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The influence of pH on the kinetics of dissimilatory nitrite reduction in *Paracoccus denitrificans*

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An almost stoichiometric conversion of nitrite to nitrous oxide was observed during the nitrite reduction by *Paracoccus denitrificans* cells in a medium of pH 6.4. The N_2O accumulated in the reaction medium and was decomposed only after nitrite had been consumed; when the pH of the medium was higher than 7.3–7.4, nitrous oxide did not accumulate. The activity of N_2O reductase was, in the whole range of pH 6.4–9.2, higher than the activity of NO_2^- reductase, both activities showing the maximum at the pH higher than 8.0. Using an artificial donor, TMPD plus ascorbate, the maximum activity of NO_2^- reductase, but not N_2O reductase was shifted by about two pH units to acidic region. The activity of nitrite reductase declined in the presence of N_2O only at higher pH values. Cytochrome *c*, as a common electron donor for both N_2O and NO_2^- reductase, was more oxidized at pH < 7.3 in the presence of NO_2^- than in the presence of N_2O, the opposite being true at pH > 7.3. The increased flux of electrons to cytochrome *c* has for a constant pH value (6.4) no effect on their distribution over NO_2^- and N_2O. The results indicate that the distribution of electrons in the terminal part is determined by the different pH optima for NO_2^- reductase and N_2O reductase, and by a mutual dependence of activities of the two reductases due to the competition for redox equivalents from a substrate.

Introduction

Denitrification processes in bacteria [1,2] form an important part of the nitrogen cycle in the biosphere. In their course, a sequential reduction of nitrate via nitrite to gaseous nitrogen oxides (mainly nitrous oxide) is brought about, leading in the final step to nitrogen . Intermediates of denitrification are believed to have a marked ecological importance: nitrite may play a role as a precursor of carcinogenic nitrosamines [3], gaseous nitrogen oxides may have a deleterious effect on the protective ozone sphere of the earth [2,4,5]. From the foregoing arguments the justification for kinetic studies of denitrification processes should be obvious, they should offer the explanation for the cause of accumulation of intermediates. Investigations of this kind are necessarily restricted, because of the poor knowledge of reaction properties of respiratory components in most denitrifying bacteria. A suitable object for these kinds of experiments may be sought in *Paracoccus denitrificans*, a biochemically well-defined bacterium, which possesses a respiratory chain similar to that of mitochondria [6,8] comprising further nitrate reductase which withdraws redox equivalents from the ubiquinone region [8–11], and nitrite and nitrous oxide reductases, into which the electrons are fed by cytochrome *c* [12,13]. The latter enzymes bring about the dissimilatory reduction of nitrate to nitrogen, creating as obligatory intermediates nitrite and nitrous oxide.

Abbreviation: TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine.

The objective of the present work is a study of kinetics of the reaction pathway nitrite → nitrous oxide → nitrogen and its partial steps, catalyzed by anaerobically grown cells of *P. denitrificans* using reaction media differing in pH. It is shown that in the control of the above pathway an inhibitory mechanism 'via respiratory chain' is involved, analogous to that which has been described explicitly in our previous study [14] (see also Ref. 9 for comparison). The results obtained make it possible to discuss factors acting on the kinetics of denitrification processes, and offer an explanation for some previous observations concerning the physiology of bacterial denitrification (see Refs. 1 and 2 for a review). A part of the results was in a preliminary form already published [15].

Materials and Methods

Microorganism and growth conditions

Paracoccus denitrificans NCIB 8944 from the Czechoslovak Collection of Microorganisms (CCM 982) was grown anaerobically in a medium described earlier [14] except that glucose was replaced with 50 mM sodium succinate as a carbon source. The cells were harvested at the early stationary stage of growth, washed with 50 mM sodium phosphate of pH 7.3 and suspended in a small volume of the same buffer.

Investigation of metabolism of terminal acceptors

Nitrous oxide concentration was continually measured with the aid of the modified Clark-type electrode described in [16] closing an electromagnetically stirred glass vessel of 2.6 ml volume tempered to 25°C. Nitrite concentration was determined colorimetrically in withdrawn samples of 20 μl [17] after deproteinization with the saturated uranyl acetate solution. Anaerobic conditions permitting the denitrification were reached by the exhaustion of oxygen from the reaction mixture, either due to the oxidase activity of *P. denitrificans* cells [16] or by the addition of a several-times-washed suspension of bakers yeast [18]. In some cases the activity of nitrite reductase was measured in sealed tubes containing 5 ml of medium devoid of oxygen by bubbling through with nitrogen. In all cases a 0.1 M sodium phosphate buffer of a given pH value containing 5 mM sodium succinate was used as a medium for activity measurement. Nitrous oxide and acetylene were introduced as water solutions, saturated at 0°C (concentrations, 58 mM and 76 mM, respectively [16]).

Cytochrome c study

The redox stage of cytochrome *c* during utilization of a given terminal acceptor was determined by means of difference spectroscopy in closed cuvettes of 3 ml volume with a spectrophotometer Cary 118 C. In the reference cuvette the substrate was omitted; the mixture was oxidized by solid potassium ferricyanide. The 100% reduction of cytochrome in the sample cuvette was achieved with the aid of sodium dithionite.

Chemicals

N,N,N′,N′-tetramethyl-*p*-phenylenediamine (TMPD) was from Fluka (Buchs, Switzerland), the other chemicals of analytical purity were obtained from Lachema (Brno, Czechoslovakia).

Results

The pH effect on the course of the pathway nitrite → nitrous oxide → nitrogen

We have followed the time-dependent changes of nitrite and nitrous oxide concentrations after the addition of nitrite to an anaerobic suspension of *P. denitrificans* cells (see Figs. 1–3, parts a). Parallel experiments were undertaken where

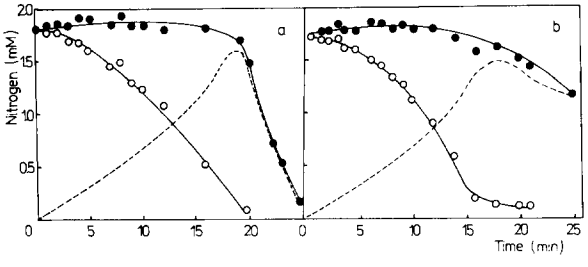

Fig. 1. The concentration dependence of nitrogenous compounds during the reduction of nitrite by the cells of *Paracoccus denitrificans* at pH 6.4. For the measurement conditions, see Materials and Methods. 4.7 mg dry weight of bacteria in 2.6 ml of reaction medium was used. The initial nitrite concentration introduced after oxygen exhaustion was 1.7 mM. Concentrations are indicated: $N\text{-}NO_2^-$ (○), $N\text{-}N_2O$ (– – –), $N\text{-}NO_2^-$ plus $N\text{-}N_2O$ (●). (a) and (b), without and with 2 mM acetylene, respectively.

acetylene in 2 mM concentration was present (see Figs. 1–3, parts b). Under these conditions the nitrous oxide reductase of the cells is effectively blocked, since its published value of K_i with acetylene (benzyl viologen as an electron donor) is 28 μM [19] (that is two orders of magnitude lower than the concentration of inhibitor in our experiments). From Fig. 1 it is evident that at pH 6.4 an almost stoichiometric accumulation of nitrous oxide proceeded simultaneously with the nitrite reduction (Fig. 1a) just similarly as appeared in an experiment where the nitrous oxide reductase was blocked by acetylene (Fig. 1b). After the nitrite was consumed, the transiently accumulated nitrous oxide was decomposed in a fast reaction.

Analogous experiments to those shown in Fig. 1 were also performed at pH 6.95 (Fig. 2) and 7.35 (Fig. 3). It is evident that the relatively small change of the pH value of the medium had a profound influence on the kinetics of the investigated process. By increasing the pH the obtainable maximum concentration of N_2O decreased. At the pH values higher than 7.3–7.4 nitrous oxide did not accumulate at all, indicating that the rate of its utilization reached the rate of its production from nitrite. Simultaneously, the higher rate of reduction of terminal acceptors NO_2^- and N_2O was detectable in the more alkaline medium. Figs. 1–3 also give the time dependences of the total analytically followed concentration of nitrogen; it is the sum of $[NO_2^-]$ and $2[N_2O]$. From the fact that in the presence of acetylene (when the nitrite reduction is blocked at the nitrous oxide level) no transient decrease of this sum could be observed, it can be assumed that during the reduction of nitrite no other nitrogenous intermediate besides nitrous oxide is accumulated in media of the given pH.

The effect of pH on the course of reactions catalyzed by nitrite reductase and nitrous-oxide reductase

In order to clarify the observed effect of pH on the kinetics of the reaction sequence nitrite → nitrous oxide → nitrogen we studied the pH dependences of the activities of nitrite reductase and nitrous oxide reductase of the cells (Fig. 4). From Fig. 4 an almost parallel course of both activity curves can be seen, both enzymes having optima in the interval pH 8–9. Nitrous oxide reductase activity was higher than the activity of nitrite reductase over the whole pH range tested, and the ratio of both activities increased with increasing pH. A similar dependence of activity on pH was also found when following the oxidase activity of cells (not shown).

Since the reduction of terminal acceptors by intact bacterial cells represents a complex event (the manifestation of which is determined by the function of a number of membrane-bound entities responsible for substrate transport across the membrane and of membrane-bound electron carriers), the observed optimal activities need not necessarily reflect the true pH optima of the terminal reductases. As can be seen from Fig. 5 this could be documented in the case of nitrite reductase. Fig. 5 shows that using an artificial electron donor TMPD plus ascorbate, which passes electrons direct to cytochrome *c* [20], the pH optimum for the nitrite conversion to nitrous oxide was shifted by about two pH units to the acidic region. No similar significant change of pH opti-

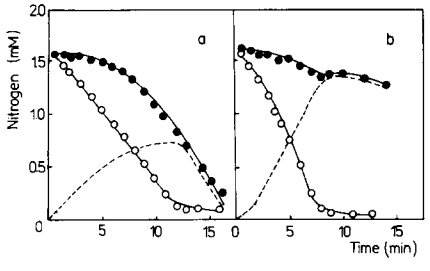

Fig. 2. The concentration dependence of nitrogenous compounds during the reduction of nitrite by the cells of *Paracoccus denitrificans* at pH 6.95. For reaction conditions and symbols, see legend to Fig. 1.

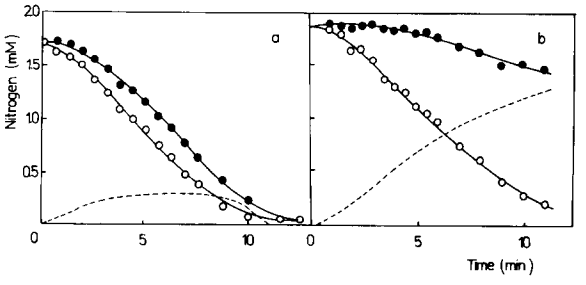

Fig. 3. The concentration dependence of nitrogenous compounds during the reduction of nitrite by the cells of *Paracoccus denitrificans* at pH 7.35. See legend to Fig. 1 for the description.

mum could be found when using an artificial electron donor with nitrous oxide reductase. This observation is consistent with previously published results [18].

Competition of terminal acceptors nitrite and nitrous oxide

As a consequence of the fact that both nitrite reductase and nitrous oxide reductase withdraw electrons from the cytochrome *c* region [12,13], competition between the terminal acceptors nitrite and nitrous oxide at the limited electron influx into the terminal region of the respiratory chain can be expected. Consequently, Alefounder et al. [9] observed that the addition of nitrous oxide strongly inhibits the reduction of nitrite by the cells of *P. denitrificans* at pH 7.3. In our experiments we found that the inhibitory effect of exogenous nitrous oxide upon the nitrite reduction was markedly dependent on the pH. Whereas at pH 8.1 similar result as in Ref. 9 could be obtained, there was no influence of exogenous nitrous oxide on nitrite reduction at pH 6.4 (not shown).

The interaction of nitrite and nitrous oxide with the respiratory chain of *P. denitrificans* at different pH values was also investigated by means of measuring he extent of cytochrome *c* reduction in a stationary state, using the same procedure as in previous studies [13,14,21,22]. The experimental results in Fig. 6 provide evidence that with increasing pH in the range of pH 6 and pH 8, the extent of cytochrome *c* reduction also increases. However, the slope of this dependence was different for individual terminal acceptors, and increased in the range nitrous oxide (nitrous oxide reductase operating), nitrite (both nitrite reductase and nitrous

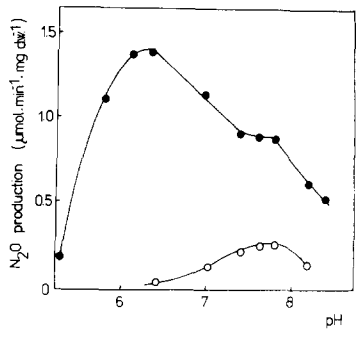

Fig. 5. The pH dependence of the conversion rate of nitrite to nitrous oxide with the physiological donor alone (succinate) and in its combination with the artificial donor (TMPD plus ascorbate). 2.6 ml of reaction medium contained 2.8 mM acetylene and 1 mg dry weight (d.w.) of bacteria. After oxygen was exhausted nitrite was added in the final concentration of 1.8 mM and the increase of nitrous oxide concentration was registered. Donors: 5 mM succinate (○), 5 mM succinate and 0.4 mM TMPD plus 7.4 mM ascorbate (●).

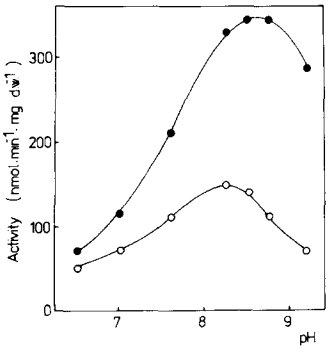

Fig. 4. The dependence of nitrite reductase and nitrous oxide reductase activities on pH. The reaction mixture (2.6 ml) contained 0.1 M sodium phosphate of a given pH value, 5 mM sodium succinate as a substrate and 5.5 mg dry weight (d.w.) of bacteria. In the following the activity of nitrite reductase (○) the initial concentration of nitrite was 1.8 mM, at measuring the activity of nitrous oxide reductase (●) nitrous oxide was introduced in the 1.2 mM concentration. For measurement conditions, see Materials and Methods.

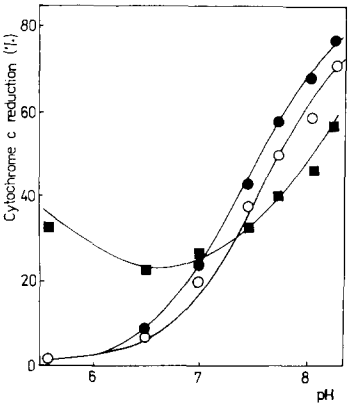

Fig. 6. The pH effect on the cytochrome *c* reduction during utilization of nitrogenous terminal acceptors. For the procedure of measurement see Materials and Methods. In the spectrophotometric cuvette was 3.5 mg dry weight of cells in 3 ml of medium. Further additions were: 1.9 mM nitrous oxide (■), 1.7 mM $NaNO_2$ (○), 1.7 mM $NaNO_2$ plus 2.5 mM acetylene (●).

oxide reductase operating), and nitrite plus acetylene (only nitrite reductase operating). Further, it is obvious from Fig. 6 that in the vicinity of pH 7.2 the extent of cytochrome *c* reduction when the cells were utilizing nitrite was very close to that when utilizing nitrous oxide. At the lower pH cytochrome *c* (and probably also other respiratory components) was kept more oxidized in the presence of nitrite, whereas at higher pH this was the case in the presence of nitrous oxide.

Does the redox state of cytochrome c affect the distribution of electron flow between NO_2^- and N_2O?

Considering the above results the question arises whether the distribution of the electron flow between NO_2^- and N_2O is determined by the redox state of cytochrome *c* in such a way that at higher reduction degrees (in an alkaline pH) the flux to nitrous oxide reductase is preferred, whereas at relatively oxidized state of cytochrome *c* (in an acidic pH) the flux to nitrite reductase prevails. On examining the possibility we performed detailed measurements of the kinetics of the transient accumulation of N_2O during the reduction of nitrite by the cells of *P. denitrificans* in an acidic medium, when, in the initial phase of the experiment, the enzymes NO_2^- reductase and N_2O reductase are saturated with their terminal acceptors. The results of the experiments are summarized in Fig. 7. In case b the double amount of cells was used in comparison to experiment a. In this way the total activities to nitrite reductase and nitrous oxide reductase were doubled while retaining their proportion; the redox stage of cytochrome *c* was the same in both a and b. It can be seen that the parallel increase in activities of both enzymes participating in the reduction of nitrite to nitrogen resulted in a 'shrinkage' of record b in the direction of the horizontal time axis in comparison to record a; no change in the record was observed in the direction of the vertical concentration axis, this means that the maximum concentration of accumulated N_2O was the same in the two cases. From the comparison of records a and c it follows that a similar effect could be obtained also at a given concentration of cells by the addition of an artificial electron donor TMPD plus ascorbate which brings about an increase in the reduction degree of cytochrome *c* (see Ref. 14 for comparison). Hence, it can be concluded that on changing the degree of reduction of cytochrome *c* in a medium at a given (constant) pH the activity ratio of nitrite reductase vs. nitrous oxide reductase does not change; consequently, there is no change in the distribution of the electron flow over NO_2^- and N_2O as terminal acceptors.

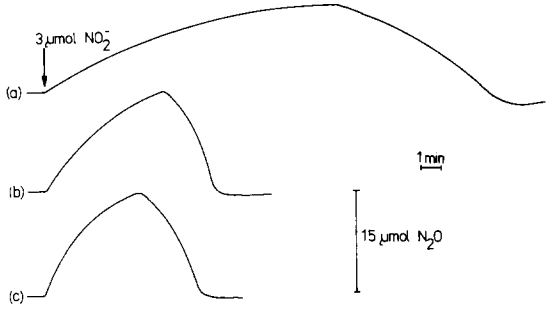

Fig. 7. The time-course of N_2O accumulation during NO_2^- reduction at various amounts of cells and in the presence of an artificial electron donor. The measurements were performed in 0.1 M sodium phosphate of pH 6.4. After oxygen had been exhausted by addition of about 1 mg dry weight of washed bakers yeast, 3.2 mg (records a, c) and 6.4 mg (record b) of *P. denitrificans* cells were introduced. In case c the mixture was supplemented with TMPD (0.05 mM) and ascorbate (10 mM). On starting the reaction with 3 μmol NO_2^- the N_2O concentration was continually registered. The response of the electrode was calibrated by additions of standard solutions of N_2O in an independent experiment.

Discussion

Two enzymes participating in the dissimilatory nitrite reduction to nitrogen were purified from anaerobically grown cells of *P. denitrificans*: nitrite reductase of the cytochrome *cd* type [23,25], and the so far less characterized nitrous oxide reductase [26]. Nevertheless, the question remains what is the product of the nitrite reductase reaction in vivo? During the reduction of nitrite catalyzed by the purified enzyme, nitrogen monoxide as a main reaction product is formed [23–25]. On the other hand, the experiments performed with isotopically labeled terminal acceptors in intact cells revealed that unlike nitrous oxide, nitrogen monoxide did not appear as a free intermediate of denitrification in *P. denitrificans* [27]. The obligatory involvement of nitrous oxide in the course of

denitrification in this bacterium was proved by using acetylene as a specific inhibitor of the nitrous oxide reductase reaction [16,28]. In keeping with these findings are also the results of the presented paper (Fig. 1–3, parts b). Hence the stoichiometric production of nitrous oxide from nitrite during the blockade of the nitrous oxide reductase with acetylene throws doubt on a transient accumulation of other free intermediates (besides nitrous oxide) in an amount exceeding some percent of the initial nitrite concentration at the used pH values.

It has already been observed by several authors that the composition of the gaseous products of denitrifying bacterial cultures is pH dependent; in general the lowering of pH leads to an increased ratio of nitrous oxide over nitrogen [1,2]. In our paper the transient accumulation of nitrous oxide in a slightly acidic medium is also shown with *P. denitrificans* (see Figs. 1–3). This finding is in qualitative agreement with the pH optima of purified nitrite reductase and nitrous oxide reductase, which are reported as 5.5–5.8 [25] and 8.0, respectively [26], and with similar values established in the presence of the artificial donor, TMPD plus ascorbate, with intact cells (Fig. 5, see also Ref. 18). However, in a discussion about this topic it should be mentioned that during utilization of a physiological substrate (succinate) the rate of reduction of terminal acceptors is markedly limited, owing to the influx of redox equivalents into the terminal part of the respiratory chain, the rate of this influx being also dependent on pH. This statement is supported by: (i) the several times higher rates of nitrite and nitrous oxide reduction observed when using the artificial donor TMPD plus ascorbate (Fig. 5, [9,16,18]), (ii) the significant shift of the apparent pH optimum of nitrite reductase of the cells in the presence of the artificial donor (compare Figs. 4 and 5), (iii) the course of pH dependences of enzyme activities and the stage of cytochrome *c* reduction (Fig. 4 and 6). The reason of the observed pH dependence of the electron influx to cytochrome *c* cannot be unequivocally established from the results presented. It seems probable that the raise in respiration on increasing the pH value of the medium is a consequence of the drop in the pH gradient across the membrane in the coupled system. Since the terminal region of the respiratory chain, comprising NO_2^- reductase, N_2O reductase and their electron donor cytochrome *c* (*c*-550), is located in the periplasmic space of *P. denitrificans* cells [9,29–31] and as it stands it does not participate in the transfer of protons across the membrane, it cannot be assumed that the alterations in magnitude of the components of protonmotive force, on changing the pH of the medium, would directly influence the distribution of the electron flow from cytochrome *c* to terminal acceptors NO_2^- and N_2O. The resulting distribution is evidently determined only by the external pH value. This conclusion is supported by the results in Fig. 7 where it can be seen that the increase in the rate of electron flow to cytochrome *c* at a constant pH value of the medium brings about only a parallel increase in activities of both reductases, while keeping constant their proportion in activities.

Another point which should be discussed is the fact that under conditions where the influx of electrons into the branching site of the respiratory chain is limited the enzymes nitrite reductase and nitrous oxide reductase probably compete for the redox equivalents and are mutually affected, similarly as in the case when blocking the nitrate reduction by oxygen [32,33] or by nitrite and nitrous oxide as denitrification intermediates [9,14]. In this way, the inhibition of nitrite reduction exerted by nitrous oxide at satisfactorily high pH values as was observed in Ref. 9 and as investigated in this paper can be explained. On the other hand it can be assumed that in the accumulation of nitrous oxide in acidic media, an inhibition of nitrous oxide reductase brought about by the parallel reduction of nitrite plays the key role. Several arguments support this idea. (1) The comparison of time dependences of nitrite and nitrous oxide concentrations in the presence and in the absence of acetylene at pH 6.4 shows (Fig. 1a and b) that during the nitrite reduction the successive reduction of nitrous oxide significantly declines. (2) The activity ratio of nitrous oxide and nitrite reductases rates in cells is, over the pH range tested, markedly higher than 0.5 (Fig. 4); this means (considering the fact that out of two molecules NO_2^-, one molecule N_2O is created) that without mutual influencing of both reductases the accumulation of nitrous oxide would not be possi-

ble. (3) At the pH values where the accumulation of nitrous oxide takes place, the respiratory chain is found to be more oxidized with nitrite (in the presence of acetylene) than with nitrous oxide, which probably indicates the preferential flow of electrons to nitrite under these conditions.

The results in this and previous papers [9,14] show clearly that individual steps of the denitrification pathway are not independent, but can be mutually affected via the change of the redox state of the respiratory components. In view of the enzyme kinetics it means that the denitrification pathway in general cannot be described as a sequence of independent successive reactions governed by the Michaelis and Menten kinetics and designated by V_{max} and K_m values, because of the fact that the maximal rate of a given reaction is dependent on the rates of other reactions catalyzed by the terminal reductases. This conclusion restricts the applicability of the mathematical model of denitrification as published in Ref. 34 which is based on the above-mentioned simplification assuming the independence of partial reactions.

References

1 Bryan, B.A. (1981) in Denitrification, Nitrification and Atmospheric Nitrous Oxide (Delwiche, C.C., ed.), pp. 67–84, John Wiley, New York
2 Knowles, R. (1982) Microbiol. Rev. 46, 43–70
3 Acros, J.C. (1978) Int. Lab. Sept./Oct., 25–38
4 Crutzen, P.J. (1981) in Denitrification, Nitrification and Atmospheric Nitrous Oxide (Delwiche, C.C., ed.), pp. 17–44, John Wiley, New York
5 Mészáros, E. (1981) Atmospheric Chemistry, pp. 62–72, Akadémiai Kiaó, Budapest
6 John, P. and Whatley, F.R. (1977) Biochim. Biphys. Acta 463, 129–153
7 Stouthamer, A.H. (1980) Trends Biochem. Sci. 5, 164–166
8 Ferguson, S.J. (1982) Biochem. Soc. Trans. 10, 198–200
9 Alefounder, P.R., Greenfield, A.J., McCarthy, J.E.G. and Ferguson, S.J. (1983) Biochim. Biophys. Acta 724, 20–39
10 Kučera, I., Křivánková, L. and Dadák, V. (1984) Biochim. Biophys. Acta 765, 43–47
11 Kučera, I., Boubíková, P. and Dadák, V. (1984) Biochim. Biophys. Acta 767, 383–388
12 Lam, Y. and Nicholas, D.J.D. (1969) Biochim. Biophys. Acta 172, 450–461
13 Boogerd, F.C., Van Verseveld, H.W. and Stouthamer, A.H. (1980) FEBS Lett. 113, 279–284
14 Kučera, I., Dadák, V. and Dobrý, R. (1983) Eur. J. Biochem. 130, 359–364
15 Kučera, I., Matyášek, R. and Dadák, V. (1984) Eur. Bioenerg. Conf. Rep. 3A, 17–18
16 Alefounder, P.R. and Ferguson, S.J. (1982) Biochem. Biophys. Res. Commun. 104, 1149–1155
17 Snell, F.D. and Snell, C.T. (1949) Colorimetric Methods of Analysis, p. 804, Van Nostrand, New York
18 Kučera, I., Boublíková, P. and Dadák, V. (1984) Collection Czechoslovak Chem. Commun. 49, 2709–2712
19 Kristjansson, J.K. and Hollocher, T.C. (1980) J. Biol. Chem. 255, 704–707
20 Willison, J.C. and John, P. (1979) J. Gen. Microbiol. 115, 443–450
21 Kučera, I., Laučík, J. and Dadák, V. (1983) Eur. J. Biochem. 136, 135–140
22 Kučera, I. and Dadák, V. (1983) Biochem. Biophys. Res. Commun. 117, 252–258
23 Lam, Y. and Nicholas, D.J.D. (1969) Biochim. Biophys. Acta 180, 459–472
24 Newton, N. (1969) Biochim. Biophys. Acta 185, 316–331
25 Timkovich, R., Dhesi, R., Martinkus, K.J., Robinson, M.K. and Rea, T.M. (1982) Arch. Biochem. Biophys. 215, 47–58
26 Kristjansson, J.K. and Hollocher, T.C. (1981) Curr. Microbiol. 6, 247–251
27 Garber, E.A.E. and Hollocher, T.C. (1981) J. Biol. Chem. 256, 5459–5465
28 Yoshinari, T. and Knowles, R. (1976) Biochem. Biophys. Res. Commun. 69, 705–710
29 Meijer, E.M., Van der Zwan, J.W. and Stouthamer, A.H. (1979) FEMS Microbiol. Lett. 5, 369–372
30 Alefounder, P.R. and Ferguson, S.J. (1980) Biochem. J. 192, 231–240
31 Boogerd, F.C., Van Verseveld, H.W. and Stouthamer, A.H. (1981) Biochim. Biophys. Acta 638, 181–191
32 Alefounder, P.R., McCarthy, J.E.G. and Ferguson, S.J. (1981) FEMS Microbiol. Lett. 12, 321–326
33 Kučera, I., Karlovský, P. and Dadák, V. (1981) FEMS Microbiol. Lett. 12, 391–394
34 Betlach, M.R. and Tiedje, J.M. (1981) Appl. Environ. Microbiol. 42, 1074–1084