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van Niel, Ed; Gottschal, JC

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Oxygen Consumption by *Desulfovibrio* Strains with and without Polyglucose

ED W. J. VAN NIEL* AND JAN C. GOTTSCHAL

Department of Microbiology, University of Groningen, 9751 NN Haren, The Netherlands

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The kinetics of oxygen reduction by *Desulfovibrio salexigens* Mast1 and the role of polyglucose in this activity were examined and compared with those of strains of *D. desulfuricans* and *D. gigas*. Oxidation rates were highest at air saturation (up to 40 nmol of O_2 min^{-1} mg of $protein^{-1}$) and declined with decreasing oxygen concentrations. Studies with cell extracts (CE) indicated that NADH oxidase was entirely responsible for the oxygen reduction in strain Mast1. In *D. desulfuricans* CSN, at least three independent systems appeared to reduce oxygen. Two were active at all oxygen concentrations (NADH oxidase and NADPH oxidase), and one was maximally active at less than 10 μM oxygen. In contrast to *D. gigas* and *D. salexigens* strains, the *D. desulfuricans* strains also contained NADH peroxidase and NADPH peroxidase activities and did not accumulate polyglucose under nonlimiting growth conditions. At air saturation, initial activities of the oxidases and peroxidases of cells harvested at the end of the log phase were on the order of 20 to 140 nmol of O_2 min^{-1} mg of $protein^{-1}$. In all strains, these enzymes were relatively stable but were susceptible to inactivation as soon as substrates were added to the assay mixture. Under those conditions, all oxidation activity disappeared after ca. 1 h of incubation. The same finding was observed with whole cells of *D. desulfuricans* CSN and *D. desulfuricans* ATCC 27774, but inactivation was less pronounced with cells of *D. salexigens* Mast1. It appeared that the presence of polyglucose in the whole cells retarded the process of inactivation of NADH oxidase, but this property was lost in crude CE. In spite of the effect of polyglucose on the oxidative potential, oxygen-dependent growth of *D. salexigens* Mast1 could be demonstrated neither in batch nor in continuous culture.

There have been only a few studies on the presence of polysaccharides in sulfate-reducing bacteria (SRB). Stams et al. (32) observed the accumulation of polyglucose in several *Desulfovibrio* species and *Desulfobulbus propionicus*. In *Desulfovibrio vulgaris* Hildenborough and *D. baculatus* HL21, polyglucose was produced when growth was limited by Fe^{2+} or NH_4^+. In *D. gigas* (32) and *D. salexigens* Mast1 (35), polyglucose accumulated in high quantities under nonlimiting growth conditions. Both organisms were able to convert polyglucose anoxically and with oxygen as an electron acceptor (29, 35).

Various SRB are aerotolerant to some degree (6, 10, 13, 28), and even after prolonged exposure to oxygen many species can resume anoxic growth. Most of them contain superoxide dismutase, and catalase has been detected in some of them (1, 2, 13, 14). Little is known about the enzymes involved in oxygen consumption in SRB. In *D. gigas*, an oxygen reduction chain consisting of an NADH oxidase (NADH rubredoxin oxidoreductase) and a rubredoxin oxygen oxidoreductase has been described (4, 5). NADH oxidase activities have also been observed in *D. desulfuricans* NCIB 8301 (1) and *D. vulgaris* Hildenborough (3). However, Hardy and Hamilton (13) observed oxygen reduction activities in several *D. vulgaris* strains but were unable to detect any NADH oxidase activity. In *D. desulfuricans* CSN, maximum oxygen consumption rates were observed below 10 μM dissolved oxygen (1, 8, 19). It was found that oxygen reduction in this organism takes places in the periplasm and is linked to cytochrome c_3 (5a), as Postgate (26) already had proposed for another *D. desulfuricans* strain.

H_2, various organic compounds, and inorganic sulfur compounds all have been identified as possible substrates coupled to oxygen reduction (7, 22, 35). Although *D. gigas* and *D. desulfuricans* CSN produces ATP under oxic conditions, the coupling of ATP formation to oxygen reduction has been observed only in the latter organism (8, 29). However, truly oxygen-dependent growth has never been demonstrated for these bacteria. In our opinion, this fact includes the recently reported oxygen-dependent growth of *D. vulgaris* Hildenborough (18), in which an approximate 50% linear increase in cell density was observed. This observation is most likely explained by growth at the expense of thiosulfate, produced by chemical reduction of oxygen by hydrogen sulfide, as was concluded much earlier for the growth of *D. vulgaris* DSM 2119 in oxygen sulfide gradient tubes (6).

In a recent paper (35), we reported that *D. salexigens* Mast1 oxidized substrates with oxygen only as long as the cells contained polyglucose. It was therefore hypothesized that *D. salexigens* Mast1, having been isolated from the oxic-anoxic layer of a microbial mat, was dependent on polyglucose to survive during oxic periods (35). We report here the presence of NADH oxidase activity in *D. salexigens* Mast1 and in several other *Desulfovibrio* strains. The NADH oxidases in all of these strains were prone to inactivation as soon as they catalyzed the oxidation of NADH. We further show that the presence of polyglucose in cells of *D. salexigens* Mast1 prolonged the activity of NADH oxidase.

MATERIALS AND METHODS

Microorganisms. The following strains were used: *D. salexigens* Mast1 (from the top layer of a marine microbial mat, Paleohori Bay, Isle of Milos, Greece; isolated from anoxic batch enrichment cultures on alanine [35]), *D. salexigens* Mast2 (same origin as strain Mast1; isolated from anoxic continuous enrichment cultures on alanine), *D. salexigens* DSM 2638 (obtained from the Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany), *D. gigas* NCIMB 9332, *D. desulfuricans* BH, and *D. desulfuricans* ATCC 27774 (kindly provided by T. Hansen), and *D. desulfuricans* CSN (kindly provided by H. Cypionka, Oldenburg, Germany).

* Corresponding author. Present address: Department of Applied Microbiology, Center for Chemistry and Chemical Engineering, Lund University, P.O. Box 124, S-221 00 Lund, Sweden. Phone: 46 46 222 8325. Fax: 46 46 222 4203. E-mail: ed.van_niel@tmb.lth.se.

Cultivation. The *D. salexigens* strains were cultivated on basal morpholinepropanesulfonic acid-buffered MPM medium (35) supplemented with various substrates (see Results). All other *Desulfovibrio* strains were grown in a bicarbonate-buffered medium (25) supplemented with trace elements (24), vitamin solution (1 ml liter^{-1}) (15), 10% yeast extract solution (10 ml liter^{-1}), and 1.25% $Na_2S \cdot 9H_2O$ solution (10 ml liter^{-1}). The carbon and energy source was either lactate or pyruvate (20 mM), unless stated otherwise. Screw-cap tubes and rubber-stoppered serum bottles (300 to 500 ml) were used for batch cultures and prepared as described by Van Niel et al. (35). When necessary, air was injected with syringes through a sterile filter (0.2 μm) to the headspace. Anoxic chemostat cultures of *D. salexigens* Mast1 were grown on 20 mM alanine at a dilution rate of 0.03 h^{-1} and a temperature of 30°C; the pH was kept at 7.4 by automatic titration with 1 N NaOH. The chemostat had a working volume of 750 ml, and contents were stirred by means of a magnetic stirrer; the headspace of the culture vessel was flushed continuously with N_2-CO_2 gas (80:20, vol/vol) freed of O_2 traces by passage over hot copper turnings. Studies of the possible growth of *D. salexigens* Mast1 under oxic conditions in continuous culture were done with two coupled chemostats. The first one was kept strictly anoxic as described above. The cells were grown on a mixture of 10 mM alanine and 2 mM glucose, and N_2-CO_2 gas was sparged through the culture to strip the sulfide. The effluent of the first fermentor was pumped continuously into the second fermentor, which was kept under oxic conditions. The oxygen concentration in this fermentor was varied by changing the ratio of air and N_2-CO_2 gas. The gas was sparged through the culture, and the oxygen concentration in the liquid was measured continuously with an oxygen electrode. Additional medium (containing 10 mM alanine) was pumped into the second fermentor. Both fermentors were run at the same dilution rate (0.03 h^{-1}).

Preparation of whole-cell suspensions and CEs. At the end of the exponential growth phase, batch cultures (200 to 500 ml) were harvested by centrifugation for 10 min at 25,000 × *g* and 4°C. After being washed with Tris-HCl buffer (50 mM, pH 7.6), the pellet was resuspended in the same buffer to a final protein concentration of 0.5 to 1 g liter^{-1} for experiments with intact cells. For cell extract (CE) preparation, the pellet was resuspended in 0.5 to 2 ml of the same buffer. The cells were broken by two successive passages through a French pressure cell. Then, part of the crude extract was centrifuged for 30 min at 18,000 rpm to obtain the soluble CE fraction. The membrane fraction was washed once with Tris-HCl buffer and finally resuspended in 1 ml of this buffer. The cell suspensions and CEs were stored on ice until use.

Enzyme assays. The rate of oxidation of NADH and NADPH in the presence of oxygen (oxidase activity) was measured spectrophotometrically at 340 nm (ε = 6,220 M^{-1} cm^{-1}). The rate of peroxidation of NADH and NADPH was also measured at 340 nm but under anoxic conditions; for this assay, the reaction was started by the addition of 2 to 4 mM H_2O_2. The assay mixture contained either crude or soluble CE diluted in Tris-HCl buffer (50 mM, pH 7.6) (final volume, 1 ml). NADH and NADPH were added at concentrations of 50 to 500 μM.

Determination of oxygen consumption rates and catalase activity. The oxygen-dependent respiration kinetics of whole cells and in CEs were determined polarographically with a biological oxygen monitor (BOM) (Yellow Springs Instruments Co., Yellow Springs, Ohio). The oxygen concentration was monitored on a paper recorder. The oxygen uptake rates were calculated from the slope of the tangents of the oxygen concentration versus time at various intervals. Catalase activity was determined by adding 2.2 mM H_2O_2 (final concentration) to an anoxic cell suspension in the BOM. The initial oxygen production rate was taken as a measure of catalase activity. The rate was corrected for the rate of chemical decomposition of H_2O_2 in the absence of cells.

Analytical techniques. Cells were counted microscopically with a Bürker-Türk counting chamber after appropriate dilution of culture samples in basal salt medium containing 1% formaldehyde. Polyglucose in washed cells was determined as glucose equivalents after hydrolysis in 2 N H_2SO_4 for 20 min at 120°C with glucose oxidase (Boehringer GmbH, Mannheim, Germany). Polyglucose was not removed from the cells prior to this determination. Ammonium and sulfide were measured colorimetrically according to Richterich (27) and Trüper and Schlegel (33), respectively. Oxygen concentrations in the input and output gases were analyzed with a gas chromatograph (Pye Unicam 104) equipped with a katharometer (thermal conductivity detector) and a Poropack Q (Waters Associates Inc., Milford, Mass.) 100/120-mesh column (4 mm by 1.2 m) as described by Gerritse et al. (11). Protein in intact cells and in CEs was measured according to the microbiuret method (12) and the Bradford method, respectively, with bovine serum albumin as a standard.

RESULTS

Growth in the presence of oxygen. The possibility of the growth of *D. salexigens* Mast1 at the expense of oxygen was studied with cultures that were pregrown under strictly anoxic conditions in both batch and continuous cultures. During the mid-exponential phase of batch cultures on 20 mM alanine, aliquots were distributed over four bottles, of which three were anoxic and one contained air. To two anoxic bottles sterile air was added to provide the cultures with oxygen concentrations of 2 and 5% in the headspace. All bottles were thoroughly shaken during the experiment. At regular intervals, growth and activity were recorded as cell counts and the formation of ammonium resulting from the conversion of alanine. After 20 h, the cells had divided only once at 2% oxygen and alanine was partly consumed (Table 1). At 5% oxygen, only a fraction of alanine was degraded, and at 21% oxygen, no activity could be detected. Since the metabolism of polyglucose in cells of *D. salexigens* Mast1 was shown to allow oxygen reduction activity for a longer time (35), oxygen-dependent growth was also attempted with cells with a high polyglucose content. For this purpose, the organism was pregrown on a mixture of alanine (10 mM) and glucose (2 mM) in an anoxic chemostat. The effluent of this culture was pumped continuously into a second chemostat that was kept under oxic conditions and was supplemented with a fresh supply of alanine. In spite of the fact that the polyglucose in the cells was partly converted (from 0.8 to 0.5 g of polyglucose g of protein^{-1}), no significant growth, measured as protein, could be detected at oxygen concentrations ranging from 1 to 10% in the gas phase.

TABLE 1. Growth and alanine degradation in batch cultures of *D. salexigens* Mast1 with 20 mM alanine after 20 h of exposure to several concentrations of oxygen[a]

O_2 (%)	Alanine (mM)	Increased cell count (10^8 cells ml^{-1})
0	11.7	8.8
2	4.4	2.2
5	0.4	0
21	0	0

[a] Each culture was started with 2.2 × 10^8 cells ml^{-1}.

Oxygen-dependent respiration. To study the kinetics of oxygen consumption of *D. salexigens* Mast1, batch cultures were grown anoxically on pyruvate. The cells were harvested at the end of the exponential growth phase. Washed cell suspensions were aerated and examined for oxygen consumption in the presence of pyruvate. The oxygen reduction rate was highest at air saturation and declined with decreasing oxygen concentrations (Fig. 1A). A similar response was found in the absence of external substrates and with other substrates, such as glycerol and alanine. Identical experiments with other *D. salexigens* strains, *D. gigas*, and several *D. desulfuricans* strains showed similar kinetics and specific oxidation rates (Table 2). Since in an earlier study by Dilling and Cypionka (8) it was shown that highest oxidation rates for several SRB, including *D. desulfuricans* CSN, occurred below 10 μM dissolved oxygen, we also checked the oxygen uptake activity of *D. salexigens* Mast1, *D. gigas*, and *D. desulfuricans* CSN at low oxygen concentrations. Washed cell suspensions were kept anoxic. *D. salexigens* Mast1 was given alanine and successive pulses of 20 μM H_2O_2. Because of high catalase activity (Table 3), the oxygen concentration increased quickly, and the initial oxygen uptake rate was determined after each pulse. The oxygen uptake rate increased with increasing oxygen concentration (Fig. 1B). The same result was obtained with anoxic cell suspensions of *D. gigas* that were sparged with air for only a few seconds. However, when anoxic cell suspensions of *D. desulfuricans* CSN were sparged with air for a few seconds, the rate of oxidation of lactate increased with decreasing oxygen concentration. Highest oxidation rates were obtained with 0 to 10 μM oxygen (Fig. 1C), confirming the observations of Dilling and Cypionka (8). In the present experiments, the oxygen concentrations were kept below 100 μM.

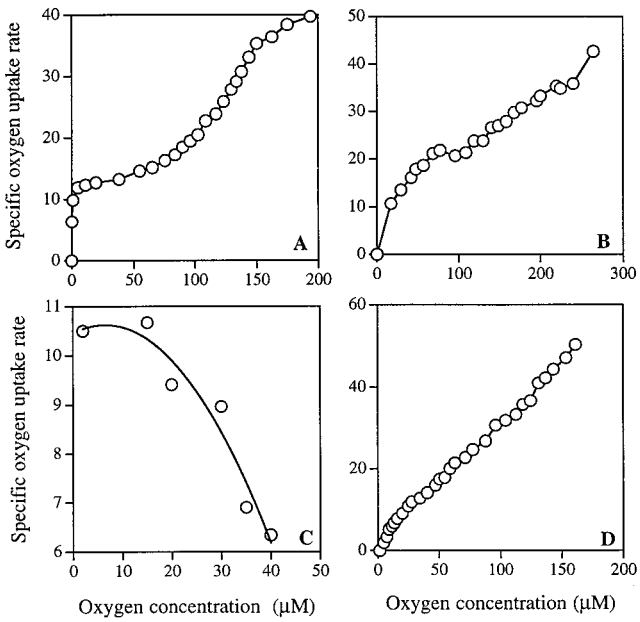

FIG. 1. (A) Oxygen uptake rate as a function of oxygen concentration in a fully aerated cell suspension of *D. salexigens* Mast1 in the presence of 4 mM pyruvate. (B) Oxygen uptake rate in a cell suspension of *D. salexigens* Mast1 in the presence of 4 mM alanine. The cell suspension was initially anoxic. The oxygen concentration was increased by adding successive pulses of 20 μM H_2O_2. (C) Lactate-dependent oxygen uptake rate in a cell suspension of *D. desulfuricans* CSN at a low initial concentration of oxygen. (D) Oxygen uptake rate in a fully aerated CE of *D. salexigens* Mast1 in the presence of 1 mmol of NADH. The rates are expressed in nanomoles of O_2 $minute^{-1}$ milligram of $protein^{-1}$.

Oxygen reduction by CEs. CEs from anoxic cultures of *D. salexigens* Mast1 grown on pyruvate or alanine showed oxygen consumption with pyruvate and NADH or with alanine and NADH, respectively. NADH oxidation activity decreased with decreasing oxygen concentration (Fig. 1D). Similar kinetics of NADH oxidation with respect to the oxygen concentration were found with the other strains. All rates were comparable to the substrate-dependent oxygen uptake rates for whole-cell suspensions (Table 2).

All strains were further examined for the presence of polyglucose as a storage polymer and for the initial specific activities of oxidases, peroxidases, and catalase (Table 3). It appeared that the strains could be divided into two groups. One group accumulated polyglucose and contained NADH oxidase activity only (except for *D. gigas*, which also contained NADH peroxidase activity). The other group did not accumulate polyglucose and contained activities of both NADH and NADPH oxidases and NADH and NADPH peroxidases. All of the organisms of the former group contained catalase activity, while from the latter group only *D. desulfuricans* ATCC 27774 contained catalase activity.

TABLE 2. Initial specific oxygen uptake rates in air-saturated suspensions of whole cells and CEs of several *Desulfovibrio* strains[a]

Organism	Oxygen uptake rate (nmol of O_2 min^{-1} mg of $protein^{-1}$) in:		
	Whole cells		CEs + NADH
	Without substrate	With substrate	
D. salexigens Mast1	12	40 (Pyruvate)	68
D. salexigens Mast2	10	25 (Pyruvate)	21
D. salexigens DSM 2638	8	20 (Pyruvate)	ND
D. gigas NCIMB 9332	10	38 (Lactate)	28
D. desulfuricans ATCC 27774	14	24 (Lactate)	30
D. desulfuricans CSN	4	40 (Lactate)	ND
D. desulfuricans BH	4	16 (Lactate)	ND

[a] Uptake rates for whole cells were measured in the presence or absence (endogenous conditions) of the indicated substrate (4 mM). NADH (1 mM) was the substrate for CEs. No oxygen consumption was present in CEs without NADH. ND, not determined.

Measuring the initial NADH consumption rates in air-saturated CEs at different NADH concentrations revealed Michaelis-Menten kinetics for NADH oxidation. The affinity constants for *D. salexigens* Mast1 and *D. gigas* were 29.6 and 5.7 μM NADH, respectively.

No oxygen reduction activity was found in the membrane fractions of any of the strains tested, and the activities were identical in both crude and soluble CEs.

Enzyme inactivation. When we were studying the kinetics of NADH oxidases, it became apparent that the activities declined in the presence of oxygen, although all substrates were present in excess. This finding was observed for CEs of all strains investigated. For example, the decline in oxygen consumption activity of *D. desulfuricans* CSN in the presence of 1 mM NADH was monitored with the BOM (Fig. 2A). After ca. 1 h, the oxygen concentration had declined from 180 to 13 μM. The activity could be restored neither by aeration nor by additional NADH. It appeared that the enzyme had become inactive. The addition of catalase or mannitol (50 mM) at the beginning of the assay did not influence the rate or extent of inactivation.

Inactivation kinetics for oxygen reduction may be described as follows (21):

$$r(O_2) = [V_{max} \times S_{O_2}/(K_{O_2} + S_{O_2})] \times e^{-kt} \quad (1)$$

where $r(O_2)$ is residual activity, V_{max} is maximum activity, S_{O_2} is the concentration of O_2, K_{O_2} is the saturation coefficient for oxygen, t is time, and k is the inactivation constant ($hour^{-1}$). Applying this formula to the NADH oxidase of *D. desulfuricans* CSN resulted in a value for k of 6.84 h^{-1} (r, 0.989) (Fig. 2A). In similar experiments with soluble and crude CEs of *D. salexigens* Mast1, values of 0.6 to 1.2 h^{-1} were found for k. Inactivation was also found with NADPH oxidase, NADH peroxidase, and NADPH peroxidase (results not shown). The addition of flavin adenine dinucleotide (FAD) (300 μmol) at the start of the assay stimulated maximum NADH oxidation activity by a factor of 1.6 to 2.0 but did not affect the process of inactivation. Product inhibition by NAD^+ was less than 10% at concentrations in the range present in the assay and therefore could be excluded.

Inactivation was not due to instability of the enzyme itself because storage of CEs under oxic conditions in the absence of oxidizable substrate at 0°C overnight resulted in a loss of initial activity of less than 10% (results not shown).

Inactivation of oxygen consumption also occurred with washed cell suspensions. For *D. desulfuricans* ATCC 27774, the inactivation constants were similar with (k, 4.83 h^{-1}; r, 0.998) and without (k, 4.74 h^{-1}; r, 0.997) 4 mM lactate in the medium (Fig. 2B). It should be emphasized that with lactate, the oxidation rate was 10 times higher than under endogenous conditions, and hence about 10 times more oxygen was consumed in total. For *D. salexigens* Mast1, the k values ranged from 0.1 to 0.6 h^{-1}. The values depended on the polyglucose content and not on the presence of external substrates (Fig. 3). The decline in the specific oxygen consumption rate for cell sus-

TABLE 3. Maximum specific activities of several enzymes involved in the reduction of O_2 and H_2O_2 in soluble CEs of several *Desulfovibrio* strains pregrown in anaerobic batch cultures[a]

Organism	Polyglucose (g g of protein^{-1})	Activity (nmol of NADH or NADPH oxidized min^{-1} mg of protein^{-1}) of:				Catalase activity (nmol of O_2 produced min^{-1} mg of protein^{-1})
		NADH oxidase	NADPH oxidase	NADH peroxidase	NADPH peroxidase	
D. salexigens Mast1	0.5	140	0	0	0	770
D. salexigens Mast2	0.1	40	0	0	0	460
D. salexigens DSM 2638	0.3	40	0	0	0	450
D. gigas NCIMB 9332	0.5	59	0	69	0	4,000[b]
D. desulfuricans ATCC 27774	0.0	75	45	120	140	280
D. desulfuricans CSN	0.0	29	25	35	38	0
D. desulfuricans BH	0.0	89	24	40	25	0

[a] O_2 reduction was measured in air-saturated conditions, and H_2O_2 reduction was measured in both air-saturated and anaerobic conditions.
[b] From Bell and LeGall (2).

pensions was clearly related to the decline in the specific NADH oxidase activity as measured in cells of cultures continuously exposed to oxygen (Fig. 3).

DISCUSSION

Despite its oxygen consumption with external substrates and intracellularly accumulated polyglucose, *D. salexigens* Mast1 showed no significant oxygen-dependent growth in either batch or continuous cultures. Only with 2% oxygen in the gas phase was this strain able to double its cell numbers (Table 1), a result similar to what had been found for two *D. desulfuricans* strains (Essex and CSN) and *Desulfobacterium autotrophicum* DSM (22). In a continuous culture, *D. desulfuricans* NCIB 8301 cell numbers increased when oxygen was introduced, with a maximum at 1.5% oxygen (1). The disadvantage of introducing oxygen to a single-chemostat system is the risk of washout. This problem was overcome with the two-chemostat system used in this study. The second, oxic chemostat was continuously supplied with active cells containing high quantities of polyglucose. However, even under these conditions, no oxygen-dependent growth of *D. salexigens* Mast1 was seen.

The strains of *D. salexigens*, *D. desulfuricans*, and *D. gigas* used in this study immediately oxidized various substrates with oxygen upon aeration. Cell suspensions of all strains showed highest oxidation rates at air saturation, but the rates were about 100-fold lower than those observed with aerobic microorganisms (20). The oxidation rates declined with decreasing oxygen concentrations (Fig. 1A). This result is in contrast to that for several *D. desulfuricans* strains (among them strain CSN) whose highest oxygen uptake activity occurred at oxygen concentrations between 0 and 10 μM (1, 8). This contrast was only apparent because the exposure of anoxic cell suspensions of *D. desulfuricans* CSN to low oxygen concentrations (Fig. 1C) confirmed the findings of Dilling and Cypionka (8). This finding was not observed with *D. salexigens* Mast1 (Fig. 1B) and *D.*

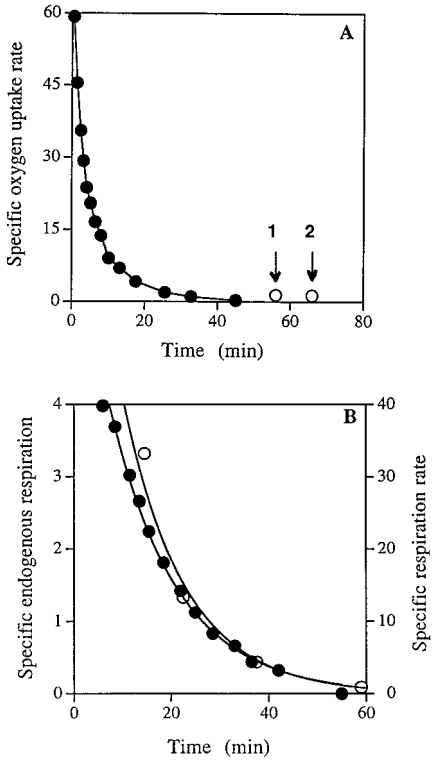

FIG. 2. (A) Time course of the inactivation of NADH oxidase activity in a CE of *D. desulfuricans* CSN in the presence of 1 mM NADH. Arrows: 1, activity after reaeration of the CE; 2, activity after the addition of more NADH. (B) Time course of the inactivation of oxygen uptake in *D. desulfuricans* ATCC 27774 under endogenous conditions (●) and in the presence of 4 mM lactate (○). The rates are expressed in nanomoles of O_2 minute^{-1} milligram of protein^{-1}.

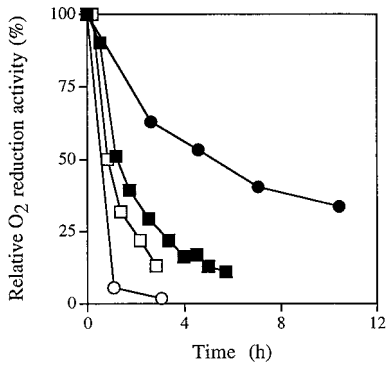

FIG. 3. Inactivation of NADH oxidase activity in cells of *D. salexigens* Mast1 exposed to 10% oxygen in closed bottles that initially contained 0.019 mg of polyglucose mg of protein^{-1} (100% activity is 80 nmol of NADH min^{-1} mg of protein^{-1}) (○) and 0.3 mg of polyglucose mg of protein^{-1} (100% activity is 43 nmol of NADH min^{-1} mg of protein^{-1}) (●) and inactivation of pyruvate-dependent oxygen uptake by cells of *D. salexigens* Mast1 exposed to oxygen in the BOM containing 0.034 mg of polyglucose mg of protein^{-1} (100% activity is 33 nmol of O_2 min^{-1} mg of protein^{-1}) (□) and 0.04 mg of polyglucose mg of protein^{-1} (100% activity is 38 nmol of O_2 min^{-1} mg of protein^{-1}) (■).

gigas. It is likely that at least three independent systems reduce oxygen in *D. desulfuricans* CSN. One system is active only at low oxygen concentrations and is inhibited by high oxygen concentrations. The other two systems are active at all oxygen concentrations. The former system is probably active in the periplasm and linked to cytochrome c_3 (5a). The latter consists of NADH and NADPH oxidases, as was further indicated by the facts that the oxidation activities of NADH and NADPH were found in soluble CEs at all oxygen concentrations and declined with decreasing oxygen concentrations (Table 3 and Fig. 1D) and that the activities were on the same order as the oxygen consumption rates for whole cells. NADH oxidase activity was present in all strains examined (Tables 2 and 3), and all responded similarly to changes in oxygen concentrations. The initial NADH oxidase specific activities for both O_2 (Table 2) and NADH (Table 3) revealed that the NADH/O_2 ratios were 2 for strain Mast1, strain Mast2, and *D. gigas* and closer to 1 for *D. desulfuricans* CSN. These results suggest that the former three organisms contain an NADH oxidase that reduces O_2 directly to H_2O but that the NADH oxidase of the latter organism reduces O_2 to H_2O_2. H_2O_2 can be further reduced to H_2O in this organism because it possesses NADH peroxidase. All of the *D. desulfuricans* strains examined here contained both NADH and NADPH oxidases and peroxidases (Table 3). Also, these strains did not accumulate polyglucose during growth with various substrates. These two properties clearly distinguish them from the *D. salexigens* strains and *D. gigas.*

In all cases, the NADH oxidase activity declined rapidly (ca. 1 h) upon addition of the substrates. The same result was seen with NADPH oxidase, NADH peroxidase, and NADPH peroxidase and with oxygen consumption by whole cells of *D. desulfuricans* strains (Fig. 2). During oxidation reactions with oxygen, it is possible that reactive intermediates which damage the enzyme are formed. Hence, it is expected that the more and the faster that oxidation is taking place, the more extensive will be the damage and thus inactivation. However, for *D. desulfuricans* ATCC 27774 (Fig. 2B), oxygen consumption activity in both the presence and the absence of lactate was inactivated at the same rate, even though the oxidation rate with lactate was 10 times higher than that under endogenous conditions and 10 times more oxygen was consumed. Therefore, it is believed that inactivation in this organism is an intrinsic property of the enzymes and their substrates rather than that it is caused by products or by-products. Inactivation only happened when both oxygen and oxidizible substrates were present. This result suggests that the configuration of the enzyme-substrate complex is prone to denaturation. One of the best-studied NADH oxidases is that of *Streptococcus faecalis*. The NADH oxidase of *D. gigas* (5) and that of *S. faecalis* (30) are quite similar in that both contain FAD and no metals and in that they share sensitivity to sulfhydryl agents. Hoskins et al. (16) found that the instability of the NADH oxidase of *S. faecalis* was due to the gradual removal of FAD. Although the addition of FAD to the CEs used in our study increased the rate of oxidation of NADH, it had no effect on the rate of inactivation.

With whole cells of *D. salexigens* Mast1 it was found that polyglucose prevented the rapid inactivation of NADH oxidase (Fig. 3). The inactivation constant was taken as a measure of the rate of inactivation. From the limited data available, a tentative relationship between the polyglucose content and the inactivation constant could be visualized (Fig. 4); it seemed that even low polyglucose contents caused substantial deceleration of the rate of inactivation of NADH oxidase. For this organism, the highest inactivation constants were found with

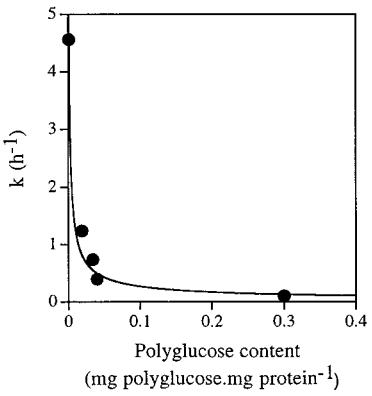

FIG. 4. Inactivation constant (k) of NADH oxidase of *D. salexigens* Mast1 as a function of polyglucose content.

CEs and were the same for crude and soluble CEs. Most of the polyglucose (96 to 98%) was detected in the cell debris after centrifugation of the crude extract. This result indicates that the protective property of polyglucose was lost once the cells were broken up.

We previously showed that *D. salexigens* Mast1 could oxidize pyruvate with oxygen only as long as polyglucose was present in the cell (35), and we could only hypothesize about an explanation for this phenomenon. Based on our present findings, it seems likely that the observed dependency originates from protection against inactivation of NADH oxidase by polyglucose. The actual mechanism still remains to be clarified. It is possible that polyglucose somehow stabilizes the enzyme-substrate complex. Interactions between polysaccharides and enzymes have been described before for other organisms (e.g., 9, 17, 23) and it was noted that such interactions altered enzymatic activity, prevented inhibition, or protected against denaturation. However, to our knowledge, no examples of protection against oxygen damage have been reported.

D. salexigens Mast1 was isolated from the oxic-anoxic interface of a microbial mat exposed to cycles of dark-anoxic and light-oxic conditions. The results of this study suggest that the organism uses polyglucose during oxic periods, in which the environment can become supersaturated with oxygen, to keep the cells in a viable state. Polyglucose is replenished during dark periods. This mechanism for surviving unfavorable periods has already been described for other microorganisms in microbial mats, such as cyanobacteria (31) and purple sulfur bacteria (34). It might explain why *Desulfovibrio* strains were found in large quantities in the top layers of a microbial mat (28). The results also suggest that aerotolerant SRB which do not accumulate polyglucose cannot survive high oxygen concentrations for a long time and hence prefer regions with permanently low or limiting oxygen concentrations. This situation has indeed been found for *D. desulfuricans* CSN (22).

Our study has revealed that polyglucose not only is used as a carbon and energy source but also protects against the rapid inactivation of NADH oxidases. Further studies should be undertaken to reveal how widely spread such a protective mechanism is among anaerobic microbes occasionally exposed to molecular oxygen.

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