In the upper point P the compound passes into the liquid state, heat being absorbed. This absorption consists of two parts, the ordinary heat of fusion and the heat evolved when a part of the liquid (endothermic) compound decomposes until the equilibrium in the liquid is reached. As at higher temperatures the quantity of the compound in the liquid is large, the second quantity of heat will be small in comparison with the first and the melting will cause absorption of heat.

At Q it is, however, just the reverse because at a low temperature there exists but little compound in the liquid and the dissociation of a large proportion of the liquid compound may evolute so much heat that this exceeds the actual heat of fusion of the solid compound. The total fusion therefore produces heat and consequently the liquid field is situated below Q.

Up to the present, however, no endothermic compounds are known in the liquid state.

Microbiology. — Professor BEIJERINCK presents a paper from himself and Mr. A. VAN DELDEN: "*On the bacteria which are active in flax-rotting*".

(Communicated in the meeting of December 19, 1903).

1. *How far flax-rotting should go.*

The object of flax-rotting is the partly solving and softening of the rind of the flax-stalk to remove the *pectose*, in consequence of which the bast-bundles are freed so that later, after drying, the fibres may easily be separated from the wood by breaking and scutching. Pectose (*pt* Fig. 1) is the substance of which the young cell-walls consist, as also the outer layers of the old cell-walls; these walls are further built up from *cellulose*, which in a good rotting does not undergo any change [1]).

By the rotting also the middle-lamellae, by which the fibres in the bast-bundles stick together, may go into solution and consequently the bast-bundles would be decomposed into the fibres proper. This is not desirable as in this case no large coherent "lints" would be got in scutching, but only loose fibres, of about 2 cm. in length.

The fibres of the bast-bundles, however, separate with much greater

[1]) For the microbes which affect the cellulose proper see OMELJANSKY, Centralb. f. Bacteriol. 2 Abt. Bd. 8 p. 193, 1901, and G. VAN ITERSON. These Proceedings 24 April 1903.

difficulty than the cells of the rind, because, in the middle-lamellae between the flax-fibres, besides *pectose*, also *lignose* is found [1]), which is not affected by the rotting (*lg* Fig. 1).

It is just by the absence of lignose that the rind is so much more easily affected by the rotting process than the bast-bundles, that the latter, in a well-conducted rotting keep together and may be obtained after the scutching as a whole.

Hence, the art of rotting consists in pushing the process on to a determined point and no further.

It is not easy to indicate where this point is situated, chiefly because the flax-stalks, which at the pulling from the field are united into sheaves for the rotting, are not all equally ripe. As now the unripe stalks are more easily rotted than the riper and tougher ones, a very unequal product is obtained by submitting all to a like process. Therefore great pains are taken at the Leie, near Courtray, as much as possible to sort the flax before the rotting, in order to form lots of the same quality. Moreover, they rot the flax there twice, which renders it possible partly to redress the irregularities originated in the first rotting.

From a theoretical point of view we assume that rotting should proceed just so far ("strong rotting") as is necessary for the easy removing of the wood (*xy* Fig. 1) from the bast-bundles (*f* Fig. 1), but not so far ("feeble rotting") as to decompose them into the elementary fibres. Therefor it is necessary that the secondary bark (*cs* Fig. 1) of the flax-stalks be quite dissolved and that the primary rind (*cp* Fig. 1) be decomposed into cells [2])

2. *Pectose and Pectine.*

Pectose is a lime compound whose composition is not yet clear. Non-reckoning its rate of lime, this substance, though chemically related to, is not identic with cellulose. According to TOLLENS and TROMP DE HAAS [3]) we find for it, after removing the lime, the

[1]) J. BEHRENS, Natürliche Röstmethoden. Das Wesen des Röstprocesses vom chemischen Standpunkte. Centralbl. f. Bacteriologie, 2te Abt. Bd. 8, pag. 161, 1902.

[2]) Whether this standpoint is right in all cases (or rather will prove to be so when the flax industry will have ceased to be a very primitive agricultural industry) is doubtful. As in a good rotting process the flax-fibre itself is not injured, it is an open question whether the spinner might not be able to spin threads of greater equality from the wholly isolated fibres, then when they are still united in bast-bundles of very unequal properties.

[3]) Untersuchungen über die Pectinstoffe, LIEBIG's Annalen der Chemie. Bd. 286 p. 278, 1895 and TOLLENS, Ueber die Constitution des Pectins. Ibid. p. 292. As in hydrolysis the pectinic substances, besides glucose and galactose, also yield pentose, TOLLENS gives as probable composition $(C^5H^8O^4)^{11}.C^5H^8O^5$.

formula n ($C^6 H^{10} O^5$) or n ($C^{12} H^{22} O^{11}$), but not exactly, a small excess of O pointing to the presence of a COOH-group, which should be substituted in the pectose (the said authors use the word pectine). TOLLENS takes the here concerned acid for gluconic acid ($C^6 H^{12} O^7$), or an acid related to it, and this would occur in the pectose as lakton or ester, that is in neutral condition. He calls pectose an oxy-plantslime, but does not mention the lime.

By treating with acids the various pectose-forms are more or less

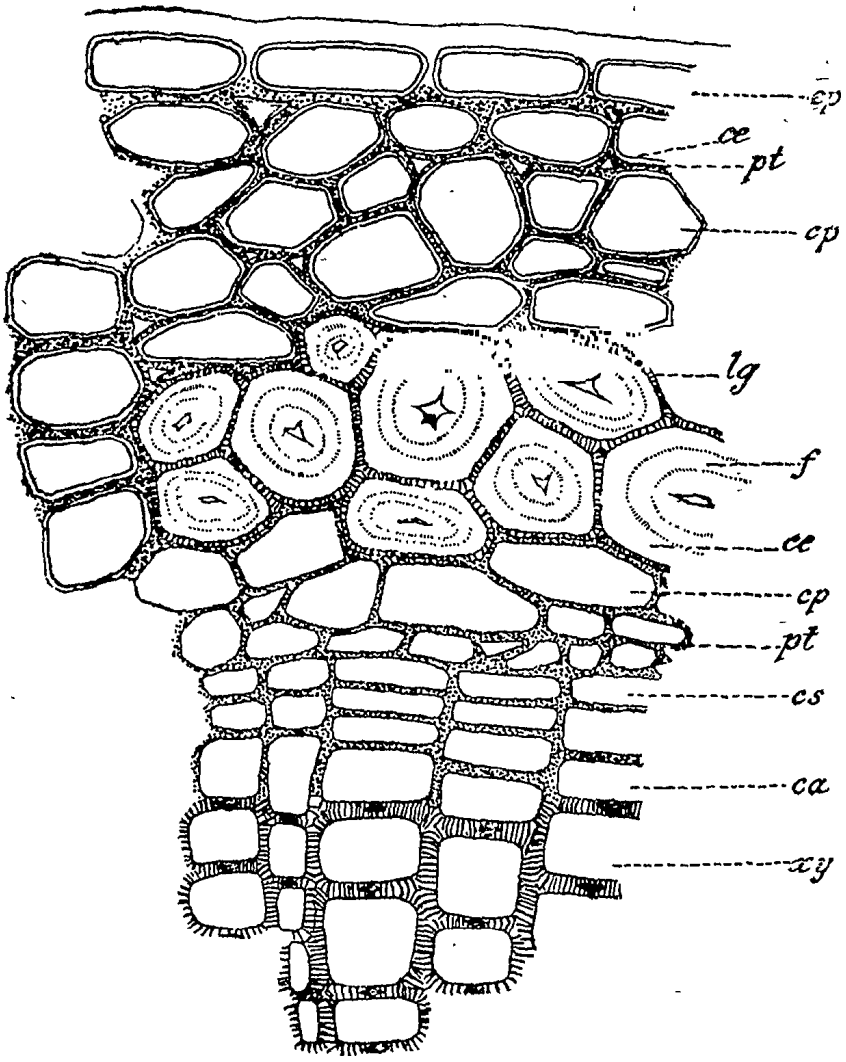

Fig. 1 (550). Transverse section of the bark and wood of a flax-stalk. *Pectose pt* dotted, *cellulose ce* left white, *lignose lg* hatched lines; *ep* epidermis, *cp* primary rind-cells with outer wall of pectose, *f* bast-fibres with outer wall of pectose + lignose, *cs* secondary rind cells, and *ca* cambiumcells whose walls quite consist of pectose, *xy* wood, with large punctations.

easily hydrolised, the pectose of the flax not so easily. Hereby first result pectine or metapectine, which have an acid character and are therefore also called pectinic acid and metapectinic acid. The pectine gelatinises in presence of lime, through the enzyme pectase, moreover through alkalies and ammonia, likewise in presence of a lime salt.

In absence of lime the compounds of alkalies with the pectine are soluble in water. Gelatinisation proper is unknown with metapectinic acid.

With continued hydrolysis, pectine and metapectine, hence, also pectose, produce galactose and pentose, and according to TOLLENS, with certain pectine kinds, dextrose and arabinose too, which sugars are easily fermented by *Granulobacter*.

By boiling with nitric acid pectose and pectine yield mucous acid.

Pectose is insoluble in cold and boiling water and in cuprioxyde-ammonia. The pectose of the flax-stalk is moreover not easily affected by dilute acids and alkalies, and remains unchanged after a short heating in water-vapour at 120° C.

Pectose can be softened by the successive influence first of an acid then of an alkali. If the flax-stalk is first extracted with dilute hydrochloric acid, by which the pectose changes into pectine, which however, as an insoluble lamella, still holds the cells together, washed out to remove the lime, salts become soluble by the hydrochloric acid, then treated with ammonia or natriumcarbonate, a considerable softening takes place. On this method, first suggested by MANGIN [1]), reposes the so-called chemical rotting after the patent of BAUER, which has, however, produced nothing of practical use, and only shows that the "inventor" did not know the requirements to which well-rotted flax should answer.

A better way of dissolving the pectose of the flax-stalks we found by placing them in a strong solution of ammonium-oxalate, but only after 3 weeks the rotting process was completely finished, so that this means has not any practical value either.

Whereas the preparation of pure pectose is troublesome in consequence of its insolubility, it is easy to make pectine.

Herefor [2]) one takes the rootstocks of *Gentiana lutea* of the chemists, grounds them finely, first extracts with H^2O and places

[1]) Although it may be read everywhere that pectose after MANGIN'S method goes "into solution", it is my experience that this is exaggeration, and that, of the decomposition of parts of plants into free cells as in rotting, there is no question in this case.

[2]) To compare BOURQUELOT and HÉRISSEY, Journal de Pharmacie et de Chimie, Sér. 6, T. 8 p. 145, 1898.

the washed material under a large quantity of 3 % H Cl, filtrates, and precipitates with alcohol. The precipitate is dissolved in boiling water, precipitated again with alcohol and this is repeated until the chlorine reaction disappears. The thus obtained pectine reacts feebly acid and solidifies with pectase + a lime salt, or with alkali + a lime salt, into a consistent transparent jelly.

3. *The rotting is caused by microbes and may be called pectose-fermentation.*

Dissolving and removing of the pectose from the rind of the flax is completely effected, without any injury of the cellulose wall of the *fibres* [1]), by some microbe species belonging to the moulds and bacteria, and hereupon the usual rotting methods are based.

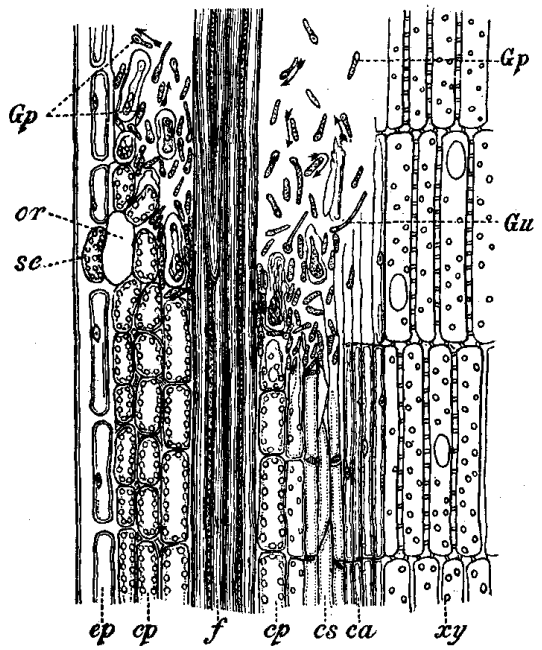

Fig. 2 (350). Rotting observed in a microscopic preparation lying in a drop of good rottingwater and consisting of a longitudinal section of the rind and the wood of a flax-stalk. Signification of the letters see Fig. 1, further: *se* cell of a stoma, *or* air chamber in the primary rind, *Gp Granulobacter pectinovorum*, the pectose bacterium proper, *Gu Granulobacter urocephalum*. The primary rind *cp* is seen to decompose into cells by solution of the pectose, and thereby tbe secondary rind *cs* and the cambium *ca* completely liquefy.

Moulds are the active agents in the very primitive so-called dew-rotting on the field; bacteria on the other hand, in the rotting after

1) But not of the cellwalls of the rind-cells from which the cellulose itself is also partly dissolved.

steeping of the flax in water, that is in the white- and the blue-rotting.

In the dew-rotting a most unequal product is obtained; this process shall not further be discussed here.

In the blue-rotting in ditches and ponds, as also in the white-rotting, a so-called anaerobic bacterium is the active agent. This highly interesting organism belongs to the genus *Granulobacter* and shall be called *G. pectinovorum* [1]) (*Gp* fig. 2). At the present moment the whole rotting industry is nothing else but a more or less rational culture-method of this bacterium.

From a theoretical point of view it is interesting that there are also some aerobic bacteria with which rotting is possible with free access of air. These are the various kinds of the so-called hay-bacteria group, also known by the name of potato-bacteria, the chief species of which are *Bacillus mesentericus vulgatus*, *B. subtilis*, and *Granulobacter (Bacillus) polymyxa* (= *B. solaniperda* KRAMER).

4. *Arrangement of the rotting experiments in the laboratory for the examination of microbes in pure culture on their power of rotting.*

In order to ascertain if any microbe can be used for rotting it is necessary to dispose of perfectly sterile flax. This is obtained by heating the flax for some time at 125—130° C. in the steam sterilisator, whereby it is seen that it is not rotted at all by this overheating.

For the laboratory experiments with the "anaerobes", thick walled test-tubes were so closely filled with flax, washed out or not, that the pressure against the glass-wall prevents it from mounting up when the tube is further filled with water. After cotton-plugging the filled tubes are sterilised in the sterilisator.

It is true that in these tubes air can penetrate from above, but if the pieces of the flax-stalks are not too short, say 20 cm., the entrance of air is not noxious to the anaerobes, provided some ordinary aerobic microbe be added, which lives at the surface and there absorbs the oxygen. We always used to this end a *Torula* yeast.

For testing the aerobic microbes the flax is spread in a thin layer at the bottom of a wide ERLENMEIJER-flask, and after immersing in a little water the whole is sterilised, after cooling infected with the species concerned and cultivated at 35°, or lower, in accordance with the microbes to be examined. After 2 or 3 days the rotting is finished.

[2]) First discovered by WINOGRADSKY (Comptes rendus T. 121, pag. 742, 1895). STÖRMER (Mittheil. der deutschen Landwirthschafts Gesellschaft Bd. 32, pag. 193 1903) used for it the name of *Plectridium pectinovorum*.

At the examination of the numerous microbes which may be obtained from flax in rotting condition, the result was negative for by far the greater part of the species. No rotting was to be observed with the various kinds of yeast, of *Mycoderma*, of *Torula*, of *Oidium* and of red yeast, nor with the lactic-acid ferments, the vinegar bacteria and the different forms of the *Aerobacter*-group, such as *A. coli* and *A. aerogenes*, which organisms are all universally found in the rotting water of natural rottings.

The aerobic bacteria of the hay-bacteriagroup (*B. mesentericus* and *B. subtilis*), which, at sufficient supply of air are on the contrary strong rotting-organisms, are rare in good rotting water. For rotting they should be kept at least at 30°.

5. *The rotting reposes on the action of the enzyme pectosinase which is secreted by the pectose-bacteria.*

The action on the flax, as well of the anaerobic *Granulobacter pectinovorum* as of the aerobic hay-bacteria and the moulds, is caused by a specific enzyme *pectosinase* [1]). This enzyme, like the acids, exerts an hydrolytic influence, first converts the pectose into pectine, and subsequently the pectine into sugars, which are fermented by *G. pectinovorum* (*Gp* fig. 2) under production of hydrogen, carbonic acid and a little butyric acid, and assimilated in case the hay-bacteria are used for the experiment.

These sugars are most probably galaciose and xylose, and (perhaps in some cases) also glucose and arabinose, which as we saw before, have been found by TOLLENS as products of the hydrolysis of the pectinic substances with acids.

Pectosinase is not easily soluble in water and can be precipitated with alcohol. In presence of chloroform and in absence of the microbes, we succeeded thereby to decompose into cells thin slices of potato, and further to liquefy with it solid plates of pectine, made by solidifying pectine from *Gentiana lutea* (see § 2) with pectase + $CaCl^2$. The action of the isolated enzyme is feeble, much feebler than when also the secreting bacteria themselves are present in living condition. This is evident from the facility with which the hay-bacteria at 37° C. decompose slices of living potatoes, whilst this gives much more trouble when effected by the enzyme prepared separately.

1) Not identic with the "pektinase" of BOURQUELOT and HÉRISSEY (Comptes rendus T. 127 pg. 191 1898) from green malt (which is identic with the "cytase" of BROWN and MORRIS (Journ. Chem. Soc. Trans. 1890, p. 458)) for with malt flax cannot be rotted.

The insolubility of pectosinase in water and, much more the fact that all the pectose bacteria examined by us lose suddenly in certain not well defined circumstances the power to secrete it render the study of this enzyme very troublesome. Particularly important is its following property.

Whilst the action of the pectosinase is favoured by a little acid, the growth of the pectose bacterium is retarded by acid.

As to the rotting-process, for which the *production* of the enzyme is clearly the essential point, one has, if not exclusively, at least chiefly to reckon with the properties of the microbes themselves, more especially with the conditions for *their production.* Hence, from this point of view only a slight production of acid will be favorable for the rotting.

From the above it follows that the chief question of flax-rotting is: what are the conditions of life of the bacteria concerned and how can their multiplication and accumulation in the flax-stalks be attained so profusely as to expel the other microbes, and, by a sufficient formation of pectosinase cause the rotting-process to go on regularly?

WINOGRADSKY has, it is true, partly answered this question by the discovery of the pectose-bacterium. But the essential point in the arrangement of a rotting experiment has quite escaped him, for he has not recognised the necessity of the water refreshing. Hence, hitherto there exists no clear method which may give rise to a natural accumulation of the bacteria specifically concerned in the rotting and accordingly neither for the really rational arrangement of the rotting-process.

This gap will be filled-up here.

6. *Fundamental experiment for the explanation of the rotting-process.*

A cylindrical glass vessel *A*, Fig. 3 is quite filled with flax *V*, so that the stalks by their pressure mutually and against the glass-wall, are prevented from floating up as the vessel is further filled with water. Thereby is obtained 5 to 10 % of weight in flax to 100 of water.

To the bottom of the vessel *A*, a glass tube *B* reaches, through which pure water can flow down from the higher placed reservoir *C*. This water flows upward through the flax-stalks, according as the washing-water flows off by the tube *D*, and thereby extracts most of the soluble substances from the flax, whilst the insoluble

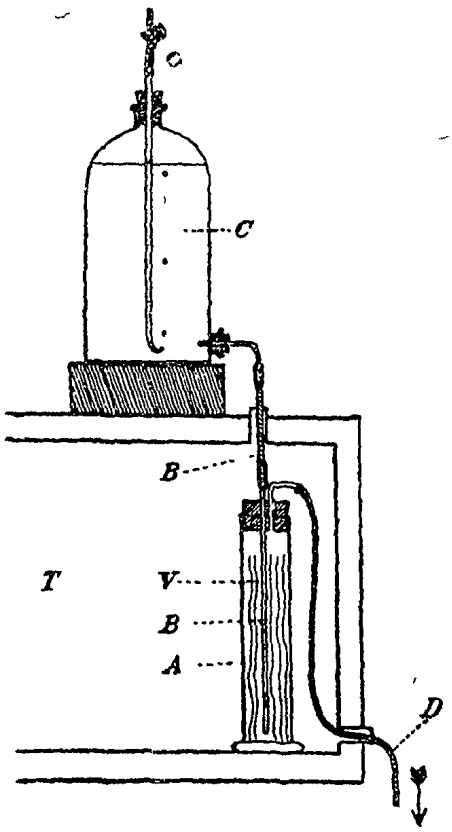

Fig. 3 Apparatus for rotting-experiment with water-current. *A* cylindrical glass jar with flax *V*, *B* tube supplying water from the reservoir *C* to the bottom of *A*, *D* tube to draw off the water. *T* thermostat.

pectose remains behind in the stalks. What is drawn off by *D* may be called "rotting-water", but this water is widely different, at the starting of the experiment, when it contains much dissolved matter and few bacteria, from that, obtained in a later period, when many bacteria and few dissolved substances occur in it.

The jar *A* is kept at a temperature of 28 to 35° C. by placing it in the thermostat *T*.

If after 2 or 3 days the flax is removed from *A* it proves to be more or less sufficiently rotted, if the water supply has been great enough to refresh the rotting-water from 5 to 10 times. Through our apparatus of 300 cc., 1.5 to 3 l. of water had thus to pass. Whilst in the Laboratory experiment the water is introduced at the bottom of the jar and drawn off at the top in order to prevent stopping of the tubes by the gases formed by the fermentation, this would be faulty with experiments on a larger scale, — in this latter case the heavier rotting-water should be drained off at the bottom.

If the rotted bark of the flax, or the pith, or the juice, contained in the rotted stalk, is microscopically examined, we find there accumulated the above mentioned very characteristic *Granulobacter pectinovorum* (Plate Fig. 1), which has supplanted nearly all other microbes and which literally fills up the intercellular spaces (*Gp* Fig. 2), in many places quite covers the surface of the fibres and has, moreover, completely dissolved the thin-walled cells of the secondary rind, and thereby freed the bast-bundles from the wood. In an iodine solution this bacterium turns deep blue over nearly its whole length in consequence of its amount of granulose.

This is a so-called anaerobe. From the experiment described, where an aerated water-current incessantly flows around the flax, it follows, however, that a fair amount of air is of no prejudice to its development; and a more minute observation shows that here, too, as with all other anaerobes, a limited aeration is not only harmless, but beneficent, and even necessary to make the growth go on in the long run.

Our observation that the water of the Leie, near and at Courtray, is strongly polluted by sulphuretted hydrogen, induced us to add c. a. 50 mgrs. of H^2S per l. to the water for our rotting-experiment, by which even in the rotting-water flowing off a little H^2S might be detected. The rotting was decidedly retarded by this addition and was less complete than in absence of sulphuretted hydrogen; yet, *G. pectinovorum* had strongly accumulated, though less profusely than commonly.

Quite otherwise was the effect of KNO^3. If 0.2 grs. of it were added per l. of the water-current, then a trace of KNO^3 might still be demonstrated in the rotting-water. Accumulation of *G. pectinovorum* and rotting proved complete in this case, so that, when Mr. PLAISIER, flax-merchant at Hendrik Ido Ambacht, came to judge of our rotted flax-samples, he classified our saltpeter-flax as "first rate". But it is clear that the presence of saltpeter is not necessary.

In fact, the accumulation of bacteria in the said experiment reposes, besides on the slight but necessary aeration, on the circumstance that by the water-current in the first 24 hours, such a complete extraction of the flax is attained, that all soluble nitrogen compounds are nearly completely expelled from it, and only the not easily soluble protoplasmatic proteid remains in the flax-cells, which substance, together with the still present carbohydrates and the pectose, prove to be the very nutriment for *G. pectinovorum* and, moreover, the food required to give rise to the secretion of pectosinase, hence, to the rotting-process. If the extraction has not taken place and the

experiment is made without the water-current going through the flax, a rich growth of all kinds of other bacteria will arise, but a real accumulation of *G. pectinovorum* and a good rotting-process is not obtained in the first two or three days.

The cause of this striking phenomenon reposes exclusively on the competition of the different microbe species. This is evident from the fact that with pure cultures of *G. pectinovorum*, even without refreshing the water, a very good rotting is possible. The substances removed from the flax by the water-current are not in themselves prejudicial to *G. pectinovorum*, but they favour the growth of the other species, in particular of the lactic-acid micrococci, so much more strongly, that *G. pectinovorum* can develop only later and with very great difficulty. It is also certain that the secretion of pectosinase in the dilute liquid is more profuse than in the more concentrated nutrient solutions. Thus we did not succeed in rotting flax by placing it in dilute sterilised malt-extract with chalk, of about 2° on the saccharometer of BALLING, which was in a vigorous fermentation by a pure culture of *G. pectinovorum*. Evidently no pectosinase secretion takes place under so favourable nutrient conditions.

Hence, there is a double reason why extraction so much promotes the rotting: the pectose bacterium gets the ascendency, and its faculty of secreting pectosinase becomes active.

If we compare the microscopic image of the bacteria (Pl. Fig. 1) of the flax, rotted after the current "experiment", with that, treated in the usual way, after the white or blue rotting methods, one is struck by the enormous difference. In the latter case hardly anything is seen but the foreign species and *G. pectinovorum* is scarcely detected; whilst in the product, obtained by the current-experiment *G. pectinovorum* is seemingly in pure culture[1]).

7. *Simplification of the current-experiment.*

When the great importance for the rotting-process of extraction of the flax-stalks and of aeration had been recognised, it was natural to try whether the current-method could be replaced by a more rational way of water-refreshing for the practice.

This was effected in the following simple manner.

After standing 24 hours on the flax, the water was completely decanted off, so that all spaces between the stalks were drained and could fill with air. Subsequently a new supply of water was provided, either of fresh water of about 30° C. or of good rotting-water of

[1]) Compare further § 12.

a previous rotting. When using fresh water it proved desirable after every 24 hours to repeat the refreshing, but when good rotting-water could be had a second renewing was not required, good rotting-water containing already a sufficient accumulation of *G. pectinovorum.*

By this treatment, too, which may be called the "decanting method", excellent rotted samples were produced in $2^1/_2$ or 3 days. It even seems that it should be preferred to the "current method", because by decanting the concentrated rotting-water will be enabled more completely to flow off from the narrow interspaces of the flax-stalks than will be possible when replacing it by slowly streaming pure water. For the same reason the *aeration* must needs be more complete anywhere in the sheaves by "decanting" than by "streaming."

On account of these experiences there is no doubt but any other method of water supply which can give rise to a sufficient extraction and aeration, can replace the "streaming" and the "decanting method", if only care be taken during the rotting not to injure the delicate and easily bruised flax-stalks.

Here once more it may be observed, that although *G. pectinovorum* belongs to the so-called obligative anaerobic bacteria, the strong aeration, described above, should be pronounced decidedly favorable to this bacterium. This is, however, quite in accordance with the experience acquired for all other well-observed anaerobes. Hence, it may be considered as a truth, confirmed by each subsequent research, that anaerobes, in the strict sense of the word, do not exist, and that the term "microaerophily" more precisely denotes the relation between such organisms and free oxygen, than the term "anaerobes".

8. *Application of the current experiment for practical rotting* [1]).

Practical rotting has until now been managed in a very primitive way. Even at the Leie, near Courtray, from whence the best flax-fibre comes to the market, even the superficial observer is struck by the numerous and great deficiencies existing there.

[1]) By "vat-rotting", the bleaching of the flax on the field through light, so essential in "white rotting", is excluded. In the flax-rotting establishments to come, it will therefore be necessary to make the rotting be followed by a chemical bleaching process. Experiments have shown that ozon or hydrogen-superoxide may be used to this end Whether hypochlorites ("electrical bleaching") will also prove applicable without weakening the fibres, will have to be made out by dynamometrical estimations. Vat-rotting will also call attention to good drying-apparatus and, no doubt, to other troublesome problems, which, completely to solve, will require surely much time and many an industrial effort.

The first step towards improvement was taken in our country already in 1892 by the Society for the Promotion of Flax-Indrustry, which tried to replace the rotting in open water by rotting in vats.

After this method the flax-sheaves are placed vertically and close to one another in a large wooden trough, in which, at some distance from the bottom, a second, perforated bottom is fitted. This false bottom supports the flax and underneath the heavier rotting-water is collected, which flows down from the flax after the vat has been quite filled with water.

Baron RENGERS at Oenkerk, too, has tried to improve the rotting of flax, by treating it after the so-called hot-waterprocess, by which he seems to have obtained very satisfactory results.

Vat-rotting and hot-waterrotting can, however, only succeed with sufficient certainty, when care is taken to provide a due refreshing of water; this may be done in various ways, but has not hitherto been sufficiently attended to.

By "vat-rotting" the following advantages may be obtained.

First. The vats can be placed in a manufactory, where the other manipulations which the flax has to undergo, can also be effected.

Second. The temperature of the rotting-water may be modified at will, by which the difference between vat-rotting and hot-water rotting disappears. Rotting will be possible throughout the year.

Third. The extraction and aeration of the flax can easily be regulated, so that the accumulation and multiplication of the pectose bacterium is made sure, and the lactic-acid micrococci, the great enemies in the rotting process, are expelled.

The theoretical requirements for vat-rotting are in general to be seen from what precedes, but it is still necessary to call attention to the following points on which the success of that process depends.

In the first place, care should be taken that the heavier water, produced by the extraction of the flax, can easily be removed. By the use of a false bottom the water collects underneath the flax, so that it is possible first quite to fill the vat, allow it to stand for 24 hours, and then to drain off all the water. The flax thereby comes quite equally into contact with the air, so that even the densest places of the sheaves are duly aerated ("decanting method").

It will be sufficient only once to refresh the water [1]).

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[1]) The experiments with pure cultures of the pectose bacterium prove that theoretically the refreshing of the water is not even once fully required, but probably the competition, particularly of the lactic-acid and butyric-acid ferments, struggling to displace the pectose bacterium, will render this ideal condition unattainable for vat-rotting on a large scale.

It is a mistake to draw off the rotting-water from the vats at the top and introduce the fresh water at the bottom. Hereby the heavier washing-water is driven back among the flax-stalks and renders a complete extraction impossible, because the rising water will always seek those places, where there is the least resistance, i. e. the open-spaces of the sheaves, and will not enter the close places, where it is most wanted. Thus the growth of the pectosebacterium is hindered and that of the lactic-acid ferments promoted. Moreover the aeration, which, when the washing water is completely drained off, takes place of itself, and quite equally and everywhere throughout the flax, would become most irregular and imperfect.

In the second place, the vat should after the first draining not be filled with fresh water only, but this water should be mixed with a fair quantity of *good* rotting-water, taken from a previous rotting. By this means the pectose bacteria are at every point introduced into the flax, which of itself harbours only a small number of these microbes, which are not at all generally distributed, neither on the flax nor in the waters.

Before commanding of good rotting-water it will be necessary once more after 24 hours, so two days after the first filling, to draw off all the water and replace it by fresh water. The pectose, bacteria have then already so strongly accumulated in the flax-stalk, that they can only for a small portion be washed away.

How easily *good* rotting-water is to be obtained follows from the description of the current-experiment.

In the third place the rotting-temperature will have to be exactly regulated. Our laboratory experiments make it evident that the most favorable temperature lies between 28° and 35° C. After $2^1/_2$ to 3 days the flax may then be removed from the vats in an excellent rotted condition (see note 1 § 8). Perhaps with a longer rotting-time the temperature might be lowered and reduced to from 25° to 27° C. Practice will have to decide whether this is desirable.

9. *Pure culture of the pectose bacterium.*

The pure culture of *G. pectinovorum*, which like all other species of *Granulobacter*, produces spores, is successfully effected as follows.

On a culture medium in a glass box, consisting of dilute malt extract of c.a. 2% BALLING, with 2% agar and 2% chalk, some material taken from the rind of a well-rotted flax-stalk, pasteurised at 90° C., is put, in order to obtain colonies of *G. pectinovorum* in streak-culture. The pasteurisation serves to kill the foreign

bacteria of which the majority produce no spores, in particular the lactic-acid ferments, but it should not be done at too high a temperature, as also most spores of the pectose-bacterium itself die already at the boiling point.

The glass-box is now placed in a well-closing exsiccator, with a three way stop-cock, in which a small dish with hydrosulphite of sodium is put. The exsiccator is evacuated by a KÖRTING pump, filled with hydrogen (or carbonic acid), and again evacuated; and this is repeated until it may be assumed that the oxygen, which can never be completely removed, is reduced to the minimum pressure tolerated by the anaerobes, wherein the hydrosulphite is also useful. The exsiccator is placed in a thermostat at about 35° C. and after 2 to 3 days the anaerobic colonies are seen to develop. They chiefly belong to the four following species of *Granulobacter:*

1. *G. pectinovorum.*
2. *G. urocephalum.*
3. *G. saccharobutyricum.*
4. *G. butylicum.*

The third of which was alluded to and the fourth described by me in a former research [1]). The two first species only, viz. *G. pectinovorum* and *G. urocephalum,* are real rotting-bacteria, the former acts strongly, the latter feebly rotting. The two last mentioned, viz. the butyric acid ferment (*G. saccharobutyricum*) and the butylic ferment (*G. butylicum*) cause no rotting at all.

The colonies of all the four kinds colour deep blue when treated with iodine solution, in consequence of their contents of granulose in thin, elongated clostridria. Besides, rodlets are found in all colonies, which do not stain blue with iodine, and which, in a former paper, have been described as "oxygen forms" of *Granulobacter* [2]). Some colonies consist of the oxygen form only, hence do not stain with iodine at all, and only contain rodlets, in which spores are seldom found.

If the material taken from the flax-stalks has not been sterilised previously to the sowing, various colonies of lactic-acid micrococci will develop on the plates, surpassing the *Granulobacter* colonies many times in dimensions and thereby easily recognisable.

10. *Description of Granulobacter pectinovorum.*

The colonies of this bacterium are recognised by the "moiré-phenomenon", figured on Plate Fig. 3. It consists in the appearance

[1]) Sur la fermentation et le ferment butyliques. Archives Néerl. T. 29, pg. 1. 1896

[2]) Fermentation butylique. pag. 35.

of characteristic, nearly rectangular dark and light fields to be observed in the colonies when obliquely illumined, which fields can interchange of tint and originate by the reflexion of light on groups of mutually parallel bacteria; of the different groups the longitudinal axes meet at nearly right angles.

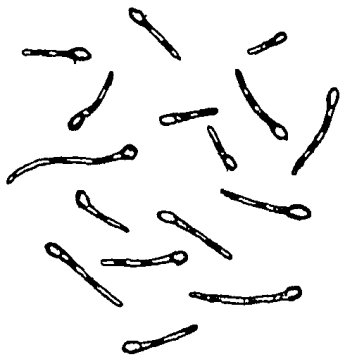
As to the bacterium itself, its description by WINOGRADSKY[1]) is in good accordance with our results. It is a rather long species, producing spores at a terminal swelling (a little beneath the end) of the very long and very thin clostridia, resembling common staves, which then look like frog-spawn (Plate Fig. 4). The rodlets are 10 to 15 μ long by 0.8 μ wide but eventually much longer. The older ones become thicker and swell at the end, to 3 μ in width; the oval spore, formed in this swelling, measures 1.8 μ by 1.2 μ.

In dilute malt-extract, with exclusion of air, a vigorous fermentation takes rise, without formation of butyric acid.

With starch[2]), inulin, mannite, erythrite, glycerin, fermentation could not be produced under any circumstances.

With peptone and with dilute broth, or albumine as sources of nitrogen, our bacterium causes fermentation in glucose, laevulose, galactose, milksugar, and maltose, with a slight production of butyric acid. Proteids and gelatin are peptonised.

With ammonium salts as source of nitrogen, fermentation cannot be produced with any of these sugars. Nitrates are not assimilated and not changed at all.



650x

Fig. 4 (650). Culture of *Granulobacter pectinovorum* in pectin-ammonium-sulphate solution. The thick ends contain oval spores; the dark spots in the rodlets indicate granulose.

[1]) Comptes rendus T. 121, p. 744, 1895.

[2]) WINOGRADSKY asserts that starch does ferment.

Pectine, prepared as indicated § 2, is decomposed, as well with albumine, peptone, or broth, as with ammoniumsalts for source of nitrogen, by which this bacterium stands by itself and is sharply distinguished, especially from the butyric-acid and the butylic ferments, which do not attack pectine at all. When the pectose is attacked, pectosinase secretion occurs.

Cellulose as filter-paper, is not in the least affected by *G. pectinovorum*. Hence, the flax-fibre as such remains quite unchanged in the rotting, but the less resistant forms of cellulose are solved quite as the pectose itself. Gum arabic remains intact. As is seen from the photogram (Plate Fig. 2), the image of the pure culture on malt-extract agar is quite different from that of the butyric-acid ferment, which latter forms thick clostridia.

This difference is not less clear in the culture liquids. Thus, in fig. 4 we see the form of the bacterium in a pectine fermentation at 35° C. in: Tap-water 100, Pectine 2, $(NH^4)^2 SO^4$ 0,05, K^2HPO^4 0,05, Chalk 2.

The dark portions represent the places where granulose is accumulated. Clostridia of the common form are completely absent. The shape of our bacterium in this or such like culture liquids is characteristic, and is not found in any other species except *G. urocephalum*.

11. *Description of Granulobacter urocephalum.*

The difference between *G. pectinovorum* (*Gp* F. 2, Plate Fig. 3) and *G. urocephalum* (*Gu* Fig. 2 Plate Fig. 4) which likewise, albeit in smaller number, accumulates in the rotting flax, consists first in the shape, which for the latter more approaches the "drumstick form", although the spores are not round, but oval, as Fig. 2 § 3 shows with great distinctness. Further, in the former secreting a much larger quantity of the rotting-enzyme pectosinase, which is the very reason why *G. pectinovorum* is more common in rotting flax than *G. urocephalum*.

Both species produce much mucus, which consists of the thickened and liquefied cell-walls of the bacteria themselves, and is found back in the so-called rotting-gum, obtained by evaporation to dryness of the rotting-water. That this species also stains deep blue with iodine is suggested by the generic name.

A characteristic difference between the two species is the following.

The colonies of *G. pectinovorum* (Pl. Fig. 3), when kept on plates of dilute malt extract agar with chalk, will relatively promptly be decomposed into a detritus, wherein only the spores can clearly be recognised, whereas the colonies of *G. urocephalum* (Pl. Fig. 4)

remain much longer unchanged and continue distinctly to show the shape of the bacteria. This phenomenon of bacteriolysis is perhaps associated with the pectosinase secretion and is also observed in the hay-bacteria.

The essential difference between *G. urocephalum* and *G. pectinovorum* is that the former with ammonium-salts as source of nitrogen, can ferment all kinds of carbohydrates, such as glucose, milk-sugar, cane-sugar and dextrine, for which *G. pectinovorum* requires peptone or broth. On the other hand, pectose is so little attacked by *G. urocephalum* that fermentation cannot be observed with this substance, even not when broth is present as source of nitrogen. Trypsine-formation is with *G. u.* about as vigorous as with *G. p.* and much more abundant than with *G. saccharobutyricum*. The secretion of diastase, on the other hand, is extremely feeble in both species and much less vigorous than in the butyric-acid ferment

12. *Accumulation experiment for G. urocephalum. Why the butyric acid- und the lactic-acid ferments disappear from good flax-rottings.*

That *G. pectinovorum* so strongly accumulates in our "current-" and "decanting experiments", reposes on the double adaptation of this ferment, on the one hand to the insoluble albuminoids of the flax-stalk by a strongly peptonising enzyme, on the other hand to the insoluble pectose by the secretion of pectosinase.

Why *G. urocephalum* also accumulates in the flax, but much less strongly, and why the common and vigorous butyric-acid ferment disappears nearly completely, was made clear by the following accumulation-experiment for *G. urocephalum*, devised by Mr. G. VAN ITERSON, at the occasion of a researeh on the butyric-acid fermentation.

If to any carbohydrate, for example insoluble starch, glucose, cane-sugar, or milk-sugar, a slight quantity of egg-albumine or peptone, or very little broth, is added as nitrogen source, in the proportion:

Tapwater 100, Glucose 5, Albumine 0.1, K^2HPO^4 0.5, Chalk 5,

infected with garden-soil and cultivated in a stoppered bottle at 35° C., there will first, that is so long as soluble carbon compounds are still present, originate a butyric fermentation, but this is soon replaced by a *Urocephalum*-fermentation.

If from the thus obtained first fermentation a small drop is transported into the same mixture, the butyric-acid ferment, indeed, does not completely disappear, but the intensity of the *Urocephalum*-fermentation becomes thereby much enhanced. If at the end of the

fermentation a new quantity of sugar (and chalk) is added, a further purification is observed.

When using a smaller dose of sugar, the soluble nitrogen compounds which occur in the albuminous matter, become more troublesome as they make the butyric fermentation more prominent.

If the same experiment is made, the albumine being replaced by an ammonium salt, *G. urocephalum* is quite expelled and the butyric ferment, *G. saccharobutyricum*, gains the victory.

That this experiment reposes only on competition, is proved by the fact that *G. urocephalum* in pure culture, can grow excellently and ferment with the said sugars and an ammoniumsalt as source of nitrogen. Further, the pure culture on dilute malt-extract-gelatin proves that *G. urocephalum*, like *G. pectinovorum*, liquefies the gelatin much more strongly than the butyricferment, and thus secretes more trypsine.

The reason why these three bacteria accumulate so unequally in the flax in the "current-experiment", and why *G. urocephalum* takes the middle between the pectose-bacterium and the butyric ferment, is thus evidently as follows.

During the extraction the insoluble nitrogen compounds are removed, so that, as source of nitrogen there remains nothing else but the insoluble vegetal proteids. This assures the victory to the strongly peptonising *G. pectinovorum* and *G. urocephalum* over the not or feebly peptonising butyric ferment.

This latter yields much more diastase than *G. pectinovorum* and *G. urocephalum*, so that its presence is a source of sugar formation, starch being never completely absent.

Hence, as soon as the butyric-ferment disappears, the insoluble carbohydrates, too, will promptly be removed by the extraction and the fermentation. The insoluble pectose only is now left behind, by which *G. pectinovorum*, which secretes much pectosinase, finally also subdues *G. urocephalum*, which produces little or no pectosinase at all.

The lactic-acid micrococci produce no enzymes which attack proteids, pectose, or carbohydrates. So, from the moment, that only insoluble proteids and insoluble carbohydrates are present, they can no more multiply and are carried off by the water-current.

EXPLANATION OF THE PLATE.

Fig. 1 (600).

Drop pressed from flax-stalk at the maximum point of a rotting during the "current expériment", stained with iodine, and showing the natural accumulation

Fig. 1.

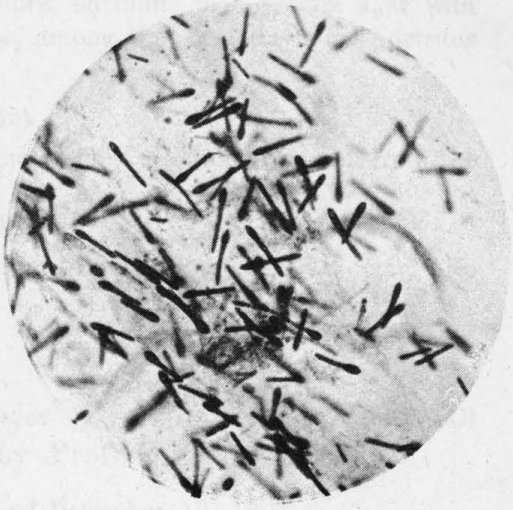


Fig 2.

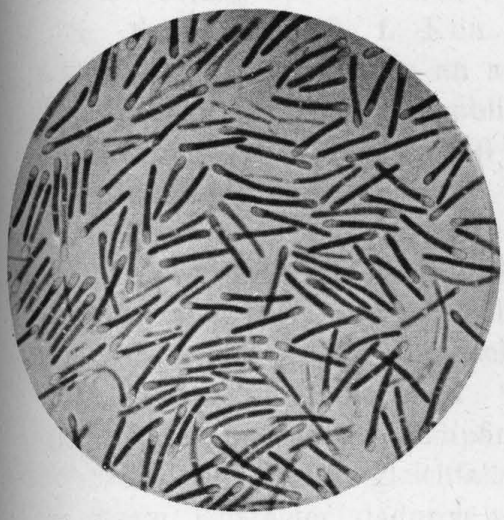


Fig. 4.

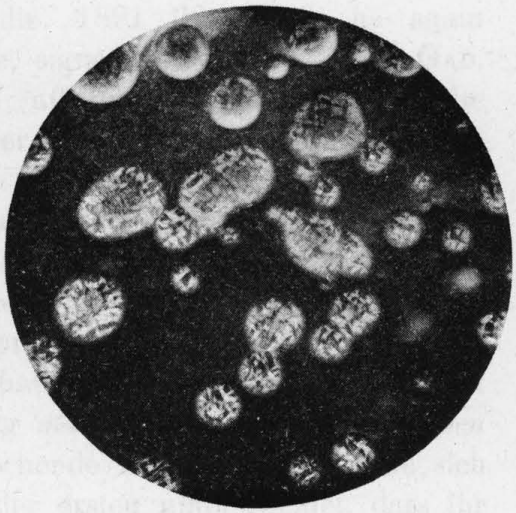


Fig. 3.

Heliotypie L. VAN LEER & Co, Amsterdam.

of *G. pectinovorum*, which is more, and of *Gr. urocephalum* which is less common. Here and there oxygen forms, which do not stain with iodine.

Fig. 2 (900).

Granulobacter pectinovorum, as pure culture, on dilute malt extract agar with chalk. Granulose stained blue with iodine, among the bacteria much detritus formed by bacteriolysis.

Fig. 3 (15).

Colonies of *G pectinovorum* on the same culture medium to demonstrate the "moiré-phenomenon."

Fig. 4 (900).

Granulobacter urocephalum, as pure culture, on dilute malt-extract agar. No detritus is found among the bacteria.

Physiology. — "*On tactual after-images*". By Prof. J. K. A. WERTHEIM SALOMONSON. (Communicated by Prof. C. WINKLER).

(Communicated in the meeting of December 19, 1903).

In 1881 the following fact was mentioned by GOLDSCHEIDER in his thesis on "die Lehre von den specifischen Energien der Sinnesorgane":

"Wenn man mit einer Messerspitze schnell, am besten die Hohlhand beruhrt, so tritt momentan nur die Tastempfindung auf, welcher dann erst der stechende Schmerz folgt. Dasselbe kann man bei einem leichten Schlag mit der flachen Messerklinge wahrnehmen."

In the Zeitschrift f. Klin. Mediz. 1891 20. 4—6, he again takes up this subject in an article, signed likewise by Prof. GAD. This article has been republished afterwards in his Gesammelte Abhandlungen Bd. 2, pag. 376 under the title. "Ueber die Summation von Hautreizen".

In this article the conditions under which the phenomenon may be observed, are defined with greater accuracy. According to GOLDSCHEIDER the best result is obtained by exerting by means of the point of a pin a short and feeble pression on the skin of the back or of the palm of the hand "so hat man ausser der ersten sofort eintretenden stechenden Empfindung *nach einem empfindungslosen Intervall* eine *zweite*, gleichfalls stechende Empfindung, welche sich in ihrem Character dadurch von der ersten unterscheidet, dass ihr nichts von Tastempfindung beigemischt ist, sie vielmehr gleichsam wie von innen zu kommen scheint. Bei massiger, noch nicht schmerzhafter Intensitat der primaren Empfindung kann die secundare schmerzhaft sein ..... Das Phanomen der secundaren Empfindung tritt schon bei sehr schwachen, vom Schwellenwerth nicht weit entfernten Reizen auf."