Microbial nitrogen cycles: physiology, genomics and applications

Rick W Ye* and Stuart M Thomas†

Genes and pathways involved in inorganic nitrogen cycles have been found in traditional as well as unusual microorganisms. These pathways or enzymes play a very important role in the adaptation or survival of these microorganisms under a variety of environmental conditions. Microbial nitrogen metabolism has industrial applications ranging from wastewater treatment to bioremediation and potential future use in biocatalysis for chemical production.

Addresses
E328/148B, DuPont Experimental Station, Route 141 and Henry Clay Road, Wilmington, Delaware 19880, USA
*e-mail: rick.ye@usa.dupont.com
†e-mail: stuart.m.thomas@usa.dupont.com

Current Opinion in Microbiology 2001, **4**:307–312

1369-5274/01/$ – see front matter

Abbreviations

anammox	anaerobic ammonia oxidation
AOB	ammonia-oxidizing bacteria
PHA	polyhydroxyalkanoates

Introduction

The metabolism of inorganic nitrogen compounds (see Figure 1) plays many important physiological roles in microorganisms. Denitrification, a process of converting nitrate to nitrous oxide or dinitrogen gas, allows microbes to use alternative electron acceptors to gain energy under oxygen-limiting conditions [1]. Chemolithotrophic nitrification derives energy from the oxidation of ammonia to nitrite [2]. Dissimilatory reduction of nitrate to ammonia under oxygen-limiting conditions serves as a process to dissipate excess reducing power [3], generates ammonia for assimilation, or supports anaerobic growth with nitrate or nitrite as the alternative electron acceptors [4]. The newly discovered anaerobic ammonia oxidation (anammox) reaction converts ammonium and nitrite to dinitrogen gas (see Figure 2). Although it is not the subject of this review, microbial nitrogen fixation converts gaseous dinitrogen to ammonia for assimilation. In addition, reactions involving inorganic nitrogen species provide a rich variety of enzymatic systems for biochemical study [5].

Microbial nitrogen metabolism also plays an important role in the global nitrogen cycle. Microbial activities, such as denitrification and anammox, are the major mechanisms that convert combined nitrogen to dinitrogen gas, thereby completing the nitrogen cycle. At the same time, microbial activities contribute to the production of greenhouse gases such as nitric and nitrous oxides in the atmosphere. These microbial activities are carried out by a wide variety of microoganisms that range from archaebacteria to proteobacteria, to Gram-positive eubacteria, to fungi.

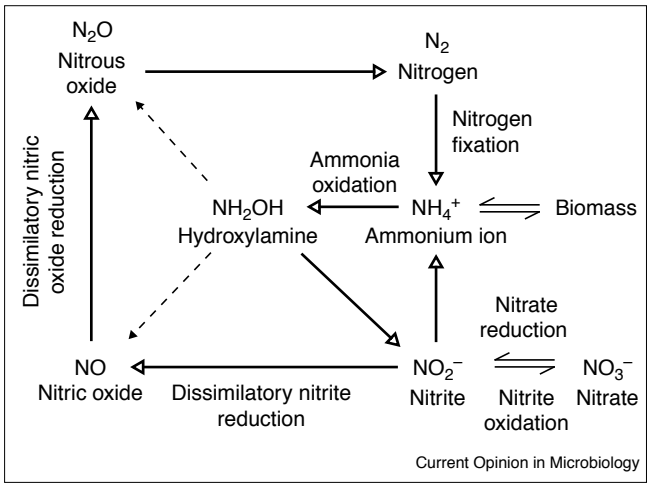
This review summarizes progress made over the past two years in the understanding of the physiology of microbial metabolism of inorganic nitrogen compounds, and highlights the advances made as a result of genome sequencing efforts. Additionally, industrial applications of microbial nitrogen metabolism will be reviewed.

Dissimilatory reduction of nitrate or nitrite to gaseous forms of nitrogen products

Dissimilatory reduction of nitrate is commonly carried out by either a membrane-bound nitrate reductase or a periplasmic nitrate reductase. The role of these two types of enzymes in the denitrification process varies, depending on the organism. In the *Pseudomonas fluorescens* YT101 strain, only the membrane-bound nitrate reductase activity is found [6]. *Paracoccus pantatropha* has both enzymes and the membrane-bound enzyme is responsible for anaerobic denitrification. The periplasmic nitrate reductase is suggested to play a role in dissipating reductant when this organism is grown on highly reduced carbons under aerobic conditions [7]. For quite a while, the role of periplasmic nitrate reductase in anaerobic denitrification was uncertain. Recently, it was demonstrated that this enzyme is required for anaerobic nitrate reduction in *Pseudomonas* sp. G-179 and *Rhodobacter sphaeroides f.* sp. *denitrificans* [8••,9]. Most nitrate reductases studied so far contain molybdenum in the form of a molybdopterin cofactor. Two catalytically distinct, molybdenum-free dissimilatory nitrate reductases, a soluble periplasmic one and a membrane-bound one, were reportedly isolated from the vanadate-reducing bacterium, *Pseudomonas isachenkovii* [10].

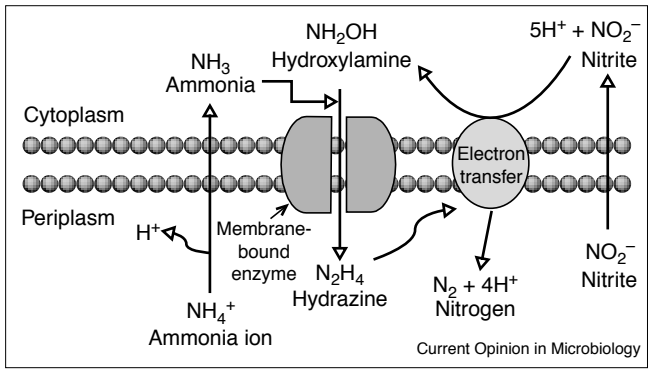
There are two types of dissimilatory nitrite reductase that catalyze the conversion of nitrite to nitric oxide in bacteria. One type is the cytochrome cd_1 nitrite reductase, and the other type is the copper-containing nitrite reductase. Based on genome sequencing information, it appears that both types of nitrite reductases are present in *Methylomonas* sp. strain 16a (JM Odom, J-F Tomb, RW Ye, K Norton, A Schenzle, S Zhang, unpublished data). This observation is the first report of a bacterium that contains both types of dissimilatory nitrite reductases. Detailed biochemical and genetic studies are needed to validate and elucidate the role of these two enzymes in this organism. The production and consumption of nitric oxide have also been reported in other strains of methanotrophic bacteria grown in nitrate-containing medium under oxygen-limiting conditions [11]. It is likely that these organisms carry out the assimilatory nitrate reduction to nitrite, which is then reduced under oxygen-limiting conditions to nitric oxide and nitrous oxide via the enzymatic activities of dissimilatory nitrite and nitric oxide reductases.

Figure 1



Microbial nitrogen cycle. Nitrate is converted to nitrite by assimilatory or dissimilatory nitrate reductases. Assimilatory reduction of nitrate to ammonia via nitrite enables microbes to use nitrate as the nitrogen source. Under oxygen-limiting conditions, nitrite can be reduced to nitric oxide or ammonia. Bacteria with the complete denitrification pathway catalyze the dissimilatory reduction of nitrate to nitrogen. In some bacteria, dissimilatory reduction of nitrate to ammonia via nitrite can support anaerobic growth or dissipate excess reducing power. Ammonia oxidizers oxidize ammonia to hydroxylamine, which is subsequently converted to nitrite. This process also leads to the production of nitric oxide and nitrous oxide. The nitrite produced can be converted to nitrate by nitrite oxidizers. The nitrification community consists of both ammonia oxidizers and nitrite oxidizers. Anaerobic ammonia oxidation (anammox) is shown in Figure 2.

Figure 2



Anaerobic ammonium oxidation (anammox) by the Planctomycetales. Anammox is coupled to nitrite reduction. Ammonia and hydroxylamine are converted to hydrazine by a membrane-bound enzyme. Hydrazine is oxidized in the periplasm. The mechanism of electron transfer for nitrite reduction is not fully known at this time. Jetten *et al.* [36] propose two potential systems: one system involves a single enzyme that is responsible for hydrazine oxidation and nitrite reduction, and the other involves a nitrite-reducing enzyme that mediates formation of hydroxylamine while an electron transport chain enzyme supplies the electrons.

A few types of nitric oxide reductases have been found in microorganisms. The most common one contains cytochrome *bc*. The cytochrome *b* nitric oxide reductase lacks the heme *c* subunit [12,13]. The DNA sequence of the cytochrome *b* enzyme has been found in *Neisseria gonorrhoeae* [14], *Neisseria meningitidis* [15], *Synechocystis* sp. PCC6803 [16], *Ralstonia eutrophus* [13] and *Methylomonas* sp. strain 16a (JM Odom, J-F Tomb, RW Ye, K Norton, A Schenzle, S Zhang, unpublished data). This enzyme is required for anaerobic growth of *N. gonorrhoeae*. In the fungus *Fusarium oxysporum*, the cytochrome P450 nitric oxide reductase is shown to be responsible for the step of nitric oxide reduction [17]. In addition, the heme–copper oxidases of *Thermus thermophilus* and cytochrome *c* nitrite reductase of *Sulfurospirillum deleyianum* can convert nitric oxide to nitrous oxide or ammonia [18,19•]. Remarkably, the cytochrome *c* nitrite reductase has a considerably broad spectrum of substrate specificity.

The existence of dissimilatory nitrite and nitric oxide reductases in nondenitrifying bacteria, as revealed by genome sequences (Table 1), clearly indicates a broader physiological role that could range from anaerobic metabolism to detoxification for these enzymes.

Dissimilatory nitrate reduction to ammonia

Investigation of anaerobic metabolism of *Bacillus subtilis* has revealed many interesting features of dissimilatory nitrate reduction to ammonia. First of all, *B. subtilis* was traditionally believed to be a strict aerobe [4]. It turns out that *B. subtilis* can carry out anaerobic dissimilatory reduction of nitrate to ammonia via nitrite. This anaerobic process has long been considered to be a way of dissipating electrons under anaerobic conditions [3]. However, *B. subtilis* is capable of using nitrate and nitrite as the alternative electron acceptors to support anaerobic growth. Anaerobic energy generation appears to be coupled to anaerobic fermentation, as mutations in *lctE* and *pta* (which encode the two enzymes required for lactate and acetate synthesis, respectively) result in a significant reduction in anaerobic fermentative and respiratory growth [20•].

With the availability of the genomic sequence for *B. subtilis* [21], the genome-wide analysis of RNA transcriptional patterns is possible. DNA microarrays, which measure mRNA levels in a high-throughput manner, have been used to investigate the changes in mRNA transcription when *B. subtilis* is grown under anaerobic conditions with nitrate or nitrite as the alternative electron acceptor [22•]. Among the 4020 genes examined, the most highly induced regions are *narGHJI* and *narK*, both of which are involved in dissimilatory nitrate reduction. Other induced regions include those involved in carbon metabolism, electron transport, antibiotic production and stress responses. Furthermore, many DNA regions with unknown functions were affected by oxygen limitation. DNA microarrays are very useful tools with which to elucidate the regulatory networks, but results of array experiments require the validation of conventional genetic and biochemical analyses. The correlation between genome-wide mRNA transcription levels and physiology has not been thoroughly investigated at the present time.

Table 1

A list of currently sequenced microbial genomes with nitrogen cycle pathways.

Species	Pathways or enzymes	References
Nitrosomonas europaea	Ammonia oxidation Dissmilatory nitrite and nitric oxide reductases	(a)
Methylomonas sp. 16a	Ammonia oxidation Dissimilatory nitrite and nitric oxide reductases	(b)
Neisseria menigitidis	Dissimilatory nitrite and nitric oxide reductases	[15]
Synechocystis sp. PCC6803	Cytochrome *b* nitric oxide reductase	[16]
Bacillus subtilis strain 168	Dissimilatory nitrate reduction to ammonia	[21]
Pseudomonas aeruginosa PAO	Denitrification	[41]
Rhodobacter sphaeroides	Denitrification Nitrogen fixation	(a)
Paracoccus denitrificans ATCC 19367	Denitrification Heterotrophic nitrification	(c)
P. denitrificans strain SANVA100	Denitrification	(c)
Azoarcus tolulyticus Tol-4	Denitrification	(c)

(a) Joint Genome Institute, URL http://spider.jgi.psf.org/JGI_microbiol/html; (b) JM Odom, J-F Tomb, RW Ye, K Norton, A Schenzle, S Zhang, unpublished data; (c) RW Ye, SM Thomas, unpublished data.

Dissimilatory nitrate reduction to ammonia in *B. subtilis* is regulated by the two-component regulatory proteins ResDE (Figure 3). Direct binding of ResD protein to upstream regions of *hmp* and *nasDEF*, which encode a flavoprotein and the nitrite reductase, respectively, has been demonstrated [23•]. Phosphorylation of ResD significantly stimulates this binding. Binding of the promoter region of *fnr*, which regulates the *nar* genes responsible for nitrate reduction, requires a higher concentration of ResD, and binding is not enhanced with ResD phosphorylation. This result appears consistent with the observation that certain levels of RNA transcripts for *nar* and *fnr* genes are induced when ResDE$^-$ mutants are grown under oxygen-limiting conditions [22•]. Furthermore, the mRNA levels for *nar* and *fnr* are reduced at the end of exponential growth in nutrient broth medium supplemented with nitrate and glucose, whereas no changes are observed for *nasDEF*, *hmp* and *resDE* at the same stage (RW Ye, unpublished data). As a result, the control of ResD on the expression of *fnr* may not be as stringent as *nasD*.

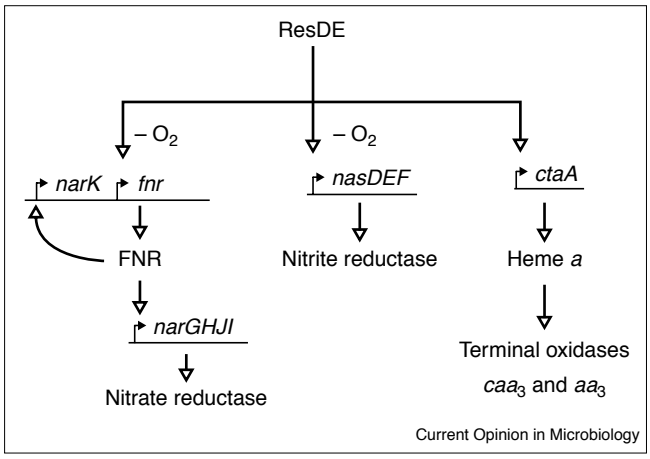
Ammonia-oxidizing bacteria

Ammonium oxidation has been observed in many bacterial species. Ammonia is oxidized by two pathways: first, ammonia is oxidized to hydroxylamine, which is then oxidized to nitrite by hydroxylamine oxidoreductase (Figure 1); second, ammonia and nitrite are anaerobically converted to dinitrogen gas (Figure 2). The aerobic chemolithoautotrophic ammonia-oxidizing bacteria (AOB) are specialists that can grow on ammonia and carbon dioxide [24] and use ammonia monooxygenase to convert ammonia to hydroxylamine. Many heterotrophic bacteria, such as *P. pantotropha* and *Alcaligenes faecalis* strain TUD [25], can carry out the same reaction. Methanotrophs are capable of converting ammonia to hydroxylamine via the methane monooxygenase, whereas the ammonium monooxygenase can oxidize methane to carbon dioxide [2]. The recently identified lithotrophic planctomycete possesses the anammox pathway, which is coupled to nitrite reduction [26•].

It is interesting to note that many AOB have either a whole or partial denitrification pathway. The genome sequencing effort of the chemolithotrophic nitrifier *Nitrosomonas europaea* is near completion (US Department of Energy Joint Genome Institute [JGI] on World Wide Web URL http://www.jgi.doe.gov/tempweb/JGI_microbial/html/index.html). Preliminary results apparently indicate the existence of copper-type dissimilatory nitrite and cytochrome *bc* nitric oxide reductases, but not dissimilatory nitrate and nitrous oxide reductases [27]. The methanotrophic AOB *Methylomonas* sp. 16a also has only these two steps of the denitrification pathway. It is observed that gaseous nitric oxide is important for ammonia oxidation activity in *Nitrosomonas eutropha* [28]. Clearly, the connection between nitric oxide, nitrite and nitrous oxide with ammonia oxidation and the role of dissimilatory nitrite and nitric oxide reductases in AOB need to be addressed.

The discovery of anammox is considered to be one of the most innovative technological advances in the removal of ammonia nitrogen from wastewater. The microorganism that carries out this process has been identified as a novel autotrophic member of the order Planctomycetales, one of the major distinct divisions of the bacterial domain [26•]. The novel planctomycete grows extremely slowly, dividing only once every two weeks, and thus cannot be cultured. Interestingly, both the purified and unpurified cells from biofilms were active only when the cell concentration was higher than 10^{10}–10^{11} cells per millilitre. These cells were reported to contain a novel type of hydroxylamine oxidoreductase, based on the amino acid sequences of several peptide fragments, electron paramagnetic resonance spectra and the possible presence of P468 cytochrome [29]. It is necessary, however, to have the complete gene sequence and to perform additional biochemical analysis in order to

Figure 3



Regulation of aerobic and anaerobic respiration in *B. subtilis* by ResDE. The expression of *narDEF* and *fnr* genes is regulated by the two-component signal transduction regulatory system ResDE. The FNR anaerobic regulator, in turn, controls the expression of *narGHJI*, which encodes membrane-bound nitrate reductase. At the same time, ResDE is required for the expression of *ctaA*, which is required for the biosynthesis of heme *a*, a component of cytochrome aa_3 and caa_3 terminal oxidases. Mutation in the *resDE* region results in an increase in mRNA levels of *cydABC*, which encode the third terminal oxidase cytochrome, *bd*. The ResDE system is also required for the expression of other aerobic and anaerobic genes [4].

enable full comparison. Within the anaerobic AOB Planctomycetales, there is evidence, based on 16S rDNA sequences, for an additional genus [30]. This second novel biofilm planctomycete, provisionally classified as *Candidatus Kuenenia stuttgartiensis*, constitutes the dominant fraction of the biofilm bacteria.

The role of nitrogen metabolism in wastewater treatment

The elimination of nutrient discharge from wastewater treatment facilities is of increasing importance, driven mainly by the need to reduce eutrophication of estuaries. The US National Oceanic and Atmospheric Administration has recently published a report describing past research, recommendations and strategies for dealing with eutrophication in the US [31]. Nitrogen-containing compounds (primarily ammonia, nitrate and nitrite) are contributors to the eutrophication of waterways. Microbial metabolism of organic and inorganic nitrogen to dinitrogen gas is commonly employed by industrial and municipal wastewater treatment facilities to meet discharge limits for these compounds. To date, commercial applications have mainly taken advantage of anammox and dissimilatory denitrification physiologies. The major applications and future directions of aerobic nitrification, anammox and denitrification are described below.

Aerobic nitrification is carried out by slow-growing autotrophic bacteria, which double every 1–5 days [32]. These microorganisms are inhibited by many heavy metals and organic chemicals, although inhibition by the latter can also be indirect and related to oxygen depletion by heterotrophs present in the system. Improved nitrogen removal and reduced inhibition of autotrophic nitrification by environmental conditions could be offset by improvements in process monitoring. For example, Caulet *et al.* [33] described an automated aeration control approach that is based on trends in oxidation reduction potential. Using this system, nitrogen removal stabilized and increased to 90%. Additionally, an offline system that determines the inhibition of nitrifying biomass has been reported by Grunditz *et al.* [34]. In this study, pure cultures of *Nitrosomonas* and *Nitrobacter* were used to determine the impact of wastestream sources and environmental conditions (such as temperature and pH) on ammonia oxidation and nitrite oxidation.

Anammox coupled to nitrite reduction offers opportunities in the area of process development of nitrogen removal systems. One of the biggest challenges is how to accelerate the slow rate of nitrogen removal from these systems (the rate is less than half that of aerobic nitrification) [35,36]. However, from a commercial applications perspective, the more challenging issue is the extremely slow growth rate (10–14 days) of the bacteria known to carry out these reactions [35,36]. Similar to aerobic nitrification, anammox is subject to inhibition. This process requires anaerobic conditions for ammonia oxidation, but inhibition by oxygen is reversible [36]. Other inhibitors of this process include acetylene and phosphate [36].

Ammonia-rich streams could be treated in a coupled nitrification/anammox system. This system would result in cost savings attributed to reduced oxygen consumption in the nitrification system, reduced carbon demand during the anaerobic period, and reduced sludge disposal costs. However, an effective partial nitrification/anammox system would require high cell density and excellent biomass retention, suggesting the necessity for a fixed film or immobilized biomass reactor configuration. Compact systems that increase the volumetric capacity of wastewater systems have recently been reviewed [37,38]. Biofilm-based reactors (fixed or mobile bed) increase the concentration of active biomass and the resistance to potentially inhibitory operating conditions. A waste treatment plant based on partial nitrification followed by anaerobic ammonia oxidation is scheduled to come online in the Netherlands in 2001 (MSM Jetten, personal communication).

Denitrifying bioreactors are used to convert waste nitrate to dinitrogen gas, although, under suboptimal conditions, the conversion of nitrate and nitrite results in nitric or nitrous oxide (greenhouse gases). One of the operating parameters in denitrifying systems is the ratio of carbon to nitrogen, expressed as COD/N (COD stands for chemical oxygen demand). The nitrogen load is commonly reported as NO_3-N (nitrate-nitrogen), which is the amount of nitrogen in the nitrate. The theoretical ratio of carbon to nitrogen (2.86 mb COD/N) is determined from the balanced chemical

equation for conversion of carbon to carbon dioxide and water with the concomitant reduction of nitrate [32]. This ratio increases either as the growth yield increases or with the formation of storage compounds such as polysaccharides and polyhydroxyalkanoates (PHA) [39]. We have demonstrated a correlation between PHA content of the biomass and denitrification activity (SM Thomas, unpublished data): as PHA levels increase above ~20%, the specific denitrification rates are reduced by three- to tenfold.

Incomplete denitrification (resulting in nitrite as the end product) could be combined with the anammox physiology to remove nitrate and ammonia in a single reactor, and potentially reduce operating costs by requiring less carbon for denitrification. Additional system variations that result in cost savings have also been described by Verstraete and Philips [40].

Anaerobic mineralization of environmental pollutants

Aerobic bacteria that are capable of oxidizing aromatic rings and halogen-containing compounds can be used to mineralize environmental pollutants (a good review of microbial metabolism related to bioremediation can be found on the World Wide Web URL http://umbbd.ahc.umn.edu/index.html). However, oxygen is often limiting in contaminated subsurface environments. Recently, bacteria that can metabolize common pollutants using nitrate as the end electron acceptor have been reported. The genus *Azoarcus* has the potential to serve as a model system, as it can metabolize toluene under anoxic conditions, and the genome sequence for this organism is being generated (RW Ye, SM Thomas, unpublished data). Additionally, the biochemistry carried out by these bacteria offer new possibilities for the development of novel biocatalysts for the production of industrial chemicals.

Bioprocess applications

The solubility limit of oxygen in aqueous solutions is very low (7.8 milligrams per litre at 30°C), and mass transfer of oxygen in high density or immobilized cell systems is usually limiting. Dissimilartory nitrate reduction to dinitrogen gas could be used in large-scale bioprocess applications to relieve oxygen limitations. Microorganisms that have been extensively studied, such as *P. denitrificans*, could serve as a production host in these types of systems, as genetic systems are well-established and genome sequence information is available (Table 1). Other advantages of this approach include lower biomass generation and the ability to use a broad range of organic feed stocks such as methanol, glucose and fatty acids. In addition, anaerobic promoters derived from genes involved in anaerobic nitrogen metabolism can be used for gene expression in established production hosts such as *B. subtilis* and *Escherichia coli*.

Conclusions and future directions

The ability to obtain full genome sequences is one of the major milestones in microbiology. Complete or partial genome sequences for several microbes that have inorganic nitrogen cycle pathways are available (Table 1). The availability of genome sequences makes functional genomic approaches, such as DNA microarrays and 2-dimensional protein gels, feasible. Applied genomic research can be used to identify genes and patterns of expression that are critical to the performance of nitrogen metabolism in industrial applications. The identification of key regulatory responses can be coupled with reporter systems (such as green fluorescent protein and bioluminescence) for the development of online measurement systems. Coupling the advances related to bacterial nitrogen metabolism with improved monitors of macroscopic performance should lead to more robust operating strategies for wastewater bioreactors. Genomic information, in combination with traditional biochemical, genetic and ecological studies, will continue to accelerate our understanding of inorganic nitrogen metabolism, and thus benefit their industrial applications.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Zumft WG: **Cell biology and molecular basis of denitrification.** *Microbiol Mol Biol Rev* 1997, **61**:533-616.

2. Hooper AB, Vannelli T, Bergmann DJ, Arciero DM: **Enzymology of the oxidation of ammonia to nitrite by bacteria.** *Antonie Van Leeuwenhoek* 1997, **71**:59-67.

3. Tiedje JM: **Ecology of denitrification and dissimilatory nitrate reduction to ammonium.** In *Biology of Anaerobic Microorganisms.* Edited by Zehnder AJB. New York: Wiley; 1988:179-244.

4. Nakano MM, Zuber P: **Anaerobic growth of a 'strict aerobe' (*Bacillus subtilis*).** *Annu Rev Microbiol* 1998, **52**:165-190.

5. Richardson DJ, Watmough NJ: **Inorganic nitrogen metabolism in bacteria.** *Curr Opin Chem Biol* 1999, **3**:207-219.

6. Ghiglione JF, Philippot L, Normand P, Lensi R, Potier P: **Disruption of *narG*, the gene encoding the catalytic subunit of respiratory nitrate reductase, also affects nitrite respiration in *Pseudomonas fluorescens* YT101.** *J Bacteriol* 1999, **181**:5099-5102.

7. Sears HJ, Sawers G, Berks BC, Ferguson SJ, Richardson DJ: **Control of periplasmic nitrate reductase gene expression (*napEDABC*) from *Paracoccus pantotrophus* in response to oxygen and carbon substrates.** *Microbiology* 2000, **146**:2977-2985.

8. •• Bedzyk L, Wang T, Ye RW: **The periplasmic nitrate reductase in *Pseudomonas* sp. strain G-179 catalyzes the first step of denitrification.** *J Bacteriol* 1999, **181**:2802-2806.

Traditionally, periplasmic nitrate reductase was not considered to be required for anaerobic denitrification. The authors demonstrate that periplasmic nitrate reductase in *Pseudomonas* sp. strain G-179 is essential for anaerobic denitrification.

9. Sabaty M, Schwintner C, Cahors S, Richaud P, Vermeglio A: **Nitrite and nitrous oxide reductase regulation by nitrogen oxides in *Rhodobacter sphaeroides f.* sp. *denitrificans* IL106.** *J Bacteriol* 1999, **181**:6028-6032.

10. Antipov AN, Lyalikova NN, Khijniak TV, L'vov NP: **Vanadate reduction by molybdenum-free dissimilatory nitrate reductases from vanadate-reducing bacteria.** *IUBMB Life* 2000, **50**:39-42.

11. Ren T, Roy R, Knowles R: **Production and consumption of nitric oxide by three methanotrophic bacteria.** *Appl Environ Microbiol* 2000, **66**:3891-3897.

12. Cramm R, Pohlmann A, Friedrich B: **Purification and characterization of the single-component nitric oxide reductase from *Ralstonia eutropha* H16.** *FEBS Lett* 1999, **460**:6-10.

13. Cramm R, Siddiqui RA, Friedrich B: **Two isofunctional nitric oxide reductases in *Alcaligenes eutrophus* H16.** *J Bacteriol* 1997, **179**:6769-6777.

14. Householder TC, Fozo EM, Cardinale JA, Clark VL: **Gonococcal nitric oxide reductase is encoded by a single gene, *norB*, which is required for anaerobic growth and is induced by nitric oxide.** *Infect Immun* 2000, **68**:5241-5246.

15. Tettelin H, Saunders NJ, Heidelberg J, Jeffries AC, Nelson KE, Eisen JA, Ketchum KA, Hood DW, Peden JF, Dodson RJ *et al.*: **Complete genome sequence of *Neisseria meningitidis* serogroup B strain MC58.** *Science* 2000, **287**:1809-1815.

16. Kaneko T, Sato S, Kotani H, Tanaka A, Asamizu E, Nakamura Y, Miyajima N, Hirosawa M, Sugiura M, Sasamoto S *et al.*: **Sequence analysis of the genome of the unicellular cyanobacterium *Synechocystis* sp. strain PCC6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions.** *DNA Res* 1996, **3**:109-136.

17. Takaya N, Shoun H: **Nitric oxide reduction, the last step in denitrification by *Fusarium oxysporum*, is obligatorily mediated by cytochrome P450nor.** *Mol Gen Genet* 2000, **263**:342-348.

18. Giuffre A, Stubauer G, Sarti P, Brunori M, Zumft WG, Buse G, Soulimane T: **The heme-copper oxidases of *Thermus thermophilus* catalyze the reduction of nitric oxide: evolutionary implications.** *Proc Natl Acad Sci USA* 1999, **96**:14718-14723.

19. • Stach P, Einsle O, Schumacher W, Kurun E, Kroneck PM: **Bacterial cytochrome c nitrite reductase: new structural and functional aspects.** *J Inorg Biochem* 2000, **79**:381-385.
This paper shows that cytochrome *c* nitrite reductase, as a protein family, has very interesting structural and functional characteristics. It can convert many substrates in addition to nitrite.

20. • Cruz Ramos H, Hoffmann T, Marino M, Nedjari H, Presecan-Siedel E, Dreesen O, Glaser P, Jahn D: **Fermentative metabolism of *Bacillus subtilis*: physiology and regulation of gene expression.** *J Bacteriol* 2000, **182**:3072-3080.
This paper shows that respiratory reduction of nitrate and nitrite is linked to fermentation process in *B. subtilis*. Interruption of the fermentation process leads to poor anaerobic growth with nitrate or nitrite as the alternative electron acceptor.

21. Kunst F, Ogasawara N, Moszer I, Albertini AM, Alloni G, Azevedo V, Bertero MG, Bessieres P, Bolotin A, Borchert S *et al.*: **The complete genome sequence of the gram-positive bacterium *Bacillus subtilis*.** *Nature* 1997, **390**:249-256.

22. • Ye RW, Tao W, Bedzyk L, Young T, Chen M, Li L: **Global gene expression profiles of *Bacillus subtilis* grown under anaerobic conditions.** *J Bacteriol* 2000, **182**:4458-4465.
The paper describes the application of a functional genomic tool, DNA microarray, to explore the gene expression patterns of *B. subtilis*, during anaerobic growth on nitrate and nitrite or during fermentation.

23. • Nakano MM, Zhu Y, Lacelle M, Zhang X, Hulett FM: **Interaction of ResD with regulatory regions of anaerobically induced genes in *Bacillus subtilis*.** *Mol Microbiol* 2000, **37**:1198-1207.
The authors demonstrate that ResD can bind to regulatory regions of anaerobic genes, including *nasDEF*, which encodes nitrite reductase, and *fnr*, which regulates dissimilatory nitrate reduction.

24. Purkhold U, Pommerening-Roser A, Juretschko S, Schmid MC, Koops HP, Wagner M: **Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and *amoA* sequence analysis: implications for molecular diversity surveys.** *Appl Environ Microbiol* 2000, **66**:5368-5382.

25. Otte S, Schalk J, Kuenen JG, Jetten MS: **Hydroxylamine oxidation and subsequent nitrous oxide production by the heterotrophic ammonia oxidizer *Alcaligenes faecalis*.** *Appl Microbiol Biotechnol* 1999, **51**:255-261.

26. • Strous M, Fuerst JA, Kramer EH, Logemann S, Muyzer G, van de Pas-Schoonen KT, Webb R, Kuenen JG, Jetten MS: **Missing lithotroph identified as new planctomycete.** *Nature* 1999, **400**:446-449.
The process of anammox is a very unique observation in microbial physiology and is considered to be one of the most innovative technological advances in the removal of ammonia nitrogen from wastewater. The identity of the responsible microoganism was unknown over the past ten years. In this paper, the authors report the isolation and identification of a new autotrophic member of the order Planctomycetales. This is one of the major distinct divisions of the bacterial domain. This group of organisms has single- or double-membrane-bounded compartments separating their chromosomes from the remainder of their cytoplasm.

27. Whittaker M, Bergmann D, Arciero D, Hooper AB: **Electron transfer during the oxidation of ammonia by the chemolithotrophic bacterium *Nitrosomonas europaea*.** *Biochim Biophys Acta* 2000, **1459**:346-355.

28. Zart D, Schmidt I, Bock E: **Significance of gaseous NO for ammonia oxidation by *Nitrosomonas eutropha*.** *Antonie Van Leeuwenhoek* 2000, **77**:49-55.

29. Schalk J, de Vries S, Kuenen JG, Jetten MS: **Involvement of a novel hydroxylamine oxidoreductase in anaerobic ammonium oxidation.** *Biochemistry* 2000, **39**:5405-5412.

30. Schmid M, Twachtmann U, Klein M, Strous M, Juretschko S, Jetten M, Metzger JW, Schleifer KH, Wagner M: **Molecular evidence for genus level diversity of bacteria capable of catalyzing anaerobic ammonium oxidation.** *Syst Appl Microbiol* 2000, **23**:93-106.

31. Bricker S, Clement C, Pirhalla DE, Orlando SP, Farrow DRG: **National estuarine eutrophication assessment: effects of nutrient enrichment in the nation's estuaries.** Atmospheric Administration, National Ocean Service, NOS, Special Projects Office, Silver Spring, Maryland 20910-3281, USA. 1999.

32. Gradly CPL, Lim H: *Biological Wastewater Treatment.* New York: Marcel Dekker, Inc; 1980.

33. Caulet P, Bujou B, Philippe JP, Lefevre F, Audic JM: **Upgrading of wastewater treatment plants for nitrogen removal: Industrial application of an automated aeration management based on ORP evolution analysis.** *Water Sci Technol* 1998, **37**:41-47.

34. Grunditz C, Dalhammar G: **Development of nitrification inhibition assays using pure cultures of *Nitrosomonas* and *Nitrobacter*.** *Water Res* 2001, **35**:433-440.

35. Strous M, Kuenen JG, Jetten MSM: **Key physiology of anaerobic ammonium oxidation.** *Appl Environ Micorbiol* 1999, **65**:3248-3250.

36. Jetten MS, Strous M, van de Pas-Schooned KT, Schalk J, van Dongen UG, van de Graaf AA, Logemann S, Muyzer G, van Loosdrecht MC, Kuenen JG: **The anaerobic oxidation of ammonia.** *FEMS Microbiol Rev* 1998, **22**:421-437.

37. Dos Santos VAPM, Tramper J, Wijffels RH: **Integrated nitrogen removal in compact systems by immobilized microorganisms: new-generation bioreactors.** *Biotechnol Annul Rev* 1998, **4**:321-394.

38. Lazarova V, Manem J: **Innovative biofilm treatment tecnologies for water and wastewater treatment.** In *Biofilm II Process Analysis and Applications*. Edited by Bryers JD. New York: Wiley-Liss; 2000:159-206.

39. Beun JJ, Verhoef EV, Van Loosdrecht MCM, Heijnen JJ: **Stoichiometry and kinetics of poly-B-hydroxybutyrate metabolism under denitrifying conditions in activated sludge cultures.** *Biotechnol Bioeng* 2000, **68**:496-507.

40. Verstraete W, Philips S: **Nitrification-denitrification processes and technologies in new contexts.** *Environ Pollut* 1998, **102**:717-726.

41. Stover CK, Pham XQ, Erwin AL, Mizoguchi SD, Warrener P, Hickey MJ, Brinkman FS, Hufnagle WO, Kowalik DJ, Lagrou M *et al.*: **Complete genome sequence of *Pseudomonas aeruginosa* PA01, an opportunistic pathogen.** *Nature* 2000, **406**:959-964.