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ABSTRACT

Title of Thesis: Microbial Phenol Degradation Utilizing a Complete-Mix Biological Reactor: The Effects of Dissolved Oxygen Content.

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Experiments were conducted using phenol as a sole carbon source in a series of completely mixed biological reactors with solids recycle (CMBR). The reactor working volume was 4 liters, and solids were recycled from 3 liter clarifiers. Dissolved oxygen concentration (DO) was varied in order to determine the impact of this important variable on system operability.

Phenol was removed at better than 99 percent efficiency during most of the runs. Filamentous growth was not observed during any run. However, bulking did occur at higher DO levels, which was the result of microbial slime production.

MICROBIAL PHENOL DEGRADATION UTILIZING A COMPLETE-MIX BIOLOGICAL REACTOR: THE EFFECTS OF DISSOLVED OXYGEN CONTENT

BY KEITH KOLLAR

THESIS SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF THE NEW JERSEY INSTITUTE OF TECHNOLOGY IN PARTIAL FUFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ENVIRONMENTAL SCIENCE (TOXICOLOGY OPTION) MAY 1988

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APPROVAL SHEET

TITLE OF THESIS: MICROBIAL PHENOL DEGRADATION UTILIZING A COMPLETE MIX BIOLOGICAL REACTOR: THE EFFECTS OF DISSOLVED OXYGEN CONTENT

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I. INTRODUCTION

Phenol is a ubiquitous environmental contaminant which arises from numerous industrial processes including direct liquefaction of coal (1), coking plant effluents, and polymeric resin production (6). Phenol is the tenth most frequently reported compound in municipal effluents (2). The high solubility of phenol (8.4% by weight in water), coupled with its low vapor pressure (0.36mm Hg) (3), merits the compound as a pollutant of concern in aquatic environments. Chronic exposure to phenol may damage the liver and kidneys as well as induce mutagenesis (4).

Previous experiments in our laboratory (5) have demonstrated the biodegradation of phenol by a heterogeneous microbial population in a complete-mix biological reactor (CMBR). However, the mixed liquor exhibited poor settling, apparently caused by filamentous organisms. In the present work, experiments were conducted to determine the factor(s) which induce bulking during the treatment of phenolic compounds in a CMBR. The focus was on the dissolved oxygen (DO) concentration in the reactors.

II. LITERATURE REVIEW

A. Kinetics

studies (47,50) had reviewed over 300 Previous references on biodegradation. Of that number, only 30 contained usable kinetic data. This information i S summarized in Table 40. Although these represent the most complete sets of literature data available, many of these references are lacking in a characterization of the microbial population used in obtaining the kinetic data. Without such characterization, it is impossible to compare the results from different references. As can be seen from the summary, there is very little consistency in the results for even a single compound. Aerobic biodegradation is a catalytic oxidation process, and in any such process it is essential to characterize the catalyst (in this case, the microbial population). For this project, the literature was also reviewed for aeration levels, to determine general operating conditions for bench-scale systems treating phenolic compounds aerobically.

B. Microbial Community

Previous studies (5,49,50) have been completed yielding a detailed description of the activated sludge and the microbial population used in the present study. These results are summarized in Tables 37-39. The mixed liquor for this study was obtained from the aeration tanks at the Livingston, New Jersey wastewater treatment plant. The Livingston plant treats 2.5 million gallons of domestic

sewage per day. Less than one percent of the incoming wastewater is of industrial origin. It has been shown in our laboratory (49,50,52) that the addition of phenol as a sole-carbon source decreases the microbial diversity of domestic mixed liquor in batch reactors. Further, studies indicated (8,53) that few of the organisms present in acclimated mixed-liquor are capable of degrading the phenol by themselves.

Typical activated sludge plants operate with an extremely diverse microbial population. The major taxonomic groups of invertebrates in activated sludge include the flagellates, motile ciliates, stalked ciliates, amoebae, rotifers and nematodes (49). High quality sludge (sludge which readily settles in sedimentation basins) is generally characterized by free swimming ciliates and stalked ciliates, with minor representation by the flagellates and rotifers (51). Fin floc and straggler floc conditions generally arise in clarifiers when certain populations (eq. flagellates and/or nematodes) increase as a result of numerous external factors including increased organic loading (high F/M ratio), varying aeration levels, temperature or pH. Free swimming ciliates (amoebae) are usually apparent when bacterial populations are high since these organisms graze on the bacteria and clarify the effluent in the process (44).

Bacteria generally form a significant fraction of the mixed liquor population. A variety of gram positive and gram

negative species have been identified in phenol acclimated mixed liquor including <u>Pseudomonas</u> <u>sp.</u> <u>Acinetobacter</u>, <u>Group</u> <u>2K-1</u>, <u>Enterobacter</u> <u>sp.</u>, and <u>Serratia</u> <u>sp.</u> (5,50). The species identified are generally mesophilic facultative heterotrophs capable of utilizing a variety of substrates for amphibolic pathways and energy generation.

Fungi and yeast are usually poorly represented in activated sludges, but are common in activated sludge operating at lower pH values or for the treatment of industrial wastes (48). Fungi and yeast are generally undesirable since many forms including Geotrichum and Penicillum are associated with filamentous growth. Filamentous growth is a characteristic of manv species of microorganisms, including bacteria, yeast, funqi, under certain conditions. Environmental and conditions such as temperature, pH (48), and mean cell residence time (46) may contribute to increased filamentous growth.

A variety of microogranisms have been identified as either phenol degraders or phenol tolerant. Many bacteria including several <u>Eseudomonads</u> (19,31,32) and <u>Bacillus</u>(24) have been shown to degrade phenol. Yeast such as <u>Candida</u>(39) can also utilize phenol. Honig et al. (35) found that <u>Chilomonas paramecium</u> is phenol tolerant.

C. Dissolved Oxygen

Conventional waste treatment aeration systems generally maintain dissolved oxygen levels in the 1-3 mg/L range (44,45,46). However, it has been shown (44) that the

treatment of of concentrated coke plant effluents, which are high in phenolics, require higher than normal dissolved oxygen levels. A common error in laboratory experimentation on activated sludge treatment is to maintain dissolved oxygen levels markedly higher in model aeration tanks than those applied in full scale treatment (44).

Beltrame, Beltrame, and Carniti (7) operated a continous stirred reactor treating phenol, nitrophenol, and cholorphenol with a DO of 7.5 mg/L at 20°C. Rozich (20), and Rozich, Gaudy and D'Adamo and Gaudy (18) biodegraded phenol in a continous stirred reactor with sludge recycle at 90% O₂ saturation. A once-through chemostat utilizing phenol as a substrate was operated at over 80% dissolved oxygen by Rozich, Gaudy and D'Adamo (25). Radhakrishnan and Sinha Ray (14) biodegraded phenol at a DO of 2.0 mg/L. Some researchers have simply reported air flow rates (9,10) without mentioning dissolved oxygen level. Results from the present study indicate that the high aeration levels reported by numerous investigators may be an important factor to monitor when treating phenol with a heterogeneous microbial population.

III. EXPERIMENTAL APPARATUS AND PROCEDURES

A. Microbial Characterization

Figure 1 shows the microbial identification procedures used to characterize mixed liquor organisms. A 10 ml sample was taken during Run 3 from CMBR-2 on day 17 and pipetted into a sterilized 25 ml glass vial with 5 mm glass beads. The vial was shaken for 10 minutes to separate bacterial clumps. A 10^{-1} dilution was made using 0.5 ml of the mixed liquor pipetted into 4.5 ml of 0.1% Tween 80 solution. A series of dilutions $(10^{-2} \text{ to } 10^{-12})$ were made with sterile distilled water. Selected dilutions (10-10 to 10^{-12}) were used for bacterial characterization, while the 10^{-1} , 10^{-3} , and 10^{-3} dilutions were used for yeast and mold characterization. Agar plates were inoculated with 0.1 ml of the selected dilutions and spread over the plate surface with an alcohol/flame sterilized glass spreader. The inoculated plates were incubated for 72 hours at room temperature (25°C).

After incubation, the bacterial colonies were counted and identified according to size, shape, and color. A representatative inoculum from each bacterial colony was isolated using the streak-plate method. Isolated bacterial colonies were then incubated on fresh nutrient agar plates for 24 hours at room temperature.

A Gram-stain was performed on each representative colony. The external features of the organism

 \dot{o}

(rod or cocci) and relative size were also noted.

Gram positive bacteria (purple) were then subjected to the catalase and coagulase tests, followed by inoculation on dextrose tryptone agar (DTA), blood agar, and Enterotubes (Hoffman-La Roche). The DTA, blood agar, and Enterotubes were then incubated for 24 hours at 37°C. The results were recorded, and Bergey's manual consulted to aid in identification (36).

Gram negative bacteria were first subjected to an oxidase test. If oxidase positive, the organism was inoculated into an Oxiferm tube (Hoffmann-LaRoche); otherwise, an Enterotube was inoculated. After incubating for 48 hours at 37°C the results were recorded and the bacteria identified using code books supplied by Hoffmann-LaRoche (37,38).

Molds were identified by morphology and color. The size and shape of the spores along with the type of hyphae were determined by microscopic analysis. The color of the mold and its growth characteristics on the agar plate were used to determine the genus of the mold. A standard reference source (39) was used to aid in identification.

Yeasts were observed microscopically to note their reproductive stages, and then isolated using the streak-plate technique. The isolated yeast colonies were incubated for 24 hours and inoculated into GBE tubes (Flow Labratories). If germ tubes were observed from a 6 hour inoculum, the yeast was known to be a member of the genus

Candida.

Germ tube positive yeast were then inoculated onto SAM slants to differentiate <u>Candida albicans</u> from <u>Candida</u> Stellatoides.

If the yeast were germ tube negative, a suspension of yeast cells was inoculated onto a UNI-YEAST TEK plate and kept at room temperature for 6 days. Observed color changes in the wells were compared to coded data, and the yeast identified according to information supplied by Flow Laboratories (40).

B. Influent Feed Preparation

The ratio of carbon:nitrogen:phosphorus for <u>E</u>. <u>Coli</u> was used in preparing the influent feed (42). However, the proportion of carbon was doubled to account for the fact that about 50 percent of the available carbon is used to provide energy to the organisms, while the other 50 percent is used for synthesis. Therefore, the influent feed had a C:N:P ratio of 100:14:3.

The influent feed was prepared in a 190 liter Nalgene tank. Phenol (Fluka Chemical Corp, Puriss sp.) was the sole carbon source for all experimental runs (250 mg/L). Nitrogen and phosphorous were provided by adding A.C.S. reagent grade ammonium carbonate and ammonium phosphate to the influent feed. The mixing of the phenol with the ammonium compounds yielded a well buffered substrate (pH 7.0). The chemicals were diluted in tapwater to provide trace nutrients.

C. Reactor Set-up and Operation

4 experimental runs were performed.

Run 1

The first run attempted to replicate results achieved in our lab by a previous investigator (5) using a modified Bio-Oxidation system (Cole Parmer, Chicago Illinois).

Operating parameters are summarized below:

Q		Influent Flow Rate		8 m1∕min
Qe		Effluent Flow Rate		8 ml/min
Qr-		Sludge Recycle Rate	= 1	ls ml∕min
Qr/	Q =	2		
Va	===	Aeration Tank Volume		4.2 liters
۷œ	-	Clarifier Volume		2.8 liters
٧t	===	System Volume		7.0 liters
So	=	Influent Substrate Con	cent	tration = 250 mg/L phenol
Н	===	Hydraulic Detention Ti	me	= 8.75 hr
A	=	Air Flow Rate		10 SCFH
Qw	==	Total System Wastage	=	500 ml/day

Using the aformentioned parameters, the modified bench-scale system exhibited unexpected instability. Previous data (5) indicated that steady-state conditions would be rapidly achieved. However, MLSS values rapidly fell, then fluctuated, while Effluent Suspended Solids levels (ESS) were very high. Initial experiments had indicated that the system pH should remain close to neutral. However, over the 15 day run the pH varied over nearly two

units (5.2-7.0). It was determined that one bioreactor was insufficient for continued experimentation. Thus, two new Lucite reactors were designed and built.

Run 2

Figures 2 and 3 show a schematic of the CMBR system (reactor, clarifier, and pumps). Each aeration tank was constructed from a six-liter Lucite cylinder (6" OD, 5" ID, 17" length) bonded via methylene chloride to a 10-inch square Lucite base. The cylinder was drilled and tapped to accomodate four-1/4" brass tubing fittings.

The aeration system provided with the Bio-Oxidation console was inadequate for two reactors, thus a new aeration system was constructed. The CMBR air supply was manifolded through a series of globe-type toggle valves from a filtered compressed air line. The filter system included an in-line oil/water trap followed by a capped stainless steel pipe containing 4 alternating sections of glass wool and fine granulated activated carbon leading to a final polishing filter (Balston DFU, grade BK). Filtered air passing through the toggle valves was regulated by three separate flow meters per reactor. Each flow meter controlled air flow to a Kimax gas dispersion tube (part # 12-c).

Solids recycling was controlled via 2 peristaltic pumps (Masterflex Model 7018-20). The influent flow rate was regulated with a 4-channel Orion Research Model 375A tubing pump. Aeration tank and clarifier effluents were collected via gravity overflow. The aeration tank overflow was routed to the clarifier via a 3/8" diameter 3 foot

length of Tygon tubing. The tubing was held in place by a clamp, and the terminus of the tubing was ten centimeters below the clarifier liquid surface to prevent system short-circuiting.

Run 2 operating parameters were established from Run 1 data. Several minor modifications were made to compensate for design changes in aeration tank and clarifier volumes. The operating parameters were as follows:

CMBR 1 and 2

 $Q = 7.6 \, \text{ml/min}$

Qe = 7.6 ml/min

Qr = 15.2 ml/min

0r/0 = 2

Va = 4.0 liters

Vc = 3.0 liters

Vt = 7.0 liters

So = 250 mg/L phenol

H = 8.75 hours

A = 9.5 SCFH

0w = 475 ml/day

A defective flow meter decreased the aeration level of CMBR 1 from day 3 to day 19, during Run 2. Both systems washed out on day 19.

Run 3

For Run 3, a third Lucite reactor was constructed and a third peristaltic pump added for solids recycle. The operating parameters for the CMBR units were as

f01]						
		CMBR-1	CMBR-2	CMBR-3		
Q		7.2 ml/min	7.2 ml/min	7.2 ml/min		
Qe	uading 	7.2 ml/min	7.2 ml/min	7.2 ml/min		
0r		15.2 ml/min	15.2 ml/min	15.2 ml/min		
Qr7(2 =	2	2	2		
Va	112	4.OL	4.OL	4. OL		
۷c		3.OL	3.OL	3.OL		
So		250 mg/L	250 mg/L	250 mg/L		
Н		8.75 hr	8.75 hr	8.75 hr		
Qw	<u></u>	400 ml/day	400 ml/day	400 ml/day		
A	==	1.5 SCFH	3.5 SCFH	9.0 SCFH		
Aer	ation lev	vels were varied	d in an attempt	to determine the		
eff	ects of di	issolved oxygen	content on prod	ess stability.		

Run 4

For Run 4, an aeration tank stand with three compartments was constructed to house magnetic stirrers. One of the magnetic stirrers was used to assist in the mixing of CMBR-2, since the aeration level (0.75 SCFH) was insufficient by itself to maintain adequate mixing. In addition, 10 ml of 1M sodium carbonate buffer solutions were added daily to each reactor to maintain a pH between 6.0 and 8.0 in the CMBR units. In previous runs, pH had varied as much as 1 full unit over a 1 day period. The additional buffering was seen as a means to decrease the likelihood that pH factors were responsible for experimental results.

Run 4 operating parameters were as follows:

		CMBR-1	CMBR-2	CMBR-3
Q	=	7.6 ml/min	7.6 ml∕min	7.6 ml∕mi⊓
Qe		7.6 ml/min	7.6 ml/min	7.6 ml/min
٥r		15.2 ml∕min	15.2 ml/min	15.2 ml∕min
Qr /	Q=	2	2	2
Va		4.0 L	4.0 L	4.0 L
۷c		3.0 L	3.0 L	3.0 L
٧t		7.0 L	7.0 L	7.0 L
So		250 mg/L	250 mg/L	250 mg/L
Н		8.75 hr	8.75 hr	8.75 hr
Qы		400 ml/day	400 ml/day	400 ml/day
A		1.5 SCFH	0.75 SCFH	9.5 SCFH

D. Hydraulic and Mixing Characteristics

The CMBR is a bench-scale adaptation of the completemix activated sludge process. In this operational mode, incoming wastes are instantaneously mixed in the aeration basin and the reactor concentration equals the effluent concentration. If through design flaws or operator error, the reactor is not completely mixed, some fraction of the wastewater may leave untreated. The percent of dead space and the amount of plug flow in the aeration basin can be determined by dye tests.

The following procedure was used to determine the fractions of dead space and plug flow in the CMBR units:

- The reactors were washed and rinsed with distilled water.
- The reactors were filled with 4.0 liters of distilled water.

- 3. A 4 ppm solution of Evans Blue Dye was prepared in 10.0 liters of distilled water.
- 4. The aeration rate to each reactor was varied (1.5,4.5 and 7.5 SCFH) to determine the effect of this parameter on mixing.
- 5. The array of 3 diffusers was raised 5 cm off the bottom of the aeration basin to determine what effect this parameter would have on mixing.
- 5. The dye solution was fed at a rate of 25.0 ml/min by a Sage Instruments Model 375A peristaltic tubing pump.
- 7. The reactor effluent was collected periodically from the moment that dye was fed, and the samples were analyzed for dye intensity on a Bausch and Laumb Spectronic 20 spectrophotometer set at 600 nanometers.
- 8. Dye recovery curves were plotted for four dye tests in Figures 4-7. The amount of dead space in a completely-mixed reactor is determined by observing at what fraction of a detention time (160 minutes) 63 percent of the dye is recovered. The fraction of dead space is equal to the remaining fraction.
- 9. The fraction of plug flow is determined by noting the y intercept of the dye concentration versus absorbance plot. A completely mixed reactor will have an intercept of 0.0.

E. Oxygen Mass Transfer Coefficient

The oxygen mass transfer coefficient was determined in accordance with Method 208 of Standard Methods for the Examination of Water and Wastewater (43). The reactor was filled with 4.0 liters of tap water, and deoxygenated by adding 80 mg/L sodium sulfite and 1 mg/L cobalt chloride catalyst. The air supply was set at the operating rate of 7.5 SCFH, and the DO in the reactor measured with a dissolved oxygen probe (Orion Research, Model 97-08). The logarithm of (Cs-C) was plotted vs. time where Cs is the saturation concentration for dissolved oxygen in distilled water at room temperature (8.0 mg/L), and C is the measured dissolved oxygen concentration. The slope of the plot is equal to the oxygen mass transfer coefficient. The experiment was repeated with killed Livingston mixed liquor instead of distilled water and the results compared.

F. DISSOLVED OXYGEN UPTAKE RATE AND DISSOLVED OXYGEN

The dissolved oxygen uptake rate (DOUR) was determined using method 213-A of Standard Methods for the Examination of Water and Wastewater (43). A 300 ml BOD bottle was filled with mixed liquor from each reactor, an Orion Research model 97-08 DO electrode was calibrated and inserted along with a magnetic stirring bar, and the bottle placed on a magnetic stirrer. DO readings were taken every minute for 15 minutes. The results were plotted against time yielding a straight line. The slope of the plot is the DOUR.

The Dissolved Oxygen(DO) content of the CMBRs' was

measured by immersing an Orion Research DO probe directly into each aeration tank. The dissolved oxygen content was read on a digital ionanalyzer (Orion Research Model 501).

G. Suspended Solids

The Mixed Liquor Suspended Solids (MLSS), Return Suspended Solids (RSS), and the Effluent Suspended Solids (ESS) were determined using a modification of Method 209-D of Standard Methods for the Examination of Water and Wastewater (43). Tared aluminum weighing dishes were used instead of ceramic crucibles. 30 ml of mixed liquor, 30 ml of return liquor, and 30 ml of effluent from the overflow tank were collected daily from each CMBR. Three-10 ml aliquots of each liquid sample were pipetted into dessicated and preweighed aluminum dishes, (9 dishes per day per CMBR). The samples were heated for 24 hours at 104°C. After cooling in a dessicator for 5 minutes, the dishes were reweighed on an analytical balance (Mettler, Type H6) and the averaged solids concentration determined by difference.

H. Temperature and pH

All 4 runs were conducted at room temperature (18-28°C). A mercury thermometer was continually immersed in each reactor. Temperature readings were made daily.

The pH of the aeration tanks were measured daily with a combination pH electrode (Orion Model 91-04) and a digital ionanalyzer (Orion Model 501). The electrode was calibrated using 3 buffer solutions (pH 4.0, 7.0 and 10.0) before each measurement. Although the ionanalyzer provided readings to 0.01 standard pH units, the results were rounded to the

nearst 0.1 pH units.

During Runs 1, 2 and 3 the pH of the CMBR units was not adjusted. However, during run 4, 10.0 ml of 1 M sodium carbonate was added every 24 hours to the aeration tanks to maintain a system pH between 6.0 and 8.0.

I. Effluent Substrate Concentration

Effluent samples (10.0 ml) were collected daily from the top of each clarifier. 1.0 ml of 10,000 mg/l copper sulfate solution was added to each sample as a biocide, and the samples stored in a refrigerator until they were analyzed by gas chromatography. The effluent concentration was not measured during Run 1.

Run 2

A Varian model 3300 gas chromatograph was used to determine effluent phenol concentrations. The GC was equipped with a Shimadzu Chromatopac model C-R3A operating in plot mode 41. GC conditions were the following:

Injection Volume		1 microliter
Injections/Sample		3
Analysis Time		3 minutes
Phenol Retention Time		1.19 minutes
Injection Port Temperature		200°C
Oven Temperature		140°C
Detector Temperature	· •••	240°C
Attenuation		26
Column	-	Alltech # 8011/2 SS 6' Chrom. W-HP

Plot integrated area vs. known concentrations of phenol standards.

Run 3 and 4

A Varian model 3760 gas chromatoraph was used to determine effluent phenol concentrations. The GC was equipped with a Hewlett Packard 3390A integrator. GC conditions were the following:

Injection Volume		1 microliter
Injections/sample		3
Analysis time		3 minutes
Phenol Retention Time		0.83 minutes
Injection Port Temperature		270°C
Column Temperature		140°C
Detector Temperature	_	300 - C
Attenuation		2
Column		Supelco: 6 ft X 1/8 inch stainless steel 10% SP2100 on 100/200 Super Coport
Calibration		Integrator using ESTD mode (external standard)

IV. RESULTS AND DISCUSSION

A. Microbial Population

Results of the microbial characterization for Run 3 are presented in Table 33 . The microorganisms identified were similar to those identified previously in our laboratory (5). <u>Pseudomonas putida</u> was identified as the organism which formed substantial aeration tank wall growth in Run 3. This adaptive behavior has been reported elsewhere (15).

Bulking due to gelatinous-like growth was observed after day 5 in all 3 clarifiers. This growth was tentatively identifed as filamentous. However, after subjecting the microbial mass to Nigrosin staining, a basic capsular dye, it was concluded that the clarifier bulking was due primarily to microbial slime layers/capsules. Many bacteria and fungi including Bacillus sp., the Lactic Acid Bacteria, Penicillium produce these extracellular products and as a means of non-specific adherence and/or defense (48). Since some of these products are known mammalian toxins (eg. those produced by Bacillus anthraeses), due care was taken while examining these structures.

The causes of bulking are poorly understood, but it is frequently associated with a variety of factors including: high C:N and C:P ratios and/or low dissolved oxygen concentrations (48); (although this is contrary to the results of the present work), high sludge volume index (51), and a pH below 6 (44).

Experimental C:N:P ratios were similar to synthetic waste streams used by several investigators (7,53) and were similar to EPA recommended levels (44,45). During Run 4, pH was maintained between 6.2 and 7.7, yet bulking was still observed. Actinomycetes were not observed in significant numbers during the 4 runs, or during previous experiments in our laboratory (5).

Microbial characterizations were not attempted during Run 1 or 2. Run 4 was prematurely terminated prior to microbial characterization.

B. Hydraulic and Mixing Characteristics

The results of the four dye mixing tests are listed in Tables 5-8 and plotted in Figures 3-6. At aeration levels between 1.5 and 7.5 SCFH, nearly complete mixing was achieved in the CMBR aeration tanks, (93.75%-99.19% complete-mix). Flug flow conditions were not seen in any of these experiments.

Diffuser location was shown to be an important factor. When the diffusers were raised 5.0 cm off the bottom of the aeration tank under identical aeration levels (7.5 SCFH), there was a 7.0% increase in dead space within the reactor. Thus, all experimental runs were conducted with the diffusers in contact with the bottom of the aeration tank.

C. Oxygen Mass Transfer Coefficient

Experiments conducted to determine the OMTC values for tapwater and killed Livingston sludge were inconclusive. It

has been reported that the OMTC is lower for wastewater than tap or distilled water because of the presence in wastewater of surface active materials. These materials include short chain fatty acids and alcohols which concentrate at the air/water interface forming a layer capable of retarding molecular diffusion (55). Results of four OMTC experiments (7.5 SCFH) yielded dramatically dissimilar results, ranging from 0.215-1.00. Since the mixed liquor used was collected on the same day, it is unlikely that varied surface active material concentrations were responsible for the observed variations. Previous experiments in our laboratory (5) used 1 mg/l of cobalt chloride catalyst. Recent data indicates (44,55) that this concentration of catalyst may be excessive, by as much as 20 times, and may have caused the observed results.

D. DISSOLVED OXYGEN UPTAKE RATE AND DISSOLVED OXYGEN

Dissolved Oxygen Uptake Rate and Dissolved Oxygen results are listed in Tables 15-23 and DOUR results are plotted in Figures 8-16.

In Runs 1 to 3 the DOUR values were 0.1-0.6 mg/L/min. In all 3 runs the DOUR values fluctuated significantly from day to day.

In Run 4, additional buffer was added to maintain aeration tank pH values of 6.0 to 8.0. The result was to increase the Suspended Solids (MLSS, RSS, ESS), in all 3 CMBR units. Additionally, the DOUR values were slightly higher on average than previous runs, with DOUR values

ranging from 0.2-1.00 mg/1/min.

While DOUR levels were indicative of microbial activity, they were not as valuable for estimating phenol degradation. In fact, the highest DOUR value recorded (1.00 mg/L/min CMBR-1, Run 4, day 1) coincided with the highest effluent phenol concentration of the run (30.8 ppm, 8% of influent concentration).

The high DOUR rates observed at the beginning of Run 4 were expected, because the microbial population was not yet acclimated to phenol as a carbon source.

The primary focus of this research was to investigate the effects of dissolved oxygen content on process stability in a bench scale activated sludge system treating phenolic compounds. For the experimental conditions studied, dissolved oxygen levels did not fluctuate in any of the CMBR units (with one exception) by more than 2 mg/L during any run.

In Run 1, the dissolved oxygen content was maintained near theoretical saturation limits (for distilled water at standard conditions) throughout the experiment. The high aeration rate was actually sufficient to move the reactor while on its tripod base. DO values ranged from 6.6-7.8 mg/L.

In Run 2, DD values ranged from 2.2-7.8 mg/L for CMBR-1 and from 6.5-7.9 mg/L for CMBR-2. The wide fluctuation seen in CMBR-1 DD values was caused by a faulty flowmeter which failed after day 3. Run 2 was continued with CMBR-1 receiving a decreased air flow rate. Both reactors washed

out on day 19. Although the units were operating under identical conditions (with the exception of the aeration rate/DD), significant variations were observed in the units. After day 3, CMBR-1 constantly maintained significantly higher DOUR and suspended solids levels while also maintaining a lower reactor pH than CMBR-2. Averaged substrate removal rates were greater than 98% for both units. These results indicated that at lower aeration levels substrate removal was not decreased and might potentially be enhanced by the higher DOUR and suspended solids concentrations.

In Run 3, CMBR units were maintained with DO levels of approximately 5.0, 6.0, and 7.0 mg/L. After an initial lag phase of two days, CMBR-1 (which had the lowest DO level) exhibited steady-state operation for 21 days, while CMBR 2 and 3 washed out on day 10 and 9 respectively.

In Run 4, three dissolved oxygen levels were chosen to simulate conditions normally found in bench-scale and fullscale activated sludge units. CMBR-3 (9.5 SCFH, DO = 6.8 to 8.0 mg/L, average DO = 7.2 mg/L), exhibited near-saturation levels of dissolved oxygen in the aeration basin. This is typical of numerous bench scale systems mentioned previously (5,7,18,19,20,21). CMBR-2 (0.75 SCFH, DO = 0.9-2.7 mg/L, average DO = 1.6mg/L), maintained a dissolved oxygen level similar to that reported in conventional full scale activated sludge plants (44,45,46). CMBR-3 (1.5 SCFH, DO = 4.0 to 6.0 mg/L, average DO = 4.6 mg/L), was operated at
an intermediate dissolved oxygen content. Results from Run 4 confirmed Run 3 data. CMBR-2, maintained the lowest DO level of the three units and had on average the highest suspended solids concentration and DOUR while maintaining the lowest aeration tank pH. In addition, CMBR-2 continously treated phenol for 31 days (when the experiment was accidentally terminated), while CMBR 1 and 3 washed out on days 24 and 12 respectively.

E. Suspended Solids

Run 1

MLSS results for Run 1 are summarized in Table 24 and plotted in Figure 17. The mixed liquor suspended (MLSS) rapidly decreased from day 0 to day 2 (3100 to 1900 mg/L). The mixed liquor changed from dark brown to a dark tan color during the two day period. With the exception of day 7, effluent suspended solids (ESS) were very high, generally greater than one-third of the MLSS value. The return suspended solids (RSS) fluctuated significantly from day to day. This was apparently caused by poorly settling solids in the clarifier which tended to adhere to the clarifier walls. Run 2

CMBR-1 was inadvertantly operated with a lower air flow rate than CMBR-2 from day 3 to day 19. CMBR-1 operated with a consistently higher suspended solids concentration for both MLSS and RSS during this time. Results for both reactors are summarized in Tables 25 and 26 and Figures 18 and 19.

Settling was improved over Run 1 in both systems,

apparently due to an increase in clarifier volume (2.8 to 3.0 liters) and a decrease in aeration rate (10 to 9.5 SCFH). With the exception of day 1, ESS were significantly lower than Run 1. In fact, ESS levels were below detectable limits (BDL) on 11 of 19 days for CMBR-1 and 10 of 19 days for CMBR-2. Day 1 ESS measurements were attributed to a population shift within the reactors by the elimination of non-phenol tolerant microorganisms.

Run 3

For Run 3, daily sludge wasting from the aeration tank was decreased from 375 ml/day to 300 ml/day in an effort to increase sludge age (mean cell residence time) thereby decreasing loss of solids in the effluent. This approach has been recommended by several authors (45,46). Results from Run 3 indicate that this effort was not successful.

Run 3 suspended solids results indicate that from day 0 to day 7 essentially the same conditions existed in all 3 CMBR units. ESS were being lost at a rate approximately 5 to 15 % of the MLSS level while MLSS and RSS rapidly decreased. The high ESS values observed on day 1 in Run 2 did not recur in Run 3. The rapid decrease in MLSS concentrations in CMBR-2 and 3 led to washout on days 9 and 8 respectively. However, CMBR-1, which was operating at the lowest DD level, reached a relatively steady-state condition and continued treating phenol for 12 more days with the following approximate suspended solids concentrations:

RSS	 900	mg∕L
ESS	 50	mg∕L

CMBR-1 washout was preceeded by a slight decrease in ESS on day 21. ESS were detected on 6 of 8 days for CMBR-3, 7 of 9 days for CMBR-2, and 9 of 21 days for CMBR-1. CMBR-1 mixed liquor had a dark tan coloration after day 8 apparently due to the low MLSS concentration in the reactor and possibly to the excess wall growth (later identified as Eseudomonas putida), in the aeration

tank.

Run 4

Suspended solids data for Run 4 are listed in Tables 30-32 Figures 23,24, and 25. Ten milliliters of 1M sodium carbonate were added daily to each aeration tank in an effort to maintain a more favorable reactor environment, pH 6.0-8.0.

The optimum pH range for many bacteria falls between 6.0-7.0. Such organisms include Escheria coli. Proteus <u>yulgaris</u>, Enterobacter aerogenes, and <u>Pseudomonas aeruginosa</u> (48). Fungi generally exhibit a wider pH range, growing well over a range of 5-9.

The additional buffering appeared to increase the suspended solids concentration over that observed in Run 3:

Run	3	CMBR-1	day	1-7	MLSS	average	=	2100	mg∕1
Run	4	CMBR-1	day	1-7	MLSS	average		2800	mg∕1
Run	Z	CMBR-3	day	1-7	MLSS	average	==	2500	mg∕1
Run	4	CMBR-3	day	1-7	MLSS	average		2800	mg∕1
Run	3	CMER-1	day	1-7	RSS a	average	H	2400	mg/l

Run	4	CMBR-1	day	1-7	RSS	average		3700	mg∕1
Run	3	CMBR-3	day	1-7	RSS	average	-	3300	mg∕l
Run	4	CMBR-3	day	1-7	RSS	average		4000	mg∕l
Run	3	CMBR-1	day	1-7	ESS	average	H	100	mg/l
Run	4	CMBR-1	day	1-7	ESS	average		500	mg∕l
Run	З	CMBR-3	day	1-7	ESS	average		200	mg∕l
Run	4	CMBR-3	day	1-7	ESS	average	=	500	mg∕l

Unfortunately, a significant fraction of the CMBR solids were being lost in the effluent of all Run 4 reactors, a problem which was not encountered during Run 3. This excess loss of solids may have resulted from the alkaline shock caused by the daily addition of the sodium carbonate buffer.

CMBR-2, which operated for 31 days, maintained the dark brown coloration typical of unacclimated Livingston mixed liquor. The wall growth seen in Run 3 was much less significant, covering only 1/3 of the aeration tank wall (primarily adjacent to the substrate influent port). Run 4 CMBR units 1 and 3 remained in operation longer than their Run 3 counterparts (24 days vs. 21 days and 12 days vs. 9 days). The instability and rapid system washout observed in Run 3 recurred in Run 4 units operating at high DO levels, (7.2 and 4.6 mg/L).

F. Temperature and pH

Temperature and pH results collected during the four runs are presented in Tables 6-14.

Run 1

Run 1 temperature data indicated that laboratory

temperatures fluctuated significantly from day to day. This resulted in mixed liquor temperatures varying as much as 6° C during a 24 hour period, (day 13-14). Reactor temperatures ranged from 15°C to 28°C, during Run 1.

Run 2,3 and 4

The CMBR units were placed in a more temperature stable area. Reactor temperatures rarely varied by more than 2°C from day to day. The stable temperature moderated the effects which even relatively small (10°C) temperature changes can have on microbial systems.

The temperature ranges and the average temperature for Runs 2,3, and 4 are summarized below:

Run	2		Range	Average
		CMBR-1	(21-27°C)	25°C
		CMBR-2	(21-25°C)	23°C
Run	3			
		CMBR-1	(22-24°C)	23-0
		CMBR-2	(20-23 °C)	22°C
		CMBR-3	(20-23 ° C)	22°C
Due	л			
RUH	-+			
		CMBR-1	(20-25°C)	23°C

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CMBR-2	(22-25°C)	24°C
CMBR-3	(21-23°C)	22°C

The results of pH measurements made during the four runs are presented in Tables 6-14 and are summarized below:

Run	2C		Range	Average
		CMBR-1	(6.2-7.5)	6.5
		CMBR+2	(6.6-7.4)	6.7
Run	3			
		CMBR-1	(5.4-7.4)	6.1
		CMBR-2	(5.7-7.7)	6.3
		CMBR-3	(5.9-7.7)	6.5
Run	4			
		CMBR-1	(6.2-7.4)	6.5
		CMBR-2	(5.7-9.6)	6.4
		CMBR-3	(6.5-7.8)	6.7

The pH of the mixed liquor during Runs 1-4 ranged between 5.4-7.8. Measurements from Run 4 indicate that when the average pH was increased from 6.1 to 6.5 in CMBR-1 and 6.5 to 6.7 in CMBR-3 by sodium carbonate buffer, both the total suspended solids (MLSS, RSS, and ESS) and DOUR increased. However, there was not a significant increase in phenol biodegradation since both systems (after an initial lag phase) removed phenol at better than 98% efficiency. The pH excursion observed during Run 4, (CMBR-2, day 31) was caused by an over-addition of sodium carbonate buffer, (50 ml vs. 10 ml). This resulted in billows of white foam in the aeration tank followed by a total loss of solids via the clarifier.

Microbial growth rates can be greatly influenced by pH, because of the nature of proteins. Because charge

interactions within polypeptide chains greatly influence both the structure and activity of enzymes, these enzymes can become inactivated at a pH outside of their optimal range (48). Researchers vary in their opinions concerning optimal pH values for aerobic activated sludge processes. Ganczarczyk reports (44) pH values ranging from 3-10 have been utilized in activated sludge systems. In general, neutral pH values are considered optimal while acidic conditions (which promote filamentous growth) are less desirable.

G. Effluent Substrate Concentration

Run 1

The effluent substrate concentration was not measured during Run 1.

Run 2

Figure 35 shows a typical chromatogram obtained during phenol analysis with a Varian Model 3300 G.C. and Shimadzu Chromatopac (Model C-R3A) in plot mode 41. The effluent substrate concentrations for Run 2 are listed in Table 34. Phenol concentrations which were below detectable levels (BDL) are listed as such in the tables.

During Run 2, CMBR 1 and 2 exhibited similar effluent substrate tendencies. On day 1, both units had detectable phenol concentrations (80.6 mg/L and 12.3 mg/L) which dropped below detection by day 3. On day 3, one of the CMBR-1 flowmeters failed which decreased both the air flow rate and dissolved oxygen in the unit. This did not have a

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significant effect on substrate removal for the remainder of the run. Phenol was detected in the effluent on days 1,2,17, and 19 in CMBR-1 and days 1,6,12,13, and 14 in CMBR-2. One tendency which was noted in both systems during Run 2 was that phenol was detected in the effluent under two different circumstances. The first occurred at the beginning of the run when mixed liquor organisms were rapidly acclimating to the inhibitory substrate and/or depleting the noninhibitory compounds within the fresh mixed liquor. The second circumstance occurred when MLSS values dropped below 1000 mg/L. Typical activated sludge units operate with MLSS values ranging from 2500-7000 mg/L (42.54). Although MLSS should not be used as a direct indicator of total microbial population, the low MLSS values did indicate that a general decrease in microbial population and/or diversity was occurring. Although MLSS values decreased to approximately one-fourth of their original concentration (4600 mg/L day 1 vs 800 mg/L day 19 CMBR-1), phenol was being removed at greater than 99% efficiency (BDL day 19 CMBR-1). This indicated that the microbial on population present during the later stages of the run was a well adapted consortium capable of utilizing phenol as a sole carbon source. In addition, the low MLSS values observed in the aeration tank during the later stages of the run indicated that a significant fraction of the substrate removal was occurring in the clarifier.

Run 3 and 4

Results from Run 3 and 4 are listed in Tables 35 and

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36. The calibration curve used in Run 2 was unsatisfactory over the range of phenol concentrations measured (correlation coefficient 0.96). A Varian Model 3760 GC with a Hewlett Packard integrator (Model 3390A) was calibrated with an external standard (ESTD mode) of 10 ppm. A typical chromatogram is illustrated in Figure 36. A one hour old influent feed preparation (250 ppm) was shown to have a 250.31 ppm phenol concentration by this method.

With the exception of CMBR-3 during Run 4, all of the units exhibited similar effluent substrate levels. After an initial lag phase of 1 or 2 days, the mixed liquor organisms readily utilized the phenol. However, the effluent substrate detected during periods of low MLSS during Run 2 did not occur during Run 3 and 4. For example, during Run 3 (CMBR-2, day 8) the MLSS fell to only 100 mg/L, yet phenol was not detected in the effluent. The extremely low MLSS value measured was caused by a build up of solids in the clarifier. Undoubtedly the majority of substrate removal at this time was occurring in the clarifier.

The efficiency of the CMBR units (substrate removal) can be determined by the following equation:

E = (So-S)/So

where So is the influent substrate concentration in mg/L and S is the averaged effluent substrate concentration over the run in mg/L. Results from Run 4, CMBR-3 (A = 9.5 SCFH) indicate that under "worst-case" conditions, (phenol detected 9 of 13 days in the effluent) CMBR efficiency for

substrate removal was 92%. During Run 4, CMBR-2 (SCFH = 0.75) had measurable effluent concentrations on 5 of 32 days with a removal efficiency in excess of 99%.

Studies both in our laboratory (5) and elsewhere (3,4,18) indicate that phenol has only a slight tendency to adsorb to biomass, exhibits only a slight tendency to bioaccumulate (Kow=1.46), and is highly soluble in water (78,960 mg/L). Results from our laboratory indicate (53) that phenol is not air stripped from batch reactors. From these results it was concluded that biodegradation represents the primary removal mechanism for phenol in the CMBR system.

The food to microorganism ratio is determined in the following manner:

F/M = So/H * X

where So is the influent substrate concentration, H is the hydraulic detention time, and X is the mixed liquor suspended solids concentration.

The F/M ratio was calculated for Run 3 and 4 using the highest and lowest MLSS concentration as well as an averaged MLSS (which is a better indicator of F/M trends):

F/M (day-1)

Run 3	with Highest MLSS	with Lowest MLSS	with Average
MLSS			
CMBR-1	0.19	0.98	0.46
CMBR-2	0.15	6.86	0.34

CMBR-3	0.14	1.14	0.26
Run 4			
CMBR-1	0.15	1,37	0.33
CMBR-2	0.17	1.37	0.34
CMBR-3	0.14	0.66	0.30
Recommend	ed F/M values	for conventi	ial activated
sludge process	es range from 0.2	to 0.6 per day	(54). Results
indicate that	the substrate lo	ading was ger	nerally within

acceptable limits. The high F/M reported in Run 3, CMBR-2 was the result of solids being retained in the clarifier prior to that systems washout.

V. Comments and Conclusions

After much trial and error, a continous flow reactor with solids recycle was operated at conditions approaching steady-state with phenol as a sole carbon source. The original microbial population came from the mixed liquor of the Livingston, N.J. wastewater treatment plant, which primarily treats domestic waste.

The phenol loading for the laboratory reactors was high (250 ppm in the influent feed), which resulted in significant stress on the microbial population.

The most important parameter was the dissolved oxygen (DO) concentration. Although stable reactor operation was obtained at an average DO of 1.6 mg/L (CMBR-2, Run 4), higher average DO levels (4.6 and 7.2 mg/L), resulted in reactor washout and considerable slime production by the bulking solids in the clarifier. The bulking was the result of the slime production rather than filamentous growth.

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ELEMENTAL CELL COMPOSITION

Element	Dry Weight Percent
Carbon	50
Oxygen	20
Nitrogen	14
Hydrogen	8
Phosphorus	3
Sulfur	1
Sodium	1
Pottasium	1
Calcium	O. 5
Magnesium	0.5
Chlorine	0.5
Iron	0.2
All Others	0.3

Source:	Gauc	łγ,	A.	F.,	et	al.		Mi	C۲	оb	iol	эgy	for
	Envi	. r or	nmen	ital	Scei	nti	st	3	аn	d	Eng:	ineer	s.
	New	Yor	-k,	NY:	McGr	aw-	Hi	11	9	19	80.		

HYDRAULIC AND MIXING DATA

A = 7.5 SCFH		
Diffuser Location		5.0 cm off bottom of tank
Qi Absorbance	:==	0.54 at 600 nm
Plug Flow	=	0
% Complete Mix	:22	93.75

TIME	ABSORBANCE	PERCENT
(min)		RECOVERY
0	0.000	0
1	0.002	0.37
2	0.005	0.93
4	0.011	2.0
8	0.022	4.1
12	0.032	5.9
15	0.039	7.2
20	0.050	9.3
60	0.138	25.6
80	0.172	31.9
100	0.210	38.9
120	0.260	48.1
140	0.278	51.5
150	0.292	54.1
160	0.320	59.3
170	0.324	60.0
180	0.331	61.3
190	0.340	63.0
200	0.355	65.7
220	0.370	68.5
240	0.380	70.4
260	0.390	72.2
280	0.415	76.9
300	0.420	77.8
320	Ŏ.44Ŏ	81.5
360	0.450	83.3
390	0.460	85.2

HYDRAULIC AND MIXING DATA

A		7.	5	SC	Fŀ	1		
Di	44	นร	er	1	oc	ation	ר ≕	bottom of tank
Qi	ê	bs	or	ba	nc	e	22	0.252 at 600 nm
F1	ιιc	F	$1 \circ$	W				0
%	Cc	mp	l e	te	٢	1i ×	==	99.19

TIME	ABSORBANCE	PERCENT
(min)		RECOVERY
0	0.000	Ö
1	0.001	0.4
2	0.002	0.8
4	0.006	2.4
8	0.012	4.8
12	0.015	6.0
15	0.019	7.5
20	0.025	9.9
40	0.052	20.6
80	0.092	36.5
120	0.132	52.4
140	0.142	56.3
150	0.152	<u> 40.3</u>
160	0.158	62.7
170	0.165	65.5
180	0.168	66.7
200	0.175	69.4
260	0.195	77.4
280	0.220	87,3
320	0.235	93.3
360	0.240	95.2
390	0.249	98.8

HYDRAULIC AND MIXING DATA

A = 4.5 SCFH		
Diffuser location		bottom of tank
Qi absorbance	==	0.260 at 600nm
Plug Flow		0
% Complete Mix		97.35

TIME	ABSORBANCE	PERCENT
(min)		RECOVERY
0	0.000	0
1	0.001	. 4
2	0.001	0.4
4	0.004	1.5
8	0.010	3.8
15	0.015	5.8
20	0.018	6.9
40	0.055	21.2
80	0.095	36.5
120	0.125	48.1
140	0.140	53.8
150	0.152	58.5
160	0.160	61.5
170	0.165	63.5
180	0.170	65.4
190	0.178	68.5
200	0.180	67.2
240	0.198	76.2
280	0.210	80.8
320	0.215	82.7
360	0.220	84.6
390	0,230	88.5

HYDRAULIC AND MIXING DATA

A = 1.5 SCFH Diffuser Location Qi absorbance Flug Flow % Complete Mix	 bottom of tank 0.255 at 260 nm 0 96.78	
TIME (min)	ABSORBANCE	PERCENT RECOVERY
0	0.000	0
1	0.002	0.8
2	0.002	0.8
4	0.008	3.1
8	0.015	5.9
12	0.020	7.3
15	0.035	13.7
40	0.052	20.4
80	0.092	36.1
120	0.135	52.9
140	0.142	55.7
150	0.149	58.4
160	0.156	61.2
170	0.162	63.5
180	0.164	64.3
190	0.170	55.7
200	0.175	68.6
240	0.188	73.7
280	0.202	79.2
320	0.210	82.4
360	0.212	83.1
390	0.222	87.5

TEMPERATURE AND pH

DAY	TEMPERATURE	pН
	(°C)	(Su)
0	15	7.0
1	26	7.0
2	25	6.1
3	26	5.9
4	23	6.2
5	21	5.9
7	21	5.8
8	21	6.0
9	20	5.9
10	21	6.1
1 1	19	6.0
12	18	6.1
13	18	6.2
14	24	6.0
15	23	5.8

TEMPERATURE AND pH

RUN 2

DAY	TEMPERATURE	рHa
	(°C)	(Su)
0	24	7.5
1	23	7.0
2	24	6.9
3	23	6.8
4	21	6.8
5		6.6
6	26	6.2
7	200	6.2
8	22	6.4
9	24	6.5
10	24	6.3
11	26	6.4
12	26	6.7
13	26	6.3
14	26	6.5
15	26	6.3
1.6	24	6.5
17	26	6.6
13	25	5.4

TEMPERATURE AND pH

RUN 2

DAY	TEMPERATURE	pHq
	(c>C)	(Su)
0	24	7.3
4	23	6.9
2	24	6.9
	23	5.9
<u>A</u>	21	6.7
	24	6.6
	25	5.6
7	23	6.6
á	21	6.7
C,	22	6.8
10	23	6.7
11	22	7.1
12	24	7.4
1 3	25	6.9
14	25	6.8
1 ==;	25	6.7
1.4	24	7.2
17	22	6.9
18	23	6.9

TEMPERATURE AND pH

RUN 3

DAY	TEMPERATURE	pH
	(c= C)	(Su)
0	22	7.6
1	24	6.7
2	23	6.3
3	22	6.3
4	24	6.2
5	22	6.2
6	23	6.O
7	22	5.9
8	23	5.9
9	24	6.1
10	277	5.4
11	24	5.6
12		5.5
13	22	5.5
14	22	5.8
15	22	6.1
16	22	5.7
17	24	6.3
18	23	6.1
19		6.2
20	23	6.3

TEMPERATURE AND pH

RUN 3

DAY	TEMPERATURE	рH
	(⇔C)	(Su)
0	20	7.7
1	23	ద.8
2	22	6.3
3	22	6.2
4	23	6.2
5		6.3
6	23	6.0
7	22	5.8
8	22	6.1
9	22	5.7

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TEMPERATURE AND pH

RUN 3

DAY	TEMPERATURE	рH
	(°C)	(Su)
0	20	7.7
1	22	7.0
2	21	6.5
3	21	6.4
4	23	6.4
5	22	6.4
6	22	6.3
7	22	5.9

TEMPERATURE AND pH

RUN 4

TEMPERATURE	pH
(°C)	(Su)
20	7.4
23	6.6
24	6.4
23	5.5
22	6.2
23	6.5
23	5.4
24	6.5
24	6.8
24	5.7
23	6.3
23	6.2
23	6.2
24	6.4
23	6.2
24	6.4
23	6.4
	6.6
23	6.3
22	6.5
24	7.3
24	6.4
25	5.6
	TEMPERATORE (°C) 20 23 24 23 22 23 23 24 24 24 24 23 23 24 23 23 24 23 23 24 23 23 24 23 23 24 23 23 24 23 23 24 23 23 24 23 24 23 23 24 23 23 24 23 23 24 23 23 24 23 23 24 23 23 24 23 23 24 23 23 24 23 23 24 23 23 24 23 23 24 23 23 24 23 23 24 23 23 24 23 23 23 23 23 23 23 23 23 23 23 23 23

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TEMPERATURE AND pH

RUN 4

DAY	TEMPERATURE	рH
	(°C)	(Su)
0	22	7.1
1	24	6.6
2	24	6.3
3	24	6.5
4	22	6.2
5	22	6.3
6	24	6.3
7	25	6.4
8	24	6.5
9	25	6.4
10	24	6.2
11	24	6.4
12	Vadata (1998)	
13	24	6.2
14	25	6.3
15	24	6.0
16	24	6.2
17	24	6.3
18	24	6.4
19	23	6.2
20	24	5.7
21	25	7.0
22	24	6.3
23	25	6.3
24	24	6.2
25	24	6.9
26	25	6.O
27	23	6.2
28	25	6.5
29	24	6.5
30	24	6.2
31	24	9.6

TEMPERATURE AND pH

RUN 4

DAY	TEMPERATURE	рH
	(°C)	(Su)
Q	21	7.8
1	22	7.2
2	22	6.9
3	22 W	6.9
4	23	6.5
5	21	7.0
6	22	6.8
7	23	6.8
8		7.4
9		7.2
10	22	7.0
11	22	6.8

DISSOLVED OXYGEN AND

DISSOLVED OXYGEN UPTAKE RATE

RUN-1

DAY	DO	DOUR
	$(m \mathfrak{g} \times 1)$	(mg/l/min)
0	á. 3	and they are provided a second
1	7.O	
2	7.1	
3		
4		where report street we have
3	7.5	0.12
6	6.8	0.16
7	7.0	0,20
8	6.8	0.23
9	చ- చ	0.44
10	7.6	0.36
11	8.1	0.24
12	8.0	0.29
13	7.8	0.27
14	6.8	0.44
15	6.7	0.52

DISSOLVED OXYGEN AND

DISSOLVED OXYGEN UPTAKE RATE

RUN 2

CMER-1

DO	DOUR
(mg/l)	(mg/l∕min)
~ ~ ~	0 70
1.1	0.20
7.4	0.18
6.4	0.15
5.7	0.21
4.6	0.19
4.6	0.26
4.9	0.27
5.3	0.24
.	0.25
4.8	0.20
4.5	0.19
4.9	0.23
4.2	o.28
4.2	0.30
4.7	0.27
4.8	0.32
4.2	0.25
4.2	o.28
4.3	0.23
	DO (mg/1) 7.8 7.7 7.4 6.4 5.7 4.6 4.5 4.6 4.5 5.5 5.5 4.8 4.9 5.3 5.5 4.8 4.5 4.9 4.2 4.2 4.2 4.2 4.2 4.2 4.3

DISSOLVED OXYGEN AND

DISSOLVED OXYGEN UPTAKE RATE

RUN 2

DAY	DO	DOUR
	(mg/1)	(mg/l/min)
O	7.6	·.28
1	7.6	0.24
2	7.4	0.22
3	7.6	0.19
4	7.4	0.20
	7.2	0.27
6	7.1	0.25
7	7.4	0.21
8	7.7	0.20
9	7.8	0.15
10	7.7	0.15
11	7.8	0.15
12	7.5	O.11
13	8.0	0.15
14	7.9	o.28
15	6.5	0.21
16	7.3	0.26
17	7.9	0.19
18	7.5	0.23
19	7.1	0.20

DISSOLVED OXYGEN AND

DISSOLVED OXYGEN UPTAKE RATE

RUN 3

DAY	DO	DOUR
	(mg/l)	(mg/l/min)
0	65 (B	0.38
1	6.7	0.40
2	5.2	0.48
3	5.9	0.31
4	5.7	0.20
5	5. <u>2</u>	0.24
6	S. 2	0.25
7	5.5	0.60
8	5.0	0.25
9	5.1	0.19
10	5.4	0.18
11	4.9	0.26
12	4.9	o.28
13	4.9	0.28
14	5.2	0.28
15	5.2	0.25
16	4.4	0.54
17	4.7	0.36
18	4.3	0.29
19	4.3	0.27
20	4,9	0.25
21	5.5	0.19

DISSOLVED OXYGEN AND

DISSOLVED OXYGEN UPTAKE RATE

RUN 3

DAY	DO (mg/l)	DOUR (mg/l/min)
O	7.5	0.49
1	6.3	0.70
2	6.4	0.47
3	6.4	0.47
4	s. 2	0.31
5	6.1	0.24
6	6.3	ം. 26
7	6.7	0.21
8	6.0	0.20
9	7.3	0.13
DISSOLVED OXYGEN AND

DISSOLVED OXGYEN UPTAKE RATE

RUN 3 CMBR-3

DAY	DO	DOUR
	(mg/l)	(mg/l/min)
0	8.i	0.48
1	7.4	0.56
2	7.3	0.47
3	7.2	0.38
4	7.2	0.32
5	7.2	0.27
6	7.2	0.27
7	7.9	0.22
8	7.9	0.17

DISSOLVED OXYGEN AND

DISSOLVED OXYGEN UPTAKE RATE

RUN 4

CMBR-1

DAY	DO	DOUR
	(mg/1)	(mg/l/min)
0	6.0	0.61
1	3. 7	1.00
<u> </u>	4.9	1.00
12	4.2	0.56
4	4.3	0.59
5	4.8	0.52
6	4.4	0.43
7	4.8	0.35
3	4.0	0.43
9	4.2	0.27
10	4.3	o.58
11	4.4	0.51
12		
13	4.2	0.32
14	4.9	0.30
15	4.6	0.31
16	4.7	0.28
17	4.2	. 38
18	4.7	0.43
19	4.2	0.35
20	4.2	0.32
21	5.0	0.28
22	4.7	0.27
23	4.6	0.22
24	5.0	0.18

DISSOLVED OXYGEN AND

DISSOLVED OXYGEN UPTAKE RATE

RUN 4

CMBR-2

DAY	DO	DOUR
	(mg/l)	(mg/l/min)
Ö	1.6	0.36
1	0.9	0.26
2	1.3	0.90
3	1.2	0.51
4	1.7	0.42
5	1.1	0.45
6	1.1	0.38
7	1.2	0.40
8	1.2	0,29
9	1.9	0.26
10	1.7	0.39
11	2.0	0.26
12		
13	2.0	0.63
14	1.3	0.76
15	2.1	0.38
16	2.0	0.20
17	1.8	0.34
18	2.0	0.57
19	2.0	0.49
20	1.8	0.42
21	2.3	0.47
22	1.9	0.29
23	1.9	0.42
24	1.2	0.65
25	1.3	0.57
26	1.3	0.42
27	1.4	0.33
28	1.5	°.46
29	1.4	0.31
30	1 - 1	0.31
$\mathbb{E}1$	2.7	o.0 9

DISSOLVED OXYGEN AND

DISSOLVED OXYGEN UPTAKE RATE

RUN 4

.

CMBR-3

DAY	DO	DOUR
	(mg/l)	(mg/l/min)
0	0 0	غدغت ال
		() a () ()
1	6.8	0.54
2	7.0	0.65
N.	7.4	0.46
4	7.5	o.38
5	6.9	0.35
6	6.8	0.25
7	7.1	0.42
3	7.1	0.51
9	6.9	0.22
10	7.8	0.19
11	7.1	0.14
12	trude state anyor	

SUSPENDED SOLIDS

	MLSS	RSS	ESS
DAY	(mg/1)	(mg/1)	(mg/l)
Ō	3100	10010 ALING 10000 BOOK	
1	2400	2100	1200
2	1900	2600	1000
	1700	2800	900
4	1300	1500	1200
5	1200	1200	1000
4	1600	1800	1100
7	1000	1900	BDL.
9	1200	900	1100
9	1000	800	900
to	900	2000	900
11	1000	1100	1200
12	1100	2200	1100
13	1200	1300	1000
14	1300	1600	900
15	1000	1300	900

SUSPENDED SOLIDS

.

	RUN 2	CMBR-1	
	MLSS	RSS	ESS
DAY	(mg/1)	(mg/l)	(mg/1)
0	4600		
1	3400	6700	3300
2	2600	3500	200
	2500	3200	BDL
4	2200	3200	EDL
	2100	2000	BDL
6	2100	1900	BDL
7	1700	1400	BDL
8	1600	1000	300
9	1300	1000	100
10	1300	1700	200
11	1700	1600	BDL
12	1300	800	BDL
13	1600	1600	200
14	1600	1200	BDL
15	1000	4200	BDL
16	1100	400	BDL
17	1000	500	BDL
18	1000	400	BDL
19	800	400	BDL

	RUN 2	CMBR-2	
DAY	MLSS	RSS	Ess
	(mg/l)	(mg/l)	(mg/l)
0	4700		
1	0025	7000	2300
2	2600	3300	120
1	2300	2700	BDL
4	2000	3000	EDL
5	2000	2300	BDL
6	1600	600	EDL
7	1500	600	200
8	1600	1200	500
9	1200	700	200
10	1000	1100	BDL
11	1200	700	BDL
12	700	1000	BDL
13	1000	900	200
14	900	1100	BDL
15	1000	4000	100
16	1000	400	BDL
17	1000	800	BDL
18	1100	400	BDL
19	600	1000	BDL

	RUN 3	CMBR-1	
	MLSS	RSS	ESS
DAY	(mg/1)	(mg/l)	(mg/1)
0	3600		
1.	3200	4100	300
2	2900	3100	100
	3000	3000	100
4	2000	2100	200
5	1500	1600	100
6	1100	1800	BDL
7	900	900	BDL
8	1100	1300	BDL
9	1000	700	100
10	1000	800	200
11	1000	900	BDL
12	1000	900	BDL
13	1000	800	BDL
14	1000	800	BDL
15	800	700	BDL
16	900	700	BDL
17	900	900	BDL
18	900	400	BDL
19	900	900	BDL
20	900	700	100
21	700	800	800

RUN 3	CMBR-2	
MLSS	RSS	ESS
(mg/1)	(mg/l)	(mg/l)
4600		
4100	4700	200
3700	4500	200
2800	4700	100
2200	3300	200
1800	2500	100
1300	1700	BDL
1000	1200	BDL
100	300	100
320	2800	100
	RUN 3 MLSS (mg/1) 4600 4100 3700 2800 2800 2200 1800 1800 1300 1000 100 320	RUN 3 CMBR-2 MLSS RSS (mg/1) (mg/1) 4600 4100 4700 3700 4500 2800 4700 2800 4700 1800 2500 1300 1700 1000 1200 100 300 320 2800

ESS
(mg/l)
terite and a seque
200
200
200
300
300
BDL
BDL
100

	RUN 4	CMBR-1	
	MLSS	RSS	ESS
DAY	(mg/l)	(mg/l)	(mg/l)
O	4700		
1	4600	4400	600
2	2900	3900	250
3	3200	4400	700
4	2800	3100	300
5	2000	3500	500
6	2700	3500	800
7	1700	3100	500
8	2400	2700	500
9	2000	2300	200
10	2000	1600	600
11	2000	2200	800
12			
13	2300	3400	300
14	2100	2000	400
15	2500	1700	400
16	1400	1600	300
17	1400	1200	300
18	1700	1800	700
19	1800	1400	300
20	1400	1000	BDL
21	1700	1900	700
22	1200	1400	200
23	1100	1100	400
24	500	700	200

SUSPENDED SOLIDS

	RUN 4	CMBR-2	
	MLSS	RSS	ESS
DAY	(mg/1)	(mg/1)	(mg/l)
Ó	4000		
1	3300	4300	400
2	3600	4500	300
	3400	6200	800
4	2700	3600	400
5	2800	3700	600
6	2700	3500	600
7	2600	3200	500
8	2400	3100	700
9	2000	2400	100
10	2300	2600	600
11	2000	3800	800
12			
13	2500	3200	BDL
14	2300	2500	500
15	2000	2500	400
16	1500	1700	200
17	1400	1500	200
18	1800	2000	500
19	1300	1500	200
20	1500	1000	BDL
21	1700	1800	900
22	1200	1300	500
23	1300	1400	400
24	1700	1000	500
25	1900	2300	700
26	1900	2500	700
27	1800	2700	400
28	1900	2000	200
29	2000	2200	350
SO	1800	1600	400
31	500 ×	500 *	900 *

* Because of an accidental pH excursion.

	RUN 4	CMBR-3	
	MLSS	RSS	ESS
DAY	(mg/1)	(mg/1)	(mg/l)
Ō	5000		-2000 1000- 0000
1	4000	4600	700
2	3600	5300	500
	2600	3900	500
4	1700	2200	200
5	2700	5000	800
6	2500	2300	600
7	2300	2000	300
8	1700	2000	700
9	1100	1200	400
10	1000	1200	400
11	1000	1000	200
12			***** ***** *****

MICROBIAL IDENTIFICATION

RUN 3 DAY 18

BACTERIA	3	1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12.	Acinetobacter anitratus Acinetobacter lwoffi Enterobacter agglomerans Group 2K — 1 (Pseudomonas — like) Proteus mirabilis Proteus yulgaris Providencia stuartii Pseudomonas aeruginosa Pseudomonas cepacia Pseudomonas fluorescens Pseudomonas maltophilia Pseudomonas putida
Yeast	2	1. 2.	<u>Candida albicans</u> Candida stellatoidea
Mold	:	1.	<u>Penicillum sp.</u>
Protists	*	1.	Amoeba sp.

EFFLUENT SUBSTRATE CONCENTRATION

CMBR-1	CMBR-2
(mg/l)	(mg/l)
BDL	BDL
80. <u>6</u>	12.3
10.01	BDL
BDL	21.24
BDL	BDL
BDL	BDL
BDL	10.0
BDL	EDL
BDL	BDL
BDL	35.4
BDL	18.3
BDL	0.5
BDL	BDL
EDL	BDL
5.8	BDL
BDL	BDL
20.2	BDL
	CMBR-1 (mg/1) BDL BOL BDL BDL BDL BDL BDL BDL BDL BDL BDL BD

EFFLUENT SUBSTRATE CONCENTRATION

	CMBR-1	CMBR-2	CMBR-3
DAY	(mg/l)	(mg/1)	(mg/1)
0	BDL	BDL	EDL
1	30,6	2.8	7.6
2	5. 2	EDL	BDL
3	EDL	BDL	BDL
4	BDL	RDL	BDL
5	BDL	BDL	BDL
6	BDL	BDL	BDL
7	BDL	BDL	BDL
8	EDL_	BDL	BDL
9	BDL	BDL	BDL
1 ()	BDL		
11	BDL		
12	EDL		
13	BDL		
14	BDL		
15	BDL		
16	EDL		
17	BDL		
18	BDL.		
19	BDL		
20	BDL.		
21	BDL		

EFFLUENT SUBSTRATE CONCENTRATION

	CMBR-1	CMBR-2	CMBR-3
DAY	(mg/1)	(mg/l)	(mg/1)
Ō	EDL	BDL	BDL
1.	30.8	63.O	24.4
2	4.3	. 8	0.1
3	BDL	6.6	1.0
4	2.1	0.6	0.9
5	BDL	BDL	1.0
6	BDL	BDL	0.6
7	BDL	BDL	BDL
8	9.0	BDL	BDL
9	BDL	BDL	72.5
10	EDL	BDL	49.2
11	BDL	BDL	80.0
12			
13	0.5	BDL	
14	0.6	EDL	
15	0.5	BDL	
16	BDL	BDL	
17	BDL	BDL	
18	BDL	BDL	
19	BDL	BDL	
20	BDL	EDL	
21	BDL	BDL	
22	BDL	BDL	
23	BDL	BDL	
24	BDL	BDL	
25		EDL	
26		EDL	
27		BDL	
28		BDL	
29		EDL	
30		BDL	
31.		BDL	

PREDOMINANT MICROBIAL GENERA IN LIVINGSTON MIXED LIQUOR

Fresh	Phenol-acclimated*	Phenol-acclimated 2-Chlorophenol**
1010 bacteria/cm ³	109 bacteria/cm ³	10° bacteria/cm³
<u>gram positive</u> gram negative *-3	5 (D. 195	0.2
Gram positive rods Gram positive cocci Pseudomonas Acinetobacter Enterobacter	(Bacillus)	> > >
Alcaligenes Serratia Providencia Escherichia coli Pasturella	> > > >	
Aeromonas.		
104 yeast cells/cm ³	³ 10 ⁶ yeast cells/cm ³	104 yeast cells/cm ³
Candida Cryptococcus Trichosporon Debaromyces Saccharomyces.		
Aspergillus Streptomyces Trichophyton. Geotrichum. Rhizopus. Rhodotorula.	>	
10° protozoa/cm³	105 protozoa/cm3	10 ⁵ protozoa/cm ³
Epistylis Vorticella Paramecium Peranema Carchesium Polychaos. Etc. (many other s	pecies occasionally observ	~>
<pre>Rotifers * 100 ppm for 10 ** 100 ppm phenol followed by 20</pre>	days for 10 days, ppm 2-chlorophenol for 10	more days

PREDOMINANT MICROBIAL GENERA IN PVSC MIXED LIQUOR

Fresb	Phenol-acclimated*	Phenol-acclimated <u>2-Chlorophenol</u> **		
1019 bacteria/cm ³	107 bacteria/cm ³	10° bacteria/cm³		
<u>gram positive</u> gram negative	c) , ۳	O. 4		
Gram positive rods ()	Bacillus)			
Gram positive cocci	(Micrococcus)	·		
Pseudomonas		na ann an an ann ann ann ann ann ann an		
Wetershieter		an man ann ann ann ann ann ann ann ann a		
Alcaliaseos				
Serratia				
Providencia				
Pasturella	·			
104 yeast cells/cm ³	10° yeast cells/cm ³	104 yeast cells/cm ³		
Candida		>		
Cryptococcus				
Trichosporen	والا المله المركز المركز المركز المركز المركز المركز المركز المركز المركز ويريز والمركز ويترز ومن ويورد ويركز المركز			
Debaromyces				
Saccharomyces.				
Hansenula.				
Penicillium				
Aspergillus				
Streptomyces				
Trichophyton.				
Geotrichum.				
10 [∞] protozoa/cm [∞]	10ª protozoa/cm³	10≅ protozoa/cm³		
Epistylis				
Vorticella.				
Paramecium.				
Peranema				
Carchesium.				
Colpidium	ال المراجع الم			
Opercularia		, 1999 مەت 1999 مەت 1999 مەت 1999 مەت 1999 مەت 1999 مەت		
Stylonichia.				
Podophyra.				
Rotifers.				
* 100 ppm for 10 d	ays			
20 ppm 2-chlorop	or 10 days, followed by henol for 10 more days			

DOMINANT BACTERIAL SPECIES IN PHENOL-ACCLIMATED FVSC MIXED LIQUOR

Investigator I

Investigator II

Acinetobacter sp. Acinetobacter lwoffii Alcaligenes faecalis Enterobacter agglomerans Providencia stuartii Pseudomonas cepacia Pseudomonas fluorescens Pseudomonas sp. Bacillus sp. Micrococcus sp. Group 2K-1 Pseudomonas-like Group 2K-1 Pseudomonas-like Serratia marcescens Staphylococcus sp.

Acinetobacter anitratus Acinetobacter lwoffii Alcaligenes faecalis Enterobacter agglomerans Providencia stuartii Pseudomonas cepacia Pseudomonas fluorescens Pseudomonas aeruginosa Bacillus sp. Micrococcus sp.

Investigator III

```
Acinetobacter lwoffii
Alcaligenes faecalis
Enterobacter agglomerans
Enterobacter cloacae
Pseudomonas cepacia
Pseudomonas fluorescens
Pseudomonas putida
Pseudomonas sp.
Bacillus sp.
Serratia liquefaciens
Klebsiella pneumoniae
```

KINETICS DATA

Monod model: $-dS/dt = U_m - [X/Y] - S/[K_m + S]$ Haldane model: $-dS/dt = U_m - [X/Y] - S/[K_m + S + S^2/K_1]$

where:

 S_{m} = initial substrate concentration, ppm S = substrate concentration, ppm X = MLSS concentration, ppm (or mg/l) t = time, hours K_{m} = Saturation Constant, ppm U = Specific Growth Rate, hr⁻¹ U_{m} = Maximum Specific Growth Rate, hr⁻¹ K_{s} = Substrate Inhibition Constant, ppm Y = Yield Coefficient, mg biomass/mg substrate k_{d} = endogenous respiration constant, hr⁻¹

Ref #	Compound(s) Tested	Conc. (ppm)	Reactor Type	Kinetic Model	Results and Comments ((pH, organisms, etc.))
, ; 9 ; ; ; ; ;	phenol	1200 	batch	Monod	$ U_{m} = 0.019 U_{m} = 0.070 $ $ K_{m} = 236 K_{m} = 236 $ $ Y = 1.21 Y = 1.21 $ $ k_{d} = 0.002 k_{d} = 0.007 $ $ 5 \circ C 23 \circ C $ $ U_{m} = 0.072 $ $ K_{m} = 236 $ $ Y = 1.21 $ $ k_{d} = 0.006 $ $ 28 \circ C $ $ $
}	2 2 2 3	; ;	2 7 7 8		<pre>(pH = 7.0; unspecified) (mixed culture fr POTW)</pre>
110	phenol	360	CSTR	Monod	U _m = 0.170 K_ = 245 Y = 0.45 20 °C; pH = 7.2 unspecified mixed culture
•		,			

行应于 	Compound(s) Tested	Conc. (ppm)	Reactor Type	Kinetic Model	Results and Comments ((pH, organisms, etc.);
	phenol		batch and continuous	Monod	$U_m = 328$ $K_m = 24.96$ $\gamma = 0.011$ $30 \text{ °C; unspecified pH}$ $Debaromyces$ L $Subalobosus$
	phenol	90 	<pre>chemostat; (no cell ; ; recycle); ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ; ;</pre>	Monod	$U_{m} = 0.287$ $K_{a} = 2.11$ $Y = 1.20$ $k_{a} = 0.01 \text{ hr}^{-1}$ $22 \text{ eC; pH} = 7.0$ $unspecified \text{ mixed}$ $culture \text{ from a POTW}$
	phenol		batch	Moned	$U_m = 0.63$ $K_a = 30$ $Y = 0.47$ $k_a = 0.05$ $24 \ ^{\circ}C; \ pH = 7.0$ $Unspecified \ mixed$ $Culture \ from \ coal \ gas$ $Wastewater \ treatment$
114	phenol 	100 to 800 	batch and CSTR	Haldane	$U_{m} = 0.270$ $K_{i} = 11.9$ $K_{m} = 19.1$ $Y = (?)$ $T = (?); pH = (?)$ $Culture (?)$
; ; ; ; ;	phenol	lup to 110	CSTR	Haldane	$U_m = 0.29$ $K_a = 0.9$ $K_i = 110$ $Y = 0.59$ $27 \text{ °C; pH} = 7.0$ $Bacterium NCIB 8250$ $Possibly Acin./Morax.$
	phenol	up to 1500 	fill-and- draw	Haldane	Um = 0.08 K = 700 K = 766 Y = .82 to 1.22 k = 3.45 × 10-4 32 to 40 =0 pH = 7.5 to 8.0 unspecified.non-meth.

Ref #	Compound(s) Tested	Conc. (ppm)	Reactor Type	Kinetic Model	Results and Comments (pH, organisms, etc.)
	phenol	100 to 800	batch and CSTR	Haldane	Um = 0.66 Um = 1.01 Km = 86.7 Km = 160 Km = 34.2 Km = 160 Y = 0.616 Y = 0.545 filamentous spherical bacteria Lacteria 28 °C; pH = 6.6
	phenol	400 to 800	batch and CSTR	Monod	U _m = 0.144 U _m = .0937 K _m = (?) K _m = (?) Y = 0.606 Y = 0.548 40 °C 30 °C <u>Bacillus cereus</u> pH = 7.0
	phenol	185 to 200	batch and CSTR	Haldane	$\begin{aligned} U_m &= 0.534 U_m &= 0.481 \\ K_m &< 1.0 & K_m &< 1.0 \\ & &= 470 & K_1 &= 840 \\ & &= 0.52 & & &= 0.52 \\ & && 200 & ppm & & 185 & ppm \\ & && batch & & & CSTR \\ & && 30 & ^{\circ}C; & pH &= 6.5 \\ & && P_{-} & putida & (ATCC & 17484) \end{aligned}$
20	phenol	30 ta 800 	batch and CSTR	Haldane	U _m = 0.464 U _m = 0.567 K _■ = 1.66 K _■ = 2.38 K _↓ = 380 K _↓ = 106 Y = 0.85 Y = 0.85 <u>1.cutaneum</u> <u>P. putida</u> pH = 4.5 pH = 6.0 30 °C
21 	phenol	120 to 800	batch	Haldane	U _m = 0.326 K ₄ = 19.2 K ₄ = 229 Y = (?) 20 °C; pH = 6.8 culture (?)
22 	phenol	50	batch and CSTR	Haldane	$U_{m} = 0.19, 0.21, 0.27$ $K_{m} = 35, 49, 67$ $K_{4} = 135, 154, 86$ Y = 1.02 $K_{d} = 0.021$ $T = 25 \circ C; pH = (?)$ Unspecified mixed Culture from primary Settler of Wilmington Del. POTW (also 18, 20)

Ref #	Compound(s) Tested	Conc. (ppm)	Reactor Type	l Kinetic Model	<pre> Results and Comments (pH, organisms, etc.)</pre>
23	phenol	50 to 1 1000	batch	Haldane	<pre>Um = 0.131 to 0.363 K = 5 to 266 K = 142 to 1199 Y = (?) 25 °C; pH = 6.9 unspecified mixed culture from POTW</pre>
24	phenol	500	CSTR	Haldane	Um = 0.181 K = 62 K = 175 Y = 1.02 K = 0.02 hr ⁻¹ T = 25 °C; pH = (?) culture (?)
25	phenol	500	batch	Haldane	Um = 0.19 K = 75 K = 449 Y = 0.83 k = 0.005 25 °C; pH = 6.9 POTW culture (?)
26	phenol	100 to 1400	CSTR	zero	k _o = 111 ppm/hr 32 °C; pH = (?) mixed culture includ. Bacillus,pseudomonad E. coli, Staph.,Citro
27	2-chlorophenol phenol nitrobenzene	120	batch	zero	$k_{\odot} = 25 \text{ mgCOD/gX}_{\odot}/\text{hr}$ $k_{\odot} = 80 \text{ mgCOD/gX}_{\odot}/\text{hr}$ $k_{\odot} = 14 \text{ mgCOD/gX}_{\odot}/\text{hr}$ $k_{\odot} = 14 \text{ mgCOD/gX}_{\odot}/\text{hr}$ $k_{\odot} = 14 \text{ mgCOD/gX}_{\odot}/\text{hr}$ $k_{\odot} = 10 \text{ days; } T = 20 \text{ eC}$ $k_{\odot} = 100 \text{ days; } T = 20 \text{ eC}$ $k_{\odot} = 100 \text{ to } 150 \text{ mg/l}$
28	phenol 2-chlorophenol 2,6-DCF	100	batch		100% degraded in 0.75 1.5 5 days T = 23 °C; pH = 7.2 soil culture (?)
29	2-chlorophenol 	100	batch		<pre>1100% degradation in 1 100% degradation in 1 1 3 days using 1 1 acclimated bacteria 1 1 T = 20 - 30 °C 1 1 pH = (?)</pre>

Ref #	Compound(s) Tested	Conc. (ppm)	Reactor Type	Kinetic Model	Results and Comments (pH, organisms, etc.)
30 	2-chlorophenol	16	batch		100% degradation in 19 days using unacclimated bacteria T = 30 °C; pH = 7.0 POTW/soil culture (?)
131	4-chlorophenol	up to 125	batch	Monod	$U_m = 0.4$ $K_m = (?)$ $Y = (?)$ $1 < S < 20$ for S > 125, complete inhibition with acclimated bacteria Pseudomonas sp. B13 30 °C; pH = (?)
1 32	2.4 -DCP	25	batch and continuous	Monod 5 or Haldane	$U_{m} = 0.12$ $K_{m} = 5.1$ $Y = (?)$ $U_{m} = 0.228$ $K_{m} = 11.7$ $K_{1} = 35.7$ $Y = (?)$ $125 \text{ PC: pH= 7.1 to 7.8}$
, ; ;	6 	} 	; ; ;		Pseudomonas NCIB 9340
	PCP	30 	batch,and fill-and- draw 		68% PCP degraded to CO_{2} in 24 hours T = 25 °C; pH = (?) soil culture (?)
 34 	phenol 2-chlorophenol 3-chlorophenol 4-chlorophenol 2,4-dichloro- 3,4-dichloro- 2,3-dichloro- PCP	125 ? ? ? ? ? ?	batch		16 ppm/hr 5 6 8 0.06 0.10 0.40 no detectable degrad. T = 23 °C; pH=7.2 river water culture(?)
 35 	2-chloro- 3-chloro- 4-chloro- benzoate	100	batch		100% degradation in: 6 days 12 days 9 days T = 25 °C; pH = 7.21 FOTW culture (?)

Ref	Compound(s) ;	Conc. 1	Reactor	Kinetic	Results and Comments
排 !	Tested	(mqq)	Гуре I	Model	(pH, organisms, etc.)
	3-chloro- 4-chloro- 3,5-dichloro- benzoate	3131 3131 3820	batch		100% degradation in: 14 hours 11 hours 29 hours T = 28 °C; pH = (?) Pseudomonas sp.WR912
137	2-chloro-	50	batch	Monad	$U_m = 0.0417$ $K_m = 5.4$ Y = 0.14
5 8 9 9 2	3-chloro- 	50	batch		$U_{m} = 0.025$ $K_{m} = 2.0$ Y = 0.14
2] } f	4-chloro- 	50	batch		$U_m = 0.050$ $K_m = 1.1$ Y = 0.25
2 7 7 8	2-chloro- 	50	CSTR		$ U_m = 0.0458$ $ K_m = 1.5$ Y = (?)
	4-chloro- 	110	CSTR		$U_{m} = 0.0458$ $K_{m} = 1.0$ V = (?)
1	2,5-dichloro- 	50	batch 	8 7 1 1 1	$U_{m} = 0.025$ $K_{m} = 1.5$ Y = 0.16
3 3 4 3 4 3	3,5-dichloro- benzoate 	50	batch 		(U _m = 0.0208 (?) (K _m = 25.3 (Y = (?)
2 5 7 1 2 2	12,4-dichloro- 1phenoxyacetate 1	98	batch 		$U_{m} = 0.0958$ $K_{m} = 5.4$ Y = 0.14
1		98 	I CSTR 		$U_{m} = 0.0917$ $K_{m} = 2.7$ Y = (?)
 	 	 			20 ⊂C; pH = 7.0 <u>Pseudomonas putida</u>

FIGURE 1



MICROBIAL IDENTIFICATION PROCEDURE







DYE RECOVERY CURVE

7.5 SCHF 93.75% COMPLETE-MIX



89

FIGURE 5 DYE RECOVERY CURVE

7.5 SCFH 99.19% COMPLETE-MIX (DIFFUSERS ON BOTTOM)





DYE RECOVERY CURVE

4.5 SCFH 97.35% COMPLETE-MIX



91

DYE RECOVERY CURVE

1.5 SCFH 96.78% COMPLETE-MIX





(umu/(/Sub) ynog



94



95




(upun/am) anou



FIGURE 13



FIGURE 14



FIGURE 15

100



FIGURE 16

FIGURE 17





FIGURE 18







日日日時



SSIN





(apumanoqL) SSIN



(epureroul) Sein







(mpresnoull) (ssu



(spumerodry)



(spuesnoul) SSM



X











S SX









SHIMADZU CHROMATOGRAM RUN 2

1

STINGS	0.923 0j.19	<u>.</u>				
CHROMAT Sample Report	OPAC C-R NO 0 NO 428	3A		FILE Method	0 41	
PKNO	TIME	AREA MK	IDNO	CONC	NAME	
1 2 3	0.405 0.923 1.19	17867 S 22 T 107 T		99.2799 0.1239 0.5962		
		17997	-	100		

221-25412

HEWLETT PACKARD CHROMATOGRAM RUN 3 AND 4

Statt							
2,62			.32				
STOP							
RUN # 103		MAR/04/88	16:12:50				
ESTD RT 0.84 1	AREA TYP(193600 P)	E CAL H B 1R	ANOUNT 50.532				
TOTAL AREA= 1193600 MUL FACTOR= 1.0000E+00							
CALIB ESTD							
REF % RTW: ESCAPE	- 0.3	3					
CALIB ESTD							
REF % RTH: % RTH:	- 0.3 3						
CAL# 1 : Ø . 2 :	RT 8 4	ANT : 5 0					
REF PK CAL#							