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MiniReview

The anammoxosome: an intracytoplasmic compartment in anammox bacteria

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Abstract

Anammox bacteria belong to the phylum Planctomycetes and perform *an*aerobic *amm*onium *ox*idation (anammox); they oxidize ammonium with nitrite as the electron acceptor to yield dinitrogen gas. The anammox reaction takes place inside the anammoxosome: an intracytoplasmic compartment bounded by a single ladderane lipid-containing membrane. The anammox bacteria, first found in a wastewater treatment plant in The Netherlands, have the potential to remove ammonium from wastewater without the addition of organic carbon. Very recently anammox bacteria were also discovered in the Black Sea where they are responsible for 30–50% of the nitrogen consumption.

This review will introduce different forms of intracytoplasmic membrane systems found in prokaryotes and discuss the compartmentalization in anammox bacteria and its possible functional relation to catabolism and energy transduction.
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1. Different forms of intracytoplasmic membrane systems in prokaryotes

Intracytoplasmic membrane systems have been found in many different prokaryotes and have different functions. One function is to provide the cell with an increased membrane surface area into which enzymes for metabolic processes may be incorporated resulting in greater metabolic activity. In this case the increased membrane surface is achieved by the extensive and complex infolding of the cytoplasmic membrane resulting in intracytoplasmic membrane systems of various morphologies (Fig. 1).

In phototrophic bacteria, the photosystems I and II are allocated in such extensive intracytoplasmic (photosynthetic) membrane systems. The intracytoplasmic membrane systems in the phylum Green Sulfur Bacteria arise in specialized non-unit membrane-enclosed structures called chlorosomes [1,2]. In the phylum Cyanobacteria, intracytoplasmic membrane systems occur as thylakoid membranes [3]. The pigments of purple bacteria belonging to the phylum Proteobacteria are also inserted in intracytoplasmic photosynthetic membrane systems [4].

Apart from the purple bacteria, the phylum Proteobacteria also contains other groups with intracytoplasmic membranes. In the aerobic chemolithoautotrophic nitrifying bacteria, the enzymes and components required for respiration are associated with intracytoplasmic membrane systems [5–7]. The nitrite-oxidizing

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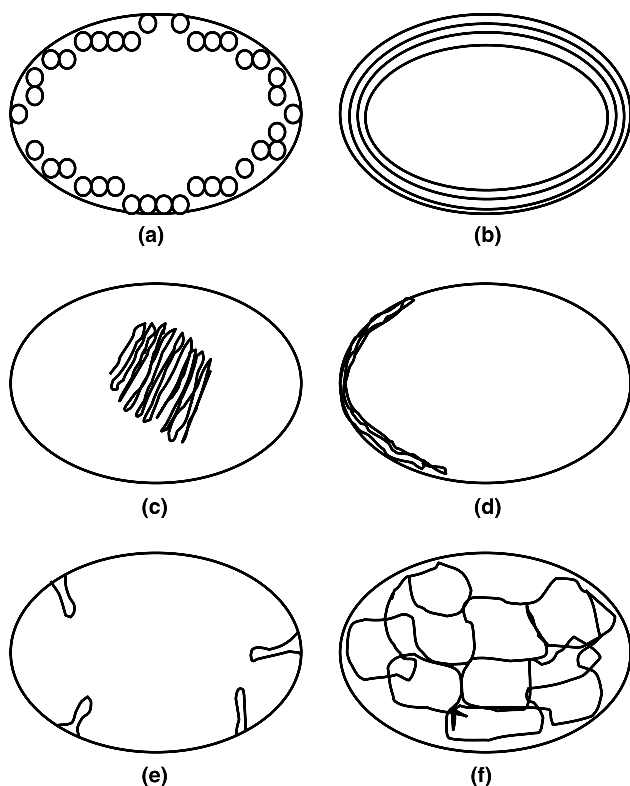


Fig. 1. Schematic representation of various morphologies of prokaryotic intracytoplasmic membranes leading to a greater surface area into which enzymes for metabolic processes may be incorporated. (a) Spherical-shaped vesicles occur in purple bacteria and as chlorosomes in Green Sulfur Bacteria. (b) Intracytoplasmic membranes running along the periphery of the cell in *Nitrosomonas*, *Nitrosococcus* and Methanotrophs type II and as thylakoid membranes in Cyanobacteria. (c) Centrally arranged membranes occurring as flat sheets/lamellar stack in purple bacteria, *Nitrosococcus* and Methanotrophs type I. (d) Peripherally arranged membranes occurring as flat sheets/lamellar stack in purple bacteria and *Nitrosococcus*, in the form of a polar cap of flattened vesicles in the peripheral region of the cell in *Nitrobacter* and random peripheral arrangement of intracytoplasmic membranes in *Nitrosomonas*. (e) Tubular intrusions of the cytoplasmic membrane in purple bacteria and also tubular intracytoplasmic membranes arranged randomly in the cytoplasm in *Nitrococcus*. (f) Random arrangement of intracytoplasmic membranes so that compartmentalization occurs in *Nitrosolobus*.

bacteria *Nitrobacter*, an α-Proteobacterium, and *Nitrococcus*, a γ-Proteobacterium, both contain intracytoplasmic membrane systems. The ammonia-oxidizing bacteria contain intracytoplasmic membranes which are the location of a key enzyme in ammonia oxidation; ammonia monooxygenase (AMO). In *Nitrosomonas eutropha*, the arrangement of the intracytoplasmic membranes is dependent on the physiological state of the cells [8]. Cells are able to grow under anaerobic conditions with hydrogen as electron donor and nitrite as electron acceptor (denitrifying cells). In this situation the AMO polypeptide concentration is very low and the intracytoplasmic membranes are arranged as circular vesicles. During recovery of ammonia oxidation, and probable de novo synthesis of AMO polypeptides, these circular forms are gradually restored to flattened peripheral membranes.

Methanotrophs contain extensive intracytoplasmic membrane systems that are related to their methane-oxidizing ability [9]. They are divided in two major groups based on intracytoplasmic cell structure. Type I methanotrophs, belonging to the γ-Proteobacteria, have intracytoplasmic membranes arranged as bundles of disc-shaped vesicles distributed throughout the cell. Type II methanotrophs, belonging to the α-Proteobacteria, have paired membranes running along the periphery of the cell.

All the intracytoplasmic membrane systems mentioned above are profitable to the organism because the extensive and complex infolding of the cytoplasmic membrane to form tubules, vesicles or flattened stacks of intracytoplasmic membranes (also called lamellae) provides a greater surface area into which enzymes for metabolic processes are incorporated; a larger membrane surface is thus provided for greater metabolic activity.

Apart from extending an existing functionality, intracytoplasmic membranes can also provide the organism with a new functionality. This applies for example to the α-Proteobacterium *Agrobacterium tumefaciens* and the γ-Proteobacteria *Thioploca* and *Thiomargarita* in which an intracytoplasmic membrane encloses a compartment that functions as a storage facility.

Volutin or metachromatic granules are subcellular entities that occur in many bacteria. The volutin granules of *A. tumefaciens* are discrete intracellular compartments that are enclosed by an intracytoplasmic membrane [10]. They are rich in phosphate, pyrophosphate, polyphosphate, magnesium, potassium and calcium. They have calcium-accumulating activity and a pyrophosphatase to generate acidity. The structural and biochemical resemblance of volutin granules of *A. tumefaciens* with eukaryotic acidocalcisomes suggests potential functional similarities as energy stores and in intracellular pH, calcium and osmotic regulation.

Thioploca and *Thiomargarita*, two sulfur-oxidizing bacteria, couple the sulfur and nitrogen cycle. They contain elemental sulfur inclusions formed by intrusions of the outer cytoplasmic membrane and a large membrane-bounded intracytoplasmic vacuole for nitrate storage [11,12]. Nitrate is taken up, stored in the vacuole with a concentration of up to 500 mM and used as an electron acceptor for sulfide oxidation.

The compartmentalization found in *A. tumefaciens*, *Thioploca* and *Thiomargarita* is not functionally related to the enlargement of the membrane surface area leading to greater metabolic activity as is the case in phototrophic, nitrifying and methanotrophic bacteria. The compartmentalization has a new, separate function: storage.

One major phylum of the domain Bacteria, the Planctomycetes, is another group in which compartmentalization is not linked to the enlargement of the membrane surface. Here, compartmentalization is linked to different, so far unknown, cellular functions. The cell compartments in Planctomycetes are divided over the two daughter cells upon division and might thus be essential to the cell's viability.

1.1. Compartmentalization in Planctomycetes

Most Planctomycetes are aerobic chemoorganoheterotrophs. Their compartmentalization is in some cases complex but always involves a single intracytoplasmic membrane defining a major cell compartment. Based on electron microscopy observations, chemical analysis and resistance to β-lactam and other cell wall-targeting antibiotics, Planctomycetes lack the otherwise universal bacterial cell wall polymer peptidoglycan [13–15]. Further, their cell wall is not surrounded by one membrane on the outer and one membrane on the inner side of the cell wall as is the case for other gram-negative bacteria. Instead, there are two membranes on the inner side and no membrane on the outer side of the cell wall. One of these membranes is closely appositioned to the proteinaceous cell wall. This membrane has been defined as the cytoplasmic membrane based on the finding of RNA in the 'paryphoplasm' compartment (see below) bounding its inner side. The other, innermost, membrane has been defined as an intracytoplasmic membrane as it is within a cytoplasm defined as any region of the cell containing RNA. A compartment – the paryphoplasm – is thus formed bounded by the cytoplasmic membrane on one side and the intracytoplasmic membrane on the other. The organization of the cell envelope of Planctomycetes is therefore very different from the other gram-negative bacteria [16].

In *Pirellula* and *Isosphaera*, the intracytoplasmic membrane defines a single interior cell compartment bounded by it which holds the cell DNA as well as ribosome-like particles [16]. In *Isosphaera*, the intracytoplasmic membrane exhibits a large invagination towards the central region of the cell. In *Gemmata* and anammox bacteria, the compartment bounded by the intracytoplasmic membrane contains yet a second membrane-bounded compartment [16,17]. In *Gemmata*, this compartment contains the cell DNA and is surrounded by a double membrane. In anammox bacteria (Fig. 2), the compartment is bounded by a single bilayer membrane. It is the site where catabolism takes place and is called the anammoxosome. The cytoplasm in anammox bacteria is thus divided into three compartments separated by single bilayer membranes: (1) the outer region, i.e., the paryphoplasm, occurs as an outer rim defined on its outer side by the cytoplasmic membrane and cell wall and on the inner side by the intracytoplasmic membrane, (2) the riboplasm, containing the nucleoid and (3) the inner ribosome-free compartment, the anammoxosome, bounded by the anammoxosome membrane.

1.2. Compartmentalization in anammox bacteria: the anammoxosome

Three genera of anammox bacteria have so far been found. The first-discovered anammox bacterium was provisionally named *Candidatus* "Brocadia anammoxidans" [18,19]. Later, two other species were found to exist: *Candidatus* "Kuenenia stuttgartiensis" [20] and *Candidatus* "Scalindua sorokinii". The latter was recently discovered in the Black Sea [21].

Anammox bacteria are coccoid bacteria with a diameter of less than 1 μm. They have a generation time of 10–30 days and are physiologically distinct from the other known Planctomycetes: they are anaerobic chemolithoautotrophs. They convert ammonium with nitrite (as the electron acceptor) to dinitrogen gas (1) with hydrazine (N_2H_4) and hydroxylamine (NH_2OH) as intermediates. This catabolic reaction is carried out 15 times to fix one molecule of carbon dioxide with nitrite as electron donor leading to the anaerobic production of nitrate in anabolism (2) [22].

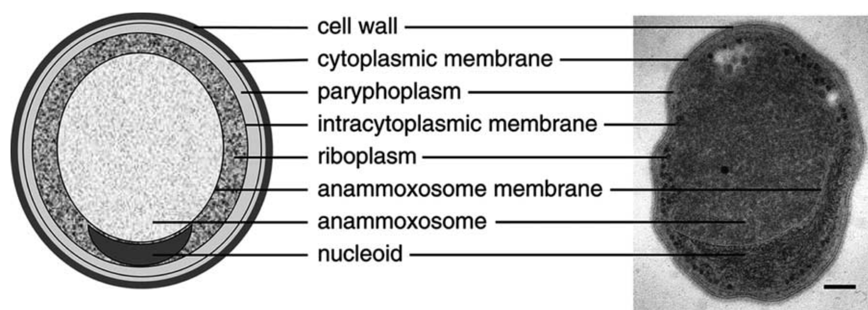
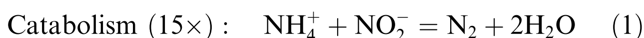
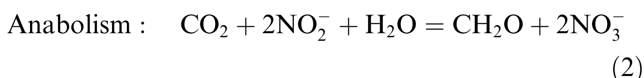


Fig. 2. Cellular compartmentalization in anammox bacteria. Left: schematic drawing, right: thin section of cryosubstituted *Candidatus* "Brocadia anammoxidans" seen via transmission electron microscopy. *Bar* 100 nm.



$$\text{Catabolism } (15\times): \quad NH_4^+ + NO_2^- = N_2 + 2H_2O \quad (1)$$



$$\text{Anabolism}: \quad CO_2 + 2NO_2^- + H_2O = CH_2O + 2NO_3^- \quad (2)$$

Anammox bacteria, like all other Planctomycetes, lack peptidoglycan and have a proteinaceous cell wall instead [13,15,23]. Anammox lipids contain a combination of ester-linked (typical of the Bacteria and Eukarya) and ether-linked (typical of the Archaea) fatty acids. Lipids are taxonomic markers and determine the membrane structure. Clearly, lipid membranes are essential to enable the existence of concentration gradients of ions and metabolites. Anammox bacteria contain a variety of abundant unconventional membrane lipids [21,24,25]. The lipids occur in a wide variety of types and derivatives. Among these, unique structures have been found. They contain one, two or both of two different ring-systems, X and Y (Fig. 3). Ring-system X is composed of three cyclobutane moieties and one cyclohexane moiety substituted with an octyl chain, which is ether-bound at its ultimate carbon atom to the glycerol unit. Ring-system Y is composed of five linearly concatenated cyclobutane rings substituted with a heptyl chain, which contains a methyl ester moiety at its ultimate carbon atom. All rings in ring-systems X and Y are fused by *cis*-ring junctions, resulting in a staircase-like arrangement of the fused rings, defined as ladderane.

Lipids containing ladderane moieties X and Y are abundant membrane lipids in anammox bacteria. They represent 34% of total lipids in *Candidatus* "Brocadia anammoxidans" [24]. The structure of the ladderane membrane lipids is unique in nature. Ladderane membrane lipids have so far been found only in anammox bacteria. This raises the question of the functional significance of the ladderane lipids.

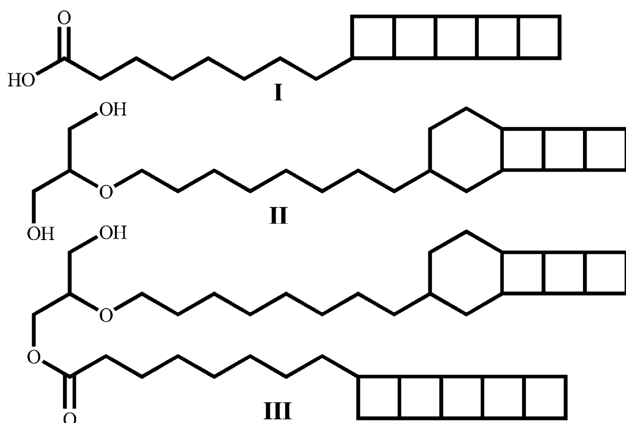


Fig. 3. Structures of three characteristic ladderane lipids: I ladderane fatty acid-containing ring-system Y. II ladderane monoalkyl glycerol ether-containing ring-system X. III ladderane glycerol ether/ester containing both ring-systems, X and Y. Lipids containing ladderane moieties X and Y are abundant membrane lipids in anammox bacteria. Adapted with permission from Jetten et al. [29].

2. Clues as to the possible functional significance of compartmentalization in anammox bacteria

2.1. Biochemical model for the build-up of a proton motive force and adenosine triphosphate (ATP) synthesis

One of the key enzymes of the anammox reaction, the hydrazine-oxidizing enzyme, was purified and immunogold labeling showed that it was situated in the anammoxosome [16]. This indicates that anammox catabolism takes place inside the anammoxosome. A biochemical model (Fig. 4) has been proposed in which ammonium and hydroxylamine are combined to hydrazine by hydrazine hydrolase (HH), the hydrazine-forming enzyme. Hydrazine is then oxidized by a hydrazine-oxidizing enzyme (HZO). HZO was shown to have some similarity to hydroxylamine oxidoreductase (HAO) of *Nitrosomonas europaea* [26]. The oxidation, taking place inside the anammoxosome, results in dinitrogen gas, four protons and four electrons. These four electrons are then used together with five protons from the riboplasm by a nitrite-reducing enzyme (NIR) to reduce nitrite presumably to hydroxylamine [18].

In the model, the anammox reaction establishes a proton gradient by the effective consumption of protons in the riboplasm and production of protons inside the anammoxosome, a mechanism known as separation of charges. This results in an electrochemical proton gradient directed from the anammoxosome to the riboplasm. This gradient contains chemical potential energy – the chemical gradient of protons results in a pH difference (ΔpH), where the riboplasm is alkaline compared to the anammoxosome – and electrical potential energy – the electrical gradient of protons results in a charge difference ($\Delta\Psi$), where the riboplasm is negatively charged compared to the anammoxosome. Both the ΔpH and the $\Delta\Psi$ have a drawing force on the pro-

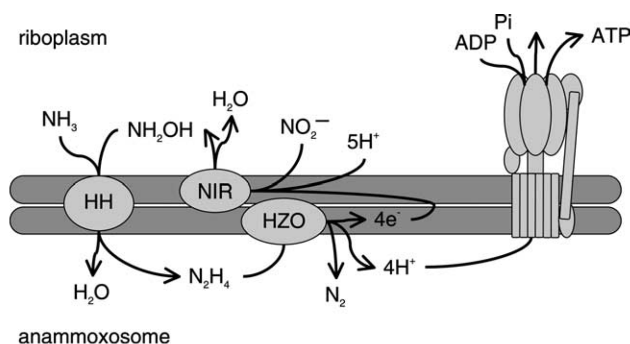


Fig. 4. Postulated anaerobic ammonium oxidation coupled to the anammoxosome membrane in anammox bacteria resulting in a proton motive force and subsequent ATP synthesis via membrane-bound ATPases. HH: hydrazine hydrolase; the hydrazine-forming enzyme, HZO: hydrazine-oxidizing enzyme, NIR: nitrite-reducing enzyme.

tons from inside to outside the anammoxosome: the proton motive force (Δp). This could be used to drive the synthesis of ATP catalyzed by membrane-bound adenosine triphosphatases (ATPases) located in the anammoxosome membrane (Fig. 4). Protons would flow passively back into the riboplasm (with the electrochemical proton gradient = downhill) through proton pores formed by the ATPases, in an analogous manner to that well known in the systems of respiratory oxidative phosphorylation and photosynthetic phosphorylation. The anammoxosome membrane-bound ATPases would be located with their globular, hydrophilic ATP synthesizing domain in the riboplasm and their hydrophobic proton translocating domain in the anammoxosome membrane. The synthesized ATP would then be released in the riboplasm.

2.2. Limitation of diffusion

Anammox bacteria depend on an electrochemical ion gradient across a membrane for sufficient ATP synthesis. Because anammox catabolism is slow, only few protons are translocated per unit of time, whereas the dissipation of the resulting electrochemical gradient by passive diffusion is independent of growth rate and proceeds at normal speed. Therefore, the passive diffusion of protons across a biological membrane is relatively more important and leads to a higher energy loss in the anammox case. For comparison, in mitochondria, the energy loss due to passive diffusion of protons is already 10% [27]. Since the proton permeability of 'normal' biological membranes is more or less constant for all forms of life [27], the proton leakage rate could be compared to the proton translocation rate for anammox bacteria, if the anammoxosome were surrounded by a 'normal' membrane. It appeared that protons would leak out of the anammoxosome more rapidly than they would be translocated in respiration (see Appendix A). Therefore, it appears that a special, less permeable membrane is essential for these cells. Furthermore, the anammox intermediates hydrazine and hydroxylamine readily diffuse through biomembranes. From a bioenergetics perspective, the energy loss associated with the loss of 1 molecule of hydrazine from the anammox cell is equivalent to 15 catabolic cycles. The reasoning is as follows: when a molecule of hydrazine is lost, the hydrazine pool has to be replenished. Presumably, hydrazine is formed via reduction of nitrite to hydroxylamine and subsequent condensation of hydroxylamine with ammonium. The necessary equivalents (four electrons) have to come from the oxidation of storage material (i.e., glycogen). Storage material is derived from carbon dioxide. Since only one molecule of carbon dioxide is fixed for 15 mole of ammonium oxidized (15 catabolic cycles), a 10% hydrazine loss would already lead to a complete loss of viability. In addition, the anammox intermediates are toxic and mutagenic due to their ability to damage DNA. For these reasons, the limitation of (proton) diffusion and confinement of anammox intermediates within the anammoxosome is extremely important for these bacteria. Since anammox catabolism takes place inside the anammoxosome, the anammoxosome membrane might be dedicated to the limitation of diffusion by means of the dense and rigid ladderane lipids (which have a lower degree of rotational freedom) as a specific adaptation to an unusual metabolism.

The unusual density of the anammoxosome membrane has been confirmed by permeability tests with fluorophores and molecular modeling of a lipid bilayer composed of ladderane lipids [24]. The density of the ladderane part of the membrane has been calculated from molecular models to be significantly higher (up to 1.5 kg/l) than for a conventional membrane (at most 1.0 kg/l). Because the model consists of only one type of ladderane lipid, the packing of the model membrane is probably still suboptimal compared to an in vivo ladderane membrane, which is much more complex with many different lipids.

The presence of the ladderane lipids in the anammoxosome membrane has been demonstrated by the enrichment of intact anammoxosomes from cells of *Candidatus* "Brocadia anammoxidans" [24]. Lipid analysis showed a strong enrichment in ladderane lipids in the enriched anammoxosome fraction: 53% of total lipids (compared to 34% in the intact cell fraction).

3. The anammoxosome as a site for catabolism, a barrier against diffusion and an efficient energy generator

The compartmentalization in anammox bacteria is, as in other prokaryotes, linked to metabolism and has a specific cellular function: catabolism. The hypothesis as to why this compartmentalization was accomplished is that dividing membrane tasks over two different types of membranes gives more freedom to the organism in the optimization of either membrane. The anammoxosome membrane can be used to generate and maintain a proton motive force for ATP synthesis and to keep as much as possible of the valuable and toxic intermediates of the anammox process away from the rest of the cell. Thus, this membrane has to be relatively impermeable as is accomplished by the presence of the rigid ladderane lipids. The cytoplasmic membrane can be used for homeostasis, such as the control of intracellular ion concentrations and transport processes, and thus has to be relatively flexible and permeable. By dividing these tasks, the cell can overcome the problem of requiring a single membrane to be both impermeable and permeable and using an intracytoplasmic compartment for ATP synthesis via this proton motive force would result in total control of the physical chemistry of the

proton motive force and thus more efficient energy transduction.

4. The anammoxosome as a potential organelle in cell biology

The anammoxosome may contain interesting features other than unique catabolic enzymes and membrane-potential formation. Tubule structures have been observed within the anammoxosome, seeming to pack the organelle in a dense mass, and these tubules are sometimes arranged in organized arrays [16]. These tubules may conceivably have cytoskeletal functions during cell division. Also, the nucleoid DNA is often attached to the anammoxosome membrane, so segregation of chromosomes during division may involve the anammoxosome itself. If these functions are confirmed, the anammoxosome would be a truly multi-functional organelle of a completely novel type, and one quite significant for understanding evolution of cell organization and function in general including possible clues to ways eukaryote-like division mechanisms may have evolved.

5. Perspective

The questions that now need to be answered are how permeable is the anammoxosome membrane compared to the cytoplasmic membrane and which membrane do anammox bacteria use to generate energy. It is of course also interesting to determine which ATPases are active in anammox bacteria. Immunogold labeling for ATPases will show where they are located. Further, the completion and annotation of the *Candidatus* "Kuenenia stuttgartiensis" genome will also supply information on the different types of ATPases that anammox bacteria possess, on genes involved in the transport of proteins and lipids to different cellular locations and on a possible role of tubules.

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Appendix A

The flux of protons, Φ in mole per second, as a result of leakage through a common lipid bilayer membrane is given by

$$\Phi = k * ([C_2] - [C_1]) * A * 10^{-3},$$

where k is the proton permeability through a lipid bilayer (10^{-5} cm/s [27]), $([C_2] - [C_1])$ the difference in proton concentration between the two sides of the membrane in mole per liter and A the surface area of the membrane in cm^2.

For the anammoxosome, assuming that the pH in the riboplasm is pH 7 and the pH in the anammoxosome is pH 3, this would give

$$\Phi = 10^{-5} * (10^{-3} - 10^{-7}) * 1.5 \times 10^{-8} * 10^{-3} = 1.5 \times 10^{-19} \text{mole } H^+/\text{s}.$$

Given that the maximum ammonium consumption rate is 55 μmol NH_4^+/g protein/min [28], that 1 NH_4^+ gives rise to the consumption of 5 H^+ in the riboplasm and the production of 4 H^+ inside the anammoxosome and that 1 anammox cell contains 3×10^{-14} g protein, the amount of protons produced inside the anammoxosome at maximum ammonium consumption rate, V_{H+max} in mole per second gives

$$V_{H+max} = (4 * 55 \times 10^{-6} * 3 \times 10^{-14})/60 = 1.1 \times 10^{-19} \text{mole } H^+/\text{s}.$$

References

[1] Cohen-Bazire, G., Pfennig, N. and Kunisawa, R. (1964) The fine structure of green bacteria. J. Cell Biol. 22, 207–225.
[2] Staehelin, L.A., Golecki, J.R., Fuller, R.C. and Drews, G. (1978) Visualization of the supramolecular architecture of chlorosomes (chlorobium type vesicles) in freeze-fractured cells of *Chloroflexus aurantiacus*. Arch. Mikrobiol. 119, 269–277.
[3] Nierzwicki-Bauer, S.A., Balkwill, D.L. and Stevens Jr., S.E. (1983) Three-dimensional ultrastructure of a unicellular cyanobacterium. J. Cell Biol. 97, 713–722.
[4] Pfennig, N. (1977) Phototrophic green and purple bacteria: a comparative systematic survey. Annu. Rev. Microbiol. 31, 275–290.
[5] Murray, R.G.E. and Watson, S.W. (1965) Structure of *Nitrosocystis oceanus* and comparison with *Nitrosomonas* and *Nitrobacter*. J. Bacteriol. 89, 1594–1609.
[6] Watson, S.W. (1971) Taxonomic considerations of the family *Nitrobacteraceae* buchanan. Int. J. Syst. Bacteriol. 21, 254–270.
[7] Watson, S.W. and Mandel, M. (1971) Comparison of the morphology and deoxyribonucleic acid composition of 27 strains of nitrifying bacteria. J. Bacteriol. 107, 563–569.
[8] Schmidt, I., Zart, D. and Bock, E. (2001) Effects of gaseous NO_2 on cells of *Nitrosomonas eutropha* previously incapable of using ammonia as an energy source. Antonie van Leeuwenhoek 79, 39–47.

[9] Hanson, R.S. and Hanson, T.E. (1996) Methanotrophic bacteria. Microbiol. Rev. 60, 439–471.

[10] Seufferheld, M., Vieira, M.C.F., Ruiz, F.A., Rodrigues, C.O., Moreno, S.N.J. and Docampo, R. (2003) Identification of organelles in bacteria similar to acidocalcisomes of unicellular eukaryotes. J. Biol. Chem. 278, 29971–29978.

[11] Jørgensen, B.B. and Gallardo, V.A. (1999) *Thioploca* spp.: filamentous sulfur bacteria with nitrate vacuoles. FEMS Microbiol. Ecol. 28, 301–313.

[12] Schulz, H.N., Brinkhoff, T., Ferdelman, T.G., Hernández Mariné, M., Teske, A. and Jørgensen, B.B. (1999) Dense populations of a giant sulfur bacterium in Namibian shelf sediments. Science 284, 493–495.

[13] König, E., Schlesner, H. and Hirsch, P. (1984) Cell wall studies on budding bacteria of the *Planctomyces/Pasteuria* group and on a *Prosthecomicrobium* sp. Arch. Microbiol. 138, 200–205.

[14] Fuerst, J.A. (1995) The planctomycetes: emerging models for microbial ecology, evolution and cell biology. Microbiology 141, 1493–1506.

[15] Liesack, W., König, H., Schlesner, H. and Hirsch, P. (1986) Chemical composition of the peptidoglycan-free cell envelopes of budding bacteria of the *Pirella/Planctomyces* group. Arch. Microbiol. 145, 361–366.

[16] Lindsay, M.R., Webb, R.I., Strous, M., Jetten, M.S.M., Butler, M.K., Forde, R.J. and Fuerst, J.A. (2001) Cell compartmentalisation in planctomycetes: novel types of structural organisation for the bacterial cell. Arch. Microbiol. 175, 413–429.

[17] Strous, M., Fuerst, J.A., Kramer, E.H.M., Logemann, S., Muyzer, G., van de Pas-Schoonen, K.T., Webb, R., Kuenen, J.G. and Jetten, M.S.M. (1999) Missing lithotroph identified as new planctomycete. Nature 400, 446–449.

[18] Kuenen, J.G. and Jetten, M.S.M. (2001) Extraordinary anaerobic ammonium-oxidizing bacteria. ASM News 67, 456–463.

[19] Jetten, M.S.M., Wagner, M., Fuerst, J., van Loosdrecht, M., Kuenen, G. and Strous, M. (2001) Microbiology and application of the anaerobic ammonium oxidation ('anammox') process. Curr. Opin. Biotechnol. 12, 283–288.

[20] Schmid, M., Twachtmann, U., Klein, M., Strous, M., Juretschko, S., Jetten, M., Metzger, J.W., Schleifer, K.H. and Wagner, M. (2000) Molecular evidence for genus level diversity of bacteria capable of catalyzing anaerobic ammonium oxidation. Syst. Appl. Microbiol. 23, 93–106.

[21] Kuypers, M.M.M., Sliekers, A.O., Lavik, G., Schmid, M., Jørgensen, B.B., Kuenen, J.G., Sinninghe Damsté, J.S., Strous, M. and Jetten, M.S.M. (2003) Anaerobic ammonium oxidation by anammox bacteria in the Black Sea. Nature 422, 608–611.

[22] Strous, M., Heijnen, J.J., Kuenen, J.G. and Jetten, M.S.M. (1998) The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. Appl. Microbiol. Biotechnol. 50, 589–596.

[23] Stackebrandt, E., Wehmeyer, U. and Liesack, W. (1986) 16S ribosomal RNA- and cell wall analysis of *Gemmata obscuriglobus*, a new member of the order Planctomycetales. FEMS Microbiol. Lett. 37, 289–292.

[24] Sinninghe Damsté, J.S., Strous, M., Rijpstra, W.I.C., Hopmans, E.C., Geenevasen, J.A.J., van Duin, A.C.T., van Niftrik, L.A. and Jetten, M.S.M. (2002) Linearly concatenated cyclobutane lipids form a dense bacterial membrane. Nature 419, 708–712.

[25] Schmid, M., Walsh, K., Webb, R., Rijpstra, W.I.R., van de Pas-Schoonen, K., Verbruggen, M.J., Hill, T., Moffett, B., Fuerst, J., Schouten, S., Sinninghe Damsté, J.S., Harris, J., Shaw, P., Jetten, M. and Strous, M. (2003) *Candidatus* "Scalindua brodae", spec. nov., *Candidatus* "Scalindua wagneri", spec. nov., two new species of anaerobic ammonium oxidizing bacteria. Syst. Appl. Microbiol. 26, 529–538.

[26] Jetten, M.S.M., Strous, M., van de Pas-Schoonen, K.T., Schalk, J., van Dongen, U.G.J.M., van de Graaf, A.A., Logemann, S., Muyzer, G., van Loosdrecht, M.C.M. and Kuenen, J.G. (1998) The anaerobic oxidation of ammonium. FEMS Microbiol. Rev. 22, 421–437.

[27] Haines, T.H. (2001) Do sterols reduce proton and sodium leaks through lipid bilayers? Prog. Lipid Res. 40, 299–324.

[28] Strous, M., Kuenen, J.G. and Jetten, M.S.M. (1999) Key physiology of anaerobic ammonium oxidation. Appl. Environ. Microbiol. 65, 3248–3250.

[29] Jetten, M.S.M., Sliekers, O., Kuypers, M., Dalsgaard, T., van Niftrik, L., Cirpus, I., van de Pas-Schoonen, K., Lavik, G., Thamdrup, B., Le Paslier, D., Op den Camp, H.J.M., Hulth, S., Nielsen, L.P., Abma, W., Third, K., Engström, P., Kuenen, J.G., Jørgensen, B.B., Canfield, D.E., Sinninghe Damsté, J.S., Revsbech, N.P., Fuerst, J., Weissenbach, J., Wagner, M., Schmidt, I., Schmid, M. and Strous, M. (2003) Anaerobic ammonium oxidation by marine and freshwater planctomycete-like bacteria. Appl. Microbiol. Biotechnol. 63, 107–114.