RICE UNIVERSITY

STUDIES ON A DUAL-ORGANISM DUAL-SUBSTRATE BIOLOGICAL REACTOR

by

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ABSTRACT

Studies on a Dual-Organism, Dual-Substrate Biological Reactor

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A dual-organism, dual-substrate microbiological reactor was studied using organisms which metabolize either phenol or methanol. The growth characteristics of yield, ratio of oxygen to substrate uptake rates ($R_{O_2}/R_S$), unit rate of oxygen uptake ($R_{O_2}/C_B$), and substrate concentration were evaluated as functions of growth rate in cultures with a single species predominating (SSP) and with a mixture of the two organisms. In both SSP cultures yield and $R_{O_2}/R_S$ were decreasing functions of growth rate, indicating an increased tendency of the organisms to excrete metabolic by-products at increased growth rates. $R_{O_2}/C_B$ in both cultures was a linear increasing function of growth rate as expected. Both yield and $R_{O_2}/R_S$ in the mixed culture were lower than in the SSP, indicating an increased tendency toward by-product excretion. The methanol organism indicated the existence of a positive interaction with the phenol organism in mixed culture. The dehydrogenase activity test was unable to predict the relative amounts of phenol and methanol organism in the mixed culture due to excessive variability in SSP cultures and inability to measure phenol organisms activity
in mixed culture. The stoichiometry of microbial oxidation of methanol and phenol was found to vary with growth rate but was complicated by possible by-product formation.
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DEDICATION

To Colleen,

"You were born together and
together you shall be forevermore
You shall be together when the
white wings of death scatter your days
Ay, you shall be together even in
the silent memory of God.
But let there be spaces in your
togetherness,
And let the winds of the heavens
dance between you."

K. Gibran
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INTRODUCTION

Specific Models for Microbial Growth

Review of Models: Previous attempts to model biological waste water treatment processes consider the biomass as a uniform culture. Analogously, the substrate is measured, irrespective of chemical species, in terms of oxygen demand, the parameter of greatest interest in waste treatment. The non-specific approach has been successful in describing the performance of the activated sludge process and has aided rational design. However, further progress will depend on a better understanding of interactions among the diverse organisms and substrates present.

A specific model describes a biological reactor in terms of concentrations of specific chemical components and distinguishable microbial groups. Examples of specific models are the models developed to describe the growth of pure bacterial cultures on a single defined substrate and the "structured" models of Fredrickson.¹ "Structured" models can be considered as a "super-specific" model in that it considers all relevant environmental influences, and the concentration of all...
intra- and extra-cellular compounds.

The extensive work by microbiologists in developing and testing specific models for pure cultures of microorganisms is exemplified by the classical study of Monod, whose formulae have been the basis for much of the research in microbial growth kinetics. The first premise of his model is that the rate of microbial growth is proportional to the number of organisms present.

\[ R^N_g = \mu N \]

- \( R^N_g \) = Rate of growth of numbers of organisms
- \( N \) = Number of organisms
- \( \mu \) = Growth rate

Generally a linear correlation exists between numbers and mass, so rate of growth is also proportional to the weight concentration of biomass.

\[ R_g = \mu C_B \]

- \( C_B \) = Weight concentration of biomass
- \( R_g \) = Rate of growth of biomass

The constant of proportionality \( \mu \), is a function of all parameters which comprise the growth environment, such as temperature, pH, and the concentrations of substrates and nutrients. Monod developed an expression relating the microbial growth rate to the concentration of the
growth limiting substrate or nutrient. The objective of biological waste water treatment is removal of dissolved organic substrates, consequently the relation of growth rate to substrate concentration is of greatest concern. If all other growth factors are supplied in excess, the growth rate can be expressed as:

\[ \mu = \frac{\mu_{\text{max}} C_s}{K_s + C_s} \]

\( \mu_{\text{max}} \) = Maximum growth rate  
\( C_s \) = Concentration of growth limiting substrate  
\( K_s \) = Saturation constant, numerically equal to substrate concentration when \( \mu = \frac{\mu_{\text{max}}}{2} \)

Although Monod developed this relationship empirically, and attempts at theoretical justification have been unsatisfactory, it has been applied to a variety of microbial systems by use of component material balances. For the chemostat, the material balance for biomass developed by Hebert\(^3\) reduces to,

\[ \frac{dC_B}{dt} = (\mu - D)C_B \]

\( D = \) dilution rate  
\( = \frac{Q}{V} \)

where \( Q = \) flow rate through reactor  
\( V = \) volume of reactor
The substrate balance in the chemostat using the Monod relation for growth rate is

\[
\frac{dC_s}{dt} = D(C_s^0 - C_s) - \frac{\mu_{\text{max}} C_s C_B}{K_s + C_s} \quad \gamma = \frac{\text{rate of microbial growth}}{\text{rate of substrate uptake}}
\]

\(C_s^0 = \text{concentration of substrate entering reactor}\)

Although widely applied, the model does not adequately describe some microbial systems, such as those exhibiting substrate inhibition effects at high substrate concentration,\(^4\) or competitive inhibition by non-metabolizable transport inhibitors.\(^5\)

Lawrence\(^6\) has developed a non-specific model for biological waste treatment by applying the Monod formulation and adding a microbial decay term which describes the variability of the yield coefficient with different growth conditions.

\[
R_s = \gamma R_s - bC_B \quad b = \text{microorganism decay coefficient}
\]

\[
R_s = \frac{kC_s C_B}{K_s + C_s} \quad \gamma = \text{true yield coefficient}
\]

\[
\mu = \frac{1}{\theta} = \frac{\gamma kC_s}{K_s + C_s} - b
\]

Most models of the activated sludge process differ in the expression for growth rate dependence on substrate concentration or in the determination of yield. Sherrard,\(^7\) for example, considers the yield as an
empirical function of growth rate, which can be derived from experimental data for any system.

\[ R_g = \gamma R_s \]

\[ \gamma = \gamma(\mu) \]

Specific models have been well developed for the limited case of a pure culture, single substrate system. For the more complex, but more practical case of multi-organism multi-substrate systems found in biological waste treatment, only non-specific models exist which can be used for design purposes. A firm understanding of microbial interactions could lead to better control and enhanced system performance. Interactions can be categorized as substrate-organism interactions or organism-organism interactions. The first is concerned with describing the effect of a particular substrate on each organism in the system, with the simplest relation being that an organism uses the substrate as a carbon or energy source to support growth. Another possible interaction occurs when an organic compound, not used as a carbon or energy source, inhibits or stimulates the growth of an organism.

**Applications of Specific Models to Industrial Waste Treatment:** A specific model for microbial growth is most relevant to biological treatment of industrial wastes containing relatively few chemical components
thereby restricting the biological diversity. A specific model of an industrial waste treatment scheme could be used to optimize the overall removal of dissolved organics or to optimize removal of certain troublesome substances, such as phenol, toxic compounds, or biological refractories which can accumulate in natural systems. Recent studies relate certain chemical compounds found in drinking water to chemical plant effluents upstream.\textsuperscript{8,9} Although considered as biologically refractory, proper system environment could lead to the removal of these compounds.

A degree of control can be exerted over the composition of waste water in certain industrial operations by separating waste streams or varying operating conditions. Modular treatment units can be used for effluents from a few select units. Decisions on separate or joint treatment of waste waters by industries with that option must be made on a regional basis. A rational decision requires data on treatment system performance in response to input parameters of specific compounds. In regional treatment studies the practice of mixing wastes and measuring non-specific parameters such as \textit{BOD}_5 before and after treatment ignores the possibilities of lower cost treatment due to interactions of microorganisms and substrates.
Biological Interactions in Microbial Systems

Organism-Organism Interactions: Mixed microbial populations often respond differently from predictions based on a simple summation of the individual populations. Only rarely will two populations not interact. Lack of interaction can usually be explained because (1) the populations are too distant from one another, (2) the populations have such different growth requirements that no organism alters that portion of the environment necessary for the growth of the others. A necessary condition is that the environment must be diverse enough to support growth of the organisms with widely divergent needs.

There is disparity among microbial ecologists over the definitions for different types of organism-organism interactions. Paynter's definitions are simple and describe the interactions of primary interest.

1) Competition - a race for nutrients and space
2) Predation - one feeds on another
3) parasitism - one steals from another
4) Commensalism - one lives off the other without helping or hurting it
5) synergism - the combination synthesizes by cooperative metabolism
6) Mutualism - each benefits the other
7) Antibiosis (Amensalism) - excretion of a factor harmful to the other.
Examples of microbial systems displaying such behavior can be found in Paynter\textsuperscript{11} and Bungay\textsuperscript{12}.

Several types of interactions are important in biological waste treatment systems\textsuperscript{13}. It is widely recognized that predators such as protozoa and ciliates exert a selective pressure on species composition, and affect sludge settleability by feeding on dispersed bacteria. Competition for substrate and nutrients necessary for growth occurs in all mixed micro-biological systems, and organisms best suited to the environment and able to grow more rapidly on available substrates will predominate. Both naturally occurring or artificially constructed biological systems are rarely at steady-state, so competition is always occurring for primary substrates and metabolic by-products, with no organism predominating for long periods. The possibility of synergistic or mutualistic interactions increases with number of organisms present and it has been observed that mixed cultures perform better than pure cultures on a dissolved organic removal basis. This can be explained in terms of synergisms and mutualisms. Pipes\textsuperscript{13} states that, "... the whole activated sludge process may be considered as a synergism of the entire population." Possible synergistic modes of action include:

1) Flocculation of organic particles by one organism so that another can metabolize them
2) Excretion of a growth factor by one organism necessary for the growth of another
3) Release of exo-enzymes which can break down polymeric compounds enabling other organisms to metabolize the degradation products
4) Growth of an organism on the metabolic by-products of another.

Substrate-Organism Interactions: Substrate-organism interactions include all effects attributable to a microbe interacting with a chemical compound present in the influent, to the exclusion of those interactions between an organism and the metabolic by-products of another organism within the system (organism-organism interactions). The most commonly reported substrate-organism interaction is diauxie, which occurs in a dual substrate system when uptake of one substrate is not initiated until the other substrate is partially or totally removed. In a multi-substrate system this phenomena is called polyauxie. Interactions in a dual substrate system probably affect yield, oxygen uptake rates and other experimental parameters when compared to single substrate systems.

Monod\(^2\) first noted diauxic behavior in a batch culture of *E. coli* which preferentially removed glucose from a glucose-xylose or glucose-sorbitol system and postulated two mechanisms of enzyme regulation to explain
the behavior; (1) the primary substrate inhibits existing enzymes necessary for metabolism of the secondary substrate or (2) synthesis of inducible enzymes necessary for metabolism of the secondary substrate is repressed. Mateles\textsuperscript{14} studied diauxic growth in continuous cultures and found the pattern of substrate uptake to be a function of growth rate. Harte\textsuperscript{15} presents similar results in both a batch system and a two stage continuous culture system of \textit{Klebsiella aerogenes}. In mixed culture studies with nitrogen deficiencies, Gaudy, et al.,\textsuperscript{16,17,18} infer that inhibition of existing enzymes is the mechanism responsible for diauxic behavior. Gaudy also explains the dependence of substrate uptake on physiological state by observing sequential substrate uptake in "young" cultures and concurrent uptake in "old" ones. Other investigations of diauxic behavior in batch systems with mixed or "natural" populations has been done by Mateles,\textsuperscript{14} Stumm-Zollinger\textsuperscript{19,20} and Mahmoud.\textsuperscript{21} The latter used a non-carbohydrate system with three ketones as substrate. Continuous culture, mixed populations experiments by Ghosh\textsuperscript{22} were used to explain diauxic behavior in terms of bacterial optimization of growth rate and yield. Population shifts were observed but not quantified. Praksam\textsuperscript{23} repeated the experiments of Gaudy and observed the population dynamics of the system, noting that after acclimation, 80\% of the population was of three types. He concluded,
like Stumm-Zollinger,¹⁹ that diverse activated sludge does not exhibit diauxie although a culture with a mixed but restricted population can.

**Biomass Measurement in Specific Models**

**Review of Techniques:** To verify a specific model, a measurement technique for amounts of each type of organism present is necessary. This is difficult in bacterial systems due to the small size of the organisms and their tendency to form aggregates. The most widely used methods ultimately depend on a plate count. A sample of bacterial suspension is homogenized by high speed mixing or ultrasonic treatment, then diluted and a measured amount applied on agar plates. After a specified incubation period, the colonies are counted and the number of viable cells per volume calculated, assuming one viable cell per colony. The technique employed most often in activated sludge microbiology²⁴⁻²⁶ involves application and isolation of organisms from a homogenized sample on non-selective agar media which will support the growth of a wide variety of microorganisms. Morphological and biochemical tests on the isolates follow. Another variation²⁶ involves plating the organisms on a variety of different types of agar media and classifying the culture by the number of isolates capable of growing on each.
Plate count procedures have the disadvantage that sterilization and media preparation procedures can be tedious, the homogenization process is not reproducible, and re-flocculation can cause variable viable cell counts. Finally, the procedure provides data in terms of the numbers of each organism type present instead of the organism mass, a more useful and more widely used parameter in engineering practice. Other means of differentiating among organisms have been used, such as an alcohol extract test, but this technique is of only limited application.

Differential Activity Concept: The differential activity concept involves a new technique of measuring specific biomass, alluded to by Farkas. Organisms are differentiated by their "activity" toward different substrates, a broader categorization than taxonomic species used in previous microbiological investigations of the activated sludge process. The technique categorizes the organisms according to the ability to metabolize a particular substrate—the characteristic of interest for engineering analyses. Weddle has reviewed techniques for measurement of activity of a culture which could be applied to a differential activity test. Some of the possible activity measures are dehydrogenase activity, oxygen uptake rate, rate of ATP formation, and unit rate of substrate uptake. Any method which
can quantify the ability of a microbiological culture to metabolize a substrate can be used. A differential activity analysis of a biological system would measure the activity of the biomass toward all substrates under the same environmental conditions occurring in the system.

The dehydrogenase activity test (DAT) measures the activity of an organism by measuring the activity of a broad class of intracellular enzymes called dehydrogenases. The enzymes mediate the transfer of hydrogen atoms in all microorganisms and are usually coupled to the nicotinamide di-nucleotides (NAD or NADP) which act as hydrogen acceptors in numerous biochemical reactions. Activity is calculated by measuring the amount of tri-phenyl tetrazolium chloride (TTC) reduced by dehydrogenase mediated reactions in a given period of time. The reduced form of TTC, tri-phenyl formazan, exhibits a bright red color and is measured spectrophotometrically. The reaction can be characterized as follows:

```
C6H5\text{-}C\quad \text{Dehydrogenase} \quad \text{C6H5}\text{-}C
\begin{align*}
\text{N} & \quad \text{N} & \quad \text{C6H5} \\
\text{Cl}^- & \quad \text{N} & \quad \text{N} & \quad \text{C6H5}
\end{align*}
\text{COLORLESS} \quad \text{2e}^- + 2\text{H}^+ \quad \text{RED} + \text{HCl}
```
TTC accepts electrons which, in normal aerobic respiration, would be passed along the electron transport chain and ultimately transferred to oxygen. Although TTC acts analogously to oxygen it probably does not accept electrons at the end of the electron transport chain since the redox potential of the salt is -0.08 volt,\textsuperscript{31} while the redox potential at the end of the chain is +0.81 volt.\textsuperscript{32}

Most workers use glucose as the substrate in the DAT,\textsuperscript{33,34} but any compound metabolized by the organisms can produce a surplus of reduced compounds to react with TTC. Previous DAT research related to waste water treatment has been primarily concerned with measuring "viable" or "active" biomass. "Active" biomass had been considered a better parameter to use in predicting biological reactor response to shock loadings or slugs of toxic compounds since the DAT responds more rapidly to environmental changes than dry weight or volatile dry weight of biomass.

Review of Pertinent Microorganisms

Methanol Metabolizing Organisms: Recent research in waste water treatment employs methanol metabolizers for de-nitrification studies since methanol is one of the cheapest carbon and energy sources for denitrifying organisms. Chalfan\textsuperscript{35} has reviewed the microbiological literature on methanol utilizers. A summary table can
be found in Appendix A-1. Methanol metabolizers are generally small, aerobic, and possibly semi-autotrophic rods with a pink pigmentation characterized by an absorbance peak near 500 m\(\mu\). Stanier\textsuperscript{36} classifies methanol utilizers as *Methanomonas methanica* if they can utilize methane but not formate or other multi-carbon compounds. Some methanol metabolizers show the ability to produce large amounts of polysaccharides,\textsuperscript{37} especially when grown on nitrate as a nitrogen source or otherwise limited to slow growth.\textsuperscript{35} A pathway for microbial degradation of methanol has been substantiated by radioactive labelling techniques\textsuperscript{38} and involves oxidation of methanol to formaldehyde which enters a modified pentose phosphate cycle (see Appendix A-2).

**Phenol Metabolizing Organisms:** Phenol removal is an important waste treatment problem since phenol, especially when chlorinated, can cause taste and odor problems in drinking water supplies. Phenolic wastes are found in a variety of industrial operations, such as the coking process associated with steel production. McKinney\textsuperscript{39} gives a review of early work in biological phenolic waste treatment. Sludge retention times vary from 5.5 days\textsuperscript{40} to 400 days\textsuperscript{41} to infinity\textsuperscript{42} and indicate the non-specificity in the systems reported. Removals range as low as a few milligrams per liter.\textsuperscript{42}
The first organism isolated and identified as a phenol metabolizer was found in the soil by Gray and Thornton\textsuperscript{43} in 1928. A listing of known phenol utilizers is given in Appendix A-3.\textsuperscript{41,43-50} The fluorescent pseudomonads\textsuperscript{44} contain several species capable of growing on phenol and are often characterized by the production of a light green, water soluble pigment. Three species comprise the fluorescent pseudomonads—\textit{Ps. aeruginosa}, \textit{Ps. putida}, and \textit{Ps. fluorescens}. The latter two species include strains capable of phenol metabolism. \textit{Ps. putida} can be further characterized by the distinct odor of triethylamine (putrid smell). Two metabolic pathways have been postulated for phenol degradation and each contains a catechol intermediate.\textsuperscript{45} Experimenters have found evidence for both in cultures grown on phenol or other aromatic compounds. Detailed diagrams of both pathways can be found in Appendix A-4.
EXPERIMENTAL SYSTEM AND TECHNIQUES

Apparatus

Reactor: The chemostat system used for all continuous culture experiments was a two liter, conical, glass reactor with an adjustable overflow line to maintain a constant volume of 0.4 to 1.2 liters. The reactor was mixed by a variable speed (225-1700 rpm) stirrer (Precision Scientific) with a maximum power consumption of 35 watts, and was baffled with four glass rods to eliminate vortexing. The reactor was completely mixed as verified by the light absorbance method used to simultaneously measure the biomass concentration in the reactor and the effluent line. Results indicated that effluent concentration equalled reactor concentration, as long as the effluent line was clear of attached biomass. A non-steady state impulse response test indicated completely mixed behavior (Appendix B-1). Consequently, the reactor contents were sampled directly during steady-state operation.

Feed System: The reactor feed was pumped by a positive displacement pump (Buchler Polystaltic) through plastic tubing from two nine liter pyrex feed tanks. One feed
tube was used at a time and provided a constant flow in the range of 150 to 500 ml/hr. The solution containing essential nutrients and substrate was fed to the reactor by allowing the liquid to drop into the reactor, preventing growth of organisms backwards into the feed tanks. Flow rate was measured by recording the time taken for 5 ml to accumulate in a 30 ml burette. Time was measured by a stopwatch with 0.2 second gradations (Security).

**Oxygen Transfer System:** Air was supplied by the laboratory compressed airline connected to the experimental system through a filter, pressure regulator, and control valve. A diffuser stone near the bottom of the reactor dispersed air to the system. The air flow rate was measured by timing the rise of a soap film in a 50 ml burette, without disturbing the air flow to the reactor. Results of non-steady state oxygen transfer tests at various air flow rates and mixing intensities are in appendix B-2. Air flow rate was one ml/sec, which was adequate to supply required oxygen for all experimental conditions. Lower flow rates were sometimes used to decrease foaming.

A theoretical first order stripping rate constant was calculated with extrapolated equilibrium data for methanol, the most volatile substrate. Assuming that the gas leaving the system was in equilibrium with
the liquid, a component balance indicated a rate constant of 0.013 (hr$^{-1}$) at a temperature of 30°C., air flow of 3.6 l/hr. and reactor volume of 1 liter. This compares favorably with an experimentally determined value of 0.018 (hr$^{-1}$) and theoretical value of 0.015 (hr$^{-1}$) evaluated at the average temperature of the experiment. No appreciable phenol stripping was measured. Water stripping could be expected since the laboratory air line was not in equilibrium with water at 30°C. This rate was about 12 ml/hr., but varied due to changing wetness of the air.

**Temperature Control:** The reactor temperature was maintained by a temperature controller (Yellow Springs Instrument Co., Themistemp, Model 71A) and a heating tape. Fluctuations in the reactor temperature were dampened by a fan blowing on the reactor continuously. All experimental data were obtained at 30 ± 0.05°C.

**Batch Growth System:** For batch growth studies a controlled environment incubator shaker was used (Brunswick Psychrotherm). This system provided a constant temperature and a shake platform for oxygen transfer and mixing. Cultures were grown in 125 ml erlenmeyer flasks, with sidearms for absorbance measurements without contaminating the culture. The system was used to grow seed for the continuous reactor, batch growth
studies on effects of nutrient concentrations and
determination of growth on secondary substrates.

**Spectrophotometers:** A ratio-recording spectrophotometer (Beckman DK-2A) with scanning ability was used to measure phenol in the ultraviolet region and to monitor the biomass concentration in the continuous reactor. A visible region spectrophotometer (Bausch and Lomb, Spectronik 70) was used in the methanol analysis and biomass analysis in the batch growth experiments.

**Experimental Techniques**

**Sampling Procedure:** The reactor was at steady-state for two detention times before sampling was initiated. The following order was followed:

1. Samples for substrate (methanol and/or phenol) were taken and filtered through three absorbant pads and a 0.45 micron membrane filter previously washed with 50-100 ml of deionized water to remove any leachable organics. Filtration occurred within 30-60 seconds after sample withdrawal from the reactor.

2. Oxygen uptake rate measured

3. Samples withdrawn for total suspended solids

4. Dehydrogenase activity test samples withdrawn.

This order was followed to minimize effects of the sampling procedure on the analysis. Each of these
tests was done in triplicate. Intermittant pH and temperature measurements indicated no significant variation.

Dehydrogenase Activity Test: A technique for measuring the dehydrogenase activity of the biomass was developed based on the methods of Ford and Lenhard. Modifications of these procedures became necessary when it was found that tri-phenyl formazan was not extracted from the cells by addition of ethanol. Direct measurement of the colored end product was made relative to a suspension of cells to which TTC had not been added. The full procedure is given in Appendix B-3.

Methanol Analysis: The analytical method for methanol in aqueous solution was a modification of the method used by Maute. The test consists of two separate reactions.

\[
\begin{align*}
(1) \text{Methanol} & \xrightarrow{\text{Potassium Permanganate} [H^+]} \text{Formaldehyde} \\
(2) \text{Formaldehyde} + \text{Chromotropic Acid} & \xrightarrow{H^+} \text{Purple Reaction Products}
\end{align*}
\]

Maute reports the 95% confidence limits for his test to be ±10 mg/l. The modification included a smaller spectrophotometer cell and involved increasing sample size, reaction time, reagent additions and concentra-
tions (Appendix B-4). Interferences with the chromotropic acid test include nitrate, nitrite, chloride, iron (III), and other oxidizing agents.\textsuperscript{53} Nitrate would not be expected to be a problem since it was not present in the feed, and biological nitrification would not be expected at the high growth rates that were maintained. The addition of bisulfite minimizes the effect of the other interferences. Phenol can interfere by reacting with formaldehyde to form polymers. Altshuler\textsuperscript{54} quantified this interference as causing a 10% error when the phenol to formaldehyde ratio was 1.0 or 2.0. A series of tests were made using the test modification with phenol concentrations of 0, 50, 100 mg/l and methanol concentration of 100 mg/l. There was no significant effect of phenol at the 95% confidence level.

**Phenol Determination:** A direct ultraviolet absorption method based on a strong absorption peak at 269 m\textmu was used to measure phenol.\textsuperscript{55} The spectrophotometer is zeroed at 300 m\textmu relative to the filtered sample, then the absorbance of that sample observed at 269 m\textmu. The method is limited to solutions which do not contain other compounds with absorption peaks in this region. Spectral scans of reactor filtrate did not indicate any unknown peaks. Interference by formation of phenoxide ions at high pH was avoided by buffering
the samples. Although not accurate below 5 mg/l, the direct ultraviolet method was compared favorably to the more sensitive 4-amino-antipyrine method in a review of phenol determination procedures by Mohler.\textsuperscript{56}

**Total Suspended Solids:** Total suspended solids were defined as those particles unable to pass a 0.45 micron membrane filter, and were assumed to be entirely biomass. The procedure used for filter handling and drying is a modification of the method reported by Eaton\textsuperscript{57} (Appendix B-5). Approximating the biomass concentration by absorbance readings at 500 \textmu{}m was a useful technique for monitoring the continuous culture and verifying attainment of steady-state.

**Oxygen Uptake Rate:** All oxygen uptake rate tests were performed with a membrane covered polarographic dissolved oxygen meter (Model 54, Yellow Springs Instrument Co.). The procedure required turning off the stirrer and air flow, clamping the effluent line and measuring dissolved oxygen concentration with time, while agitating the probe. Two sources of experimental error were recognized in this procedure---reaeration due to probe agitation and probe lag in response to a changing dissolved oxygen concentration. The first was shown experimentally to cause less than a 10% error under the worst conditions. The uptake rates measured were
significantly smaller than those reported by Benedick as causing appreciable probe lag error.

**Temperature and pH:** Temperature was measured by a thermometer (Taglibue ASTM Extreme Precision Grade) with 0.1°C divisions and an accuracy of +0.02°C at 30°C. Two pH meters (Beckman Zeromatic, Coleman Companion) were used with a prepared buffer (Fisher Chemical Co.) pH 6.89 at 30°C.

**Reactor Maintenance:** Reactor maintenance procedures were developed to minimize effects of foaming and extraneous biological growth on walls, feed tubes, and effluent lines. Foaming was controlled with a silicone type antifoam agent (Sigma Antifoam "A" Concentrate). Wall growth was removed by periodically scraping the sides of the reactor with a soft rubber-tipped glass rod, and by emptying and cleaning the reactor daily. The effluent line tended to clog with flocculated organisms, especially the methanol metabolizers, and was blown clear regularly. The feed lines were regularly cleaned with 5% sodium hypochlorite and tube growth was not observed.

**Feed Preparation:** The feed solution was prepared and sterilized for 30 minutes at 121°C. in two separate containers, one with phosphate buffer, the other with the remaining nutrients. This procedure was necessitated
due to formation of a precipitate when the two solutions were autoclaved together. Deionized water was sterilized and used as a make-up for evaporation losses. The phosphate buffer was prepared from dry, reagent grade chemicals and adjusted to pH 7.0 with $4N \text{H}_2\text{SO}_4$. Nutrient solution was prepared from dry ammonium chloride and stock nutrient solutions. Substrates were added after both nutrient solutions were cooled and mixed. Methanol was added as reagent grade liquid and phenol was added as a concentrated (5%) aqueous solution.

**Organism Isolation and Maintenance:** The microorganisms used in this study were isolated from the Houston Ship Channel in enrichment cultures containing the standard nutrients plus 100 mg/l substrate carbon (methanol or phenol from stock solutions). The standard nutrient media is a modification of that used by Schroeder$^{50}$ (Appendix B-6). The organisms were isolated by two successive platings on agar media composed of standard nutrients, 200 mg/l substrate carbon, and 20 g/l agar. After isolation the organisms were maintained as a pure culture on agar slants and were transferred every month or two.
EXPERIMENTAL RESULTS

Preliminary Results

Organisms: The methanol organism was a small rod (0.5 - 1.0 x 1.0 - 3.0 micron) which formed aggregates, especially in low shear environments. The organism was white during growth but turned red after standing in the effluent receiving tank. The organism required no growth factors in addition to the standard media. Large amounts of polysaccharide were produced when the organism was grown on nitrate as the sole source of nitrogen.

The phenol organism was a rod (0.7 - 1.0 x 1.5 - 3.0 micron) which grew singly and in pairs, producing a light green water soluble pigment. After isolation the organism grew well initially, but growth became scanty after several transfers. In continuous culture, the organisms washed out after approximately ten detention times, indicating lack of a necessary growth factor in the feed solution. Tabak⁶⁰ reported using Vitamin B₁₂ as a growth factor for phenol metabolizers. Cobalt, a constituent of Vitamin B₁₂, was added as a nutrient supplement and stimulated vigorous growth.
Initially cobalt was not added to the methanol cultures, which necessitated experiments to determine the cobalt concentration which allowed the phenol metabolizers to grow without adversely affecting the methanol utilizers. The methanol organisms showed a decreased batch growth rate in the presence of 0.5 mg/l CoSO₄—the concentration used in the phenol cultures. A concentration of 0.025 mg/l CoSO₄ caused no significant difference (95% confidence level) in batch growth rates of the methanol organisms compared to a cobalt-free media. The phenol organisms grew equally well at either concentration (Appendix C-1). Consequently, mixed culture media contained 0.025 mg/l CoSO₄. Batch growth tests indicated that neither organism would grow on the primary substrate of the other organism (Appendices C-2, C-3).

Results of Single Species Predominant Cultures

Introduction: Continuous cultures inoculated with a single species and fed one substrate were called single species predominate (SSP) cultures. SSP cultures were not pure in microbiologist's terms but competitive pressures did not allow appreciable numbers of other organisms to become established. No extraneous organisms were observed during regular microscopic examination. An exception was the phenol SSP culture at growth rates lower than 0.300 (hr⁻¹) when less than 5% of the biomass
was a larger rod. The predominance of a single species implied that variation in measurements with changes in growth rate were not caused by a change in the types of organism in the reactor.

**Methanol SSP Cultures:** The methanol SSP culture was troublesome due to foaming and wall growth which necessitated constant scraping and antifoam addition. After all steady state data was taken (Appendix C-4), phenol toxicity tests on the methanol organisms were performed and indicated no adverse effects. Negligible amounts of methanol were measured in the reactor at all growth rates (Figure 1). The maximum growth rate was obtained by adding an excess of methanol to the continuous culture and measuring the response by the light absorbance technique. A growth rate was calculated from the non-steady state material balance and the flow rate adjusted to a higher dilution rate. The procedure was repeated over the period of a week until a maximum growth rate was obtained (Appendix C-5). The Monod kinetic constants, calculated by using the measured maximum growth rate and fitting the curve to intersect the experimental point with the highest growth rate, were \( \mu_{\text{max}} = 0.51 \) (hr\(^{-1}\)) and \( K_s = 0.25 \) (mg/l). The saturation constant compares favorably with the value of 0.65 mg/l obtained by Harrison. The data for yield, ratio of oxygen to methanol uptake rate \( (R_{O_2}/R_m) \), unit oxygen uptake rate,
Figure I - Methanol Concentration vs Growth Rate for SSP Experiments, using $\mu_{\text{max}}$ from Appendix C-5
and dehydrogenase activity test are shown as functions of growth rate in Figures 2, 3, 4, and 5 respectively. The stoichiometry for the microbial oxidation of methanol was computed as a function of growth rate (Figure 5) by using the following equation, incorporating the cell composition formula of Porges.\textsuperscript{62}

\[
\text{CH}_3\text{OH} + (\text{AO}_2)\text{O}_2 + (A_B)\text{NH}_3 \rightarrow (A_B)\text{C}_5\text{H}_7\text{NO}_2 + (\text{AcO}_2)\text{CO}_2 \\
+ (A_{\text{H}_2\text{O}})\text{H}_2\text{O}
\]

The equation was balanced with respect to carbon, oxygen and nitrogen using experimental values of yield and oxygen uptake rate. This method enabled an error function to be computed by calculating the imbalance of hydrogen atoms in the otherwise balanced equation.

**Error Function** = \( \frac{\text{Hydrogen atoms on left side}}{\text{Total number of hydrogen atoms}} \) - \( \frac{\text{Hydrogen atoms on right side}}{\text{Total number of hydrogen atoms}} \)

**Phenol SSP Cultures**: Data from phenol SSP cultures (Appendix C-6) produced a classic Monod function for substrate concentration dependence on growth rate (Figure 7). The kinetic constants were obtained by plotting the inverse of growth rate versus the inverse of substrate concentration. The slope of a straight line drawn through the three highest growth rate points was \( K_s/\mu_{\text{max}} \), and the intercept \( 1/\mu_{\text{max}} \). These values
Figure 2 - Yield vs Growth Rate for Methanol SSP Experiments
Figure 3 - Ratio of Oxygen Uptake to Methanol Uptake vs Growth Rate for Methanol SSP Experiments
Figure 4 - Unit Rate of Oxygen Uptake vs Growth Rate for Methanol SSP Experiments
Figure 5 - Dehydrogenase Activity ($DAT_m$) vs Growth Rate for Methanol SSP Experiments
Figure 6 - Stoichiometric Coefficients vs Growth Rate for Methanol SSP Experiments
Figure 7 — Phenol Concentration vs Growth Rate for SSP Experiments
were $u_{\text{max}} = 0.728 \text{ (hr}^{-1})$ and $K_S = 25.5 \text{ (mg/l)}$. Data for yield ($y$), ratio of oxygen to phenol uptake rate ($R_{O_2}/R_p$), unit rate of oxygen uptake, and dehydrogenase activity ($DAT_p$) are shown as functions of growth rate (Figures 8, 9, 10, and 11). The stoichiometric coefficients (Figures 12 and 13) were calculated as before to balance the following equation:

$$C_6H_6O + (A_{O_2})O_2 + (A_B)NH_3 \rightarrow (A_B)C_5H_7NO_2 + (A_{CO_2})CO_2$$

$$+ (A_{H_2O})H_2O$$

**Mixed Culture:** Data was obtained for six growth rates with the mixed continuous culture (Appendix C-7). The measured substrate concentrations were compared to the predicted values given by the Monod function obtained in the SSP cultures (Figure 14). The differential dehydrogenase activities ($DAT^*$, Figure 15) cannot be directly compared to SSP activities because the mixed culture DAT* is expressed on a basis of mixed biomass composed of methanol and phenol organisms.

One of the primary goals of the research was to predict behavior of a specific dual organism, dual substrate system from behavior of each separate single organism, single substrate system. The biomass in the mixed culture was predicted as a function of growth rate and compared to the measured concentration (Figure 16). The predicted value was obtained from,
Figure 8 - Yield vs Growth Rate for Phenol SSP Experiments
Figure 9 - Ratio of Oxygen Uptake to Phenol Uptake vs Growth Rate for Phenol SSP Experiments
Figure 10 - Unit Rate of Oxygen Uptake vs Growth Rate for Phenol SSP Experiments
Figure II— Dehydrogenase Activity vs Growth Rate for Phenol SSP Experiments

Dehydrogenase Activity (Absorbance Units) vs Growth Rate (hr⁻¹): Experiments with high and low Replicates.
Figure 12 - Stochiometric Coefficients $A_B$, $A_{H_2O}$ vs Growth Rate for Phenol SSP Experiments
Figure 13 - Stochiometric Coefficients $A_{O_2}$, $A_{CO_2}$ and the Error Function vs Growth Rate for the Phenol SSP Experiments
Figure 14 - Concentration of Phenol and Methanol vs Growth Rate for Mixed Culture Experiments with Monod Function from SSP Experiments
Figure 15 — Differential Dehydrogenase Activity vs Growth Rate for Mixed Culture Experiments
Figure 16 — Predicted and Actual Biomass Concentration vs Growth Rate for Mixed Culture Experiments
\((C_B)_{\text{predicted}} = \gamma_m^* (C_m^o - C_m^*) + \gamma_p^* (C_p^o - C_p^*)\)

where; \(\gamma_m^*\), \(\gamma_p^*\) are the methanol and phenol yields obtained in SSP culture

\(C_m^*, C_p^*\) are the substrate concentrations predicted from SSP data

\(C_m^o, C_p^o\) are the influent substrate concentrations used in the mixed culture.

The sharp decline in the biomass concentration is due to predicted washout of the methanol organism at a growth rate equal to 0.51 \((\text{hr}^{-1})\). To determine if the mixed culture yields were the same as in the SSP culture, biomass concentrations were predicted using the following equation and plotted against the actual concentrations (Figure 17).

\((C_B)_{\text{predicted}} = \gamma_m^* (C_m^o - C_m^*) + \gamma_p^* (C_p^o - C_p^*)\)

\(C_m^o, C_m^*, C_p^o, C_p^*\) are influent and effluent substrate concentrations in mixed culture

\(\gamma_m^*, \gamma_p^*\) are yields from SSP cultures.

Points falling on the 45\(^o\) line would indicate no variation in yields between the mixed and SSP cultures.

Prediction of oxygen uptake rates was made analogously to the biomass prediction (Figure 18).
Figure 17 – Measured Biomass Concentration vs Biomass Concentration Prediction by Assumption of Invariant Yield (Growth Rate in parenthesis)
Figure 18 — Predicted and Measured Oxygen Uptake Rates vs Growth Rate for Mixed Culture Experiments
\[(Ro_2)_{\text{predicted}} = \frac{Ro_2^*}{R_m}(C_m^o-C_m^*)D + \frac{Ro_2^*}{R_p}(C_p^o-C_p^*)D\]

\[\frac{Ro_2^*}{R_m}, \frac{Ro_2^*}{R_p}\] are ratios of oxygen to substrate uptake rates obtained in SSP cultures.

\[D = \frac{Q}{V}\] is the dilution rate.

A graph of measured rates versus rates predicted using mixed culture substrate uptake rate data and \(Ro_2^*/R_m\), \(Ro_2^*/R_p\) from SSP cultures was used as a check to determine if the oxygen to substrate ratios were the same in both systems (Figure 19).

\[(Ro_2')_{\text{predicted}} = \frac{Ro_2^*}{R_m}(C_m^o-C_m')D + \frac{Ro_2^*}{R_p}(C_p^o-C_p')\]

Development of reaction stoichiometry as a function of growth rate in the mixed culture was complicated by changes in the relative amounts of each substrate consumed. Changes in apparent stoichiometry could indicate changes in the relative substrate uptake rates, instead of changes in metabolic patterns. If stoichiometry is constant, however, there should be a linear relationship between stoichiometric coefficients and ratios of phenol to methanol uptake rates. Mixed culture stoichiometric coefficients were calculated in the same manner as the SSP cultures for the following equation.

\[\text{CH}_3\text{OH} + (A_p)\text{C}_6\text{H}_5\text{O} + (A_0)\text{O}_2 \rightarrow (A_B)\text{C}_5\text{H}_7\text{NO}_2 + (A_{CO_2})\text{CO}_2
+ (A_{H_2O})\text{H}_2\text{O}\] (1.0)
Figure 19 — Measured Oxygen Uptake Rate vs Oxygen Uptake Rate Predicted by Assuming Invariant $\frac{R_{O_2}}{R_m}$, $\frac{R_{O_2}}{R_p}$ (Growth Rate in Parenthesis)
Figures 20 and 21 show the coefficients plotted versus $A_p$---the molar ratio of phenol metabolized to methanol metabolized. The error function showed a definite correlation with growth rate (Figure 22).
Figure 20 - Stoichiometric Coefficients $A_B$, $A_{O_2}$, $A_{CO_2}$ vs Stoichiometric Coefficient $A_p$ for Mixed Cultures Experiments
Figure 21 — Stoichiometric Coefficient $A_{H_2O}$ and the Error Function vs Stoichiometric Coefficient $A_P$ for the Mixed Culture Experiments.
Figure 22 - Error Function for Mixed Culture Stoichiometry vs Growth Rate
DISCUSSION

The four parameters obtained by independent measurement at each steady state were dilution rate (growth rate), substrate concentration, oxygen uptake rate and biomass concentration. Relationships between these parameters are predictable from theory and experimental results in continuous culture. The parameters calculated were yield \([(C^o_s - C_s)/C_B]\), ratio of oxygen to substrate uptake rate \([R_{o_2}/D(C^o_s - C_s)]\), and unit rate of oxygen uptake \([R_{o_2}/C_B]\). Inspection of these parameters plotted as functions of growth rate often indicate points which do not coincide with established trends. Comparison among data generated at the same growth rate can indicate which point is incorrect. For example, if the measured values of unit oxygen uptake rate and yield at a given growth rate do not correlate with others, but the ratio of oxygen to substrate uptake rates appears reasonable, then the inconsistent point can be attributed to an erroneous biomass measurement. Although all experimental points are reported, the functional relationship to growth rate was determined based on the validity of each point.

-56-
The Monod function for growth rate dependence on substrate concentration was chosen as a model due to its widespread use and applicability to diverse systems. The Monod saturation constant $K_s$ is particularly useful as a good estimate of substrate concentration remaining in a waste treatment effluent.

Yield is also of direct interest to waste water treatment since it determines the size of sludge handling facilities which is often the most costly unit process in the entire system. Yield was originally considered constant, but increased use of continuous culture techniques has produced reports of yield variation with growth rate. Yield is usually reported as an increasing function of growth rate and explained in terms of maintenance energy requirements. The concept of maintenance energy is that a microorganism has two separate energy needs. One, the maintenance energy, is constant and is that energy needed to replace cellular components, provide for active transport and other energy demanding control mechanisms. The other need is energy for growth which is proportional to growth rate. As growth rate increases the fraction of energy obtained from the substrate, needed for maintenance decreases, allowing more energy to be used for synthesis, thereby increasing yield. This concept has been applied to activated sludge models. Decreases
in yield with growth rate have been reported and are explained by increased formation of metabolic by-products at higher growth rates,\textsuperscript{70,71} or by changing cell composition.\textsuperscript{3,72} Cell composition changes are usually associated with storage of reserve materials such as polysaccharides, or poly-β-hydroxy-butyric acid.  

The ratio of oxygen to substrate uptake rate relates the amount of metabolic activity occurring per substrate molecule entering the organism. The fraction of substrate used for energy generating metabolism can be found by relating the measured to the maximum \( \frac{R_{O_2}}{R_S} \) derived from the equation for total chemical oxidation of substrates

\[
(1.0) \text{Substrate} + \left( \frac{R_{O_2}}{R_S} \right)_{\text{max}} \overset{O_2}{\longrightarrow} \left( \frac{R_{CO_2}}{R_S} \right)_{\text{max}} \text{CO}_2 + \text{H}_2\text{O}(\text{H}_2\text{O})
\]

\% Substrate used for metabolism = \( \frac{R_{O_2}/R_S}{(R_{O_2}/R_S)_{\text{max}}} \times 100\% \)

The rate of carbon dioxide formation per mole of substrate is in the same relation to its maximum as is \( R_{O_2}/R_S \)

\[
\frac{R_{CO_2}}{R_S} = \frac{R_{O_2}}{R_S} \left( \frac{R_{CO_2}/R_S}{R_{O_2}/R_S} \right)_{\text{max}}
\]

The maintenance energy concept predicts a slight decrease in \( R_{O_2}/R_S \) and \( R_{CO_2}/R_S \) with growth rate.
The unit rate of oxygen uptake \( (R_o^2/C_B) \), like unit rate of substrate uptake, is expected to be a linear function of growth rate if cellular composition and size do not change. \( R_o^2/C_B \) expresses the amount of energy producing processes per unit cell mass. When plotted versus growth rate the extrapolated intercept at zero growth is used to measure the maintenance energy coefficient. The dehydrogenase activity, using an exogenous substrate, is reported to increase with growth rate,\(^{33,73}\) possibly because of a change in the numbers of viable cells per unit cell mass.\(^{29}\)

The stoichiometric coefficients obtained for the microbial oxidation of phenol and methanol are based on the following assumptions: (1) a constant, correct formula for biomass composition, (2) no products formed except cells, carbon dioxide and water, and (3) ammonia being the form of nitrogen used by the organism. The formula for cell composition used in this work was derived for a mixed culture growing on a skim milk media.\(^{62}\) \( C_6H_9.6N_1.25O_1.5 \) is a formula derived for \( E. coli \) and \( C_5H_10N_1.25O_2.75 \) has been found to be the cell composition of a methanol utilizer grown in continuous culture.\(^{74}\) Of the stoichiometric coefficients only \( A_B \) and \( A_{O_2} \) are parameters obtained from actual measurements, independent of the other coefficients. \( A_{H_2O} \) is obtained by a carbon balance and \( A_{H_2O} \) by an oxygen
balance. If cell composition changes with growth rate due to a decrease in storage products $A_B$ would be expected to decline, $A_{O_2}$ to increase, causing $A_{CO_2}$ and $A_{H_2O}$ to react according to the relative changes in $A_B$ and $A_{O_2}$. $A_{CO_2}$ and $A_{H_2O}$ are particularly sensitive to changes in $A_B$ because of the five carbon atoms in the cell composition formula. If metabolic by-products are released to the media $A_B$ and $A_{O_2}$ would decrease, causing $A_{CO_2}$ to increase and $A_{H_2O}$ to decrease.

**System Results**

**Methanol SSP Cultures:** Negligible amounts of methanol were measured at all growth rates which necessitated an alternative method for measuring the maximum growth rate which resulted in $\mu_{\text{max}} = 0.51 \pm 0.03 \ (\text{hr}^{-1})$. Previously reported yields for methanol metabolizing organisms are $20-40\%$ lower than those measured.\textsuperscript{35,74} The decrease in yield with growth rate could be caused by (1) less polysaccharide storage products, (2) more metabolic by-products released to the media, (3) loss of autotrophic metabolism. The decrease of $R_{O_2}/R_m$ with increasing growth rate indicates a smaller fraction of methanol is being used as an energy source at higher growth rates. This behavior is predicted by the maintenance energy concept or production of by-products, but contradicts a postulated decrease in storage
materials. The unit rate of oxygen uptake produced an excellent linear relationship with growth rate with the exception of one point. This same point did not follow the pattern established by other yield or $R_{O_2}/R_m$ measurements either, so it cannot be determined whether the oxygen uptake rate or biomass measurement is in error. The excellent fit of the $R_{O_2}/C_B$ data would discredit autotrophic metabolism as an explanation of yield data. The extrapolated intercept at growth rate equal to zero (0.03 mg $O_2$/mg Biomass-hr) validates the existence of a maintenance energy.

Stoichiometric analysis presents an opportunity to examine all growth parameters at once. The stoichiometric coefficients for the methanol SSP cultures are all linear functions of growth rate, including the error function. The major anomaly is the concurrent decrease of $A_{O_2}$ and increase in $A_{CO_2}$. The two are linked by biochemical considerations and their ratio should be constant. The behavior could be due to formation of by-products which are released to the media. This would cause erroneously high $A_{CO_2}$ values and cause $A_{O_2}$ and $A_B$ to be measured as reported. A postulated decrease in storage products cannot explain the decreasing $A_{O_2}$ and good fit of $R_{O_2}/C_B$ with growth rate.
Phenol SSP Cultures: Inspection of the yield and unit oxygen uptake rate variation with growth rate indicates an inconsistent point for a growth rate equal to 0.59 (hr\(^{-1}\)). The \(\text{Ro}_2/\text{R}_p\) value at 0.59 hr\(^{-1}\) is consistent with other \(\text{Ro}_2/\text{R}_p\) values, indicating an erroneously high biomass measurement at this growth rate. Data for a growth rate of 0.50 (hr\(^{-1}\)) displays a similar but smaller error. High biomass concentration could be measured due to wall growth dislodged during the measurement procedure prior to solids sampling.

The Monod model for the phenol organism gave an excellent fit at higher growth rates but predicted higher substrate concentrations than measured at the lower growth rates. The small degree of culture contamination found at the lower growth rates probably contributed to this effect. No effects of substrate inhibition were evident at this phenol concentration.

The yield data for the phenol SSP cultures and methanol SSP cultures are similar. By-product formation is again a valid explanation for this behavior as indicated by the visible, excreted pigment. The production of a light green pigment indicated the organism was a fluorescent pseudomonad, known to be phenol metabolizers. Absence of tri-ethylamine odor indicates that the organism is a strain of *Pseudomonas fluorescens*. Cell composition changes due to storage product
accumulation can be discounted for the phenol organism since it has been reported that fluorescent pseudomonads do not synthesize any specific reserve material.\textsuperscript{58}

\( \text{RO}_2/R_p \) measurements were nearly constant with a slight decrease at higher growth rates. The unit rate of oxygen uptake was linear with the growth rate when the inconsistent points were ignored. The combination of visible pigmentation, decreasing yield, and decreasing \( \text{RO}_2/R_p \) indicate the effect of by-product excretion.

The stoichiometry for phenol SSP cultures does not indicate the linear trends exhibited in methanol SSP data. The points were scattered, especially at the highest growth rate where the error approached 50\%, presumably due to by-product formation.

**Mixed Culture:** Investigation of behavior of phenol and methanol organisms in mixed culture was a primary interest of this research. Examples of organism-organism interaction might reasonably be expected on the basis of previous experience with mixed cultures.\textsuperscript{10} This was found to be the case since this methanol organism was able to grow in mixed culture at growth rates significantly higher than the maximum obtained in SSP culture. Affinity of the organism for methanol remained the same as demonstrated by the negligible substrate concentration at lower growth rates. The phenol concentrations in mixed culture were generally
lower than predicted from SSP data, but were scattered to such a degree that no meaningful inferences can be drawn as to the effect of mixed culture growth or the organism's affinity for substrate or its maximum growth rate.

The oxygen uptake rate and biomass concentrations were not rigorously predictable from SSP data. Measured biomass concentrations were lower than predicted except at growth rates where methanol organisms were not expected to be able to grow. This is caused by higher yields in the SSP cultures as compared to mixed, since substrate concentrations were the same or less than predicted. Prediction using actual substrate concentration data indicate lower yields in the mixed system, contrary to other reports on pure mixed cultures. The ratio of oxygen consumed to substrate removal was also lower in the mixed system, especially at high growth rates. The data indicate that the mixed culture has an accelerated tendency to excrete organic by-products with increasing growth rate.

Dehydrogenase Activity Test

Introduction: The dehydrogenase activity test used in a differential activity analysis in mixed cultures was unable to provide a means for measuring the relative amounts of methanol and phenol organisms in the system.
The large variability in the test at different growth rates and failure of the phenol DAT in mixed culture to give non-zero results at most growth rates is not explainable at present. The measurement error as indicated by the high and low replicate values is negligible compared to variation with growth rate. The DAT technique used varies from those reported in the following manner:

(1) Cells were centrifuged to remove substrate from the sample
(2) There was no filtration step after the TTC reaction, a blank was used to correct the absorbance
(3) A non-glucose substrate (methanol or phenol) was used
(4) The pH (7.0) was lower than in other tests (7.6 - 8.5).

Changes in the technique which might improve reproducibility include:

(1) Centrifuge cells at reduced temperatures at higher speeds for shorter times
(2) Investigate the effect of reaction time on variability
(3) Run the test in the dark
(4) De-oxygenate test media, use nitrogen atmosphere during test
(5) Develop a functional filtration step.

Results: No plausible explanation for the lack of phenol DAT in mixed culture exists except the excretion of some inhibitor substance by the methanol organisms. The only difference between the mixed and SSP tests was the presence of the methanol organisms. The inconsistencies are not readily explainable. The average methanol DAT in the mixed system (50) was higher than in the SSP culture (57) when it is considered that 30-40% of the biomass in the mixed system was not able to metabolize methanol. There was no difference in the mixed system DAT when methanol or methanol and phenol were added as substrate, even when a significant phenol DAT was measured. The variability and erratic behavior indicate more research is necessary before the DAT is applicable to a differential activity analysis. The reproducibility of unit oxygen uptake rate indicate that it would be a better choice for a differential activity analysis in this system.
CONCLUSIONS

1. The methanol organisms were able to grow at significantly higher growth rates in the mixed culture than SSP cultures, indicating a positive interaction.

2. By-product excretion is a plausible explanation for observed decrease in yield and $R_{O_2}/R_S$ in the phenol and methanol cultures.

3. The concentrations of phenol and methanol measured in the mixed system were generally equal to or less than those in the SSP cultures.

4. Yield and $R_{O_2}/R_S$ in the mixed system were lower than in the SSP cultures, indicating an accelerated tendency toward by-product excretion.

5. The dehydrogenase activity test as conducted is not an adequate activity measurement for differential activity analysis in this system.

6. Stoichiometric equations for biological oxidation of methanol and phenol were developed.

RECOMMENDATIONS FOR FURTHER RESEARCH

1. Validate and explain the mechanism of by-product excretion by the phenol and methanol organisms.
2. Apply SSP-mixed culture analysis to other industrial wastes.

3. Investigate application of unit oxygen uptake rate as a technique for differential activity analysis.

4. Investigate reasons for DAT variability and erratic behavior in mixed culture.
### APPENDIX A-1

Review of Methanol Metabolizers (After Chalfan, 35)

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<tr>
<td>Doubling Time (hrs.)</td>
<td>2</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>4 - 10</td>
</tr>
<tr>
<td>Growth Temp. (C)</td>
<td>28 - 40</td>
<td>30</td>
<td>25 - 30</td>
<td>30 - 37</td>
<td>30</td>
</tr>
</tbody>
</table>

Sole Carbon Source

- methane
- methanol
- formaldehyde
- formate
APPENDIX A-1 (continued)

<table>
<thead>
<tr>
<th>Reference</th>
<th>(35)</th>
<th>(75)</th>
<th>(76)</th>
<th>(71)</th>
<th>(78)</th>
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</thead>
<tbody>
<tr>
<td>ethanol</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>glucose</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>fructose</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>starch</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>peptone</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Autotrophic</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>+</td>
</tr>
<tr>
<td>metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

++ strong growth   + weak growth   * variable   - no growth   * not reported
## APPENDIX A-1 (continued)

<table>
<thead>
<tr>
<th>Reference</th>
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<th>(80)</th>
<th>(81)</th>
<th>(82)</th>
<th>(80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Ps. PRL-W4</td>
<td>Vibrio extorquens</td>
<td>Methyllococcus capsulatus</td>
<td>Methanomonas methanooxidans</td>
<td>Meth. methano.</td>
</tr>
<tr>
<td>Morphology</td>
<td>rod, rounded ends</td>
<td>rod, single polar flag.</td>
<td>diplococcus capsule</td>
<td>rods</td>
<td>rods, as rosettes</td>
</tr>
<tr>
<td>Size (microns)</td>
<td>0.5 - 1.2</td>
<td>*</td>
<td>1.0 x 1.0</td>
<td>1.0x1.5-3.0</td>
<td>1.0-1.5x4.0</td>
</tr>
<tr>
<td>Pigmentation (Abs. max., μm)</td>
<td>pink (455,488,520)</td>
<td>pink</td>
<td>pink</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Doubling Time (hrs.)</td>
<td>7.2</td>
<td>*</td>
<td>3.5 - 13</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Growth Temp. (°C)</td>
<td>30</td>
<td>28</td>
<td>30 - 50</td>
<td>*</td>
<td>*</td>
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<tr>
<td>Sole Carbon Source</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>methane</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>*</td>
</tr>
<tr>
<td>methanol</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>*</td>
</tr>
<tr>
<td>formaldehyde</td>
<td>-</td>
<td>*</td>
<td>*</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>formate</td>
<td>-</td>
<td>+</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
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### APPENDIX A-1 (continued)

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<th>(81)</th>
<th>(82)</th>
<th>(80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ethanol</td>
<td>-</td>
<td>++</td>
<td>±</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>glucose</td>
<td>++</td>
<td>-</td>
<td>±</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>fructose</td>
<td>-</td>
<td>+</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>starch</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>peptone</td>
<td>*</td>
<td>+</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Autotrophic metabolism</td>
<td>+</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

++ strong growth  + weak growth  ± variable  - no growth  * not reported
Pathway of MeOH Degradation, (76), (38)

\[
\text{CH}_3\text{OH} \xrightarrow{\text{H}_2\text{O}_2, \text{alcohol peroxidase}} \text{H} \cdot \text{CHO} + 2\text{H}_2\text{O}
\]

APPENDIX A-2

Ribose-5-Phosphate

Ribose-6-Phosphate

Allulose-6-phosphate

Fructose-6-phosphate

Fructose 1,6-di-phosphate

ATP

ADP

Glyceraldehyde phosphate

Xylose phosphate

Sedoheptulose phosphate

Erythrose phosphate

Glyceraldehyde phosphate

Xylose phosphate

Dihydroxy Acetone Phosphate
APPENDIX A-3

Bacteria Able to Metabolize Phenol, with references

*Ps. fluorescens* (41, 44, 46)
*Ps. putida* (44, 45)
*Ps. rathones* (43, 47)
*Ps. acidovorans* (44)
*Ps. multivorans* (44)
*Ps. desmolytica* (48)
*Ps. fragi* (48)
*Ps. dacunahae* (48)
*Ps. cruciuae* (41)
*Vibrio cuneata* (43, 47)
*Micrococcus varians* (48)
*Micrococcus sphaeriodes* (47)
*Mycobacterium crystallo* (47)
*Bacillus subtilis* (49)
*Zooglea ramigira* (49)
*Streptomyces griseas* (49)
*Norcardia sp.* (41)
*Achromobacter sp.* (50)
Appendix A-4
Metabolic Pathways for Phenol Degradation (45)

Phenol

Phenol

Catechol

2 hydroxy-
muconic
semialdehyde

Pyruvate

Acetaldehyde

Formate

4-hydroxy-
2 keto valerate

Benzoate

Phenol

Catechol

Cis-Cis Muconate

Muconolactone

Succinate

Acetyl CoA

HS-CoA

β-keto Adipate

β-keto Adipate

enol lactone
Appendix B-1

Impulse Response Test for Reactor System

- Measured Absorbance of Tracer
- Response function of a Completely Mixed Reactor with
  \[ \frac{V}{Q} = 126 \text{ (min.)} \]

**Test Conditions**
- \( V = 1,030 \text{ (mL)} \)
- \( Q = 7.73 \text{ (mL/min)} \)
- \( \frac{V}{Q} = 133 \text{ (min.)} \)
## Oxygen Transfer Test

Results as $K_{LA}$ (min$^{-1}$) at 30°C.

<table>
<thead>
<tr>
<th>Stirrer setting (arbitrary units)</th>
<th>7</th>
<th>8</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>air flow (mls/sec)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.1</td>
<td>2.80</td>
<td>3.32</td>
<td>3.08</td>
</tr>
<tr>
<td>4.3</td>
<td>2.46</td>
<td>2.82</td>
<td>2.71</td>
</tr>
<tr>
<td>2.1</td>
<td>1.17</td>
<td>1.59</td>
<td>1.82</td>
</tr>
<tr>
<td>1.1</td>
<td>0.77</td>
<td>1.02</td>
<td>1.06</td>
</tr>
</tbody>
</table>
APPENDIX B-3

Procedure for Dehydrogenase Activity Test

(a) Procedure

(1) centrifuge sample for 30 minutes at 2000 rpm (1400 g)
(2) wash cells with standard nutrient solution
(3) centrifuge at 2000 rpm for 30 minutes
(4) re-suspend cells with standard nutrient solution
(5) add 20 ml of washed cell suspension to a 125 ml flask and place in water bath at 30°C
(6) after temperature equilibrium has been reached, add 1 ml of TTC-substrate reagent to each flask except the blank, replace in water bath
(7) after 60 minutes add 30 ml of ethanol
(8) read absorbance at 485 μm, as soon as possible, use deionized water as a reference
(9) measure total suspended solids of washed cell suspension

(b) Reagents: Tri-phenyl tetrazolium chloride---substrate reagent
10 gm/l Tri-phenyl tetrazolium chloride
4 gm carbon/l substrate
balance is deionized water

(c) Calculations

\[ \text{D.A.T.} = \frac{\text{Absorbance of sample} - \text{Absorbance of blank}}{\text{TSS (gm/20 ml)}} \]
APPENDIX B-4

Methanol Test

(a) Procedure

(1) add 5 ml of sample to a 125 ml Erlynmeyer flask
(2) add 1.0 ml of potassium permanganate solution
(3) after 1 minute add 1.0 ml of bisulfite solution
(4) add 5 ml of Chromotropic Acid Reagent
(5) immediately after addition of Chromotropic acid add 50 ml of concentrated H₂SO₄
(6) after 5 minutes cool to room temperature
(7) measure absorbance at 578 m\textmu

(b) Reagents

(1) Potassium Permanganate Reagent
3 gm KMnO₄
15 ml H₃PO₄
100 ml H₂O

(2) Bisulfite Solution—-12.5% (w/w) aqueous solution

(3) Chromotropic Acid Reagent
1% (w/w) solution of Chromotropic acid (4-5, Dihydroxy 2-7 Napthalenedisulfonic acid) in H₂SO₄
APPENDIX B-5

Total Suspended Solids Procedure
(a) use Gelman Metrical filters type GN-6, or Millipore HAWP
(b) dry filters for 3 hrs. at 80°C., place in dessicator for 15-30 min.
(c) remove from dessicator, expose to atmosphere for 3-5 minutes, weigh, replace in dessicator
(d) use two filters from the same lot for every test, filter on bottom is a control for leaching
(e) after filtration dry filters for 2 hours at 80°C.
(d) dessicate and weigh as before
APPENDIX B-6

Standard Media

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Stock gm/l</th>
<th>Reactor mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na₂MoO₄ • 2H₂O</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>MnSO₄ • H₂O</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>MgSO₄ • 7H₂O</td>
<td>22.5</td>
<td>22.5</td>
</tr>
<tr>
<td>FeCl₃ • 6H₂O</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>27.5</td>
<td>27.5</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td></td>
<td>300</td>
</tr>
<tr>
<td>Phosphate Buffer [AsPO₄³⁻]</td>
<td></td>
<td>1,720</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td></td>
<td>1,300</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td></td>
<td>510</td>
</tr>
<tr>
<td>Na₂HPO₄ • 7H₂O</td>
<td></td>
<td>2,000</td>
</tr>
<tr>
<td>CoSO₄</td>
<td>0.05</td>
<td>0.025</td>
</tr>
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</table>

mixed culture

0.50 phenol SSP

0 methanol SSP
APPENDIX C-1

Batch Growth Rates (hr⁻¹)

<table>
<thead>
<tr>
<th></th>
<th>with 0.025 mg/l CoSO₄</th>
<th>with 0.5 mg/l CoSO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>0.318</td>
<td>0.315</td>
</tr>
<tr>
<td>Organism No. 2</td>
<td>0.299</td>
<td>0.294</td>
</tr>
<tr>
<td>No. 3</td>
<td>0.314</td>
<td>0.304</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>without CoSO₄</th>
<th>with 0.025 mg/l CoSO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>0.441</td>
<td>0.427</td>
</tr>
<tr>
<td>Organism No. 2</td>
<td>0.433</td>
<td>0.425</td>
</tr>
</tbody>
</table>
APPENDIX C-2

Methanol Organism Ability to Grow on Phenol

Six batch cultures were used, each sterilized and seeded from pure culture of methanol metabolizers. Two replicates were used---one pair with methanol alone, one pair with phenol alone, one pair with phenol and methanol, all at approximately 100 mg/l substrate carbon.

**ABSORBANCE**

<table>
<thead>
<tr>
<th>Time</th>
<th>Methanol</th>
<th>Phenol</th>
<th>Methanol and Phenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>(hr)</td>
<td>No. 1</td>
<td>No. 2</td>
<td>No. 1</td>
</tr>
<tr>
<td>0</td>
<td>0.108</td>
<td>0.11</td>
<td>0.095</td>
</tr>
<tr>
<td>4</td>
<td>0.17</td>
<td>0.18</td>
<td>0.095</td>
</tr>
<tr>
<td>34</td>
<td>0.50</td>
<td>0.48</td>
<td>0.089</td>
</tr>
<tr>
<td>98</td>
<td>0.42</td>
<td>0.43</td>
<td>0.082</td>
</tr>
<tr>
<td>121</td>
<td>----</td>
<td>----</td>
<td>0.082</td>
</tr>
<tr>
<td>178</td>
<td>----</td>
<td>----</td>
<td>0.079</td>
</tr>
</tbody>
</table>
Phenol Organism Ability to Grow On Methanol

Pure cultures of phenol metabolizers were grown in batch cultures with phenol, methanol, or phenol and methanol as organic substrate. Each experiment was done in duplicate and growth measured by light absorbance at 500 μu.

**ABSORBANCE**

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Phenol No. 1</th>
<th>Phenol No. 2</th>
<th>Methanol No. 1</th>
<th>Methanol No. 2</th>
<th>Methanol No. 1</th>
<th>Methanol No. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.039</td>
<td>0.043</td>
<td>0.038</td>
<td>0.045</td>
<td>0.040</td>
<td>0.046</td>
</tr>
<tr>
<td>7</td>
<td>0.72</td>
<td>0.76</td>
<td>0.020</td>
<td>0.025</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>21</td>
<td>----</td>
<td>----</td>
<td>0.012</td>
<td>0.018</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>116</td>
<td>----</td>
<td>----</td>
<td>0.018</td>
<td>0.015</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>248</td>
<td>----</td>
<td>----</td>
<td>0.008</td>
<td>0.010</td>
<td>----</td>
<td>----</td>
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</table>
Summary of Methanol SSP Cultures

<table>
<thead>
<tr>
<th></th>
<th>0.193</th>
<th>0.280</th>
<th>0.379</th>
<th>0.455</th>
</tr>
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<tbody>
<tr>
<td>[MeOH]_out, (mg/l)</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>[MeOH]_in, (mg/l)</td>
<td>625</td>
<td>536</td>
<td>530*</td>
<td>530*</td>
</tr>
<tr>
<td>Biomass (Mg dry weight/l)</td>
<td>348</td>
<td>285</td>
<td>281</td>
<td>253</td>
</tr>
<tr>
<td>Yield (gm Biomass/gm MeOH)</td>
<td>0.556</td>
<td>0.532</td>
<td>0.530</td>
<td>0.478</td>
</tr>
<tr>
<td>O₂ Uptake (mg O₂/l-min.)</td>
<td>1.76</td>
<td>2.02</td>
<td>2.32</td>
<td>2.87</td>
</tr>
<tr>
<td>Ratio oxygen to methanol uptake</td>
<td>0.880</td>
<td>0.806</td>
<td>0.692</td>
<td>0.714</td>
</tr>
<tr>
<td>Rates (MgO₂/MgMeOH)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unit rate of oxygen uptake (Ro₂/C_B)</td>
<td>0.304</td>
<td>0.425</td>
<td>0.496</td>
<td>0.681</td>
</tr>
<tr>
<td>(Mg-O₂/Mg-Biomass-hr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dehydrogenase Activity Test (absorbance units/gm biomass)</td>
<td>47</td>
<td>71</td>
<td>58</td>
<td>52</td>
</tr>
</tbody>
</table>

*This value computed, not measured.
Maximum Growth Rate Measurement for Methanol SSP Culture

$\mu_{\text{max}} = 0.5 \text{ hr}^{-1}$
APPENDIX C-6

Summary of Phenol SSP Cultures

<table>
<thead>
<tr>
<th></th>
<th>0.180</th>
<th>0.398</th>
<th>0.504</th>
<th>0.588</th>
<th>0.626</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth Rate (hr⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[PhOH]_{in}, (mg/l)</td>
<td>238</td>
<td>245</td>
<td>243</td>
<td>245</td>
<td>241</td>
</tr>
<tr>
<td>[PhOH]_{out}, (mg/l)</td>
<td>2</td>
<td>13</td>
<td>54</td>
<td>107</td>
<td>163</td>
</tr>
<tr>
<td>Biomass concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg Dry weight/mg phenol)</td>
<td>199</td>
<td>159</td>
<td>130</td>
<td>116</td>
<td>43</td>
</tr>
<tr>
<td>Yield (gm Biomass/gm phenol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.805</td>
<td>0.685</td>
<td>0.706</td>
<td>0.840</td>
<td>0.551</td>
</tr>
<tr>
<td>Oxygen Uptake Rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg/l - min)</td>
<td>0.922</td>
<td>2.08</td>
<td>2.08</td>
<td>1.65</td>
<td>0.925</td>
</tr>
<tr>
<td>Ratio of oxygen to</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>phenol uptake rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(MgO₂/MgPhOH)</td>
<td>1.30</td>
<td>1.35</td>
<td>1.35</td>
<td>1.22</td>
<td>1.22</td>
</tr>
<tr>
<td>Unit rate of oxygen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uptake (R₀₂/Cₜ)</td>
<td>0.278</td>
<td>0.785</td>
<td>0.961</td>
<td>0.853</td>
<td>1.290</td>
</tr>
<tr>
<td>(MgO₂/Mg-Biomass-hr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dehydrogenase Activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test (absorbance units/gm Biomass)</td>
<td>47</td>
<td>34</td>
<td>64</td>
<td>40</td>
<td>----</td>
</tr>
</tbody>
</table>
APPENDIX C-7

Summary for Mixed Cultures

<table>
<thead>
<tr>
<th></th>
<th>Growth Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.193</td>
</tr>
<tr>
<td>[PhOH]_{in}' (mg/l)</td>
<td>126</td>
</tr>
<tr>
<td>[PhOH]_{out'} (mg/l)</td>
<td>13</td>
</tr>
<tr>
<td>[MeOH]*_{in}' (mg/l)</td>
<td>264</td>
</tr>
<tr>
<td>[MeOH]_{out'} (mg/l)</td>
<td>1</td>
</tr>
<tr>
<td>Biomass concentration</td>
<td>198</td>
</tr>
<tr>
<td>(mg Dry weight/l)</td>
<td></td>
</tr>
<tr>
<td>Oxygen Uptake Rate (mg/l-min)</td>
<td>1.34</td>
</tr>
<tr>
<td>Dehydrogenase Activity Test</td>
<td></td>
</tr>
<tr>
<td>with Phenol (absorbance)</td>
<td></td>
</tr>
<tr>
<td>units/gm Biomass)</td>
<td>34</td>
</tr>
<tr>
<td>DAT with Methanol, (A.U./gm)</td>
<td>48</td>
</tr>
<tr>
<td>DAT with Methanol and Phenol (A.U./gm)</td>
<td>44</td>
</tr>
</tbody>
</table>

*This number computed, not measured.
FOOTNOTES


NOMENCLATURE

A  Stoichiometric Coefficient
C  Concentration in mg/l
D  Dilution rate (hr$^{-1}$)
K$_s$ Saturation constant in Monod formula (mg/l)
N  Number of microorganisms
R  Rate of uptake or formation
SSP Single species predominating
Y  Yield
μ  Growth Rate
θ  Sludge Age (inverse of Growth Rate)

Subscripts

B  biomass
CO$_2$ carbon dioxide
g  biological growth
H$_2$O water
m  methanol
max maximum
O$_2$ oxygen
p  phenol
pred. predicted value
s  substrate
Superscripts

\(o\) parameter evaluated in influent

\(*\) value obtained in another system

\('\) predicted value for \(\gamma\) or \(R_o/R_g\) in variance test