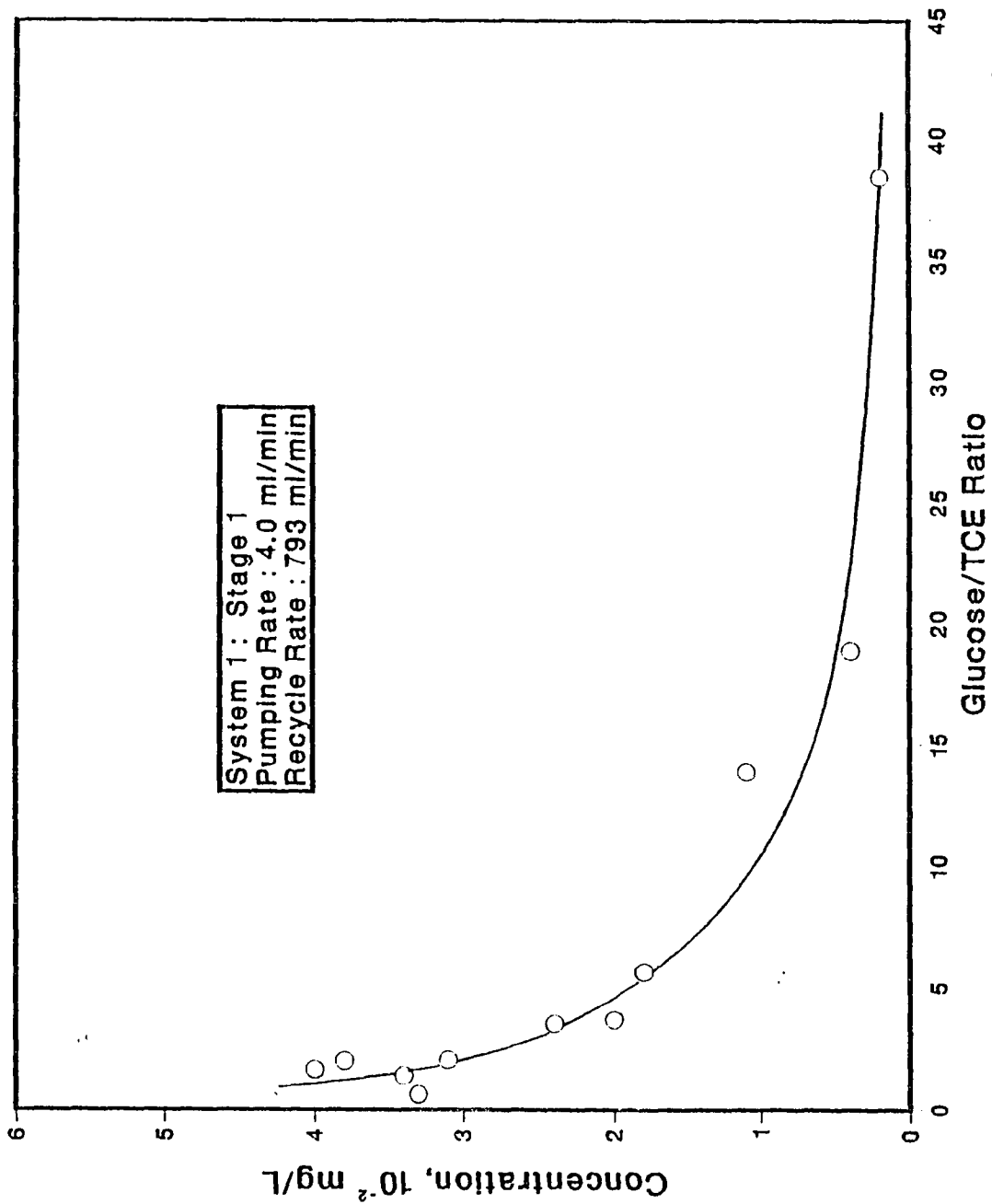
FIGURE 25   EFFECT OF GLUCOSE/TCE RATIO ON EFFLUENT TDCE CONCENTRATION

System 1 : Stage 1
Pumping Rate : 4.0 ml/min
Recycle Rate : 793 ml/min

Glucose/TCE Ratio

Concentration, 10^{-2} mg/L
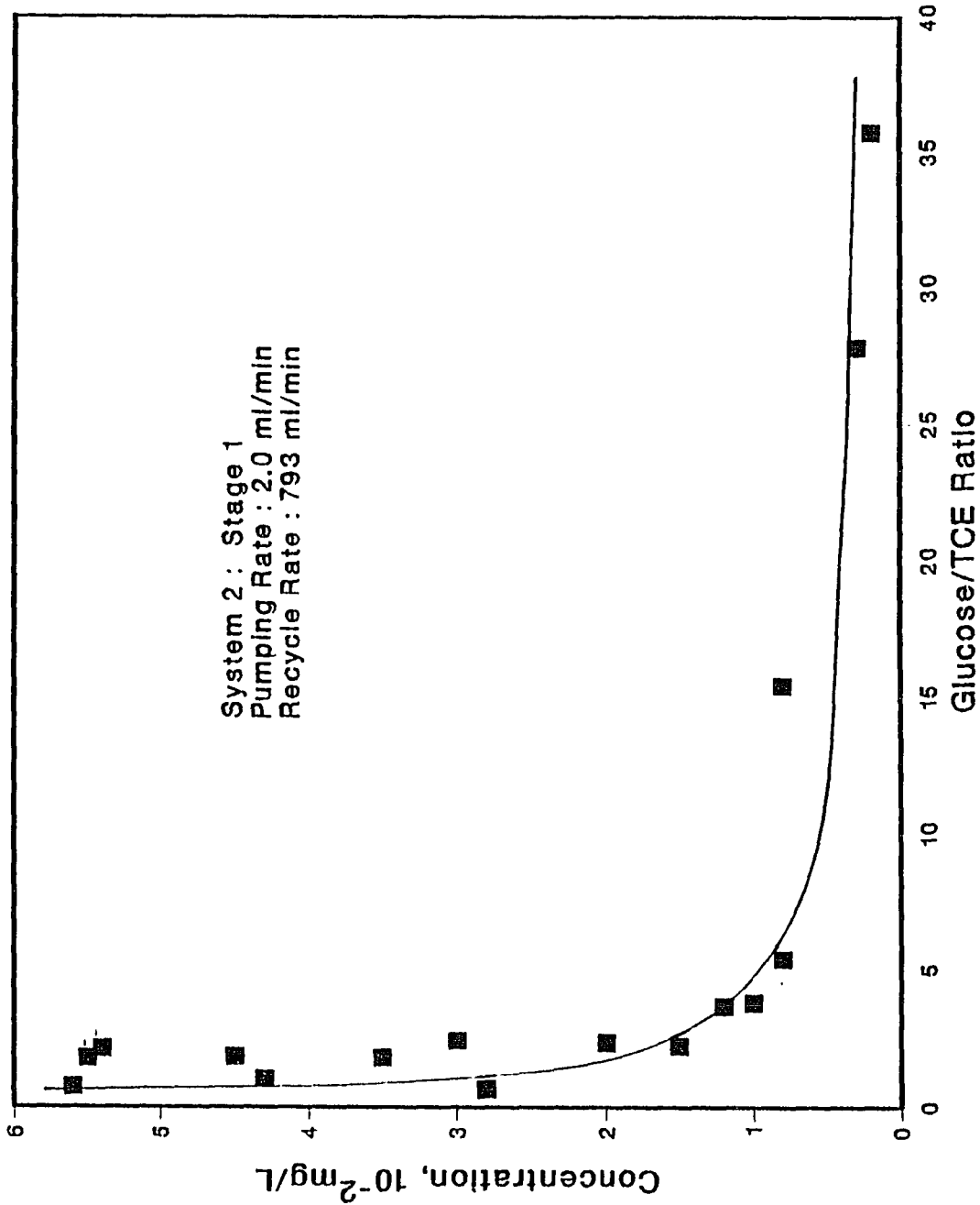
FIGURE 26   EFFECT OF GLUCOSE/TCE RATIO ON EFFLUENT TCE CONCENTRATION (2)
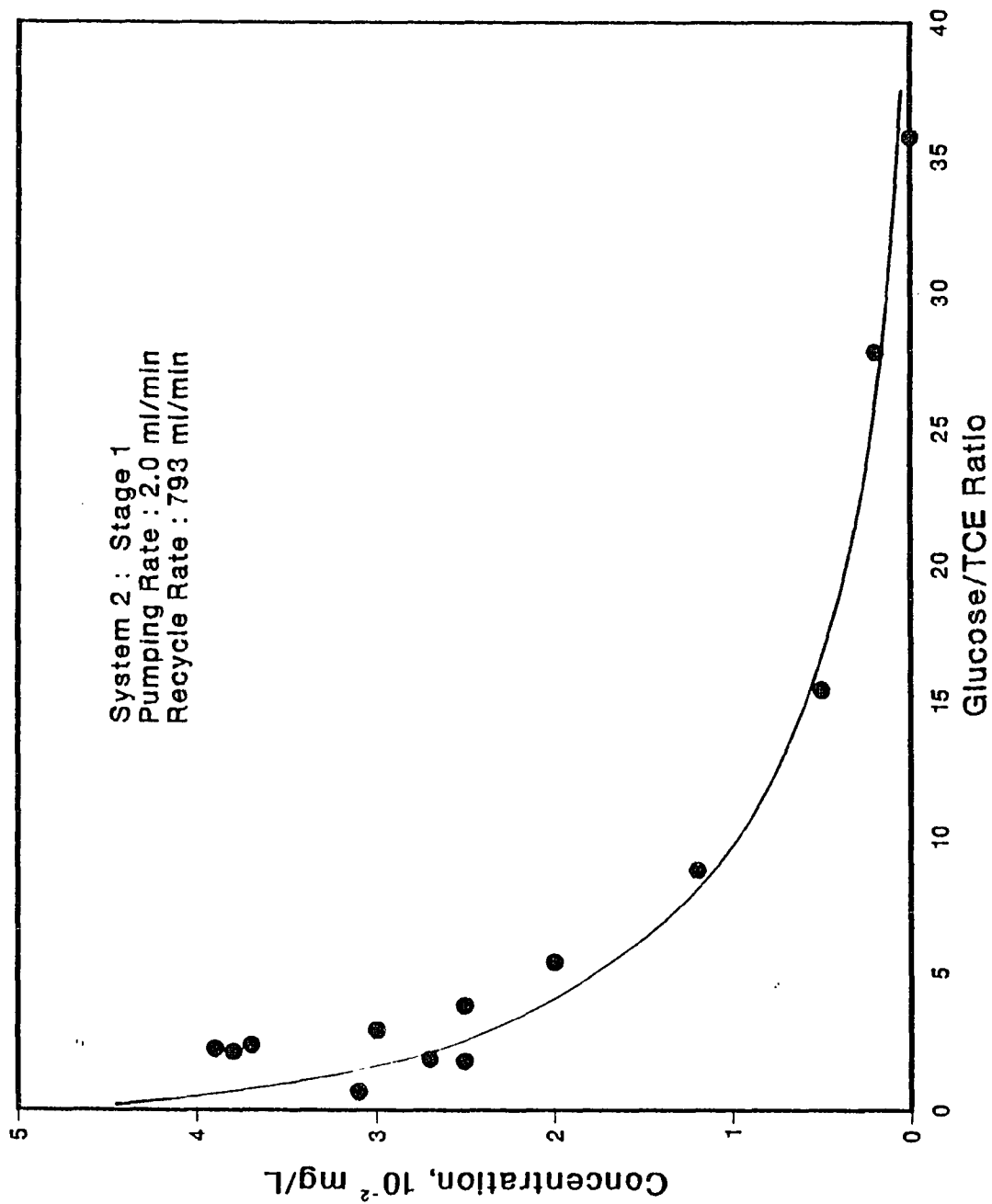
FIGURE 27   EFFECT OF GLUCOSE/TCE RATIO ON EFFLUENT DCA CONCENTRATION
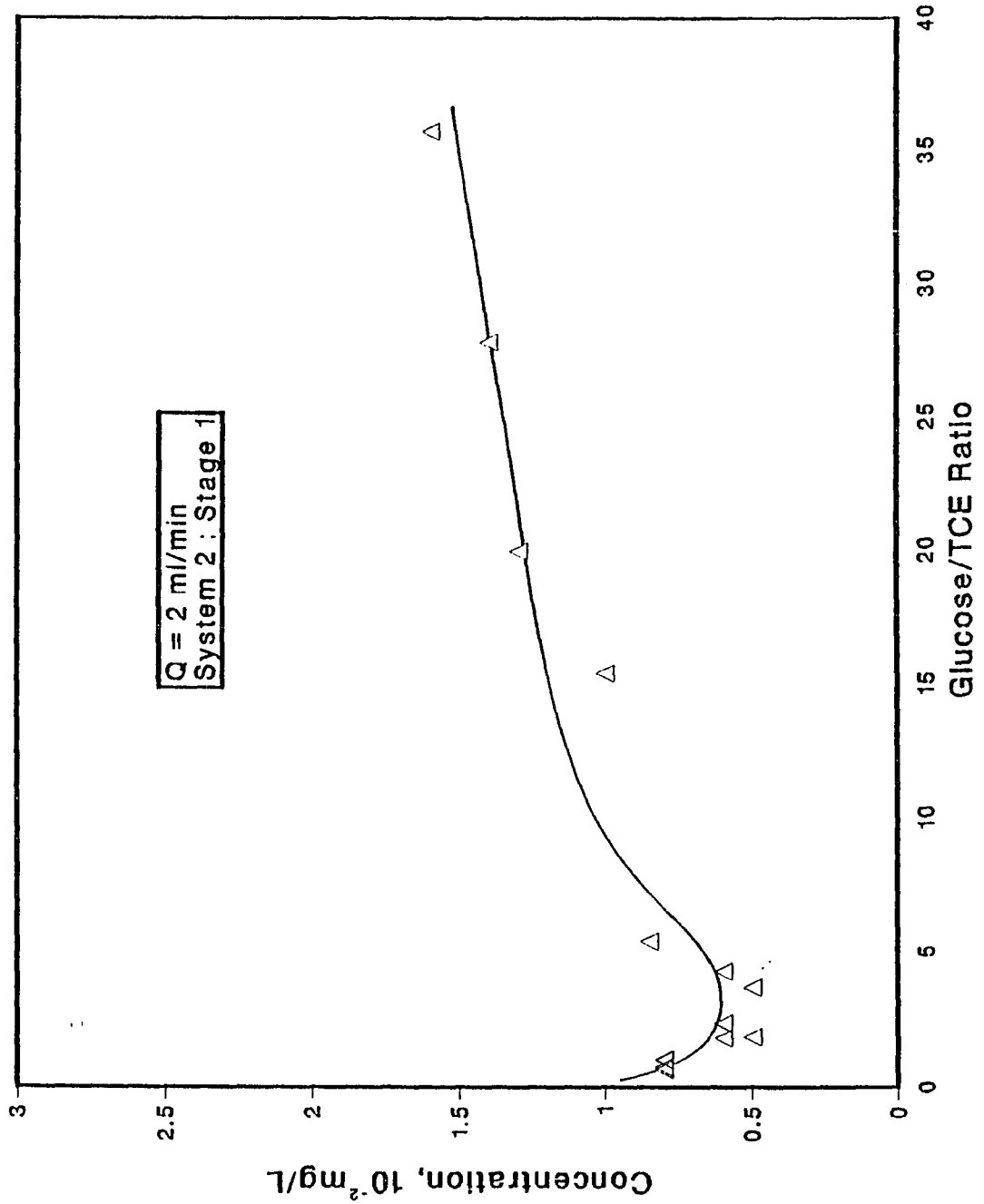
FIGURE 28   EFFECT OF GLUCOSE/TCE RATIO ON EFFLUENT CA CONCENTRATION

For the percent reduction of TCE, the two systems have the same trend, i. e., a region where no effect of the ratio was observed followed by a significant increase of reduction with decreasing ratio. (See Figures 29 and 30). This is due to the elevated TCE concentration in the solution at low ratio values, which resulted in a greater driving force towards the further transformation of the reactant TCE and subsequent production of more intermediate compounds. However, System 2 performed better, with a percent reduction of over 87 and reaching a maximum of as high as 98%. The percent reduction attained in System 1 ranged from 80 to 95%. The foregoing further reiterated the important role played by the feed rate, Q, on the process performance.

One of the reasons behind the phenomenon of decreasing effluent concentration with increasing ratio is quite obvious: At a higher ratio, i.e., at a lower influent TCE concentration, less TCE was available for transformation to TDCE and less TCE eluting from the system. However, careful examination of the resulting composition of the gas samples obtained from the reactor headspace (Figure 31) revealed that the effect of the ratio is not as simple as it may first appear. Although there was an initial decline in the concentration of VC as the ratio increased from about 2 to 5.5, an abrupt increase in its concentration was registered beyond the ratio of 5.5. This strongly implies that there is a certain range of the ratio within which optimal performan-
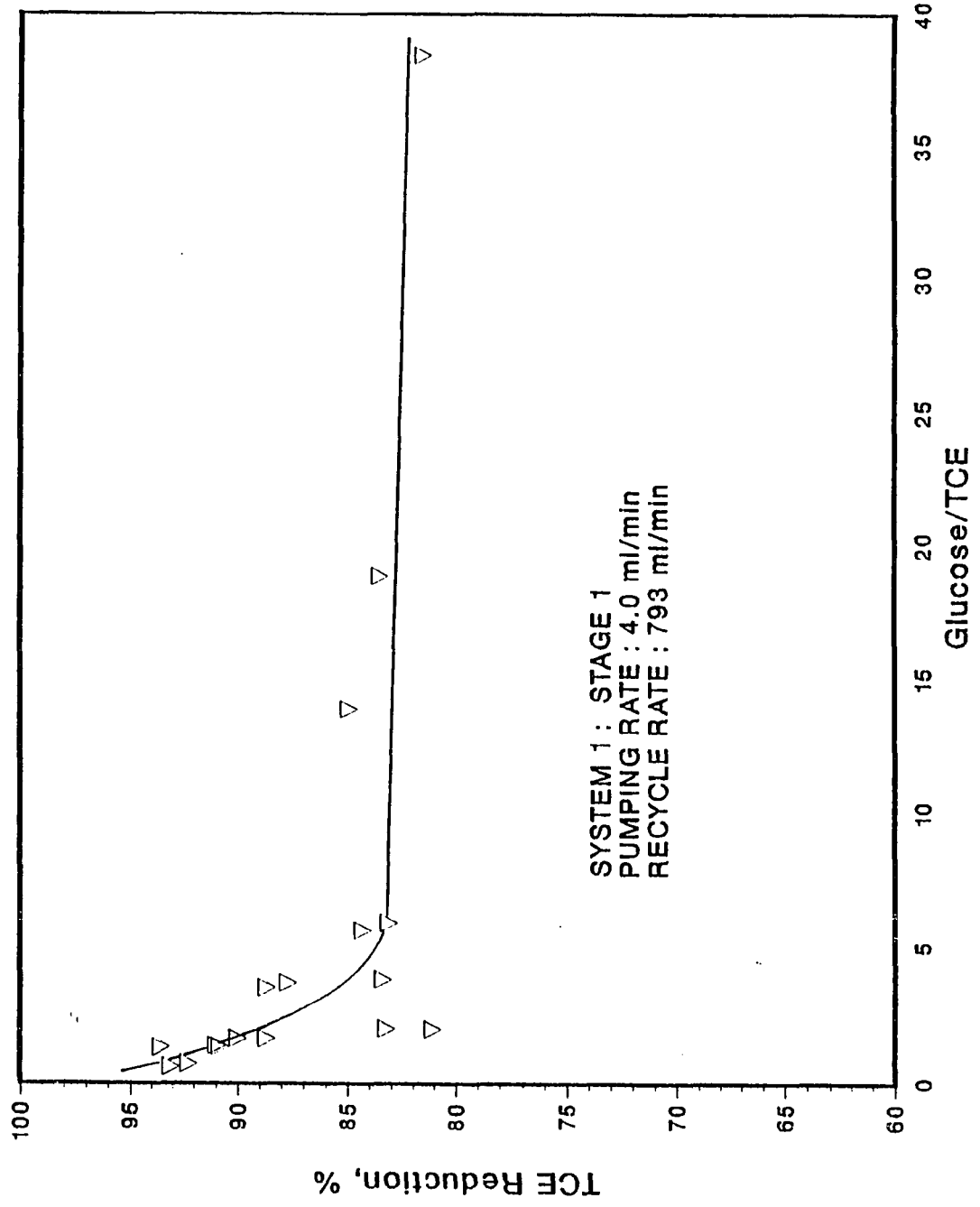
FIGURE 29 EFFECT OF GLUCOSE/TCE RATIO ON TCE REDUCTION (1)

SYSTEM 1 : STAGE 1
PUMPING RATE : 4.0 ml/min
RECYCLE RATE : 793 ml/min

Glucose/TCE
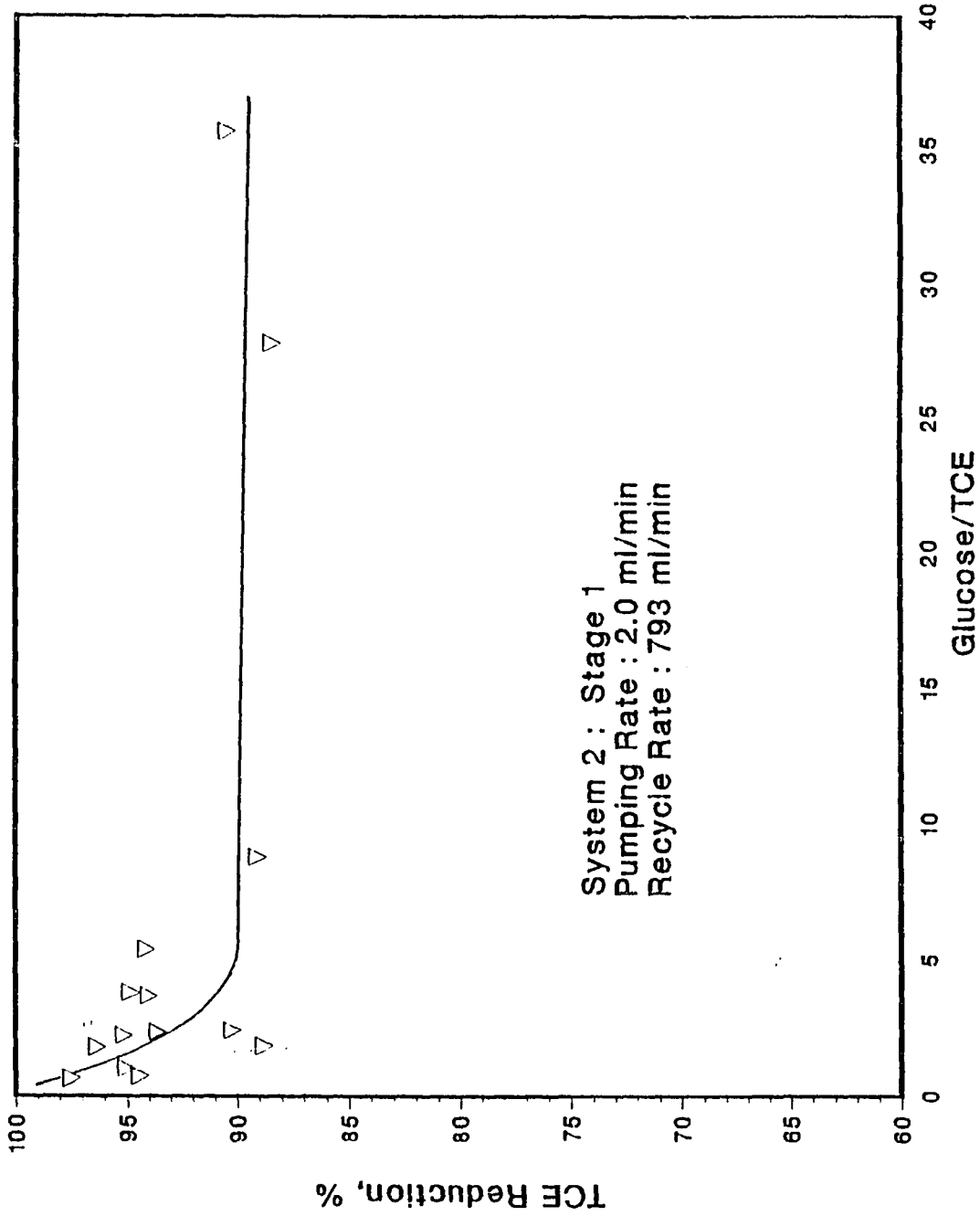
TCE Reduction, %

FIGURE 30   EFFECT OF GLUCOSE/TCE RATIO ON TCE REDUCTION (2)

System 2 : Stage 1
Pumping Rate : 2.0 ml/min
Recycle Rate : 793 ml/min

Table 14: Effect of Glucose/TCE Ratio on Effluent Composition (1)

System 1:   Pumping Rate, $Q = 4.0$ ml/min
                                   Recycle Rate, $Q_r = 793$ ml/min

G/TCE	Sid	TCE(1)	Reduction	TDCE(1)	TDCE(2)
mg/L	mg/L		% (TCE)	mg/L	mg/L
38.46	0.038	0.007	81.58	0.002	0.000
18.87	0.079	0.013	83.54	0.004	0.002
13.89	0.106	0.016	84.91	0.011	0.004
5.92	0.249	0.042	83.13	0.015	0.006
5.65	0.261	0.041	84.29	0.018	0.005
3.83	0.385	0.064	83.38	–	–
3.70	0.398	0.053	87.70	0.020	0.008
3.53	0.418	0.054	88.70	0.024	0.008
2.03	0.727	0.122	83.22	0.031	0.018
2.00	0.736	0.139	81.10	0.038	0.020
1.65	0.895	0.111	90.00	0.042	0.008
1.65	0.892	0.107	88.00	0.040	0.015
1.39	1.063	0.096	90.97	0.034	0.008
1.36	1.089	0.181	90.80	–	–
1.34	1.099	0.071	93.54	–	–
0.73	2.028	0.157	92.26	–	–
0.63	2.341	0.162	93.08	0.033	0.004

Note : (1) means first stage;
       (2) means second stage;
       G/TCE = Glucose/TCE Ratio.

Table 15: Effect of Glucose/TCE Ratio on Effluent Composition (2)

System 2:   Pumping Rate, Q = 2.0 ml/min

Recycle Rate, Q_r = 793 ml/min

Glucose/TCE	$(Si)_d$ mg/L	TCE(1) mg/L	Reduction, %	DCA(1) mg/L	CA(1) mg/L
35.71	0.021	0.002	90.48	0.000	0.016
27.78	0.026	0.003	88.46	0.002	0.014
15.38	0.048	0.005	89.50	0.005	0.010
8.76	0.220	0.024	89.10	0.012	–
5.38	0.137	0.008	94.16	0.020	0.008
3.80	0.194	0.010	94.84	0.025	0.021
3.68	0.201	0.012	94.03	–	0.005
2.40	0.307	0.030	90.22	–	–
2.35	0.313	0.020	93.61	0.037	0.006
2.22	0.310	0.015	95.16	0.039	–
2.11	0.320	0.040	87.50	0.038	–
1.83	0.402	0.045	88.80	0.027	0.005
1.78	0.414	0.035	96.36	0.025	0.006
1.77	0.380	0.040	89.47	0.025	–
1.02	0.723	0.043	94.05	–	0.008
0.73	1.007	0.056	94.44	–	0.008
0.66	1.124	0.028	97.51	0.031	0.008

MeOH Loading = 10.0 - 23.0 mg/L-hr
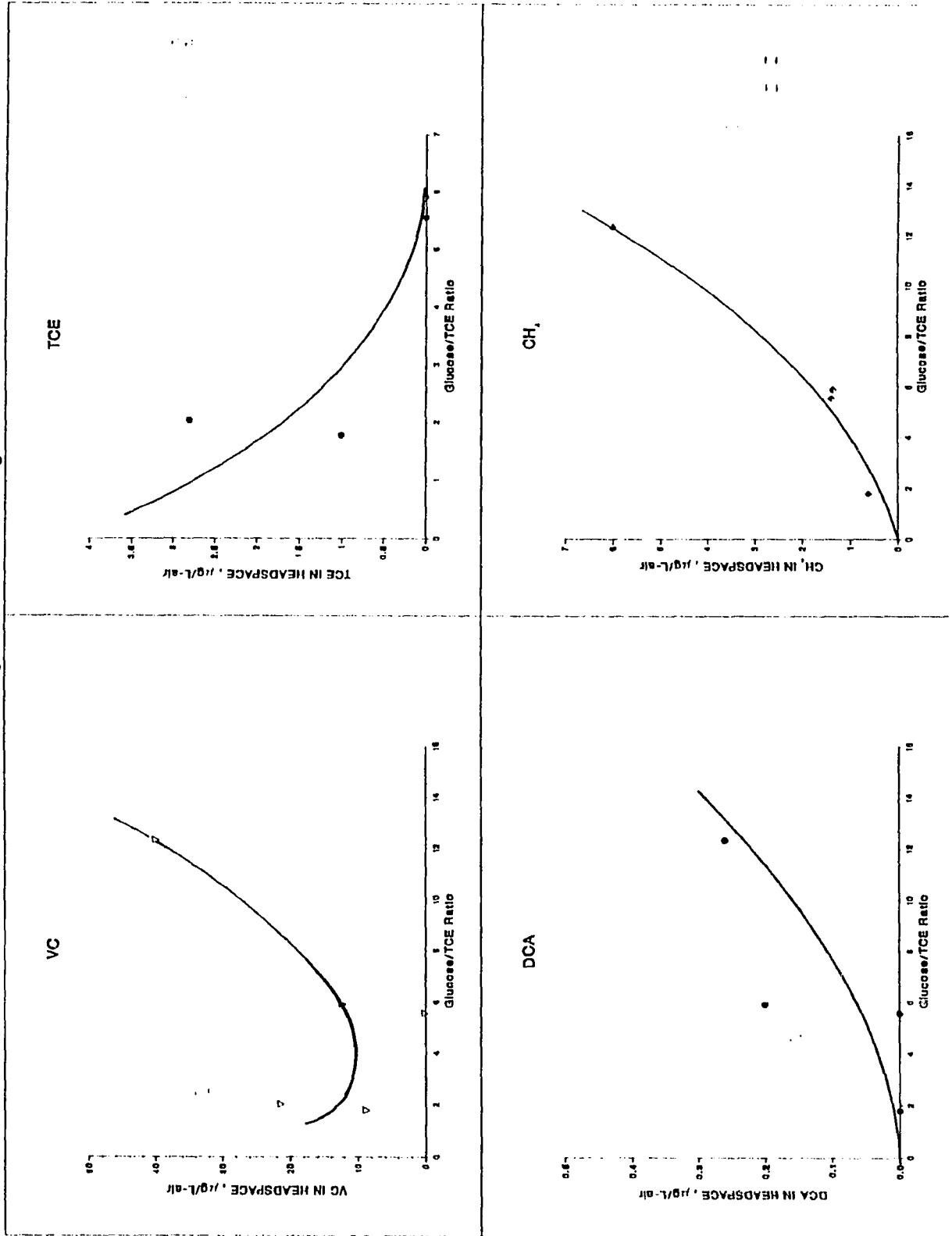
FIGURE 31 EFFECT OF GLUCOSE/TCE RATIO ON GAS COMPOSITION (STAGE 1)

Table 16:   Effect of Glucose/TCE Ratio On Gas Composition

MeOH Loading = 10 - 23 mg/L-hr

Glucose Concentration = 300 mg/L

Glucose/TCE	VC	TCE	DCA	CH_4
		[ug/L-air]		
1.78	8.80	1.00	nd	0.62
2.03	21.34	2.80	0.30	6.83
5.55	nd	nd	nd	1.40
5.91	12.30	nd	0.20	1.37
12.33	40.00	8.80	0.26	6.02

ce of the process can be achieved. As can be seen from Figure 27, any increase in the ratio beyond 5.5 does not contribute much to the process performance because the concentrations of both the reactant TCE and the intermediates reach asymptotic value and level off. On the contrary, ratio higher than 5.5 may be detrimental, for increasing production of VC has been detected as suggested in Figure 31. The production of methane increased gradually with increasing ratio due primarily to the increase in glucose concentration relative to that of TCE.

According to the proposed TCE degradation pathway, CA originates from VC and/or DCA. In both cases, three hydrogen are needed. However, the distribution of the required hydrogen is not the same for each step: When CA originates from DCA, two hydrogen are needed initially to rupture the double bond. This is followed by the attachment of one more hydrogen to form CA. On the other hand, when CA originates from VC, the sequence is reversed: One hydrogen is needed first in transforming TDCE to VC. Then two more hydrogen are used in breaking the double bond in the VC to form CA. It can be seen from Figure 28 that initially there is a decline in the concentration of effluent CA with increasing glucose/TCE ratio, the trend being the same as for the other intermediates, such as DCA in this system and TDCE in System 1. This is due to the decrease in the concentration of the reactant TCE with an increase in the ratio. With less reac-

tant available for reaction, less conversion to products resulted. In this region there is an accompanying decrease in the amount of VC detected in the headspace, as shown in Figure 31.

However, at a glucose/TCE ratio in excess of about 4, there is a gradual increase of effluent CA concentration as well as the VC level as the ratio increases while the level of DCA continues to decline. The increase in the glucose concentration has lead to the enhanced growth of the fermenters, which in their course of metabolism, release hydrogen to oxydize a reduced electron carrier such as the enzyme NADH$_2$. This hydrogen is utilized by the methanogens in dechlorinating TCE and the subsedquent intermediate products. Since in this region the DCA level dropped to near zero while the VC in the headspace increased markedly, it could be deduced that at elevated glucose concentration relative to TCE, the transformation to CA via DCA was favored over that via VC. Hence, circumstantial evidence exists which points to the role of glucose in effecting a biotransformation of TCE to CA via a route other than the conventional sequential reductive dechlorination. This is attributable to the more energy provided by the hydrogen at relatively high glucose/TCE ratio, which has effected the cleavage of the double bond in the DCEs at the early stage of the process.

In the author's opinion, from the standpoint of ease of degradability, DCA is preferred to VC. DCA has been found to be almost completely biodegraded to nonvolatiles such as chloride ions and carbon dioxide in both aerobic [89] and anaerobic environments [52].

Relative Importance Of Removal Pathways And Mass Balance

As stated before, there are three pathways whereby TCE can be eliminated from the aqueous phase in this process. The influent TCE first encountered the GAC bed where most of it was presumably adsorbed. This initial adsorption step served to concentrate the TCE for subsequent microbial utilization. However, due to the nature of the reaction, complete biotransformation of the TCE could not be achieved in the limited time of the experiment. Therefore some of the TCE would remain unchanged and be detected as residual TCE on the GAC particles. The fraction of TCE that did not adsorb would have escaped to the headspace of the column and thus could hardly be available for microbial action. Still another fraction of the TCE might escape adsorption and eluted with the effluent stream from the system. In order to obtain an assessment of the relative importance of the three removal pathways, the relative amounts of TCE in the different phases should be determined. Using TCE loading as a parameter, a mass balance accounting for the relative distribution of TCE in the various phases was carried out as

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follows:

(1) The difference between the concentrations of TCE in the influent and the effluent was calculated and designated as the overall TCE depleted in the reactor. This overall depletion thus includes the fraction of the TCE that has been removed from the aqueous phase via volatilization to the headspace, adsorption onto the GAC particles, and/or biodegradation.

(2) The concentration of TCE found in the headspace of the column was converted to the corresponding hypothetical liquid phase concentration with Henry's law and plotted with the overall TCE depletion obtained in (1), as shown in Figure 32. It must be noted that only a negligibly small fraction (about 1%) of the overall TCE removed volatilized to the headspace of the reactor. The headspace volume (0.128 L) constitutes only about 5% of the total reactor volume (3 L). Since this region of the reactor is not open to the atmosphere, once equilibration of TCE in the gas and the liquid phases has been attained and dynamic equilibrium has been established, no further increase in the headspace TCE concentration can be expected. In fact, the volatilization of TCE in the column needs not concern us not only because of its minute amounts but also in the actual application, the entire system is a closed-loop system and the gas in the headspace is rarely exposed to the environment.

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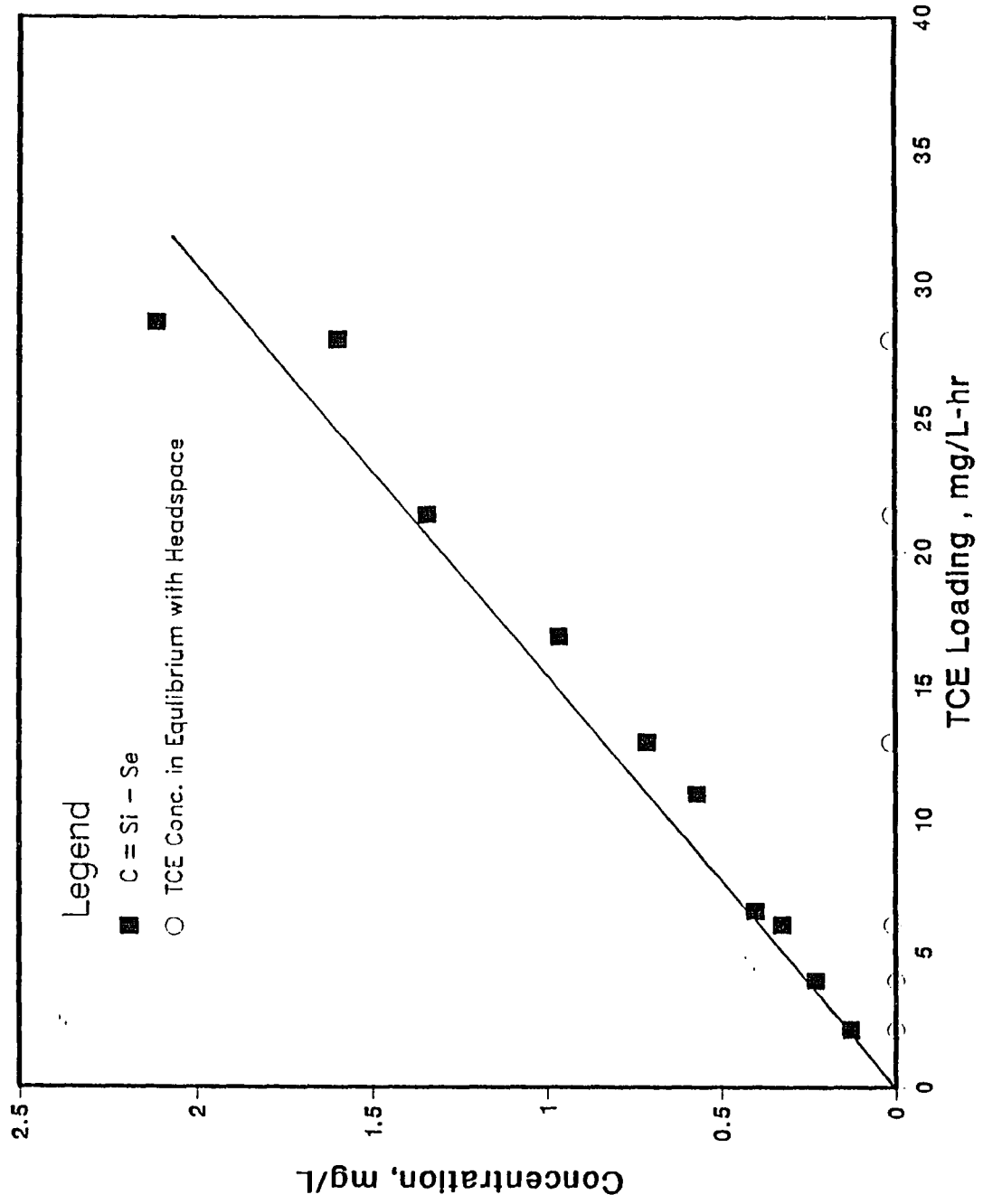
FIGURE 32 COMPARISON BETWEEN TCE VOLATILIZATION AND REMOVAL

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(3)   The difference in the ordinates of the two curves in Figure 32 is the amount of TCE adsorbed on the GAC particles and subsequently biotransformed to other compounds or remained unaltered on the particles. To account for the relative significance of the residual TCE, this difference was plotted with the concentration of TCE detected on the GAC samples in Figure 33.   The conversion of units was carried out on the assumption that the adsorption isotherm followed the Freundlich's adsorption model.   Again, the fraction of TCE that escaped microbial degradation and remained unchanged on the solid phase is negligibly small, as indicated in the figure.

(4)   Based on the data obtained above, it is clear that most of the TCE introduced into the system has been removed through biodegradation.   Because the series of reactions following the dechlorination of TCE to TDCE is slow, the major intermediate product eluting from the first stage is TDCE.   Figure 34 shows the relative concentration of the overall TCE transformed and the TDCE that eluted from the first stage.   The difference between the ordinates of the two curves is the fraction of the TDCE that underwent further degradation to the subsequent intermediate products such as DCA, VC, and CA.

(5)   Figure 35 presents the concentrations of the different intermediate products originated from the reduc-
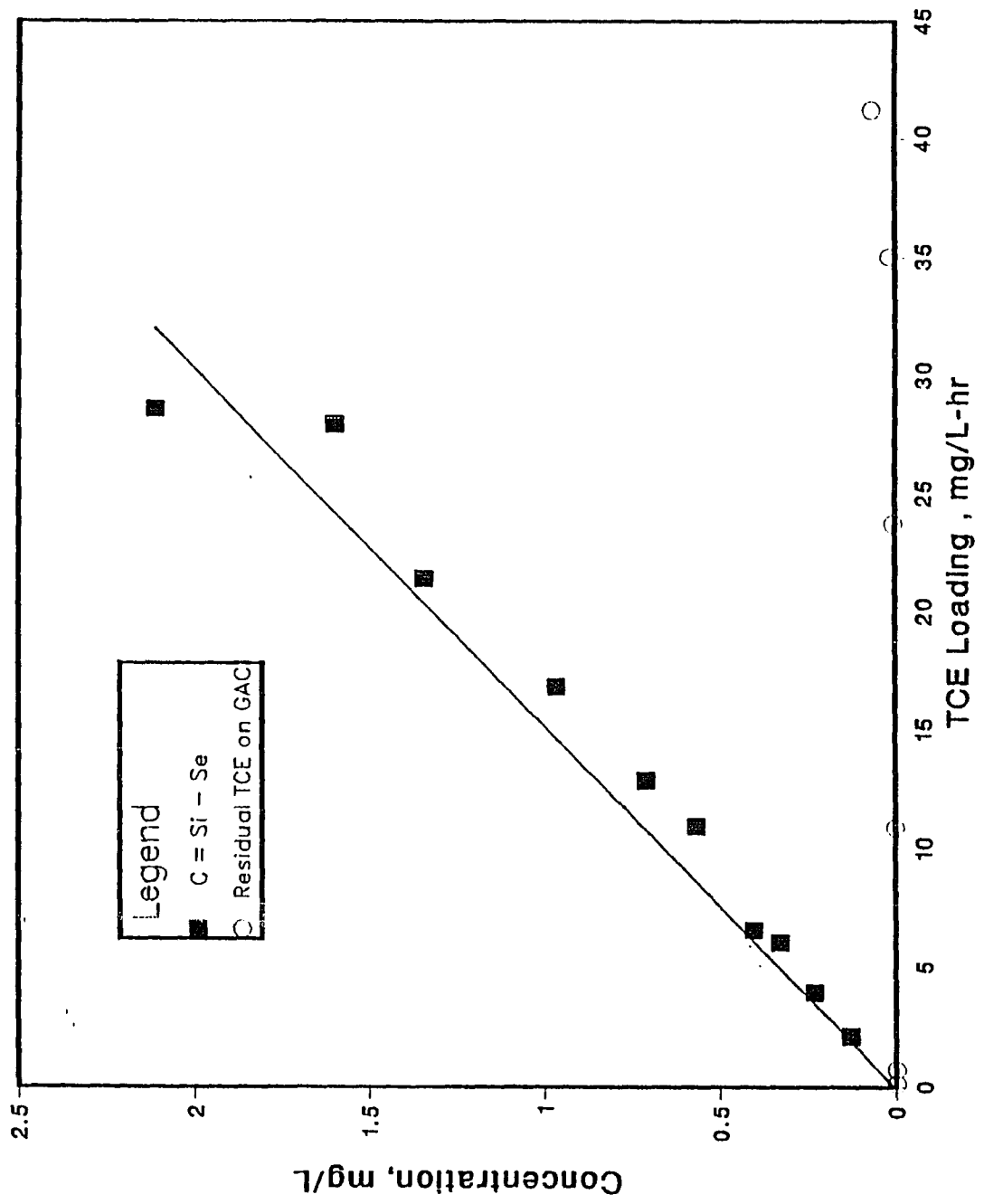
FIGURE 33 COMPARISON BETWEEN REMOVAL AND TCE ON GAC PARTICLES
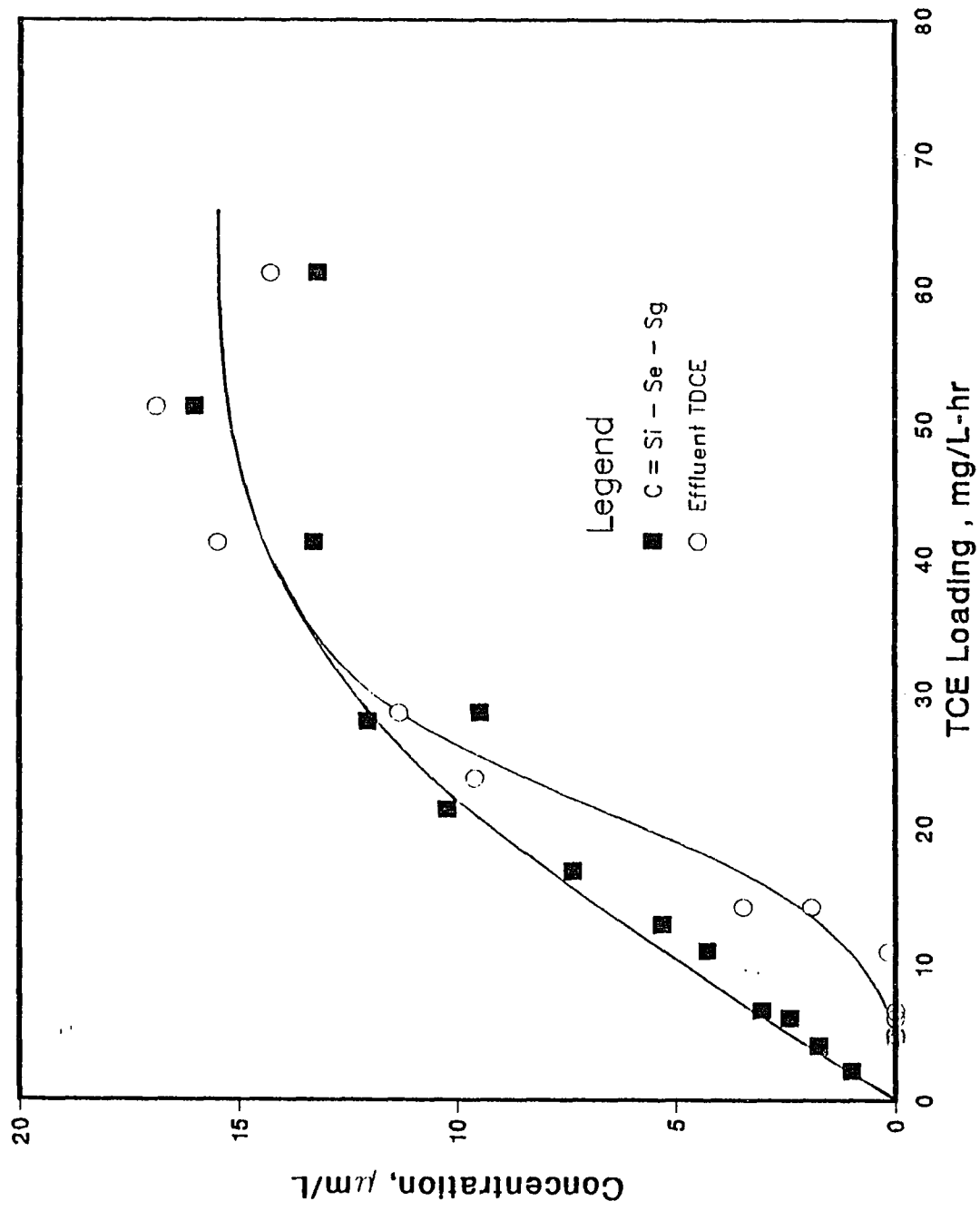
FIGURE 34   COMPARISON BETWEEN TCE CONVERSION AND EFFLUENT TDCE
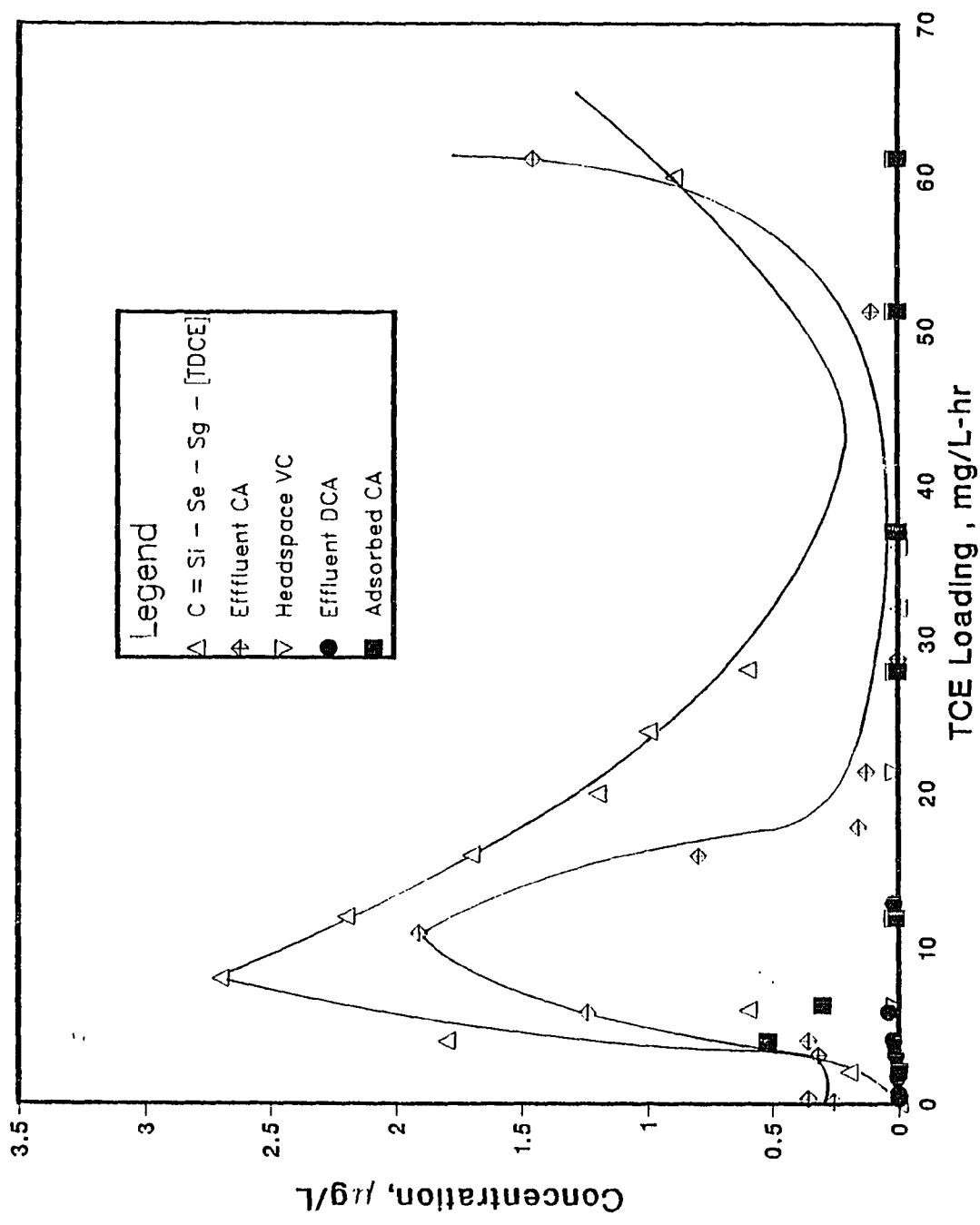
FIGURE 35   COMPARISON OF CONVERSIONS

tive dechlorination of TDCE. It is evident that most of the TDCE that underwent further degradation was transformed to CA.

The results obtained in the mass balance revealed that in the range of influent TCE concentration of 0.02 to 4.64 mg/L for one-stage operation, about 1% of the TCE introduced into the system volatilized to the reactor headspace, a maximum of 2% remained unaltered on the GAC particles, and a maximum of 35% escaped biotransformation and eluted with the first-stage effluent. Over 62% was biotransformed to the DCEs, 75% of which was further degraded to CA. This part of the experiment clearly illustrated that the process performance in the present study can well be approximated, without undue generality, by considering only the difference between the concentration of TCE introduced to the system and that eluted off the system.

Reaction Kinetics

Overall TCE Depletion

In order to derive pertinent kinetic parameters such as the reaction rate constant, the effluent TCE concentrations were plotted, in Figure 36, against the rates of TCE biodeg-radation, as computed by taking the difference between the concentration of overall TCE depletion and that due to volatilization as well as the residual TCE on the GAC parti-
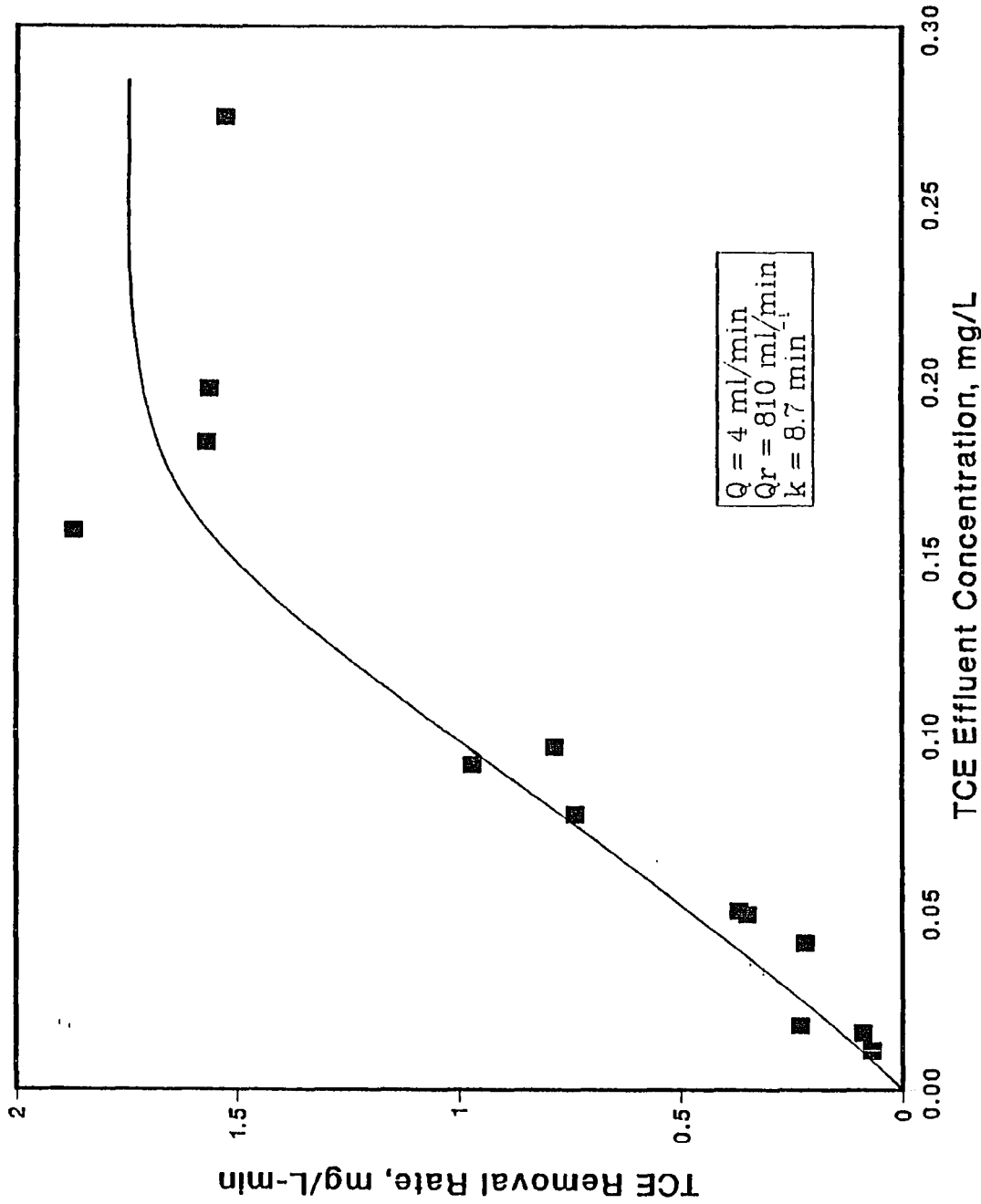
FIGURE 36 EVALUATION OF FIRST-ORDER RATE CONSTANT FOR TCE

cles and dividing by a reaction time of 1 minute. As shown in the figure, the overall rate of depletion of TCE from the aqueous phase follows a Michaelis-Menten type of kinetics. This is quite rational since, as shown in the preceding analyses, of the three removal pathways operative in this process, biotransformation is the most important one. Therefore in the overall kinetics, the biological behavior will be controlling. The Michaelis-Menten equation describes enzymatic kinetics and applies as well to surface-catalyzed reactions, which characterize most biological reactions [71, 90].

The linear portion of the curve was then used in evaluating the first-order rate constant, k. The value obtained was 8.72 min^{-1}, which was orders of magnitude greater than values reported in the literature [28, 66, 69, 71, 75]. Perhaps it is due primarily to the difference in the reactor configuration. In fluidized bed, intensive agitation provides an intimate contact between the substrate and the microorganisms capable of decomposing it. Figure 37 is the Lineweaver-Burke plot of the TCE removal data. The maximum removal rate and the half-velocity constant, calculated by linear regression, are 1.63 mg/L-min and 0.11 mg/L, respectively.

It is envisioned that in this process TCE was initially adsorbed on the activated carbon, which effectively concen-

System 1: Stage 1
Recycle Rate : 800 ml/min
Pumping Rate : 3.80--17.64 ml/min

$1/R_r$, $[mg/L-hr]^{-1}$

$1/S_e$, $[mg/L]^{-1}$
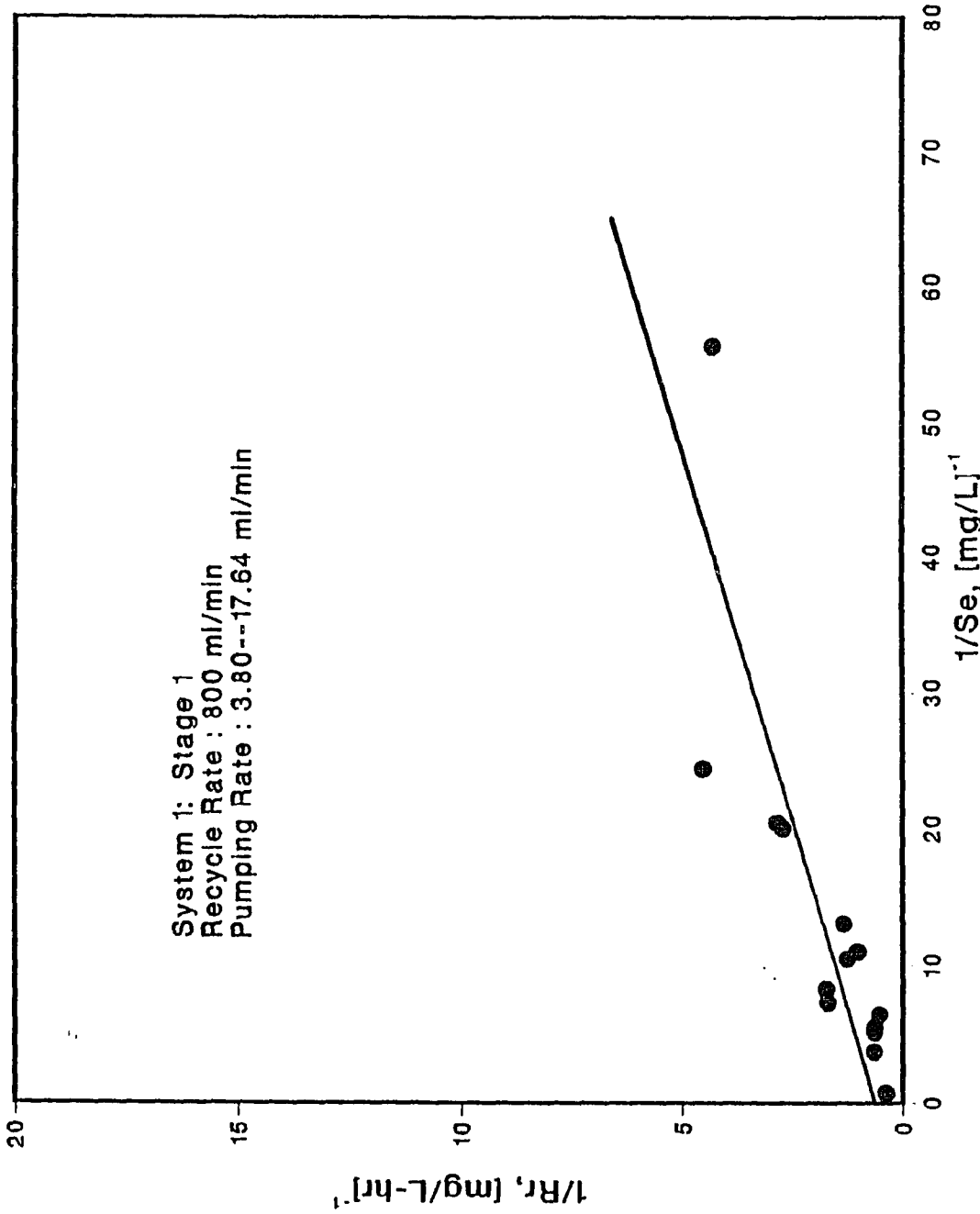
FIGURE 37   LINEWEAVER-BURKE PLOT OF TCE REMOVAL RATE

Table 17: Lineweaver-Burke Plot of TCE Removal Rate

System : 1   Stage 1

Recycle Rate   Qr = 810 ml/min

Pumping Rate   Q = 3.8 - 17.64 ml/min

(Si)d	Se	1/Se	Removal Rate, Rr	1/Rr
mg/L	mg/L	L/mg	mg/L-min	L-min/mg
0.249	0.018	55.56	0.231	4.32
0.261	0.041	24.39	0.220	4.54
0.398	0.049	20.41	0.349	2.86
0.418	0.050	20.00	0.368	2.72
0.818	0.077	12.99	0.741	1.35
0.894	0.096	10.42	0.788	1.27
1.063	0.091	10.99	0.972	1.03
2.028	0.157	6.37	1.871	0.53
0.722	0.138	7.25	0.598	1.71
0.691	0.121	8.26	0.570	1.75
0.650	0.108	4.59	0.432	2.31
1.751	0.182	5.49	1.569	0.64
1.802	0.274	3.65	1.528	0.65
1.759	0.197	5.08	1.562	0.64
4.036	1.425	0.70	2.611	0.38

trated the TCE and prevented its escape to the aqueous effluent. The rate of TCE depletion obtained in this study more resembled that of adsorption rather than mere biodegradation.

It must be noted that the disagreement can be attributed to two factors: (1) Activated carbon has a considerably greater adsorption capacity than the soil used in other's work, and (2) The cells inoculated into our system (about 100 ml of digester sludge) is 50 times larger than that reported by Barrio-Lage. If it is assumed that all of the microorganisms in the 100 ml inoculum were actively involved in the biodegradation of TCE, and the density is taken as 1.03 [85], the maximum rate of TCE degradation in the 3-L reactor volume will be 47.5×10^{-6} mg-TCE/mg-cell-min. This value is ten times greater than that reported by Fogel [66] for the rate of TCE degradation by the methane-utilizing mixed aerobic culture. This partly demonstrates the advantageous feature offered by the fluidized bed bioreactor in concentrating large amounts of bacterial population.

Kinetics Of TDCE Degradation

The V_m and K_m values for the degradation of TDCE were found to be 0.059 mg/L-min and 0.096 mg/L, respectively. The first order reaction rate constant was 0.3 min^{-1} (See Figure 38), which was orders of magnitude smaller than that

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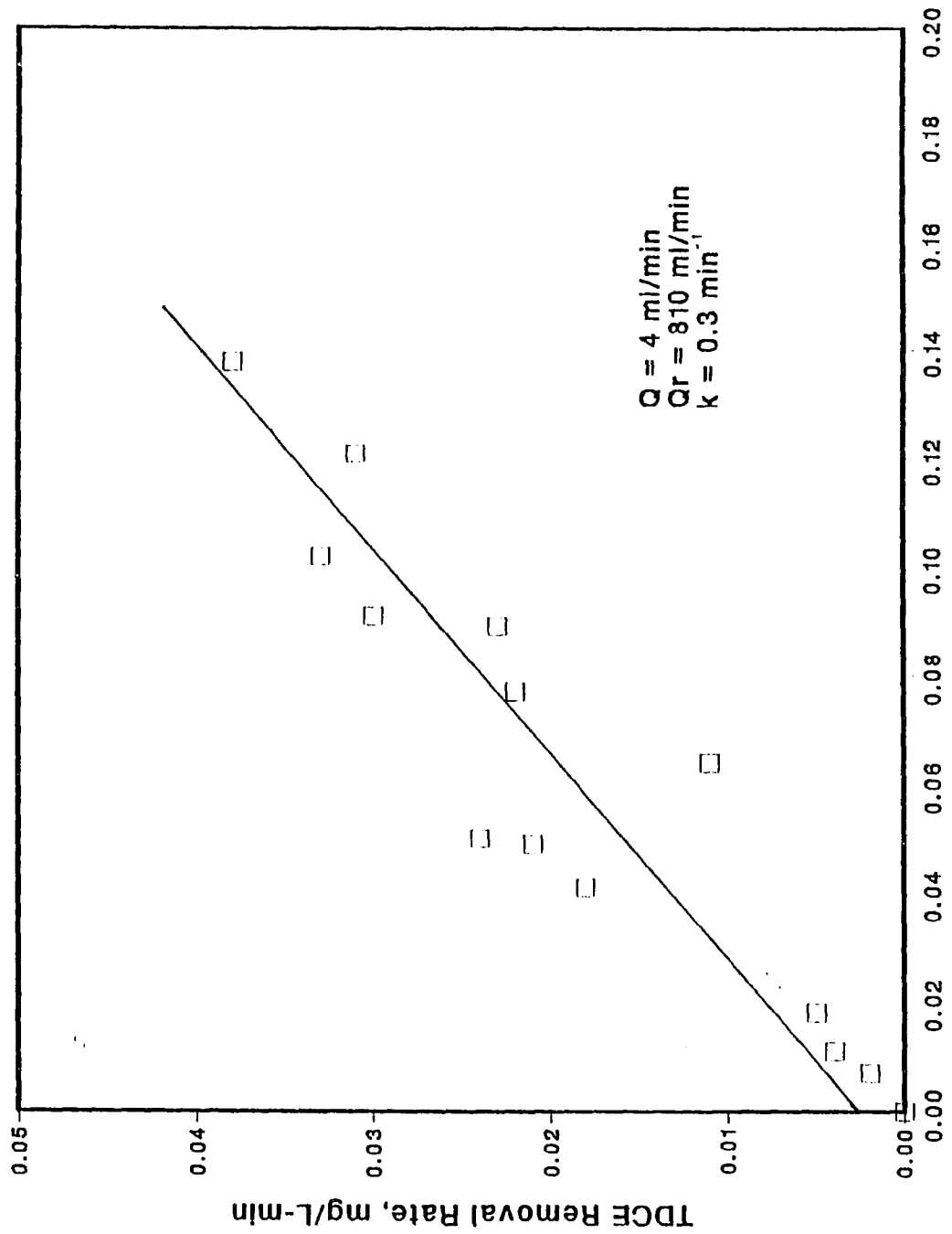
FIGURE 38   EVALUATION OF FIRST-ORDER RATE CONSTANT FOR TDCE

of TCE. This is in good agreement with results reported in
the literature that the rates of transformation decrease as
the chlorine is removed in the reductive dechlorination
process.

Proposed Biodegradation Pathway

Based on our experimental data, the anaerobic biodegra-
dation pathway of TCE in the two-stage fluidized bed process
is proposed as shown in Figure 39. Accordingly, the inter-
mediate product from the first reductive dechloriantion of
TCE involved all three geometric isomers 1,1-DCE, TDCE, and
CDCE. Our data indicated that TDCE is the predominant
species, based on peak identification using standard solu-
tion of TDCE. No attempt was made to differentiate between
TDCE and CDEC. Parsons et. al. [29] were able to show
isomer specificity, i.e., CDCE was favored over TDCE, proba-
bly because more energy would be involved in eliminating the
chlorine at the trans position than at the cis position.
1,1-DCE was detected occassionally in our system, specially
in the latter part of the study, i.e., at relatively high
TCE loading. The phenomenon was attributed to the impair-
ment of the metabolic rate caused by excessively high TCE
concentration. As noted before, the transformation from TCE
to the DCEs occured in a considerably faster rate than that
of the subsequent transformation to the lower chlorinated
compounds. Hence, an accumulation of the 1,1-DCE was regis-
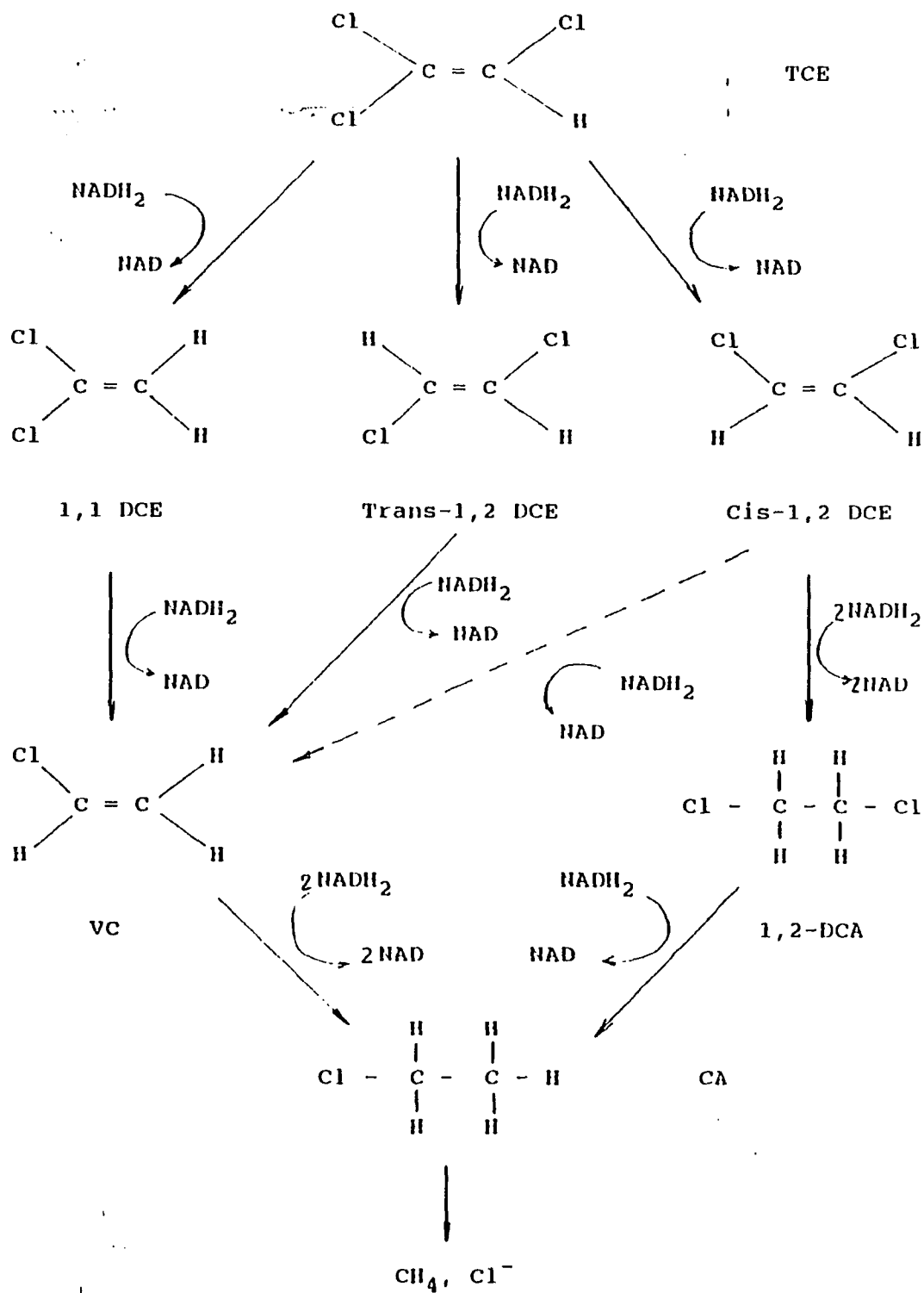
Figure 39: Proposed Anaerobic Biodegradation Pathway Of TCE

tered.   Contrary to this, Kleopfer et. al. [70] stated explicitly that no 1,1-DCE was found in their investigation on the anaerobic biodegradation of TCE in soil.

The second intermediate product in the reductive dechlorination pathway of TCE is VC.   This has been proven by many investigators [29, 33, 70].   VC was also found in the gas-phase samples, as well as on the GAC particles in our systems.   However, in addition to this, a transient production of DCA was also noted.   To the author's knowledge, this is the first report implicating DCA as one of the integral parts in the reductive biodegradation pathway of TCE.

CA is the last chlorinated intermediate product so far detected in the process.   Baek and Jaffe [33] claimed that CA had originated from VC, the double bond of which was ruptured by the attachment of 2 hydrogen atoms.   This is in great contrast to Barrio-Lage's [72] finding where it was postulated that CA originated directly from CDCE: CA was found only in microcosms spiked with CDCE but not in those containing any of the other isomers.   They therefore concluded that mechanisms other than reductive dechlorination might have taken place.   Our results on the effect of glucose/TCE ratio suggest that both claims are possible. With some circumstantial evidences, Vogel and McCarty [32] were able to show that further breakdown of VC was possible

under reductive environment, leading to the mineralization of TCE to CO_2.

In Vogel's work [32] where partial mineralization of TECE under anaerobic environment to CO_2 was observed, it was speculated that the intermediate product, VC, formed during the process was degraded in a way similar to that of DCA. Accordingly, DCA was initially oxidized yielding 1,2-dichloroethanol, which decomposed spontaneously to HCl and 2-chloroacetaldehyde. The latter compound was then further oxidized to chloroacetate, which was dehalogenated to glycolate, a metabolite readily utilizable as a carbon source by many bacteria. The suggested mechanism was based on the detection of a NAD-dependent 2-chloroacetaldehyde dehydrogenase activity in the extracts of cells grown on DCA. Baek and Jaffe [33] had shown the anaerobic biodegradation of TCE to CH_4 via the formation of VC and CA and had also suggested the same pathway. In our study, no attempt was made in identifying the intermediate products in the transformation from DCA to CA. However, based on the mechanism illustrated above, the final breakdown of DCA to CA yielded Cl^-, i. e., dechlorination followed by attachment of hydrogen had taken place.

Generalized Correlation

In view of the significant effect of the influent TCE concentration on the treatment efficiency of the present

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process, the experimental data were arranged in terms of the pertinent dimensionless parameters discussed before. In the range of $F/V_r G_m$ = 0.066 to 0.077, the dimensionless inlet concentration parameter, C_i/K_m, was plotted against the treatment index, $C_e/_i$ in Figure 40. A computer program written in Fortran was developed to find the best line of fit using the nonlinear convergence method (See Appendix 1). The resulting generalized correlation is:

$$C_e/C_i = 0.0395 \ e^{[0.2904(Ci/Km)]}$$

with a standard deviation of + 0.14.

Another computer program was developed to incorporate the effect of the flowrate, as represented by the dimensionless flow parameter, $F/V_r G_m$. A polynomial equation was obtained with a correlation coefficient of 0.94:

$$C_e/C_i = 6.89 - 0.12(C_i/K_m) - 182.2(F/V_r G_m) +$$
$$2.93(C_i/K_m)(F/_r G_m) + 1172.92(F/V_r G_m)^2$$

A very good agreement between the experimental and the theoretical data is evident from Figure 42.

The significance of these correlations lies on the fact that given a desired TCE level in the finished water, the influent concentration can be monitored in order to meet the treatment objective without undue delay provided the values for Q, G_m, V_r and K_m are fixed. Conversely, given a value

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of the influent TCE concentration, the TCE level in the effluent can be adequately predicted from the model. Of course, more work is still needed especially in incorporating a wider range of values for the parameter $F/V_r G_m$ and the parameters associated with the microbial growth and transport processes through the immobilized biomass.

$F/(V_r \times G_{max}) = 0.066 - 0.077$

MODEL EQUATION : $Ce/Ci = 0.0395 \exp[0.2904(Ci/Km)]$

Legend
○ EXPERIMENTAL DATA
● CALCULATED DATA

C_e°/C_i

C_i/K_m
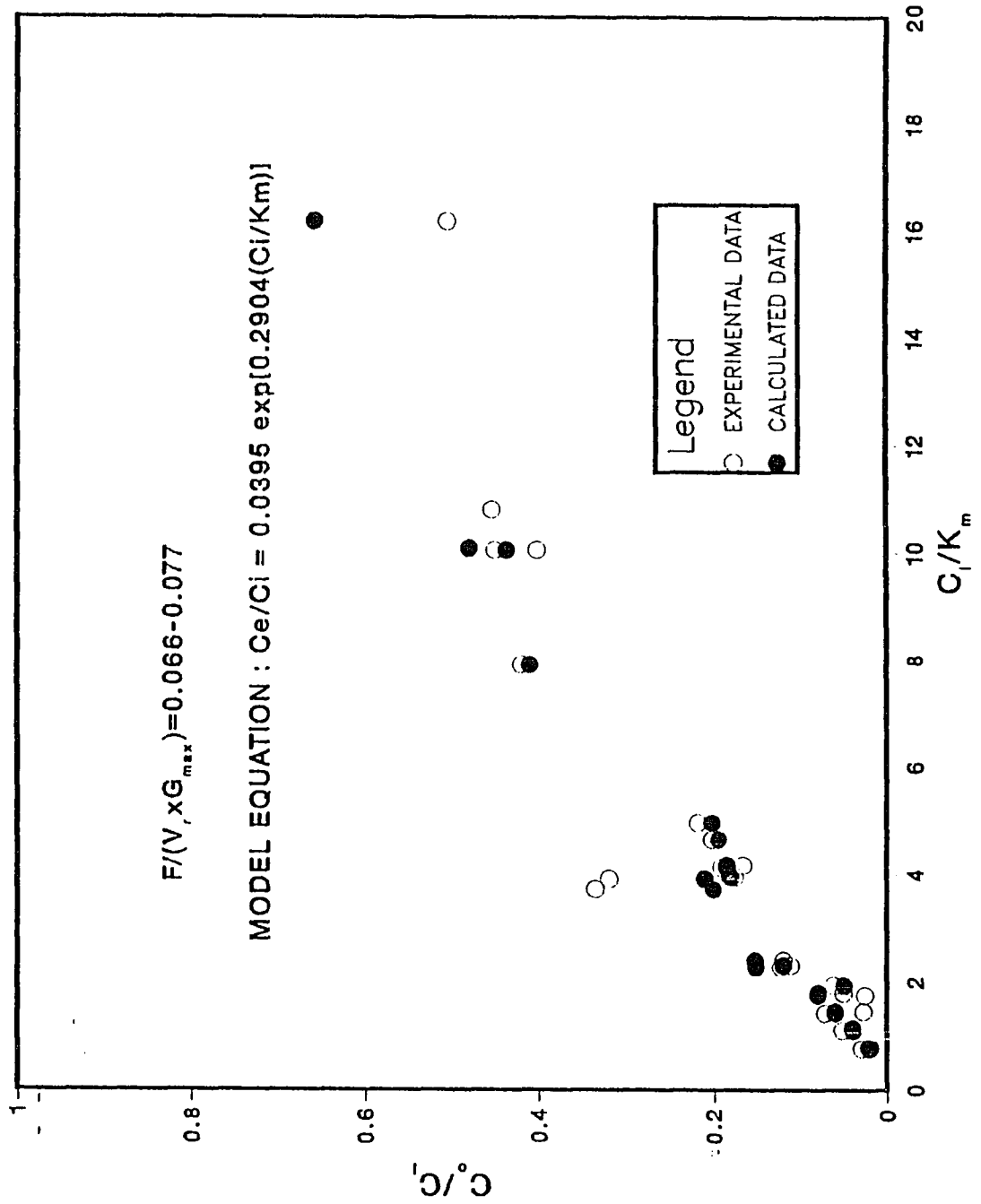
FIGURE 40 DIMENSIONLESS PARAMETER PLOT OF TCE CONVERSION INDICES (1)

121
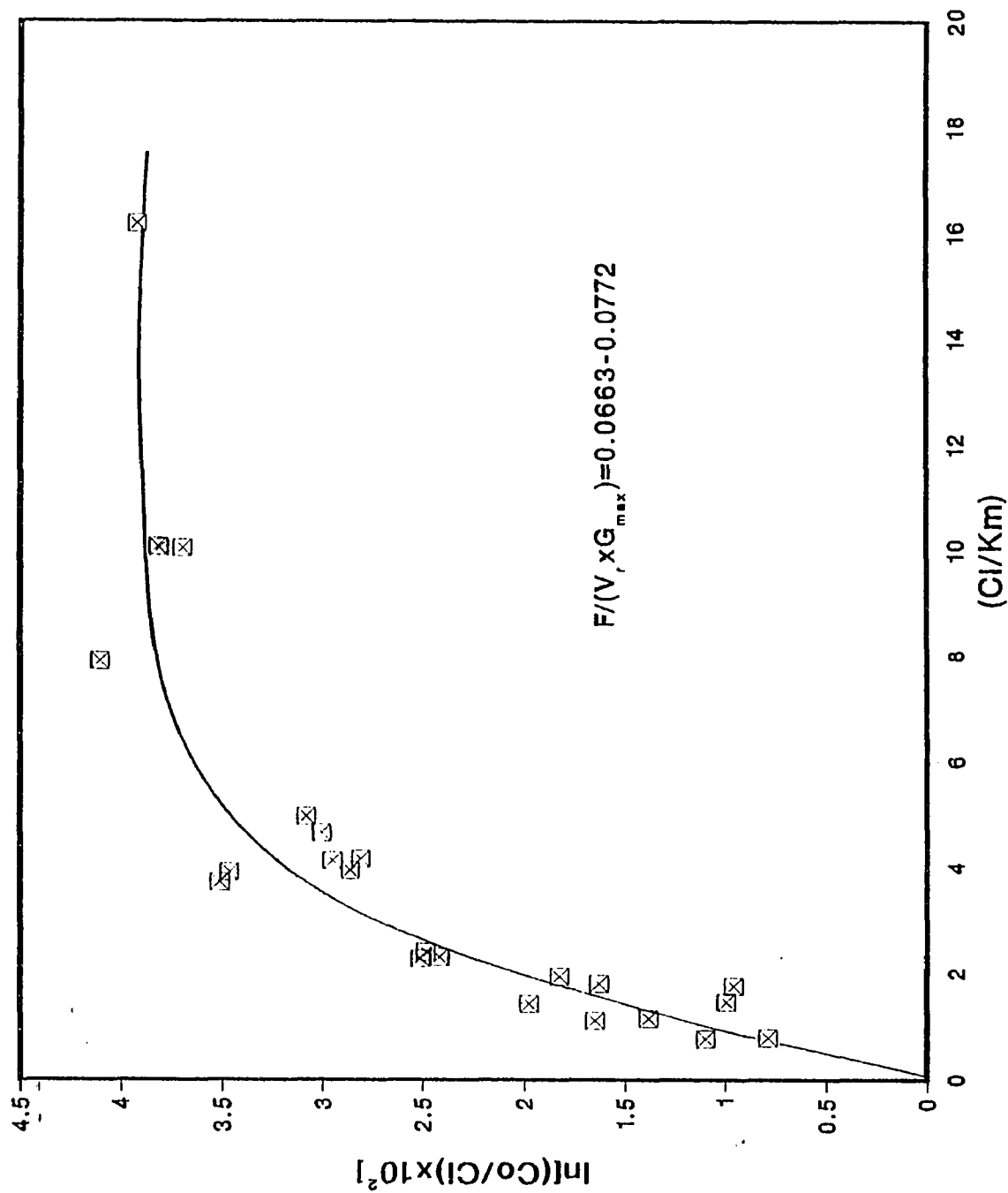
FIGURE 41 DIMENSIONLESS PARAMETER PLOT OF TCE CONVERSION INDICES (2)

$F/(V_r \times G_{max})=0.0663-0.0772$

(Cl/Km)

$ln[(Co/Cl) \times 10^2]$

122
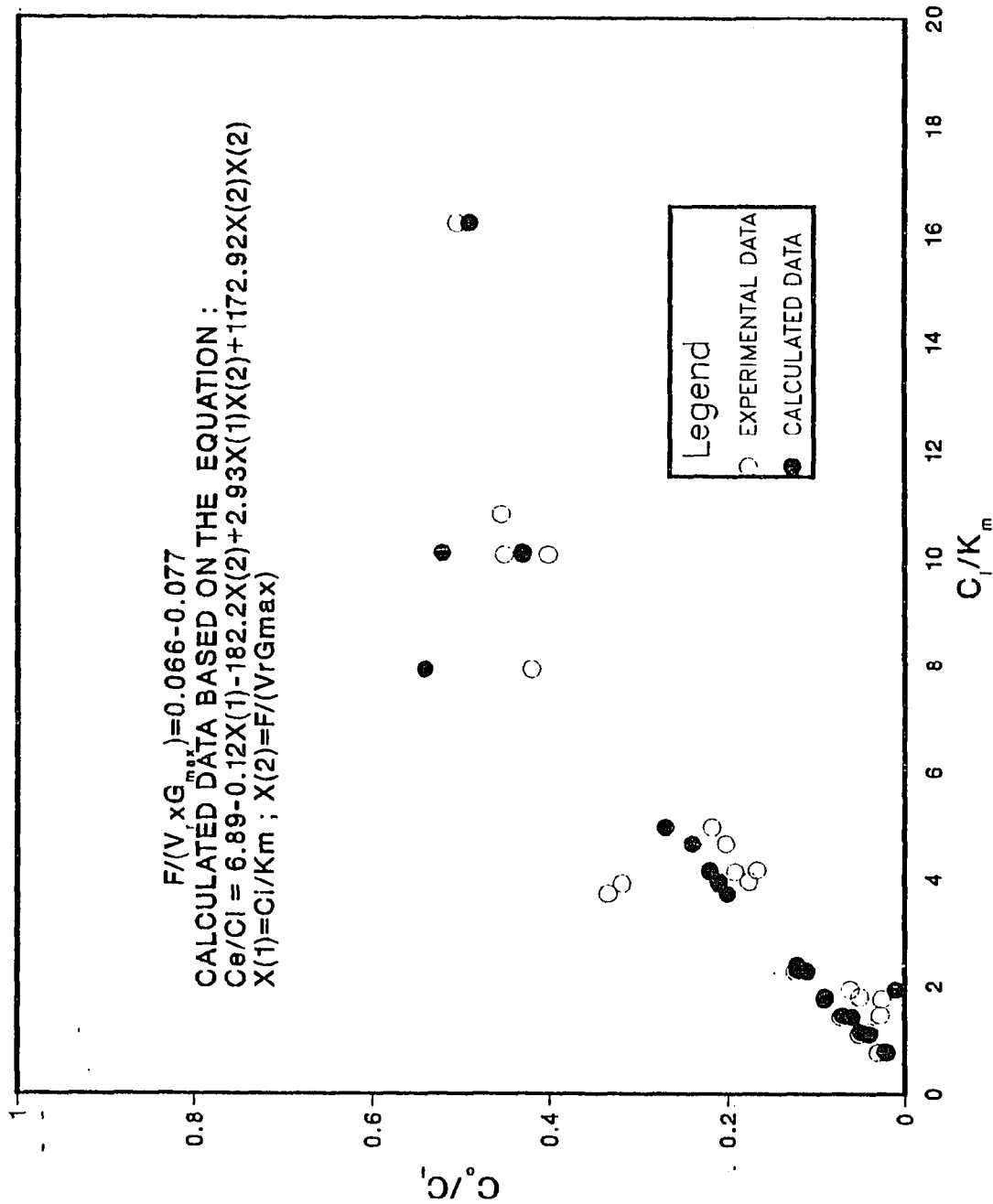
FIGURE 42   DIMENSIONLESS PARAMETER PLOT OF TCE CONVERSION INDICES (1)

$Ce/Ci = 6.89-0.12X(1)-182.2X(2)+2.93X(1)X(2)+1172.92X(2)X(2)$
$X(1)=Ci/Km$; $X(2)=F/(VrGmax)$
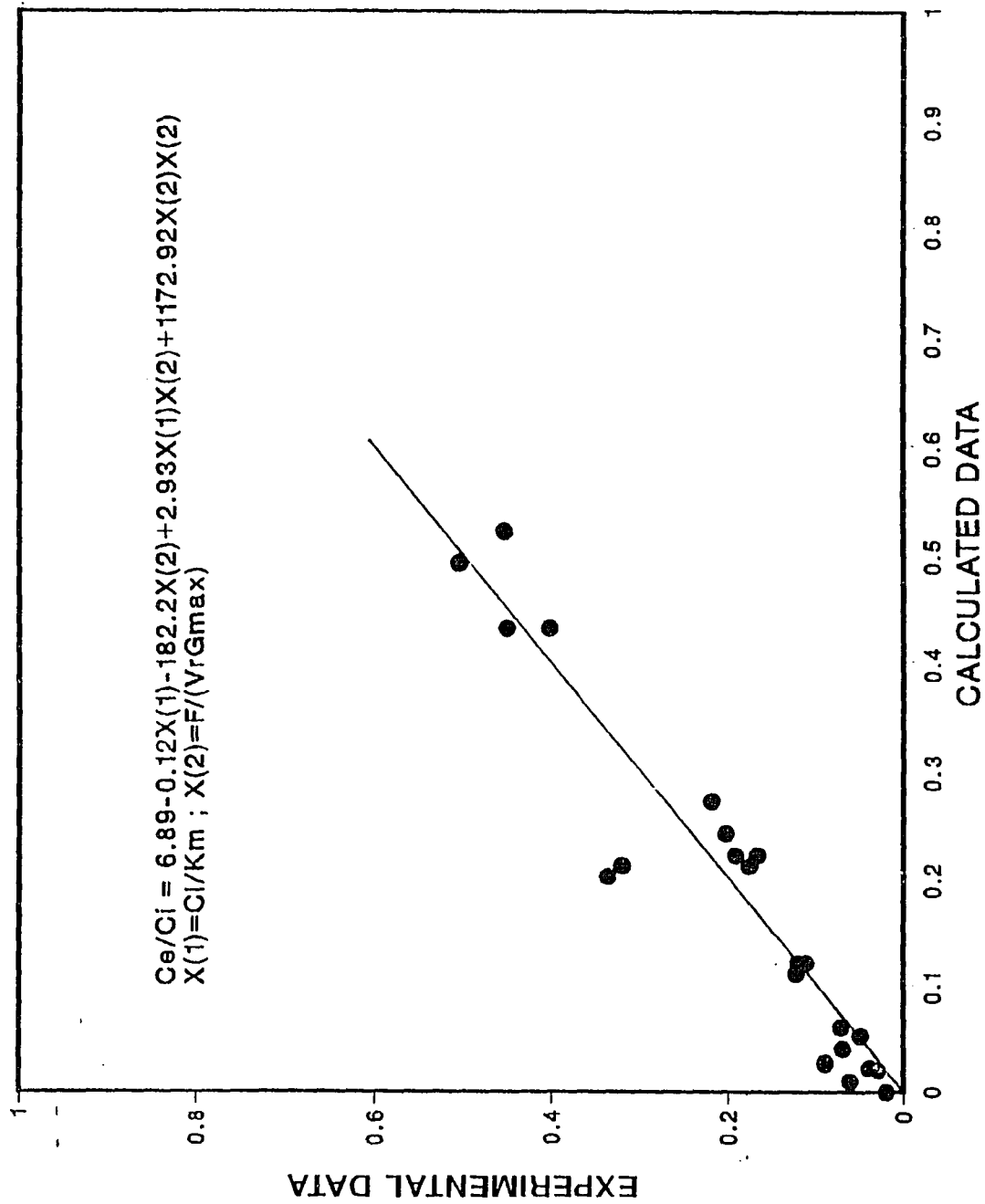
FIGURE 42   COMPARISON BETWEEN EXPERIMENTAL DATA AND CALCULATED DATA

Table 18: Dimensionless Parameter Plot of TCE Conversion Indices

Flow Rate Parameter: $F/(V_rG_{max}) = 0.066-0.077$

$K_m = 0.11$ mg/L $V_{max} = 1.63$ mg/L-min

Volume of Column V = 3088.9 ml

Effective Volume of Bed = 1029.6 ml

Qr	Q	F	(Si)d	Se	X	Y	ln(Y*100)
	ml/min			mg/L			
793.0	17.60	810.6	2.821	1.425	16.18	0.505	3.922
793.0	10.20	803.2	1.751	0.789	10.05	0.451	3.809
793.0	10.20	803.2	1.759	0.704	10.05	0.402	3.694
793.0	4.70	797.7	0.812	0.164	4.66	0.202	3.006
921.0	4.70	925.7	0.867	0.189	4.97	0.218	3.082
921.0	4.70	925.7	0.338	0.021	1.94	0.062	1.825
666.0	4.70	670.7	1.882	0.855	10.80	0.454	3.816
666.0	4.70	670.7	1.379	0.836	7.91	0.606	4.104
793.0	4.00	797.0	0.249	0.018	1.43	0.072	1.974
793.0	4.00	797.0	0.398	0.049	2.28	0.123	2.510
793.0	4.00	797.0	0.418	0.050	2.40	0.120	2.485
793.0	3.80	796.8	0.722	0.138	4.14	0.191	2.950
793.0	3.80	796.8	0.650	0.218	3.73	0.335	3.512
793.0	3.80	796.8	0.691	0.121	3.96	0.175	2.862
793.0	3.80	796.8	0.727	0.121	4.17	0.166	2.809
793.0	3.80	796.8	0.684	0.218	3.92	0.319	3.463
793.0	2.00	795.0	0.133	0.004	0.76	0.030	1.099
793.0	2.00	795.0	0.137	0.003	0.79	0.022	0.789
793.0	2.00	795.0	0.194	0.010	1.11	0.052	1.649
793.0	2.00	795.0	0.201	0.008	1.15	0.040	1.386
793.0	2.00	795.0	0.255	0.007	1.46	0.027	0.993
793.0	2.00	795.0	0.307	0.008	1.76	0.026	0.956
793.0	2.00	795.0	0.313	0.016	1.80	0.051	1.629
793.0	2.00	795.0	0.402	0.045	2.31	0.112	2.416

Note :

$X = (Si)_d / K_m$

$Y = S_e / (Si)_d$

CHAPTER V

CONCLUSIONS

Based on our data and observations, the following conclusions may be drawn:

(1) The removal of TCE from the aqueous phase in this process is essentially complete at an initial level of about 0.3 to 480 mg/l with no consideration of flow recirculation effect. The relative distribution of TCE in the different phases is as follows: 1% volatilized to the headspace, a maximum of 2% remained unaltered on the GAC particles, a maximum of 35% eluted from the first stage of the system, and about 62% converted to intermediate products.

(2) In general, there is an increase in the effluent TCE level with increasing influent concentration, loading, and feed rate. The optimum feed rate was found to be about 4 ml/min. The optimal recycle rate ranged from 750 to 850 ml/min.

(3) Both the rates of removal of TCE from the aqueous phase and the appearance of TDCE in the effluent follow Michaelis-Menten type of kinetics. The first-order reaction rate coefficients are 8.7 min^{-1} and 0.3 min^{-1} for TCE and TDCE, respectively. For TCE, V_m = 1.63 mg/L-min, K_m = 0.11 mg/L. For TDCE, V_m = 0.06 mg/L-min and K_m = 0.1 mg/L.

(4) Since it is postulated that the sequential dehalo-genation of TCE to VC and 1,2-DCA is a result of the deposition of hydrogen produced during the metabolism of glucose by the nonmethanogenic fermenters, manipulation of the glucose concentration in the feed may effect a process selectivity favoring the formation of the more acceptable intermediate product, i.e., 1,2-DCA. In the range of glucose/TCE ratio investigated, a ratio greater than 4 seemed to favor the production of CA via DCA.

(5) The production of an appreciable amount of CA is an indication that attainment of complete mineralization of TCE anaerobically in this process is possible since CA has been found to be considerably more susceptible to microbial decomposition than TCE.

APPENDIX 1

COMPUTER PROGRAM FOR CONVERGENCE METHOD

```
C        ********************** LCRA2.FOR ********************************
C
C
C
C        PROGRAM CRVREG  (NON-LINEAR REGRESSION ANALYSIS)
C                        (CONVERGENCE METHOD)
C
C
C
C                SS---- SUM OF SQUARE;
C                B1---- THE CONSTANT OF BIO-REACTION PROCESS;
C                AK---- THE CONSTANT OF BIO-REACTION PROCESS;
C                BO---- THE INTERCEPT OF REGRESSION EQUATION;
C                SSR--- SUM OF SQUARE OF REGRESSION;
C                SE---- STANDARD ERROR OF B1;
C                SM---- SUM;
C                SSQ--- MEAN SQUARE OF RESIDUAL;
C                FTS----- FISHER VARIANCE RATIO.
C                K----- NUMBER OF THE MODEL EQUTION.
C
C                MODEL1:  Y=Bo-B1(1/t);
C                MODEL2:  1/(1-Y)=Bo+B1t;
C                MODEL3:  lnY=Bo+B1lnt;
C                MODEL4:  ln(Y)=Bo+B1X;
C                MODEL5:  lnCa=Bo+B1t;
C                MODEL6:  1/Ca=Bo+B1t.
C
C
C
C        ***************************************************************
C
         DIMENSION SUMX(50),SUMY(50),SUMXX(50),SUMXY(50),SUMYY(50)
         DIMENSION SSX(50),SSXY(50),SSY(50),SSR(50),SSRE(50)
         DIMENSION SSQ(50),S(50),AVX(50),AVY(50),B1(50),BO(50)
         DIMENSION VB(50),SE(50),F(50),YCAL(50),YEC(50),YECSQ(50)
         DIMENSION SMYEC(50),X(50),Y(50),XI(50),XO(50),FL(50)
         DIMENSION FTS(50)
         COMMON K,N
         WRITE (29,90)
   90    FORMAT (20X,'TABLE     THE RESUTS OF ANALYSIS')
         WRITE (29,91)
         WRITE (29,91)
   91    FORMAT (50X)
         WRITE (29,92)
   92    FORMAT (10X,'BO',13X,'B1',14X,'STANDARD ERROR')
         WRITE (29,91)
         WRITE (29,91)
         WRITE (29,91)
         WRITE (101,98)
   98    FORMAT (10X,'COMPARISON BETWEEN (Ce/Ci)exp AND (Ce/Ci)cal')
         WRITE (101,91)
         WRITE (101,91)
         WRITE (101,91)
         WRITE (101,97)
   97    FORMAT (8X,'Ci/Km',10X,'Ce/Ci',15X,'Ce/Ci(cal)',10X,'F/Vr Gmax')
         WRITE (101,91)
         WRITE (101,91)
         WRITE (101,91)
         BKM=0.1743
         VMAX=2.03
         VR=3088.9
         GMAX=VMAX/BKM
```

```
C     ***************** NONLES.FOR **************************
C
C
C          PROGRAM CRVREG (CURVILINAR REGRESSION ANALYSIS)            *
C
C
C
C               SS---- SUM OF SQUARE;
C               Bij---- THE CONSTANT OF QUADRATIC EQUATION;
C               BO---- THE INTERCEPT OF THE TESTED EQUATION;
C               SSR--- SUM OF SQUARE OF REGRESSION;
C               STDAV---- STANDARD DEVIATION OF REGRESSION;
C               SM---- SUM;
C               SSQ--- MEAN SQUARE OF RESIDUAL;
C               CF----- REGRESSION COEFFICIENT;
C               N----- NUMBER OF THE DATA POINT;
C               K=1:  X=LOADING, (mg/L-hr), Y=REMOVAL RATE
C               K=2:  X=Ci,(mg/L), Y=REMOVAL RATE,(mg/L-min.)
C               K=3:  X1=Ci/Km,  X2=F/(VrGmax); Y=Co/Ci.
C
C
C
C          MODEL EQUATION:
C
C               Y = Bo + B X +B X + B X + B X + B X X
C
C
C     *****************************************************
C
C
C     ***********************************************************
C
C
C          TO CALCULATE THE SUM OF PARAMETERS
C
C
C
C     ***********************************************************
      DIMENSION Y(50),X1(50),X2(50),X1SQ(50),X2SQ(50),X1X2(50)
      DIMENSION X1X2SQ(50),X1SQX2(50),X1X2P2(50),X1P3(50)
      DIMENSION X2P3(50),X1X2P3(50),X1P3X2(50),X1P4(50),X2P4(50)
      DIMENSION X1Y(50),X2Y(50),X1SQY(50),X2SQY(50),X1X2Y(50)
      DIMENSION YSQ(50),SMX1(50),SMX2(50),SMX3(50),SMX4(50)
      DIMENSION SMX5(50),SMX6(50),SMX7(50),SMX8(50),SMX9(50)
      DIMENSION SMX10(50),SMX11(50),SMX12(50),SMX13(50),SMX14(50)
      DIMENSION SMX15(50),SMX16(50),SMX17(50),SMX18(50),SMX19(50)
```

129

```
      DO 95 N=1,1
      DO 95 I=1,1
      GO TO (120,121,122),N
120   P=24.0
      GO TO 123
121   P=7.0
      GO TO 123
122   P=6.0
123   K=4
      CALL SUMCAL (SMX,SMY,SMXX,SMXY,SMYY)
      SUMX(I)=SMX
      SUMY(I)=SMY
      SUMXX(I)=SMXX
      SUMYY(I)=SMYY
      SUMXY(I)=SMXY
      AVX(I)=SUMX(I)/P
      AVY(I)=SUMY(I)/P
      SSX(I)=SUMXX(I)-(SUMX(I)*AVX(I))
      SSY(I)=SUMYY(I)-(SUMY(I)*AVY(I))
      SSXY(I)=SUMXY(I)-(SUMX(I)*AVY(I))
      B1(I)=SSXY(I)/SSX(I)
      BO(I)=AVY(I)-B1(I)*AVX(I)
      SSR(I)=SSXY(I)*B1(I)
      SSRE(I)=SSY(I)-SSR(1)
      SSQ(I)=SSRE(I)/(P-2.0)
      S(I)=SQRT(SSQ(I))
      FTS(I)=SSR(I)/SSQ(I)
      VB(I)=SSQ(I)/SUMXX(I)
      SE(I)=SQRT(VB(I))
      B11=B1(I)
      BOO=EXP(BO(I))
108   CALL CONV(BOO,B11,SY0,SXY,SY1,SY2,SX2Y,SY2Y1,SY2XY1,F)
      BYEX=BOO*SY2XY1
      BX2Y=(BOO**2)*SX2Y
      BXY=BOO*SXY
      AVY0=BXY/SY0
      AVY2Y1=SY2Y1/SY0
      SSX1=BX2Y-BXY*AVY2Y1
      SSXY1=BYEX-BXY*AVY2Y1
      B1D=SSXY1/SSX1
      BOD=AVY2Y1-B1D*AVY0
      ER=10E-30
      IF(B1D.LE.ER.AND.BOD.LE.ER) GO TO 93
      BOO=BOD+BOO
      B11=B1D+B11
      GO TO 108
93    M=24
      REWIND 103
      DO 94 L=1,M
      READ (103,*) XI(L),XO(L),F(L)
      X(L)=XI(L)/BKM
      Y(L)=XO(L)/XI(L)
      YCAL(L)=BOO*EXP(B11*X(L))
      YEC(L)=YCAL(L)-Y(L)
      YECSQ(L)=(YEC(L))**2
      IF(L.GT.1) GO TO 109
      SYEC=0.0
109   SMYEC(L)=SYEC+YECSQ(L)
      SYEC=SMYEC(L)
      FL(L)=F(L)/(VR*GMAX)
```

```
      DIMENSION SMX20(50),SMX21(50),YCAL(50),XX1(50),XX2(50),YY(50)
      N=23
      DO 95 L=1,N
      READ (104,*) X1(L),X2(L),Y(L)
      X1SQ(L)=X1(L)**2
      X2SQ(L)=X2(L)**2
      X1X2(L)=X1(L)*X2(L)
      X1X2SQ(L)=X1(L)*X2SQ(L)
      X1SQX2(L)=X1SQ(L)*X2(L)
      X1X2P2(L)=X1SQ(L)*X2SQ(L)
      X1P3(L)=X1(L)*X1SQ(L)
      X2P3(L)=X2(L)*X2SQ(L)
      X1X2P3(L)=X1(L)*X2P3(L)
      X1P3X2(L)=X1P3(L)*X2(L)
      X1P4(L)=(X1SQ(L))**2
      X2P4(L)=(X2SQ(L))**2
      X1Y(L)=X1(L)*Y(L)
      X2Y(L)=X2(L)*Y(L)
      X1SQY(L)=(X1SQ(L))*Y(L)
      X2SQY(L)=X2SQ(L)*Y(L)
      X1X2Y(L)=X1X2(L)*Y(L)
      YSQ(L)=Y(L)**2
      IF(L.GT.1) GO TO 107
      SX1=0.0
      SX2=0.0
      SX3=0.0
      SX4=0.0
      SX5=0.0
      SX6=0.0
      SX7=0.0
      SX8=0.0
      SX9=0.0
      SX10=0.0
      SX11=0.0
      SX12=0.0
      SX13=0.0
      SX14=0.0
      SX15=0.0
      SX16=0.0
      SX17=0.0
      SX18=0.0
      SX19=0.0
      SX20=0.0
      SX21=0.0
107   SMX1(L)=SX1+X1(L)
      SMX2(L)=SX2+X2(L)
      SMX3(L)=SX3+X1SQ(L)
      SMX4(L)=SX4+X2SQ(L)
      SMX5(L)=SX5+X1X2(L)
      SMX6(L)=SX6+X1X2SQ(L)
      SMX7(L)=SX7+X1SQX2(L)
      SMX8(L)=SX8+X1X2P2(L)
      SMX9(L)=SX9+X1P3X2(L)
      SMX10(L)=SX10+X1X2P3(L)
      SMX11(L)=SX11+X1P3(L)
      SMX12(L)=SX12+X2P3(L)
      SMX13(L)=SX13+X1P4(L)
      SMX14(L)=SX14+X2P4(L)
      SMX15(L)=SX15+Y(L)
      SMX16(L)=SX16+X1Y(L)
```

```fortran
      SMX17(L)=SX17+X2Y(L)
      SMX18(L)=SX18+X1SQY(L)
      SMX19(L)=SX19+X2SQY(L)
      SMX20(L)=SX20+X1X2Y(L)
      SMX21(L)=SX21+YSQ(L)
      SX1=SMX1(L)
      SX2=SMX2(L)
      SX3=SMX3(L)
      SX4=SMX4(L)
      SX5=SMX5(L)
      SX6=SMX6(L)
      SX7=SMX7(L)
      SX8=SMX8(L)
      SX9=SMX9(L)
      SX10=SMX10(L)
      SX11=SMX11(L)
      SX12=SMX12(L)
      SX13=SMX13(L)
      SX14=SMX14(L)
      SX15=SMX15(L)
      SX16=SMX16(L)
      SX17=SMX17(L)
      SX18=SMX18(L)
      SX19=SMX19(L)
      SX20=SMX20(L)
   95 SX21=SMX21(L)
C     **************************************************************
C
C         CALCULATION OF SUM OF SQUARES
C
C     **************************************************************
      AVX1=SX1/N
      AVX2=SX2/N
      AVX3=SX3/N
      AVX4=SX4/N
      AVX5=SX5/N
      AVX15=SX15/N
      A1=SX3-AVX1*SX1
      A2=SX5-AVX1*SX2
      A3=SX11-AVX1*SX3
      A4=SX6-AVX1*SX4
      A5=SX7-AVX1*SX5
      A6=SX16-AVX1*SX15
      B1=SX4-(AVX2*SX2+(A2/A1)*A2)
      B2=SX7-(AVX2*SX3+(A2/A1)*A3)
      B3=SX12-(AVX2*SX4+(A2/A1)*A4)
      B4=SX6-(AVX2*SX5+(A2/A1)*A5)
      B5=SX17-(AVX2*SX15+(A2/A1)*A6)
      C1=SX13-(AVX3*SX3+(A3/A1)*A3+(B2/B1)*B2)
      C2=SX8-(AVX3*SX4+(A3/A1)*A4+(B2/B1)*B3)
      C3=SX9-(AVX3*SX5+(A3/A1)*A5+(B2/B1)*B4)
      C4=SX18-(AVX3*SX15+(A3/A1)*A6+(B2/B1)*B5)
      SD1=SX14-(AVX4*SX4+(A4/A1)*A4+(B3/B1)*B3+(C2/C1)*C2)
      SD2=SX10-(AVX4*SX5+(A4/A1)*A5+(B3/B1)*B4+(C2/C1)*C3)
      SD3=SX19-(AVX4*SX15+(A4/A1)*A6+(B3/B1)*B5+(C2/C1)*C4)
      E1=SX8-(AVX5*SX5+(A5/A1)*A5+(B4/B1)*B4+(C3/C1)*C3+(SD2/SD1)*SD2)
      E2=SX20-(AVX5*SX15+(A5/A1)*A6+(B4/B1)*B5+(C3/C1)*
     3 C4+(SD2/SD1)*SD3)
C     **************************************************************
C
```

```
C
C
C              CALCULATION OF THE CONSTANTS OF QUADRATIC EQUATION
C
C
C      ***********************************************************
C
       B12=E2/E1
       B22=(SD3/SD1)-B12*(SD2/SD1)
       B11=(C4/C1)-B12*(C3/C1)-B22*(C2/C1)
       BB2=(B5/B1)-B12*(B4/B1)-B22*(B3/B1)-B11*(B2/B1)
       BB1=(A6/A1)-B12*(A5/A1)-B22*(A4/A1)-B11*(A3/A1)-B2*(A2/A1)
       BO=AVX15-B12*AVX5-B22*AVX4-B11*AVX3-B2*AVX2-B1*AVX1
       WRITE (40,200) BO,BB1,BB2,B11,B12,B22
       WRITE (40,205)
       WRITE (40,205)
       WRITE (40,205)
       WRITE (40,205)
       REWIND 104
       DO 195 L=1,N
       READ (104,*) XX1(L),XX2(L),YY(L)
       YCAL(L)=BO+BB1*XX1(L)+BB2*XX2(L)+B11*XX1(L)**2+B22*XX2(L)**2+
     1 B12*XX1(L)*XX2(L)
       WRITE (40,211) XX1(L),XX2(L),YY(L),YCAL(L)
       WRITE (40,205)
  195  CONTINUE
       WRITE (40,205)
       WRITE (40,205)
       WRITE (40,205)
       WRITE (40,205)
C
C
C      ***********************************************************
C
C
C              ANALYSIS OF THE REGRESSION   RESULTS
C
C
C      ***********************************************************
C
       SSRB12=E2*(E2/E1)
       SSRB22=SD3*(SD3/SD1)
       SSRB11=C4*(C4/C1)
       SSRB2=B5*(B5/B1)
       SSRB1=A6*(A6/A1)
       CF=AVX15*SX15
       SSY=SX21-SX15*AVX15
       STDAV=SQRT(ABS(SSY)/(N-1))
       WRITE (40,202) SSRB11,SSRB22,SSRB12,SSRB2,SSRB1,CF,
     4 SSY,STDAV
  200  FORMAT (6F10.2)
  211  FORMAT (4F10.2)
  202  FORMAT (8F10.2)
  205  FORMAT (50X)
       STOP
       END
```

```
        SMXY=0.0
        SMYY=0.0
97      SX(L)=SMX+BX(L)
        SY(L)=SMY+BY(L)
        SXSQ(L)=SMXX+XSQ(L)
        SYSQ(L)=SMYY+YSQ(L)
        SXY(L)=SMXY+XY(L)
        SMX=SX(L)
        SMY=SY(L)
        SMXX=SXSQ(L)
        SMXY=SXY(L)
95      SMYY=SYSQ(L)
        RETURN
        END
```

APPENDIX 2

COMPUTER PROGRAM FOR CURVILINEAR REGRESSION OF

POLYNOMIAL EQUATION

```
            WRITE (101,96) X(L),Y(L),YCAL(L),FL(L)
      94    CONTINUE
            STERR=SQRT(SYEC/(M-1))
            WRITE (29,99) BOO,B11,STERR
      95    CONTINUE
      96    FORMAT (1X,F12.4,3X,F12.4,10X,F12.4,8X,F12.4)
      99    FORMAT (1X,F12.4,3X,F12.4,10X,F12.4)
            STOP
            END
C
C
C           ************************************************************
C
C
C           THIS IS THE SUBROUTINE BY CONVERGENCE METHOD TO ESTIMATE THE
C            NON-LINEAR PARAMETERS
C
C
C           ************************************************************
            SUBROUTINE CONV(BOO,B11,SYO,SXY,SY1,SY2,SX2Y,SY2Y1,SY2XY1,F)
            DIMENSION Y(50),X(50),YO(50),XY(50),Y1(50),Y2(50)
            DIMENSION X2Y(50),Y2Y1(50),XI(50),XO(50)
            DIMENSION Y2XY1(50),SMYO(50),SMXY(50),SMY1(50)
            DIMENSION SMY2(50),SMX2Y(50),F(50)
            DIMENSION SMY2Y1(50),SMY2XY1(50)
            BKM=0.1743
            M=24
            REWIND 103
            DO 106 J=1,M
            READ(103,*) XI(J),XO(J),F(J)
            X(J)=XI(J)/BKM
            Y(J)=XO(J)/XI(J)
            YO(J)=EXP(2*B11*X(J))
            XY(J)=X(J)*YO(J)
            Y1(J)=EXP(B11*X(J))
            Y2(J)=Y(J)-BOO*Y1(J)
            X2Y(J)=(X(J)**2)*YO(J)
            Y2Y1(J)=Y1(J)*Y2(J)
            Y2XY1(J)=X(J)*Y2Y1(J)
            IF(J.GT.1) GO TO 107
            SYO=0.0
            SXY=0.0
            SY1=0.0
            SY2=0.0
            SX2Y=0.0
            SY2Y1=0.0
            SY2XY1=0.0
      107   SMYO(J)=SYO+YO(J)
            SMXY(J)=SXY+XY(J)
            SMY1(J)=SY1+Y1(J)
            SMY2(J)=SY2+Y2(J)
            SMX2Y(J)=SX2Y+X2Y(J)
            SMY2Y1(J)=SY2Y1+Y2Y1(J)
            SMY2XY1(J)=SY2XY1+Y2XY1(J)
            SYO=SMYO(J)
            SXY=SMXY(J)
            SY1=SMY1(J)
            SY2=SMY2(J)
            SX2Y=SMX2Y(J)
            SY2Y1=SMY2Y1(J)
            SY2XY1=SMY2XY1(J)
```

```
106       CONTINUE
          RETURN
          END
C
C
C         ***************************************************************
C
C         THIS IS THE SUBROUTINE TO CALCULATE THE SUM OF SQUARES
C
C
C         ***************************************************************
C
          SUBROUTINE SUMCAL(SMX,SMY,SMXX,SMXY,SMYY)
          DIMENSION Y(50),X(50),CA(50),BY(50),XSQ(50),XY(50)
          DIMENSION SX(50),SY(50),SXSQ(50),SXY(50),F(50)
          DIMENSION YSQ(50),SYSQ(50),BX(50),XI(50),XO(50)
          COMMON K,N
          BKM=0.1743
          REWIND 103
          IF(N.GT.1) GO TO 300
          NN=24
          DO 95 LL=1,NN
          L=LL
300       IF(N.EQ.1) GO TO 302
          IF(N.GT.2) GO TO 301
          NO=7
          DO 95 LN=1,NO
          L=LN
301       IF(N.EQ.2) GO TO 302
          NI=6
          DO 95 LM=1,NI
          L=LM
302       IF(K.LE.4) GO TO 98
          READ (100,*) X(L),Y(L)
          IF(K.EQ.5) GO TO 103
          BY(L)=1.0/Y(L)
          GO TO 104
98        READ (103,*) XI(L),XO(L),F(L)
          X(L)=XI(L)/BKM
          Y(L)=XO(L)/XI(L)
          GO TO (99,100,101,102),K
99        X(L)=1.0/X(L)
          BY(L)=Y(L)
          GO TO 104
100       BY(L)=1.0/(1.0-Y(L))
          GO TO 104
101       BY(L)=ALOG(Y(L))
          BX(L)=ALOG(X(L))
          GO TO 104
102       BY(L)=ALOG(Y(L))
          BX(L)=X(L)
          GO TO 104
103       BY(L)=1.0/(Y(L))
          BX(L)=1.0/(X(L))
104       XSQ(L)=(BX(L)**2)
          XY(L)=BX(L)*BY(L)
          YSQ(L)=(BY(L))**2
          IF(L.GT.1) GO TO 97
          SMX=0.0
          SMY=0.0
          SMXX=0.0
```

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