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Effects of cell retention time, butyrate loading rate and *p*H on oxidation of butyrate in aerated lagoons

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The treatability of butyrate in aerated lagoon was investigated in continuous laboratory-scale aerated reactors at 35°C. The effects of cell retention time, organic loading rate and *p*H on cellular growth and oxidation rate of butyrate were investigated. The cell retention time was varied in series of bioreactors from 1.5 to 8.0 days and influent *p*H was varied from 4.0 to 7.0. Butyrate loading rate of up to 3 kg/m^3/d as soluble organic carbon was investigated. The analysis showed that the maximum specific growth rate of heterotrophic bacteria was 2.1 d^{-1}, yield coefficient was 0.51, bacterial decay coefficient was 0.133 d^{-1} and cell maintenance coefficient was 0.262 d^{-1}. The maximum specific substrate utilization rate, half-rate saturation constant and substrate inhibition constant were 1.22 mg SOC/mg POC/d, 7.8 mg SOC/L, and 5900 mg SOC/L, respectively. Butyrate removal rate was influenced by loading rate, but was independent of the mode of variation of butyrate loading rate. No evidence was found to support the view that total butyrate concentration influenced the density of heterotrophic bacteria. However, the concentration of undissociated butyrate was found to significantly affect the density of heterotrophic bacteria. In the alkaline *p*H range, heterotrophic bacteria increased as butyrate concentration increased. Densities of heterotrophic bacteria as high as 1.1×10^{10} cfu/mL were observed when effluent *p*H was 8.4, but decreased to 3×10^3 cfu/mL when effluent *p*H decreased to 4.3.

Aerobic systems such as aerated lagoons and oxidation ponds can be used as a sole wastewater treatment process, secondary treatment, tertiary treatment, pre-treatment, balancing influent quality or for agriculture. When used as a sole treatment system lagoons may incorporate anaerobic ponds where influent contains high strength organic wastes. In addition, in recent years the interest in anaerobic treatment system for treatment of wastes has been growing because the energy stored in organic matter can be recovered in form of methane. When waste stabilization ponds are used for secondary or tertiary treatment of effluents from anaerobic systems they receive ammonia and volatile fatty acids (VFA) which are produced under anaerobic conditions. These products may also be found in the bottom sections of facultative ponds.

Aerobic systems such as aerated lagoons, oxidation ponds and activated sludge have often been used for secondary treatment of effluents from anaerobic treatment processes. They are usually used even when more sophisticated physico-chemical treatment (PCT) are applied. This is because although PCTs are very effective in removing fats, oils, phosphates, proteins, carbohydrates, colour and suspended and colloidal solids[1], but their ability to removal organics is low. Narkis *et al.*[2] found that because of their high hydrophobicity and low absorption energies, volatile fatty acids were almost not affected by chemical treatment and by activated carbon and accounted for 70-90% of COD and BOD in the effluent from activated carbon treatment. For these reasons aerobic treatment systems have often been used for improving the quality of anaerobic effluents.

Viswanathan *et al.*[3] did analysis of fatty matter in raw sewage and in aerobic and anaerobic conditions and found a considerable amount of fatty acids in raw sewage. They reported saturated long-chain fatty acids (Lauric, Myristic, Palmitic, Stearic) and unsaturated long-chain fatty acids (Oleic, Linoleic, Linolenic) of 548.5 and 591.4 mg/L, respectively. Noike and Matsumoto[4] in their research work on night soil reported the total

concentration of volatile fatty acids of about 3500 to 4000 mg/L. Oxidation pond sediments have been reported to have a potential of producing up to 8800 mg/L of volatile acids[5]. In thermophilic aerobic digestion of sludge, production of volatile fatty acids of between 950 to 10000 mg/L have been observed[6,7]. These VFAs are known to be inhibitory to microorganisms in anaerobic treatment processes either in unionized form[8,9], or total concentration of acids[10]. Among the VFAs, propionate[11], acetate[8], butyrate[12] and long-chain fatty acids[13] influenced the specific growth rate, lag time and activities of anaerobic bacteria. Eilersen *et al.*[14] in their work on inhibition by VFA on the nitrification activity of activated sludge concluded that the fatty acids of the *iso*-configuration were more inhibitory than fatty acids of the *n*-configuration. Volatile fatty acids have also been shown to affect the growth of alga *Chlorella vulgaris* and aerobic heterotrophs[15,16].

Loehr and Roth[17] concluded that the structural configuration of fatty matter affected the metabolism of the compound. They further reported that BOD test does not appear to determine the oxygen demand of some fatty acids because of lag times that occur with non-acclimated seed and with small number of active organisms. Biological treatability is conventionally evaluated by assessment of biodegradability rates of BOD and/or COD, without taking into consideration the composition of organics. Although effluents from anaerobic processes are treated in aerobic systems, little is known about their degradability characteristics and effects on aerobic bacteria. The aim of this research was to study the fate of volatile organic acid during treatment in a laboratory simulation of an aerated lagoon. The specific objectives of this study are to investigate the effects of butyrate on the growth of aerobic heterotrophs, and the degradation characteristics of butyrate in aerobic condition.

Methods and Materials

Experimental set-up—Laboratory-scale aerated lagoon reactors were constructed from a transparent acrylic plastic. The reactors, each of 5 L working volume, were provided with a sampling port near the bottom, overflow port near the top and a cover with air release ports (Fig. 1). Substrate was provided from feed tanks by means of variable multi-channel peristaltic pumps, which were also used to control sludge retention time. The reactors were incubated at 35°C controlled by thermostat which automatically switched on/off refrigerator or heater. The cultures were continuously mixed by magnetic stirrers and oxygen was supplied by bubbling the air with air pumps. Prior to sampling, biomass attached on the reactor walls was carefully re-suspended by swirling the culture contents.

Medium—The nutrient composition shown in Table 1 was used. Tap water was used to prepare all the medium, to which trace minerals were supplemented. The *p*H of the medium was controlled by adjusting with 1 *N* HCl or 1 *N* NaOH solutions. Butyric acid was used as the sole source

Table 1—Nutrient composition

Chemical	Amount
$MgSO_4.7H_2O$	250 mg
K_2HPO_4	110 mg
KH_2PO_4	110 mg
NaCl	100 mg
$CaCl_2.2H_2O$	10 mg
EDTA-Na	16 mg
Tap water	998 mL
Fe solution	1 mL
Trace minerals	1 mL
Fe solution	
$FeSO_4.7H_2O$	500 mg
Distilled water	250 mL
Trace minerals	
H_3BO_3	286 mg
$MnSO_4.4H_2O$	130 mg
$ZnSO_4.7H_2O$	320 mg
$CuSO_4.5H_2O$	183 mg
Na_2MoO_4	2.1 mg
Distilled water	100 mL

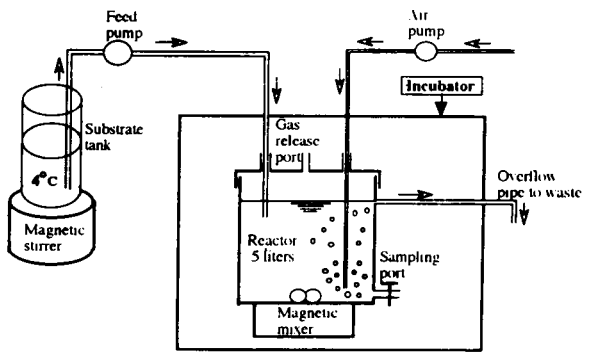


Fig. 1—Schematic diagram of experimental apparatus

of organic carbon. The concentration of substrate was prepared and varied in accordance with experimental design. Nitrogen was provided as nitrate to maintain carbon to nitrogen ratio of 4:1 by weight. The preparation of substrate was done daily and substrate was stored in continuously mixed feed tanks which were maintained at 4°C.

Microorganisms—The original seed heterotrophic bacteria were collected from the final sedimentation basin of an activated sludge wastewater treatment plant and was maintained in the laboratory at 35°C on butyrate-fed seed culture. Nutrient composition similar to that used for experiments was used to maintain bacterial seed culture. Each set of experiments was run until steady-state conditions were attained before experimental conditions were varied.

Analytical methods—Biomass concentration was measured by cell organic carbon determined at 680°C with an aid of a Total Organic Carbon (TOC) analyzer (Shimadzu, model TOC-5000, Japan). Before the measurement of cell organic carbon, the biomass was disrupted by a sonifier (Branson Sonic Power, model 185, USA) for 5 min to break the cells into small pieces and extend the settling time during measurements. Soluble organic carbon (SOC) was analyzed after removing the particulate matter through 0.45 μm filters. Inorganic carbon was analyzed by a TOC analyzer using phosphoric acid.

Heterotrophic bacterial population was determined in quadruplicate by pour-plate count on serial dilution of samples mixed with tryptone glucose extract agar as laid in the Standard Method[18]. Sterilization was done in accordance with the Standard Methods. Other researchers have recommended incubation at 35°C for 72 h[19,20]. Although Standard Methods[18] recommended incubation time of 48 h, this incubation time is insufficient for detection of cells capable of forming colonies (Fig. 2). Jen and Bell[21] reported that at 95% confidence limit the accuracy of enumeration of heterotrophic bacteria using pour plate method is within ±20% when sample size is 10. In field conditions usually plates are measured in triplicate or quadruplicate. For this sample size the accuracy of enumeration of heterotrophic bacteria using pour plate method at 95% confidence limit is within ±30%. This means that the minimum incubation time can be determined as duration after which heterotrophic bacterial count exceeds 70% of the number of cells capable of forming a colony. The incubation time of 72 h, a multiple of 24 h period was adopted because it meets this criteria. If 48 h had been adopted as recommended by Jen and Bell[21] and Standard Method[18], only about 32.5% of the colonies grown in butyrate may be recorded.

Volatile fatty acids were determined by injecting acidified supernatants into a Shimadzu GC-8A gas chromatograph equipped with a Shimadzu C-R6A integrator. Separation took place in a glass column, packed with 50-80 mesh porapak T and porapak Q. The operational temperatures for the injector and detector were 180 and 160°C, respectively. The precision of the measurements was determined using standard volatile fatty acids solution and the observed standard deviation of data was below 1%.

Dissolved oxygen was measured by a DO meter (TOA Electronics, DO-1B) and *p*H was measured by a *p*H meter (TOA Electronics, HM-18E). Heterotrophic bacteria were enumerated in triplicate using tryptone glucose beef extract agar[18].

Results and Discussion

Effect of hydraulic retention time

Removal of organic matter—The function described by Eq. (1) was used to describe the removal efficiency of soluble organic matter.

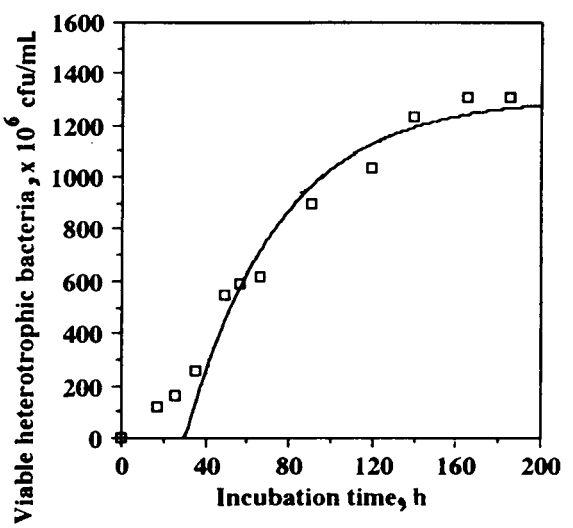


Fig. 2—Effect of incubation time on the colony forming units

$$\ln(E_o - E) = \ln E_o - \beta(t - t_{min}) \quad ...(1)$$

where E_o is the maximum removal efficiency, E is the removal efficiency observed at time t and β is a constant (d^{-1}).

The uptake of organics is possible only when the mean hydraulic retention time, t, is greater than the minimum hydraulic retention time, t_{min}. This minimum hydraulic retention time is defined as the retention time at which bacteria have their maximum growth rate, μ_{max}. When hydraulic retention time of bioreactor is shorter than t_{min}, bacteria will be washed out from the bioreactor and therefore no degradation will take place. The constant β in Eq. (1) can be calculated from the linear regression of $\ln(E_o - E)$ on t. Since t_{min} is not zero, the intercept γ obtained from the linear regression of $\ln(E_o - E)$ on t, will differ from $\ln E_o$. Therefore, t_{min} can be calculated as:

$$t_{min} = \frac{\gamma - \ln E_o}{\beta} \quad ...(2)$$

The parameter β and γ in Eq. (2) were calculated as 0.843 d^{-1} and 4.972, respectively. From these values t_{min} was calculated from Eq. (2) as 0.47 d. Fig. 3 shows that the determined parameters predict well the removal efficiency as a function of hydraulic retention time. Over 95% of the organic matter was removed when hydraulic retention time was longer than 4 d, but only about 50% when hydraulic retention time was decreased to 1.33 d. Prolonged incubation above 4 d slightly caused an increase in soluble organics. This is probably due to regeneration of organics from decomposing microorganisms.

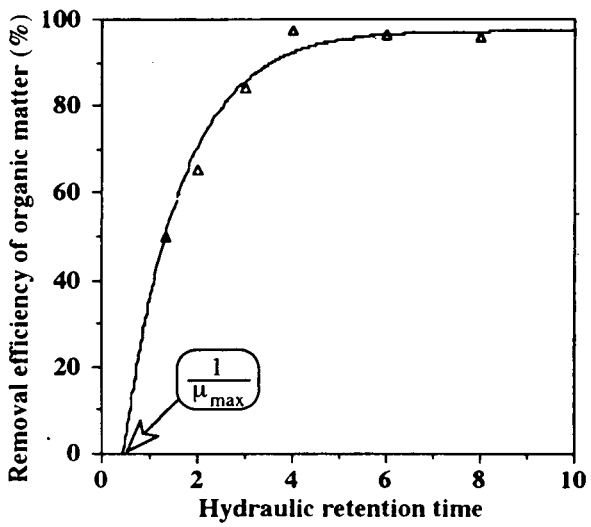


Fig. 3—Effect of hydraulic retention time on removal efficiency of soluble organic matter

The maximum growth rate of bacteria in the bioreactor is related to the minimum hydraulic retention time according to Eq. (3). Since the bioreactor was assumed to be completely mixed, average cell retention time, θ_c was assumed to be equal to hydraulic retention time, t. Therefore, the maximum growth rate of bacteria, μ_{max}, was calculated from Eq. (3) and was obtained as 2.1 d^{-1}.

$$\mu_{max} = \frac{1}{t_{min}} \quad ...(3)$$

It is also worthwhile to note that the maximum growth rate of heterotrophic bacteria is relatively similar to those grown in different systems and on different substrates (Table 2). Carberry and Greene[25] observed a value of 2.0 d^{-1} in algal-bacterial clay system. In an earlier study in batch cultures of *Chlorella vulgaris*-heterotrophic bacteria a maximum growth rate of 1.70 d^{-1} was obtained[16]. In contrast, the growth rate of aerobic bacteria on butyrate was much higher than anaerobic bacteria on the same substrate. This suggests that butyrate and perhaps other fatty acids can be oxidized much faster in aerobic systems than in anaerobic systems. Therefore, assuming the same sludge retention time, the effluent from anaerobic system may contain higher concentration of fatty acids than the effluent from aerobic system. These results are in agreement with Viswanathan *et al.*[3] who researched on the

Table 2—Comparison of growth rates of aerobic and anaerobic bacteria

Substrate	System	μ_{max} (d^{-1})	Temp., °C	Reference
Butyrate	Anaerobic	0.37	35	Lawrence and McCarty[22]
Butyrate	Anaerobic	0.86	37	Massey and Pohland[23]
Butyrate	Anaerobic	0.18	35	Chang *et al.*[24]
Butyrate	Aerobic	2.10	30	This study
Glucose	Algal-bacterial (Aerobic)	1.70	30	Mayo and Noike[16]
Glucose	Algal-bacterial clay (Aerobic)	2.00	30	Carberry and Greene[25]

degradation of saturated and unsaturated fatty acids. They concluded that the absence of fatty matter in the effluent of aerobic sludge, after treatment for 3 days, indicate that aerobic systems have a remarkable efficiency in oxidation of fatty matter.

The pH of the effluent—The *p*H in the reactors depended on the detention time of the water, as shown in Fig. 4. The shorter the detention time, the lower is the *p*H in the reactors. In all the runs, however, the *p*H was alkaline and varied from about 8.0-8.2 in a reactor controlled at 1.33 d to about 8.9-9.1 when hydraulic retention time as increased to 8 days.

Counts of viable heterotrophic bacteria—The effect of hydraulic retention time on counts of viable heterotrophic bacteria is presented in Fig. 4. The density of heterotrophic bacteria during steady state conditions was about 2×10^{10}/mL and did not vary significantly with hydraulic retention time. These results are not surprising because as discussed earlier the washout of bacteria may start when hydraulic retention time is below 11.2 h.

No evidence was found to support the view that elevated *p*H influenced the counts of heterotrophic bacteria. This is not surprising because the critical *p*H for die-off of most bacteria is above 9.0. Pearson *et al.*[26] using buffer solution for investigation of the effect of *p*H on mortality of faecal coliforms concluded that *p*H values above 9.0 were detrimental. In their research in field waste stabilization ponds in India, Parhad and Rao[27] found that *Escherichia coli* were removed rapidly at *p*H above 9.3. Previous work done on batch cultures containing *Chlorella vulgaris* and heterotrophic bacteria showed no significant effect of alkaline *p*H on the density of heterotrophic bacteria at *p*H below 10.0[16,28]. However, the mortality rates of heterotrophic bacteria were accelerated at elevated *p*H above 10.0. In fact, the alkaline *p*H values were beneficial to this system since the residue butyrate is known to be inhibitory to bacteria at low *p*H values because of the increased concentration of undissociated acid[8].

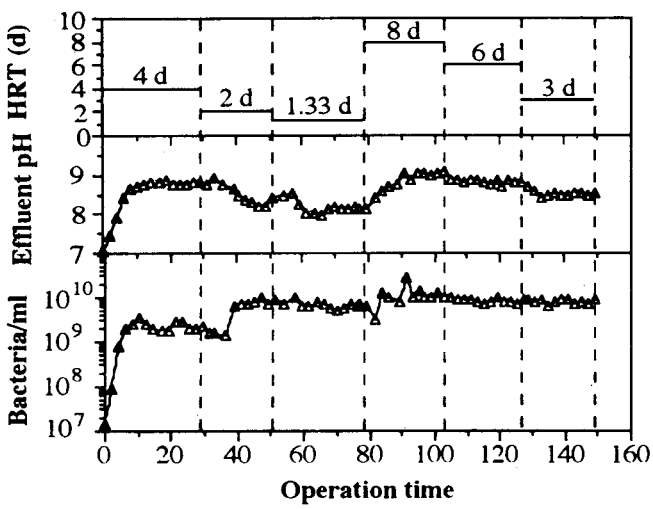


Fig. 4—Effect of hydraulic retention time on the effluent *p*H and density of heterotrophic bacteria. Influent concentration of *N*-butyrate was 86.2 mM

Maintenance energy—Servizi and Bogan[29] proposed that microbial cell yield in mixed cultures, should be proportional to the free energy of substrate degradation according to the relationship (4).

$$Y=kS \qquad \ldots(4)$$

where Y is the yield coefficient (mg biomass synthesized/mol. Substrate utilized), k is the proportionality constant, and S is the substrate concentration. It has been generally accepted that the effective microbial cell yield is a function of specific growth rate and the cellular maintenance requirements. A mathematical model relating to these parameters was developed by Lawrence and McCarty[30] for activated sludge system as shown by Eq. (5).

$$\frac{1}{\theta_c} = Y_{max}\nu - k_d \qquad \ldots (5)$$

Where θ_c is the average cell residence time (d); $(1/\theta_c)=\mu$ is the specific growth rate of biomass (d^{-1}); k_d is the biomass decay coefficient (mg biomass which die away/unit biomass, d); Y_{max} is the maximum cell yield (mg biomass formed/mg substrate removed), i.e., in the absence of cell maintenance requirements; and ν is the substrate removed/mg biomass/unit time.

The cell yield and cell maintenance requirements in axenic cultures may be described in accordance with Eq. (6), which was developed by Pirt[31].

$$\nu = \frac{\mu}{Y} = \frac{\mu}{Y_{max}} + m \qquad \ldots(6)$$

where m is the cell maintenance coefficient (mg substrate required for the maintenance of 1 mg of

biomass in a unit time). Eqs (5) and (6) are primarily related according to Eq. (7).

$$m = \frac{k_d}{Y_{max}} \quad \ldots(7)$$

Fig. 5 shows the dependence of specific substrate utilization rate on the hydraulic retention time. The kinetic values obtained from this figure were Y_{max}=0.51 mg POC/mg SOC, k_d=0.133 d^{-1}, and m=0.262 mg SOC/mg POC/d. To permit comparison, data from the literature on the maximum biomass yield coefficient were converted to carbon units. Most of the data available in literature express biomass yield in g VSS/g COD utilized. The conversion factor used was g VSS=1.415 g COD and was based on a well-established biochemical expression shown in Eq. (8). Table 3 shows the maximum biomass yield, maintenance coefficient and decay rate constant of bacteria in some aerobic and anaerobic systems.

$$C_5H_7O_2N+5O_2=5CO_2+NH_3+2H_2O \quad \ldots(8)$$
(biomass)

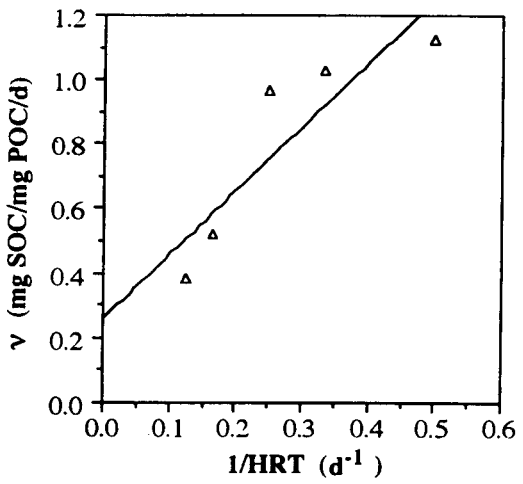


Fig. 5—Maximum yield and maintenance energy coefficient of heterotrophic bacteria

It appears that the maximum biomass yield coeffficient for aerobic systems varies from 0.51 and 0.89, while the maintenance coefficient falls between 0.048 and 0.262 d^{-1}. Surprisingly, these kinetic parameters together with decay coefficient, k_d obtained in this study for butyrate were closely related to those reported by various authors for synthetic sewage than those obtained when glucose or domestic sewage was used. It is difficult to tell from the available information whether this closeness has any practical implication. Regardless of the substrate, aerobic systems seem to have high biomass yields and low maintenance coefficients than anaerobic systems, which suggests that microorganisms will gain most energy by carrying out an aerobic process than anaerobic process. This finding further supports the previous discussion that degradation of organic matter is faster in aerobic system than in anaerobic system.

Effect of butyrate concentration on the density of viable bacteria

To determine the effect of butyrate loading rate on the population of heterotrophic bacteria, series of experiments were carried out in continuous bioreactors at hydraulic retention time of 4 days.

Table 3—Maximum biomass yield, maintenance coefficient, and decay rate constant on different substrates in aerobic systems

System	Substrate	Temp. °C	Y_{max}	m d^{-1}	k_d d^{-1}	Reference
Aerobic	Domestic sewage	20	0.66~0.89	0.048~0.072	0.043~0.064	Lawrence & McCarty[30]
	Domestic sewage	20	0.70	0.072	0.050	Surucu *et al.*[32]
	Glucose	20	0.66	0.072	0.048	Lawrence & McCart[30]
	Butyrate	30	0.51	0.262	0.133	This study
	Synthetic sewage	20	0.86	0.216	0.186	Lawrence & McCarty[30]
	Synthetic sewage	22	0.51	0.036	0.018	Pipyn and Verstraete[33]
	Synthetic sewage	22	0.59	0.192	0.113	Humenik and Ball[34]
Anaerobic	Butyrate	35	0.067	0.406	0.027	Lawrence & McCarty[22]
	Butyrate	35	0.031	0.161	0.005	Chang *et al.*[24]
	Mixed acids	35	0.042	2.332	0.099	Lin *et al.*[35]
	Acetate	35	0.058	0.326	0.019	Lawrence & McCarty[22]
	Propionate	35	0.059	0.169	0.010	Lawrence & McCarty[22]
	Acetate	35	0.153	1.850	0.283	Chang *et al.*[24]
	Propionate	35	0.061	1.508	0.092	Chang *et al.*[24]

The counts of heterotrophic bacteria increased as butyrate concentration increased (Fig. 6). In the low load stage of 300 mg SOC/L, the population of heterotrophic bacteria was 1.0×10^5-4.5×10^5 cfu/mL, but as influent soluble organic carbon was increased to 11820 mg/L, the densities of bacteria increased to 6.3×10^9 to 1.1×10^{10} cfu/mL. The counts of heterotrophic bacteria found in this study at high loading rates are significantly higher than those observed in other aerobic systems. Lemmer *et al.*[36] summarized the data of varies researchers on activated sludge in Germany and reported the typical range of 2×10^6 to 4.8×10^8 cfu/mL. In stabilization ponds the counts of viable bacteria of 1×10^6 to 4×10^8 cfu/mL were also reported[19,37,38], but the organic loading rate in ponds is usually low.

No evidence was found to support the view that total acid concentration influenced the population of heterotrophic bacteria. Although the concentration of total acid in the effluent increased to as high as 130 mM, the highest concentration of undissociated butyric acid was only 0.02 mM. This was because the increase in butyrate loading rate was accompanied by increase in *p*H values of the bioreactor contents. It is possible that inhibition of bacteria is influenced mainly by undissociated acid. Volatile fatty acids have been demonstrated to easily penetrate through the membrane of microorganisms in undissociated form than in anion form[8,39].

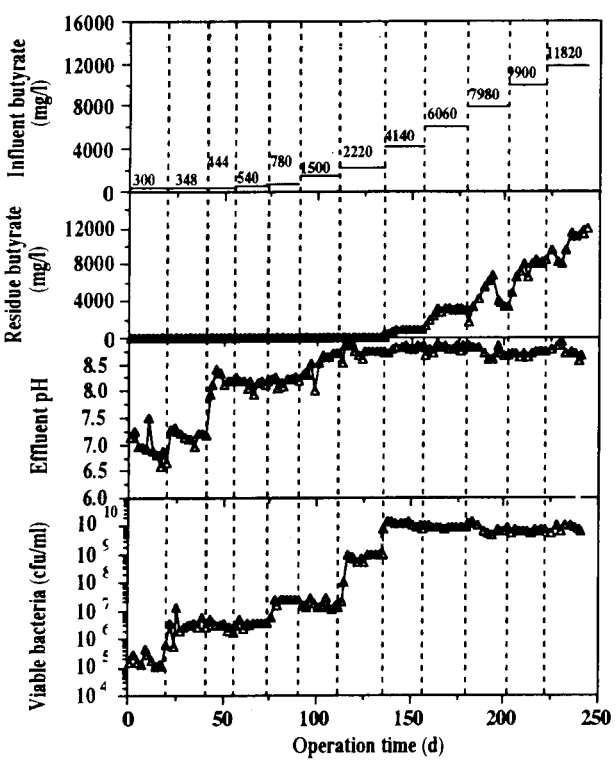


Fig. 6—Effect of butyrate loading rate on the density of viable bacteria

Changing organic loading rate by butyrate concentration and hydraulic retention time

The kinetics of the effect of butyrate concentration on the specific butyrate utilization rate was analyzed from a series of experiments performed at twelve different influent butyrate concentrations and six hydraulic retention time values. Eq. (9) was chosen for analysis of data because the experimental specific substrate utilization rates versus effluent substrate concentration showed a bell-shaped curve.

$$\nu = \frac{\nu_{max} S}{S + K_s + (S^2 / K_i)} \qquad \ldots(9)$$

where ν_{max} is the maximum specific substrate utilization rate (mg SOC/mg POC/d), S is the substrate concentration (mg SOC/L), K_s is the half rate saturation constant (mg SOC/L), and K_i is the substrate inhibition constant (mg SOC/L).

This equation was based on assumption that at high concentration, substrate concentration, S, activated the inhibition mechanism in the active side of the enzyme, and therefore K_i is inversely dependent upon S. The kinetic constants ν_{max}, K_s, and K_i, were established using a non-linear regression analysis with a computer software (Microsoft Excel version 4.0 software; Trade secret, Soft-Art, Inc.). The values of kinetic constants obtained are shown in Table 4 and were used to fit the experimental data as shown in Fig. 7. The solid curve, which was plotted from Eq. (9) was calculated after substitution of the kinetic constants determined experimentally. A good agreement was obtained between the predicted and experimental data, as seen from Fig. 7.

Table 4—Effect of hydraulic retention time on kinetic constants

	ν_{max} (mg SOC/mg POC/d)	K_s (mg SOC/L)	K_i (mg SOC/L)
HRT=4 d	1.26	8.3	4586
All data	1.22	7.8	5900

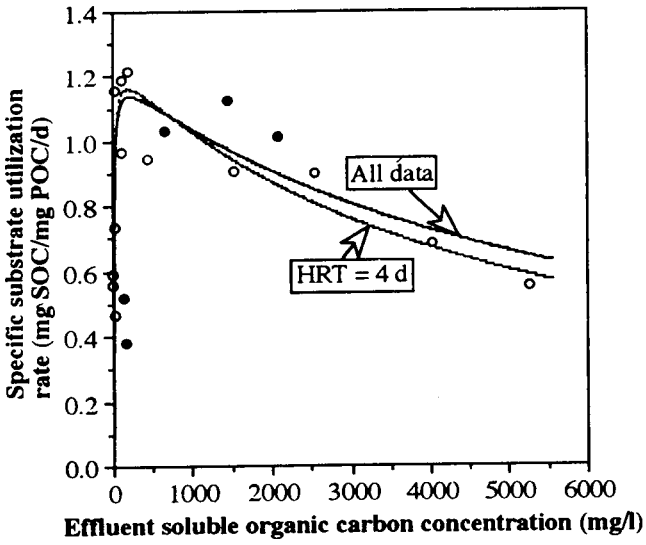


Fig. 7—Effect of substrate concentration on specific substrate utilization rate

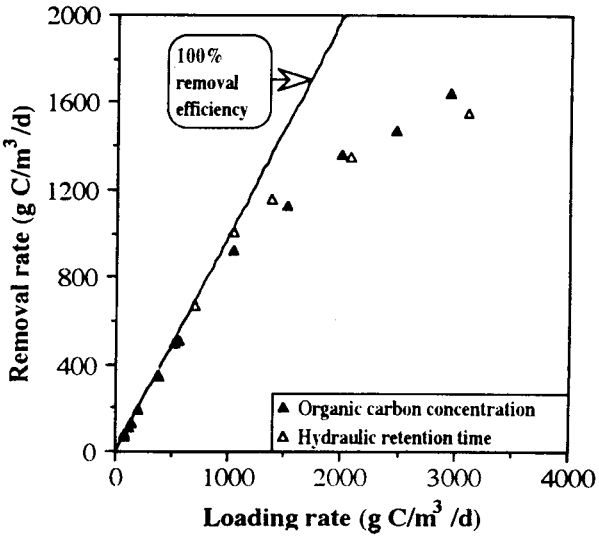


Fig. 8—Effect of butyrate loading rate on removal rate. Hydraulic retention times as 4 days when organic loading rate was varied by butyrate concentration and when hydraulic retention time was varied butyrate concentration was kept at 86.2 mM

Changes in predominance of the mixed microbial population may occur with different hydraulic retention times, which may influence the kinetic constants[40].Changing the loading rate by hydraulic retention time or organic carbon concentration did not seem to influence the kinetic constants (Table 4). The kinetic constants, ν_{max}, K_s and K_i for the experimental data collected when hydraulic retention time was maintained at 4 days, varied by 3.2, 7.8 and 22.3% of the mean for all the data. This discrepancy is not significant, but it seems to appear at low loading rates. Qualitative observations of the bacterial colour on the plates and microscopic observation of the morphology did not show any predominance of bacteria population throughout the experiments.

Fig. 8 shows the effect of butyrate loading rate on butyrate removal rate. It is evident that the removal rate is influenced by loading rate, but is independent of mode of variation of butyrate loading rate. Variation of butyrate loading rate by hydraulic retention time and butyrate concentration produced very similar removal rates.

Effect of *p*H on the rate of oxidation of butyrate and population of heterotrophic bacteria

The effects of *p*H values on the oxidation rates of butyrate and population densities of heterotrophic bacteria were investigated in continuous completely mixed bioreactors. Influent butyrate concentration of 126 mM was used and hydraulic retention time was maintained at 4 days. The *p*H of the influent was adjusted to *p*H 7.0 by addition of 1 *N* HCl or 1 *N* NaOH, until steady-state conditions were attained. The *p*H of the influent was then gradually decreased from 7.0 to 4.0 over a period of two weeks. This was followed by a gradual increase of *p*H of influent from 4.0 to 7.0 over a similar period.

The results show that *p*H of the effluent was generally higher than the *p*H of the influent. The effluent *p*H was 4.3 and 8.4 for influent *p*H of 4.0 and 7.0, respectively. The increase in *p*H was lower for low *p*H values because of reduced bacterial activities. The results also indicated that densities of viable heterotrophic bacteria was influenced by the *p*H of the reactor contents (Fig. 9). Surprisingly, for the same *p*H of the effluent, the density of viable bacteria was higher on decreasing *p*H from 7.0 to 4.0, than on increasing *p*H from 4.0 to 7.0. These findings suggest that recovery of bacterial population is considerably slow.

Fig. 9 also shows that the optimum *p*H for heterotrophic bacteria is between 6.5 and 8.5, but the density of heterotrophic bacteria decreased significantly when *p*H of the media was decreased

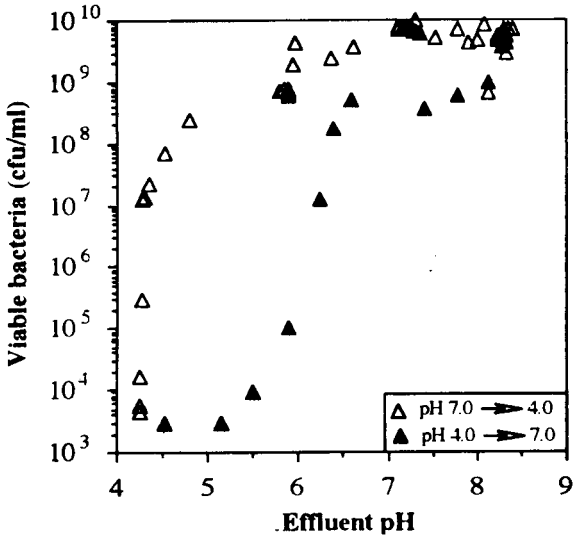


Fig. 9—Effect of *p*H on the density of viable heterotrophic bacteria. Influent butyrate concentration was 126 mM

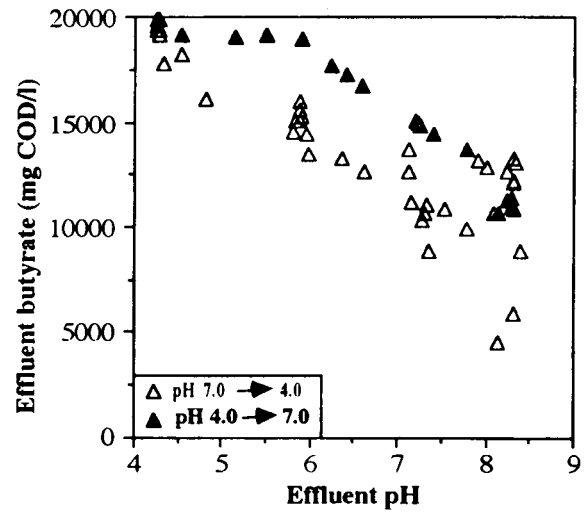


Fig. 10—Effect of effluent *p*H on effluent butyrate concentration

to below 6.0. Mayo and Noike[20] in their experiments with glucose as a sole source of organic carbon, found that *p*H as low as 4.0 did not significantly affect the density of heterotrophic bacteria. The inhibitory characteristics of *p*H on cellular growth (Fig. 9) and oxidation rates (Fig. 10) was because of increased concentration of undissociated acid, which easily penetrates through the membrane of bacteria in undissociated form than in anion form. The mean concentration of undissociated butyrate was only 0.02 and 8.2 mM when the effluent *p*H was 8.3 and 6.0, respectively, but increased to 115 mM at effluent *p*H of 4.3. Fig. 10 further shows that for the same effluent *p*H , the efficiency of removal of COD was lower on increasing *p*H from 4.0 to 7.0 than on decreasing *p*H from 7.0 to 4.0 because of low bacteria densities.

Conclusions

From the findings of this work, the following conclusions are made:

1 The following kinetic constants were obtained for heterotrophic bacteria fed with butyrate: Maximum specific growth rate, μ_{max}=2.1 d^{-1}; Yield coefficient, Y_{max}=0.51; Bacteria decay coefficient, k_d=0.133 d^{-1}; Cell maintenance coefficient, m=0.262 d^{-1}; maximum specific substrate utilization rate, v_{max}=1.22 mg SOC/mg POC/d; Half-rate saturation constant, K_s=7.8 mg SOC/L; and Substrate inhibition constant, K_i=5900 mg SOC/L.

2 No evidence was found to support the view that total volatile acid concentration influenced the density of heterotrophic bacteria. However, at low *p*H values, the bacterial density was significantly reduced because of increased concentration of undissociated butyrate which is known to easily penetrate the membrane of bacteria.

3 Butyrate removal rate was independent of the mode of variation of butyrate loading rate. Variation of butyrate loading rate by hydraulic retention time and butyrate concentration produced very similar removal rates.

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