Enhanced Production of Formic Acid by *Halobacterium halobium* MMT_{22} by Reduction of Hydrogencarbonate in Aqueous Solution

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Enhanced production of formic acid was observed by reduction of hydrogencarbonate in aqueous solution by *Halobacterium halobium* MMT_{22}. A one and half fold increase in the production of formic acid was observed in the presence of ascorbic acid as electron donor and $[Ru(Bipy)_3]^{2+}$ as photosensitizer.

Since 1970's there has been a considerable research effort directed toward harnessing photochemical reactions for solar energy conversion and storage.

Fixation of carbon dioxide has bee extensively studied[1] because of its importance in photosynthesis as a source of C_1 chemicals.[2] Moreover the catalytic, reduction of CO_2 to organic molecules may be fundamentally important to cope with the rapid increase of the concentration of carbon dioxide in the atmosphere as a pollutant resulting from the combustion of fossil fuels.[3–6]

In a previous paper[7] we have reported photobiological as well as photoelectrochemical conversion of CO_2 to formic acid by *Halobacterium halobium* MMT_{22}. As the hydrogencarbonate ion in solution can act as a source of CO_2 in solution where concentrations can be studied over a wide range we report in this paper its reduction to formic acid by *H. halobium* MMT_{22}. The yield of formic acid obtained was 0.42 M (mg cells)$^{-1}$ h^{-1} which increasrs to 0.532 M (mg cells)$^{-1}$ h^{-1} in presence of ascorbic acid and to 0.646 M (mg cells)$^{-1}$ h^{-1} in the presence of ascorbic acid and $[Ru(Bipy)_3]^{2+}$ (1 M=1 mol dm^{-3}).

Experimental

$[Ru(Bipy)_3]^{2+}$ was prepared by literature procedure.[8] All the other chemicals used were of analytical grade.

The extreme halophile, *H. halobium* MMT_{22} was isolated from the brine obtained from the salt farm of CSMCRI in a nutrient medium at pH 7.0. Incubation was carried out at 40 °C under illumination.

Photocatalytic experiments were carried out in a double walled Pyrex glass cylindrical reaction vessel (40 ml) with a flattened surface positioned perpendicular to the incident light (exposed area 10 cm^2, 250 W Xe lamp from Applied Photo Physics).

For the optimization of reaction conditions, different sets of experiments were carried out. In a typical photocatalytic experiment an aqueous system containing 25 ml 25% NaCl; *H. halobium* MMT_{22} as biocatalyst (1—5 mg wet weight); $NaHCO_3$ as a source of CO_2 (0.2—2.0 M) was irradiated with a monochromatic excitation spectral line (420—480 nm). Water at (10—50 °C) was continuously circulated through a water jacketed cell containing the reaction mixture to maintain the desired temperature within ±0.2 °C.

In other sets of experiments investigations were carried out to study the effect of electron donor and photosensitizer on formic acid production.

In a similar experimental system containing 25.0 ml 25% NaCl, 1 mg *H. halobium* MMT_{22}, 1.0 M $NaHCO_3$; with $[Ru(Bipy)_3]^{2+}$ as photosensitizer (10^{-4} M) and ascorbic acid as electron donor (10^{-1}, 10^{-2}, 10^{-3} M) was irradiated with a monochromatic excitation spectral line (460 nm). Water at 40 °C was continuously circulated through a water jacketed cell containing the reaction mixture.

Samples were taken out at various time intervals and made free of bacterial cells. The product analysis was done by HPLC.

Results and Discussion

Under strictly anaerobic conditions on illumination, each bacteriorhodopsin cycling in the purple membrane of *H. halobium*, release 2—3 protons which are released on the exterior of the cells. Simultaneously carbon uptake is greatly increased as CO_2 is fixed through the reductive pathways and NADH is produced during illumination by reversal of electron transport. Thus NADH functions as electron donor in the system.[9–11]

In our previous paper we have reported photobiological as well as photoelectrochemical conversion of CO_2 to formic acid by *H. halobium* MMT_{22}. The yield of formic acid increased considerably by regenerating NADH by cathodic reduction at −0.60 V or in the

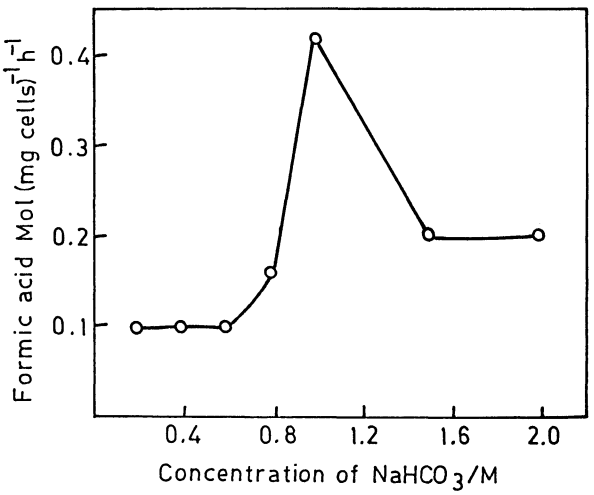


Fig. 1. Formic acid production at different substrate concentration.

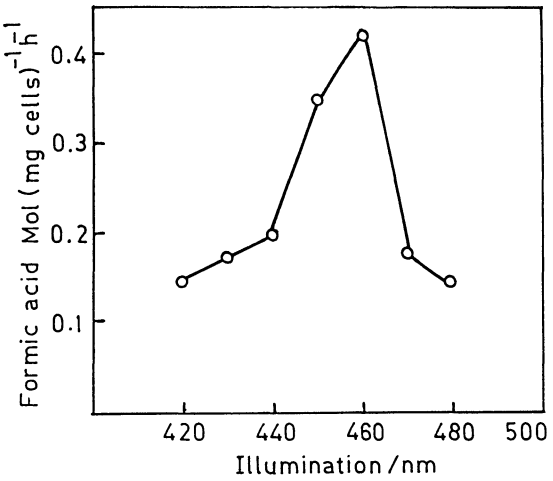


Fig. 2. Formic acid production at different wavelengths when $NaHCO_3$ was used as a source of CO_2.

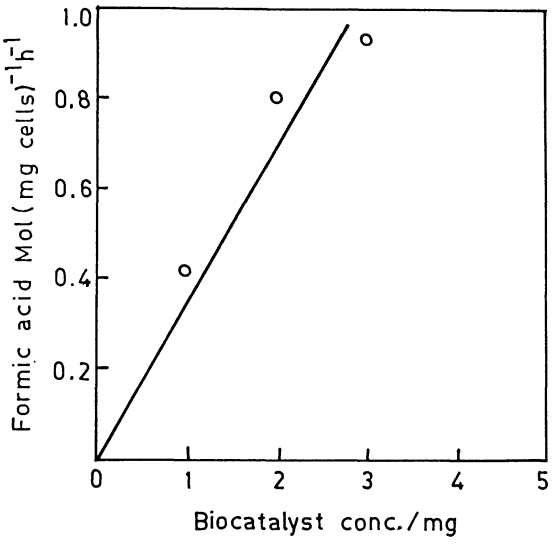


Fig. 3. Formic acid production at different biocatalyst concentrations.

presence of the two electron donor ascorbic acid. Under photoelectrochemical reduction, a maximum yield of 0.2 mol of HCOOH (mg cells)$^{-1}$ h^{-1} was obtained.[7]

In our present studies $NaHCO_3$ was used as a substrate for formic acid production. Optimization of the reaction conditions revealed the following results. 1 M $NaHCO_3$ concentration (Fig. 1), monochromatic excitation spectral line at 460 nm at 40 °C (Fig. 2) were found to be optimum conditions for formic acid production. An increase in the biocatalyst concentration causes a linear increase in formic acid production (Fig. 3).

Tris(bipyridyl)ruthenium complexes $[Ru(Bipy)_3]^{2+}$ have been intensively tested as sensitizers for photochemical water splitting using visible light.[12] There are many reports which describe application of such complexes to CO_2 fixation.[12] Using $[Ru(Bipy)_3]^{2+}$ as a photosensitizer in visible light and ascorbic acid as a sacrificial electron donor and *H. halobium* as the biocatalyst, formic acid was found to be the sole organic product in our present investigations.

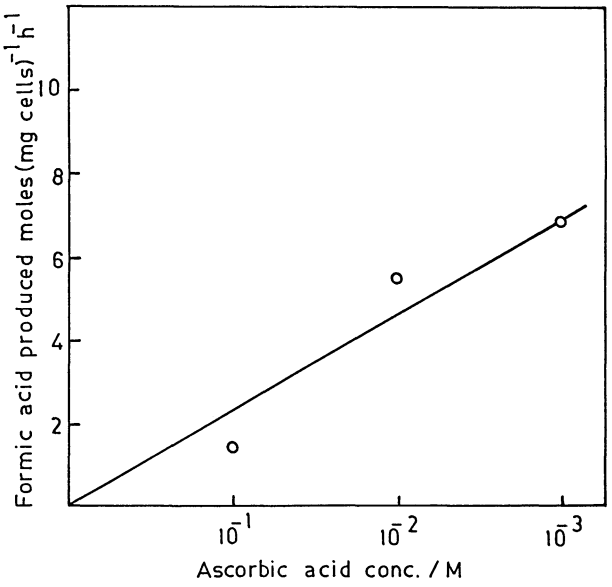


Fig. 4. Effect of ascorbic acid on formic acid production.

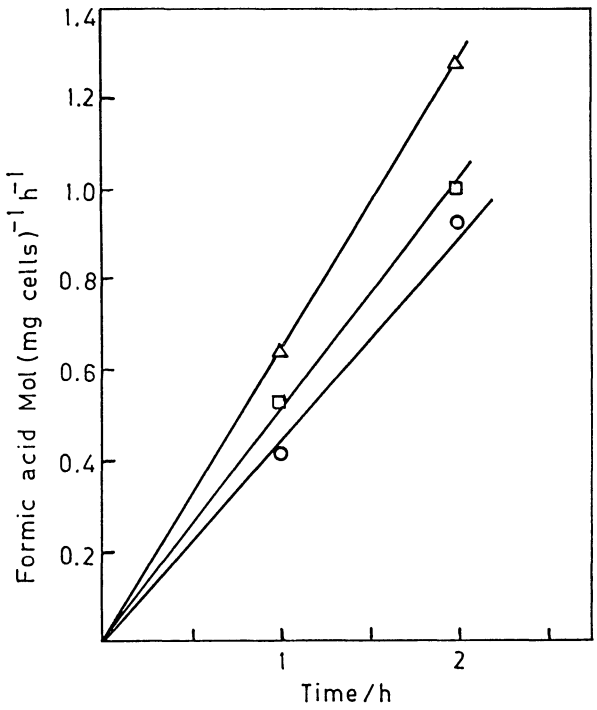
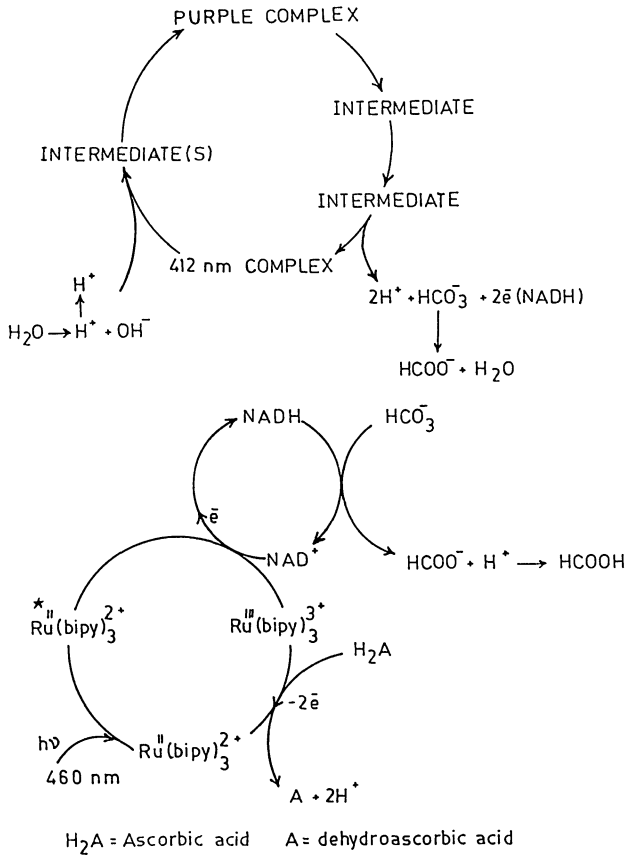


Fig. 5. Formic acid production by *Halobacterium halobium* MMT_{22} in presence of electron donor and photosensitizer.

In the earlier report[7] *H. halobium* MMT_{22} showed continuous production of HCOOH at the rate of 0.2 M (mg cells)$^{-1}$ h^{-1}. In the present studies using hydro-

Table 1. Reduction of Hydrogencarbonate in Aqueous Solution to Formic Acid by *Halobacterium halobium* MMT_{22}

Time / h	Formic acid produced / M h^{-1} (mg cells)$^{-1}$		
	Conditions		
	H. halobium MMT_{22} in hydrogencarbonate solution	*H. halobium* MMT_{22} +ascorbic acid	*H. halobium* MMT_{22} +accorbic acid+ $[Ru(Bipy)_3]^{2+}$
0	0.0	0.0	0.0
1	0.42	0.532	0.646
2	0.81	1.042	1.30



Scheme 1.

gencarbonate in solution an enhanced production of formic acid was observed in aqueous solution. The rate of formation of formic acid is 0.45 M h^{-1}. Figure 4 shows the effect of ascorbic acid concentration on formic acid production. In presence of ascorbic acid the rate increases to 0.55 M h^{-1}. Here ascorbic acid serves as two electron donor which enhances the rate of CO_2 reduction by maintaining the NADH/NAD^+ cycle. In presence of $[Ru(Bipy)_3]^{2+}$ formic acid production was found to be maximum with a rate of 0.66 M h^{-1}. Figure 5 and Table 1 compares the rates of formic acid production when ascorbic acid and $[Ru(Bipy)_3]^{2+}$ were used for the investigations. A 1.5 times increase in formic acid production was observed when $[Ru(Bipy)_3]^{2+}$ was used as a photosensitizer. The mechanism of the reaction is shown in Scheme 1.

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