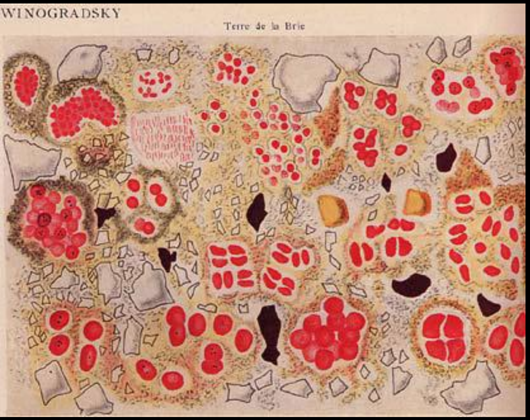
Sergej Nikolaevitch Winogradsky (Sergei Nikolaevitch Vinogradskii; (1856–1953)) a pioneer in Enviromental Microbiology

Sergej N. Winogradsky in the 1880s (From Selman Waksman, Sergei N. Winogradsky: His Life and Work [New Brunswick, N.J.: Rutgers University Press, 1953] p. 49).

Sergej Nikolaevitch Winogradsky (1857–1953), photographed around 1925. 130 H. G. Schlegel

Vinogradskii in 1923 (From Selman Waksman, Sergei N. Winogradsky: His Life and Work [New Brunswick, N.J.: Rutgers University Press,1953], p. 50).



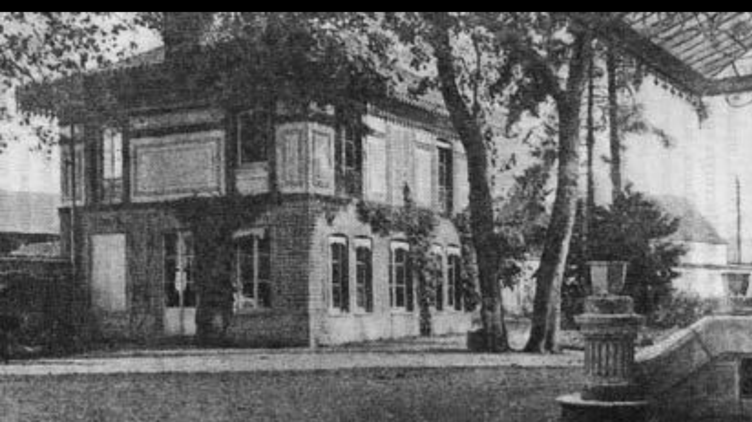
Vinogradskii's Drawing of a Soil Landscape. A Brie Sample. From Serge Winogradsky, Microbiologie du Sol: Problemes et Methodes – Cinquante Ans de Recherches [Paris: Masson et Cie, 1949], p. 859.

Curriculum Vitae I

- born on September 1, 1856, in Kiev, Russia, of a rich and aristocratic family.
- At 10 years of age, the young Sergei entered the Second Gymnasium.
- 1873, Sergej began studying law at the University of Kiev. He found the law uninteresting and transferred to the division of natural science. This was also boring and Sergej stopped attending classes. Subsequently, he studied music but this was "without any activity of the brain."
- 1877, Winogradsky quit music and returned to the natural sciences at the University of St. Petersburg.
- 1879, he married Zinaida Tichotzkaia; they remained married for 60 years and had four daughters.
- 1881, Winogradsky graduated with a diploma in science and became a candidate for a professorship at the University.
- 1885, Winogradsky accepted a position at the University of Strasbourg, Botanical Institute under Anton de Bary
- After de Bary's death in 1887, Winogradsky settled in Zurich, working at the University of Zürich
- 1891, Winogradsky spent a few months in Paris at the Pasteur Institute.

Curriculum Vitae II

THE ENCYCLOPAEDIA OF SAINT PETERSBURG
http://www.encspb.ru



The Laboratory of Agricultural Microbiology at Brie-Compte-Robert (From Selman Waksman, Sergei N. Winogradsky: His Life and Work [New Brunswick, N.J.: Rutgers University Press, 1953], p. 49).

❖ 1891, Winogradsky returned to St. Petersburg to organize a division of general microbiology at the newly established Institute of Experimental Medicine. He was later made director of the institute and editor of Archives of Biological Sciences.

❖ From 1905 onwards Winogradsky settled permanently on his estates in the Ukraine and lived the life of a scientifically interested landowner.

❖ The first World War had broken out by 1914, and the Ukraine became strongly affected by revolutions.

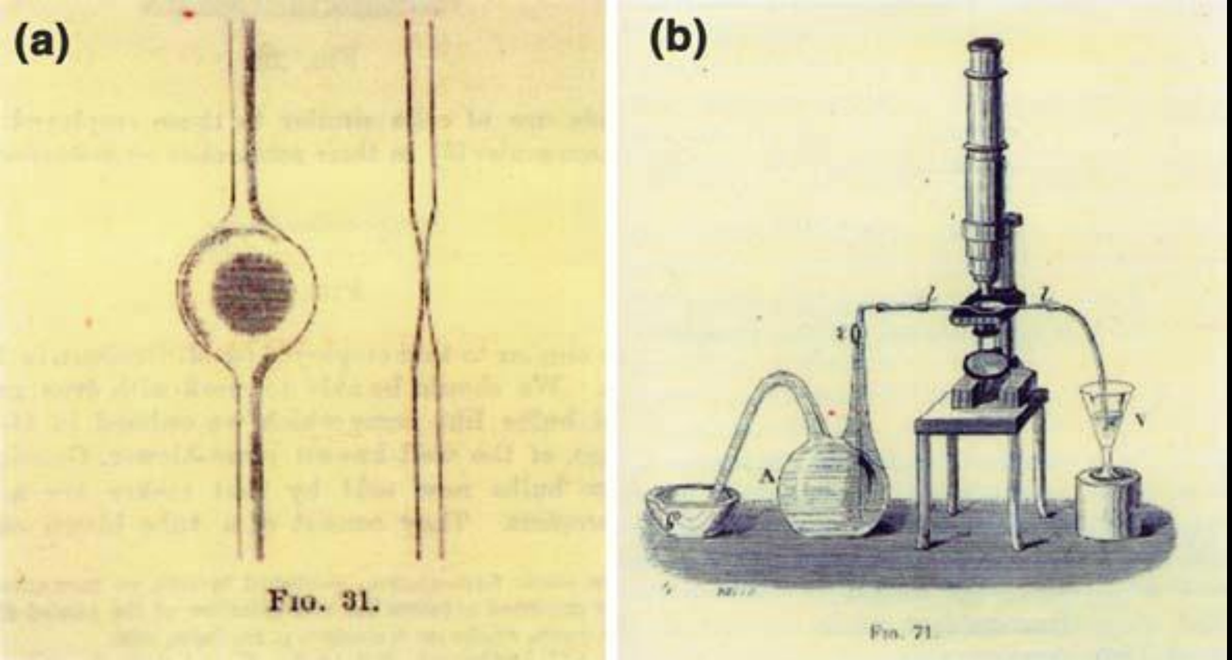
❖ Winogradsky left southward, left Odessa with a French warship which brought him to Marseilles.

❖ 1921 he arrived at his villa in Switzerland. He was then appointed to a professorship at the Agricultural Institute of the University of Belgrade.

❖ February 1922 from the director of the Pasteur Institute in Paris, Dr. E. Roux, inviting him to come to Paris and organize a division of agricultural bacteriology at the Institute. The division suited him very well, it was a small estate at Brie-Comte-Robert, 30 km from Paris.

❖ 1949 his comprehensive book ‘Microbiologie du sol, Problems et methods was published 1953.

❖ Sergej N. Winogradsky died on February 24, 1953, at the age of 97 in Paris.



(a) Geissler Chamber, These chambers are flattened glass tubing that allow for culture liquids to pass slowly though, perhaps influencing the development of the microorganisms living in the chambers, (b) Geissler Chamber with a microscope. [Louis Pasteur, E´ tudes sur le vinaigre: sa fabrication, ses maladies, moyens de les pre´venir : nouvelles observations sur la conservation des vins par la chaleur [Paris: Gauthier- Villars V. Masson et fils, 1868])

He first grew ‘‘normal appearing’’ Mycoderma cultures in ‘‘nutritive liquids, the chemical make-up of which were precisely known.’’ Then, varying these liquids by only one nutritive component, while keeping the remaining conditions of the culture strictly uniform, he observed the organisms for deviations in shape and life cycle. Borrowing from Pasteur’s publications, Winogradsky used an apparatus – Geissler chambers – that permitted ‘‘attentive’’ and prolonged microscopical observation.

Winogradsky discovered:

Beggiatoa minima, Clostridium pasteurianum, Cytophaga hutchinsonii, Nitrosococcus nitrosus, Nitrosocystis javaensis, N. coccoides, Nitrosomonas europaea, Nitrosospira briensis, N. antarctica, Nitrobacter

The Doctrine of Monomorphism (1885 at the University of Strasbourg)

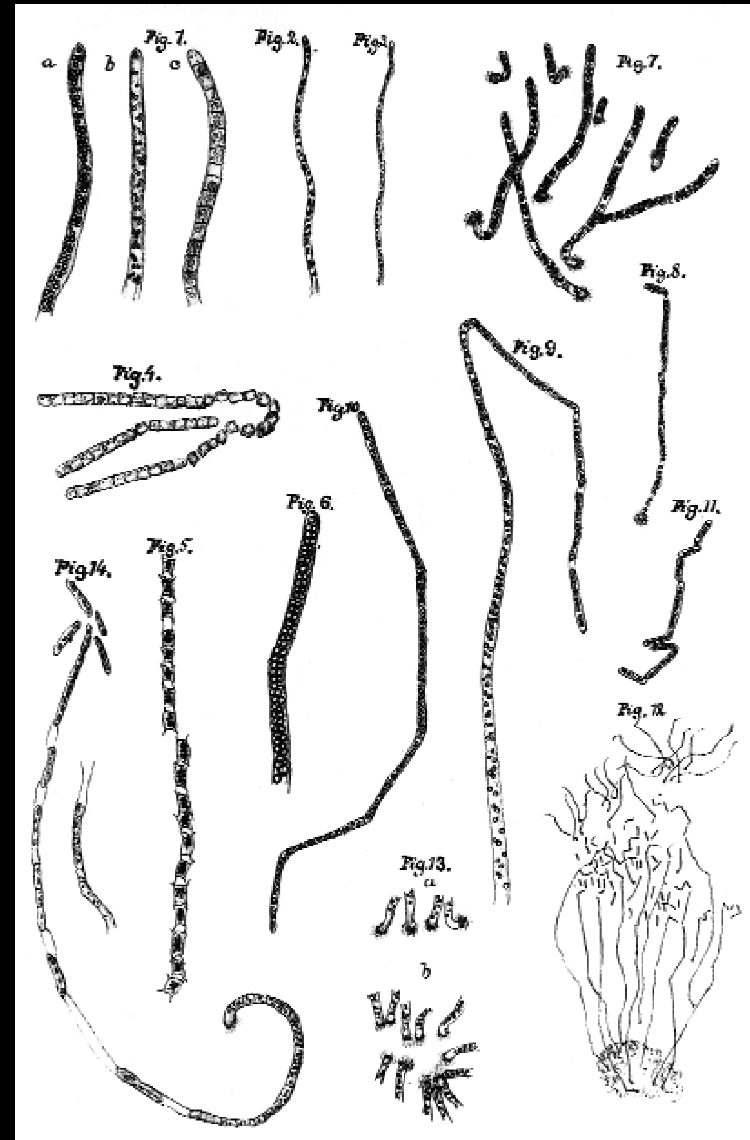
- He began his investigations on *Beggiatoa* (was surprisingly well known at the time.)
- Considering that it had never been grown in pure culture.
- Researchers knew about *Beggiatoa* because it formed large mats in sulfurous waters
- *Beggiatoa* would not grow on the conventional nutrient media developed by Koch
- Mixed cultures provided arguments for the proponents of pleomorphism—the notion that bacteria change shape.

- Winogradsky developed a method of culturing *Beggiatoa* by imitating its natural environment on glass slides and silica gel.

- With respect to his original question on the stability and constancy of the shape of *Beggiatoa* the results were unequivocal: the threads never changed their diameter or formed agglomerates of cocci or spirilla → they were not pleomorphic.
He clearly distinguished *Beggiatoa* where he saw rodlike gonidia, slowly moving and attaching to surfaces.

- His observations affirmed the doctrine of monomorphism. He later wrote "bacteria are…like other organisms, characterized by morphological types that can and should be systemically grouped into genera and species.“

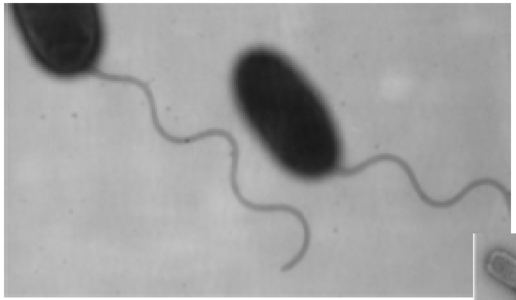
Filaments of Beggiatoa (Phase contrast micrograph); the golden granules within the tubes are sulfur. Image from Microbial Diversity 1997 (aus Rolf Schauder Gradient organisms. Microbial Diversity Course, 1997, MLB, Woods Hole.

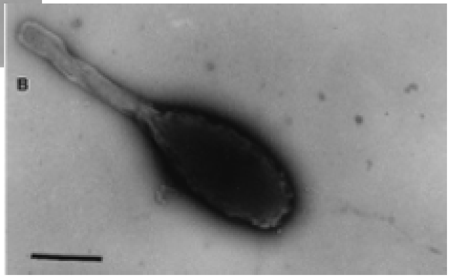


Beggiatoa and Thiotrix (Part of a table, first published in Winogradsky S.N. (1888) Beiträge zur Morphologie und Physiologie der Bacterien. Arthur Felix Verlag, Leipzig**).**

Classical methods for identification of microorganisms

- Phenotypic analysis
  - Morphology
    - Shape of bacterial cell
    - Aggregation
    - Colony morphology
    - Gram staining properties





  - Physiology
    - Aerobic or anaerobic
    - Energy production: respiration or fermentation or photosynthesis
    - Temperature and pH optima
    - Nutrient requirements (for example, carbon source)
    - Storage products & pigments

The Discovery of Chemolithotrophy

He managed to grow *Beggiatoa, Thiothrix* and various purple sulfur bacteria in the laboratory.

"I usually proceeded in the way that I put some pieces of a fresh *Butomus* rhizome together with the mud attached to it into a deep 3 to 5 liter water holding vessel and added a few grams of gypsum. After standing for 5 to 7 days at room temperature H_2S evolution starts…after 3 to 6 weeks already by microscopical investigation one can without efforts find some forms of sulfur bacteria; sometimes they grow enormously rich" (Winogradsky S.N. (1888) Über Eisenbakterien. *Bot Ztg* ***46:*** 261–270 1888).

The main result of the careful observations concerned the metabolism of sulfur.

Winogradsky formed the following conclusions:

(i) *Beggiatoa* is not involved in sulfate reduction and H_2S evolution

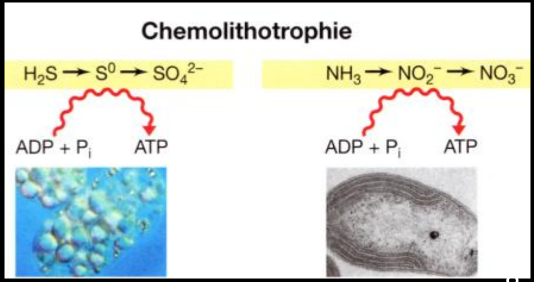
(ii) sulfur is accumulated in the plasma of the *Beggiatoa* cells due to the oxidation of H_2S.

The oxidation of H_2S corresponds to the process of respiration. The energy is exclusively derived from the process of sulfur oxidation.

When this conclusion became known in Strassbuorg his colleagues congratulated Winogradsky With the words "You have discovered a new *modus vivendi*!"

$$2H_2S + O_2 \rightarrow 2S^0 + 2H_2O$$

$$2.7H_2S + O_2 \rightarrow 2.7S^0 + 2H_2O + 1.4H^+$$



Chemoorganotrophes

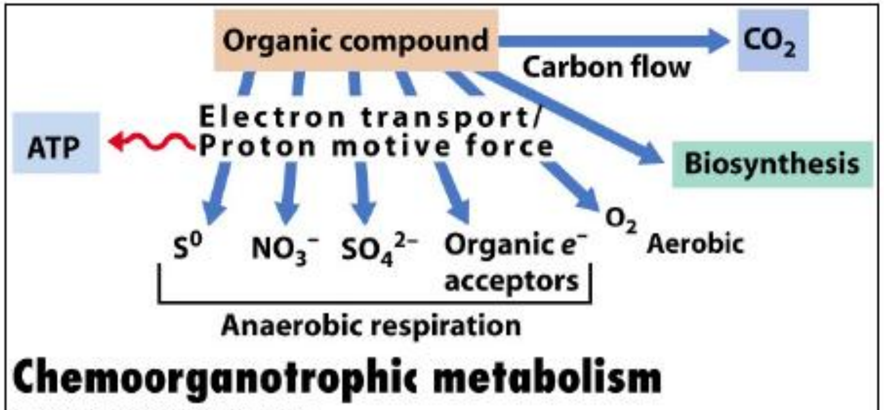


Figure 5-23a Brock Biology of Microorganisms 11/e

Organic substrates as energy- i.e. electron source.

Chemolithotrophes

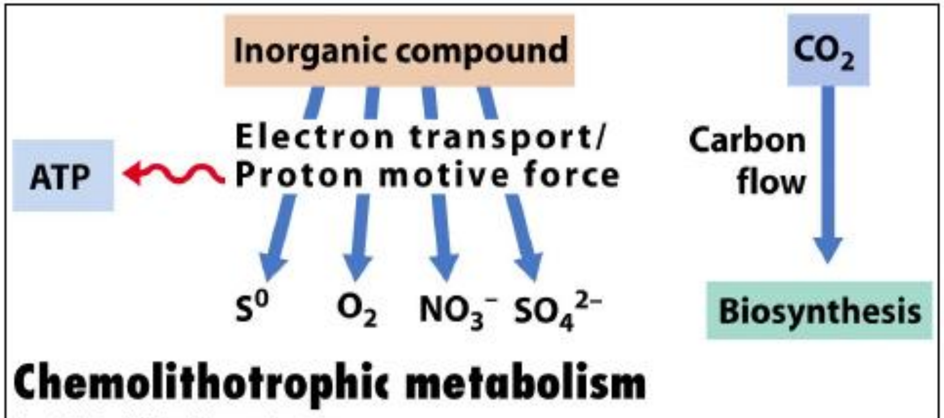


Figure 5-23b Brock Biology of Microorganisms 11/e

Inorganic substrates as energy- i.e. electron source.

Differentiation according to Carbon source

Heterotrophic: Organic compounds as C-source

Autotrophic: Inorganic compounds; CO_2 as unique C-source.

The discovery of chemoautotrophy

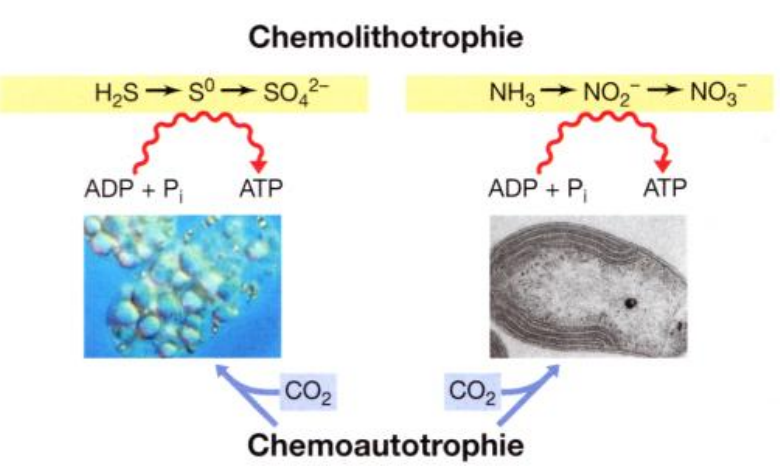
• The discovery of autotrophy was not the result of a preconceived theory but came as a byproduct of work on bacterial diversity. It is an outstanding example for serendipity, the faculty in making unexpected discoveries by accident.

• Winogradsky had worked with groups of bacteria which used an inorganic substance as an energy source.

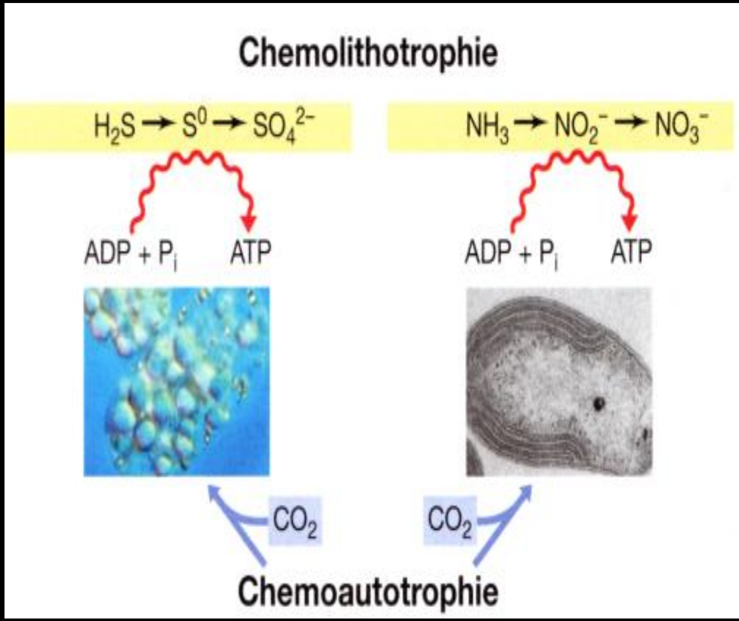
• He asked the main question "from which kind of carbon compound did the organisms derive the carbon necessary to build their bodies" .

• By growing from nitrifier in liquid culture he had already determined nitrite, nitrate and by wet combustion the assimilated carbon, and had calculated the stoichiometry of oxidized nitrogen and assimilated carbon: on average one part of carbon was assimilated per 35 parts of oxidized nitrogen.

In the paper ´Sur les organismes de la nitrification. *(Compt Rend Acad Sci (Paris)* ***110: 1013–1016***) published in May 1890 he assumed that the source of carbon in cells was carbon dioxide and that the process of assimilation was comparable to carbohydrate synthesis of chlorophyllous plants.



The discovery of chemoautotrophy



• *Nitrosomonas* and *Nitrobacter* in addition to the sulfur oxidizers were unable to use any other source of energy and carbon.

• Organic matter was unnecessary or even harmful to them.

• Thus, they were considered to be obligate autotrophic.

• The term "autotrophic" was not used by Winogradsky.

• Winogradsky's concept and called the new type of metabolism "Anorgoxydation" (inorganic oxidation).

• The combination between inorganic oxidation and autotrophy is now called lithoautotrophy.

• Winogradsky reviewed his work "Eisenbakterien als Anorgoxydanten"! (*Centbl Bakt (II)* ***57: 1–21***)

• In a footnote he remarked that bacteria, which utilize thiosulfate, gaseous hydrogen, carbon monoxide, or methane, also probably belong to the inorganic oxidants.

• Winogradsky's papers are models of observation, mature consideration, cautious conclusions and a far-sighted way of thinking

Nitrogen oxidation

- In the time in Zürich his investigations were concentrated on the oxidation of nitrogen in the soil.

- He proved that the oxidation was a two step process:

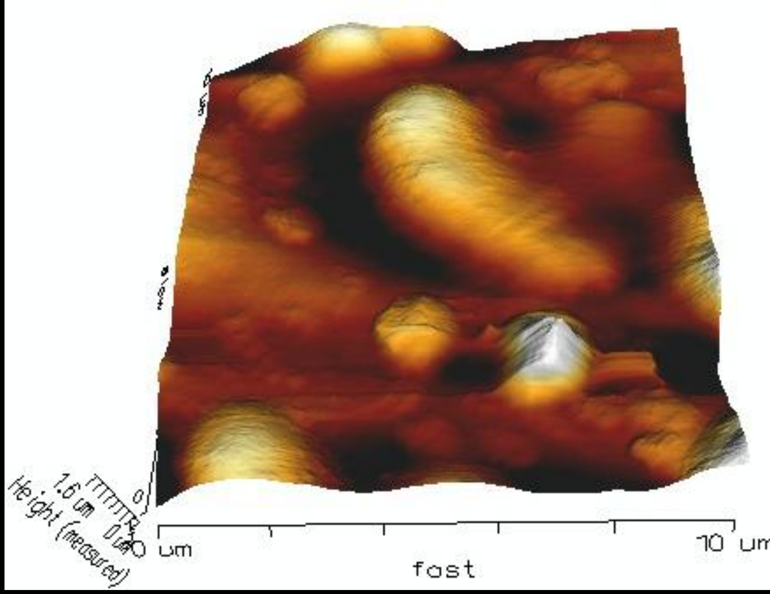
  $NH_3^+ \rightarrow NO_3^-$ and $NO_3^- - \rightarrow NO_2^-$

  each step was performed by different organisms.

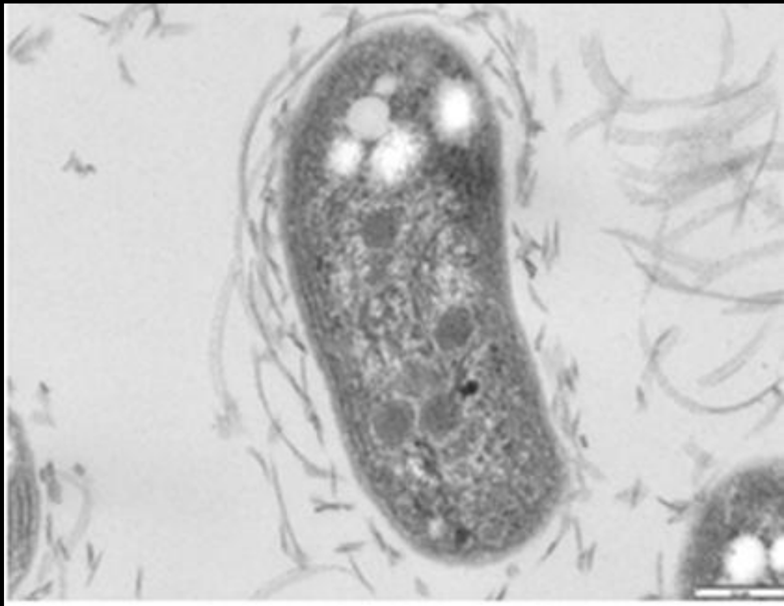
- Winogradsky's interest in nitrification continued throughout his career and he isolated several genera of nitrifying bacteria.
- The type species for the genus *Nitrobacter*, *N. winogradskyi* (ATCC 24391) was named for Sergei Winograsky.

- Years later, Winogradsky's daughter Helen would work on nitrogen-oxidizing bacteria at the Pasteur Institute.

- She isolated and described the new genera *Nitrosogloea* and *Nitrosocystis*; and in 1933, coauthored a paper with her father on *Nitrosospira*.



3D-AFM image of *Nitrobacter* 311 attached at PP-folil (BSc Jürgen Schrötz (2008) unpublished)



TEM-image of Nitrbacter winogradskyi Nb-255 (W.J. Hickey, Universizy of Wiconsin-Madison, 2006)

Clostridium pasteurianum

- Winogradsky took up the problem of nonsymbiotic nitrogen fixation.

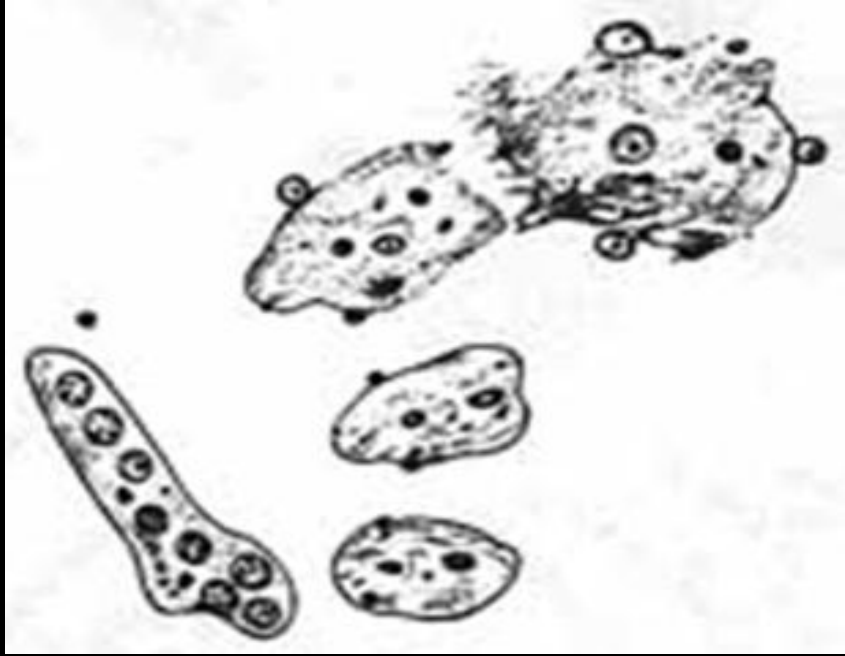
- He inoculated liquid carbohydrate, nitrogen-free media with soil.

- After a pellicle of aerobic bacteria grew on the surface, he discovered and isolated a nitrogen-fixing obligate anaerobe growing below the surface.

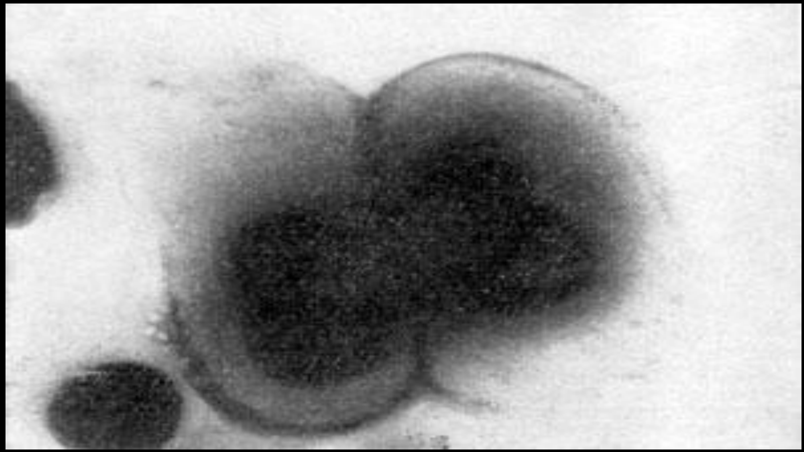
- Winogradsky identified the organism as Clostridium pasteurianum.

- The pellicle-forming aerobe was also capable of fixing nitrogen.

- Beijerinck later isolated and identified it as *Azotobacter chroococcum*.



Formation of rengenerative bodies inside sweollen and dying cells of Azotobacter (http://www.soilandhealth.org/01aglibrary/010112Krasil/010112krasil.pt1.sect2.html)



Azotobacter chroococcum (http://zr.molbiol.ru/ba_stroenie.html

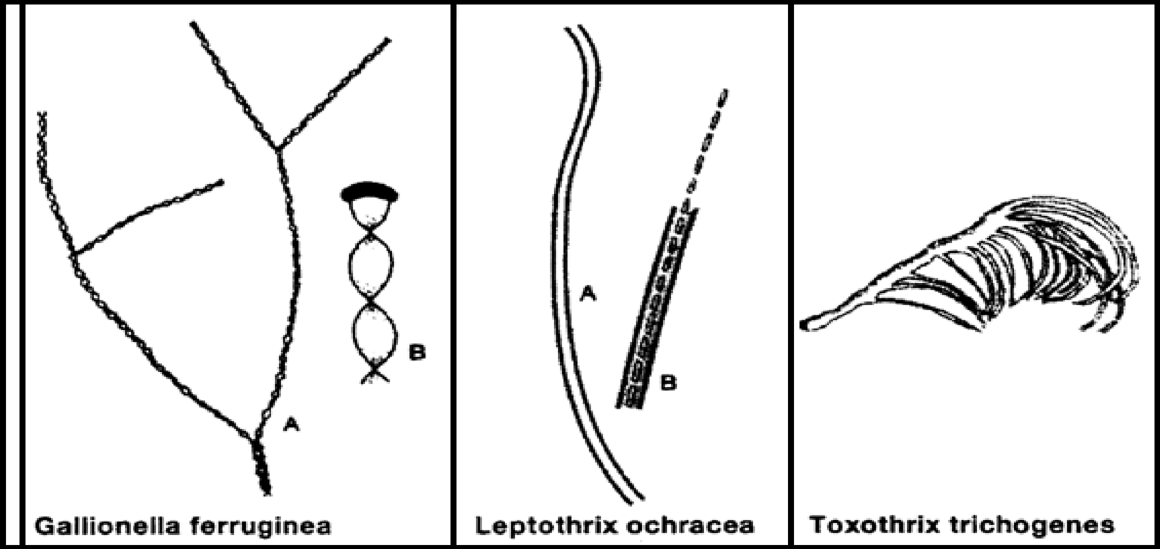
Iron Bacteria

• In the Institute at Belgrade he find a copy of "*Centralblatt für Bakteriolge*", from which he could learn about discoveries during the past.

• To his surprise, he found that very little had been done with the autotrophic bacteria.

• He immediately prepared a paper, "Iron Bacteria as Inorganic Oxidants" which was accepted and published in the "*Centralblatt*" in 1922.

• Winogradsky used the same techniques that he had used with *Beggiatoa* to show that bacteria could oxidize Fe^{2+} to Fe^{3+}. ($2Fe^{2+} + 0.5\ O_2 \rightarrow 2Fe^{3+} + H_2O$)



Iron Bacteria (http://www.trinkwasserspezi.de/enteis.html)

Winogradsky column

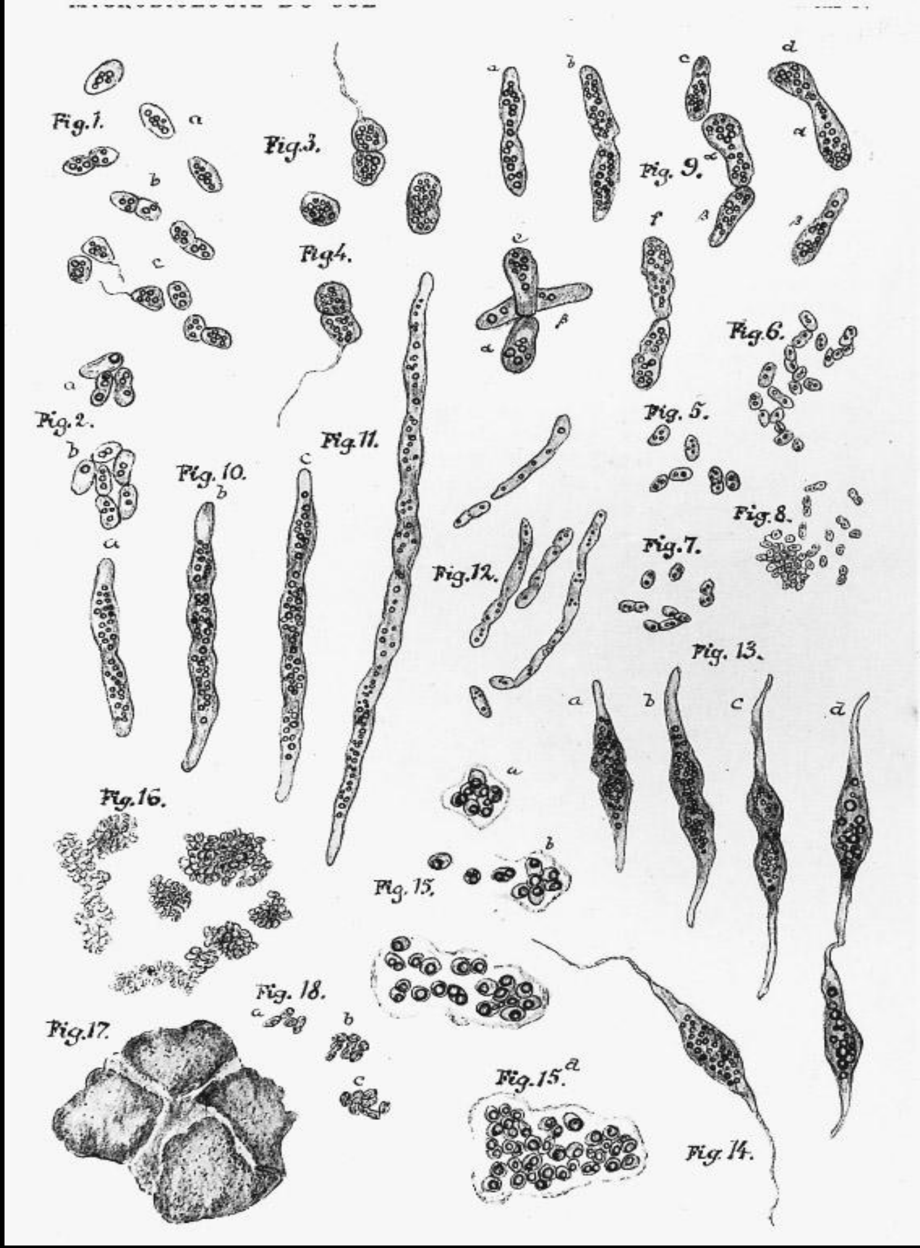
• The glass cylinder, which Winogradsky used to grow *Beggiatoa*, is nowadays known as the "Winogradsky column" and became a standard cultivation method in general microbiology.

• The column is suited to impress students with the beauty of bacteria, to introduce them into the selective culture methods, into anoxygenic photosynthesis and the world of anaerobic bacteria.

• Winogradsky described in his monograph

"die rothen Schwefelbakterien"

Thiodictyon, Thiothece gelatinosa, Thiocapsa roseo-persicina, Thiopedia rosea, Thiocystis, Chromatium okenii, C. vinosum, C. warmingii, Rhabdomonas rosea, Rhabdochromatium and various other species.
(Winogradsky S.N. (1888) *Beiträge zur Morphologie und Physiologie der Bacterien. Arthur Felix Verlag, Leipzig*)



Purple sulfur bacteria observed in Winogradsky Winogradsky S.N. (1888) *Beiträge zur Morphologie und Physiologie der Bacterien. Arthur Felix Verlag, Leipzig*)

The **Winogradsky Column**

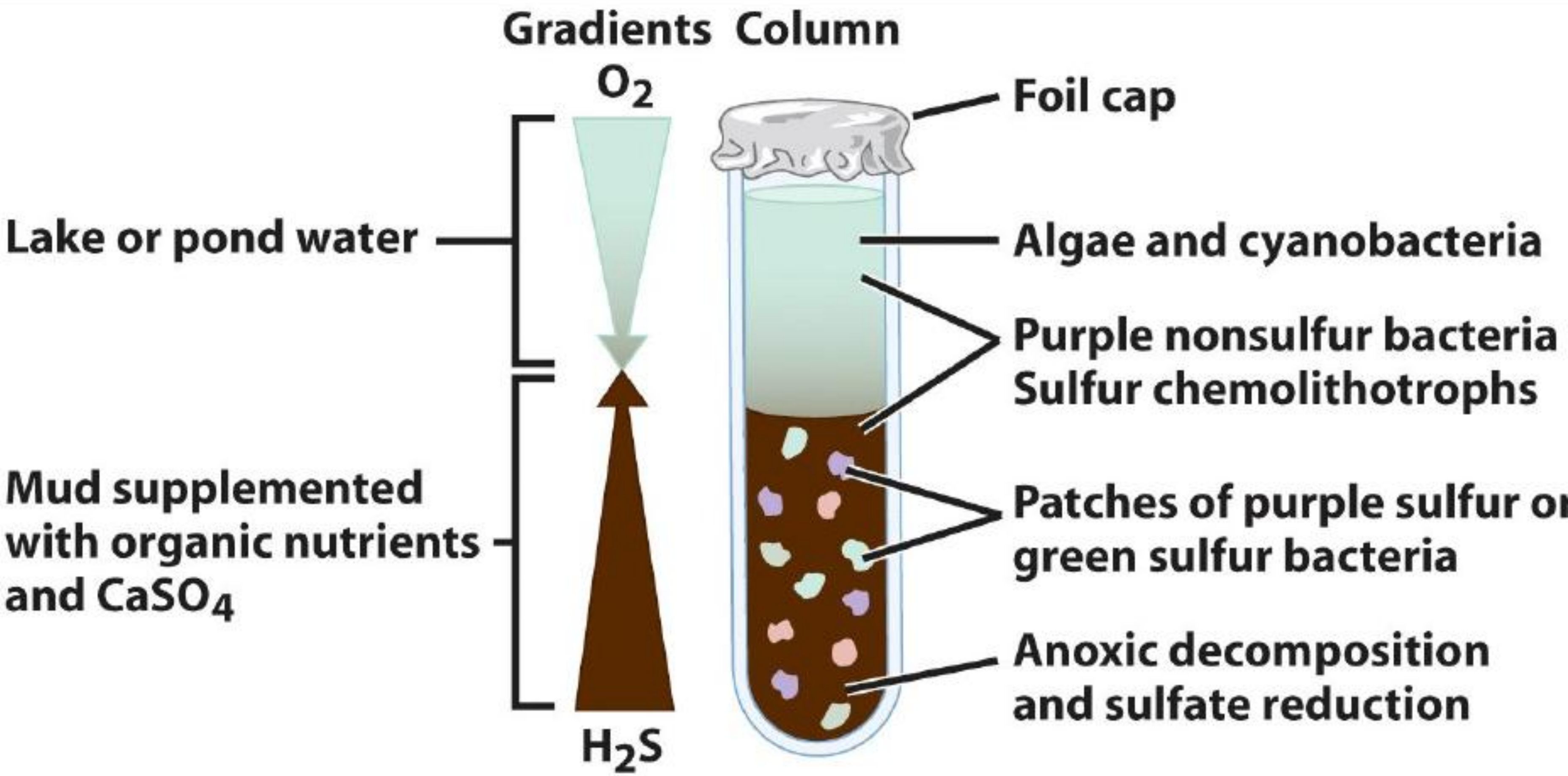


Figure 18-2a Brock Biology of Microorganisms 11/e

Winogradsky column

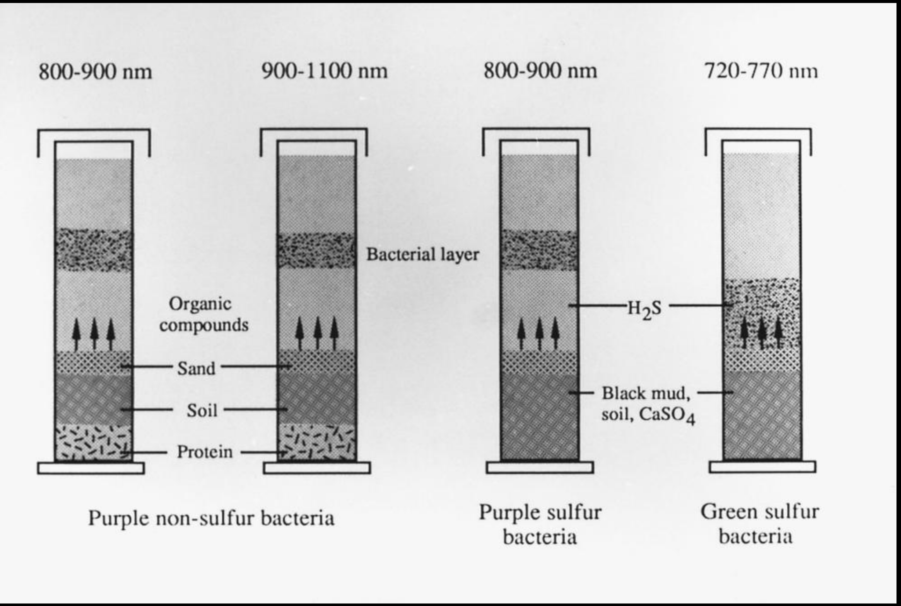
• Winogradsky observed the accumulation of the red bacteria at the light-exposed side of the glass cylinder.

• Thus, he understood that the purple bacteria were light-sensitive and were responding to light.

• But he did not consider the possibility of an anaerobic photosynthesis.

• As far as he was concerned, the metabolism of the purple sulfur bacteria was chemolithotrophic, the same type as the metabolism of *Beggiatoa*.

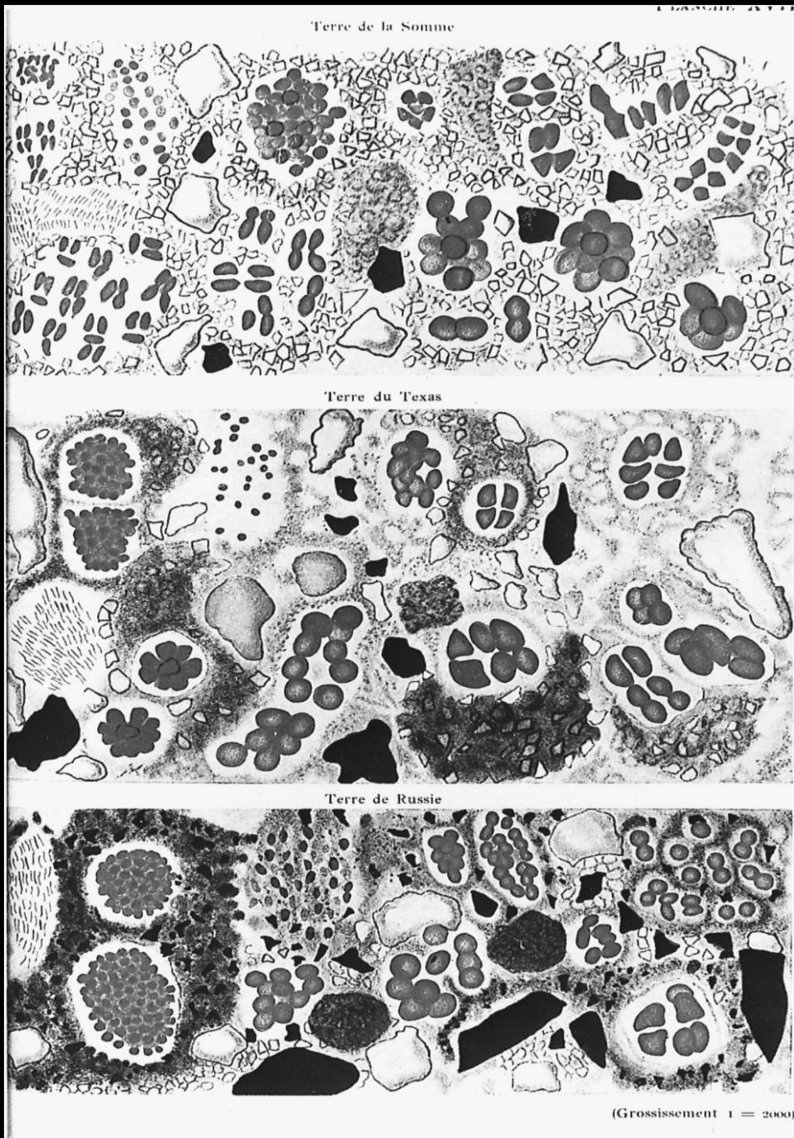
• In the minds of Engelmann and Winogradsky, photosynthesis was obligately linked with oxygen evolution.



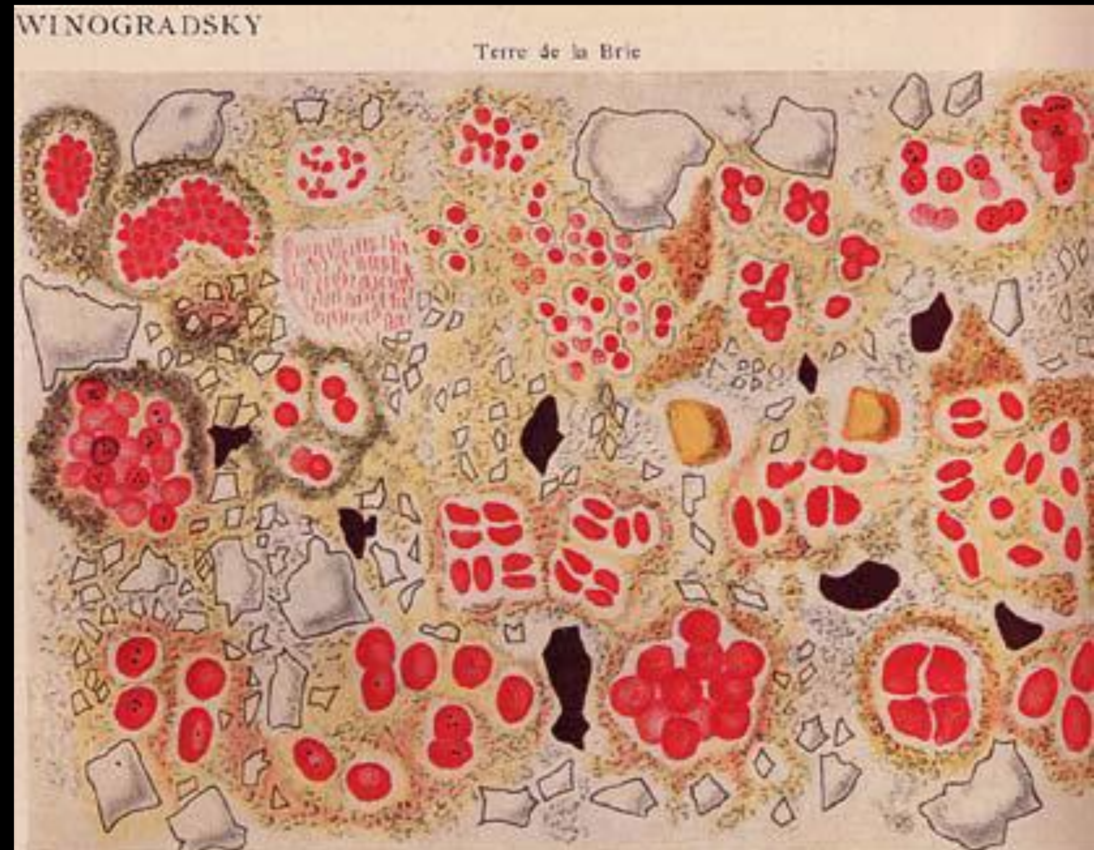
Enrichment cultures ('Winogradsky columns') for anoxygenic phototrophic bacteria. The spectral transmission regions of the light
filters, behind which the cited bacteria species and groups preferentially develop, are shown at the top. Some days after start of the culture
and inoculation with pond water and mud, red and, respectively, green bacterial layers form in the water columns (modified from Schlegel H.G. (1993) General Microbiology. 7th Edn Cambridge University Press, Cambridge

• Winogradsky left the discovery that the purple bacteria were representatives of a new metabolic type characterized by the assimilation of carbon dioxide or organic substances in the light under anaerobic conditions.

• This concept of anoxygenic photolithoautotrophy was later specified by C. B. van Niel .



Drawing of microorganisms in the soil as observed by microscopical inspection (Winogradsky S.N. (1949) *Microbiologie du Sol. Masson et Cie,*Edn)



Winogradsky´s Drawing of a Soil Landscape. A Brie Sample.
(From Serge Winogradsky, Microbiologie du Sol: Problemes et Methodes – Cinquante Ans de Recherches [Paris: Masson et Cie, 1949], p. 859).

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