

(6) EFFECT OF GLUCOSE LOADING RATE ON CARBON DISTRIBUTION IN ALGAL-BACTERIAL SYSTEM

藻類-細菌混合培養系における炭素収支に及ぼすグルコース負荷の影響

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ABSTRACT; This paper presents the effect of organic loading rate on the carbon distribution in mixed algal-bacteria fed batch culture. Glucose used as a source of organic carbon was fed daily in granular form to provide input concentration ranging from 25~700 mg/l. Results show that glucose was largely converted to biomass in aerobic reactors. In anaerobic reactors however, glucose was mainly converted to gases and volatile fatty acids (VFAs). The growth of biomass in anaerobic reactors were affected by accumulation of VFAs. The maximum biomass specific growth rate, μ_{\max} , and substrate saturation constant, K_s , were 0.44 d^{-1} and 135 mg/l , respectively. The specific dissolved inorganic carbon accumulation increased as glucose loading rate increased. However, because of VFA accumulation in anaerobic reactors, pH dropped resulting in shift of equilibrium between H_2CO_3^* ($\text{H}_2\text{CO}_3 + \text{CO}_{2(\text{aq})}$), HCO_3^- and CO_3^{2-} in favor of H_2CO_3^* production, most of which was released as $\text{CO}_{2(\text{g})}$ to the atmosphere.

KEY WORDS; Algal-bacterial system, glucose loading rate, *Chlorella vulgaris*, heterotrophic bacteria, carbon mass balance.

1. Introduction

Algae play a major role as producers in waste stabilization ponds and other polluted aquatic habitats. Because of this characteristic, algae has been used extensively for treatment of domestic effluents and has found application in industrial wastewater treatment systems. In waste stabilization ponds, symbiotic relationship of algae and bacteria is used with algae providing oxygen to bacteria whose oxidative activity is more efficient (Martinez *et al.*, 1987). The major role of algae in waste stabilization ponds is production of oxygen which is required for oxidation of organic matter by bacteria. Its ability to remove nutrients such as nitrogen and phosphorus, responsible for eutrophication of receiving waters, as well as uptake of some heavy metals is of great importance in wastewater treatment.

While aerobic bacteria produce CO_2 for algae through aerobic degradation of organic wastes, algae in turn produce oxygen photosynthetically to be used for bacterial respiration (Oswald, 1973). Under the dark conditions such as during the night or in pond's lower layers, both algae and bacteria consume oxygen for respiration and produce CO_2 . For its growth, while bacteria utilize organic matter as a sole source of carbon (Azov *et al.*, 1982), algae utilize carbon from bicarbonate alkalinity, CO_2 released by bacterial degradation of organic matter as well as CO_2 dissolved in the pond from the atmosphere. Partially degraded organic compounds may also be utilized by algae through photo-assimilation (Neilson and Lewin, 1974) and through algal heterotrophism (Abeliovich and Weisman, 1978). Martinez *et al.* (1987) reported that acetate affected the photoautotrophic growth of *Pseudoanabaena catenata*,

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but was assimilated by *Chlorella* sp., *Scenedesmus quadricauda* and *Stichococcus bacillaris*. Sucrose was observed to be assimilated well by *Chlorella* sp. and *Stichococcus bacillaris* but *Scenedesmus quadricauda* showed lower rates than the controls under photo- and chemoheterotrophic conditions. *Chlorella* was reported to assimilate glucose well.

In a natural environment, CO₂ for algal growth is obtained from bacterial degradation of organic matter. This work was carried out to investigate the behavior and distribution of carbon in both aerobic and anaerobic conditions. Glucose was provided as a sole source of organic material in fed batch reactors inoculated with heterotrophic bacteria and algae.

2. Method and Materials

2.1 Nutrient composition and reactor operation

The nutrient composition shown in Table 1 was used. After the nutrient composition was mixed, pH was adjusted to about 7.0 by 20% hydrochloric acid. The reactors each of 5 liters were made from acrylic plastic and were provided with sampling port near the bottom and a cover with air release ports. Light intensity provided was 6000 lux by cool white fluorescent lamps, and was operated at 12 hour alternating photo periods of light and darkness controlled by a timer. This light intensity may not be a growth limiting factor because saturation light intensity for growth of algae is less than 5400 lux (Goldman; 1979). The cultures were incubated at the temperature of 30°C and mixing of culture was provided by magnetic stirrers. Algae used for the experiments was *Chlorella vulgaris* and bacterial inoculum used to seed the chemostat reactors was collected from the final sedimentation tank of the Sendai city activated sludge wastewater treatment plant. Glucose was added daily to provide input concentrations shown in Table 1. For the easy clarity of abbreviation reactors were named according to the amount of glucose they received. Prior to sampling, biomass attached on the reactor walls was carefully re-suspended.

Table 1: Nutrient composition and glucose loading rate.

Chemical	Amount	Fe solution	Reactor	Glucose loading rate (mg/l/d)
KNO ₃	1000 mg	FeSO ₄ ·7H ₂ O = 500 mg		
MgSO ₄ ·7H ₂ O	250 mg	Distilled water = 250 ml		
K ₂ HPO ₄	250 mg	Trace mineral composition	R25	25
NaCl	100 mg	H ₃ BO ₃ = 286 mg	R50	50
CaCl ₂ ·2H ₂ O	10 mg	MnSO ₄ ·4H ₂ O = 130 mg	R75	75
EDTA-Na	16 mg	ZnSO ₄ ·7H ₂ O = 320 mg	R100	100
Distilled water	998 ml	CuSO ₄ ·5H ₂ O = 183 mg	R150	150
Fe solution	1 ml	Na ₂ MoO ₄ = 2.1 mg	R300	300
Trace minerals	1 ml	Distilled water = 100 ml	R700	700

2.2 Sample analyses

Particulate organic carbon (POC), inorganic carbon (IC) and dissolved organic carbon (DOC) were analyzed by TOC analyzer (Shimadzu, model TOC-5000). Prior to measurement of particulate organic carbon, biomass was first disrupted by sonifier (Branson Sonic Power, model 185) for 5 minutes in order to break the particles to small pieces and extend the settling time during measurement. Dissolved organic matter (DOC) was analyzed after removing particulate matter by centrifuging at 3500 rpm for 15 minutes.

Algal biomass was measured by chlorophyll *a* as laid in 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 spectrophotometric method (Hitachi, model U-1100). Heterotrophic bacteria were determined by pour-plate count method on serial dilution of samples mixed with tryptone glucose

extract agar medium (APHA *et al.*, 1985). The plates were incubated at 35°C for 48 h.

Volatile fatty acids were measured using gas chromatography (Shimadzu, GC-8A) equipped with integrator (Shimadzu, C-R6A). The operational temperature for the injection block and column were 180 and 140°C, respectively. Samples were prepared by centrifuging at 3500 rpm for 15 minutes. To 1 ml samples prepared from the supernatant in appropriate dilutions, 0.25 ml of 25% metaphosphoric acid was added and thoroughly mixed. Standard solutions of acetic acid, propionic acid, iso-butyric acid and normal butyric acid were prepared from dilutions ranging from 50 to 1000 mg/l and were processed through the same procedure.

Glucose concentration was determined by procedure proposed by Dubois *et al.* (1956). Dissolved oxygen was measured by DO meter (TOA Electronics, DO-1B) and pH was measured by pH meter (TOA Electronics, HM-18E).

3. RESULTS AND DISCUSSION

3.1 Effect of glucose loading rate on dissolved oxygen

Figure 1 shows DO variation with glucose loading rate. The general behavior indicates that dissolved oxygen level is higher in reactors with low glucose loading rate. As glucose addition exceeds 150 mg/l, reactors became anaerobic. R300 and R700 were devoid of DO and became anaerobic just after operation was started and maintained anaerobic conditions in spite of presence of algae. Some level of algal activity was observed in these reactors in spite of its anaerobic nature. Complete lack of oxygen in anaerobic reactors in the first two weeks seems to indicate that oxygen produced by algae was immediately consumed by bacterial oxidation of glucose. In R150, DO gradually decreased as biomass increased and reactors became anaerobic after 15 days of operation.

Analysis of diurnal fluctuation of DO and pH revealed a rise when lights were switched on and fall of these parameters when lights were switched off or glucose was added. However, as might be expected, the concentration of glucose added to the reactors played an important role in diurnal DO fluctuation (Fig. 2). The higher the glucose concentration added, the higher the fall in DO concentration and the longer time it took to recover to the original DO concentration. As soon as glucose was added, DO in all reactors declined rapidly because the rate of bacterial metabolism of glucose was faster than the rate algae photosynthesized more oxygen and the trend reversed when glucose was almost depleted.

3.2 Effect on biomass production

In analyzing the kinetic parameters, glucose was assumed to be the only biomass growth limiting substrate. The variations in the total biomass and culture volume in a fed batch reactor may be defined

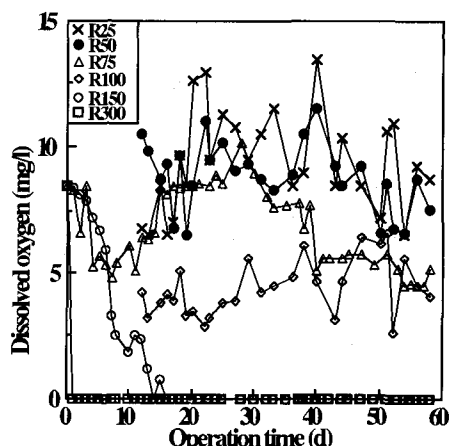


Fig. 1: Variation of dissolved oxygen concentration with glucose loading rate.

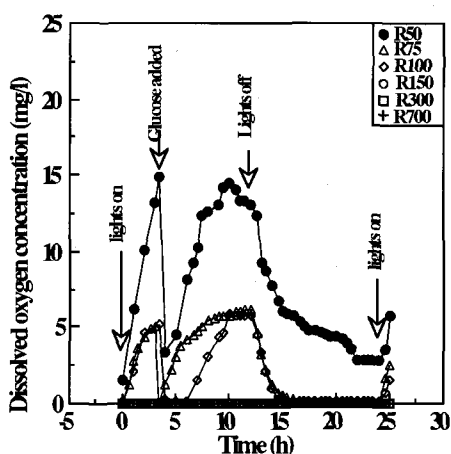


Fig. 2: Typical diurnal fluctuation of dissolved oxygen concentration. Data of day 37

by the following mass balance equations assuming Monod kinetics is valid.

For biomass $\frac{d(V \cdot X)}{dt} = \mu \cdot V \cdot X$ eq. 1 and For culture volume $\frac{dV}{dt} = V_s - V_e - V_a$ eq. 2

where μ = specific growth rate (d^{-1}), X = biomass concentration (mg POC/l), t = operation time (d), V = culture volume (l), V_s = volume of substrate added daily (l), V_e = volume of culture lost through evaporation (l) and V_a = volume of sample taken for analysis (l).

Because glucose was added daily in solid form, the influence of V_s on the total culture volume is negligible. Sample volume does not also contribute to the change in biomass concentration nor to substrate degradation rate, because substrate was added based on the volume of culture remaining. Evaporation was assumed negligible compared to volume of culture. Therefore biomass mass balance may be re-written as:

$$\frac{dV}{dt} \cdot X + \frac{dX}{dt} V = \mu \cdot V \cdot X \quad \text{.....eq. 3}$$

Combining equation 2 with equation 3 and neglecting V_e then:

$$\frac{dX}{dt} = \mu \cdot X = \frac{\mu_{\max} \cdot S \cdot X}{K_s + S} \quad \text{.... eq. 4}$$

where μ_{\max} = maximum specific growth rate (d^{-1}), S = concentration of growth limiting substrate (mg/l) and K_s = saturation constant (mg substrate/l).

The value of specific biomass growth rate, μ , was determined as the slope of the natural logarithm of biomass versus time during the exponential growth phase. Non-linear regression of Monod equation was used for analysis of maximum biomass specific growth

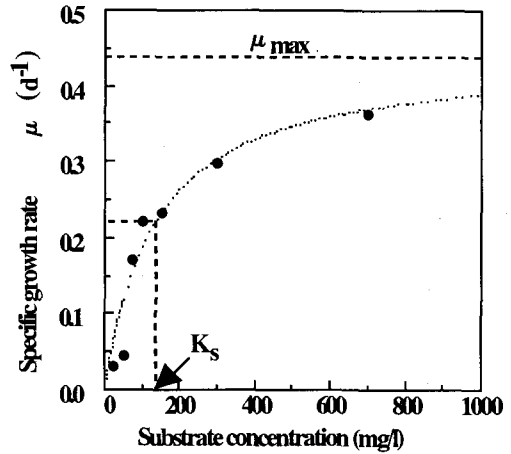


Fig. 3: Monod plot of kinetic parameters.

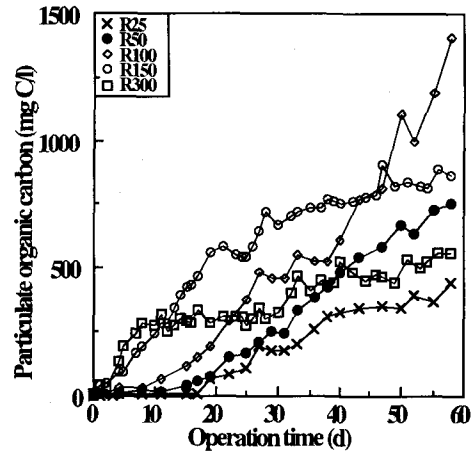


Fig. 4: Variation of particulate organic carbon with glucose loading rate.

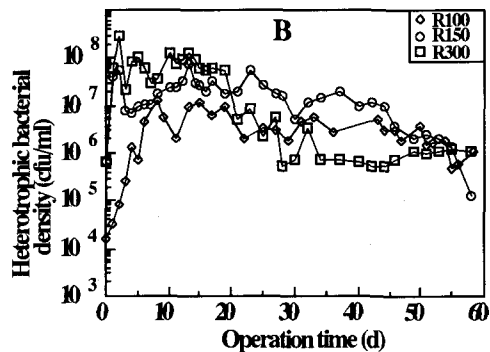
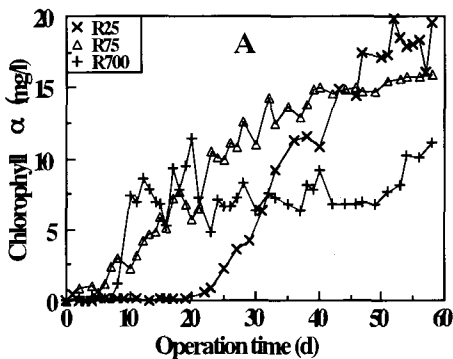


Fig. 5: Effect of glucose loading rate on growth of algae (A) and bacteria (B).

rate, μ_{\max} , and substrate saturation constant, K_s (Fig. 3). The resulting maximum specific biomass growth rate, μ_{\max} , and glucose saturation constant, K_s , were 0.44 d^{-1} and 135 mg/l , respectively.

Figure 4 shows that biomass production measured as POC increased with increasing glucose loading rate. However, biomass growth was significantly affected in R300 and R150 from day 10 and day 27, respectively. Slow down in biomass growth rate in these reactors might be traced to accumulation of VFAs which seems to inhibit growth of microorganisms.

Interestingly, algae survived and even grew in anaerobic condition for the entire duration of the experiments (see R700 in Fig. 5) but the concentration was lower than in aerobic reactors. This behavior is not quite unexpected because algae have been detected in damp dune slack areas (Stewart; 1967) and estuarine muds at E_h values of -100 mV (Baas-Becking and Wood; 1955 a,b) where anaerobic or microaerophilic conditions exist. *Chlorella*, *Ankistrodesmus*, *Scenedesmus*, *Coelastrum*, *Chlamydomonas*, *Porphyridium* (Knobloch; 1966), *Anabaena flos-aquae* and *Nostoc muscorum* (Stewart and Pearson; 1974) have been observed to grow in presence of sulfide. Martinez *et al.* (1987) also reported *Chlorella* sp. to assimilate acetate well in photoautotrophic conditions, a substrate common in anaerobic conditions. Figure 5 also shows the heterotrophic

bacterial density increased as glucose loading was increased. However, under anaerobic condition, bacteria were affected by accumulation of VFAs and this explained declining bacterial densities after about three weeks of operation. Although accumulation of VFA resulted in drop of pH, in absence of VFAs, low pH does not significantly inhibit growth of *Chlorella vulgaris* (Table 2) and bacteria (Fig. 6). The results shown in Table 2 and Fig. 6 were collected from reactors operated at 30°C , 6000 lux and glucose loading rate of 75 mg/l/d . Because at this glucose loading rate no VFAs were formed, the results shown in Table 2 and Fig. 6 represent the effect of pH alone. Physical and microscopic observation showed that the shape and color of colonies were different at different pH values, which suggests that different species grew at different pH values. A comparison of results in Fig. 5 and those in Table 2 and Fig. 6 suggest that VFAs might have played a major inhibition role to growth of bacteria and algae in anaerobic reactors.

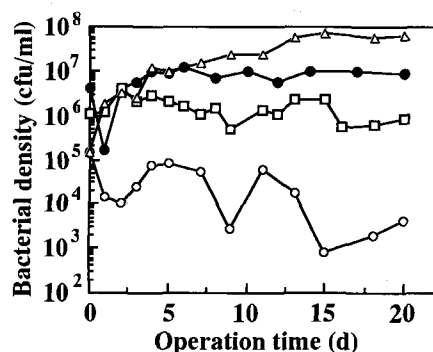


Fig. 6: Effect of pH on bacterial density at pH 3.5 (●), pH 4.5 (△), pH 7.0 (□) and pH 11.0 (○). Glucose was fed at the rate of 75 mg/l/d . No VFAs were detected.

Table 2: Effect of pH on *Chlorella vulgaris* growth rate.

pH	3.0	3.5	4.5	5.5	7.0	8.0	9.5	10.4	10.7	11.0
μ (d^{-1})	0.33	0.44	0.51	0.50	0.49	0.48	0.40	0.33	0.26	0.25

3.3 Effect on dissolved organic carbon accumulation

Figure 7 shows dissolved organic carbon (DOC) accumulation as a function of glucose loading rate. For the entire duration of the experiments, DOC in aerobic reactors was low. In the first 27 days, dissolved organic carbon in the aerobic reactors ranged from 15 to 40 mg/l but steadily increased to $20 \sim 80 \text{ mg/l}$. The concentration of glucose that was not consumed was less than 3 mg C/l in aerobic reactors, and is therefore not an important contributing factor to DOC. Part of the soluble organic matter is probably contributed by dead microorganisms which in the course of time decomposes to non-particulate matter. DOC in aerobic reactors might have been caused by excretion of dissolved organic substances by algae particularly during the stationary phase. Similar behavior has earlier been reported by Lee *et al.* (1992) who observed excretion of dissolved organic substances by algae during the stationary phase.

In anaerobic reactors, the DOC increased because of VFAs accumulation. DOC concentration rose to about 100 times in R300 and R700 compared to the average concentration in aerobic reactors. The contribution of unused glucose was about 10% of DOC observed in anaerobic reactors. Excretion of extracellular products by algae from alive cells is a minor factor because algal concentration in these reactors was relatively low. Part of dissolved organic matter might have been regenerated from dead cells particularly because biomass growth was observed to decrease. The major key contributing factor to DOC was observed to be production of VFAs. In R150 for instance, contribution of acetic acid, propionic acid and iso-butyric acid to dissolved organic carbon were 51~60%, 17~24% and 0~3%, respectively whereas glucose contributed only 7~12%.

The specific DOC accumulation was determined when VFA started to accumulate, and was calculated as an initial slope of the DOC accumulation versus glucose input rate (Fig. 8). The specific DOC accumulation was not constant, but decreased with increasing glucose concentration. At high glucose input rate, carbon was largely released as gases. This might have been the reason for decreasing specific DOC accumulation with increasing glucose loading rate.

3.4 Effect on dissolved inorganic carbon (DIC) production

Inorganic carbon production from organic carbon by algal-bacterial system was found to be influenced by the amount of glucose added to the system (Fig. 9). The specific DIC accumulation was not constant, but increased as glucose loading rate increased. The reasons that contribute to increasing specific DIC accumulation with increasing glucose loading rate are two folds. One major reason is probably decreasing specific POC accumulation rate with increasing glucose loading rate. This means that at high organic loading rate, excess amount of CO_2 that is released by bacterial degradation of glucose and algal respiration is not converted to particulate organic matter but remains in the water in various forms of inorganic carbon. Another contributing factor is that bioreactors with high glucose loading rate tend to have high pH values (as

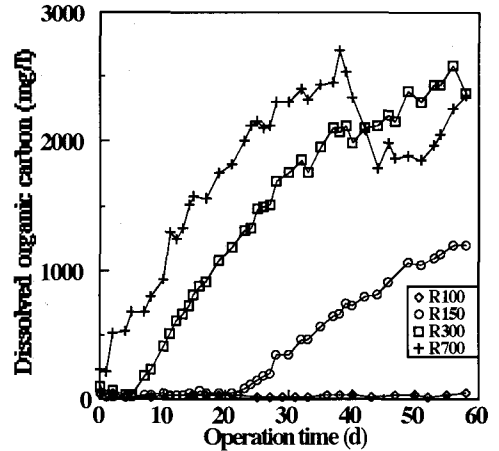


Fig.7: Effect of glucose concentration on dissolved organic carbon accumulation.

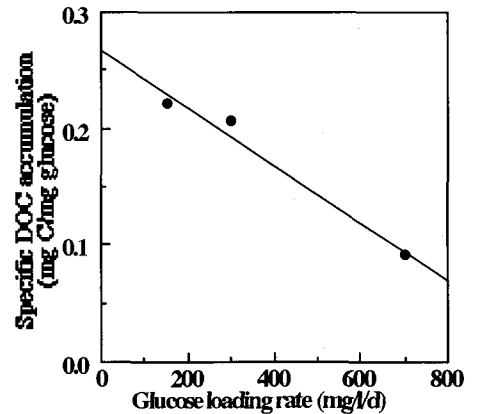


Fig.8: Specific dissolved organic carbon accumulation in anaerobic reactors when volatile fatty acids production begins.

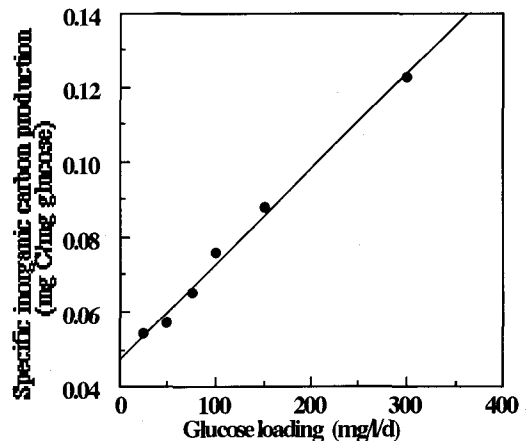
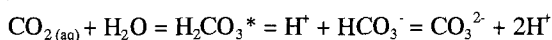


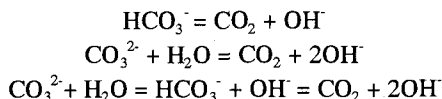
Fig.9: Effect of glucose loading on specific inorganic carbon accumulation.

long as VFAs are not produced) during the biomass exponential growth phase. The high pH values favors HCO_3^- and CO_3^{2-} production which are easily retained in the water body than $\text{CO}_{2(g)}$.

Figure 10 shows a build-up of pH and DIC in four reactors. As expected in R25, the build-up of DIC was slow because of the concentration of glucose added. As glucose concentration was increased such as the case of R75, inorganic carbon accumulation increased. Excessive loading however, did not favor DIC accumulation as shown by results of R700. It can be noticed that when pH dropped to 4.7 in R700 in the first 2 days of operation, DIC did not accumulate until pH recovered between day 2 and 8. When pH fell once again from day 8 because of VFAs accumulation, so did inorganic carbon. This mechanism could be explained by the following chemical equation:



At 25°C H_2CO_3^* dominate at $\text{pH} < 6.3$, HCO_3^- at $6.3 < \text{pH} < 10.3$ and CO_3^{2-} at $\text{pH} > 10.3$. The equilibrium constant for carbonic acid, $K = 10^{-3.5}$ which suggests that the concentration of H_2CO_3^* is usually in form of $\text{CO}_{2(aq)}$. The total concentration of HCO_3^- and CO_3^{2-} will start to decrease at $\text{pH} 8.3$, which suggests that part of HCO_3^- and CO_3^{2-} converted to H_2CO_3^* at pH below 8.3 would be released to the atmosphere as $\text{CO}_{2(g)}$, thus reducing total inorganic carbon in the solution. This also explains why when pH dropped in R25 from day 37 to 49 (Fig. 10), inorganic carbon did not decline. If algal consumption of CO_2 was a reason for low DIC concentration in R700 then pH should have increased according to the following chemical equations:



The fact that pH decreased seems to suggest reasons other than algal consumption of CO_2 . In any case at acidic pH values observed in R700, the concentration of CO_3^{2-} is nil and that of HCO_3^- is negligible to bring any remarkable influence on inorganic carbon concentration.

Even when no glucose is added, small concentration of inorganic carbon may be absorbed from the surrounding atmosphere to the system (Fig. 9). This suggests that in a natural environment algae may still be able to fix CO_2 from the atmosphere if carbon is a growth limiting factor.

3.5 Effect on gas release

Gas release rates to the surrounding atmosphere were calculated by subtracting total carbon recovered from the known amount of supplied carbon. The total carbon recovery was calculated as the sum of the dissolved inorganic carbon, particulate organic carbon (biomass) and dissolved organic carbon (unused glucose, VFA and other intermediate soluble carbon). Figure 11 shows that loss of carbon through gas release is higher in bioreactors fed with high glucose concentration. At the start of the operation, the loss was high and increasing probably because bacterial activities were faster than the

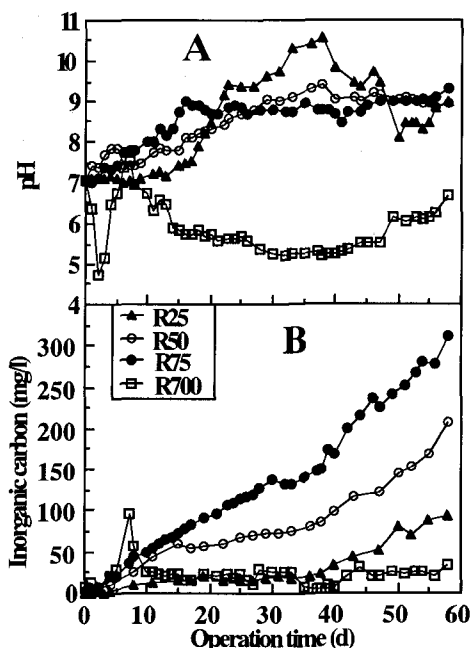


Fig. 10: Effect of glucose input rate on pH (A) and dissolved inorganic carbon accumulation (B).

ability of algae to consume CO_2 . This means that CO_2 produced by bacterial degradation of glucose was not all consumed by algae. Approximately on day 10~15 the trend reversed and carbon loss was smaller as time increased in aerobic reactors. Loss of carbon in R75 was unexpectedly lower than in R25 and R50. This behavior might have been caused by presence of *Scenedesmus* sp. contributing about 40% of algae cells in R75 most of which seems to be *Scenedesmus actus* and *Scenedesmus acumunatus* whereas other reactors contain almost entirely *Chlorella vulgaris*. These results are in agreement with Azov (1982) and Goldman *et al.* (1982) who reported that *Scenedesmus* sp. has high ability of inorganic carbon uptake than *Chlorella vulgaris*. Azov (1982) observed substantial difference in steady state biomass yields for *Chlorella vulgaris* and *Scenedesmus* sp. grown at the same pH values. At pH 7.5 (High CO_2), and pH 9.5 (Low CO_2), biomass yields for *Chlorella vulgaris* were 16% and 26% lower than those for *Scenedesmus* sp., respectively. *Scenedesmus* sp. are known to be less sensitive to alkaline pH than *Chlorella vulgaris* (Goldman *et al.*; 1982). The presence of *Scenedesmus* sp. in R75 where pH was about 9.0, might have influenced the uptake of inorganic carbon, thus reducing gas release to the atmosphere.

Gas release rates in anaerobic reactors however, increased as glucose was continued to be added. At the end of operation, gas release rates were 38, 55 and 79% of the total carbon in R150, R300 and R700, respectively. Such level of release of carbon is not uncommon. Gang *et al.* (1992) reported CO_2 release ranging from 82.8 to 100% of input in thermophilic oxic process treating high strength food processing wastewater. Carbon loss was high in anaerobic reactors because probably part of carbon was lost through methane production which can not be re-utilized by algae and partly because algal concentration and activities in anaerobic reactors were significantly affected by VFAs accumulation.

3.6 Volatile fatty acids (VFA) production

To describe anaerobic nature of algal-bacterial system in our study, VFAs accumulation was employed as a criterion. VFA in these experiments were observed only in reactor with glucose loading rate exceeding 100 mg/l/d. The first sign of reactors turning anaerobic was observed by complete loss of dissolved oxygen, followed by simultaneous decline in pH, increase in dissolved organic carbon and odorous characters. The strong odorous smell typical of alcohol fermentation in all anaerobic reactors persisted only in the first 5

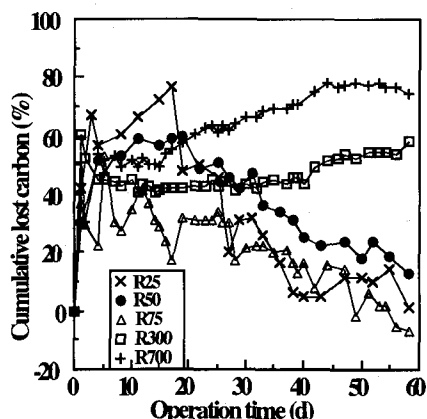


Fig. 11: Effect of glucose loading rate on gas release.

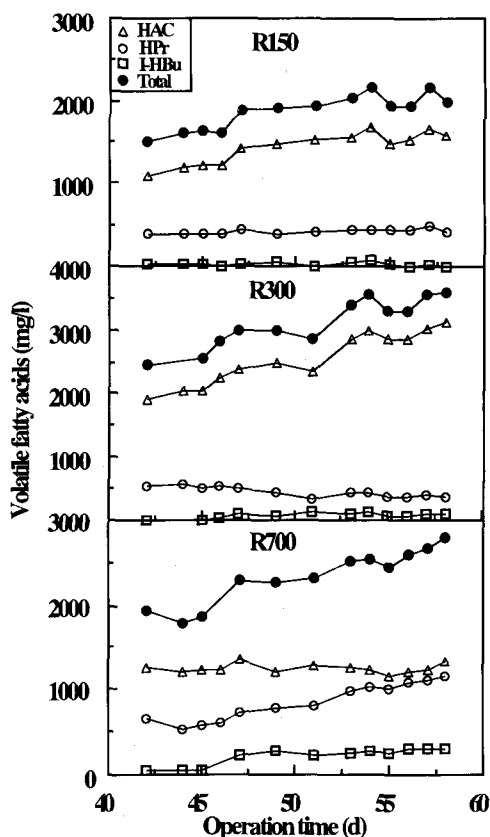


Fig.12: Volatile fatty acids accumulation.

days after pH started to decline but became mild thereafter.

The major dominating VFAs were acetic and propionic acid with minor contribution from iso-butyric acid (Fig. 12). This was consistent with other findings in the literature (Brockett; 1976). In R300 and R150 at the time VFA sampling was started, acetic acid accumulation was still progressing but propionic acid was rather stable at about 425 mg/l in R150 and even declined in R300 from 549 mg/l on day 42 to 369 mg/l on day 58. In R700, propionic acid and iso-butyric acid concentration were still increasing but acetic acid was rather uniform at about 1250 mg/l. This behavior is quite different to the one observed in R150 and R300 and seems to suggest that the mechanisms of VFA production change with increasing glucose loading rate. As expected VFAs were not recovered in the aerobic reactors.

3.7 Carbon mass balance

Earlier discussion has demonstrated that biomass growth was higher in reactors receiving largest glucose concentration but excessive organic carbon loading was detrimental to biomass growth (Fig. 4). However, Figure 13 indicate that organic matter is more efficiently converted to biomass under aerobic condition than anaerobic condition. The distribution of carbon in aerobic and anaerobic reactors was observed to be different. The loss of carbon in the anaerobic reactors were relatively constant. However, in the aerobic reactors loss of carbon decreased as biomass increased, an indication that utilization of CO_2 by algae was progressing well. In both aerobic and anaerobic reactors IC was less than 20%, but as explained earlier IC was lost in anaerobic reactors as pH declined because of VFAs accumulation. Although glucose was efficiently degraded in both cases, the increase in dissolved organic carbon (DOC) in the anaerobic reactors were observed. The accumulation of DOC in anaerobic reactors was mainly because of accumulation of VFAs. This work has demonstrated that carbon distribution in oxidation ponds is significantly affected by the organic loading rate.

4. Conclusions

Based on the results obtained in this work, the following conclusions can be made.

- (1) Organic material is more likely to be converted to biomass in aerobic than in anaerobic conditions.
- (2) Dissolved inorganic carbon (DIC) concentration in the system increased as organic loading rate increased. However, because pH governs the proportion of free CO_2 , HCO_3^- and CO_3^{2-} , its variation will influence the concentration of inorganic carbon. DIC was low at low pH values, regardless of glucose loading rate.
- (3) The release of carbon in form of gases is higher under anaerobic conditions than aerobic conditions. This behavior was observed to be influenced by algal concentration, glucose loading rate and pH.
- (4) Glucose degradation in both aerobic and anaerobic conditions was good. Dissolved organic carbon (DOC) in anaerobic conditions was observed to be contributed largely by volatile fatty acids. The specific DOC accumulation in anaerobic reactors was observed to increase as glucose loading rate decreased.

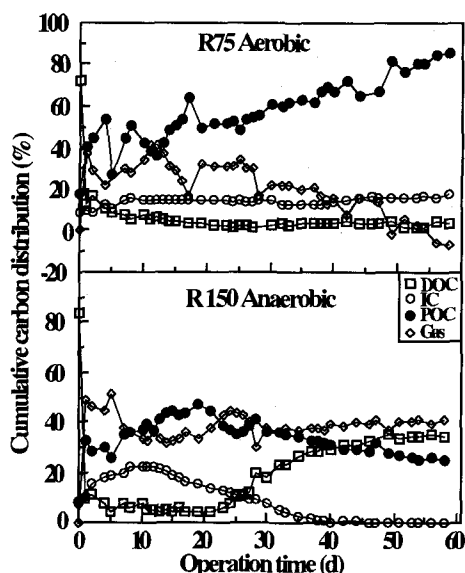


Fig. 13: Relative distribution of POC, IC, DOC and Carbon lost through gas release in aerobic and anaerobic reactors.

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