BIOKINETIC PARAMETERS OF A PHOTOSYNTHETIC WASTE STABILIZATION PROCESS

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ABSTRACT

Biokinetic parameters, characteristic of an innovative/alternative wastewater treatment process, were determined in order to demonstrate economical wastewater stabilization and the simultaneous generation of useful biomass. The Algal Bacterial Clay Treatment (ABCT) reactor was designed to be coupled with a mollusc filter feeder clarifier. Michaelis-Menten and Monod models were characterized experimentally for the ABCT reactor under varying conditions of mixing rate, aeration rate, light intensity, temperature, presence or absence of clay particles, and initial substrate concentrations under batch conditions in order to simulate a plug flow reactor. A final two-week continuous flow "steady state," complete mix experiment was also conducted. Under the latter condition, data acquired for pH, CO₂, and O₂ diurnal fluctuations, using a data acquisition system and PC attached to the biostat reactor, documented the periodic CO₂ limitation and O₂ limitation. The lack of severe effects due to the diurnal limitations indicated that the ABCT strategy is efficient and potentially productive of useful biomass.

KEYWORDS

Wastewater; innovative treatment; energy-efficient; microbial biomass

INTRODUCTION

Recent USEPA interest in innovative and alternative wastewater treatment strategies due to rising energy costs, limitations of conventional treatment strategies, and a need for economic advanced waste treatment strategies has led to development of the Algal Bacterial Clay Treatment Pond (ABCT). Precursors of the ABCT system include the High Rate Algal Pond (HRAP) (Oswald et al., 1957b; Oswald and Gotaas, 1957; Oswald, 1962; Soeder, 1980) and the Intensive Algal Wastewater Treatment System (IAWTS) (Shelef et al., 1968; Shelef et al., 1970; Shelef et al., 1973). These photosynthetic ponds were designed to stabilize wastewater without the aeration expense normally encountered in conventional waste treatment practices. The HRAP strategy included the following advantages over the conventional bio- and physico-chemical treatment schemes: lower capital cost; a plug-flow hydraulic regime requiring minimal aeration at only the reactor inlet; lower overall energy requirements for oxygenation throughout the length of the plug flow reactor due to solar-produced oxygen via photosynthesis; the concurrent production of a usable protein-rich biomass; essentially no sludge accumulation in the reactor; tertiary treatment due to nutrient stripping by algae; virtual absence of pathogenic bacteria/viruses in the effluent; and ammonia stripping as a result of high pH's encountered in the reactor. The later IAWTS was optimized by decreasing pond depth to maximize light utilization by the microalgae, thus providing conditions for increased pond loadings and minimal aeration in the form of a cage aerator. This latter pond was composed of folding channels and had a detention time ranging from 2-8 days. The reduced
pond depth of 35-50 cm allowed for greater BOD loadings and optimized photosynthetic algal production of oxygen. As a result of an increased standing crop of algae within the reactor, metabolic nutrient requirements of the algae were much greater than in previous pond designs and, therefore, nutrient removal was significant. Removal capabilities of 95% for total phosphorus and 70% for total nitrogen were comparable to tertiary treatment nutrient removals (Shelef and Halperin, 1970; Shelef et al., 1976; Shelef et al., 1977; Okuyama, 1983; Gordin, 1986; Huang and Gloyna, 1984). Dark photoperiod respiration of algae and bacteria drove DO levels to critically low concentrations requiring a cage aerator perpendicular to the channel direction at the influent.

The present alterations Carberry has developed within the treatment train include: the addition of clay to the reactor and the design of an alternative clarification system. This design has been classified as an Algal-Bacterial-Clay Treatment Pond (ABCT). This modified pond has been studied in order to optimize the generation of algae from bacterial degradation of wastewater. Work in collaboration with Pruder (Pruder and Carberry, 1981) has examined the utilization of molluscs for clarifying the ABCT effluent. This paper describes the determination of biokinetic parameters for the ABCT system.

BACKGROUND
In natural standing surface waters, in a lake for example, the exchange of CO₂ between the atmosphere and the water, the carbonate buffer system of the lake, and various other CO₂ sources are in relative equilibrium. In the engineered ABCT, however, during photosynthetic periods, CO₂ is withdrawn from the aqueous medium by the enormous algae biomass at such an increased rate that all other CO₂ inputs to the system are negligible. As a result, dissolved CO₂ concentrations drop. During dark periods when algae respire, the reverse scenario exists; the rapid expulsion of metabolically-produced CO₂ during darkness results in increased dissolved CO₂ concentrations. Large fluctuations in CO₂ concentrations produce a CO₂ rate-limiting condition. Because of this rate-limiting condition, the carbonate buffer system of the photosynthetic pond can be considered a "closed" system. The CO₂ fluctuations cause pH fluctuations. As the dissolved CO₂ is consumed by the algae, the pH rises, and, conversely, the pH will decrease during dark periods when both bacteria and algae respire. Since many of the algal pond treatment systems throughout the literature have been noted for high pH's during algal photo-active periods, it has been postulated that the high pH's may result in ammonia stripping (Soeder, 1980).

The by-product of algal photosynthesis, oxygen, is a microbial respiration reactant; and conversely, products of microbial respiration, carbon dioxide and nitrogen compounds, are reactants of algal photosynthesis. The resulting commensalistic relationship existing between the bacteria and algae reportedly occurs for only gram-negative bacteria (Zagallo, 1953; Simidu et al., 1971; Chrost, 1972; Godlewska-Lipowa and Jablonska, 1972; Caldwell, D.E. and Caldwell, S.J., 1978; Jones, 1982). Such reports of gram-negative bacterial "selection" by algae are complemented by literature describing bacterial production of chemical agents which deter algal proliferation (Pratt et al., 1944; Shiaris and Morrison, 1976; Berland et al., 1972; Delucca and McCracken, 1977; Berger et al., 1979). In addition, Dor, noted that Chlorella and Scenedesmus were the numerically dominant inhabitants of most stabilization ponds (Dor, 1975 and 1976). Chlorella and E. aerogenes were chosen, therefore, as the model algal and bacterial species for this study.

The addition of low concentrations of clay in the ABCT reactor was studied in order to determine the effects of clay to dampen shock loadings to the plug flow reactor and to enhance mollusc feeding in the subsequent clarifier. It was postulated that the clay would adsorb some of the organics from a shock load at the influent junction of the reactor, and the organics would then slowly desorb as the wastewater traveled downstream in the plug-flow reactor. Previous work by Stotzky found that certain species of clay stimulated bacterial respiration (Stotzky and Rem, 1966a and b). Specifically, montmorillonite, and attapulgite stimulated bacterial respiration apparently due to the pH maintenance within optimum growth range due to the clays' cation exchange capacity (CEC). Furthermore, Pruder had found that particulates admixed with algal feed enhanced growth rates of molluscs (Ali and Pruder, 1982) proposed to act as filter feeders in the clarification stage of the wastewater treatment plant for Lewes, DE. Such a plan would combine the expertise of oyster cultivation at the College of Marine Science, University of Delaware, located in Lewes, and qualify for innovative technology funding by USEPA. In addition, the normal problems of sludge disposal would be eliminated and a useful biomass produced.
MATERIALS/METHODS

Axenic cultures of *Enterobacter aerogenes* were purchased from Carolina Biological Supply Company, Burlington, North Carolina, and cultured in 40 ml test tubes containing medium shown in Table 1. Once a substantial population was established, several test tubes were aseptically transferred to a 250 ml Erlenmeyer flask. Culture media was then changed to a synthetic waste solution (Table 1) and used both as testing medium and for sustenance between test periods. The 250 ml Erlenmeyer culture was fed daily until the concentration was high enough to transfer to a 4 liter Plexiglas cylinder. This stock culture was maintained throughout the study on the synthetic medium. Mixing and aeration were provided by a magnetic stirrer and two air diffusers connected to an air compressor. Routine streak plating was used to check contamination. Plating and microscopic inspection of the culture showed *E. aerogenes* to be the dominant bacterial population throughout the study. The culture was fed synthetic waste every other day, and testing always began on a feeding day. Due to periodic solids build up, the culture was drained to approximately one-third its volume once a week, and filled with distilled water. Acclimation to the waste occurred two weeks prior to initial testing.

Carolina Biological Supply Company provided the bacteria-free *Chlorella vulgaris* source on agar slants. The algae were initially transferred to 40-ml test tubes containing Bristol’s Solution (Stein, 1973). This inorganic nutrient solution diminished the chance of bacterial contamination, and *Chlorella* was reported to respond well to this solution (Starr, 1960). After a two-week acclimation and growth period, the algae were aseptically transferred to 250 ml Erlenmeyer flasks containing the synthetic waste medium described in Table 1, with sodium bicarbonate as the only carbon substrate for the algae. To avoid light-limiting conditions once algal population densities increased, the algal cultures were placed in front of an illumination bank, comprised of 3 panels with two 20-watt *Sylvania* cool white fluorescent bulbs per panel. All six lights illuminated provided 142 watts per m².

| TABLE 1: Synthetic Waste Used Throughout the Batch Testing |
|-----------------|-----------------|
| (NH₄)₂SO₄        | 250 mg/l        |
| MgSO₄·6H₂O       | 50 mg/l         |
| MnSO₄·H₂O        | 5 mg/l          |
| CaCl₂·2H₂O       | 3.75 mg/l       |
| FeCl₂·6H₂O       | 0.25 mg/l       |
| 2M Phosphate Buffer | 10 mg/l      |
| Tapwater         | 20 mg/l         |

for Bacteria

| Glucose          | 383 mg/l        |

for Algae

| 1M NaHCO₃        | 90 mg/l         |

Attapulgite PFI-1 was obtained from the Clay Mineral Society Source Clay Repository, Department of Geology, University of Missouri. Prior to usage, attapulgite was dried at 105°C for 24 hours and then run through a series of sieves to produce a size range of 75-125 μm equivalent diameter.

A Braun Biostat-E fermentation unit was used as the process reactor. This instrument simultaneously recorded 6 separate parameters and displayed the measured values on both a multipoint continuous recorder and a digital display module. The Biostat culture vessel had a 5-liter volume. Temperature was controlled and mixing speed was kept constant at 150 rpm using 5 impeller blades. The pH of the culture was controlled throughout most of the study. Calibrated probes measured oxygen partial pressure, carbon dioxide partial pressure, and pH. The measured value outputs of the Biostat were fed to a Keithley data acquisition system. At pre-determined time intervals, the continuously-acquired data was transferred from the buffer of the Keithley system to an IBM Enhanced AT. This network allowed for instant and continual system analysis. Other instruments used included the Yellow Springs Instrument’s Dissolved Oxygen and Salinity-Conductivity-Temperature (SCT) meters. HACH kits were used for determination of nitrate, orthophosphate and alkalinity.

After initial algal-bacterial compatibility tests, a series of batch experiments was conducted to determine the effect of several process parameters on algal growth, bacterial growth and substrate utilization. Table 2 lists the conditions for each test. For all
tests, the following analyses were conducted. A sample was removed from the biostat reactor, stirred, and then split into two sub-samples. One sub-sample was measured for mixed liquor suspended solids (MLSS). The other sub-sample was measured for chlorophyll A content, using the extraction method of Strickland and Parsons (1968). Prior to testing, a correlation curve was developed between axenic algal suspended solids samples at various concentrations and the respective chlorophyll A content. Chlorophyll A content variations reported in the literature between 1-2% by cell weight, were ignored due to the small resultant error in relation to the error assumed with suspended solids measurement (up to 10%). The algal biomass concentration was subtracted from the MLSS measurement to determine the bacterial biomass concentration, as shown in Equation 1:

\[ \text{MLSS} = \text{SSalgae} - \text{SSbacteria} (\text{mg/l}) \]  

(1)

The Hexokinase Enzymatic method by Sigma Chemical Company was used to determine glucose concentrations, using a Bausch and Lomb Spectronic 21 at 520 nm. For the experiments using real wastewater, chemical oxygen demand (COD) was determined using the HACH Vial Method.

The Biostat oxygen probe measured partial pressure of oxygen within the reactor. Dissolved oxygen measurements were taken using a YSI DO meter and the corresponding oxygen partial pressures were correlated. The conductivity was also measured for each correlation in order to account for different dissolved oxygen/partial pressure correlations at different conductivities. The biostat carbon dioxide probe output was in millibars and these readings were converted to concentration of carbon dioxide in units of mg/l by converting the millibar reading to atmospheres and substituting this value into Henry’s Law.

Batch tests were conducted in the Biostat in a sequence in which the process parameters under consideration could be looked at singly in order to develop a better understanding of their effects upon the overall system. Initially, irradiance variations and the resultant impact to the system were experimentally observed. Next, concentrations of clay were added to the system and its influence was examined. Then, tests with substrate-limiting conditions were conducted. High and low temperature extremes were then imposed on the system. Finally, both a batch test and a continuous-flow study were conducted using real wastewater as the experimental substrate to compare with results from all previous experiments with synthetic substrate. Wastewater was obtained from the Elkton, Maryland treatment facility after primary treatment consisting of screening, grit removal, and primary sedimentation. The wastewater sample was centrifuged and the centrate was used as the test medium. Biomass measurements were conducted the same as in the batch studies since only the soluble portion of the wastewater was used as substrate. Chemical oxygen demand (COD) was used to determine the waste strength. In the last two-week experiment, the Biostat was set up to simulate a continuous flow complete mix reactor by using a Braun FE211 peristaltic pump to feed Elkton
wastewater refrigerated to eliminate any measurable biodegradation of the influent prior to its use. Another synchronized peristaltic pump withdrew the effluent from the reactor in order to operate without any measurable change in volume within the reactor. This test was divided into two sections. During the first five days of the test, the reactor was fed continuously, light and temperature were controlled, and all other process parameters were monitored. For the second portion of the test (the last nine days), the waste feed was turned off, therefore permitting determination of the nonbiodegradable portion of the Elkton wastewater.

Thirteen batch tests were used to determine the specific growth rates of the bacteria biomass, i.e., the growth rate per unit of biomass, expressed as follows:

\[
\frac{dX}{dt} = \mu X
\]  

(2)

where \( \mu \) is equal to the specific growth rate, \( \frac{dX}{dt} \) is the change in bacterial biomass per unit time, and \( X \) is the bacterial biomass. Equation 2 can be integrated, linearized and rearranged as follows:

\[
\mu = \frac{\ln X - \ln X_0}{t}
\]  

(3)

The cell yields for each batch test were determined as:

\[
Y = \frac{-dX}{-X_0 - X} = \frac{X - X_0}{S_0 - S}
\]  

(4)

where \( Y \) is the mass of cells per unit of substrate removed, \( S_0 \) is the initial substrate concentration when time equals zero, and \( S \) is the substrate concentration at time equal to \( t \).

The Monod equation relates specific growth rate, \( \mu \), as a function of substrate concentration:

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

(5)

where \( \mu_{\text{max}} \) is the maximum specific growth rate (time\(^{-1}\)), and \( K_s \) is the substrate saturation constant (mass/volume). This equation was used to determine the biokinetic constants \( \mu_{\text{max}} \) and \( K_s \) using the Lineweaver-Burke technique.

The Michaelis-Menton equation, which quantifies bacterial substrate utilization, was used to characterize the wastewater biodegradation within the reactor as follows:

\[
-r = \frac{k_o S}{K_m + S}
\]  

(6)

where \( r \) is the bacterial specific substrate uptake rate defined as the change in substrate per unit time per unit of biomass, \( k_o \) is the maximum specific substrate uptake rate, and \( K_m \) is the half velocity constant. From Equations 4, 5 and 6,

\[
\mu_{\text{max}} = k_o Y
\]  

(7)

To determine \( k_o \) and \( K_m \) from the Michaelis-Menton relationship, the linearization technique of Hanes was used (Hanes, 1935). For the Hanes method, the inverse of Equation 6 is multiplied by \( S \):

\[
S = \frac{S}{r} = \frac{S}{k_o} + \frac{K_m}{k_o}
\]  

(8)

For the continuous flow study, mass balances were written for the biomass and substrate at steady state within the reactor. Since the dilution rate, \( D \), of a complete mix reactor is equal to:

\[
D = \frac{Q}{V}
\]
where $Q$ is the volumetric low rate and $V$ is the reactor volume, then, the rate of change in the biomass, $X$, for such a reactor is:

$$\frac{dX}{dt} = \mu X - DX$$

where $\mu X$ represents the increase in cell mass within the reactor due to cell growth and $DX$ represents the cell decrease due to removal from the reactor via the effluent. Assuming a steady state, $\frac{dx}{dt} = 0$ and

$$\mu = D - \frac{Q}{V}.$$  \hspace{1cm} (9)

The specific growth rate of the biomass can, therefore, be determined knowing the flow rate of the system.

Writing a mass balance for the biomass within the reactor will lead to a predictive equation allowing for the determination of $K_s$, the substrate saturation constant of the Monod equation. The mass balance for $X$ in the reactor is:

$$V \frac{dX}{dt} = \mu XV - QX$$

Substituting the Monod equation for $\mu$, substituting $Q/V$ for $D$, and re-arranging yields:

$$K_s = \frac{\mu_{max} - \frac{D}{\mu}}{D}.$$  \hspace{1cm} (10)

The value for $\mu_{max}$ was determined from the batch tests and the continuous flow study yielded the dilution rate, $D$, and the effluent substrate concentration, $S$.

Next, developing a mass balance for the substrate concentration, $S$, within the reactor will result in an equation which can be used to determine the cell yield:

$$\frac{dS}{dt} = QS_i - QS_e - \frac{\mu XV}{Y}$$

where $S_i$ and $S_e$ represent the influent and effluent substrate concentrations, respectively, and $\mu XV/Y$ represents the change in substrate concentration due to the bacterial consumption. Assuming steady state and solving for $X$, yields:

$$X = Y(S_i - S_e).$$  \hspace{1cm} (11)

This predictive equation results in the determination of cell yield for a continuous flow study with a known biomass, influent, and effluent substrate concentration.

Stoichiometric relationships were developed to predict the algal biomass production using the following method. Total bacterial respiration for a simple organic substrate ($C_6H_{14}O_2N$) was developed by Reynolds (1982). This equation accounts for both cell synthesis and respiration components of bio-oxidation:

$$C_6H_{14}O_2N + 3.36O_2 \rightarrow 1.61CO_2 + 0.12NH_4 + 0.12H^+ + 0.88C_3H_7O_2N$$  \hspace{1cm} (12)

where $C_3H_7O_2N$ represents the chemical formula for bacterial biomass. Using the $O_2/CO_2$ molar ratio resulting from this bio-oxidation reaction, the $CO_2$ production from a given waste can be predicted. The conversion of the $BOD$ of a given wastewater to the mass of $CO_2$ produced as a result of the wastewater’s bio-oxidation is:

$$\left[\frac{BOD \text{ gm } O_2}{\text{cubic meter}}\right] \left[\frac{\text{mole } O_2}{32 \text{ gm } O_2}\right] \left[\frac{1.61 \text{ moles } CO_2}{3.36 \text{ moles } O_2}\right] \left[\frac{44 \text{ gm } CO_2}{\text{mole } CO_2}\right] \left[0.66 \text{ (BOD)}\right] - \frac{X \text{ gm } CO_2}{\text{cubic meter}}$$

where $1.61 \text{ moles } CO_2/3.36 \text{ mole } O_2$ is the molar ratio taken from Equation 12. For this derivation, the nitrogenous oxygen demand was assumed to be negligible, since nitrification is not significant when reactor detention times are less than four to five days.

Since a pH increase was measured during photosynthesis for all testing involved in this study, a photosynthetic reaction with increasing pH was utilized (Stumm and Morgan, 1981).
where \( C_{106}H_{263}O_{110}N_{16}P \) represents the chemical formula for algal biomass. The mass of algae produced per gram of \( CO_2 \) in photosynthesis would then be:

\[
\frac{1 \text{ mole algae}}{106 \text{ moles } CO_2} \times \frac{1 \text{ mole } CO_2 \text{ algae}}{44 \text{ gm } CO_2} \times \frac{3550 \text{ gm algae}}{\text{ mole algae}} = 0.76 \text{ gm algae/gm } CO_2
\]

From a known influent BOD concentration, one could predict the expected gross algae production. Predictions of algal biomass production were made using this method and then compared to production in the batch studies.

**RESULTS/DISCUSSION**

Six initial batch tests were conducted to investigate the effects of irradiance, mixing, and aeration variations, on the reactor process. Table 2 lists the variations for each test. Results indicated that bacteria grew and degraded the substrate under all conditions. Table 3 lists the varying results from this series, with Test 5 serving as standard conditions without clay. Proper conditions for algal photosynthesis and growth required minimal mixing and no supplemental aeration. Under diurnal conditions created by alternating the light bank operation and otherwise darkened laboratory conditions, the algae grew at a slower overall rate than when continuously illuminated.

<table>
<thead>
<tr>
<th>TEST</th>
<th>( \mu ) (hr(^{-1} ))</th>
<th>( Y )</th>
<th>( k_0 ) (hr(^{-1} ))</th>
<th>( K_m ) (mg/l)</th>
<th>( \mu_{\text{max}} ) (hr(^{-1} ))</th>
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<td>0.18</td>
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<tr>
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<td>0.34</td>
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In one completely darkened test, Test 6, the algae grew at 50% of the previous diurnal rate, and bacterial biomass production was 30% lower due to the resulting anoxic conditions. These results are presented in Figure 1. This "phenomenon" of algal growth in total darkness has been reported previously. Wiedeman (1962) conducted algae growth studies under a variety of environmental conditions and found a variety of algal genera which were capable of utilizing simple pre-formed organics as a carbon source during conditions of total darkness, both in the presence of oxygen and under anoxic conditions. He classified algae with this capability, such as *Chlorella*, as "euheterotrophs".

Bacterial yields and specific growth rates for tests investigating effects of clay particles, Tests 7 and 8, showed no significant change when compared to tests which had the same experimental conditions without the addition of clay. Algal specific growth rates showed no significant change relative to tests without clay as shown in Table 4. Average results from two tests with clay and a test without clay are illustrated in Figure 2.
The next two tests, Tests 9 and 10, were compared to the test containing clay and 1000 mg/l initial substrate concentration in order to observe the effect of lower initial substrate concentrations on the system. The effect on algal specific growth rates was unclear since one test resulted in the highest rate and the other the lowest rate. Bacterial growth rates, however, were significantly lower with lower initial substrate concentration. This latter result would indicate that the specific growth rate for bacteria was in a first order zone. Average results of these tests are shown in Figure 3 and can be compared to Figure 2.

Test 11 was conducted to determine what effect extremely high temperatures imposed on the system. The optimum temperature for *E. aeroogenes* growth is reported to be 30° C. (James, 1983). In support of this, the specific growth rate determined for this test was the highest recorded for all testing. The yield also was high, when compared to the range developed from previous tests. For this test, however, the algal biomass seemed to be stressed. Though the specific growth rate was within the range developed from previous testing, the net algae cell yield was much lower than any previous testing, including the net cell yield recorded for the test conducted in total darkness. The next test was conducted at 16° C. The specific growth rate for the bacteria was greatly lowered in comparison to the previous test. The algal specific growth rate was, however, higher in comparison, suggesting that algal biomass may prefer cooler water temperatures. The net algal cell yield was comparable to previous testing. Results of temperature variation are illustrated in Figure 4.
Test 13 was conducted with wastewater obtained from the Elkton, Maryland treatment facility to compare with tests previously conducted using a synthetic waste medium. Bacterial specific growth rate remained within the range of growth rates developed from previous testing. Algal specific growth rate was also comparable to previous values. Results for real and synthetic wastewaters are illustrated in Figure 5 and indicate that the synthetic waste used in previous tests served as an appropriate model.

The last test, a continuous flow study, used wastewater from the Elkton treatment facility. Biomass results, influent COD concentrations, and effluent COD concentrations are illustrated in Figure 6. To determine values for the kinetic constants from this continuous flow study it was assumed that the system reached steady state conditions one day after the test initiation. Average values for influent substrate concentration, effluent substrate concentration, and bacterial biomass were used in the biokinetic parameter estimations. The specific growth rate, μ, was obtained as described in the kinetic analyses section of materials and methods using Equation 9. Since the flow rate was .36 l/hr used with a reactor volume of 5 liters, the specific growth rate was .072 hr⁻¹.

Following the 4 day monitoring of biomass, and substrate concentrations, the reactor was run as a batch reactor for an additional 9 days. This facilitated the determination of the nonbiodegradable portion of the Elkton wastewater. This was determined to be 27 mg COD/l.

As illustrated in Table 5, initial tests with aeration maintained substantial DO concentrations for the duration of each test. In non-aerated experiments, considerable drops in DO occurred and anoxic conditions existed for periods of each test but the process was not affected detrimentally.
TABLE 5: Dissolved Oxygen (mg/l)

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<tr>
<th>Test</th>
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<th>8 hr</th>
<th>12 hr</th>
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</tbody>
</table>

Typical dissolved oxygen and carbon dioxide concentrations for Tests 10-14 can be found in Figure 7. Predictably, an immediate increase in dissolved CO$_2$ concentrations was seen until peak concentrations occurred no later than 7 hours into the test. This increase in CO$_2$ can be accounted for by the "surge" of bacterial biodegradation. In all of these batch tests except at 25°C., the dissolved CO$_2$ concentrations were reduced to negligible concentrations due to the increase in algal biomass. Alkalinity measurements taken throughout the entire study indicated minimal fluctuations in comparison to the changes measured in dissolved CO$_2$ concentrations. See Table 6. This indicated that inorganic carbon forms, specifically bicarbonate and carbonate, were not being utilized to any measurable extent by the algae. As a result, dissolved CO$_2$ can be considered the "preferred" algal carbon substrate species.

The computer sampling system was used to acquire CO$_2$/O$_2$ data around the clock for the 4 day study. However, a malfunction was encountered with the CO$_2$ measurement and relay system. Therefore, only daytime values for CO$_2$ were recorded.

Figure 8 illustrates a daily pattern of CO$_2$/O$_2$ fluctuations after the second day of testing in the continuous flow mode. During daylight hours there was a steady increase in dissolved oxygen values and concurrently a rapid decrease in dissolved CO$_2$ concentrations. Just the opposite scenario most likely occurred during dark, unrecorded periods. The DO values recorded during dark periods dropped off to approximately zero by 3:00 a.m. The CO$_2$ values most probably increased as a result of algal/bacterial respiration. This variation was supported by the pH fluctuations.

TABLE 6: Alkalinity

<table>
<thead>
<tr>
<th>Test</th>
<th>0 hr</th>
<th>Time</th>
<th>8 hr</th>
<th>24 hr</th>
<th>48 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>718</td>
<td>613</td>
<td>635</td>
<td>650</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>600</td>
<td>540</td>
<td>587</td>
<td>507</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>689</td>
<td>630</td>
<td>652</td>
<td>628</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>1030</td>
<td>940</td>
<td>975</td>
<td>923</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 7. Typical Oxygen and Carbon Dioxide Concentrations in Batch Experiments

Fig. 8. Continuous Flow DO and CO$_2$ Concentrations
Biokinetic parameters

pH was continuously recorded for the length of the continuous flow study. The pH also fluctuated on a diurnal cycle; rising during the day and falling during the night. As the CO₂ concentration decreased during the day, the pH rose. This, in all likelihood, resulted from an increase in CO₂ concentration due to biological respiration. This result concurs with predictions of Buhr and Miller (1983), using a dynamic model which illustrated operational characteristics of the high-rate algal-pond. From this simulation they concluded, that the operating parameters (pH, DO, CO₂) change on a diurnal pattern, and that appreciable variation may also occur along the length of the reactor’s channel.

The method for stoichiometric prediction of algal yield was presented. Using this method and the batch test results, predictions were made and compared to the actual findings of each test in Table 4. As shown in the table, the predictions are often quite accurate. The accuracy for these predictions in comparison to the measured values is ± 17% error.

Measured nitrate concentrations for all of the tests listed in Table 4, were less than 1 mg/l, the minimum detection level for the analytical test method employed. The assumption that no measurable nitrification took place was therefore satisfied.

CONCLUSIONS

In both synthetic and real low-strength wastewaters, high algal concentrations were generated despite substrate limitations. In high strength wastes, light may be limiting, since attenuation occurs rapidly at high algal concentrations. Under high strength wastewater conditions, then, severe O₂ limitations may cause adverse effects on the treatment process unless mixing is increased. No such adverse effects were observed in these studies at glucose concentrations up to 1000 mg/liter, despite negligible DO concentrations during dark periods. Temperature variations affected both algal and bacterial performance. The presence of clay particles did not affect the treatment process adversely, and may, in fact, tend to dampen shock loading and attenuate diurnal pH variations in the plug-flow reactor.

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REFERENCES


