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Article

The Relevance of the CO_2 Partial Pressure of Sodium Bicarbonate Solutions for the Mass Cultivation of the Microalga *Spirulina*

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O meio de cultivo na base de bicarbonato de sódio usado para produzir a microalga *Spirulina* em massa e preparado seguinte ZARROUK[1] é meta-estável em contato com a atmosfera. Este fato causa uma grande perda de CO_2. Com cálculos e experiências foi determinada a relação entre a pressão parcial de CO_2 de soluções de bicarbonato de sódio e o pH da solução. Com pH 10.2 existe um equilíbrio com a concentração de CO_2 no ar e desta maneira não há perda de CO_2.

It is demonstrated by experiments and calculations that the medium used for growing the microalga *Spirulina* (prepared according to ZARROUK[1] with a resulting pH of 8.7) is highly meta-stable in contact with the atmosphere and is thus loosing considerable amounts of CO_2. This economic problem can be avoided by raising the pH of the culture medium to 10.2 where its partial pressure of CO_2 corresponds to the partial pressure of CO_2 in the atmosphere. The pH shift has practically no influence on the growth of the algae.

Keywords: Spirulina, *CO_2, sodium bicarbonate*

Introduction

Commercial production of the “magic” blue-green microalga *Spirulina* , a desired health food, is steadily increasing around the world. Most producers use in the growth medium a salt composition as given by Zarrouk[1] with sodium bicarbonate as carbon source which counts for at least 60% of all nutrient costs. Reducing the original amount of 16.8 g/L of bicarbonate, as given by Zarrouk, is one attempt to reduce these costs. The algae assimilate during their growth CO_2 from the medium so that recovery of the carbon source in the medium is done by adding CO_2. The consumption of CO_2, however, as many producers report, is at least 2-3 times higher than the theoretically necessary amount, as calculated from the carbon content in the algae. This fact is for the *Spirulina* production economically a burden which, however, can easily be eliminated.

Little attention has so far been paid to the fact that the medium commonly employed for growing *Spirulina* is strongly meta-stable due to the fact that it has a CO_2 partial pressure exceeding that of the atmosphere by a large factor. This causes a permanent evasion of CO_2 to the atmosphere. How fast this evasion process is, depends on the type and intensity of mixing in the culture. Agitation in ponds with paddle wheels at modest speed causes a relatively slow loss, while mixing with air bubbles or air-lift pumps causes a dramatic loss. Air mixing, however, receives recently more and more attention, as it provides with less energy consumption and in a much more efficient way the desired vertical mixing in the medium[2]. The water flow in a paddle wheel agitated pond presents soon after the paddle wheel a strong tendency to a laminar flow pattern which is not appropriate for obtaining a high algal productivity. Air mixing, though very effective for high algae productivity, requires special attention, in order not to flush out fast CO_2 from the medium. We studied this effect in detail and found good agreement between experimental results and calculated data.

Theoretical Approach

To simplify the complex ionic situation as it exists in the real medium, we considered for the calculations a pure bicarbonate solution, *i.e.* neglecting ionic interactions with other species present in the medium. This simplification seems to be justified by the overwhelming amount of bicarbonate in the ZARROUK medium, compared to the other constituents (16.8 g/L bicarbonate, 2.5 g/L nitrate, and a total of less than 2 g/L of sulfate, phosphate, potassium, calcium, magnesium). In spite of this simplification, our experimental results coincide well with the calculated data.

Starting from the following equations:

$$HCO_3^- \rightleftharpoons H^+ + CO_3^{2-} \quad (K_2 = 5.62 \times 10^{-11})$$

$$OH^- + CO_{2\,aq} \rightleftharpoons HCO_3^- \quad (K_h = 2.9 \times 10^{-8})$$

and using for the 2-phase equilibrium

$$[CO_2]_{atm} : [CO_2]_{aq} = 25.8$$

leads to the final result

$$P_{CO2} = 7.481 \times 10^4 \; TC \frac{[H^+]^2}{([H^+]+K_2)} \text{ [atm]}$$

for the CO_2 partial pressure of a bicarbonate solution. TC (total carbon) stands for $[CO_2]_{aq} + [HCO_3^-] + [CO_3^{2-}]$

For experimental realization, variations of pH in the medium are realized by adding NaOH. Figure 1 shows the calculated relationship between P and pH for the two most commonly used values of TC. It turns out that at pH values of 10.15 (TC = 0.1 M) or 10.35 (TC = 0.2 M), respectively, the CO_2 partial pressure of the bicarbonate solution is in equilibrium with the partial pressure in the amosphere. At lower pH, CO_2 will escape, at higher pH values, CO_2 from the atmosphere will be dissolved in the medium.

A *Spirulina* medium prepared according to the recipe of ZARROUK (with 16.8 g/L of bicabonate) has a pH of 8.7; practically the same pH results, if the bicarbonate amount is reduced to one half. CO_2 will then be removed from the medium by (a) assimilation by the algae cells, and (b) by escape to the atmosphere due to its high partial pressure. Process (b) alone will stop, when the equilibrium pH is reached, while photosynthesis in *Spirulina* cells can then still go on leading to even higher pH values. In a culture with air mixing, the process of flushing out CO_2 is much faster than the CO_2 fixation by the cells, see Fig. 3. This means: reaching the high equilibrium value of pH in case of air mixing is principally due to the loss of CO_2 to the atmosphere. Figure 2 shows, how big this loss is. The curve shows the relationship $[CO_2]_{removed}$ (as fraction of the initial amount of TC present in the solution) *vs.* pH and was calculated according to the following equation:

$$[H^+] = \frac{K_2 \left(1 - 2\frac{[Co_2]^*}{TC_o} - \frac{K_2}{K_2 + [H^+]_0}\right)}{\frac{K_2}{K_2 + [H^+]_0} + \frac{[CO_2]^*}{TC_o}}$$

where $[CO_2]^*$ = amount of CO_2 removed from a solution that originally contained TC_o = 0.2 M of total carbon; $[H^+]_0$ = initial value in the solution before starting removal of CO_2. In the Zarrouk medium (pH = 8.7), $[H^+]_0$ is 2×10^{-9}. Our experimental results coincide well with the curve in Fig. 2.

It turns out that 35-40% of the total carbon in the Zarrouk medium has to be removed as CO_2 , until the CO_2 partial pressure of the solution reached that of the atmosphere. This removal is largely a loss to the atmosphere which can be avoided, if the pH of the freshly prepared

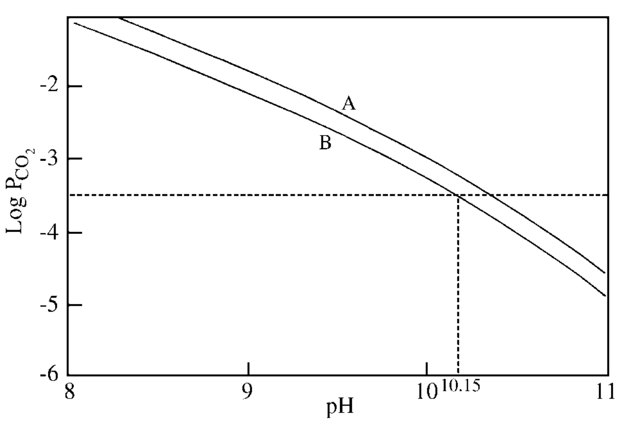

Figure 1. P_{CO2} [atm] of sodium bicarbonate solutions as function of the pH. A: 0.2 M TC, B: 0.1 M. Broken line: CO_2 partial pressure of the atmosphere. Solution B is in equilibrium with the atmosphere at pH 10.15.

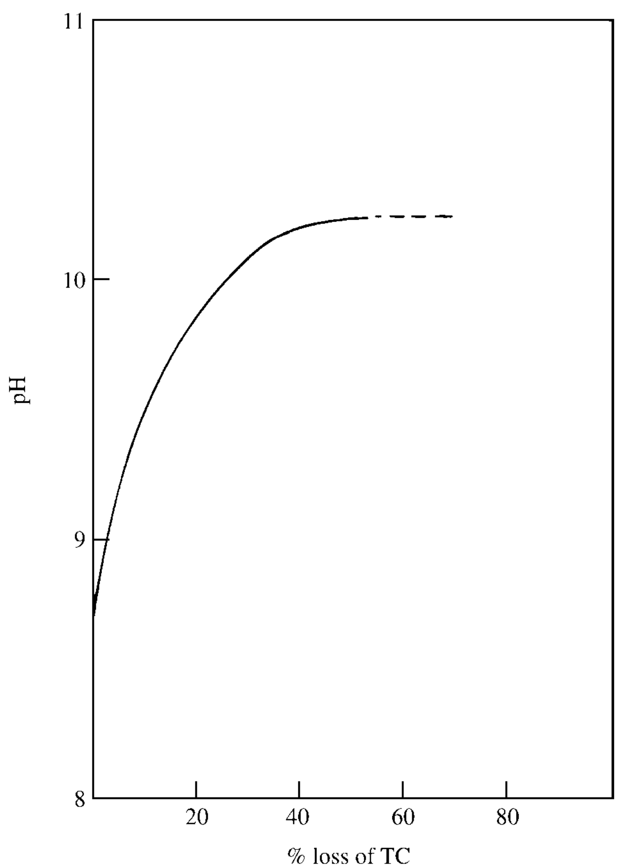

Figure 2. The effect of loss of CO_2 on the pH of the solution. Starting from a 0.2 M sodium bicarbonate solution, the curve shows the changing pH as function of the percentage of removed TC.

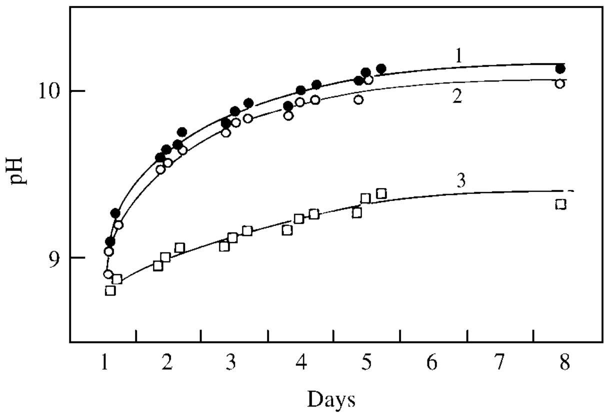

Figure 3. Removal of TC by various processes and the resulting changes of pH. Curve 1: fast loss of CO_2 in an air-mixed solution. Curve 2: same as 1, but with an algae culture. Its liberation of CO_2 from respiration keeps the curve below curve 1. Curve 3: slow evasion of CO_2 from a medium stirred without braking the water/air interface.

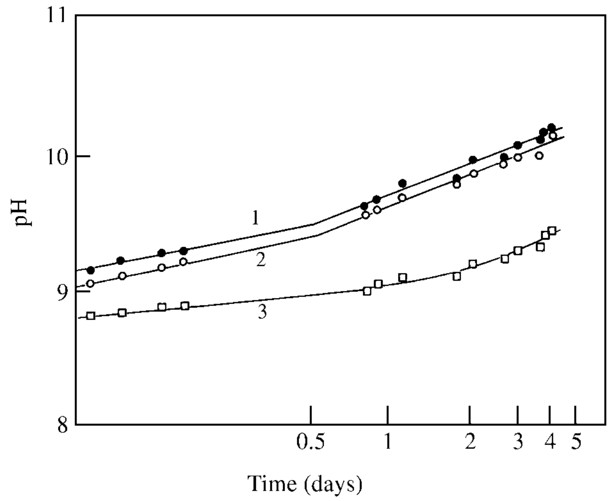

Figure 4. Semi-log plot of the data of Fig. 3 demonstrating that the evasion process is still going on after 11 days.

solution was raised to the equilibrium value. The effect of this pH shift upon the growth rate of *Spirulina* is neglegible (see below). Maintaining the pH artificially low by continuous addition of CO_2 (a common practice) means a permanent loss of CO_2.

Experimental

The phenomenon discussed above was studied using solutions containing all mineral components as given in the recipe of Zarrouk. Each experiment was done with 10 L of solution in open buckets. The experiments were run partly using the pure solution, partly with the solution containing *Spirulina platensis*. The agitation was done in two different ways: (a) by air flushing (about 1 L/min) using a porous stone as outlet that produced air bubbles of about 3 mm diameter; (b) with a propeller-type agitator in the water, in this way not breaking the water/air interface. The results are shown in Figs. 3, 4, and 5.

Results and Discussion

Figure 3: Curve 1 shows the asymptotic increase of pH in an air mixed medium without cells. From a semi-log plot (Fig. 4) it is evident that the increase of pH would still go on, beyond the measured period. The experiment of curve 2 is made under same conditions (air mixing), but with a culture of algae in the medium that grew steadily up to 600 mg/L. This curve is always below curve 1 due to the permanent addition of CO_2 resulting from algal respiration. From the biomass increase (from initially 200 mg/L to 600 mg/L at the end; the biomass containing 45% of C) follows that 1.8 g of C = 7.5% of the total carbon initially present in the solution was fixed into biomass. From the variation of pH (from initially 8.9 to finally 10.1) and using the curve of Fig. 2 it can be concluded that a total of 26.6% of the initial total carbon was removed from the solution. This means: 26.6-7.5 = 19.1% went out into the air.

In experiment 3, the solution contained also an algae culture, but it differed from the others by the way of mixing: stirring inside the liquid so that the interface water/air was not broken. This means a slow release of CO_2 controlled by molecular diffusion through the stagnant micro layer at the surface of the water phase. From the increase in biomass during the experiment (from initially 200 mg/L to finally 500 mg/L) and the increase in pH it can be concluded (as above) that 5.6% of the total carbon were fixed into biomass, while in this case only 2.3% escaped to the air.

Figure 5 presents the results of still another series of experiments, all with an algae culture. They were started at different pH values. Curve 1 shows again the initially fast approach to the equilibrium pH. The culture was mixed with an air flow. In experiment 2, the initial pH was raised by adding NaOH to 10.7, *i.e.* above the equilibrium value (in the late afternoon). The clture was also mixed with air. Next morning, the pH value was at 10.0, *i.e.* below the equilibrium level. This fact can be attributed to the libera-

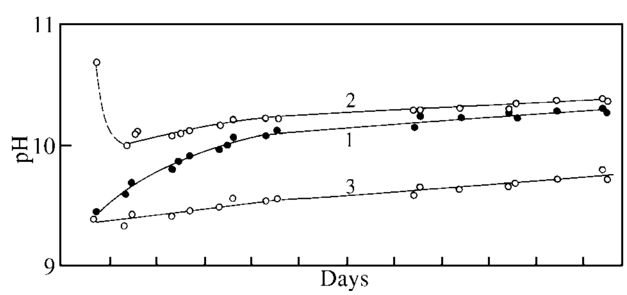

Figure 5. All curves represent cultures with algae. 1 and 2: air-mixed, 3 stirred without braking the interface. The pH of experiment 2 was initially raised by adding NaOH to a pH above the equilibrium value. Note that curves 1 and 2 approach asymptotically each other. Curve 3 shows again the slow evasion of CO_2 from a solution, when the interface is kept stable.

tion of CO_2 by respiration of the algae. Within a few hours, the pH went then up to 10.1, and increased further over the next days approaching the equilibrium level. Both curves (1 and 2) which had started at quite different pH values, finally met together. Curve 3 shows again the slow evasion of CO_2, while the medium was being mixed with an agitator inside the medium. The growth of the algae was equal in all three cultures demonstrating that the influence of pH on the growth rate of *Spirulina* is very small. This means that the proposed initial pH shift to the equilibrium value can be done without loosing productivity.

A final remark: mixing a large-scale culture intensively without breaking the surface is technically, according to the state of the art, not possible. *I.e.* the case of curve 3 in Fig. 5 can presently not be realized at technical scale.

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