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Microbiology. — "*Oxidation of manganocarbonate by microbes*".

By Prof. Dr. M. W. BEIJERINCK.

In experiments on nitrification, manganocarbonate being used as indicator of oxidation, it was found that the nitrifying microbes themselves could not attack this substance, but that other organisms possess this power in a high degree.

When two pieces of filter paper, with a little manganocarbonate between them and moistened with a dilute solution of ammonium-chloride and kaliumfosfate, are infected with garden soil and kept at about 25° C., dark brown or black spots of a manganicompound will appear upon them after some days.

The reactions characteristic of the thus produced manganicompound, are the sudden decomposition of hydrogenperoxide and the oxidation of hydroiodic acid under secretion of iodium, which reactions are not caused by the manganocarbonate itself.

In the microscopic field the newly formed manganicompound is sometimes precipitated as a detritus, in other cases in the shape of black sferites, no doubt chiefly consisting of Mn_2O_3 or MnO_2 .

When examining the related microbes, bacteria and various species of mould were recognised as cause of this oxidation.

The best way to obtain these organisms in a form fit for further experiments, proved to be the sowing of the material, developed on the filter paper, on plates obtained by dissolving agar in water to which, likewise as to the filter paper, had been added, besides c.a. 1% manganocarbonate, 0,05% kaliumfosfate and 0.05% ammoniumchloride or as much nitrate. With an equable distribution of the carbonate such plates are as white as paper. When cultivating thereon the oxidising microbes, the bacteria form a deep brown granulous deposit, partly at the glass wall, whilst the mould species produce large black spots, whereby in the agar black sferites are formed, which will sometimes grow out to $\frac{3}{10}$ millimeter diameter and are then visible to the naked eye.

As to the bacteria, the manganicompound mostly remains diffusedly precipitated in the extensive colonies, without producing distinct sferites.

It is true that in these brown spots, which after some weeks attain one or two cm. in diameter, small grains are found, but these prove to consist of clumps of bacteria, enclosed in a brown or black envelope or film of manganioxides. The capsulated bacteria have the shape of very thin, short rodlets; the loose ones, occurring in the same spots, that of micrococci.

With much trouble I obtained pure cultures of these bacteria on agarplates containing 0.05% to 0.1% manganolactate, whereon appear extremely small, capsulated colonies, which, after being crushed, prove to consist of very delicate, quickly moving rodlets, whilst the brown wall of the capsula again gives the usual manganireactions.

Agar alone proved to be the best food for these bacteria. The said rate of manganolactate could be assimilated, but on media, richer in organic food no development was observed. If, however, organic substances are quite absent the bacteria cannot oxidise the manganolactate. Although to my opinion, they are closely related to the nitrate ferment, they are not able to convert nitrites into nitrates, nor can they oxidise ammonium salts to nitrites.

By their motility they are distinguished from the genus *Siderocapsa*¹⁾ described by MOLISCH.

The mould species which oxidise manganocarbonate to mangani-oxides, and which can likewise be easily grown on filterpaper plates from garden soil, grow, like the manganese bacteria, also very well on agarplates containing nothing but manganocarbonate and some mineral salts. On pure agar, without manganocarbonate, they also develop, but less quickly, so that the carbonate evidently serves as food, and not only as a catalyser.

On plates of broth or malt extraction they likewise grow very well, but more slowly than ordinary moulds, and they quite lose thereupon their characteristic properties.

They can, however, also be cultivated on various other media where they produce much mycelium and sometimes fructify, but only oxidise the added manganocarbonate in the presence of a very slight concentration of the dissolved organic nutrient substances.

These moulds belong to very different groups of the Fungi, but all seem to be real inhabitants of the soil. So I have found species of the genera *Botrytis*, *Sporocybe*, *Trichocladium*, and in particular of *Mycogone*, which I knew already as common moulds of fertile garden soil. As might be expected, new forms also appeared, among which a new species of *Mycogone* with tetrahedrally arranged brown spores, common in the garden of the Laboratory for Microbiology.

Somewhat more minutely I examined a *Papulospora*, which I will call *P. manganica*, and a *Sporocybe* to which, for its common occurrence on the filterpaper, the name of *S. chartoikoon* is given.

¹⁾ Die Eisenbakterien, S 10, Jena 1910.

Our *Papulospora* is very much like *P. sepedonioides* PREUSS,¹⁾ but differs in some respects from the description given of it by SACCARDO.²⁾ The very fine mycelium remains in the substrate and produces only extremely short hyphae bearing small spore-heads, which do not grow out of the surface of the medium and under the microscope easily divide into the oblong spores. When these spores are cultivated in dilute broth they form a fine, branched, multi-cellular mycelium, which produces isolated spores on shorter or longer hyphae, and this is quite in accordance with the description in the literature of the genus *Monosporium*.

When sown on agarmanganocarbonate plates, at 25 to 30° C. the spores produce, after few days already a very delicate, strongly branched mycelium, soon followed by brown-colouring of the surroundings. Shortly after spherites appear in the brown field, first brown, but later jetblack. If cultivating on agar with about $\frac{1}{10}$ % manganolactate, the spherites come later and are at first colourless, but finally they also grow black. Besides as spherites, the manganic compound is also deposited as a brownish black precipitate at the mycelial threads. The fact that the spherites may be colourless, proves that they must contain something else than manganioxides only, and cautious dissolving in hydrochloric acid of the black substance always leaves a spherical substrate wherein the manganese is precipitated.

Hence, the spherites remind very strongly of the calcospherites of shells and egg-shells, described by HARTING³⁾ in 1872, and also of the spherites of calcium-carbonate, artificially precipitated in gelatin or albumin, all consisting of an organic substance, in which calcium-carbonate or calciumfosfate, or both, are deposited. HARTING thinks that this substance, in case the spherites form in gelatin, chemically differs from the gelatin itself and calls it "calcoglobuline". Evidently a similar conception may be applied to the manganese spherites of *Papulospora* found in agar. The formation of spherites in the agar can continue for months successively, so that evidently the mould does not produce noxious substances. It is then observed that the

¹⁾ ENGLER's Pflanzenfamilien, Bd. 1, Abt. 1 S. 428, Fig. 221 D.

²⁾ SACCARDO, Sylloge fungorum, Bd. 4, Pag. 59, 1885.

³⁾ Recherches de morphologie synthétique (Acad. Royale des sciences Néerland.), Amsterdam, v. D. Post 1872. Very beautiful spherites of ironfosfate may be obtained by allowing ferroammoniumsulfate to diffuse against sodiumfosfate. They likewise consist of a spherical substrate in which the ferrisalt is deposited. The late Prof. VAN 't HOFF, who long ago examined these spherites for me, came to the conclusion that the ironfosfate occurs in it as ultramicroscopic crystal needles or trichites, belonging to the monoclinic system, radiating from the centre and arranged in layers.

deposition is periodical. At first I thought that in this periodicity light is concerned, but the formation of rings also occurs in the dark, so that they seem to be of the same nature as the rings of LIESEGANG, which, for example, are formed when silvernitrate diffuses in plates of chromate gelatin, wherein the silverchromate precipitates in rings, separated by rings without silverchromate.

As to the way in which the manganic compound originates from the manganocarbonate, it appears that this may take place as well within as without the mycelium. That this can indeed occur in the cells or at least in the cell-walls, is shown by the great accumulation in the wall, and this is quite in accordance with the character of nutrient substance of the carbonate, referred to above. The increase of the sferites, however, takes place in a way suggesting the idea that also to a fairly great distance from the mycelium an oxidising agent is spread, able to transmit the oxygen of the air to the carbonate. I infer this from the fact that the formation of sferites often occurs in the midst of manganocarbonate plates, which remain snow-white in the vicinity, and I suppose that if the blackish brown oxide originated from the rather far distant mycelium, the way between should be coloured brown by a solution of that oxide. It must, however, be noted that such a brown-colouring of the white culture medium is observed in other cases, as with *Sporocybe chartoikoon*, and furthermore that I have not been able to oxidise manganocarbonate by means of oxidase and peroxidase of different origin, of which we should first of all think as causing the said distance-reaction. That peroxidase could not be concerned was to be expected, as for its action hydrogenperoxide must be present, which directly reduces the manganic compound. But that this oxidation could neither be enacted by oxidase was not to be foreseen.

In this relation I wish moreover to remark that the manganocarbonate is kept by me as a diluted pap in stoppered bottles under water, in order always to have it ready for experiments. It then remains white for years without any precaution, whilst likewise my manganocarbonate agarplates continue unchanged for months, nay, even for more than a year, at free accession of air. Hence the statement met with in some manuals for chemistry, that this substance oxidises already at the air, requires a nearer explanation of the circumstances accompanying this change, for example strong drying over a BUNSEN flame, or presence of alkaline vapours.

To the organic substances that can oxidise manganocarbonate, chinon belongs. Formation of sferites I have not observed, and there is not the least reason to accept that the described moulds

and bacteria would produce this compound, easily recognisable by its colour. So, if later it should be proved that the manganese moulds really produce a substance causing oxidation out of the cells, it may now already be asserted that it can neither be oxidase nor chinon.

From the preceding we see that with the oxidation of mangano-carbonate by microbes, many questions are related, worth a nearer examination, especially with regard to the conversions which these very common microbes cause in the soil.

Physics. — *“On the law of partition of energy.”* IV. By Prof. J. D. VAN DER WAALS JR. (Communicated by Prof. J. D. VAN DER WAALS.)

§ 12. In my previous communications on this subject I started from the earlier formula of PLANCK, in which no zero-point energy was assumed. In fact the assumption of zero-point energy involves great difficulties. In my opinion the supposition that a vibrator vibrating with slighter energy than νh would not emit energy¹⁾ is not so much responsible for these difficulties — something similar would already be found in a charge, which moved in a perfectly conducting inclosure — but rather the assumption that radiation coming from the outside yet acts on the electron in a normal way, and sets it vibrating.²⁾

In spite of these difficulties PLANCK's later formula for the energy of a vibrator has of late been preferred by different physicists. The quantitative grounds adduced for this, seem to be still pretty un-

¹⁾ Strictly speaking it might be said that PLANCK's vibrators in a certain sense do radiate, also when they contain less energy than a quantum. For they absorb energy, and absorption is a kind of emission. If e.g. we imagine a source of light and a black screen and investigate the light behind the screen by means of electro-magnetic potentials, we find darkness there, only because the contributions to those potentials, yielded by the electrons of the screen, just cancel the contributions furnished by the electrons of the source of light. If the electrons of the screen did not emit potentials and forces derived from them, we should have to observe the direct light of the source behind the screen. So PLANCK's supposition does not really come to this that vibrators when they do not possess exactly a whole number of quanta, do not radiate, but that they radiate in a particular way unilaterally.

²⁾ It is remarkable that it is assumed here that the elementary process of absorption is not reversible, whereas by the cooperation of many suchlike processes reversible observable phenomena do originate.