Electronic Journal of Biotechnology 48 (2020) 36–45

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

Review

Respiration in *Azotobacter vinelandii* and its relationship with the synthesis of biopolymers

Tania Castillo [a], Andrés García [b], Claudio Padilla-Córdova [c], Alvaro Díaz-Barrera [c], Carlos Peña [a,*]

[a] Departamento de Ingeniería Celular y Biocatálisis, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Apdo. Post. 510-3, Cuernavaca 62250, Morelos, Mexico
[b] Laboratorio de Biotecnología Ambiental, Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos, Av. Universidad 1001, Col. Chamilpa, Cuernavaca, Morelos 62209, Mexico
[c] Escuela de Ingeniería Bioquímica, Facultad de Ingeniería, Pontificia Universidad Católica de Valparaíso, Av. Brasil 2147, Casilla 4059, Valparaíso, Chile

ARTICLE INFO

Article history:
Received 20 April 2020
Accepted 27 August 2020
Available online 4 September 2020

Keywords:
poly(3-hydroxybutyrate)
Azotobacter vinelandii
Alginate
Biopolymers
Gram-negative
Nitrogen fixation
Oxygen
PHB
Respiration
Soil bacterium

ABSTRACT

Azotobacter vinelandii is a gram-negative soil bacterium that produces two biopolymers of biotechnological interest, alginate and poly(3-hydroxybutyrate), and it has been widely studied because of its capability to fix nitrogen even in the presence of oxygen. This bacterium is characterized by its high respiration rates, which are almost 10-fold higher than those of *Escherichia coli* and are a disadvantage for fermentation processes. On the other hand, several works have demonstrated that adequate control of the oxygen supply in *A. vinelandii* cultivations determines the yields and physicochemical characteristics of alginate and poly(3-hydroxybutyrate). Here, we summarize a review of the characteristics of *A. vinelandii* related to its respiration systems, as well as some of the most important findings on the oxygen consumption rates as a function of the cultivation parameters and biopolymer production.

How to cite: Castillo T, García A, Padilla-Córdova C, et al. Respiration in *Azotobacter vinelandii* and its relationship with the synthesis of biopolymers. Electron J Biotechnol 2020;48. https://doi.org/10.1016/j.ejbt.2020.08.001

1. Introduction

Respiration occurs in higher organisms, animals, and plants, as well as most bacteria. Respiration can be defined as the physiological process in which reduced organic substrates are oxidized to release energy, and it entails the flow of electrons through membrane-associated transport systems from a donor to an electron acceptor [1]. The process in eukaryotes occurs in the mitochondria, whereas in prokaryotes, it occurs in the cytoplasmic membrane and involves the donation of electrons by low-redox-potential electron donors, such as NADH [2]. This is followed by electron transfer through a range of redox cofactors bound to integral membrane or membrane-associated protein complexes. The process terminates with the reduction of the high-redox-potential electron acceptor, oxygen. The energy released during this electron transfer process is used to drive the translocation of protons across the membrane to generate a transmembrane proton electrochemical gradient or proton motive force that can drive the synthesis of ATP [3]. The respiration processes of bacteria and archaea are often highly flexible. In these organisms, a diverse range of electron acceptors can be utilized, including elemental sulfur [4,5], nitrogen oxyanions and nitrogen oxides [6], halogenated organics [7], metalloid oxyanions such as selenite, arsenate, Fe(III) and Mn(IV) [8] and radionuclides such as U(VI) and Tc(VII) [9]. This respiratory diversity has contributed to the ability of prokaryotes to colonize many of Earth's most hostile microoxic and anoxic environments.

Azotobacter vinelandii is an obligate aerobe characterized by its high respiration rate in comparison with other prokaryotes under similar growth conditions. This bacterium exhibits a very high respiration rate, and it also fixes nitrogen (N_2), even when it is exposed to high oxygen concentrations [10,11,12,13,14]; this process is performed by the nitrogenase complex, which is highly oxygen sensitive [15,16,17]. Under conditions of nitrogen fixation and high oxygen, the main mechanism proposed to prevent nitrogenase inactivation is respiratory protection. In this mechanism, the oxygen consumption rate is increased, allowing the maintenance of low intracellular oxygen concentrations [18,19].

A. vinelandii exhibits wide versatility in its respiratory system because *A. vinelandii* can regulate its oxygen consumption rates using different terminal oxidases as a function of the culture conditions [20]. This review aims to summarize the recent literature related to the respiration process in several strains of *A. vinelandii* grown under different cultivation conditions from a genetic and physiological point of

* Corresponding author.
E-mail address: carlosf@ibt.unam.mx (C. Peña).
Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

https://doi.org/10.1016/j.ejbt.2020.08.001

view, as well as the technological perspectives based on the understanding of the different mechanisms that operate in the bacterium.

2. Respiration in *A. vinelandii*

A. vinelandii is a soil bacterium characterized for its biotechnological potential, since this microorganism produces alginate and poly(3-hydroxybutyrate) (P3HB) and has the ability to fix atmospheric nitrogen [17,21,22,23]. On the other hand, respiration is tightly controlled at different levels in response to nitrogen and oxygen availability [24,25]. Five terminal oxidases have been identified in the genome of *A. vinelandii*: two copies of cytochrome bd and one copy of cytochromes c, o and cbb3 [12]. These terminal oxidases can be coupled with three different NADH dehydrogenases, which are able to transfer reducing equivalents to ubiquinone-8: NADH dehydrogenase I (NDHI), NADH dehydrogenase II (NDHII) and sodium-translocating NADH dehydrogenase (Na^+-NQR) [26].

2.1. NADH dehydrogenases

As in other bacteria, the NDH-I complex of *A. vinelandii* is formed by the Nuo complex that is formed by at least 13 subunits [12,27], which are encoded by genes from Avin28440 to Avin28560 [12]. *A. vinelandii* NDH-I oxidizes NADH and dNADH but not NADPH [28]. The electron transfer from NADH to Ubiquinone-8 through NDH-I is coupled to proton exchange with a total H^-/e^- yield of 3 (one from NADH oxidation and 2 from the Q-cycle) [29,30]. In *A. vinelandii,* it has been suggested that this dehydrogenase is coupled to the high-efficiency respiratory chains of the o, c or cbb_3 types.

In contrast to NDH-I, the enzyme NDH-II can oxidize NADH and NADPH but not dNADH, although the affinity of this enzyme for NADPH is very low [28]. This enzyme is composed of a single subunit lacking an energy coupling site [27]. In *A. vinelandii,* this enzyme is encoded by the gene *ndhII*, which is downregulated by CydR and is expressed when *A. vinelandii* is grown under high oxygen concentrations and diazotrophic conditions [28].

Finally, the sodium-translocating NADH dehydrogenase (Na^+-NQR) has six subunits (NqrA-F) that are encoded by the *nqr* operon [31]; from these subunits, NqrF shows homology to several flavin-containing oxidoreductases, and it also has an N-terminal domain with a [2Fe-2S] cluster; thus, it has been suggested that NqrF has NADH:ferredoxin oxidoreductase and ferredoxin [32]. Na^+-NQR oxidizes NADH, transferring two electrons to ubiquinone and pumping out 2 sodium ions; thus, the energy conservation of this oxidoreductase is lower than that generated by the NDH-I oxidoreductase [32].

2.2. Respiratory chains

2.2.1. Cytochrome bd and the uncoupled respiratory chain

Azotobacter sp. has been widely studied due to its ability to fix nitrogen by the oxygen-sensitive enzyme nitrogenase, even when grown under high oxygen concentrations. One of the most important molecular mechanisms that protect the nitrogenase complex in this bacterium is the process known as “respiratory protection”, which involves an increase in the respiration rate when exposed to high oxygen concentrations and diazotrophic conditions [33,34,35,36]. This is due to the activity of the uncoupled respiratory chain, which is composed of cytochrome bd and NDH II dehydrogenase [18].

Although two copies of cytochrome bd oxidase have been identified in the genome of *A. vinelandii*, only the complex Cyd bdI, encoded by the genes Avin19880 and Avin1990, has been widely studied [12]. This respiratory complex is characterized by its low oxygen affinity (Km of 0.052 μM) but high oxygen consumption rates and low energetic yields; it has been reported to produce a total yield of only 1 H-/e- [37]. This cytochrome is essential for growth under diazotrophic conditions and high oxygen concentrations, and its expression increases when *A. vinelandii* is grown under these conditions [38,39]. The structural genes of this respiratory chain (*ndhII, cydAI* and *cydBI*) are under the negative control of CydR, which is positively regulated by the protein AlgU ([39]; Fig. 1). Therefore, mutant strains impaired in *cydR* overexpress the genes *cydAI* and *cydBI* [39]; in this regard, the OP strain, which has a spontaneous mutation in *algU*, overexpresses the gene *cydAI* in contrast with its derivative *algU +* [40].

2.2.2. Cytochromes o, c and cbb_3 and the coupled respiratory chains

In addition to the uncoupled respiratory chains, based on the *A. vinelandii* genome, three terminal oxidases have been identified: cytochrome c (Cdt oxidase; Avin00950 to Avin01020), cytochrome o (Cox oxidase; Avin11170 to Avin11180) and cytochrome cbb_3 (Cco oxidase; Avin19940 to Avin20010) [12]. These oxidases can undergo ubiquinol-oxygen reduction in a single step pathway by transferring electrons directly from the ubiquinone complex to one of the terminal oxidases or through a two-step pathway that involves electron transfer through cytochrome c reductase [12].

The difference between cytochrome bd and the o-type cytochrome is the higher oxygen affinity (Km of 0.03 μM); cytochrome bd is expressed when the oxygen concentration is low, suggesting that this cytochrome contributes to maximizing energy conservation [38] due to the transport of one electron from NADH to oxygen through the chain formed by NADH dehydrogenase I, ubiquinone-8 and cytochrome type-o, allowing translocation of up to 5 H^+ (Fig. 1). Another important characteristic is that this cytochrome is not required for growth under diazotrophic conditions even in the presence of air [41].

3. Design of mutant strains of *A. vinelandii* as a tool to understand respiratory features

The construction of mutant strains of *A. vinelandii* has been useful for understanding the respiratory chains, as well as the efficiency of energy conservation. In addition, the high respiration rates achieved by this bacterium have been a disadvantage for its use at the industrial level; consequently, an alternative means of dealing with the high respiration rates of this bacterium is to design mutant strains with low respiration rates. As previously mentioned, the respiratory chains of *A. vinelandii* include five terminal oxidases. Table 1 summarizes some of the mutant strains with the changes made on these oxidases. In this context, Kelly et al. [42] evaluated the parental strain UW136 and its derivative strains with three different phenotypes: expressing all the cytochromes (MK1), with an impairment in cytochrome d (MK5) and a mutant with increased content of cytochrome d (MK8). The study observed that the parental strain and the MK1 strain grew well on either Burk-Sucrose medium with ammonium (BSN) or Burk-Sucrose medium without a fixed nitrogen source (BS) and incubated in either air or under an atmosphere of 1.5% O_2 [42]. In contrast, cytochrome d-deficient mutants (MK5) grew on BSN at high oxygen concentrations, whereas under N_2-fixing conditions, this mutant was able to grow only in an atmosphere with a low oxygen concentration (1.5%), showing a higher sensitivity to oxygen when fixing nitrogen than its parental strain (UW136) and the mutant strain MK1. Interestingly, as shown in Table 1, the respiration rates with NADH and malate for mutant MK5 were approximately 10- and 25-fold lower than those for the parent strain UW136 [42]. In contrast, the mutant MK8, which showed higher levels of cytochrome d (3-fold) but lower concentrations of cytochrome c (60%) compared with the wild-type strain, failed to grow in an atmosphere with low oxygen concentrations (1.5%) on either BSN or BS medium [42]. In this line, McInerney et al. [43], by chemical mutagenesis, generated a mutant strain, TZN200, with a higher content of cytochrome d (5-fold) and lower content of cytochrome c (46%) and cytochrome o (71%) compared to the parental strain (OP). Using NADH and succinate as substrates, the respiration rates of this mutant were 2.3- and 2.5-fold

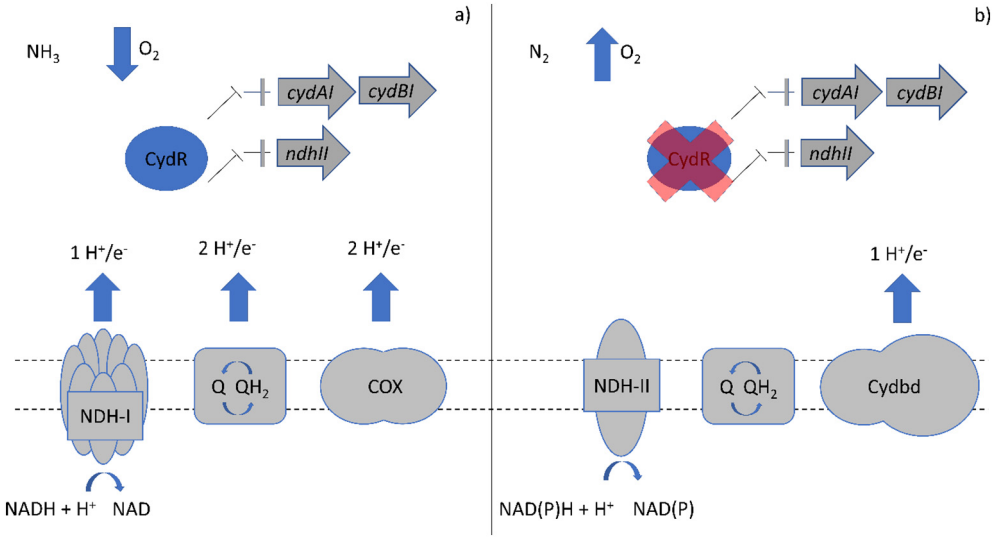

Fig. 1. In panel (a) a scheme of the coupled respiratory chain is shown. This is active in presence of NH_3 and at low oxygen concentrations, due to expression of *cydAB* is repressed by CydR; this chain has a global H^+/e^- yield of 5. The uncoupled respiratory chain of *A. vinelandii* is represented in panel (b), this is expressed in presence of atmospheric nitrogen and high oxygen concentrations, due to the inactivated of the negative regulator CydR.

higher, respectively, than the parental strain (OP) using the same substrates; however, the respiration rate using Ascorbate + $Ph(NMe_2)_2$, which is dependent on cytochrome c, was 94% lower in the TZN200 strain than in the parental strain. The growth rate and the H/O ratio were similar for both strains (OP and the mutant TZN200) when they were grown under nonlimiting oxygen conditions, confirming that the growth of *A. vinelandii* under these growth conditions does not require c- or o-type cytochromes. On the other hand, Hoffman et al. [44] reported a mutant strain of *A. vinelandii* lacking cytochrome c (AV-90); the respiration rate (Table 1) and the H/O quotient of this strain with diverse substrates did not show significant differences compared to that of the OP strain, suggesting that this cytochrome does not play an important role in the oxidative metabolism or in energy conservation of *A. vinelandii*. Regarding the cytochrome o-type oxidase, Leung et al. [41] inactivated the gene *cyoB*, which encodes the o-type oxidase. In the resultant strain (DL10), the respiration rate decreased 47% with respect to its parental strain UW136, suggesting that this cytochrome oxidase could play an important role in oxidative metabolism. It has been proposed that due to its high affinity to oxygen, under microaerobic conditions or during stationary growth phase, the type-o cytochrome could be functionally more important than cytochrome bd; in this line, at the early stationary growth phase of cultivation, the cytochrome d content of the UW136 strain decreases 4-fold, whereas the cytochrome d content of the cytochrome o-impaired mutant increased two-fold.

4. Culture parameters affecting respiration in *A. vinelandii*

One of the main challenges of the cultivation of *A. vinelandii* at larger scales is the inability to reach a high biomass because of its characteristically high respiration rates. The respiration rates of *A. vinelandii* are determined by the strain, the specific growth rate, the oxygen availability, and the nitrogen source (Table 2). The nitrogen source is one of the most important parameters that could have an impact on the respiration rates of *A. vinelandii* due to the high energetic cost that implies the cellular maintenance of cultures grown under diazotrophy and at high oxygen concentrations because of respiratory protection [17,23].

As shown in Table 2, several authors have reported that *A. vinelandii* strains exhibit high respiration rates (qO_2), specifically when they were grown under diazotrophy and high oxygen concentrations (dissolved oxygen tension (DOT) ≥ 10%) [25,45,46]. In chemostat cultivations, Post et al. [25] observed a linear increase in qO_2 values within a DOT range of 0 and 30%. With DOT values above 30%, no significant changes in qO_2 values were achieved, and this behavior was observed in cultures with NH_3 or under diazotrophic conditions. These authors observed that, at low concentrations of sucrose (3 g L^{-1}) and a DOT of 25%, the qO_2, under diazotrophic conditions, was two-fold higher than the qO_2 in the presence of NH_3 [25]; however, when *A. vinelandii* OP was grown with a higher sucrose concentration (15 g L^{-1}), similar qO_2 values were achieved with N_2 or NH_3, suggesting that under the latter conditions, the C/N ratio has an important role. Similarly, Bühler et al. [45] found that at low DOT (5%), the qO_2 value in cultures of the OP strain was only 24 mmol g_P^{-1} h^{-1}, and it was unaffected by changes in the C/N ratio, which ranged between 5 to 10. In contrast, when the same strain was exposed to a DOT of 60%, the qO_2 increased by 45% from 120 mmol g_P^{-1} h^{-1} at a C/N ratio of 5 mol_S mol_{NH4}^{-1} to 174 mmol g_P^{-1} h^{-1} at a C/N ratio of 10 mol_S mol_{NH4}^{-1}. In this regard, Inomura et al. [47] modeled the respiration rates and nitrogen fixation of *A. vinelandii* at different C/N ratios and DOT values and found three metabolic stages based on laboratory data: a carbon limited phase, which is present for low C/N ratios and characterized by low respiration rates; ammonium-limited growth for intermediate C/N ratios, in which there is an increase in the respiration rates as a function of the C/N ratio; and for high C/N ratios, growth based on nitrogen fixation at which the highest respiration rate is achieved and remains constant as the C/N ratio increases. In this model, the C/N ratio at which nitrogen fixation occurs is oxygen dependent; thus, at 30% DOT, the C/N ratio at which nitrogen fixation occurs is 5, whereas at 60% DOT, nitrogen fixation starts with a C/N ratio of 7 [47].

The impact of the oxygen concentration on the respiration rates of *A. vinelandii* has also been described by Bühler et al. [45] and Linkerhagner and Oelze [46]. Bühler et al. [45] explained that the qO_2 values in the cultivations conducted at the highest DOT (60%) were up to 10-fold higher than those achieved at the lowest DOT (5%) (Table 2). On the other hand, Linkerhagner and Oelze [46] reported that in cultivations conducted under diazotrophic conditions, an increase in the DOT from 27 to 54% was reflected in an increase in the qO_2 from 168.6 mmol g_P^{-1} h^{-1} to 258 mmol g_P^{-1} h^{-1}, respectively. In a related study, Lozano et al. [48] reported that the qO_2 value increased 3-fold as a function of the DOT (within a range between 0.5 and 5%) in batch cultivations when the agitation rate was kept constant at 300

Table 1
Strains of *A. vinelandii*.

Strain	Genotype	Phenotype	Main characteristics	*In vitro* respiration rates	Reference
O	Native strain	Mucoid	N.D.	N.D.	[87]
OP	Natural *algU*- mutant of O strain	Non mucoid	This strain is able to grow under diazotrophy at high oxygen concentrations.	Quantified in membrane vesicles with malate: 66.6 mmol g^{-1} h^{-1}; with NADH: 40.8 mmol g^{-1} h^{-1}	[44]
				Quantified in membrane vesicles with succinate: 14.4 mmol g^{-1} h^{-1}; with NADH: 72 mmol g^{-1} h^{-1}	[43]
OPN	OP derivative ptsN::Km ^{r}	Non mucoid	P(3HB) overproducer	N.D.	[88]
OPNA	Double OP mutant strain *ptsN*::*Km*^{r} and *rsmA*::*Sp*^{r}	Non mucoid	P(3HB) overproducer	N.D.	[86]
OP AlgU+	OP derivative *algU::Km* ^{r}	AlgU+ Mucoid	This strain shows a similar alginate production as ATCC9046	N.D.	[40]
UW136	Natural rifampin-resistant mutant of OP	Non mucoid	High recombination index	Quantified in membrane vesicles with malate: 15 mmol g^{-1} h^{-1}; with NADH 15 mmol g^{-1} h^{-1}	[42]
				Quantified in fresh cell suspension with glucose: 0.52 mmol g^{-1} h^{-1}.	[41]
MK1	UW136:Tn5-B20 *Rif*^{R} *Km*^{R}		This strain showed a similar content of cytochromes as compared to its wild type.	N.D.	[42]
MK5	UW136 *cydB::Tn5-B20 Rif*^{R} *Km*^{R}	Cyd-	This strain was unable to grow in BS under high oxygen concentrations.	Quantified in membrane vesicles with malate: 0.6 mmol g^{-1} h^{-1}; NADH: 1.5 mmol g^{-1} h^{-1}	[42]
MK8	UW136 *cydR::Tn5-B20 Rif*^{R} *Km*^{R}	Cyd+	Showed a higher level of cytochrome d was able to grow in air on BSN and BS medium. It did not grow in an atmosphere of 1.5% O_2 on either BSN or BS medium.	N. D.	[42]
DL10	UW136 *cyoB::Tn903 Rif*^{R} *Km*^{R}	cytochrome o^{-}	Showed a high level of cytochrome bd but no detectable amounts of cytochrome o, this mutant was able to fix dinitrogen in presence of air	Quantified in fresh cell suspension with glucose: 0.27 mmol g^{-1} h^{-1}.	[41]
AV-90	Ph(NMe_2)$_2$-oxidase-	cytochrome c^{-}	This mutant was able to grow under diazotrophic conditions in presence of air; showed a lower growth at low oxygen concentrations as compared to its parental strain.	Quantified in membrane vesicles with malate: 58.2 mmol g^{-1} h^{-1}; with NADH: 43.2 mmol g^{-1} h^{-1}	[44]
TZN200	Mutant isolated after treatment with N-methyl-N′-nitro-N nitrosoguanidine.	Cyd+ Unable to reduce Tetrazolium red	In this mutant, concentration of cytochrome d showed an increase of 5-fold and a decrease of 46% of cytochrome c with respect to the wild type OP.	Quantified in membrane vesicles with succinate: 36.6 mmol g^{-1} h^{-1}; with NADH: 164.4 mmol g^{-1} h^{-1}	[43]
ATCC 9046	Native strain	Mucoid	High alginate production	N.D	[89]
AT9	ATCC 9046 *mucG::Tn5*	Mucoid	Hypermucoid-colony phenotype	N.D	[14]
ATCN4	ATCC 9046 *nqrE::Tn5*	Mucoid	Hypermucoid-colony phenotype	N.D	[26]
AEIV Also known as strain E	Native strain	Mucoid	Low alginate production	N.D	[26]
GG9	AEIV *mucG::Tn5*	Mucoid	Hypermucoid-colony phenotype	N.D	[71]
GG101	AEIV *ubiA::Tn5*		Hypermucoid-colony phenotype	N.D	[74]
DSM 93-541b	Native strain	Mucoid Non-flagellated	High alginate production	N.D	[49]

rpm. In contrast, these authors did not observe significant differences in the qO_2 value within the same DOT range but used an agitation rate of 700 rpm. In accordance with these results, Sabra et al. [49] observed a linear increase in the qO_2 values achieved by *A. vinelandii* in chemostat cultivations for DOT values within a range of 0.5 to 5% (Table 2); however, no significant differences were found for DOT values in the range of 5 to 10% [49]. It must be emphasized that under these DOT values (5–10%), the dilution rate in chemostat cultivations influences the respiration rate. Sabra et al. [49] used diazotrophic and phosphate-limited chemostats and observed that the qO_2 value increased 2.33-fold as a function of the dilution rate within a range from 0.08 to 0.26 h^{-1} (Table 2).

On the other hand, the effect of the phosphate concentration on the qO_2 value was evaluated by Linkerhagner and Oelze [46], who found that, at the same DOT (54%), the highest qO_2 (258 mmol g_P^{-1} h^{-1}) was reached with a low phosphate concentration (5.8 mg L^{-1}); while an increase in the phosphate concentration to 580 mg L^{-1} was reflected by a reduction in the qO_2 value to 171 mmol g_P^{-1} h^{-1}. A similar behavior was described when the DOT of cultivations decreased to 27% (68 μM); at this oxygen concentration, the qO_2 was 168.6 mmol g_P^{-1} h^{-1} when the phosphate concentration was 5.8 mg L^{-1}, but the qO_2 decreased to 82.2 mmol g_P^{-1} h^{-1} in those cultivations developed at the highest phosphate concentration (580 mg L^{-1}) [46].

From these results, it is clear that, in addition to the nitrogen fixation and the high oxygen concentrations, the respiration rates of *A. vinelandii* are also affected by the phosphate concentration, as well as by the C/N ratios or the specific growth rates, and these could be parameters for the design of cultivation strategies that would allow the minimization of oxygen consumption rates, ensuring that cultivations were not phosphate or nitrogen limited either in chemostat or batch cultivations [50]. In this regard, Díaz-Barrera et al. [51] reported on the cultivation of two different *A. vinelandii* strains (OP and ATCC9046) grown in bioreactors with a complex nitrogen source (yeast extract) and oxygen limitation (DOT ≈ 0%), and qO_2 values of 9.5 and 13.3 mmol g^{-1} h^{-1} were obtained, respectively; these values are within the range reported for other bacterial strains such as *Bacillus thuringensis* [52], *Escherichia coli* [53] and *Pseudomonas* [54].

Table 2
Comparison of specific oxygen consumption rates (qO_2) in *A. vinelandii* strains grown at different growth conditions. C/N ratios are expressed as mol of sucrose per mol of NH_4. Values of qO_2 are expressed as mmol per gram of biomass per hour and (*) is for those qO_2 expressed as mmol per gram of protein per hour.

Strain	Nitrogen source	Growth conditions	qO_2 (mmol g^{-1} h^{-1})	Reference
Chemostat cultivations				
DSM 93-541b	Diazotrophy	D = 0.08 h^{-1} DOT = 0.5%	7.5	[49]
		D = 0.26 h^{-1} DOT = 0.5%	15	
		D = 0.08 h^{-1} DOT = 5–10%	30	
		D = 0.26 h^{-1} DOT = 5–10%	70	
ATCC9046	Diazotrophy	300 rpm (1 vvm) D = 0.08 h^{-1}	2.2	[90]
		500 rpm (1 vvm) D = 0.08 h^{-1}	4.8	
ATCC9046	Diazotrophy	600 rpm (1 vvm) D = 0.09 h^{-1}	15	[91]
ATCC9046	NH_4	300 rpm (1 vvm) D = 0.08 h^{-1}	3.7*	[85]
		700 rpm (1 vvm) D = 0.08 h^{-1}	20*	
ATCC9046	NH_4	DOT of 8% D = 0.1 h^{-1}	45.3	[72]
OP	Diazotrophy	DOT = 25% Sucrose concentration = 3 g L^{-1}	120*	[25]
		DOT = 25% Sucrose concentration = 15 g L^{-1}	187*	
OP	Diazotrophy	DOT = 27% Phosphate = 580 mg mL^{-1} D = 0.1 h^{-1}	82.2*	[46]
		DOT = 27% Phosphate = 5.8 mg mL^{-1} D = 0.1 h^{-1}	168.6*	
		DOT = 54% Phosphate = 580 mg mL^{-1} D = 0.1 h^{-1}	171*	
		DOT = 54% Phosphate = 5.8 mg mL^{-1} D = 0.1 h^{-1}	258*	
OP	NH_4	DOT = 5% C/N = 5	≈ 24*	[45]
		DOT = 5% C/N = 10	≈ 24*	
		DOT = 60% C/N = 5	≈ 120 *	
		DOT = 60% C/N = 10	≈ 174 *	
OP	NH_4	DOT = 25% Sucrose concentration = 3 g L^{-1}	≈ 60*	[25]
		DOT = 25% Sucrose concentration = 15 g L^{-1}	≈ 195*	
Batch cultivations				
ATCC9046	Diazotrophy	200 rpm 10 mL of filling volume/250 mL of total volume	55	[40]
ATCC9046	NH_4	DOT of 5% (300 rpm)	24	[48]
		DOT of 0.5% (300 rpm)	8.5	
		DOT of 5% (700 rpm)	29	
		DOT of 0.5% (700 rpm)	28	
ATCC9046	Yeast extract	200 rpm 100 mL of filling volume/500 mL of total volume	12	[60]
		100 rpm 100 mL of filling volume/500 mL of total volume	5.2	
ATCC9046	Yeast extract	600 rpm (1 vvm)	13.3	[51]
OP	Diazotrophy	200 rpm 10 mL of filling volume/250 mL of total volume	80	[40]
OP	NH_4	DOT of 4% 450 rpm 1.5 L min^{-1}	148.9	[61]
OP	Yeast extract	600 rpm (1 vvm)	9.5	[51]
OPNA	Yeast extract/Peptone	DOT = 4% 700 rpm (1 vvm)	20*	[86]

5. Alginate and P(3HB) production as a function of oxygen consumption and the relationship with their chemical properties

A. vinelandii is a microorganism of biotechnological interest because of its ability to produce alginate, an extracellular polysaccharide with properties as a viscosifyer and gelling agent. Alginate is formed by units of mannuronic acid and its epimer guluronic acid, and mannuronic units can be acetylated. It is important to point out that the viscosifying and gelling capabilities of alginates largely depend on the chemical structure, the relative content of the two monomers (G and M), the degree of acetylation, and the mean molecular weight (MMW) of the polymer [55,56].

During alginate biosynthesis, per monomeric unit, one ATP molecule and one GTP molecule are consumed, and two molecules of NADH are released. Thus, although this process has a high demand for energy, it also contributes to reducing power generation, and it has been suggested that alginate biosynthesis imposes a high rate of oxygen consumption on the cell, correlated to the energy demand for the synthesis of this polymer [57].

On the other hand, P(3HB), an intracellular biopolyester, can be used as a substitute for conventional plastics derived from the petrochemical industry. P(3HB) is a polymer composed of units of 3-hydroxybutyrate. Each monomeric unit is synthesized from the condensation of two molecules of acetyl-CoA into acetoacetyl-CoA and then reduced to 3-hydroxybutyryl-CoA by oxidation of one molecule of NADPH.

It is important to point out that the genes *algU* and *cydR*, which are involved in the transcriptional regulation of the genes *cydA* and *cydB*, are also involved in the transcriptional regulation of the alginate and P (3HB) structural genes. While alginate biosynthetic genes are positively regulated by AlgU, CydR negatively regulates the genes related to P(3HB) biosynthesis [58]. Thus, a *cydR*- mutant in *A. vinelandii* causes overexpression of β-ketothiolase and acetoacetyl-coA reductase, promoting the accumulation of P(3HB) throughout the exponential growth rate [59], and in *algU*- mutants in *A. vinelandii* strains are defective in alginate production. Therefore, the biosynthesis of both polymers is highly related to energetic metabolism, in which oxygen has an important role, and the evaluation of respiratory parameters could be of great importance for understanding the distribution of carbon usage in this bacterium.

Along this line, several studies have demonstrated the importance of oxygen availability and oxygen consumption on alginate and P(3HB) biosynthesis ([14,48,60,61,62,63,64,65,66,67]; Fig. 2). Table 3 shows data reported on biopolymer production by *A. vinelandii* growing under different qO_2 conditions.

Specifically, alginate production has been widely studied within a range of DOT values from 0.5 to 10% [48,49,62,68,69]. In addition, alginate production has been studied as a function of the maximum oxygen transfer rate (OTR_{max}), which under constant DOT conditions ($dO_2/dt \approx 0$) is equal to the maximum oxygen consumption rate (OUR_{max}) [48,65,70]. Interestingly, these parameters (OTR_{max}/OUR_{max}) and therefore the qO_2 value not only determine the concentration but also affect the mean molecular weight (MMW) of alginate produced by *A. vinelandii* [14,65,71]. Sabra et al. [49] used chemostat cultivations conducted at a constant DOT of 5% and different dilution rates and observed that the specific alginate production (q_{Alg}) increased from 0.04 g_{Alg} g^{-1} h^{-1} at a qO_2 value of 30 mmol g^{-1} h^{-1} to 0.21 g_{Alg} g^{-1} h^{-1} when the qO_2 value was 70 mmol g^{-1} h^{-1} (Table 3). These authors also observed that the MMW of the alginate increased from 400 to 800 kDa within a qO_2 range from 7.5 to 30 mmol g^{-1} h^{-1} ([49; Table 3). In this line, Díaz-Barrera et al. [61] used chemostat cultivations conducted at 0% DOT and observed that the MMW of the alginate decreased from 1350 to 869 kDa when the qO_2 value increased from 2.2 to 4.8 mmol g^{-1} L^{-1}. In contrast, when DOT was controlled in chemostat cultivations at 8% DOT, the qO_2 value increased from 10.9 to 45.3 mmol g^{-1} L^{-1}, and the MMW increased from 450 to 800 kDa ([72]; Table 3).

In batch cultivations, Díaz-Barrera et al. [70] found that at 0% DOT, the OTR_{max} increased, and therefore the OUR_{max}, which increased

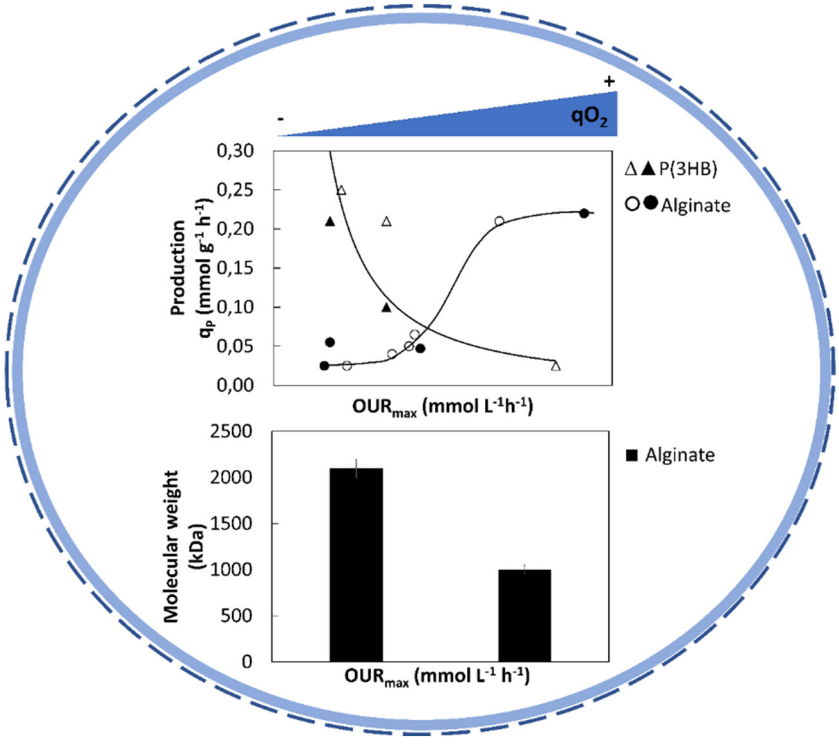

Fig. 2. Model of alginate and P(3HB) production as a function of the oxygen uptake rates in *Azotobacter vinelandii*, in which is observed the direct correlation between the OUR_{max} and alginate production, as well as the inverse correlation between OUR_{max} and P(3HB) production and alginate MMW. (○,△) batch cultures; [40,48,51] (●,▲) chemostat cultures; [49,85,90,91].

Table 3
Comparison of biopolymer production in *A. vinelandii* strains grown at different growth conditions. Values of qO_2 are expressed as mmol per gram of biomass per hour and (*) is for those qO_2 expressed as mmol per gram of protein per hour.

Strain	Type of cultivation	Product	Specific product production (g g^{-1} h^{-1})	MMW (KDa)	qO_2 (mmol g^{-1} h^{-1})	Reference
DSM 93-541b	Chemostat	Alginate	0.04	400	7.5	[49]
		Alginate	0.04	800	30	
		Alginate	0.21	N. D.	70	
ATCC9046	Chemostat	Alginate	0.016	1350	2.2	[90]
		Alginate	0.051	869	4.8	
ATCC9046	Chemostat	Alginate	0.06	500	15	[91]
ATCC9046	Chemostat	Alginate	1.01	N. D.	3.7*	[85]
		P(3HB)	1.15			
		Alginate	1.50	N. D.	20*	
		P(3HB)	0.07			
ATCC9046	Chemostat	Alginate	0.2	450	10.9	[72]
		Alginate	0.18	800	45.3	
ATCC9046	Batch	Alginate	0.018	150	24	[48]
		Alginate	0.02	150	8.5	
		Alginate	0.032	200	29	
		Alginate	0.025	550	28	
ATCC9046	Batch shaken flasks	Alginate	0.5	N. D.	55	[40]
ATCC9046	Batch shaken flasks	Alginate	0.05	2170	13.6	[14]
AT9			0.052	3112	16.4	
GG9			0.037	2300	12.9	
OPALgU+			0046	2100	15.2	
ATCC9046	Batch shaken flasks	Alginate	1.14	1750	12	[60]
		Alginate	0.83	1850	5.2	
OP	Batch	P(3HB)	0.2	N. D.	9.5	[51]
OP	Batch	P(3HB)	0.42	N. D.	49.5	[61]
		P(3HB)	0.63	N.D.	11.6	
OPNA	Batch	P(3HB)	0.25	N. D.	20*	[86]

from 3 to 9 mmol L^{-1} h^{-1}, positively affected alginate production; however, there was an inverse correlation between the MMW of the alginate and the OUR_{max}, and the highest MMW value (1560 kDa) was reached at the lowest OUR_{max} (3 mmol L^{-1} h^{-1}). Similarly, Lozano et al. [48] found that in bioreactor batch cultivations conducted at the same DOT (5%) but different OUR_{max}, the highest alginate production (3 g L^{-1}) was achieved in cultivations conducted at the highest OUR_{max} (100 mmol L^{-1} h^{-1}; $qO_2 = 29$ mmol g^{-1} h^{-1}), although the highest MMW value (550 kDa) was achieved when the OUR_{max} was only 17 mmol L^{-1} h^{-1} ($qO_2 = 28$ mmol g^{-1} h^{-1}) (Table 3). This behavior was also observed by Peña et al. [60] and Gómez-Pazarín et al. [73] in shaken flask cultivations, in which they found a direct correlation between alginate production and OUR_{max} but an inverse correlation between this parameter and the alginate MMW (Table 3). More recently, García et al. [14] found that in shaking flask cultures grown at high OTR_{max} (5 mmol L^{-1} h^{-1}), the highest qO_2 value was achieved in cultures with the AT9 strain; this value was 25% higher than the qO_2 value reached by cultures of GG9 and ATCC 9046 and 8% higher with respect to the culture of the OPAlgU + strain.

Interestingly, *A. vinelandii* mutant strains impaired in the respiratory complex showed an improvement in alginate biosynthesis [26,74,75]. In this regard, the mutant strain ATCN4 (Table 1), which has suppressed Na^+-NQR complex activity due to an insertion within the gene *nqrE* encoding a subunit of this oxidoreductase, showed an alginate-overproducing phenotype [26]. Similarly, the mutant strain GG101 (Table 1) has an interruption in the *ubiA* gene, which encodes the enzyme of the second step of ubiquinone 8 (Q_8), and this mutation reduces the Q_8 content that correlates with a decrease in the OUR_{max} of 8% compared with the wild-type strain. Surprisingly, this mutation was reflected in an increase in the specific alginate production of up to 14-fold [74]. Although this positive effect on alginate production in both mutants could be directly related to a decrease in oxygen consumption, the molecular mechanisms that are involved remain unknown.

Some reports have been published on the influence of oxygen availability on the acetylation degree [76,77]. Castillo et al. [76] used chemostat cultures and showed that the acetylation degree of alginates was higher in the steady-state at 9% DOT than those developed at a low DOT (1%). Díaz-Barrera et al. [72] reported similar evidence in chemostat cultures of *A. vinelandii*. Those authors reported that regardless of the dilution rate, the highest acetylation degree (6% w w-1) was obtained in the chemostat cultures under a DOT of 8% compared to 1%. It is possible that under low oxygen concentrations (and lower oxygen consumption), some enzymes of the tricarboxylic acid cycle (TCA), including citrate synthase and isocitrate dehydrogenase (ICDH), could be downregulated by the accumulation of NADPH + and NADH +, reducing the flux of acetyl-CoA through the TCA cycle [78] and increasing the availability of acetyl-CoA for alginate acetylation processes.

In shaking flask cultures, Peña et al. [60] demonstrated that in cultures conducted at a lower OTRmax (cultures at 100 rpm), an alginate with a high degree of acetylation (5.8% w w^{-1}) was obtained. In contrast, in cultures with a higher OTRmax (cultures at 150 and 200 rpm), the acetylation of the alginate was found to be 4.2% w w^{-1} and 3.5% w w^{-1}, respectively. Using metabolic flux analysis, Castillo et al. [77] revealed that changes in oxygen availability have a considerable impact on metabolic fluxes, which is reflected, among other factors, in the degree of acetylation of alginate. Under low aeration conditions, it was found that the tricarboxylic acid cycle was downregulated, while the phosphoenolpyruvate/pyruvate/acetyl-CoA nodes and the glyoxylate shunt were upregulated. These responses exerted an important effect on both alginate and P(3HB) production, as well as on alginate acetylation.

Studies about the influence of oxygen consumption on the G-M content in alginate are limited [49,79,80]. Sabra et al. [49] used continuous culture and demonstrated that an increase in the DOT led to the formation of alginate with a higher molecular weight and a greater guluronic acid content. Similarly, in cultures of *A. vinelandii* grown to a high DOT (10%), the guluronic acid content was dominant (65–100%) in the alginate structure [80]. Similarly, Moral and Sanin [79] conducted batch cultures of *A. vinelandii* in a bioreactor and demonstrated that GG blocks dominated at both low oxygen transfer rate (cultures at 200 rpm) and high oxygen transfer rate (cultures at 700 rpm).

On the other hand, several studies related to P(3HB) biosynthesis have proposed that oxygen limitation leads to high concentrations of NADH and NADPH, inhibiting the citrate synthase and isocitrate

dehydrogenase activities of the tricarboxylic acid cycle and increasing the concentration of acetyl-CoA available for P(3HB) biosynthesis [78,81]. In addition, iron scarcity has been reported to increase the P (3HB) levels in *A. vinelandii* [82,83]. In this regard, Page and Knosp [84] reported that the mutant strain UWD, which has a mutation in respiratory NADH oxidase, resulted in the ability of the strain to accumulate P(3HB) during the exponential phase without the need for nutrient limitation, supporting the fact that an imbalance in reducing power leads to P(3HB) accumulation [61].

More recently, it was confirmed that in chemostat cultivations of *A. vinelandii* ATCC9046 under oxygen-limiting conditions and therefore under low qO_2 values (3.7 mmol g_P^{-1} h^{-1}), the metabolic flux from pyruvate towards acetyl-CoA is enhanced, showing an increase in P (3HB) production of up to 10-fold compared to the condition of nonoxygen limitation with a qO_2 of 20 mmol g_P^{-1} h^{-1} ([85]; Table 3). It has also been observed that in cultures of the OP strain, a lower qO_2 (at least 30%) was obtained with respect to the alginate producer strain ATCC 9046 cultured under the same growth conditions [51]. This behavior was also observed in the OPNA strain, a P(3HB) overproducer mutant, when cultured in a bioreactor batch under controlled oxygen conditions (4% of DOT) and with a complex nitrogen source. García et al. [86] reported qO_2 values 2.5-fold lower than those obtained with the ATCC9046 strain grown under similar DOT conditions, suggesting that under those growth conditions, the P (3HB) producer strains had a lower demand for oxygen than the alginate producer strain (Table 3).

6. Concluding remarks

This review highlights the importance of analyzing respiration during the cultivation of *A. vinelandii* because this bacterium exhibits rates of oxygen consumption higher than those determined for other prokaryotes, offering a challenge for the cultivation of this bacterium on a large scale. The respiratory system in *A. vinelandii* is very complex and includes cytochromes and dehydrogenases, which are activated in a synchronized way under conditions of limitation and not oxygen limitation, as well as fixation and nonfixation of nitrogen, affecting the energetic efficiency of the bacteria. It has been shown that among the factors of cultivation that influence oxygen consumption in *A. vinelandii*, one of the most important factors is dissolved oxygen tension and the C/N ratio of the medium; it was found that under nonoxygen limitation and nitrogen fixation, the respiration rate and the activity of the uncoupled respiratory chain, which is composed of cytochrome bd and NDH II dehydrogenase, of the bacteria increase. On the other hand, cultures with low C/N ratios are characterized by low respiration rates, whereas for high C/N ratios, growth based on nitrogen fixation and high respiration rates are achieved and remain constant as the C/N ratio increases. It is important to point out the close relationship between respiration and the synthesis of alginate and P(3HB). In the case of alginate production, the bacterium requires high oxygen consumption due to the energy demand for the synthesis of this polymer. However, at low oxygen consumption rates, the accumulation of P(3HB) in the bacterium increases.

Undoubtedly, understanding the regulatory mechanisms controlling respiration in *A. vinelandii* has helped some researchers construct mutants that exhibit very low respiration rates similar to other bacterial strains, such as *Bacillus thuringensis*, *Escherichia coli* and *Pseudomonas*. For the OP and OPNA strains, P(3HB) producers exhibit at least 30% less oxygen consumption than native alginate-producing strains (ATCC9046).

From a commercial viewpoint, it is evident that the production of alginate using A. vinelandii requires a high level of aeration to achieve good polymer yield. This would require the use of energy-expensive sparging techniques or advanced fermenters, such as overpressurization, in which oxygen solubility increases by pressurizing the bioreactor. In this sense, new culture strategies based on the use of mutant strains that exhibit a low consumption of oxygen coupled to cultivation under nutritional conditions that allow the bacterium to reach a better energetic balance in terms of the aeration conditions required during the process can be designed for the production of both biopolymers. In this way, the production costs will be reduced, making the process more competitive and feasible to scale up for commercial use. On the other hand, the possibility of manipulating the chemical characteristics of alginate and P(3HB), and therefore their functional properties, by controlling the availability of oxygen in the culture, offers an alternative with potential for custom-made biopolymers, which can be used for biomedical and pharmaceutical applications.

Conflict of interest

The authors declare that they have no conflict of interest.

Financial support

This work was supported by the project CONACYT-FONCICYT (277600), DGAPA-UNAM (AG200219), and FONDECYT Grant 1170896.

References

[1] Richardson DJ. Bacterial respiration: A flexible process for a changing environment. Microbiology. 2000;144:551–71. https://doi.org/10.1099/00221287-146-3-551.
[2] Hamilton WA. Bioenergetics of sulphate-reducing bacteria in relation to their environmental impact. Biodegradation. 1998;9:201–12. https://doi.org/10.1023/A:1008362304234.
[3] Trumpower BL, Gennis RB. Energy transduction by cytochrome complexes in mitochondrial and bacterial respiration: The enzymology of coupling electron transfer reactions to transmembrane proton translocation. Annu Rev Biochem. 1994;63:675–716. https://doi.org/10.1146/annurev.bi.63.070194.003331.
[4] McAlpine AS, McEwan AG, Bailey S. The high resolution crystal structure of DMSO reductase in complex with DMSO. J Mol Biol. 1998;275:613–23. https://doi.org/10.1006/jmbi.1997.1513.
[5] Lie TJ, Godchaux W, Leadbetter ER. Sulfonates as terminal electron acceptors for growth of sulfite-reducing bacteria (*Desulfitobacterium* spp.) and sulfate- reducing bacteria: Effects of inhibitors of sulfidogenesis. Appl Environ Microbiol. 1999;65:4611–7https://doi.org/10.1128/aem.65.10.4611-4617.1999.
[6] Berks BC, Richardson DJ, Reilly A, et al. The *napEDABC* gene cluster encoding the periplasmic nitrate reductase system of *Thiosphaera pantotropha*. Biochem J. 1995;309:983–92. https://doi.org/10.1042/bj3090983.
[7] Louie TM, Mohn WW. Evidence for a chemiosmotic model of dehalorespiration in *Desulfomonile tiedjei* DCB-1. J Bacteriol. 1999;181:40–6. https://doi.org/10.1128/jb.181.1.40-46.1999.
[8] Lovley DR. Dissimilatory Fe (III) and Mn (IV) reduction. Microbiol Rev. 1991;55:259–87.
[9] Lovley DR, Phillips EJP. Reduction of uranium by *Desulfovibrio desulfuricans*. Appl Environ Microbiol. 1992;58:850–6. https://doi.org/10.1128/aem.58.3.850-856.1992.
[10] Post E, Golecki JR, Oelze J. Morphological and ultrastructural variations in *Azotobacter vinelandii* growing in oxygen-controlled continous culture. Arch Microbiol. 1982;133:75–82. https://doi.org/10.1007/BF00943773.
[11] Oelze J. Respiratory protection of nitrogenase in *Azotobacter* species: Is a widely held hypothesis unequivocally supported by experimental evidence? FEMS Microbiol Rev. 2000;24:321-233. https://doi.org/10.1016/S0168-6445(00)00029-2.
[12] Setubal JC, Dos Santos P, Goldman BS, et al. Genome sequence of *Azotobacter vinelandii*, an obligate aerobe specialized to support diverse anaerobic metabolic processes. J Bacteriol. 2009;191:4534–45. https://doi.org/10.1128/JB.00504-09.
[13] Natzke J, Noar J, Bruno-Bárcena JM. *Azotobacter vinelandii* nitrogenase activity, hydrogen production, and response to oxygen exposure. Appl Environ Microbiol. 2018;84(16):e01208-1218. https://doi.org/10.1128/AEM.01208-18.
[14] García A, Castillo T, Ramos D, et al. Molecular weight and viscosifying power of alginates produced by mutant strains of *Azotobacter vinelandii* under microaerophilic conditions. Biotechnol Rep. 2020;26:e00436. https://doi.org/10.1016/j.btre.2020.e00436.
[15] Carter IS, Dawes EA. Effect of oxygen concentration and growth rate on glucose metabolism, poly-β-hydroxybutyrate biosynthesis and respiration of *Azotobacter beijerinckii*. J Gen Microbiol. 1979;110:393–400. https://doi.org/10.1099/00221287-110-2-393.
[16] Noar JD, Bruno-Bárcena JM. *Azotobacter vinelandii*: The source of 100 years of discoveries and many more to come. Microbiology. 2018;164:421–36. https://doi.org/10.1099/mic.0.000643.
[17] Barney BM. Aerobic nitrogen-fixing bacteria for hydrogen and ammonium production: Current state and perspectives. Appl Microbiol Biotechnol. 2020;104:1383–99. https://doi.org/10.1007/s00253-019-10210-9.
[18] Bertsova YV, Bogachev AV, Skulachev VP. Two NADH:ubiquinone reductases of *Azotobacter vinelandii* and their role in the respiratory protection. Biochim Biophys Acta. 1998;1363:125–33. https://doi.org/10.1016/S0005-2728(97)00094-7.

[19] Kuhla J, Oelze J. Dependency of growth yield, maintenance and Ks-values on the dissolved oxygen concentration in continuous cultures of *Azotobacter vinelandii*. Arch Microbiol. 1988;149:509–14. https://doi.org/10.1007/BF00446753.
[20] Galindo E, Peña C, Núñez C, et al. Molecular and bioengineering strategies to improve alginate and polydydroxyalkanoate production by *Azotobacter vinelandii*. Microb Cell Fact. 2007;6:1–16. https://doi.org/10.1186/1475-2859-6-7.
[21] Kennedy C, Toukdarian A. Genetics of Azotobacters: Applications to nitrogen fixation and related aspects of metabolism. Annu Rev Microbiol. 1987;41:227–58. https://doi.org/10.1146/annurev.mi.41.100187.001303.
[22] Hamilton TL, Ludwig M, Dixon R, et al. Transcriptional profiling of nitrogen fixation in *Azotobacter vinelandii*. J Bacteriol. 2011;193:4477–86. https://doi.org/10.1128/JB.05099-11.
[23] Inomura K, Bragg J, Follows MJ. A quantitative analysis of the direct and indirect costs of nitrogen fixation: A model based on *Azotobacter vinelandii*. ISME J. 2017;11:166–75. https://doi.org/10.1038/ismej.2016.97.
[24] Drozd JW. Respiration and energy conservation in *Azotobacter vinelandii*. FEMS Microbiol Lett. 1978;3:47–9. https://doi.org/10.1111/j.1574-6968.1978.tb01881.x.
[25] Post E, Kleiner D, Oelze J. Whole cell respiration and nitrogenase activities in *Azotobacter vinelandii* growing in oxygen controlled continuous culture. Arch Microbiol. 1983;134:68–72. https://doi.org/10.1007/BF00429410.
[26] Núñez C, Bogachev AV, Guzman G, et al. The Na^+-translocating NADH:ubiquinone oxidoreductase of *Azotobacter vinelandii* negatively regulates alginate synthesis. Microbiology. 2009;155:249–56. https://doi.org/10.1099/mic.0.022533-0.
[27] Yagi T, Yano T, Di Bernardo S, et al. Procaryotic complex I (NDH-1), an overview. Biochim Biophys Acta. 1998;1364:125–33. https://doi.org/10.1016/s0005-2728(98)00023-1.
[28] Bertsova YV, Bogachev AV, Skulachev VP. Noncoupled NADH:ubiquinone oxidoreductase of *Azotobacter vinelandii* is required for diazotrophic growth at high oxygen concentrations. J Bacteriol. 2001;183:6869–74. https://doi.org/10.1128/JB.183.23.6869-6874.2001.
[29] Bertsova YV, Bogachev AV, Skulachev VP. Generation of protonic potential by the *bd*-type quinol oxidase of *Azotobacter vinelandii*. FEBS Lett. 1997;414:369–72. https://doi.org/10.1016/s0014-5793(97)01047-8.
[30] Bertsova YV, Baykov AA, Bogachev AV. A simple strategy to differentiate between H^+- and Na^+-transporting NADH:quinone oxidoreductases. Arch Biochem Biophys. 2020;681. https://doi.org/10.1016/j.abb.2020.108266.
[31] Fadeeva MS, Yakovtseva EA, Belevich GA, et al. Regulation of expression of Na^+-translocating NADH:quinone oxidoreductase genes in *Vibrio harveyi* and *Klebsiella pneumoniae*. Arch Microbiol. 2007;188:341–8. https://doi.org/10.1007/s00203-007-0254-5.
[32] Verkhovsky MI, Bogachev AV. Sodium-translocating NADH:quinone oxidoreductase as a redox-driven ion pump. Biochim Biophys Acta. 2010;1797:738–46. https://doi.org/10.1016/j.bbabio.2009.12.020.
[33] Dalton H, Postgate JR. Effect of oxygen on growth of *Azotobacter chroococcum* in batch and continuous cultures. J Gen Microbiol. 1969;54:463–73. https://doi.org/10.1099/00221287-54-3-463.
[34] Jones CW, Brice JM, Wright V, et al. Respiratory protection of nitrogenase in *Azotobacter vinelandii*. FEBS Lett. 1973;29:77–81.
[35] Haaker H, Veeger C. Regulation of respiration and nitrogen fixation in different types of *Azotobacter vinelandii*. Eur J Biochem. 1976;63:499–507. https://doi.org/10.1111/j.1432-1033.1976.tb10253.x.
[36] Poole RK, Hill S. Respiratory protection of nitrogenase activity in *Azotobacter vinelandii* — Roles of the terminal oxidases. Biosci Rep. 1997;17:303–17. https://doi.org/10.1023/A:1027336712748.
[37] D'Mello R, Purchase D, Poole RK, et al. Expression and content of terminal oxidases in *Azotobacter vinelandii* grown with excess NH_4^+ are modulated by O_2 supply. Microbiology. 1997;143:231–7. https://doi.org/10.1099/00221287-143-1-231.
[38] D'Mello R, Hill S, Poole RK. Determination of the oxygen affinities of terminal oxidases in *Azotobacter vinelandii* using deoxygenation of oxyleghaemoglobin and oxymyoglobin: Cytochrome *bd* is a low-affinity oxidase. Microbiology. 1994;140:1395–402https://doi.org/10.1099/00221287-140-6-1395.
[39] Wu GS, Hill MJ, Kelly G, et al. The *cydR* gene product, required for regulation of cytochrome *bd* expression in the obligate aerobe *Azotobacter vinelandii*, is an Fnr-like protein. Microbiology. 1997;143:2197–207. https://doi.org/10.1099/00221287-143-7-2197.
[40] Castillo T, López I, Flores C, Peña C, et al. Oxygen uptake rate in alginate producer (algU+) and non-producer (algU-) strains of *Azotobacter vinelandii* under nitrogen-fixation conditions. J Appl Microbiol. 2018;125:181–9. https://doi.org/10.1111/jam.13760.
[41] Leung D, Van der Ost J, Kelly M, et al. Mutagenesis of a gene encoding a cytochrome *o* like terminal oxidase of *Azotobacter vinelandii*: A cytochrome *o* mutant is aerotolerant during nitrogen fixation. FEMS Microbiol Lett. 1994;119:351–8. https://doi.org/10.1111/j.1574-6968.1994.tb06912.x.
[42] Kelly MJ, Poole RK, Yates MG, et al. Cloning and mutagenesis of genes encoding the cytochrome *bd* terminal oxidase complex in *Azotobacter vinelandii*: Mutants deficient in the cytochrome *d* complex are unable to fix nitrogen in air. J Bacteriol. 1990;172:6010–9. [https://dx.doi.org/10.1128/jb.172.10.6010-6019.1990].
[43] McInerney MJ, Holmes KS, Hoffman P, et al. Respiratory mutants of *Azotobacter vinelandii* with elevated levels of cytochrome *d*. Eur J Biochem. 1984;141:447–52. https://doi.org/10.1111/j.1432-1033.1984.tb08212.x.
[44] Hoffman PS, Morgan TV, DerVartanian DV. Respiratory properties of cytochrome-*c*-deficient mutants of *Azotobacter vinelandii*. Eur J Biochem. 1980;110:349–54. https://doi.org/10.1111/j.1432-1033.1980.tb04874.x.
[45] Bühler T, Monter U, Sann R, et al. Control of respiration and growth yield in ammonium-assimilating cultures of *Azotobacter vinelandii*. Arch Microbiol. 1987;148:242–6. https://doi.org/10.1007/BF00414819.
[46] Linkerhägner K, Oelze J. Nitrogenase activity and regeneration of the cellular ATP pool in *Azotobacter vinelandii* adapted to different oxygen concentrations. J Bacteriol. 1997;179:1362–7. https://doi.org/10.1128/jb.179.4.1362-1367.1997.
[47] Inomura K, Bragg J, Riemann L, et al. A quantitative model of nitrogen fixation in the presence of ammonium. PLoS One. 2018;13:1–16. https://doi.org/10.1371/journal.pone.0208282.
[48] Lozano E, Galindo E, Peña C. Oxygen transfer rate during the production of alginate by *Azotobacter vinelandii* under oxygen-limited and non oxygen-limited conditions. Microb Cell Fact. 2011;10:13. [https://dx.doi.org/10.1186/1475-2859-10-13].
[49] Sabra W, Zeng AP, Lünsdorf H, et al. Effect of oxygen formation and structure of *Azotobacter vinelandii* and its role in protecting nitrogenase. Appl Environ Microbiol. 2000;66(9):4037–44.
[50] Zapata-Vélez AM, Trujillo-Roldán MA. The lack of a nitrogen source and/or the C/N ratio affects the molecular weight of alginate and its productivity in submerged cultures of *Azotobacter vinelandii*. Ann Microbiol. 2010;60:661–8. https://doi.org/10.1007/s13213-010-0111-7.
[51] Díaz-Barrera A, Andler R, Martínez I, et al. Poly-3-hydroxybutyrate production by *Azotobacter vinelandii* strains in batch cultures at different oxygen transfer rates. J Chem Technol Biotechnol. 2016;91:1063–71. https://doi.org/10.1002/jctb.4684.
[52] Rowe GE, Margaritis A, Wei N. Specific oxygen uptake rate variations during batch fermentation of *Bacillus thuringiensis* subspecies *kurstaki* HD-1. Biotechnol Prog. 2003;19:1439–43. https://doi.org/10.1021/bp030018o.
[53] Andersen KB, Von Meyenburg K. Are growth rates of *Escherichia coli* in batch cultures limited by respiration? J Bacteriol. 1980;144:114–23. https://doi.org/10.1128/jb.144.1.114-123.1980.
[54] Gomez E, Santos VE, Alcon A, et al. Oxygen-uptake and mass-transfer rates on the growth of *Pseudomonas putida* CECT5279: Influence on biodesulfurization (BDS) capability. Energy Fuel. 2006;20:1565–71. https://doi.org/10.1021/ef050362y.
[55] Geddie J, Sutherland IW. The effect of acetylation on cation binding by algal and bacterial alginates. Biotechnol Appl Biochem. 1994;20:117–29.
[56] Peña C, Hernández L, Galindo E, et al. Manipulation of the acetylation degree of *Azotobacter vinelandii* alginate by supplementing the culture medium with 3-(N-morpholino)-propane-sulfonic acid. Lett Appl Microbiol. 2006;43:200–4. https://doi.org/10.1111/j.1472-765X.2006.01925.x.
[57] Mærk M, Jakobsen ØM, Sletta H, et al. Identification of regulatory genes and metabolic processes important for alginate biosynthesis in *Azotobacter vinelandii* by screening of a transposon insertion mutant library. Front Bioeng Biotechnol. 2020;7:1–14. https://doi.org/10.3389/fbioe.2019.00475.
[58] Muhammadi Ahmed N. Genetics of bacterial alginate: alginate genes distribution, organization and biosynthesis in bacteria. Curr Genomics. 2007;8:191–202. https://doi.org/10.2174/138920207780833810.
[59] Wu G, Cruz-Ramos H, Hill S, et al. Regulation of cytochrome *bd* expression in the obligate aerobe *Azotobacter vinelandii* by CydR (Fnr): Sensitivity to oxygen reactive oxygen species and nitric oxide. J Biol Chem. 2000;275:4679–86. https://doi.org/10.1074/jbc.275.7.4679.
[60] Peña C, Galindo E, Büchs J. The viscosifying power, degree of acetylation and molecular mass of the alginate produced by *Azotobacter vinelandii* in shake flasks are determined by the oxygen transfer rate. Process Biochem. 2011;46:290–7. https://doi.org/10.1016/j.procbio.2010.08.025.
[61] Díaz-Barrera A, Urtuvia V, Padilla-Córdova C, et al. Poly(3-hydroxybutyrate) accumulation by *Azotobacter vinelandii* under different oxygen transfer strategies. J Ind Microbiol Biotechnol. 2019;46:13–9. https://doi.org/10.1007/s10295-018-2090-9.
[62] Peña C, Trujillo-Roldán M, Galindo E. Influence of dissolved oxygen tension and agitation speed on alginate production and its molecular weight in cultures of *Azotobacter vinelandii*. Enzyme Microb Technol. 2000;27:390–8. https://doi.org/10.1016/S0141-0229(00)00221-0.
[63] Sabra W, Zeng AP, Deckwer WD. Bacterial alginate: Physiology, product quality and process aspects. Appl Microbiol Biotechnol. 2001;56:315–25. https://doi.org/10.1007/s002530100699.
[64] Trujillo-Roldán MA, Moreno S, Espín G, et al. The roles of oxygen and alginate-lyase in determining the molecular weight of alginate produced by *Azotobacter vinelandii*. Appl Microbiol Biotechnol. 2004;63:742–7. https://doi.org/10.1007/s00253-003-1419-z.
[65] Flores C, Díaz-Barrera A, Martínez F, et al. Role of oxygen in the polymerization and de-polymerization of alginate produced by *Azotobacter vinelandii*. J Chem Technol Biotechnol. 2015;90:356–65. https://doi.org/10.1002/jctb.4548.
[66] Blunt W, Sparling R, Gapes DJ, et al. The role of dissolved oxygen content as a modulator of microbial polyhydroxyalkanoate synthesis. World J Microbiol Biotechnol. 2018;34(8):106. https://doi.org/10.1007/s11274-018-2488-6.
[67] Farias G, Fabregas E, Díaz-Barrera A, et al. Automatic control for the production of alginate by *Azotobacter vinelandii*. IEEE Access. 2019;7:168606–12. https://doi.org/10.1109/ACCESS.2019.2954180.
[68] Flores C, Moreno S, Espín G, et al. Expression of alginases and alginate polymerase genes in response to oxygen, and their relationship with the alginate molecular weight in *Azotobacter vinelandii*. Enzyme Microb Technol. 2013;53:85–91. https://doi.org/10.1016/j.enzmictec.2013.04.010.
[69] Jiménez L, Castillo T, Flores C, et al. Analysis of respiratory activity and carbon usage of a mutant of *Azotobacter vinelandii* impaired in poly-β-hydroxybutyrate synthesis. J Ind Microbiol Biotechnol. 2016;43:1167–74. https://doi.org/10.1007/s10295-016-1774-2.
[70] Díaz-Barrera A, Peña C, Galindo E. The oxygen transfer rate influences the molecular mass of the alginate produced by *Azotobacter vinelandii*. Appl Microbiol Biotechnol. 2007;76:903–10. https://doi.org/10.1007/s00253-007-1060-3.
[71] Ahumada-Manuel CL, Guzmán J, Peña C, et al. The signaling protein MucG negatively affects the production and the molecular mass of alginate in *Azotobacter vinelandii*. Appl Microbiol Biotechnol. 2017;101:1521–34. https://doi.org/10.1007/s00253-016-7931-8.

[72] Díaz-Barrera A, Maturana N, Pacheco-Leyva I, et al. Different responses in the expression of alginases, alginate polymerase and acetylation genes during alginate production by *Azotobacter vinelandii* under oxygen-controlled conditions. J Ind Microbiol Biotechnol. 2017;44:1041–51.

[73] Gómez-Pazarín K, Flores C, Castillo T, et al. Molecular weight and viscosifying power of alginates produced in *Azotobacter vinelandii* cultures in shake flasks under low power input. J Chem Technol Biotechnol. 2016;91:1485–92. https://doi.org/10.1002/jctb.4747.

[74] Núñez C, Peña C, Kloeckner W, et al. Alginate synthesis in *Azotobacter vinelandii* is increased by reducing the intracellular production of ubiquinone. Appl Microbiol Biotechnol. 2013;97:2503–12. https://doi.org/10.1007/s00253-012-4329-0.

[75] Gaytán I, Peña C, Núnez C, et al. *Azotobacter vinelandii* lacking the Na^+-NQR activity: A potential source for producing alginates with improved properties and at high yield. World J Microbiol Biotechnol. 2012;28:2731–40. https://doi.org/10.1007/s11274-012-1084-4.

[76] Castillo T, Galindo E, Peña CF, et al. The acetylation degree of alginates in *Azotobacter vinelandii* ATCC9046 is determined by dissolved oxygen and specific growth rate: Studies in glucose-limited chemostat cultivations. J Ind Microbiol Biotechnol. 2013;40(7):715–23. https://doi.org/10.1007/s10295-013-1274-6.

[77] Castillo T, Heinzle E, Peifer S, et al. Oxygen supply strongly influences metabolic fluxes, the production of poly(3-hydroxybutyrate) and alginate, and the degree of acetylation of alginate in *Azotobacter vinelandii*. Process Biochem. 2013;48:995–1003. https://doi.org/10.1016/j.procbio.2013.04.014.

[78] Senior PJ, Dawes EA. The regulation of poly-b-hydroxybutyrate metabolism in *Azotobacter beijerinckii*. Biochem J. 1973;134:225–38. https://doi.org/10.1042/bj1340225.

[79] Moral ÇK, Sanin FD. An investigation of agitation speed as a factor affecting the quantity and monomer distribution of alginate from *Azotobacter vinelandii* ATCC®9046. J Ind Microbiol Biotechnol. 2012;39:513–9. https//doi:https://doi.org/10.1007/s10295-011-1043-3.

[80] Moral ÇK, Doğan Ö, Sanin FD. Effect of oxygen tension and medium components on monomer distribution of alginate. Appl Biochem Biotechnol. 2015;176:875–91. [doi:10.1007/s12010-015-1617-z].

[81] García A, Pérez D, Castro M, et al. Production and recovery of poly-3-hydroxybutyrate [P(3HB)] of ultra-high molecular weight using fed-batch cultures of *Azotobacter vinelandii* OPNA strain. J Chem Technol Biotechnol. 2019;94:1853–60. https://doi.org/10.1002/jctb.5959.

[82] Pyla R, Kim TJ, Silva JL, et al. Overproduction of poly-β-hydroxybutyrate in the *Azotobacter vinelandii* mutant that does not express small RNA ArrF. Appl Microbiol Biotechnol. 2009;84:717–24. https://doi.org/10.1007/s00253-009-2002-z.

[83] Pyla R, Kim TJ, Silva JL, et al. Proteome analysis of *Azotobacter vinelandii* Δ*arrF* mutant that overproduces poly-β-hydroxybutyrate polymer. Appl Microbiol Biotechnol. 2010;88:1343–54. https://doi.org/10.1007/s00253-010-2852-4.

[84] Page WJ, Knosp O. Hyperproduction of poly-b-hydroxybutyrate during exponential growth of *Azotobacter vinelandii* UWD. Appl Environ Microbiol. 1989;55:1334–9.

[85] García A, Ferrer P, Albiol J, et al. Metabolic flux analysis and the NAD(P)H/$NAD(P)^+$ ratios in chemostat cultures of *Azotobacter vinelandii*. Microb Cell Fact. 2018;17:10. https://doi.org/10.1186/s12934-018-0860-8.

[86] García A, Segura D, Espín G, et al. High production of poly-b-hydroxybutyrate (PHB) by an *Azotobacter vinelandii* mutant altered in PHB regulation using a fed-batch fermentation process. Biochem Eng J. 2014;82:117–23. https://doi.org/10.1016/j.bej.2013.10.020.

[87] Bush JA, Wilson PW. A non-gummy chromogenic strain of *Azotobacter vinelandii*. Nature. 1959;184:381. https://doi.org/10.1038/184381a0.

[88] Peña C, López S, García A, et al. Biosynthesis of poly-β-hydroxybutyrate (PHB) with a high molecular mass by a mutant strain of *Azotobacter vinelandii* (OPN). Ann Microbiol. 2014;64:39–47. https://doi.org/10.1007/s13213-013-0630-0.

[89] Martínez-Salazar JM, Moreno S, Nájera R, et al. Characterization of the genes coding for the putative sigma factor AlgU and its regulators MucA, MucB, MucC and MucD in *Azotobacter vinelandii* and evaluation of their roles in alginate biosynthesis. J Bacteriol. 1996;178:1800–8.

[90] Díaz-Barrera A, Aguirre A, Berrios J, et al. Continuous cultures for alginate production by *Azotobacter vinelandii* growing at different oxygen uptake rates. Process Biochem. 2011;46:1879–83. https://doi.org/10.1016/j.procbio.2011.06.022.

[91] Díaz-Barrera A, Martínez F, Guevara Pezoa F, et al. Evaluation of gene expression and alginate production in response to oxygen transfer in continuous culture of *Azotobacter vinelandii*. PLoS One. 2014;9(8):e105993. https://doi.org/10.1371/journal.pone.0105993.