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RESPONSE OF MIXED CULTURES OF *CHLORELLA VULGARIS* AND HETEROTROPHIC BACTERIA TO VARIATION OF pH

A. W. Mayo* and T. Noike**

** Department of Civil Engineering, University of Dar es Salaam, P.O. Box 35131, Dar es Salaam, Tanzania*
*** Department of Civil Engineering, Tohoku University, Aoba, Sendai 980, Japan*

ABSTRACT

The effects of hydrogen ion concentration on the growth of heterotrophic bacteria and alga *Chlorella vulgaris*, one of the dominant species in waste stabilization ponds were investigated in fed batch cultures. Glucose used as a source of organic carbon was fed daily in granular form and pH was controlled between 3.0 and 11.5 by addition of either HCl or NaOH. Heterotrophic bacterial densities were highest between pH 4.0 and 5.5, but the growth rate of *Chlorella vulgaris* was optimum at pH 4.5~8.0. *Chlorella vulgaris* was sensitive to alkaline pH values, with maximum growth rates, μ_{max}, of 2.03, 1.12 and 0.75 d^{-1} at pH 7.0, 9.0 and 10.0, respectively. The saturation constants, K_s, for the same pH values were 181, 157 and 52 mg/l, respectively. The optimum total biomass yield was 0.381 mg POC/mg glucose and was observed at pH 5.5~8.0, but the magnitude was strongly influenced by the pre-culture pH conditions. Glucose metabolism was significantly affected at alkaline conditions than at acidic conditions. These results also suggest that in a mixed culture with bacteria, algae are largely responsible for glucose metabolism, with apparent saturation constants at pH 7.0 of 27 mg/l and 181 mg/l for bacteria and *Chlorella vulgaris,* respectively.

KEY WORDS

Algal growth rate; bacterial density; biomass yield; *Chlorella vulgaris*; degradation rate; glucose: heterotrophic bacteria; pH; waste stabilization ponds.

INTRODUCTION

Understanding the effects of pH on the growth of algae and bacteria, and the subsequent impact on the degradation of organic material, is important in waste stabilization ponds. The role of pH in oxidation ponds is complex. It has been established that pH influences the relative fraction of CO_3^{2-}, HCO_3^-, and free CO_2. Above pH 8, inorganic carbon is almost entirely in form of HCO_3^- and CO_3^{2-}. The source of carbon which most algal species prefer for photosynthesis is unionized dissolved CO_2 (Tsuzuki *et al.*, 1980; Azov *et al.*, 1982), but other findings suggest active assimilation of HCO_3^- by algae (Ip *et al.*, 1982; Miller and Colman, 1980; Radmer and Ollinger, 1980). Other indirect effects of pH include toxicity of ammonia to living cells in which pH influences the ratio of free NH_3 and ammonium ion (NH_4^+) (Azov and Goldman, 1982), and availability of phosphorus to algae (Bogan *et al.*, 1960). The pH was also reported to influence biomass regulation (Goldman *et al.*, 1982a), algal species competition (Goldman *et al.*, 1982b) and algal photosynthesis (Emerson and Green, 1938).

Usually, growth rate of most microorganisms is maximum at pH values near neutral and falls quickly at too

high and too low pH values. The search for the best pH is important in designing wastewater treatment systems for degradation of organic wastes. Understanding the effect of pH on heterotrophic bacteria and algal growth as well as its impact on organic matter degradation is important in waste stabilization ponds where wide ranges of pH values are common.

In this paper, the effects of hydrogen ion concentration on the growth of an alga (*Chlorella vulgaris*) and heterotrophic bacteria at pH between 3 and 11.5 are reported. To reduce possible interference of NH_3, S^{2-} and volatile fatty acids, the experiments were carried out with a low glucose concentration fed daily in granular form to maintain aerobic conditions. In addition, nitrogen was provided in the form of NO_3-N and sulphate was provided in low concentration. Our experience has shown that at glucose concentration of 75 mg/l/d, the culture remains aerobic, thus avoiding a reducing environment.

METHOD AND MATERIALS

Nutrient Composition and Reactor Operation

The nutrient composition shown in Table 1 was used. Distilled water was used to prepare all the medium, to which trace metals were added. The pH of the medium was controlled by adjusting with 1N HCl or 1N NaOH. Glucose was added daily in granular form at 10:00 a.m. (3 hours after lights were switched on) to provide an input concentration of 75 mg/l (unless otherwise stated) of the remaining volume of culture.

TABLE 1. NUTRIENT COMPOSITION

Chemical	Amount
KNO_3	1000 mg
$MgSO_4.7H_2O$	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
Distilled water	998 ml
Fe solution	1 ml
Trace minerals	1 ml

Fe solution	
$FeSO_4.7H_2O$	= 500 mg
Distilled water	= 250 ml

Trace mineral composition	
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

Bacteria inoculum was first obtained from the final sedimentation basin of the Sendai City activated sludge wastewater treatment plant and was maintained in the laboratory on glucose-fed seed culture. Nutrient composition similar to that used for culture experiments was used to maintain bacterial culture. About 400 mg/l was fed daily and oxygen was provided by bubbling in air with an air pump. They were passaged once every two weeks to maintain good viability and stability of heterotrophic bacterial populations. Actively growing bacterial cells from a newly prepared culture were acclimatized at 30°C in a 500 ml conical flask containing 200 ml liquid medium. After incubation for about 24~36 h while continuously mixed with magnetic stirrers, the inoculum was used to inoculate cultures for the pH effect examination. Initial bacterial density was approximately 10^5 cfu/ml.

Alga *Chlorella vulgaris* was used for the experiments. Algae were air-grown at 30°C and 6000 lux in a 5 liters conical flask containing about 2 liters of liquid medium with nutrient composition shown in Table 1. The media for the experiments were allowed to adjust to culture temperature prior to inoculation of algae and bacteria. The pH of algae system used for seeding, though not controlled, was stable at near pH 8.5, which was termed as pre-culture pH in this work.

The reactors, each of 5 liters were made of acrylic plastic and were provided with a sampling port near the bottom and a cover with air release ports. Light intensity provided was 6000 lux by cool white fluorescent lamps, operated at 12 hour alternating photo periods of light and darkness controlled by a timer. This intensity may not be a limiting factor for algal growth because the saturation light intensity for algal growth is less than 5400 lux (Goldman, 1979). The cultures were incubated at 30°C controlled by refrigerator/heater, and were continuously mixed by magnetic stirrers. Lighting system, timer, heater, refrigerator and defroster all in one incubator, were programmable (Eyela, Model FLI-301N). Prior to sampling, biomass attached on the reactor walls was carefully re-suspended by swirling the culture contents. All the samples were collected at 10:00 a.m. before daily glucose inputs were made.

Sample Analyses

Algal biomass was measured by chlorophyll *a* method in accordance with the standard methods of examination of water and wastewater samples (APHA *et al.*, 1985). Chlorophyll *a* was extracted by 90% acetone and was measured by a spectrophotometric method (Hitachi, model U-1100). Heterotrophic bacterial populations were determined by pour-plate count on serial dilution of samples mixed with tryptone glucose extract agar (APHA *et al.*, 1985). The plates were incubated at 35°C for 48 h.

The total biomass in the reactors was measured by particulate organic carbon (POC) analyzed by TOC analyzer (Shimadzu, model TOC-5000). Prior to measurement of particulate organic carbon, the biomass was first disrupted by sonifier for 5 minutes in order to break the biomass to fine particles and extend the settling time during measurement. Total carbon (TC) and inorganic carbon (IC) were analyzed from two samples, one with particulate matter as well as soluble organic matter (DOC), and the second sample with particulate matter removed by centrifuging at 3500 rpm for 15 minutes.

Glucose concentration was determined by the phenol-sulfuric acid method (Dubois *et al.*, 1956), and was measured spectrophotometrically at 490 nm (Hitachi, model 100-20). Glucose concentration in the samples was then determined by comparing the optical density reading with standard glucose solution calibration curves. Dissolved oxygen was measured by DO meter (TOA Electronics Limited, model DO-1B) and pH was checked by pH meter (TOA Electronics Limited, model HM-10P).

RESULTS

Effect of pH on the Growth of Heterotrophic Bacteria

Figure 1 shows the effect of pH on the growth of heterotrophic bacteria. Peak heterotrophic bacterial growth was between pH 4.0 and 5.5, steady bacterial numbers were observed between pH 7.0 and 10.0, with rapid decline at pH above 10.0. The peak growth at pH 4.0~5.5 might have been influenced by substrate availability as algal competition for glucose was reduced.

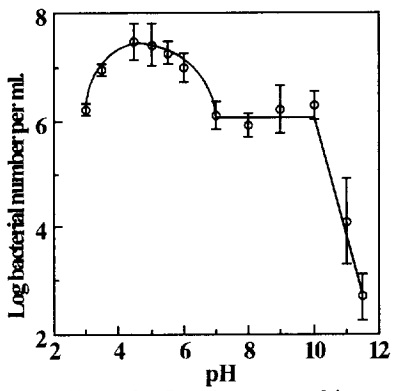

Fig. 1: Effect of pH on heterotrophic bacterial density. The vertical bars are standard deviations of 13 samples for each run taken from day 5 to 20.

Effect of pH on Algal Growth

The criteria used for evaluation of the growth of *Chlorella vulgaris* in these experiments was chlorophyll *a*. The growth curves for *Chlorella vulgaris* for selected pH values are shown in Figure 2. Lag time was observed for pH values below 4.5 and above 10.0, and was longer at too low or too high pH values. At pH values near neutral, chlorophyll *a* approaches its saturation :h 1 values much faster than at other values. Saturation started to appear at chlorophyll *a* concentration of around 13~15 mg/l. Saturation was probably caused by light becoming a growth-limiting factor as increased turbidity causes more light shading to cells.

At pH 11.5, *Chlorella vulgaris* growth was not detected indicating that specific growth rate was close to zero. Growth of algae was however observed at pH as low as 3.0, unlike Carberry and Brunner (1991) who reported that pH value of about 3.0 inactivated *Chlorella vulgaris*. It is possible that reasons other than pH alone might have influenced their results because *Chlorella vulgaris* grew at the same pH in these experiments.

Figure 2 shows the low ability of *Chlorella vulgaris* to survive for long duration at pH values above 10. Because pH was controlled by addition of NaOH solution, it was suspected that Na^+ ions might have affected the growth of algae. To check if Na^+ ions had influenced results, on day 20, the contents of the reactor controlled at pH 10.7 were split into 4 parts without diluting. In one of the reactors, pH was controlled at 10.7 and in the other three parts was adjusted by HCl to and controlled at pH 8.0, 7.0 and 5.5.

Glucose at a concentration of 75 mg/l continued to be added daily. The chlorophyll *a* results presented in Figure 3 revealed a lag phase of about 1 day for pH 7.0 and 8.0 followed by an exponential growth. The growth rates of algae after the initial lag phase were 0.335 and 0.332 d^{-1} at pH 7.0 and 8.0, respectively. These values were reasonably similar to 0.373 and 0.370 d^{-1}, respectively for the same pH values in the previous experiments.

These results show that hydroxyl ions and not Na^+ ions were largely responsible for algal growth inhibition at pH 10.7, and seem to suggest *Chlorella vulgaris* could not live long at pH values above 10. At pH 5.5, however, chlorophyll *a* declined even faster than in the control reactor (pH 10.7) and was not detectable after 3 days. These results were surprising because *Chlorella vulgaris* grew well at the same pH (Fig. 2). Two possible reasons for the decline in chlorophyl *a* at pH 5.5 were either the high pH gradient shock to the algal cells, or the high concentration of Cl^- ions, input when pH was reduced from 10.7 to 5.5. using HCl. These effects are discussed below.

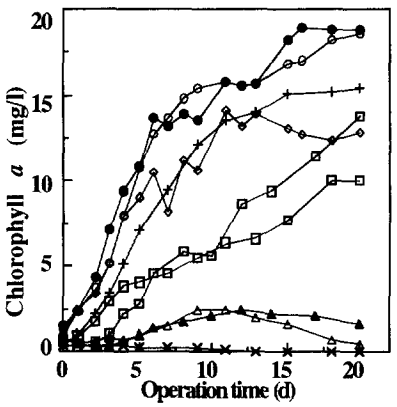

Fig. 2: Effect of pH on growth of *Chlorella vulgaris*
(□) pH 3.0, (+) pH 4.5, (○) pH 5.5,
(●) pH 7.0, (◇) pH 9.0, (□) pH 10.0,
(▲) pH 10.7, (△) pH 11.0, (×) pH 11.5.

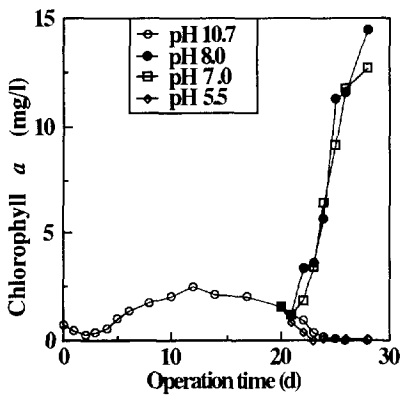

Fig. 3: Recovery of high pH grown *Chlorella vulgaris* on reduction of pH.

System Recovery After Prolonged High pH

To check if Cl^- ions had significant influence, the experiments were repeated at pH 11.0 and cells were collected after 20 days of incubation as in the previous case. The cells were then cultured in fresh media controlled at pH 5.5 and 8.0. Glucose at the rate of 75 mg per litre of culture continued to be added daily, and a comparison with a pre-culture pH of 8.5 at the same pH values was made. Figure 4 illustrates the effect of prolonged culture at pH 11.0 on recovery of algae and bacteria. Heterotrophic bacteria quickly recovered from about 10^4 cfu/ml to 2 x 10^8 and 3 x 10^7 cfu/ml in 2 days at pH 5.5 and 8.0, respectively (Fig. 4B). The semi-logarithmic plot of algal growth indicates that algae exhibited a lag time which increased as pH gradient between the original and new culture pH increased. The observed lag times at pH 5.5 and 8.0 were 10 and 2 days, respectively (Fig. 4C). The comparison of the pre-culture pH at 8.5 and 11.0 seems to indicate that algal chlorophyll *a* was much lower for pre-culture pH of 11.0 as previously discussed. The bacteria, however, exhibited higher densities for pre-culture pH of 11.0 than pH 8.5. This difference might have been caused by abundant substrate (see Fig. 4A) during algal lag growth phase.

TABLE 2. BIOMASS YIELD AS A FUNCTION OF pH

Pre-culture pH	Incubation pH	Biomass yield (mg POC/mg glucose)	r^2
8.5	5.5	0.378	0.987
	8.0	0.383	0.994
11.0	5.5	0.081	0.896
	8.0	0.169	0.934

The biomass yields observed after prolonged high pH were much lower than those at pre-culture pH 8.5

(Table 2), indicating that the pre-culture pH has a significant influence on the biomass yield.

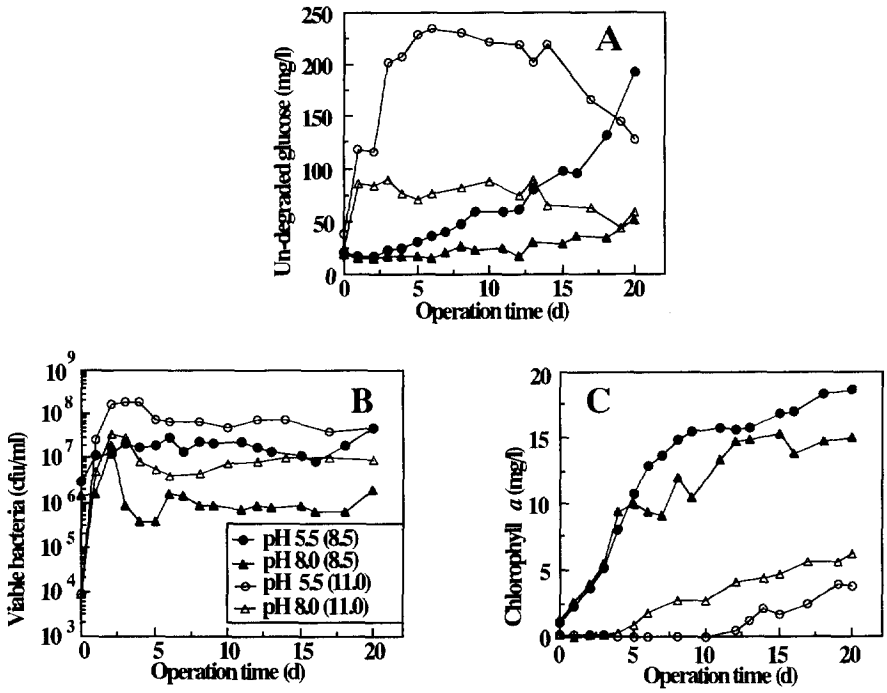

Fig. 4: Effect of pH stress on glucose uptake (A), bacterial growth (B) and algal growth (C). Values in parenthesis are pre-culture pH. Symbols shown in Fig. 4B were also used for Figs. 4A and 4C.

Kinetics of H^+ Inhibition

The effect of pH on the biomass growth may be directly or indirectly attributed to hydrogen ion concentration in the medium. In most microbial cultures, hydrogen ion has been observed to be a non-competitive inhibitor to growth near neutral pH values but may inhibit metabolic activities and substrate uptake rates when hydrogen ion concentration is too low or too high. The effect of $[H^+]$ on the specific growth rate may be described by the Haldane equation as shown by equation (1):

$$\mu = \frac{\mu_o [H^+]}{[H^+] + K_{OH} + [H^+]^2/K_H} \quad (1)$$

where μ_o is the specific maximum growth rate at a certain substrate concentration, K_{OH} and K_H are rate constants. It may be noticed that hydrogen ion concentration is considered as a substrate when medium is alkaline but as an inhibitor when it is acidic. The pH-dependence of equation (1) causes substrate concentration rate maxima to shift as hydrogen ion concentration varies.

A non-linear regression analysis was used to fit a model described by equation (1),between pH 3.0 and 11.0, for the total biomass and algal chlorophyll *a* obtained from mixed culture algal-bacterial systems. The specific biomass growth rate, μ was determined as the slope of natural logarithm of biomass versus time during the exponential growth phase. The best values for the rate constants were observed to be μ_o = 0.357 d^{-1}, K_{OH} = 3.271 x 10^{-11} M and K_H = 1.49 x 10^{-3} (r = 0.943) for total biomass and μ_o = 0.386 d^{-1}, K_{OH} = 9.05 x 10^{-12} M and K_H = 6.54 x 10^{-3} M (r = 0.986) for algal chlorophyll *a* and were used to fit the data shown in Figure 5.

At pH values above 8, the term $[H^+]^2/K_H$ in equation (1) will be much smaller than K_{OH}, and may therefore be neglected. Equation (1) may therefore be reduced to equation (2), which is similar to the Monrod equation:

$$\mu = \frac{\mu_o [H^+]}{[H^+] + K_{OH}} \quad (2)$$

A double reciprocal plot of equation (2) is shown in Figure 6. The maximum biomass growth rate, μ_o, was 0.311 d^{-1} and $K_{OH} = 2.22 \times 10^{-10}$ M, with correlation coefficient of 0.989. These estimates appear to slightly underestimate μ_o and overestimate K_{OH}, and is thought to be largely influenced by error enlargement when Lineweaver-Burke plot is made. Figure 6 also shows that hydrogen ion is a strong inhibitor of biomass growth at pH values above 8 in *Chlorella vulgaris*-bacterial systems.

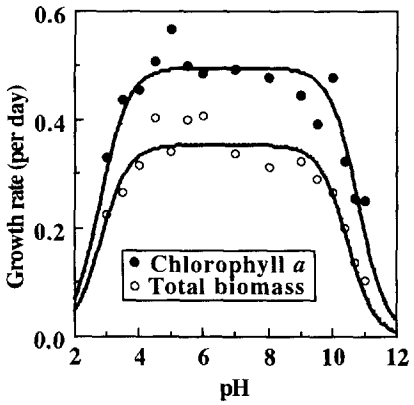

Fig. 5: Effect of pH on growth rate of *Chlorella vulgaris* and total biomass.

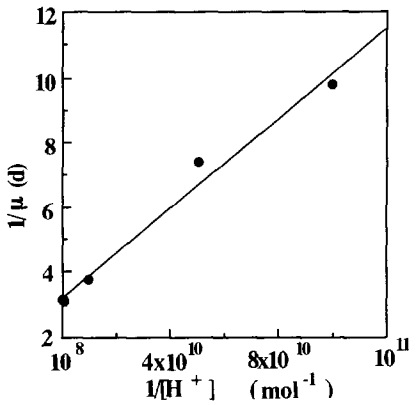

Fig. 6: Lineweaver-Burke plot of the effect of pH on total biomass at pH above 8.0.

The optimum pH from the model would be equal to $(pK_H + pK_{OH})/2 = 6.66$ and 6.82 for total biomass and *Chlorella vulgaris*, respectively. These values are very close to the pH range of 6.0 to 6.5 observed to be the optimum for *Chlorella* sp. by Krauss (1964) (cited by Abid, 1983) and pH 6.85 for *Chlorella vulgaris* observed as the optimum pH for maximum protonation of proton carrier (Kommor and Tanner, 1974).

The optimum pH for total biomass growth rate was also close to that observed for algae. Heterotrophic bacterial density was, however, highest between pH 4.0 and 5.5, which seems to indicate that algal growth is probably a dominant contributor to the total biomass. The growth rates of algae were also higher than the growth rates of the total biomass indicating the system contained some slow growing microorganisms.

The kinetic growth parameters for *Chlorella vulgaris* were determined from batch culture at glucose concentrations of 25~1500 mg/l and are presented in Table 3. The highest values for the maximum specific growth rate, μ_{max}, and saturation constant, K_s, were at pH 7.0 and decreased as pH increased.

TABLE 3. EFFECT OF pH ON μ_{max} AND K_s

pH	μ_{max} (d^{-1})	K_s (mg/l)
7.0	2.03	181
9.0	1.12	157
10.0	0.75	52

Effect of pH on Biomass Yield

The total biomass growth yield factor, Y, was determined from the growth data at the exponential phase by plotting biomass concentration as particulate organic carbon versus cumulative glucose consumed; the slope of the resulting line equals the total biomass yield. Biomass yield kinetic parameters were estimated by a non-linear regression analysis similar to the one shown by equation(1)(with Y and Y_o replacing μ and μ_o, respectively). As shown in Figure 7, biomass growth yield was significantly influenced by the pH of the

medium. The best values for the kinetic parameters were $Y_o = 0.381$ mg POC/mg glucose, $K_{OH} = 5.62 \times 10^{-11}$ M and $K_H = 1.32 \times 10^{-3}$ M. The optimum yield was at pH 5.5~8.0. Goldman *et al.* (1982a) found that the growth rate of freshwater algae *Chlorella vulgaris* and *Scenedesmus obliquus* in continuous CO_2-fed cultures assessed by the maximum steady state biomass was highest at pH 7.9. Their work was, however, based on a pH range of 7.9~10.6 but did not study values outside this range. The low yields at extreme pH values could be attributed by high cell death rates at unfavorable pH values.

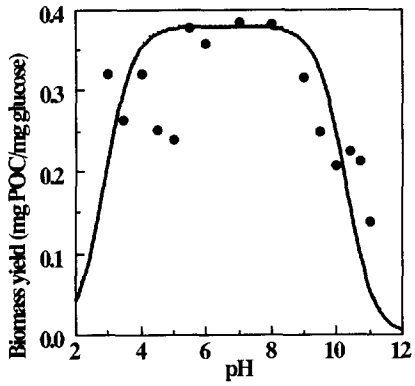

Fig. 7: Effect of pH on total biomass yield.

The results of this work show a broad yield factor between pH 5.5 and 8.0. Constant yields over a wide range of pH have also been reported by Olivero *et al.* (1982) in their research on batch culture of *Saccharomyces cerevisiae* fermentation of maltose, and Vairo *et al.* (1981) in continuous culture of sugarcane molasses. Other research findings such as those reported by Lallai *et al.* (1988) on mixed cultres of different kinds of bacteria (*Achromobacter, Aeromonas, Flavobacterium, Pseudomonas* etc.) in batch culture for degradation of phenol and Eroshin *et al.* (1976) on *Saccharomyces cerevisiae* in continuous culture,however, revealed sharp peak yield factors.

Effect of pH on Glucose Uptake in Fed Batch Cultures

Glucose uptake by the algal-bacterial system was calculated as a slope of cumulative glucose consumed against glucose added and the results obtained are presented in Figure 8. The uptake of glucose is pH-dependent with a broad optimum from pH 6.0 to 8.0, a steep decline above pH 8.0 and relatively mild decline below pH 6.0. The decrease in influx velocity with increasing pH could be because of declining affinity for glucose by the uptake system. An acidic pH was less detrimental than an alkaline pH. At pH 11.0, for instance, glucose uptake was about 30% less than at optimum conditions but was only about 5% less for pH 3.0.

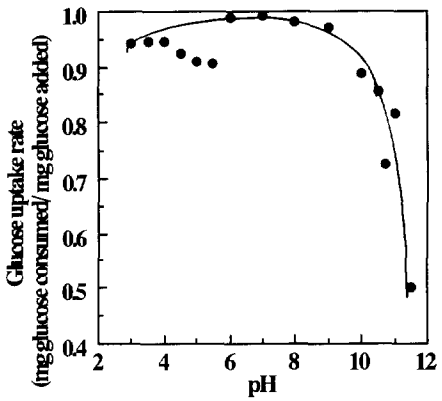

Fig. 8: Effect of pH on glucose uptake rate in fed batch cultures.

Effect of pH on K_m in Batch Cultures

To determine the cause of low degradation at high pH, a batch culture study was made with glucose under controlled, constant pH. The biomass used for the batch cultures was collected at the end of the exponential growth phase and the input biomass provided was about 550 mg POC/l (range 546~558 mg POC/l). By using the data from batch culture, the maximum specific glucose degradation rate, ν_{max}, and half rate saturation coefficient, K_m, were determined according to a generic Michaelis-Menten rate law (equation 3), using analytical approach developed by Halwach (1978) shown in equation (4):

$$\nu = \frac{\nu_{max} \cdot S}{K_m + S} \quad \text{.... (3)} \quad \text{and} \quad \frac{t}{U} = \frac{Km}{\nu max\ Xo}\left[\frac{1}{U}\ln\left(\frac{1}{1-U}\right) - 1\right] + \frac{So + Km}{\nu max\ Xo} \quad \text{.... (4)}$$

where $U = [(S_o - S)/S_o]$, S_o is the initial substrate concentration, S is the substrate concentration at time, t, and X_o is the initial biomass concentration (mg/l). Biomass concentration changes were assumed negligible compared to the initial biomass concentration, X_o, over the period of experimentation (about 12 hours). By plotting the graph of (t/U) against [(1/U) * ln[{1/(1-U)}-1]], the slope $[K_m/(\nu_{max}X_o)]$ and vertical intercept

$[(S_o + K_m)/(\nu_{max} X_o)]$ were determined. K_m and ν_{max} were calculated from the determined slopes and vertical intercepts.

The apparent half rate constants, K_m calculated from equation (4) were pH-dependent, with the high affinity uptake observed near neutral pH. By increasing or decreasing hydrogen ion concentration, the apparent K_m increased showing that the microorganisms affinity for glucose was decreasing (Fig. 9). Kommor and Tanner (1974) observed half maximal protonation of carrier achieved at pH 6.85 for pure cultures of *Chlorella vulgaris* grown in sugar. This value surprisingly similar to 6.87 observed as pH value for high glucose affinity for a mixed batch of culture of *Chlorella vulgaris*-heterotrophic bacteria estimated by a second-degree polynomial function plot of K_m versus pH. It is also worth mentioning that the specific glucose rate showed a bell-shaped curve with optimum uptake near pH 7.0.

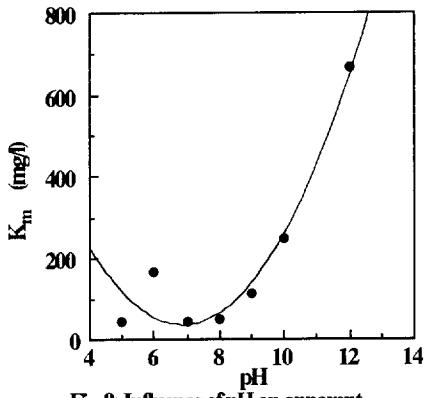

Fig. 9: Influence of pH on apparent glucose saturation constant.

Response of *Chlorella Vulgaris* Under Uncontrolled pH

To see how *Chlorella vulgaris* behaves when pH is not controlled, fed batch cultures were made with daily input glucose concentrations of 25 and 75 mg per liter of culture. The results for pH and algal concentration are shown in Figure 10. On the basis of these results, it is intuitively obvious that the maximum tolerable pH without significantly affecting activity of *Chlorella vulgaris* is around 9.0. When the pH in the bioreactor fed daily with 25 mg glucose/l rose to around 10.6 for instance, a rapid decline was observed and only started to rise again when pH was around 8.5. Attempts to culture *Chlorella vulgaris* at other glucose concentrations (50, 100 and 150 mg/l/d) and air-grown cultures also showed the steady state pH of between 8.5 and 9.2, the range which was also found to be a turning point for optimum growth of this alga (Fig. 5).

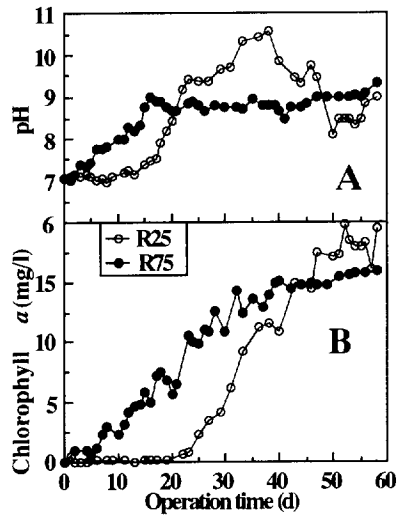

Fig. 10: Fluctuation of pH (A) and growth of *Chlorella vulgaris* (B) without pH control.

DISCUSSION

Although bacterial densities were higher for pre-culture pH of 11.0 than for pre-culture pH 8.5, the un-utilized glucose concentration increased until algal growth picked up (Fig. 4). The decline in glucose utilization rate above pH 8.0 (Fig. 8), also corresponds to reduced *Chlorella vulgaris* growth rates (Figs. 2 and 5) but not heterotrophic bacterial densities which were steady up to pH 10.0 (Fig. 1). These results suggest that algae were largely responsible for glucose metabolism rather than bacteria. Earlier work has shown that alga and bacterial half-rate saturation constants, K_s were 181 and 27 mg/l, respectively (Mayo and Noike, 1994). The research work by Wright and Hobbie (1965) showed the apparent K_s value for natural glucose-fed bacterial cultures in oligotrophic environments to be 7 μg/l, further supporting minor role of bacteria in glucose assimilation. It was also observed that the optimum pH for bacterial growth was between pH 4.0 and 5.5, yet the maximum total biomass yield was at pH 5.5~8.0 (Fig. 7), which further suggests the role of bacteria in glucose utilization was minor compared to that of algae.

Chlorella vulgaris was more sensitive to alkaline pH than acidic pH (Fig. 2). Such a response is in

agreement with other research findings (King, 1970; Ip *et al*., 1982). King (1970) suggested that green algae are less efficient at utilizing low concentrations of CO_2 than blue-green algae and that, under alkaline conditions, green algae would not predominate. Ip *et al*. (1982) also reported that unicellular green algae such as *Chlorella*, *Scenedesmus*, etc. were predominant in high CO_2 and low pH samples. Wringley and Toerien (1990) limnological study of mixed species of algae in small-scale sewage ponds in series observed that at least 14 genera were dominant in their 21 month study. Their data indicated that *Chlorella* dominated occasionally in the first two ponds, but never in the third pond. Although they did not present pH data for the third pond, pH in pond 4 varied between 8.6~9.2, the range corresponding to declining growth for *Chlorella vulgaris* (Fig. 5 and 7). The failure of *Chlorella* to dominate in pond 3 might have been influenced by pH.

The results presented in our work cannot however, be related solely to availability of free CO_2 in the medium because *Chlorella vulgaris* is known to utilize sugars (Kommor and Tanner, 1974; Martinez *et al*., 1987). However, *Chlorella vulgaris*-heterotrophic bacterial uptake of glucose was lower at alkaline pH than acidic pH (Fig. 8). Earlier discussion (also see Fig. 4)suggested that *Chlorella vulgaris* is responsible for more glucose assimilation than heterotrophic bacteria. It might be assumed, therefore, that the growth of *Chlorella vulgaris* at alkaline pH was affected not only by free CO_2 availability, but also by the direct effects of pH on metabolic activities.

Chlorella vulgaris, although sensitive to alkaline pH, has been observed in maturation ponds (Neel *et al*., 1961), where pH values are normally over 9.0. The ability of *Chlorella vulgaris* to compete with high pH resistant species is probably because of the toxic long chain fatty acids it discharges when under stress such as at high pH (Pratt and Fong, 1940), making it able to compete for the limited nutrients in maturation ponds, particularly if they were dominant initially (Goldman *et al*., 1982b). The ponds however, do maintain high pH values only during mid-daytime hours, and pH is normally low during the night, early morning and late evening hours. It was observed that *Chlorella vulgaris* may in fact survive or even grow at high pH values provided such pH values are not prolonged (Fig. 10).

CONCLUSIONS

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

(1) The optimum growth of *Chlorella vulgaris* was at pH near 7.0, but that of heterotrophic bacteria was at pH 4.5. Alkaline pHs were more unsuitable for glucose metabolism than acidic pHs.

(2) *Chlorella vulgaris* was largely responsible for the observed metabolism of glucose rather than the heterotrophic bacteria

(3) The maximum pH at which *Chlorella vulgaris* could survive was close to pH 11.5, but prolonged high values above 10.0 were detrimental to growth.

(4) The recovery of *Chlorella vulgaris* after prolonged high pH values was much slower than heterotrophic bacterial recovery at the same culture conditions.

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