original sample of gas. Pure oxygen was used in the explosion, and the excess after it could be absorbed and a residue, if any, measured. In all cases there was a residue of from one to five cubic centimeters, hardly enough for proof of the presence of nitrogen.

In order to prove the presence of nitrogen in the residue, a large fermentation tube was procured and a culture made in this, using the necessary precautions. Smith's fermentation fluid, +1% of glucose, was used. The gases, CO_2, CO, H and O, were absorbed as described before. The residue, amounting to some 20 c. c., was kept in a pipette over water; 10 c. c. of this residue was transferred to a eudiometer provided with platinum electrodes and mixed with 22 c. c. of oxygen prepared from the electrolysis of water. This mixture was sparked. In the course of three hours the volume showed some signs of diminution, and after six hours the volume had been reduced to less than half. The current was turned off and the gas allowed to stand for 24 hours, with no practical change in results.

The contraction in volume of the gas when sparked with oxygen showed the presence of nitrogen in the formation of an oxide of nitrogen which was soluble in water.

Micro-Organisms in Flour. By Carleton G. Ferris.

Flours have been studied from the chemical standpoint with considerable care, but comparatively little has been done as regards investigation from the bacteriological standpoint. Although the chemical side of the question has been considered the most important, as it undoubtedly is, there are certain changes occurring in dough made from chemically pure flour which cannot be attributed to the chemical side of the question. For example, bread made from a flour which chemically contained a proper quantity of gluten, etc., may be spoiled. The point might be raised by some that the bad bread was not the result of using a certain flour, but that the micro-organisms present were found in the water used and in the surrounding air, or possibly from an impure yeast. Experiment has proven, however, that bad bread can be obtained even when sterilized, distilled water and pure yeast are used. As the growth of bacteria does not commence in the dough until nearly all fermentation has ceased, it is reasonable to assume that the changes in the dough and in the bread must arise from some agency present in the flours themselves and independent of the chemical side of the question. Again, in salt-rising bread, where the sponge contains nothing but a chemically pure flour and water, an active fermentation takes place which must be due to the presence of organisms in the flour. Bearing these points in mind, the writer made a series of investigations to determine the micro-organisms found in the flours sold on the local market. A careful canvass of the market was made and samples of the flours sold secured, together with data concerning each. In securing these samples every precaution was used to prevent contamination and to preserve similar conditions throughout. The flours examined include, first, the leading patent flours, most of which are Minnesota products; second, medium-grade flours; third, low-grade flours, commonly called "seconds" by bakers. In these tests the effort was made to determine the condition of each flour under as nearly similar conditions as possible. Each experiment was repeated a sufficient number of times to secure accuracy and overcome abnormal conditions. The ordinary culture media were used, with the addition of blood serum and flour and starch paste. Plate cultures were made, using agar, wort gelatine and broth gelatine. Owing to the fact that many forms liquefy the broth gelatine plates, the number of colonies produced could not be determined, as the plate soon became a mass of mixed forms. Owing to the acidity of the wort gelatine, some forms of bacteria would not grow in it; others liquefied the gelatine, giving the same objection as was applied to the broth gelatine, but to a less degree. Again, moulds grew so rapidly in the wort gelatine that they sometimes crowded the bacteria out. Agar, on account of its nonliquefying properties and comparatively unfavorable condition for the growth of moulds, gave the best results. In occulating for plate cultures, on an average of .0022 grammes of flour were used in each case, hence the comparative number of organisms in each flour could be determined. The names of the flours used are withheld for obvious reasons. The names of the various forms of bacteria found were not always obtainable, as no descriptions could be found in manuals. Of the twelve flours sold on the local market, seventeen distinct species of bacteria were found, one yeast and three moulds—Mucor mucedo, Aspergillus glaucus and Penicillium glaucum. These flours differed greatly as to the number of species obtained from each. Some contained but one form, while others contained as many as seven. Some contained little or no moulds, while others abounded in them. No corroded starch grains were found, showing the flours to be in good condition. The number of bacteria found per gram in the samples by counting the colonies produced on agar plates afford the means of some rather interesting comparisons. For example, Nos. 1, 4, 6, 9 and 11, high-grade patent flours, gave the following number of organisms per gram:

	Bacteria.	*Moulds.*
No. 1	4,090	2,272
No. 4	4,545	909
No. 6	2,727	000
No. 9	4,545	4,090
No. 11	6,363	000

Nos. 3, 7 and 12, medium grade products, gave the following number per gram.

	Bacteria.	*Moulds.*
No. 3	14,545	1,363
No. 7	15,909	2,727
No. 12	18,136	909

Note the increase of organisms produced using the common flours.

Using Nos. 8 and 10, low-grade flours:

	Bacteria.	*Moulds.*
No. 8	222,727	000
No. 10	254,545	909

The above figures show beyond a doubt that the high-grade patent flours are much freer from bacteria than the medium and common-grade products. The process seems to affect the number of bacteria in flours. For example: No. 10, manufactured under a poor process, contains more bacteria than Nos. 8, 12 and 3 flours, manufactured under very careful processes.

The number of moulds found in these flours does not vary to the appreciable extent that the bacteria do. Grade seems to be no guide here. Among the 17 different species of bacteria there is one which was found in three of the flours which is a peculiar form, and although its properties seem to be very distinctive, yet no description could be found to answer it. The colonies are hard, dry, and pure white in color, and grow upward from the agar, forming a solid, round and slightly wrinkled mass, which cannot be torn apart by the needle. When the surface is scraped with a needle, the white covering is removed, revealing a brown substratum. This form grows rapidly in agar, wort gelatine, blood serum, starch and flour pastes. The form liquefies wort gelatine slowly, and hard, white lumps float on the surface. This bacterium is a facultative anaerobic bacillus, very short and possessing no movement. Its size is $1 \times 1\frac{2}{3}\ \mu$. Old cultures give a distinct odor of old hay. The cultures of starch and flour pastes containing this form were tested with Fehling's solution to determine the diastasic action, with the result that both the starch and flour pastes showed a very marked action of the changing of starch to sugar, the action in the flour paste being more marked. Eighty-five per cent. of the 17 species grew well in blood serum, the form described above being the most luxuriant grower. It is a curious fact that bacillus subtilis, a form appearing in large numbers on grain, does not appear in the flour. There is not sufficient heat produced in the roller process to kill the bacillus, hence its elimination cannot be traced to that. It has been suggested that possibly the form lived on the husk or bran and clung to it and was thus eliminated. This is only a supposition, however. About 40 per cent. of the different species produced a marked diastasic action in flour and starch pastes.

Briant, Walsh, and Waldo, English chemists, made investigations on this subject, and the conclusions arrived at by them are as follows: The lower the grade of flour, the greater the number of organisms present. That under similar conditions, sourness is far more likely to occur in bread made from low-grade flours.

The writer's investigations of flours confirm the above results.

DESCRIPTION OF FORMS.

Flour No. 1 contains 3 forms: a (1), B (1) (c).

a (1) Is a facultative anaerobic, non-liquefying micrococcus. Grows well in agar but not so well in wort gelatine. Has a grayish color grown on agar. Colonies oily, and smooth. Size is $1\frac{1}{2}\ \mu$. Arranged in twos and fours, and possesses no movement. Does not grow on blood serum.

B (1) Is a facultative anaerobic, non-liquefying bacillus. Grows well in agar, but poorly in wort gelatine. The colonies are grayish white in color, dull, dry and floury, with convoluted edges. Possesses a slow, waddling movement. Size, $3-9 \times 1\ \mu$.

c Is a facultative anaerobic, non-liquefying bacillus. Grows very well in agar, but does not grow at all in wort gelatine. The colonies are yellow with a greenish tinge, growing in concentric layers. The colonies are smooth and oily. This form does not grow in blood serum. Has a waddling movement straight ahead. Size, $6 \times 1\ \mu$.

Flour No. 2 contains 3 forms: a (2) a (3) (c′).

a (2) Is a facultative anaerobic, liquefying bacillus. Grows slightly more in agar than in wort gelatine. Full description has been given in the body of this article.

a (3) Is a facultative anaerobic, liquefying bacillus. Grows well in agar and wort gelatine. Liquifies gelatine and produces a marked white film over the surface. The colonies are oily, smooth and regular, of a grayish color. This form is $3 - 9\ \mu \times 1\ \mu$, and possesses no movement.

c (1) Facultative anaerobic, non-liquefying coccus form, groups of two or more. Grows well in agar, but does not grow at all in wort gelatine. The colonies are smooth and oily, with smooth edges. Pink in color. Is $1\frac{1}{2} - 1\ \mu$ diameter. Possesses no movement.

Flour No. 3 contains 4 forms: a (1) a (2) c (1), same as No. 2 (c′) and d (1).

a (1) Aerobic, liquefying, bacillus, growing singly and in filaments. This form grows very well in both wort gelatine and agar. The colonies are tough and slimy, with smooth edges. Yellow in color. Size is $3 \times \frac{3}{4}\ \mu$, and possesses no movement.

a (2) Facultative anaerobic, liquefying bacillus. Grows well in agar; not so well in wort gelatine. The colonies are grayish yellow in color, oily and slimy, with irregular edges. This bacillus is $3 \times \frac{3}{4}\ \mu$, and possesses no movement.

c (1) Pink variety same as No. 2 c (1).

d (1) Facultative anaerobic, non-liquefying bacillus. Grows well in agar, and very slightly in wort gelatine. The colonies are yellow, tree-like in form, with firm edges, with the center slimy and oily. Form is $3 \times 4\ \mu$ in size and has a slow, waddling movement.

No. 4 flour contains 6 forms and one yeast: a (2) e (1) b (2) a (1) c (1) d (1) and yeast f (1).

a (2) Facultative anaerobic, non-liquefying bacillus. This form grows well in agar, but does not grow at all in wort gelatine. The colonies are grayish yellow, oily, slimy, and have smooth edges. Size, $3 - 4\frac{1}{2} \times \frac{3}{4}\ \mu$. Has a burrowing movement.

T (1) Turns out to be a red yeast twice as long as broad, differing from all red yeasts previously noted. Has granular contents vacuoles, large and vary from 1 — 5 in number. Gross appearnce, very dull, firm, pink on agar. Also grows well in gelatine. Some cells show spore like bodies at each end. Size, $6 \times 3\ \mu$.

e (1) White variety. Same as No. 2 a (2).

a (1) Same as No. 1 a (1).

B (2) Facultative anaerobic, liquefying bacillus. Grows well in agar and wort gelatine. The colonies are yellow, iridescent smooth, oily, with smooth edges. Size is $3 \times \frac{3}{4} \mu$, and possesses slight jerky movement.

c (1) Facultative anaerobic, liquefying bacillus. Grows well in agar and wort gelatine. The colonies are yellow, smooth, oily with smooth edges. Size, $1\frac{1}{2} \times \frac{3}{4} \mu$. Has no movement.

d (1) Facultative anaerobic, liquefying bacillus. Grows well in agar and wort gelatine. Colonies, yellowish white, slimy, oily, irregular edges. Liquefies gelatine entirely, forming a white and firm film over the surface, also a heavy sediment is formed. The gelatine becomes red brown in color, on agar an emerald green color appears near surface of the agar. Size, $2 \times \frac{3}{4} \mu$. Has a movement in circles.

Flour No. 5 contains two forms c (2) B (2).

c (2) Facultative anaerobic, non-liquefying, zooglœa mass. Very luxuriant grower in both agar and wort gelatine. Colonies are smooth and oily, with smooth edges. This form is yellow in color.

B (2) Facultative anaerobic, liquefying bacillus. Good grower in wort gelatine and agar. Colonies are yellow, smooth and slimy, with smooth edges. Produces a little gas. Size, $4\frac{1}{2} \times 1 \mu$. Possesses no movement.

Flour No. 6 contains but one bacterium, same as No. 3 d (1).

Flour No. 7 contains 4 forms: a (1) a (3) b (1) b (3).

The white form a (3) same as No. 2 a (2) also b (1) same as No. 5 b (2).

a (1) Facultative anaerobic, liquefying bacillus. Good grower in wort gelatine and agar. Colonies are dark yellow in color, slimy and oily, with smooth edges. Size, $2\frac{1}{4} \times 1 \mu$. Possesses no movement.

D (3) Facultative anaerobic, liquefying bacillus. Good growths in agar and wort gelatine. Colonies are yellow in color, smooth, oily, with regular edges. Size, $2\frac{1}{2} \times 1 \mu$. Possesses no movement.

Flour No. 8 contains 3 forms:

a (3) Same as No. 2 a (2).

a (2) Same as No. 3 d (1).

a (1) Same as No. 5 B (2).

Flour No. 9 contains 2 forms:

a (1) Same as No. 5 b (2).

a (2) Same as No. 2 a (2).

Flour No. 10 contains one form:

b (2) Same as No. 4 d (1).

Flour No. 11 contains 2 forms.

a (1) Same as No. 1 a (1).

a (2) Same as No. 3 a (1).

Flour No. 12 contains 3 forms.

a (1) Same as No. 7 b (3).

b (1) Same as No. 4 b (2).

c (1) Same as No. 2 a (3).

No.	No. of Forms.	Bac. Per Gram.	Moulds Per Gram.	Grade of Flour.
1	3	4090	2272	High.
2	3	2727	2727	High.
3	4	14545	1363	Medium.
4	6–1 yeast.	4545	909	High.
5	2	9090	909	Medium.
6	1	2727	000	High.
7	4	15909	2727	Medium.
8	3	222727	000	Poor.
9	2	4545	4090	High.
10	1	254545	909	Poor.
11	2	6363	0000	Medium.
12	3	18136	909	Poor.

This work was done under the direction of Miss Katherine E. Golden.

The Number of Micro-Organisms in Air, Water and Milk as Determined by Their Growth Upon Different Media.

By A. W. Bitting.

While conducting a series of experiments to determine the number of bacteria and moulds in milk, some variations in number were found when the tests were made upon different culture media. This led to an experiment to determine the number of micro-organisms present in air, water and milk, using agar agar, glycerine agar, beef gelatine, and wort gelatine. The results of the test were somewhat of a surprise. The agar agar, glycerine agar and beef gelatine were neutral, while the work gelatine was slightly acid, but the degree of acidity not determined. Ten exposures were made with each media, using petri dishes and kept as close together as possible. The conditions were evidently as nearly alike as it is possible to obtain.