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FACTORS AFFECTING GERMICIDAL PROPERTIES OF SULPHUR DIOXIDE IN WET-STARCH-PROCESS WATERS

By

John Elwood Killinger

A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject - Food and Sanitary Chemistry

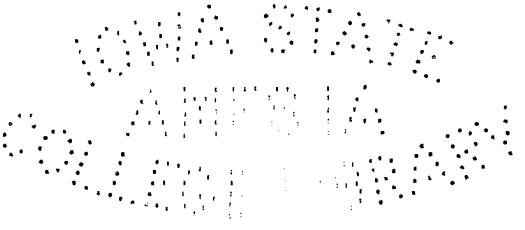

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TABLE OF CONTENTS

Page

INTRODUCTION

Corn Starch is used in the manufacturing processes of more than thirty modern industries. The separation of starch from the other constituents of the kernel is basic for a large industry, operating fourteen plants in the United States and processing from 60 to 80,000,000 bushels of shelled corn each year.

The Corn Industries Research Foundation (1) published a pamphlet entitled "Tapping the Treasure in Corn". The following diagramatic scheme, page 6, reprinted from this pamphlet, shows the various uses to which products from corn are put.

The Corn Kernel - Its Substance

The accompanying sketch, page 7, shows the location of the various constituents of the corn kernel, each of them storing a different material or mixture of materials.

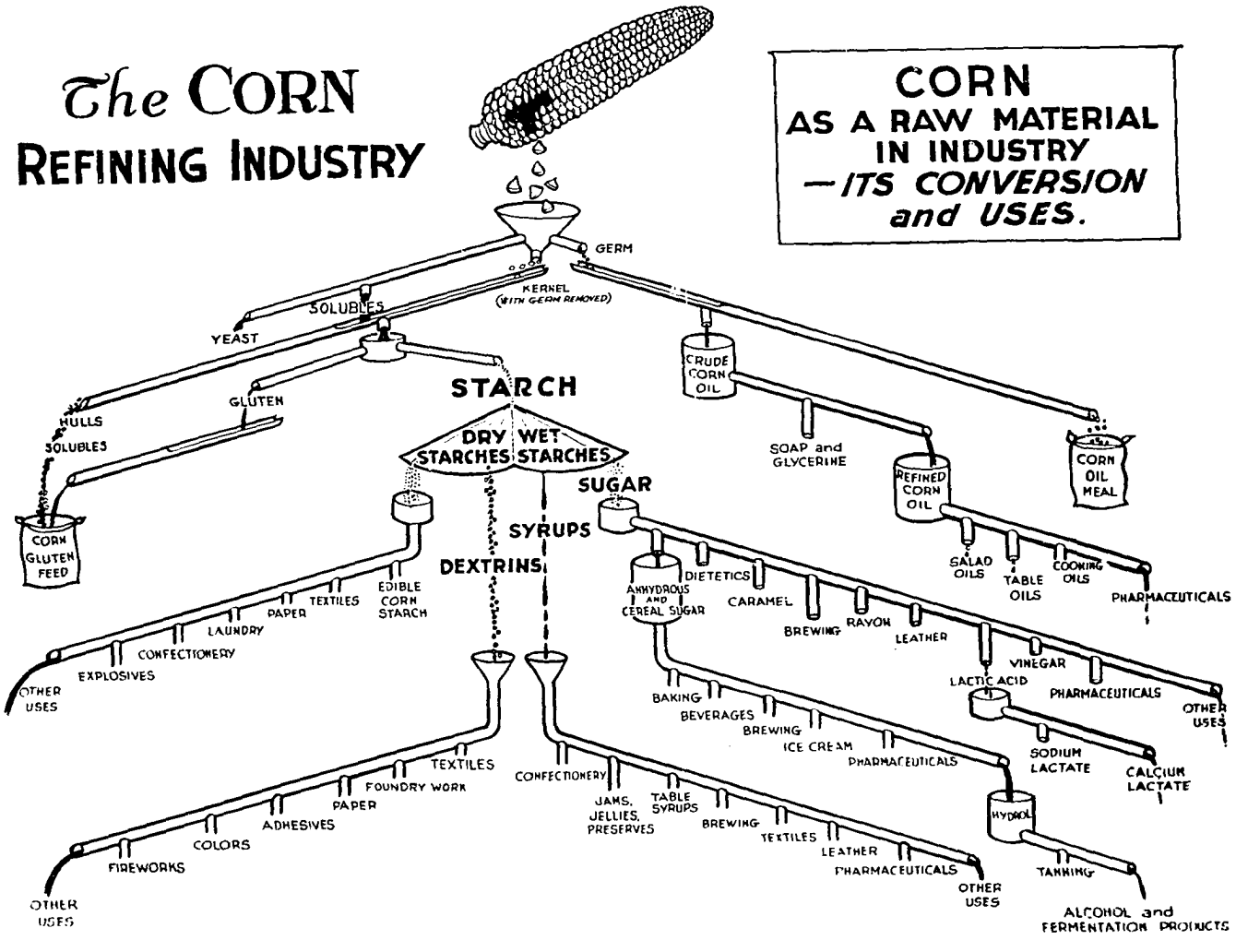
The hull, or thin outer skin, is fibre.

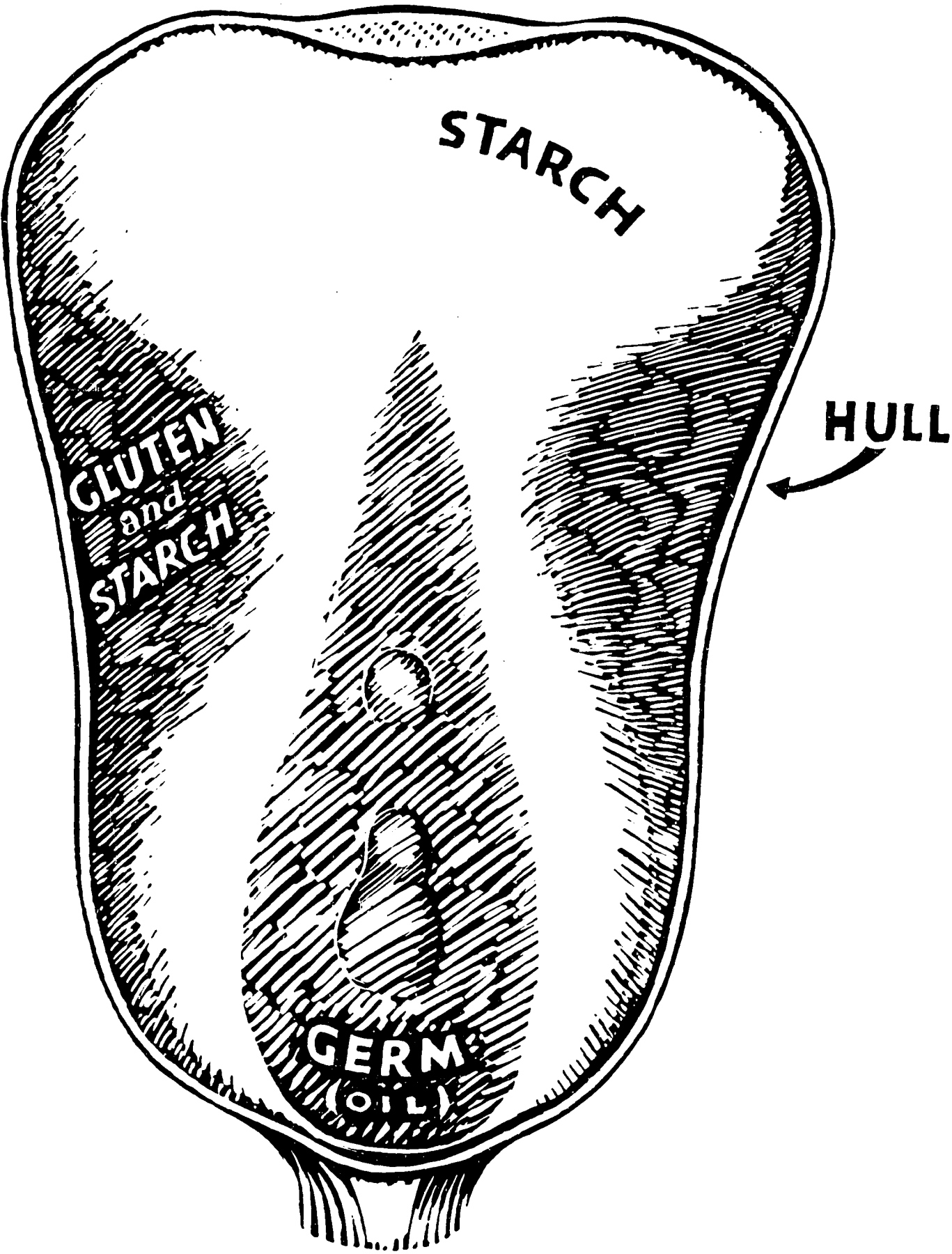
Next to the hull is a shallow layer of gluten, a substance rich in protein.

On the sides and back of the kernel, inside the layer of gluten, a mixture of starch and gluten bulges in towards the center.

Filling the upper part of the kernel and extending down-

The CORN REFINING INDUSTRY




STARCH
HULL
GLUTEN and STARCH
GERM (OIL)

ward to partly surround the germ, is the white starchy part of the kernel.

The germ itself is level with the flat "front" of the kernel and contains protein, most of the oil and a large share of the minerals.

These five parts can be seen clearly in a kernel that is softened in water and cut open with a razor blade. Although they are distinct, they are not made up, exclusively, of utterly different substances. The gluten layer, for example, contains a percentage of starch and the white starchy part contains a trace of oil and from five to eight per cent of protein.

Chemically, (2) from 15 to 20 per cent of the kernel is water, and the remainder is made up about as follows:

Starch (and other carbohydrates) . . .	80.0%
Protein	10.0%
Oil	4.5%
Fibre	3.5%
Ash	2.0%

The term "ash" refers to the mineral content. The ash of the corn kernel contains salts of calcium, magnesium, phosphorous, aluminum, iron, sodium, potassium, and chlorine.

The process of manufacturing corn starch, corn syrup, corn oil, corn feeds and products derived from these materials is based upon a most complete disintegration of the corn kernel

and the separation of the various parts, one from the other.

The process is both exceedingly simple and exceedingly complex; simple in that it is merely the separating of the parts of the corn and using these in the manufacture of a large variety of materials; complex in that the proper separation and treatment require very extensive equipment and an endless amount of detail.

Milling of the Corn

Cleaning. The corn used in the manufacture of corn starch, corn syrup and allied products is received in the shelled condition, and before being used is thoroughly cleaned, thus removing chaff, dirt, cracked corn, wheat, oats, bits of cobs, etc. The thoroughly cleaned corn is then ready for the milling process.

Steeping. The cleaned corn is run into large tanks called steeps, where water containing a small amount of sulphur dioxide is added until the corn is covered. The temperature of the water is then raised to the desired degree, the exact temperature being dependent upon the character of the corn and the length of time it is to be soaked or steeped. An average temperature is 120°F. The corn is maintained at this temperature for a period of time sufficient to soften so that

the germ can be torn loose from the rest of the corn; the starch and gluten removed from the hull, and the corn thus separated into its component parts, i.e., germ, hull, gluten and starch.

Separation of the Germ. After the corn has been steeped, it is passed through mills which tear it apart without grinding, thus liberating the germ from the rest of the kernel. The torn corn is mixed with water containing enough starch to give a starch milk heavy enough to float the oil laden germ, while the rest of the corn sinks to the bottom. The germs are allowed to float off the top while the heavier part of the kernel is continuously pumped from the bottom of the separators.

The germ, with the accompanying starch and water, is passed through reels or over screens where the starch and water are removed. The cleaned germ is ready to send to the oil house.

Separation of the Hull. The corn, freed from the germ, after being drawn from the bottom of the separators is ground in stone mills which thoroughly disintegrate the harder portions of the corn, and rubs the starch from the hull. This permits of the subsequent separation of the hull from the starch and gluten, by means of reels or shakers. These devices are covered with silk bolting cloth, through the pores of which the microscopic particles of starch and gluten pass,

while the fragments of the hull remain on the silk and are discharged at the end of the reels or shakers.

Separation of the Starch from the Gluten. The mixture of gluten and starch suspended in water is then run on to so-called starch tables or starch runs. These consist of long flat troughs from 24 to 30 inches wide, about 8 inches deep, and about 100 feet long. The mixture of gluten, starch, and water, containing something like 8 to 10 per cent solid matter, is run on to these tables in a slow stream which spreads into a thin film. The tables have a gentle slope so that the water runs slowly to the lower end carrying with it the gluten in suspension, but dropping the starch. The starch builds up on the tables, and finally the flow is stopped. The starch remaining on the tables is then ready for removal, to be used in the manufacture of dry or modified starch, or for the manufacture of corn syrup or corn sugar. The gluten is pumped to the feed house for the manufacture of gluten meal, or mixed with the hull for the manufacture of gluten feed.

Recovery of the Corn Solubles. The water in which the corn is soaked or steeped dissolves certain substances from the corn kernel. These consist chiefly of the soluble nitrogenous compounds or proteins, mineral matter, organic phosphates and a trace of sugars. By concentrating the steep water in vacuum pans, it is possible to save the corn solubles by adding them

to the gluten feed thus putting back into the feed materials which are of considerable value in animal nutrition.

Since this investigation deals only with the process waters in the initial separation of the constituents, the true manufacturing process will not be considered in this thesis.

It is seen that the separation of corn into its constituents is one of grinding, sieving, flotation and washing. All of these operations are carried out in water. The re-use of this tremendously large volume of water is necessary from an economic standpoint. The re-circulation of these waters also solves an otherwise complex and costly sewage disposal problem.

It was the purpose of this investigation to study the microbiological problems in the re-use of starch process waters. It is of importance to describe somewhat more in detail the main two types of re-circulated water.

Gluten Water

The name gluten water characterizes the water which is siphoned from the gluten settler tanks.

For every bushel of corn ground (3) there are approximately 36 gallons of gluten water returned to the process. These 36 gallons are redistributed approximately as follows: about 11 gallons go to the steeps; about 25 gallons to the mill house for washing, flushing, suspending the ground corn,

and separating it from the hulls.

The following table (4) gives the average per cent composition and content of gluten water:

Solids	1100-1200 grains per gallon
Protein	60% on dry substance basis
Sulphur	0.03-0.07% as SO_2
pH	4.1-4.5
Nitrogen free extract.	40%

Starch Wash Water

The name starch wash water is given to that water which results from washing, re-puddling and re-washing the starch cake on the filters. This water originally is fresh water and picks up its solids which are the impurities washed from the starch. Since the starch has been carried in gluten water prior to this time, it follows that the solids, both dissolved and suspended, are of a similar nature to those in gluten water.

There is an average of 10-11 (5) gallons of starch wash water returned to the mill house for every bushel of corn ground.

Average per cent composition and content of starch wash water (6):

Solids	350-400 grains per gallon
Protein	60%
Sulphur	0.02-0.04% as SO_2
pH	3.5-4.0
Nitrogen free extract.	40%

It should be bornein mind that the solids, in these waters came originally from the ground corn. Without some means of sterilization, the solids would in a short time be attacked by microorganisms which abound in the water. This perishability is inherent in the process of starch manufacture wherein the crushed and ground corn is suspended in a large amount of water. It, therefore, is of prime importance for the manufacturer to provide adequate means for prevention of excessive growths. If this is not done, serious losses will be entailed.

The loss of starch due to consumption by microorganisms is one reason for careful processing of these waters. The chief loss, without adequate means of sterilization, would occur in improper separations and sedimentation. Starch to be a saleable commodity must be quite free from protein. Cattle feed which comprises a large proportion of the material derived from corn is sold on a nitrogen percentage basis. Therefore, it is seen that an improper separation results in a part of the more expensive starch being mixed with the feed.

Because of the nature of the materials and necessity for maintaining suitable working conditions it is not possible to maintain the plant absolutely free from microorganisms, nor is

it necessary. It is sufficient merely to apply sterilization methods to such a degree that growths are checked so as not to permit development of the deleterious conditions referred to above.

There are several methods which might be employed to check the growth of micro-organisms. The most common antiseptic, and the one in use by most corn starch plants today, is sulphur dioxide.

For every organism there exists an optimum temperature for growth, a minimum temperature below which it will not grow, and a maximum temperature above which growth will not occur. In the early days of corn starch manufacture it was recommended that the manufacturing processes be carried out at very low temperatures to check bacterial and yeast deterioration, i.e., to maintain temperatures approaching the minimum growth temperatures. Obviously it would be equally or more effective to raise the temperature of the liquors up to or beyond the maximum growth temperatures of the micro-organisms responsible for fermentation, provided these temperatures are below the point at which heat deterioration of the product would ensue. Furthermore, should chemicals (such as sulphur dioxide) be employed, the elevated temperatures would enhance their germicidal efficiency. The addition of chemicals (such as a highly ionized acid) to a liquor containing sulphur dioxide which has had a chance to combine with the organic solids present should

make the sulphur dioxide more effective.

Observations were carried out as indicated above on the influence of temperature, sulphur dioxide, and a highly ionized acid on the growth of microorganisms found in starch wash and gluten waters.

Experiments were made to learn if there were a method of determining the inhibitory effectiveness of sulphur dioxide without actually making a bacteriological examination.

The bacteriological phases of this problem will be considered first. By this work, the nature of the organisms present in the process waters has been determined. The effects that SO_2, temperature, and hydrogen-ion concentration have on these microorganisms were observed.

The second section of this paper will deal with the determination of sulphur dioxide and a method for determining that which has combined with the organic solids present in corn starch process waters. It will be shown that a correlation exists between uncombined SO_2 and germicidal effectiveness.

HISTORICAL

The only data found in the literature pertinent to this problem are that of Dr. Max Levine's direct testimony (7) in the patent infringement suit of Penick and Ford, Ltd., Inc., vs Corn Products Refining Company. This litigation concerned the sterilization and re-use of corn starch process waters. It was the author's good fortune to have materially aided in the collection and presentation of these data. A goodly portion of the bacteriological data in this thesis has been taken from this brief. Indeed it is doubtful if this thesis would have been presented by the author had it not been for recognition of the necessity for further work on this subject.

Throughout this thesis, reference is made to combined SO_2 in the waters. That sulphur dioxide does combine with ketones, aldehydes and amines has been shown by Feigel, F. (9). Seventeen compounds were prepared by treating various amines with different ketones and SO_2. The compounds so produced correspond to the general formula:

Divers and Ogama (10) state that ammonia and SO_2 combine and form various compounds. Thus, with an excess of SO_2 amino sulfinic acid ($NH_2 \cdot SO_2H$) is always formed but with an excess of

NH_3 either white ($NH_2 \cdot SO_2NH_4$) or red ($NH_4N(SO_2NH_4)_2$ are formed.

William F. Pond (11) describes an apparatus which takes a definite quantity of SO_2 and measures the residual gas after solvents such as CCl_4, and C_6H_6 have been saturated.

Lloyd (12) has determined the solubility of SO_2 in several solvents including benzene. He found 44.0 cc. SO_2 (standard pressure temperature) per cc. of saturated solution. From these data it was learned that SO_2 was soluble enough in benzene to attempt extractions of SO_2 from process waters.

EXPERIMENTAL

Bacteriological

1. Microorganisms in Starch Process Waters.

During the summer of 1929, samples were taken at various points in the Penick and Ford plant at Cedar Rapids, Iowa, and examined bacteriologically. A number of organisms were fished from wort agar plates for further examination.

Some of the characteristics of 39 cultures are shown in Table I, pages 20 and 21. The organisms are yeasts and closely related forms. Microscopical examination showed them to be spherical, oval or elongated cells. The latter frequently formed chains or filaments. The filamentous forms generally produced heavy surface growths (films or membranes) on liquid media, and dry and tenacious growths on wort and gluten water agar. The other types generally produced moist grayish growths on wort agar, films on liquid media were relatively uncommon.

In addition to yeast-like forms, bacteria were occasionally found. They were usually spore forming, starch digesting types. The acidity of the process waters furnishes an unfavorable condition for most bacteria.

The photomicrographs, pages 22 and 23, show the three general types of yeast-like forms found.

TABLE I. CHARACTERISTICS OF MICROORGANISMS FROM PLAN
(96-98°F. 48 hrs.)

Lab. No.	Isolation No.	Source	Growth on wort agar	Malt Broth Growth	Gas %	Film	Morpholog
1	23	Silk reel	++++	++	-	+	Oval, irr
2	23a	Silk reel	++++	++	-	-	Oval
3	57	Steep	++++	+	-	-	Oval
4	64	Starch wash water	++++	++++	-	+	Elong. ov stain
5	66	Starch wash water	++++	+++	-	+	Elong. ov stain
6	95	Gluten from table	++++	++	-	-	Spherical
7	30a	Silk reel	++++	+++	-	+	Elongated shaped
8	42	Silk reel	++++	++++	-	+	Elongated shaped
9	44	Silk reel	++++	++	-	+	Elongated shaped
10	48	Silk reel	++++	+++	5	+	Elongated shaped
11	65	Gluten settler water	++++	+++	-	+	Elongated shaped
12	8	Silk reel	++	+++	3	-	Spherical
13	19	Silk reel	++	+++	6	-	Oval, sph
14	78	Starch wash water	++++	-	-	-	Spherical
15	79	Starch wash water	++++	+	-	-	-
16	10	Silk reel	++++	+	-	-	-
17	61	Gluten from table	++++	++++	-	+	Elong. ov stain
18	83	Starch cake	++++	+	-	-	Spherical

ACTERISTICS OF MICROORGANISMS FROM PLANT LIQUORS
(96-98°F. 48 hrs.)

wth wort ar	Malt Broth Growth	Gas %	Film	Morphology from wort agar	Appearance on wort
+++	++	-	+	Oval, irregular stain	Gray, dull, pasty
+++	++	-	-	Oval	Gray, glistening, pasty
+++	+	-	-	Oval	Like No.2
+++	++++	-	+	Elong. oval club, irr. stain	Like No.1
+++	+++	-	+	Elong. oval club, irr. stain	Like No.1
+++	++	-	-	Spherical	Dull, slightly wart-like
+++	+++	-	+	Elongated and club-shaped	Cretaceous, coral-like, whitish
+++	++++	-	+	Elongated and club-shaped	Like No.1
+++	++	-	+	Elongated and club-shaped	Like No.7
+++	+++	5	+	Elongated and club-shaped	Like No.7
+++	+++	-	+	Elongated and club-shaped	Like No.7
++	+++	3	-	Spherical	Like No.2
++	+++	6	-	Oval, spherical	Like No.2
+++	-	-	-	Spherical	Like No.2
+++	+	-	-	-	Gray, glistening, butyrous
+++	+	-	-	-	Gray, glistening, butyrous
+++	++++	-	+	Elong. oval club, irr. stain	Like No.1
+++	+	-	-	Spherical	Like No.15

TABLE I. (continued)

Lab. No.	Isolation No.	Source	Growth on wort agar	Malt Broth growth	Gas %	Film	Morpholo
19	98	Starch wash water	++++	++	-	±	Oval, irr
20	5	Silk reel	++++	++++	10	+	Oval, sph
21	110	Starch cake	++++	+++	5	±	Elongated
22	118	Table slime	++++	+++	100	-	Oval
23	75	Gluten from table	++++	+	-	-	Oval
24	84	Starch cake	+++	+++	10	-	Spherical
25	112	Starch cake	+++	+++	5	±	Spherical
26	85	Steep corn	+++	+++	-	+	Irregular
27	86	Steep corn	++++	+++	-	+	Irregular
28	54	Silk reel	++++	++	-	-	Long fila
29	56	Silk reel	++++	+	-	-	Long fila
30	40	Silk reel	++++	+++	-	+	Long irre
31	94	Gluten from table	++++	++++	-	+	Oval, irr
32	12	Silk reel	++++	+++	-	+	Oval, sma
33	15	Silk reel	++++	++	-	+	Long, irr
34	24	Silk reel	+++	+++	-	+	Club, irr
35	34	Silk reel	+++	+++	-	+	Spherical
36	39	Silk reel	+++	+++	-	+	Long fila
37	101	Gluten settler water	++++	+	-	+	Oval and
38	113	Starch cake	++++	+++	-	+	Long, fil
39	117	Table slime	+++	+++	-	+	Chains, r

TABLE I. (continued)

h rt	Malt Broth growth	Gas %	Film	Morphology from wort agar	Appearance on wort
+	++	-	±	Oval, irregular stain	Like No.6
+	++++	10	+	Oval, spheres	Like No.19, but growth more spreading
+	+++	5	±	Elongated	Like No.19
+	+++	100	-	Oval	Creamy, glistening, butyrous
+	+	-	-	Oval	Like No.15
+	+++	10	-	Spherical, small	Like No.15
+	+++	5	±	Spherical, small	Like No.15, but slightly brown
+	+++	-	+	Irregular long fila	Dry, white, adherent to medium
+	+++	-	+	Irregular long fila	Like No.26
+	++	-	-	Long filaments	Tenacious colonies
+	+	-	-	Long filaments	Like No.28
+	+++	-	+	Long irregular stain	Like No.36
+	++++	-	+	Oval, irregular stain	Dry, wrinkled, gray, pasty
+	+++	-	+	Oval, small	Like No.36
+	++	-	+	Long, irregular	Dull, gray, slightly raised
+	+++	-	+	Club, irregular stain	Like No.36
+	+++	-	+	Spherical and fila	Like No.33
+	+++	-	+	Long filaments	Spongy, gray, crateriform colonies, adherent
+	+	-	+	Oval and miscellaneous	
+	+++	-	+	Long, filamentous	Like No.36, but white
+	+++	-	+	Chains, rectangular	Like No.26

TYPES OF MICROORGANISMS FROM STARCH PLANT OF PENICK AND FORD AT CEDAR RAPIDS, IOWA

Group 1. Oval or spherical cells; singly or in irregular masses; rarely produce surface growths in liquid media; growth on solid media, moist, pasty, resembling the common yeasts in consistency; generally fail to grow or are killed at 107-109°F.

Gram stain. Magnified: 500 Diameters.

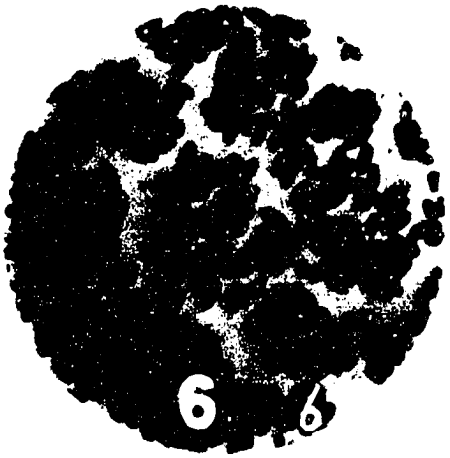
Group 2. Elongated oval or irregularly shaped cells; frequently form chains; generally produce heavy surface growths on liquid media; growth on solid media generally dry, membranous, chalky, and wrinkled; generally grow at 107-109°F.

Gram stain. Magnified: 500 Diameters.

Group 3. Filamentous organisms; cells long, sometimes branches; slimy, tenacious growth in liquid media, often with heavy membranes; growth on solid media mold-like and adheres tenaciously to the media; markedly inhibited or do not grow at temperatures above 98°F.

Unstained live preparation. Gram stain.

Magnified: 500 Diameters.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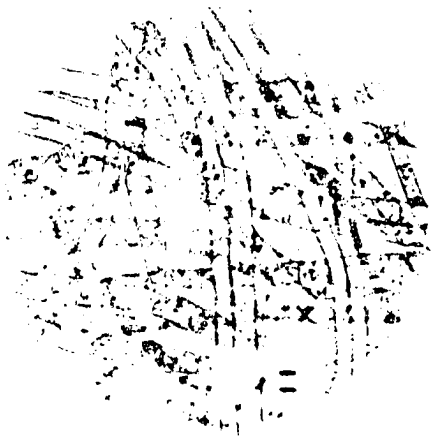


2. Effect of Temperature of Incubation on Growth of Organisms on Wort Media.

Thirty-eight cultures, which were obtained from various places in the plant, were streaked on wort agar slants. They were incubated at 96-98°F. and 107-109°F. for two days and relative vigor of growths ascertained. The results are summarized in Table II, below.

TABLE II. SHOWING EFFECT OF TEMPERATURE OF INCUBATION ON GROWTH OF MICROORGANISMS ON WORT AGAR AND MALT EXTRACT BROTH.

Grew equally well at 96-98°F. and 107-109°F.	Growth inhibited at 107-109°F.	No growth at 107-109°F. 48 hours.	Number of organisms observed.
Observations with Wort Agar			
14 (36.9)	7 (18.4)	17 (44.7)	38
Observations with Malt Extract Broth			
11 (29)	9 (23.7)	18 (47.3)	38

Figures indicate number of organisms.
Figures in parentheses indicate per cent of organisms.

It is evident that on the very favorable wort agar medium, the higher temperature (107-109°F.) was detrimental to the growth of a large proportion of the organisms. Thus, 44.7% which grew luxuriantly at 96-98°F. failed to show any growth at 107-109°F. in two days.

A larger percentage failed to show growth or were distinctly inhibited by exposure to 107-109°F. on the very favorable wort agar medium.

3. Effect of Temperature of Incubation on Growth of Organisms on Gluten Water Agar.

In Table II, page 24, the results of the inhibitory effect of temperature were given on a very favorable medium. The same experiment was conducted using a medium which was not so favorable for growth. The medium was made by adding 2% agar agar to gluten water. On this medium it was found that 60% of the organisms which grew well at 96-98°F. were inhibited or failed to grow at all at 107-109°F. Of these which failed to show growth at 107-109°F., 45% failed to grow on subsequent incubation at a favorable temperature (70-75°F.) showing that exposure to 107-109°F. was germicidal for these strains.

4. Effect of SO_2 on Growth of Organisms on Gluten Water Agar.

To gluten water agar, prepared as described above, were added various quantities of SO_2 and the materials tubed aseptically ; after cooling, the agar slants were inoculated from 24-hour malt extract broth cultures of the various test

organisms and incubated at 96-98°F. The results are shown in Table III, below.

TABLE III. EFFECT OF ADDITION OF SO_2 TO GLUTEN WATER AGAR ON GROWTH AT 96-98°F.

% SO_2 Added	No. or Slight inhibition of growth	Distinct inhibition of growth	No growth	Number of test organisms
None	20 (100)	—	—	20
.009	19 (95)	1 (5)	1 (5)	20
.018	14 (70)	2 (10)	4 (20)	20
.028	6 (30)	5 (25)	9 (45)	20

Figures indicate number of cultures.
Figures in parentheses indicate percentage of cultures tested.

It is seen from Table III that as the concentration of added SO_2 increased, a larger proportion of cultures failed to grow. Addition of 0.016 to 0.018% SO_2 markedly inhibited or completely prevented growth of 20% of the cultures. Addition of 0.024 to 0.030% SO_2 effected marked inhibition of growth of 25% of cultures, whereas, complete prevention of growth was effected in 45% of the test cultures.

In consideration of the effect of addition of SO_2, it should be noted that the organisms inoculated on the surface of agar media are not exposed to the full force of the SO_2 present as would be the case if they were completely immersed in a liquid medium to which the same quantities of SO_2 had been

added. The organisms were all found to be aerobes (air loving) and therefore find more favorable conditions for growth on the surface of an agar slant than would be the case in less well aerated liquid media. The concentrations of added SO_2 which were found to be inhibitory (0.016 to 0.030%) are therefore considered in excess of what would be necessary to effect a similar inhibition in gluten water or similar liquid media.

5. Effect of Temperature on Sterilizing Action of SO_2. (Observations with Gluten Water Agar).

To ascertain the influence of temperature on the germicidal or antiseptic effects of added SO_2, it is necessary to employ organisms which, in the absence of freshly added SO_2, grow about equally well on gluten water agar, at the temperatures under consideration. Observations on seven organisms which fell in this category will be considered. The relative vigor of growth at 96-98°F. and 107-109°F. on gluten water agar containing various concentrations of added SO_2 is indicated in Table IV, page 28.

TABLE IV. EFFECT OF TEMPERATURE AND SO_2

% SO_2 Added	None		0.009		0.018		0.026		
Temp. of Inc.	96-98	107-109	96-98	107-109	96-98	107-109	96-98	107-109	
Culture	Relative Vigor of Growth (48 hours)								
5	+++	+++	+++	+++	+++	-	-	-	*
7	+++	+++	+++	+++	+++	+	+++	-	
10	+++	+++	+++	+++	+++	+	+++	-	
11	+++	+++	+++	+++	+++	+	+++	-	
31	+++	+++	+++	-	+++	-	+++	-	
32	+++	+++	+++	+++	+++	+	++	-	
36	+++	+++	+++	+++	+++	+	+	-	
% Inh.	0	0	0	14.3	0	71.5	28.6	-	
% N.G.	0	0	0	0	0	28.6	14.3	100	
% Killed	0	0	0	0	0	0	0	100	

*Culture killed as no growth developed on subsequent storage at 96-98°F.

Number of + indicates relative vigor of growth; - indicates no growth.

Inh. indicates inhibited.

N.G. indicates no growth.

6. Effect of Temperature of Incubation on Growth of Isolated Organisms in Gluten Water.

In the experiments with solid media, the effects of various agents on growth of a given organism could be determined only qualitatively by observing the relative vigor of growth. Quantitative data may be obtained by employing liquid media. Several experiments with isolated cultures were performed in the following manner.

Small flasks containing 100 cc. of gluten water were sterilized. Some were stored at 96-98°F., and others at 107-

109°F. On the following day they were inoculated with known quantities of 48 hour wort cultures of various organisms, previously isolated from plant liquors. The flasks were replaced in their respective incubators, and the number of live organisms ascertained after 24 and 48 hours storage by plating on wort agar (48 hours, 96-98°F.).

Illustrative results are shown in Table V, below. It is evident that organisms are present which are adversely affected by exposure to temperatures of 107-109°F. in gluten water. In general, the long chain forming types which produce heavy surface growth in liquid media grew well at both temperatures, or were somewhat inhibited by the higher temperatures; the oval or spherical types generally died at a temperature of 107-109°F.

TABLE V. EFFECT OF TEMPERATURE OF INCUBATION ON GROWTH OF ISOLATED ORGANISMS IN GLUTEN WATER.

Period of Storage (Days)	Organisms per cc. in Flasks Stored at 97-98°F.	Organisms per cc. in Flasks Stored at 107-109°F.
Organisms I P.F. (Elongated yeasts chain. Heavy surface growth)*		
0 (Initial)	900	900
1 day	850,000	980,000
2 days	8,400,000	16,000,000
Organisms II P.F. (Large irregular shaped. Heavy surface growth)*		
0 (Initial)	1,600	1,600
1 day	5,000,000	480,000
2 days	18,000,000	2,000,000
Organisms III P.F. (Oval yeast)*		
0 (Initial)	700	700
1 day	5,000,000	100
2 days	10,000,000	1,300

* Observations in gluten water (pH 4.5; SO_2, 0.004%).

7. Effect of Concentration of SO_2 on Growth of Isolated Organisms in Gluten Water.

Flasks or large tubes containing gluten water were sterilized and, after cooling, inoculated with known quantities of organisms from malt extract broth. Various quantities of SO_2 were then added, the tubes incubated at 96-98°F., and the number of viable organisms determined by plating on wort agar. The results of a number of experiments are shown in Table VI, page 30a.

It is evident that the organisms grew very well in the gluten water when stored at 96-98°F., but that addition of SO_2 markedly inhibited or even killed off the organisms. It is also of interest to note that the sterilizing action of SO_2 is most effective when freshly added and disappears as it becomes old. Thus, considering the results in the mixture of organisms (A6, A12, A13, A17) it was found that an initial count of 30,000 rose to 2,500,000 after 24 hours, when no fresh SO_2 was added, whereas, addition of 0.010% SO_2 kept the count down to 40,000 or practically no growth. Extending the incubation period to 48 hours, resulted in a count of 33,000,000 in the flask without added SO_2 and 12,000,000 in that to which 0.010% SO_2 had been added. The addition of this small quantity of fresh SO_2 (0.010%) was very effective during the first day but thereafter its sterilizing effect was gone, because it had

TABLE VI. EFFECT OF ADDITION OF SO_2 ON GROWTH OF ISOLATED ORGANISMS IN GLUTEN WATER STORED AT 96-98°F.

SO_2 % Added	pH	Viable organisms per cu. cm. Initial	After 24 hrs. at 96-98°F.	After 48 hrs. at 96-98°F.
		Organism X_{15}*		
.000	4.3	600	640,000	
.012	4.3	600	300,000	
.028	4.3	600	6,000	
		Organism X_{20}*		
.000	4.3	180	150,000	
.012	4.3	180	8,000	
.028	4.3	180	36	
		Mixture Organisms Nos. A1, A8, A14, A20**		
.000	4.3	20,000	1,200,000	3,200,000
.010	4.3	20,000	10,000	2,000,000
.020	4.2	20,000	1,600	6,300
.028	4.1	20,000	3	3
		Mixture Organisms Nos. A6, A12, A13, A17**		
.000	4.3	30,000	2,500,000	33,000,000
.010	4.3	30,000	40,000	12,000,000
.020	4.2	30,000	20,000	99,000
.028	4.1	30,000	0	44
		Mixture of Organisms Nos. A3, A5, A7, A23		
.000	4.3	17,000	200,000	5,000,000
.010	4.3	17,000	60,000	330,000
.020	4.2	17,000	16,000	29,000
.028	4.1	17,000	1,800	4,600
		Organism I P.F.+		
.000	4.6	800	—	7,900,000
.020	4.4	800	—	870,000
		Organism I P.F.++		
.000	4.5	900	850,000	8,400,000
.034	4.0	900	600	3,300
		Organism III P.F.+		
.000	4.6	30,000	—	16,000,000
.020	4.4	30,000	—	530,000
		Organism III P.F.++		
.000	4.5	700	5,000,000	10,000,000
.034	4.2	700	10	110

*Gluten water with initial (old) SO_2 of .030% employed.
**Gluten water with initial (old) SO_2 of .028% employed.
+Gluten water with initial (old) SO_2 of .024% employed.
++Gluten water with initial (old) SO_2 of .004% employed.

disappeared as free active SO_2.

With addition of larger quantities of SO_2 the sterilizing action was greater and persisted for longer times. Thus, with 0.020% and 0.029% SO_2, the counts after 24 hours were 20,000 and zero, and after 48 hours, only 99,000 and 44 cubic centimeters respectively, whereas without SO_2 addition, the count had risen to 33,000,000.

8. Observations on Effect of Storing Gluten Water.

In order to ascertain whether gluten water would remain sweet or become foul on storage, a number of samples were collected and observed as to changes taking place on standing. The observations consisted of determining the number of living organisms (capable of growing on wort agar) before and after storage and changes in the physical appearance of the sample. These data will be found in Table VII, page 32.

TABLE VII. EFFECT OF STORAGE ON THE NUMBER OF VIABLE ORGANISMS IN GLUTEN WATER.

Date	SO_2% old	Organisms per cc. after storage at 96-98°F. Initial	24 Hours
6/30/29	0.055	40,000	92,000
7/19/29	0.044	58,000	2,700,000
7/22/29	0.044	120,000	1,100,000
5/ 8/30	0.062	93,000	83,000
5/ 9/30	0.057	56,000	2,400,000
5/ 9/30	0.057	41,000	5,600,000
5/ 9/30	0.057	60,000	5,600,000
5/10/30	0.056	200,000	4,000,000
5/11/30	0.064	36,000	850,000
5/11/30	0.064	24,000	110,000

It is apparent that the number of living organisms increased materially on storage of gluten water. Generally, after 24 hours, a surface growth or film developed on the liquid; and in 48 to 72 hours, the liquid became putrid when stored in vessels to which air had access. The return of such a contaminated and putrescible liquor to the process constitutes a serious hazard.

9. Effect of Air Supply and Stirring on Growth of Microorganisms in Gluten Water.

It was noted in some of the observations with gluten water that there was a decrease instead of growth, if storage was in closed containers. It was felt that the availability of air might explain this phenomenon, and in view of the fact that the

gluten water returned to the system is subjected to constant aeration and agitation, on the reels, the influence of these factors on growth of microorganisms was ascertained as described below.

Samples of gluten water were collected in sterile Mason jars (pints). In the first series one jar was filled about nine-tenths full, and the lid screwed on tightly, while another jar was filled about one-fourth full and the lid left slightly ajar, so that air could enter. The closed jar will be referred to as anaerobic, the open jar as aerobic. The second series was similar except that two aerobic jars one-third full were employed and one of these was supplied with a stirrer.

The results which are tabulated in Table VIII, page 34, show clearly that air and agitation markedly favor the growth of microorganisms in gluten water.

In the anaerobic (closed) jars, considerable decreases in counts were generally obtained. In the aerobic (partially open) jars the counts rose very rapidly. Thus, a sample of fresh gluten water with an initial count of 630,000 per cubic centimeter showed only 480,000 after storage in a closed jar for two days at 96-98°F., whereas, the same gluten water stored in the presence of air gave a count of 48,000,000.

It is apparent that the presence of air and agitation favors the growth of microorganisms in gluten water. The con-

TABLE VIII. EFFECT OF AIR SUPPLY AND STIRRING ON GROWTH OF MICROORGANISMS IN GLUTEN WATER.

% SO_2 Old	Initial count	Organisms per cubic centimeter after 48 hours at 96-98°F.		
		Anaerobic	Aerobic	Aerobic (stirred)
Observations with Gluten Water No.1				
0.022	630,000	480,000	48,000,000	—
0.034	1,400,000	460,000	88,000,000	—
0.043	13,000	250,000	32,000,000	—
0.042	44,000	390,000	54,000,000	—
Observations with Gluten Water No.2				
0.046	1,200,000	640,000	8,000,000	61,000,000
0.045	1,600,000	100,000	23,000,000	21,000,000
0.045	1,300,000	110,000	32,000,000	43,000,000
0.045	1,900,000	410,000	12,000,000	24,000,000

Anaerobic = jars sealed; air kept out.
Aerobic = jars not sealed; air admitted.

ditions on the reels, because of the abundant air supply, are therefore conducive to growth of microorganisms, introduced with returning gluten water, unless some factors such as increased temperature or concentration of SO_2, are made operative to counteract this favorable influence.

10. Effect of Heating Gluten Water.

Gluten water samples were heated to various temperatures as indicated below and the number of viable organisms capable of growing on wort agar at 96-98°F. ascertained.

Samples of gluten water entering and leaving the heaters were collected in sterile jars, and one cubic centimeter por-

tions immediately transferred to sterile dilution water. The heated sample was quickly placed in a water bath at a temperature of the heated gluten water and samples withdrawn into dilution water at one or two minute intervals as shown in Table IX. The samples were plated on wort agar and the plates counted after 48 hours at 96-98°F. The results are detailed in Table IX, below, summarized in Table IXa, page 38, and plotted on Plot I, page 39.

TABLE IX. EFFECT OF PASSING GLUTEN WATER THROUGH PLANT HEATER ON VIABILITY OF MICROORGANISMS.

Time elapsed	Temp. °F.	% SO_2	Surviving organisms per cubic centimeter	Per cent reduction
September 5, 1930 (9:45 A.M.)				
Before heating	82	0.035	190,000	—
0 Min.	110	0.035	150,000	21.0
2 "	108	—	130,000	31.5
4 "	108	—	83,000	56.4
6 "	107	—	84,000	55.8
8 "	107	—	73,000	61.6
September 5, 1930 (11:00 A.M.)				
Before Heating	82	0.038	310,000	—
0 Min.	111	0.038	240,000	22.5
2 "	111	—	240,000	22.5
4 "	111	—	180,000	41.8
6 "	111	—	150,000	51.6
8 "	111	—	150,000	51.6

TABLE IX (continued)

Time elapsed	Temp. °F.	% SO_2	Surviving organisms per cubic centimeter	Per cent reduction
September 5, 1930 (1:15 P.M.)				
Before heating	82	0.035	250,000	—
0 Min.	111	0.030	190,000	24.0
2 "	111	—	150,000	40.0
4 "	111	—	110,000	56.0
6 "	111	—	110,000	56.0
8 "	111	—	100,000	60.0
September 9, 1930 (9:30 A.M.)				
Before heating	82	0.021	420,000	—
0 Min.	110	0.021	300,000	28.5
2 "	110	—	310,000	26.2
4 "	110	—	270,000	35.7
6 "	110	—	210,000	50.0
8 "	110	—	190,000	54.7
September 9, 1930 (10:30 A.M.)				
Before heating	82	0.020	580,000	—
0 Min.	110	0.020	330,000	43.0
2 "	110	—	360,000	38.0
4 "	110	—	290,000	50.0
6 "	110	—	230,000	60.4
8 "	110	—	200,000	65.6
September 5, 1930 (2:00 P.M.)				
Before heating	82	0.034	360,000	—
0 Min.	120	0.034	210,000	41.7
1 "	120	—	82,000	77.2
2 "	120	—	68,000	81.2
3 "	120	—	38,000	89.5
4 "	120	—	29,000	91.9

TABLE IX (continued)

Time elapsed	Temp. °F.	% SO_2	Surviving organisms per cubic centimeter	Per cent reduction
September 5, 1930 (3:15 P.M.)				
Before heating	82	0.032	280,000	—
0 Min.	120	0.032	170,000	39.2
1 "	120	—	30,000	89.3
2 "	120	—	34,000	87.8
3 "	120	—	22,000	92.1
4 "	120	—	16,000	94.3
September 5, 1930 (4:30 P.M.)				
Before heating	82	0.032	290,000	—
0 Min.	120	0.032	130,000	55.1
1 "	118	—	29,000	90.0
2 "	118	—	17,000	94.1
3 "	118	—	11,000	96.2
4 "	118	—	9,500	97.2
September 9, 1930 (1:30 P.M.)				
Before heating	82	0.020	480,000	—
0 Min.	125	0.020	49,000	89.8
1 "	125	—	17,000	96.4
2 "	125	—	22,000	95.4
3 "	125	—	23,000	95.2
4 "	125	—	36,000	92.5
September 9, 1930 (3:00 P.M.)				
Before heating	82	0.021	350,000	—
0 Min.	125	0.021	64,000	81.7
1 "	125	—	27,000	92.3
2 "	125	—	25,000	92.7
3 "	125	—	29,000	91.7
4 "	125	—	25,000	92.7

Reaction of all samples was pH 4.5.

TABLE IXa. SUMMARY OF EFFECT OF TEMPERATURE AND TIME OF HEATING ON VIABLE ORGANISMS IN GLUTEN WATER.

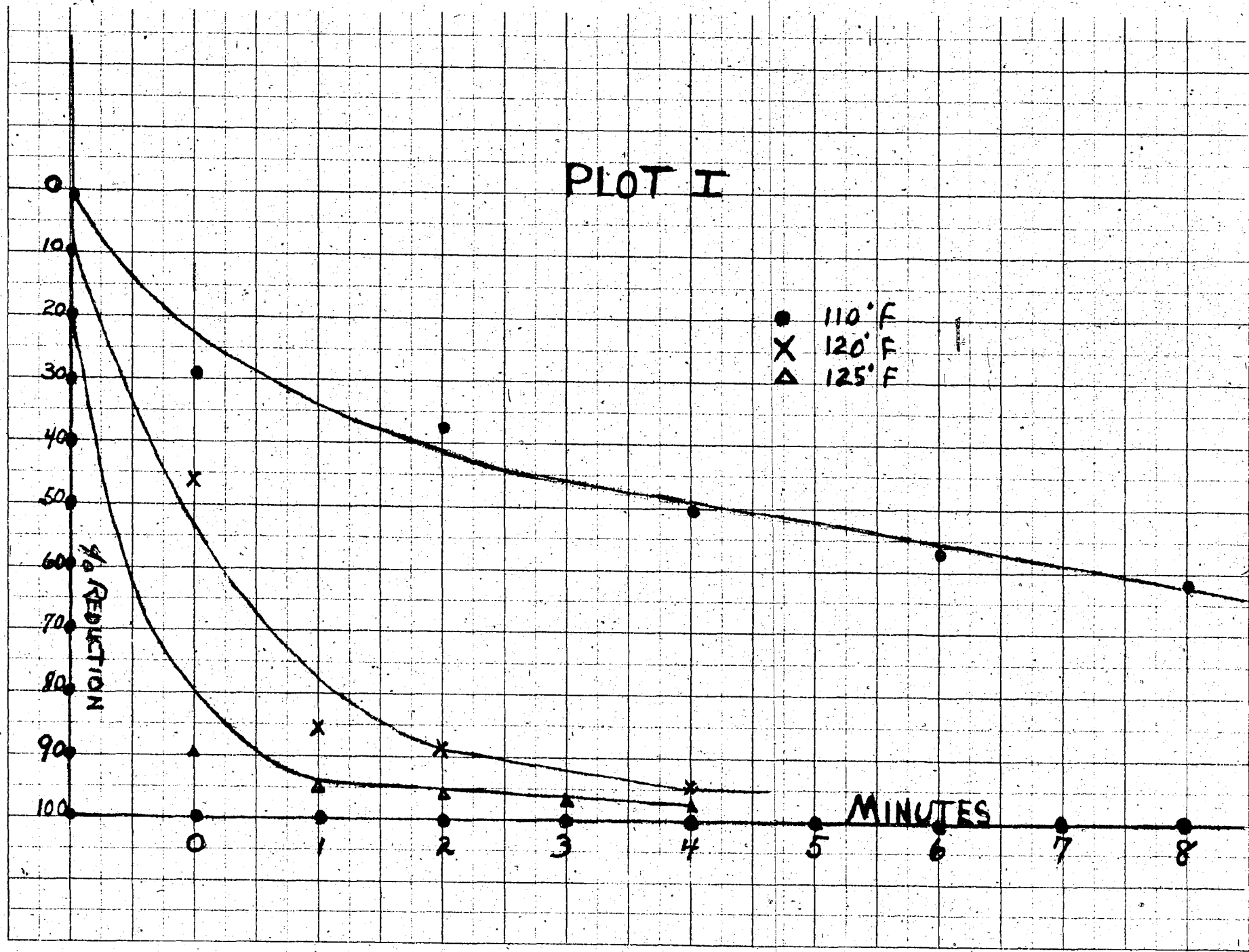
Period of exposure at indicated temperature.	Per cent reduction at 110°F.	120°F.	125°F.
0*	27.8	45.3	85.8
1 Min.	—	85.5	94.3
2 "	30.6	87.7	94.1**
3 "	—	92.6	93.5**
4 "	47.8	94.5**	95.0**
6 "	54.8		
8 "	58.7		

*Brought to the indicated temperature from an initial temperature of 82°F. by passage through heater.

**Surviving organisms were all bacteria.

In general, it was found that heating the gluten water (with a titratable SO_2 content of 0.020 to 0.038%) to 110°F. effected reductions of about 25%; that maintaining this temperature for four minutes resulted in approximately 48% reductions, while exposure for eight minutes effected decreases of about 59% in viable yeast-like organisms.

The higher temperatures effected much more rapid destruction of the microorganisms in gluten water. Thus, when the gluten water was heated to 120°F., the effluent from the heater contained 45% less organisms than that going in. The drop in the number of viable organisms averaged 87.7% after


PLOT I
● 110° F
X 120° F
△ 125° F
0
10
20
30
40
50
60
70
80
90
100
% REDUCTION
0
1
2
3
4
5
6
7
8
MINUTES

two minutes and 94.5% after four minutes exposure at 120°F.

The gluten water issuing from the heaters at 125°F. contained 85% less viable organisms than the influent. After one minute additional storage at this temperature there was a reduction of 94%. The surviving organisms were bacteria.

11. Effect of Addition of Chemicals on Growth of Organisms and Keeping Qualities of Gluten Water at Different Temperatures.

The observations here described will be considered under the following heads:

a. Effect of addition of SO_2 to the plating medium.

b. Observations on the relative sterilizing efficiency of fresh and old SO_2.

c. Effect of temperature and SO_2 on growth, reaction, and film formation in gluten water.

All observations in this section were on a laboratory scale and as the techniques were different for each set of experiments, these will be described somewhat in detail.

a. Effect of addition of SO_2 to the Plating Medium. The determination of the number of viable organisms in a liquid consists in placing known quantities of the test liquid in sterile containers (petri dishes) adding a suitable solidifiable nutrient medium, and after storage (incubation) for a

designated period, counting the number of colonies of organisms which develop. Each colony is considered to be the progeny of one organism, so that if the quantity of the test material in the petri dish is known, the number of colonies is taken as a measure of the number of organisms originally present, and the number in any given quantity of the test medium may be readily calculated. Thus, if 1/1000 of a cubic centimeter of gluten water is placed in a petri dish, a suitable medium such as wort agar is added, and 75 colonies develop; then there were at least 75 organisms in the 1/1000 cc. test sample or 75,000 per cubic centimeter of gluten water. The determination is dependent on the nutrient medium being suitable for growth of the organisms. If addition of SO_2 to such a medium prevented growth, the proportion of colonies thus prevented from developing becomes a measure of the sterilizing action of SO_2.

The number of viable organisms in three samples of gluten water was ascertained by plating out in the usual manner using wort agar as the nutrient medium. At the same time counts were made employing wort agar to which different quantities of SO_2 had been added. This was done by cooling the wort agar to 122-131°F., adding the desired quantity of SO_2, then cooling the medium to 109-113°F., pouring the plates and finally titrating the excess medium in the bottle with iodine for a

measure of the SO_2 present. The plates thus prepared were incubated for two days at 96-98°F., after which they were counted with the results shown in Table X, below.

TABLE X. EFFECT OF ADDITION OF SO_2 TO PLATING MEDIUM (WORT AGAR) ON THE NUMBER OF ORGANISMS IN GLUTEN WATER.

% SO_2 Added	Organisms per cubic centimeter gluten water		
	Sample I	Sample II	Sample III
None	1,200,000	670,000	230,000
0.010	—	—	58,000
0.015-0.018	625,000	1,000	2,400
0.024-0.025	230	0	—
Per cent of organisms failing to develop in presence of SO_2 added			
0.010	—	—	74.8
0.015-0.018	48.0	99.8	99.0
0.024-0.025	99.9	100.0	—

It is seen that the addition of 0.015-0.018% SO_2 resulted in a decrease of 48% in the number of organisms developing from one of the samples and 99% of the organisms in the other two samples of gluten water were prevented from growing.

b. Relative sterilizing efficiency of fresh and old SO_2. In the course of the various experiments it became apparent that the titratable SO_2 was not a direct measure of the sterilizing efficiency. It seemed that freshly added SO_2 was more effective than an equal quantity of SO_2 already present. An experiment was designed to throw some light on the question. In view of the fact that it is necessarily quite complicated it is described in detail.

One hundred cubic centimeter portions of sterilized filtered gluten water were placed in each of 30 blake bottles and resterilized in the autoclave. After cooling to about room temperature (95°F.) they were subdivided into six series of five bottles each; marked A, B, C, D, E, and F series respectively. To one bottle of each of the A, B, C, and F series was now added the following quantities of an approximately 0.8% SO_2 solution: -0, 1.0, 2.0, 3.0, and 4.0 cc.

The A, B, and C series of bottles containing added SO_2 and the D, and E series to which no SO_2 had been added, were all placed in the incubator at 98°F., and the F series immediately titrated to determine concentrations of SO_2, and discarded.

The following day the "C" series of bottles was titrated to determine the amount of SO_2 present and discarded. It was found that there was no change in the titratable SO_2 from that observed in the "F" series the previous day. The titrations

in the "C" series of bottles were taken as measures of the apparent SO_2 contents of the corresponding bottles of the A, and B series.

To each of the bottles of the "B" series, one cubic centimeter of gluten water was added, and the bottles replaced in the 98°F. incubator. The count in this gluten water was 700,000 per cubic centimeter on both plain and wort agar.

The "A" series of bottles remained in the 98°F. incubator as controls for pH, and SO_2 as well as to determine the sterility of the technique.

To each bottle of the "D" and "E" series one cubic centimeter of fresh gluten water was added and then to one bottle of each of the "D", and "E" series was added the following quantities of an approximately 0.8% SO_2 solution; namely 0, 1.0, 2.0, 3.0, and 4.0 cc.

The "D" series of bottles was placed in the 98°F. incubator along side the "A", and "B" series. The "E" series of bottles was immediately titrated for SO_2 and pH and the bottles discarded. These results, which were taken as measures of these constituents in the corresponding bottles of the "D" series were found to be practically identical with those observed for the "B" series previously referred to.

There was now present in the 98°F. incubator three series (A, B, and D) of bottles of sterilized gluten water, the dif-

ferent bottles of each series containing various concentration of SO_2 as shown in Table XII.

The "A" series, or control, was not inoculated with fresh gluten water.

The "B" series had received its quota of SO_2 one day and was inoculated with 1.0 cc. of fresh gluten water the following day.

The "D" series was inoculated with 1.0 cc. of gluten water and a few minutes (2 to 5 minutes) thereafter was added the SO_2 solution.

The titrations disclosed that the pH and SO_2 concentration in the "B" and "D" series were practically identical, but whereas the "B" series received its inoculum of fresh gluten water 24 hours after the addition of SO_2; the "D" series was inoculated a few minutes before the SO_2 was added. The organisms in the inoculum were thus exposed to "old" SO_2 in the "B" series and to "fresh" SO_2 in the "D" series. The total titratable concentration of SO_2 being the same, and other conditions being apparently identical, the differences observed in the "B" and "D" series are attributed to the difference in time of addition of the SO_2.

The number of viable organisms in each bottle of the "A", "B", and "D" series was determined after 24 hours storage on wort and plain agar (as was also the reaction pH and SO_2 content).

The results are detailed for the "B", and "D" series in Table XI, below, and illustrated on Plot II, page 47.

TABLE XI. EFFECT OF PRESENCE OF OLD AND FRESH SO_2 ON GROWTH OF MICROORGANISMS IN GLUTEN WATER.

Sample series	pH	SO_2% Added	SO_2% Total at start	SO_2% Total after 24 hours	Organisms per cubic centimeter gluten water storage, 24 hours at 98°F. Plain agar	Organisms per cubic centimeter gluten water storage, 24 hours at 98°F. Wort agar
Series B.	SO_2 added 24 hours before gluten water inoculum. Stored 24 hours at 98°F., then plated.					
B_0*	4.6	0	0.030	0.027	870,000	620,000
B_1*	4.5	0.008	0.038	0.033	400,000	320,000
B_2**	4.4	0.017	0.047	0.043	320,000	310,000
B_3+	4.3	0.025	0.055	0.051	33,000	52,000
B_4	4.2	0.033	0.063	0.060	22,000	32,000
Series D.	SO_2 added immediately after gluten water inoculum. Stored 24 hours at 98°F., then plated.					
D_0*	4.6	0	0.029	0.024	615,000	645,000
D_1+	4.5	0.008	0.037	0.034	30,000	52,000
D_2	4.4	0.018	0.047	0.043	5,800	8,100
D_3	4.4	0.026	0.055	0.051	5,300	7,900
D_4	4.3	0.033	0.062	0.061	4,000	7,400

Initial count 7000 per cubic centimeter, on both plain and wort agar.

* Heavy surface growth on bottles B_0, B_1, and D_0 after 24 hours at 98°F.

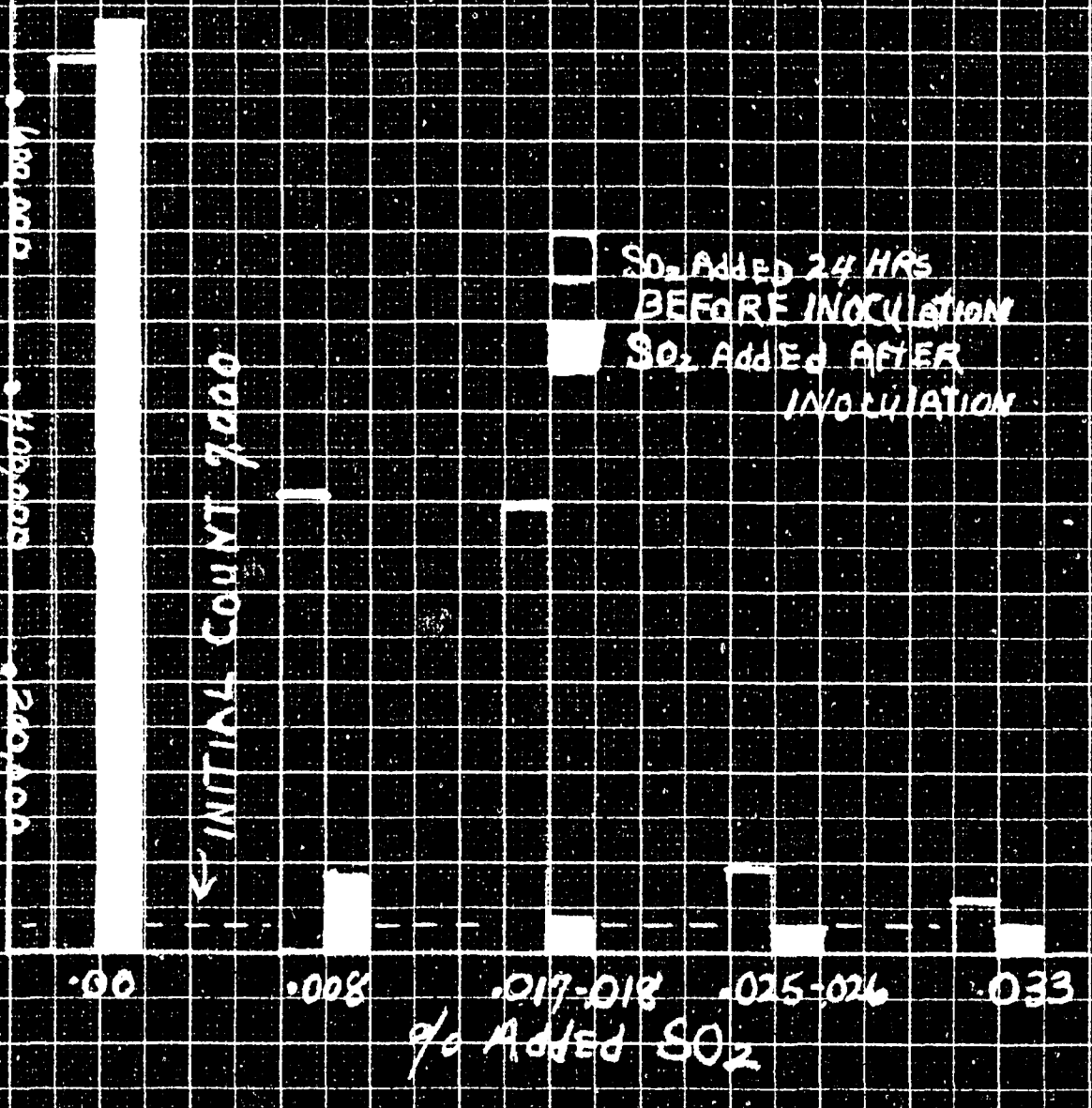
** Surface growth covered one-half area of liquid in bottles.

\+ Extremely small area (2%) of surface growth.

PLOT II

EFFECT of OID AND FRESH SO_2

INITIAL SO_2 .029%



All of the bottles of the "A" series (uninoculated) were found to be sterile, and the SO_2 values showed no significant change during incubation, a maximum drop of 0.003% being observed.

A perusal of Table XI, page 46, shows very clearly the fact that it is not merely the total titratable or apparent SO_2 that needs to be considered but that the proportion of the SO_2 which is freshly added is of paramount significance. The bottles marked B_0 and D_0, which contained no added SO_2 beyond that present in the gluten water itself (0.030 and 0.029%), were really duplicates and they showed practically identical wort agar counts of 620,000 and 650,000, respectively. The effect of adding 0.008% SO_2 a day before the inoculum (B_1 bottle) is indicated by a slight reduction in the wort agar count to 320,000 but the addition of the same quantity of SO_2 immediately after the inoculum (D_1 bottle) resulted in a count of but 52,000. Similarly in the B_2 and D_2 bottles to which were added 0.017% and 0.018% SO_2, a day before and immediately after the inoculum, respectively, the counts of the gluten water stored for 24 hours at 96-98°F. were 310,000 and 8,100, respectively. Thus, of two bottles containing the same apparent SO_2 (0.047% as indicated by titration) there were present only one-fortieth as many organisms in the bottle receiving 0.018% fresh SO_2 (D_2 bottle) as there were in the bottle (B_2 bottle) which

received the same amount of SO_2 24 hours before inoculation. To put it another way, in the bottle with freshly added SO_2 there was no multiplication, whereas in the one with old SO_2 the number of organisms increased forty fold. It is felt that the difference may be explained by the action of uncombined H_2SO_3 on yeasts. Titration with iodine includes not only the SO_2 present as H_2SO_3 but SO_2 in loosely bound compounds. Sulphur dioxide, which is bound, although it may be detected in whole by the iodine titration, is not as available for germicidal or antiseptic action on yeasts as free SO_2. The concentration of freshly added and uncombined SO_2 is therefore a determining factor with respect to its germicidal or antiseptic action in gluten water.

c. Effect of temperature and SO_2 on growth, change in pH and film formation in gluten water. The effect of additions of SO_2 and the influence of temperature on the sterilizing efficiency of SO_2 when added to gluten water were observed by introducing organisms into sterilized gluten water to which were then added various concentrations of SO_2, duplicate samples, being stored at various temperatures as indicated below in the detailed descriptions of the experiments.

(1) Observations on temperature and SO_2 with gluten water. Portions (50 cc.) of gluten water were placed

in 150 cc. extraction flasks and sterilized. One set of flasks was inoculated with a mixture of organisms X20, X24, and X29. Various quantities of SO_2 were then added to each of two flasks, one of which was incubated at 96-98°F. and the other at 107-109°F. A similar experiment was performed employing gluten water as the inoculum in place of the isolated cultures. The results are summarized in Table XII, page 51.

Considering the experiment with the isolated cultures it will be observed that in the absence of freshly added SO_2, a surface growth (film) developed very rapidly at 96-98°F., the entire surface being covered after 24 hours, whereas at 107-109°F., only about 20% of the surface showed a film. On addition of 0.014% SO_2 the surface growth after 24 hours at 96-98°F. was reduced to less than 10% of the surface area, whereas, at the higher temperature (107-109°F.) there was no growth at all, even after three days incubation. With 0.029% freshly added SO_2 there was no surface growth at either temperatures after three days. It is apparent, therefore, that, as respects film formation by these organisms, the addition of 0.014% fresh SO_2 exerted a retarding effect at 96-98°F., and complete inhibition at 107-109°F., while with the higher fresh SO_2 content (0.029%) inhibition was complete at both temperatures.

A consideration of the change in reaction shows similar

TABLE XII. EFFECT OF TEMPERATURE AND SO_2 ON GROWTH AND CHANGE OF REACTION IN GLUTEN WATER

% SO_2 Added:	Reaction pH				Area % Surface film			Organisms per cc.	
	Initial:	1 day	2 days	3 days	1 day	2 days	3 days	Initial:	48 hour
Experiment with mixtures of cultures X20, X24 and X29, as inoculum									
Flasks kept at 96-98°F.									
.000:	4.3	4.3	7.0	7.7	100	100	100	3,500	14,000,000
.014:	4.1	4.1	6.4	7.3	10	100	100	3,500	10,000,000
.029:	3.7	3.7	3.7	3.8	0	0	0	3,500	420
Flasks kept at 107-109°F.									
.000:	4.3	4.2	6.6	7.4	20	100	100	3,500	730,000
.014:	4.1	4.1	4.1	4.1	0	0	0	3,500	100
.029:	3.7	3.7	3.7	3.7	0	0	0	3,500	10
Experiment with gluten water as inoculum									
Flasks kept at 96-98°F.									
.000:	4.3	4.3	7.0	7.9	50	100	100	2,800	1,800,000
.014:	4.1	4.1	4.5	6.3	5	100	100	2,800	1,600,000
.029:	3.7	3.7	3.7	3.7	0	0	0	2,800	200
Flasks kept at 107-109°F.									
.000:	4.3	4.2	5.0	6.7	5	75	100	2,800	160,000
.014:	4.1	4.1	4.2	4.1	0	0	0	2,800	100
.029:	3.7	3.7	3.7	3.7	0	0	0	2,800	10

Initial SO_2 = .028%

It will be noted that:

(1) A small quantity of freshly added SO_2 (.014%) retarded development of alkaline reactions and surface films through inhibition of growths of microorganisms at 96-98°F.

(2) The larger quantity of freshly added SO_2 (.029%) completely prevented alkinization and film formation by its germicidal action on the microorganisms.

(3) The sterilizing action of SO_2 was distinctly greater at the higher temperature. A concentration of SO_2 (.014%) which was inhibitory at 96-98°F. was germicidal at 107-109°F.

results. The flasks without freshly added SO_2 became progressively more alkaline; those kept at 96-98°F. going from an initial reaction of pH 4.3 to pH 7.0 after two days and pH 7.7 after three days; the flask at 107-109°F. showed reactions of pH 6.6 and 7.4, respectively, after corresponding periods of incubation. The higher temperature thus retarded the rate of alkalinization.

The addition of 0.014% SO_2 markedly retarded alkalinization. Thus, comparing the results of the flask at 96-98°F., containing no fresh SO_2 with that to which 0.014% SO_2 was added, the reactions after one day were pH 4.5 and pH 4.1, after two days pH 7.0 and pH 6.4, and after three days, pH 7.7 and 7.3 respectively. Change in reaction was completely arrested for 24 hours by the addition of 0.014% SO_2 at 96-98°F. The effect of SO_2 was much more marked at 107-109°F., where no change in reaction was observed even after three days incubation.

Another measure of the influence of temperature and SO_2 which may be employed is the determination of the number of viable organisms. An initial count of 3,500 rose to 14,000,000 after 48 hours at 96-98°F., and to but 725,000 at 107-109°F. in gluten water to which no fresh SO_2 had been added. The inhibitory effect of temperature is evident.

A much more marked effect was observed in a comparison of the flasks containing 0.014% fresh SO_2. In this series the

count after 48 hours at 96-98°F. was 10,000,000, whereas, that at 107-109°F. fell to 100. A concentration of 0.014% fresh SO_2 was inhibitory at 96-98°F., but germicidal at 107-109°F.

It is apparent that for the organisms in question both the higher temperatures (107-109°F.) and the addition of 0.014 to 0.029% SO_2 served to inhibit growth, and that the effect of SO_2 was much more marked at the higher temperature.

In the series of observations employing gluten water as the inoculum, confirmatory results were obtained.

The addition of 0.014% retarded film formation at 96-98°F., and completely inhibited this phenomenon at a temperature of 107-109°F.

As regards alkalinization, an initial reaction of pH 4.3 in the flasks without fresh SO_2 rose to pH 7.0 in two days at 96-98°F., as compared with pH 5.0 at 107-109°F. After three days incubation the respective reactions were pH 7.9 and 6.7. The addition of 0.014% SO_2 served to markedly retard the rate of alkalinization at 96-98°F., the reaction after two days being pH 4.5 and after three days pH 6.3, whereas. at 107-109°F., no change whatever in reaction was observed even after three days of storage. The addition of 0.014% SO_2 was therefore very effective in preventing the destruction of the acids in the gluten water by yeast growth, particularly at the temperature of 107-109°F.

The change in counts also illustrates the detrimental ef-

fects of increased temperature and SO_2. An initial count of 2,800 rose in 48 hours to 1,800,000 at 96-98°F., and to but 156,000 at 107-109°F. in gluten water to which no SO_2 had been added. In the series containing 0.014% fresh SO_2 the counts after 48 hours were 1,600,000 and 100 at 96-98°F. and 107-109°F. respectively. Thus, this small quantity of SO_2 served to slightly retard the rate of growth at 96-98°F., but acted as a germicide at 107-109°F. Increasing the fresh SO_2 content to 0.029% resulted in a decrease of viable cells even at 96-98°F., the initial count of 2,800 falling to 200.

The results of these series of observations show that the combined action of fresh SO_2 and elevated temperature was particularly efficacious against the growth of the organisms in gluten water.

12. Observations on Contamination and Condition of Silks.

As stated above it was thought that serious losses would ensue if the excessive growth of microorganisms was unchecked in the process waters. The starch and gluten, after being disintegrated and freed from the hull, pass through the small openings in the silk reels. Should the interstices become clogged by corn deposits or by the bodies of microorganisms some of the starch and gluten would pass through the reels and be separated out with the hulls. In order to substantiate this

contention one reel of each set of reels from the grit reels to the fifth fine feed reel was marked for experimental purposes. In order to have conditions under different plant operation as comparable as possible, these reels had new silks put on them at the beginning of each change. The exact period of exposure of the silks (age of silk) was thus known at any time a panel was removed for examination. Aside from the silk replacement, there was no change in the treatment accorded the reel. It was washed twice daily with the regular hydrochloric acid wash. Panels were taken for analysis seven to eight hours after the HCl wash. The examination consisted of the determination of the number of microorganisms adhering to the water sprayed silk and a microscopic examination of the silk proper.

a. Number of organisms on silk. The extent of contamination of the silk was determined in the following manner. About seven to eight hours after the regular HCl wash, the feed to the test reel was stopped and the water spray applied for several minutes to remove any substances which would normally be dislodged by the water spray wash. The reel was then stopped and the tier, which contained the section of silk to be removed, was again sprayed with water. After draining a few minutes, a section of silk was removed aseptically, folded four times (inner faces of folded silk in contact), and placed in a large Mason jar. The silk was immediately brought to the

laboratory, placed on a wooden block, and a set of disks cut out. An inner pair of silk disks was now removed and placed in a sterile flask containing 75 cc. of water and some glass beads. After thorough shaking, the disks were picked up with a sterile forceps and cut up into small pieces with a sterile scissors. The material was then again thoroughly shaken and plated out on wort agar. The results are calculated on the basis of the number of organisms per square inch of inner surface of silk and are shown in Table XIII, below.

TABLE XIII. EFFECT OF REDUCING STERILIZING AGENTS ON CONTAMINATION OF SILK REELS.

Silk	Reel	P.F. (Reduced Sterilization) SO_2 not added at reels. Gluten water not added.	P.F. (Normal) SO_2 added at reels. Gluten water heated.
		Organisms per square inch of inner silk surface	
F (17)	Grit	3,300,000*	950+
A (17)	1st Fine	1,700,000*	23,000
B (9)	2nd "	3,500,000*	1,800+
C (9)	3rd "	2,100,000*	1,900+
D (9)	4th "	17,000,000*	1,400+
E (9)	5th "	3,200,000*	1,400+

*Colonies good size, yeast-like.
+Over 90% of the colonies very small or pin point size.

b. Microscopic appearance of silks. Unused portions of silks employed for the microorganism counts were washed under

the tap to remove adhering bits of gluten or other debris, then cut into strips and stained by Gram's method. Hand washing of the silks under the tap was found necessary because they did not stain properly if the gluten, etc., was allowed to remain. Small portions of these stained silks were mounted in Canada balsam and examined microscopically.

Their appearance is indicated in the accompanying photomicrographs. It is noted that during the periods of reduced sterilization the silks contained yeasts and yeast-like forms which frequently formed chains or masses growing out into and across the openings.

These filamentous types of yeast-like forms are very tenacious and they are not readily removed from the silks once they have gained a foothold.

There is another way whereby the growth of yeasts on silks may produce clogging. In experiments previously described, it was noted that some of the yeasts growing in gluten water produced an alkaline reaction of pH 5.6 in 24 hours and pH 7.4 to pH 7.6 in 48 hours at 98°F. If these yeasts grow on silks, there may be produced a relatively alkaline zone which when coming in contact with the acid liquors will tend to alkalinize them at the point of contact, causing precipitation to occur. This precipitation could build up to such an extent that clogging would occur.

WASHED SILKS
(P.F. Normal)

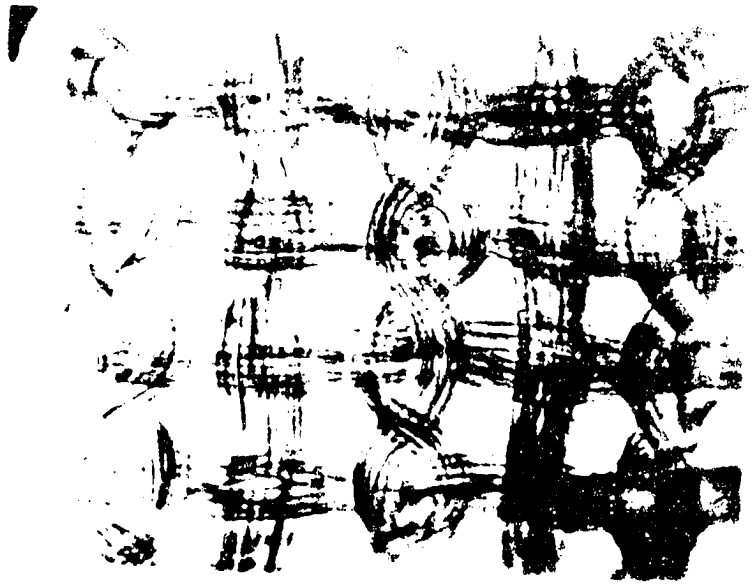
First Fine Feed Reel

No.17 Silk in use twenty days.
(Magnified 125 diameters)

Fourth Fine Feed Reel

No.9 Silk in use eight days.
(Magnified 125 diameters)

Remarks: Silk clean; no evidence of clogging.



WASHED SILK
(P.F. Reduced sterilization)

Grit Reel

No.17 Silk in use ten days.
(Magnified 125 diameters) View 1

Grit Reel

No.17 Silk in use ten days.
(Magnified 250 diameters) View 2

Remarks: Silk partially clogged; filamentous organisms not evident.


F

WASHED SILK
(F.F. Reduced sterilization)

First Fine Feed Reel

No.17 Silk in use ten days.
(Magnified 125 diameters) View 1

First Fine Reed Reel

No.17 Silk in use ten days.
(Magnified 250 diameters) View 2

Remarks: Silk partially clogged; filamentous organisms not evident.


A

WASHED SILK
(P.F. Reduced sterilization)

Second Fine Feed Reel

No.9 Silk in use ten days.
(Magnified 125 diameters) View 1

Second Fine Feed Reel

No.9 Silk in use ten days.
(Magnified 250 diameters) View 2

Remarks: Filamentous organisms still not evident.


B


B

WASHED SILK
(P.F. Reduced sterilization)

Third Fine Feed Reel

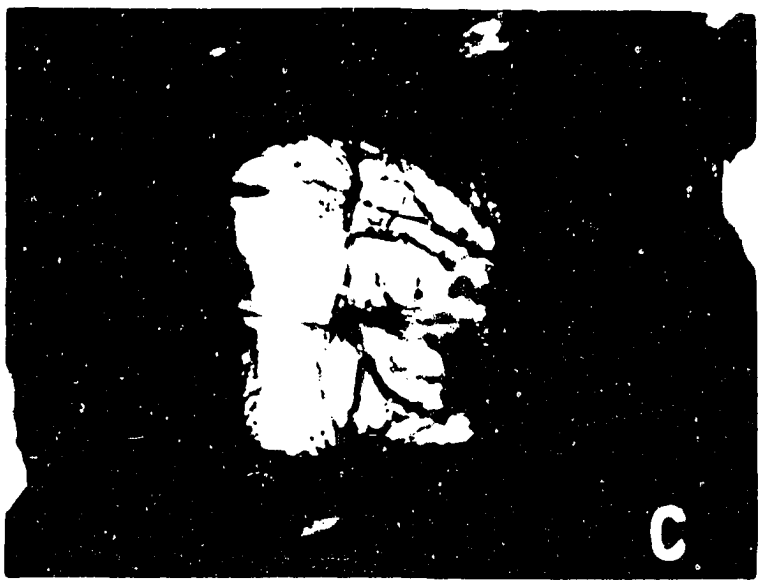
No.9 Silk in use ten days.
(Magnified 125 diameters) View 1

Third Fine Feed Reel

No.9 Silk in use ten days.
(Magnified 250 diameters) View 2

Remarks: Clogging due to filamentous organisms distinctly evident.


C


C

WASHED SILK
(P.F. Reduced sterilization)

Fourth Fine Feed Reel

No.9 Silk in use ten days.
(Magnified 125 diameters) View 1

Fourth Fine Feed Reel

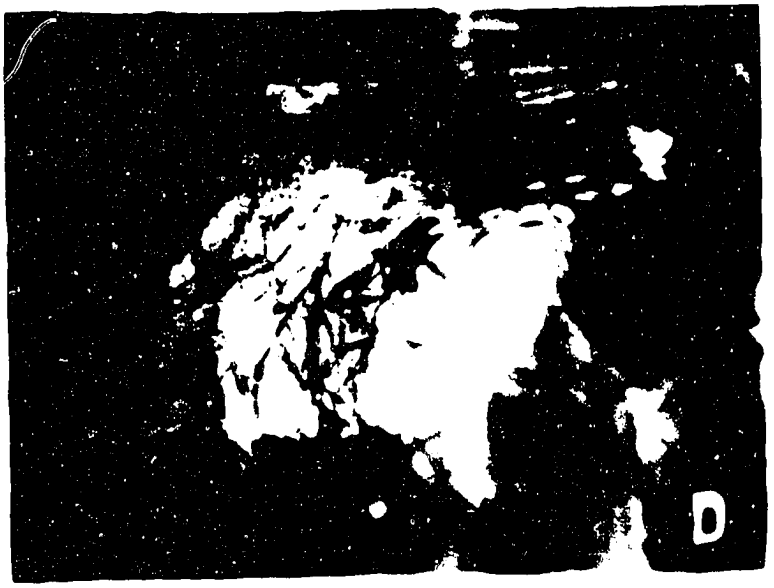
No.9 Silk in use ten days.
(Magnified 250 diameters) View 2

Fourth Fine Feed Reel

No.9 Silk in use ten days.
(Magnified 500 diameters) View 3

Remarks: Considerable clogging due to filamentous organisms.


D


D


D

WASHED SILK
(P.F. Reduced sterilization)

Fifth Fine Feed Reel

No.9 Silk in use ten days.
(Magnified 125 diameters) View 1

Fifth Fine Feed Reel

No.9 Silk in use ten days.
(Magnified 250 diameters) View 2

Fifth Fine Feed Reel

No.9 Silk in use ten days.
(Magnified 500 diameters) View 3

Remarks: Considerable clogging due to filamentous organisms.


E


E


E

13. Effect of Addition of a Highly Ionized Acid on the Growth or Death of Microorganisms in Normal Gluten Water.

The relative efficiency of fresh and old SO_2 has been discussed under heading 11-b. It was found that sulphur dioxide lost some of its potency as a germicide after it had been in gluten water for some time. The iodine titration reveals that it has not volatilized to any great extent. Therefore, it is not unreasonable to assume it has combined in an unoxidized state with some constituent of gluten water. In all probability, it combines with the aldehydes always found present. It may combine with the degredated proteins which result from the steeping and other operations.

It has long been known that most sulphite addition compounds are unstable in acid or alkaline solutions and are regenerated to the original compounds.

Likewise, it has been shown (8) that SO_2 to be effective as a germicide must not be combined as the sodium salt of sulphurous acid. This then leaves but one possibility for reactivating the sulphur dioxide which has combined with the solids in gluten water.

Various amounts of hydrochloric acid (10%) were added to unsterilized normal gluten water. The reaction (pH) and the growth in 24 and 48 hours were noted.

The acid was diluted in tap water and found to be sterile.

Large necked 16 ounce sterile cotton stoppered bottles were used to store the various samples of gluten water. They were stored at 96-98°F. for 24 and 48 hours and plated on wort agar, incubated 24 hours at 96-98°F.

The pH of the different bottles was determined with a Coleman electrometer which was checked against standard buffer solutions and found to be accurate. The results of this experiment are tabulated in Table XIV, page 74.

Throughout this latter work the hydrogen-ion concentration was determined with this instrument. It is of interest to note the reactions tabulated, for the earlier bacteriological studies on this problem were determined by indicators which were later found to give a more alkaline reaction than the electrometer. The difference noted ranged from one to two-tenths of a pH.

TABLE XIV. NORMAL GLUTEN WATER AND HYDROCHLO

Date	Water cc.	% SO_2	Acid cc.	pH	Initial count	24 Hour count	48 Hour count	Ave. pH	Ave. SO_2
5/13/37	300	.0617	0.00	4.20	110,000	1,500,000	15,000,000		
5/15/37	300	.0646	0.00	4.10	60,000	1,200,000	13,700,000		
5/17/37	300	.0576	0.00	4.15	90,000	1,150,000	12,000,000		
5/18/37	300	.0601	0.00	4.20	100,000	9,000,000	22,000,000		
5/21/37	300	.0640	0.00	4.10	58,000	900,000	10,500,000	4.16	.0616
5/17/37	300	.0576	0.30	4.05	90,000	1,300,000	10,000,000		
5/18/37	300	.0601	0.30	4.15	100,000	1,360,000	10,100,000		
5/21/37	300	.0640	0.30	4.13	58,000	850,000	9,800,000	4.13	
5/17/37	300	.0576	0.60	3.85	90,000	300,000	3,500,000		
5/18/37	300	.0601	0.60	3.90	100,000	360,000	4,200,000		
5/21/37	300	.0640	0.60	3.85	58,000	230,000	3,500,000	3.87	
5/17/37	300	.0576	0.90	3.75	90,000	6,000	900,000		
5/18/37	300	.0601	0.90	3.85	100,000	51,000	800,000		
5/21/37	300	.0640	0.90	3.80	58,000	75,000	700,000	3.80	
5/17/37	300	.0576	1.20	3.65	90,000	1	0		
5/18/37	300	.0601	1.20	3.78	100,000	200	60,000		
5/21/37	300	.0640	1.20	3.75	58,000	0	200	3.72	
5/15/37	300	.0646	1.50	3.55	60,000	0	0		
5/17/37	300	.0576	1.50	3.50	90,000	0	0	3.52	

ORMAL GLUTEN WATER AND HYDROCHLORIC ACID

4 Hour count	48 Hour count	Ave. pH	Ave. SO_2	Ave. initial	Ave. 24 Hour count	% Increase	Ave. 48 Hour count	% Increase
500,000	15,000,000							
200,000	13,700,000							
150,000	12,000,000							
000,000	22,000,000							
800,000	10,500,000	4.16	.0616	83,600	2,750,000	3,293	14,650,000	17,500
500,000	10,000,000							
560,000	10,100,000							
550,000	9,800,000	4.13		82,700	1,170,000	1,412	9,970,000	12,050
500,000	3,500,000							
360,000	[illegible],200,000							
280,000	3,500,000	3.87		82,700	280,000	339	3,730,000	4,510
6,000	900,000							
51,000	800,000							
75,000	700,000	3.80		82,700	6,200	0	800,000	967
1	0							
200	60,000							
0	200	3.72		82,700	67	0	20,000	0
0	0							
0	0	3.52		75,000	0	0	0	0

A perusal of this table shows quite clearly that small amounts of hydrochloric acid added to a normal gluten water containing sulphur dioxide are quite inhibitory. Larger amounts actually exert a killing effect on the organisms present.

Since inhibition of growth of micro-organisms is found with but a slight change in pH it is not unreasonable to surmise that the acid has activated the SO_2. This point will be further discussed later in this paper.

14. Effect of a Highly Ionized Acid on the Growth or Death of Micro-organisms in Normal Starch Wash Water.

A similar series of experiments as in No.13 was made with normal starch wash water. Quite similar results will be found by inspecting Table XV, page 76. The chief difference between Nos.13 and 14 which exists is a matter of degree. The starch wash water has a much lower buffering value than gluten water. This can be seen by comparing the amounts of acid necessary to get the same pH with the two waters. It is also believed that the lower percentage increase in growth from the initial is due to the fact that starch wash water contains normally more active SO_2 than gluten water even though the total percentage of SO_2 is much lower in starch wash water.

TABLE XV. NORMAL STARCH WASH WATER AND

Date	Water cc.	% SO_2	Acid cc.	pH	Initial count	24 Hour count	48 Hour count	Ave. pH	Ave. SO_2	I
5/13/37	300	.0352	0.00	3.60	1,000	5,000	10,000			
5/15/37	300	.0346	0.00	3.60	650	6,500	28,000			
5/17/37	300	.0320	0.00	3.65	1,800	5,000	48,000			
5/18/37	300	.0326		3.65	1,000	5,000	10,000	3.625	.0336	
5/18/37	300	.0326	0.05	3.60	1,000	3,000	20,000	3.60		
5/18/37	300	.0326	0.10	3.55	1,000	60	14,000	3.55		
5/18/37	300	.0326	0.15	3.50	650	2,000	6,000			
5/17/37	300	.0320	0.15	3.50	1,800	5,000	15,000			
5/18/37	300	.0326	0.15	3.45	1,000	70	4,000	3.48		
5/18/37	300	.0326	0.20	3.40	1,000	50	2,000	3.40		
5/13/37	300	.0352	0.30	3.25	1,000	0	15			
5/15/37	300	.0346	0.30	3.30	650	1,000	4,500			
5/17/37	300	.0320	0.30	3.30	1,800	500	1,600	3.28		
5/15/37	300	.0346	0.45	3.15	650	20	200			
5/17/37	300	.0320	0.45	3.10	1,800	350	5,000	3.12		
5/17/37	300	.0320	0.60	3.00	1,800	30	710	3.00		
5/13/37	300	.0352	0.75	2.90	1,000	0	0			
5/17/37	300	.0320	0.75	2.90	1,800	0	5	2.90		

V. NORMAL STARCH WASH WATER AND HCl

4 Hour count	48 Hour count	Ave. pH	Ave. SO2	Ave. Initial	Ave. 24 Hour count	% Increase	Ave. 48 Hour count	% Increase
5,000	10,000							
6,500	28,000							
5,000	48,000							
5,000	10,000	3.625	.0336	1,110	5,370	483	24,000	2,162
3,000	20,000	3.60		1,000	3,000	300	20,000	2,000
60	14,000	3.55		1,000	0	0	14,000	1,400
2,000	6,000							
5,000	15,000							
70	4,000	3.48		1,720	2,360	131	8,330	484
50	2,000	3.40		1,000	1,500	150	2,000	200
0	15							
1,000	4,500							
500	1,600	3.28		1,150	500	0	2,038	175
20	200							
350	5,000	3.12		1,220	185	0	2,600	213
30	710	3.00		1,800	30	0	710	0
0	0							
0	5	2.90		1,400	0	0	3	0

Here, as in the case with gluten water, the addition of small amount of acid to a sulphured process water very materially decreases the growth rate of the existing microorganisms. That this difference is due to something other than pH will be shown later.

15. Effect of pH on Growth or Death of Microorganisms in Gluten Water Free from SO_2.

In order to determine the effect of pH on the growth rate of the organisms in starch process waters it was necessary to use a water free from SO_2.

Sulphur dioxide free process water does not exist. When the plant was operated with reduced sterilization there was some sulphur dioxide in the waters.

Gluten water was boiled for a long period of time and it was found there remained a considerable amount of titratable SO_2. This further substantiates the belief that some of the SO_2 in gluten water is combined with the organic matter present.

It was found that the addition of no less than 125 cc. of 10% hydrochloric acid to three liters was necessary to liberate the SO_2. With this amount of acid it was possible to boil out the SO_2. Bubbling air into the boiling water expedited the removal. With this method it was found that the gluten

water could be diluted back to its original volume and carefully neutralized to the original pH with either soda ash or dilute caustic soda. If soda ash was used it was found necessary to aerate for a long time to dispel the carbon dioxide formed in the neutralization. The neutralization must be done at a temperature not much above room temperature. If it is carefully done, there will be little change in the physical appearance of the liquor.

With a water of this type it was possible to add an inoculum and study the effect of pH on the growth or death of the inoculum. The inoculum which was used came from untreated gluten water and was a characteristic mixture which had been grown in a malt extract broth.

The water free from SO_2 was transferred asceptically to sterile 16 ounce wide mouth bottles. Various amounts of hydrochloric acid (10%) were added and a few minutes later inoculated with the malt extract broth culture. These bottles were loosely plugged with cotton and stored at 96-98°F. for 24 and 48 hours. It should also be noted that the bottles were about two-thirds full. One series of experiments was run with the bottles about one-eighth full and it was found the growth rates were abnormally high. This, it was felt, was due to the low ratio of surface exposed to total volume. The viable organisms were determined by plating on wort agar at 96-

98°F.

The results are summarized in Table XVI, page 80.

This table shows at a glance that the microorganisms present in gluten water can grow at reactions as low or lower than pH 2.68. This pH is far lower than that at which no growth was found in the case of sulphured gluten water.

16. Effect of pH on Growth or Death of Microorganisms in Starch Wash Water free from SO_2.

Starch wash water was treated quite similarly to gluten water to free it from SO_2. In this case it was found that a much smaller amount of 10% hydrochloric acid was necessary to liberate the combined SO_2. In the case of gluten water it took 125 cc. of 10% HCl for three liters, whereas, with starch wash water, 40 cc. were found to be ample for the same amount.

The inoculum came from starch wash water and was grown in a malt extract broth. The results are tabulated in Table XVII, page 81.

This table shows that the organisms found in starch wash water are capable of growth at a pH as low as 1.8. A comparison of this with sulphured starch wash water shows quite clearly that sulphur dioxide is activated by HCl.

TABLE XVI. GLUTEN WATER FROM SO_2 -

Date	Water cc.	10% HCl cc.	pH	Initial count	24 Hour count	48 Hour count	Ave. pH	Av Init cou
5/17/37	300	0.00	4.10	15,000	1,300,000	20,000,000		
5/18/37	300	0.00	4.20	4,330	600,000	3,000,000		
5/19/37	300	0.00	4.13	10,000	800,000	4,000,000	4.15	9,7
5/ 7/37	300	0.45	3.92		1,000,000	11,700,000		
5/18/37	300	0.45	4.05		750,000	2,300,000		
5/19/37	300	0.45	4.10		800,000	4,000,000	4.02	9,7
5/17/37	300	0.90	3.75		670,000	4,270,000		
5/18/37	300	0.90	3.88		380,000	3,300,000		
5/19/37	300	0.90	3.90		510,000	3,100,000	3.84	9,7
5/17/37	300	1.80	—		—	1,900,000		
5/18/37	300	1.80	3.53		900,000	1,800,000		
5/19/37	300	1.80	3.55		600,000	2,000,000	3,54	9,7
5/17/37	300	3.70	2.95		400,000	1,800,000		
5/18/37	300	3.70	2.93		42,000	1,600,000		
5/19/37	300	3.70	2.98		60,000	1,200,000	2.95	9,7
5/17/37	300	4.50	2.50		400	62,000		
5/18/37	300	4.50	2.75		8,600	63,000		
5/19/37	300	4.50	2.80		1,000	98,000	2.68	9,7

VI. GLUTEN WATER FREE FROM SO_2 — HCl

Hour count	48 Hour count	Ave. pH	Ave. Initial count	Ave. 24 Hour count	% Increase	Ave. 48 Hour count	% Increase
00,000	20,000,000						
00,000	3,000,000						
00,000	4,000,000	4.15	9,770	900,000	9,210	9,000,000	92,100
00,000	11,700,000						
50,000	2,300,000						
00,000	4,000,000	4.02	9,770	850,000	8,800	6,000,000	61,400
70,000	4,270,000						
80,000	3,300,000						
10,000	3,100,000	3.84	9,770	520,000	5,310	3,560,000	36,500
—	1,900,000						
00,000	1,800,000						
00,000	2,000,000	3,54	9,770	750,000	7,660	1,900,000	19,450
00,000	1,800,000						
42,000	1,600,000						
60,000	1,200,000	2.95	9,770	167,000	1,710	1,530,000	15,690
400	62,000						
8,600	63,000						
1,000	98,000	2.68	9,770	3,300	0	74,300	760

TABLE XVII. STARCH WASH WATER NO SO_2 —

Date	Water cc.	Acid cc.	pH	Initial count	24 Hour count	48 Hour count	Ave. pH	Ave. Initial count
5/17/37	300	0.00	3.95	80,000	4,400,000	6,000,000		
5/18/37	300	0.00	4.10	14,700	5,000,000	6,600,000		
5/19/37	300	0.00	4.00	30,000	4,500,000	7,000,000	4.01	41,600
5/17/37	300	0.15	3.77		3,000,000	5,900,000		
5/18/37	300	0.15	3.90		4,000,000	—		
5/19/37	300	0.15	3.75		3,700,000		3.80	41,600
5/18/37	300	0.30	3.70	14,700	2,600,000	5,000,000		
5/19/37	300	0.30	3.70	30,000	3,000,000	6,500,000	3.70	41,600
5/17/37	300	0.30	3.70	80,000	2,800,000	5,750,000		
5/17/37	300	0.45	3.28		3,200,000	4,200,000		
5/18/37	300	0.45	3.51		2,900,000	—		
5/19/37	300	0.45	3.30		2,800,000	3,800,000	3.36	41,600
5/17/37	300	0.60	3.10		3,500,000	4,000,000		
5/18/37	300	0.60	3.32		1,400,000	3,800,000		
5/19/37	300	0.60	3.20		2,000,000	4,000,000	3.20	41,600
5/17/37	300	0.90	2.80		1,500,000	1,900,000		
5/18/37	300	0.90	3.05		1,300,000			
5/19/37	300	0.90	2.95		1,000,000	3,000,000	2.93	41,600
5/17/37	300	1.20	2.52		200,000	2,000,000		
5/18/37	300	1.20	2.75		520,000	2,000,000		
5/19/37	300	1.20	2.68		800,000	1,200,000	2.65	41,600
5/18/37	300	1.80	2.25		280,000	2,000,000		
5/19/37	300	1.80	2.25		70,000	300,000	2.25	
5/17/37	300	3.00	1.80		10,000	135,000	1.80	41,600

VII. STARCH WASH WATER NO SO_2 — HCl

Hour count	48 Hour count	Ave. pH	Ave. Initial count	Ave. 24 Hour count	% Increase	Ave. 48 Hour count	% Increase
00,000	6,000,000						
00,000	6,600,000						
00,000	7,000,000	4.01	41,600	4,630,000	11,129	6,530,000	15,690
00,000	5,900,000						
00,000	—						
00,000		3.80	41,600	3,600,000	8,650	5,900,000	14,180
00,000	5,000,000						
00,000	6,500,000	3.70	41,600	2,800,000	6,730	5,750,000	13,820
00,000	5,750,000						
00,000	4,200,000						
00,000	—						
00,000	3,800,000	3.36	41,600	2,970,000	7,148	4,000,000	9,600
00,000	4,000,000						
00,000	3,800,000						
00,000	4,000,000	3.20	41,600	2,300,000	5,540	3,930,000	9,430
00,000	1,900,000						
00,000							
00,000	3,000,000	2.93	41,600	1,266,000	3,040	2,450,000	5,880
00,000	2,000,000						
20,000	2,000,000						
00,000	1,200,000	2.65	41,600	507,000	1,218	1,730,000	4,160
80,000	2,000,000						
70,000	300,000	2.25		175,000	420	1,150,000	2,760
10,000	135,000	1.80	41,600	10,000	0	135,000	324

17. Regrouping and Summarizing Sections 13, 14, 15, and 16.

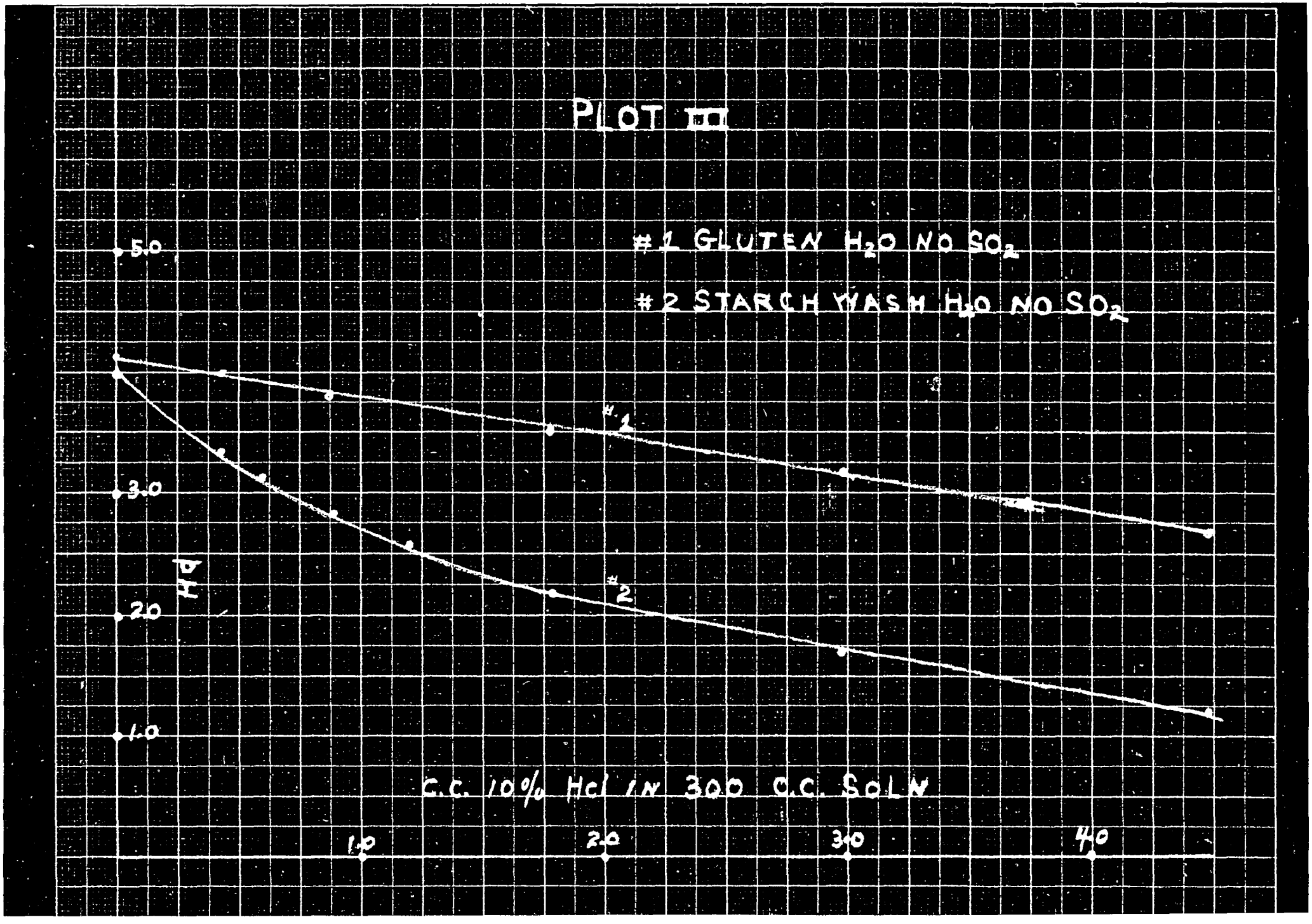
In order to further clarify the results obtained with activation of sulphur dioxide, the results were combined and plotted to scale.

The first point to stress is the difference in the amount of acid necessary to produce like reactions in gluten and starch wash water.

The results are shown on Plot III, page 83.

The points on these curves were obtained from the waters which were used in experiments discussed under section 15 and 16. It is to be noted that a much larger quantity of acid is necessary to produce a given pH in gluten water than in starch wash water.

A comparison of the increase in numbers of microorganisms in gluten water with and without sulphur dioxide is made in Plots IV and V, pages 84 and 85, respectively. The data for these figures is contained in Tables XIV and XVI, pages 74 and 80, respectively.


PLOT III
#1 GLUTEN H_2O NO SO_2
#2 STARCH WASH H_2O NO SO_2
5.0
3.0
2.0
1.0
pH
#1
#2
c.c. 10% Hcl IN 300 c.c. SOLN
1.0
2.0
3.0
4.0

PLOT IV

% INCREASE IN 48 HRS GLUTEN WATER NO SO_2

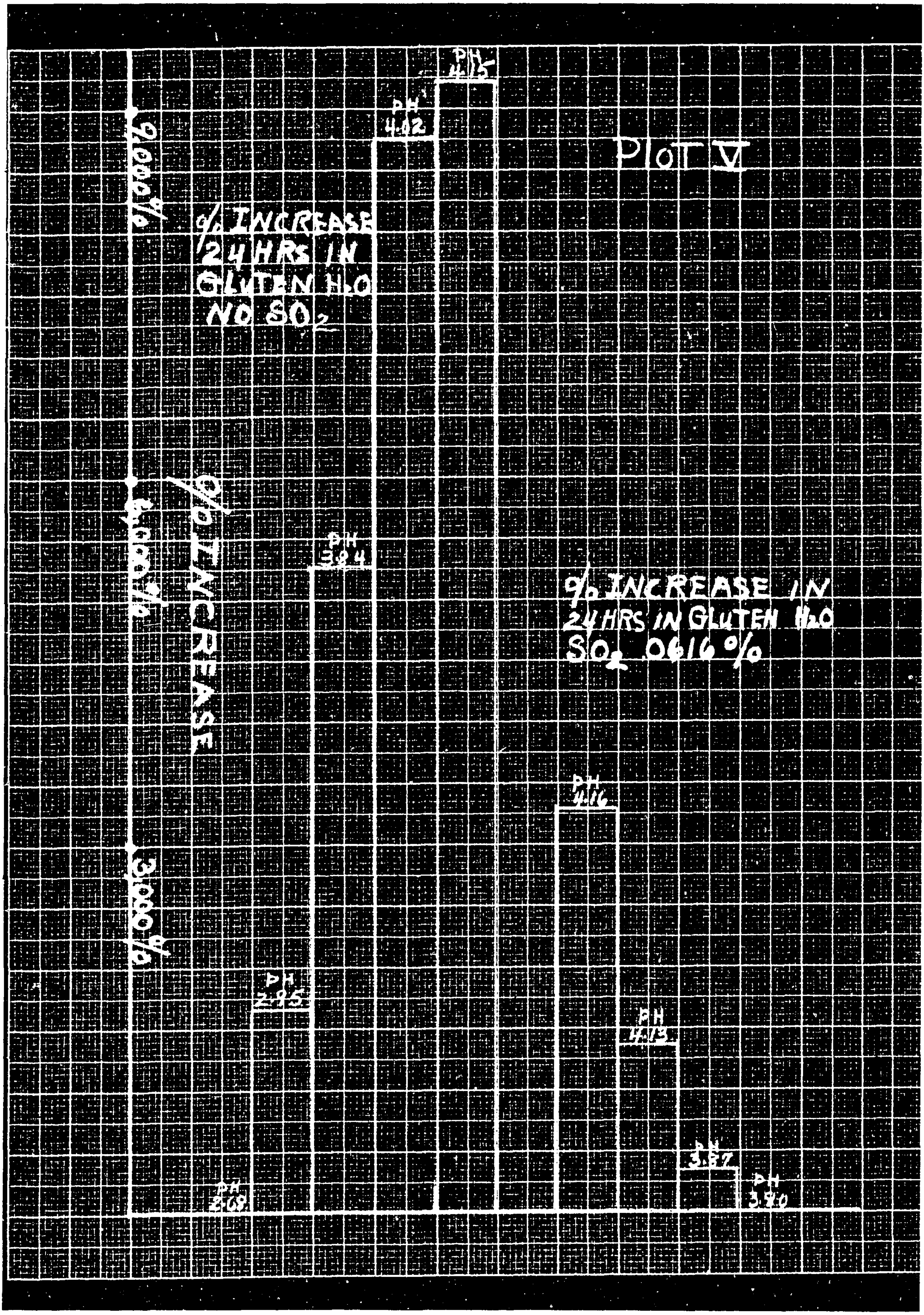
% INCREASE IN 48 HRS TIME GLUTEN WATER .016% SO_2

90,000

50,000

% INCREASE

10,000


PLOT V
% INCREASE 24 HRS IN GLUTEN H2O NO SO2
% INCREASE IN 24 HRS IN GLUTEN H2O SO2 .0616 %
% INCREASE
9,000%
6,000%
3,000%
PH 2.68
PH 2.95
PH 3.84
PH 4.02
PH 4.15
PH 4.16
PH 4.13
PH 3.87
PH 3.80

Both the effects of combined and free SO_2 as inhibitory agents are shown. Sulphur dioxide, in the combined form, does inhibit growth. This is shown by comparing the percentage increase from the initial in the absence of any added acid. In the case of the 48 hour incubation tests it is seen that the organisms in the gluten water free from SO_2 at pH 4.15 increased 92,100 per cent. The organisms in gluten water having an average 0.616% old or combined SO_2 at pH 4.16 increased to but 17,500 per cent. In other words, the sulphur dioxide which was in the latter gluten water, even though it was combined, inhibited the growth of microorganisms to a very marked extent. The 24 hour incubation tests show the per cent increase in the unsulphured gluten water 9,210 while in the case of the sulphured water the increase was 3,293.

The effect of freeing or activating the sulphur dioxide is shown by comparing the growth increase at similar reactions between the sulphured and unsulphured sets of experiments. In each case it is noted that the sulphured gluten water retarded the growth of microorganisms to a much more marked extent than in the unsulphured gluten water.

In the 48 hour series, the following percentage increases were obtained:

pH	% Increase in Gluten Water, no SO_2	% Increase in Gluten Water, 0.616% SO_2
3.80	—	967%
3.84	36,500%	—
3.87	—	4,510%
4.02	61,400%	—
4.13	—	12,050%

A comparison of the growth percentage increase in starch wash water with and without SO_2 is made in Plots VI and VII, pages 88 and 89, respectively.

Here, as with gluten water, the effect of combined and free SO_2 is graphically illustrated. The same general findings exist. The chief difference between starch wash water and gluten water is one of degree.

In Plots VIII and IX, pages 90 and 91, respectively, the per cent growth of microorganisms in unsulphured gluten and starch wash water is shown. In Plot VIII (48 hour experiments) it is noted that the gluten water is the better medium for growth. Plot IX (24 hour experiments) shows about the same number of organisms growing in both media. The 24 hour counts on starch wash water are only a little lower than the 48 hour counts on this same medium. It is felt the food supply in the starch wash water is a limiting factor. From the average analysis of the two waters it is seen that the gluten water contains three to four times more organic solids than starch wash water.

Plot VII

% INCREASE IN 4 HRS. STARCH WASH WATER Ag SO_4

PH 4.01

3.80

3.70

3.20

2.93

2.65

2.25

1.80

% INCREASE IN 4 HRS STARCH WASH WATER .0334% SO_4

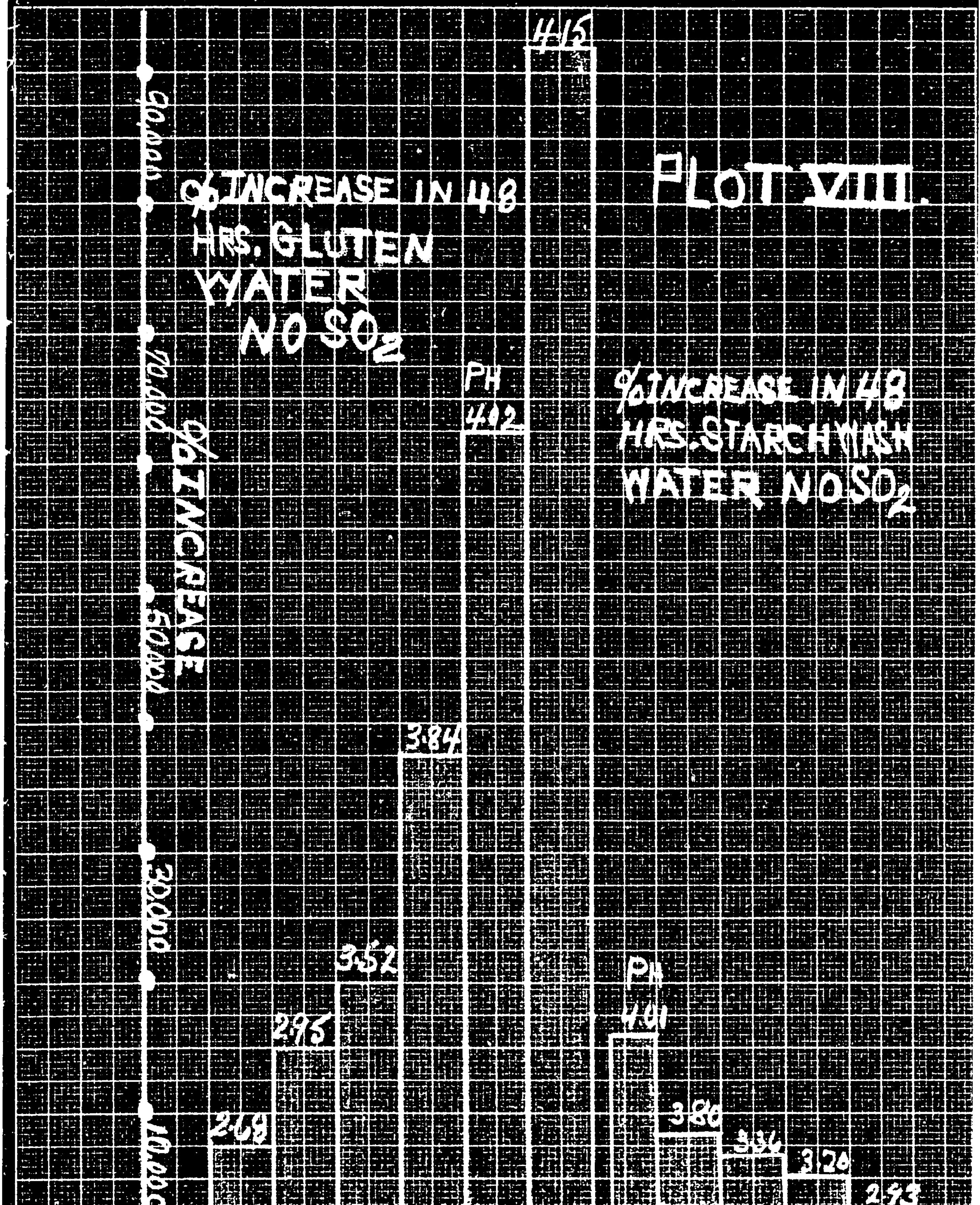
PH 3.62

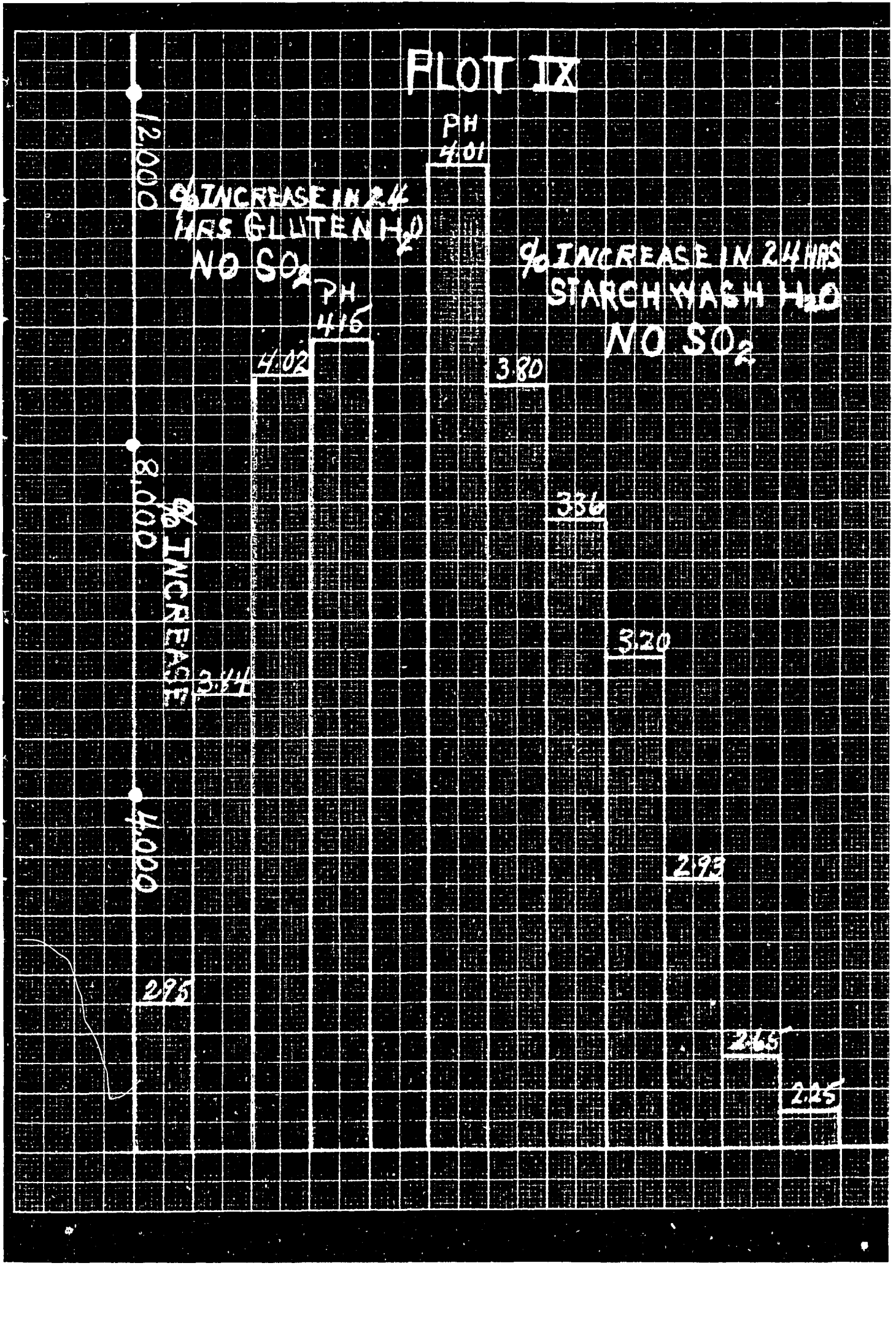
3.60

3.55

3.48

3.44


PLOT VIII.
% INCREASE IN 48 HRS. GLUTEN WATER NO SO2
% INCREASE IN 48 HRS. STARCH WASH WATER NO SO2
% INCREASE
90,000
70,000
50,000
30,000
10,000
PH
2.68
2.95
3.52
3.84
4.12
4.15
PH
4.01
3.80
3.34
3.20
2.93


PLOT IX
12,000
8,000
4,000
% INCREASE
PH 4.01
% INCREASE IN 24 HRS GLUTEN H2O NO SO2
PH 4.16
4.02
3.80
% INCREASE IN 24 HRS STARCH WASH H2O NO SO2
3.36
3.20
3.44
2.93
2.95
2.85

18. Gluten and Starch Wash Water Freed from SO_2 Containing Various Amounts of Added SO_2.

Both process waters were treated with acid and boiled until free from SO_2. The liquors were diluted back to their original volumes, the pH adjusted to 3.9, and various amounts of SO_2 water added. The results are shown in Table XVIII, below.

TABLE XVIII. EFFECT OF DIFFERENT AMOUNTS SO_2 IN PROCESS WATERS.

H_2O	% SO_2	pH	Initial count	24 Hour count
300 cc. Gluten water	0.01	3.9	18,460	900,000
300 cc. Gluten water	0.02	3.9	17,300	200,000
300 cc. Gluten water	0.03	3.8	16,000	80,000
300 cc. Gluten water	0.04	3.8	15,000	400
300 cc. Gluten water	0.05	3.7	14,000	10
300 cc. Starch wash water	0.00	3.9	40,600	1,200,000
300 cc. Starch wash water	0.01	3.6	37,700	111,000
300 cc. Starch wash water	0.02	2.4	35,200	0
300 cc. Starch wash water	0.03	3.2	33,000	0

Gluten water (0.02-0.03% SO_2) at pH 3.9 was inhibitory and with starch wash water 0.02% SO_2 at pH 3.4 was germicidal.

Chemical

1. Iodine Titration of SO_2 in Corn Starch Process Waters.

Throughout the entire bacteriological section of this paper the per cent of sulphur dioxide in the waters under consideration has been given. It has also been stated that the total amount of SO_2 present was determined by the iodine titration method.

A description of this method follows.

Reagents

Iodine solution. The 0.02 N iodine solution was prepared by dissolving 10 grams of KI (free from iodic acid) in a liter flask, using as little water as possible. To this solution 2.54 g. resublimed iodine were added and dissolved by shaking. The solution was diluted to the liter mark with H_2O. The iodine was standardized against a $Na_2S_2O_3$ solution that had recently been standardized against a $K_2Cr_2O_7$.

Starch solution. Two to three grams of potato starch or five grams of soluble starch were stirred with 100cc. of 1% salicylic acid solution, then 300-400 cc. of boiling water were added and the whole boiled until the starch was gelatinized. It was then diluted to one liter.

Method

A definite volume of the water under consideration (usually 10 cc.) is delivered to a small beaker or bottle. The iodine solution is run into the sample fairly rapidly (with very little stirring) until about three-fourths of the necessary iodine has been added. At this point, a drop or two of starch solution is added, then more iodine until there persists a deep blue color which does not fade out in one-half minute.

This method is reliable for determining the SO_2 in corn starch process waters which ranges from 0.01 to 0.08%.

There are several precautions which must be observed in order to obtain accurate results. The iodine solution must be standardized at least twice a day. When titrating samples which contain over 0.08% SO_2 it is necessary to dilute the sample with water which has been previously titrated with iodine for any reducing substance present. Vigorous stirring is to be avoided because of the volatility of the sulphur dioxide.

In the regular routine testing of SO_2 in corn starch process liquors the acidity of the sample is first determined. This same sample is titrated for SO_2. In this case the solution is alkaline to phenolphthalein and most of the iodine is added before it is acidified with dilute hydrochloric acid. When determining SO_2 in this manner it is not necessary to be so careful about excess stirring.

The first method has been checked against the second and both against the official method (13) of A.O.A.C. For all practical purposes either the first or second method yields results which are very well in accord with the official method.

Sulphur dioxide in corn syrup is combined in such a manner that the iodine titration value does not check with the official distillation method.

The methods used by R. R. Tatlock and Thompson (14) for determining SO_2 in corn syrup are as follows:

Method I. The method consists essentially of distilling rapidly, 50 g. of sample acidified with HCl and receiving the distillate in water containing 2 cc. of a filtered 1% starch solution and a few drops of N/20 iodine. As soon as the iodine is decolorized, the distillate is continuously titrated with N/20 iodine while the distillation is going on. This is continued until the color due to 0.1 cc. N/20 iodine persists for more than two minutes.

Method II. Fifty grams of syrup are dissolved in 50 cc. of water at 50°C., cooled to 15°C., and 20 cc. of 5% NaOH added and allowed to stand 15 minutes. Then a mixture of 30 cc. of 20% H_2SO_4 with 100 cc. of water is added, and the liberated SO_2 titrated at once with N/20 iodine, until a blue starch color, permanent for at least one minute, is produced. Method II is ordinarily employed, but where an excess of SO_2 is found, the results are always checked by the first method.

The total and combined sulphur dioxides in wines are determined by the French workers using the Ripper Method (15). In this method the free SO_2 is determined by direct titration with iodine in a dilute sulphuric acid solution; the total SO_2 by titration after treatment with potash, and the aldehyde combined SO_2 by difference. The statement is made by Ripper that aldehyde sulphur acids will not oxidize with iodine.

The total sulphur dioxide content of gluten and starch wash water is determined by the regular iodine titration method without addition of acid or alkali. It has been shown, from a bacteriological analysis, that all of the SO_2 in gluten and starch wash water is not of the same germicidal strength. It is seen, from the results above with corn syrup and wines, that the SO_2 which has combined with aldehyde must first be liberated by caustic and then titrated in an acid solution. It follows that the combination of SO_2 with the organic constituents of corn starch process waters is of a different nature. It is thought that the combination in the case of gluten and starch wash water is a weak chemical addition product with the degradated protein or amino acids present.

It has been shown that very small amounts of hydrochloric acid caused the SO_2 which was contained in the water to become much more toxic towards the microorganisms present.

If the acid liberated the SO_2 from some combined form and

thus freed it as SO_2 or as H_2SO_3 then it should be extractable in a solvent which is immissible with water but one which dissolves SO_2.

2. Benzene Extraction of SO_2 from Distilled Water.

The technique for determining the extractability of uncombined SO_2 was worked out using sulphur dioxide dissolved in distilled water.

Small well stoppered bottles were used. A definite volume of a known strength SO_2 solution was shaken with various quantities of benzene, (C_6H_6). The benzene used was C.P. benzene.

a. Determination of equilibrium time or time necessary to shake solutions of sulphur dioxide in distilled water and gluten water for constant results. The results are tabulated below in Table XIX.

TABLE XIX. DETERMINATION OF EQUILIBRIUM TIME.

Temperature	Time shaken and allowed to stand	% SO_2 Removed by 40 cc. benzene from 20 cc. solution: Gluten water	% SO_2 Removed by 40 cc. benzene from 20 cc. solution: SO_2 in distilled water
85°F.	5 Minutes	5.32	47.4
85°F.	30 Minutes	4.48	47.5
85°F.	1 Hour	3.61	47.5
85°F.	2 Hours	2.32	47.5
85°F.	12 Hours	2.32	47.4

It is to be noted that in the case with gluten water, the results obtained were not constant until after two hours had elapsed. These results are believed to exist due to the slowness of separation of the foam and froth which occur when gluten water is shaken with benzene. It was noted that the aliquot portions were not clear and free from benzene until they had stood practically two hours.

In the work with all waters, the procedure followed in regards to time was: the mixtures were shaken for about five minutes, allowed to stand 5 to 10 minutes and shaken again for five minutes. This was repeated at least five times and then the resulting emulsions were allowed to stand until the aqueous layer was free from benzene.

All experiments were performed in a room where the temperature did not vary more than five degrees from 80°F.

After thorough shaking and an equilibrium was reached the bottles were allowed to stand until two distinct layers formed.

The amount of SO_2 and the volume of the original water were known. A titration of an aliquot portion of the aqueous layer after extraction revealed the amount of SO_2 remaining in the water. By simple calculation, it was possible to determine the percentage of SO_2 that the various amounts of benzene extracted.

A check on the above method, SO_2 in aliquot portions of

benzene was determined. This was done by transferring the benzene to a glass stoppered bottle and shaking it with a large excess of distilled water, titrating it at the same time with iodine. Since the iodine is quite soluble in benzene, great care must be exercised in reading the end point. It is possible to do this by shaking after each addition until the benzene shows no red color. The SO_2 in the benzene could also be determined by adding an excess of bromine and determining the sulphate so produced as barium sulphate.

From the data used in determining the percentage SO_2 removed by the benzene the distribution coefficient can also be determined. The calculations for both per cent removed and the distribution coefficient follow.

b. Per cent SO_2 removed.

Original iodine titration for 10 cc. of SO_2 water = 8.3 cc.

Mixture = 20 cc. SO_2 solution plus 60 cc. benzene.

Iodine titration for 10 cc. of aqueous layer after extraction = 3.65 cc.

$8.3 \times \frac{20}{10}$ = 16.6 cc. titration for all of SO_2 originally present in 20 cc.

$3.65 \times \frac{20}{10}$ = 7.30 cc. titration for all of SO_2 remaining in 20 cc. of aqueous layer.

16.6 - 7.3 = 9.3 cc. titration for the SO_2 which is in the 60 cc. of benzene.

$\frac{16.6}{9.3}$ = 56.02% SO_2 removed by 60 cc. benzene.

c. Distribution coefficient.

Let W = cc. of solution containing X_o g. solute.

NL = cc. of extracting solvent.

X_1 = Residue unextracted.

Then $\frac{X_1}{W}$ = Concentration of extracted solution.

$\frac{X_o - X_1}{NL}$ = Concentration of extracting solvent.

$$\text{Coefficient} = K = \frac{\frac{X_1}{W}}{\frac{(X_o - X_1)}{NL}}$$

$$\frac{NL}{KW} = \frac{(X_o - X_1)}{(X_1)}$$

$X_1 = \frac{KW}{NL + KW} X_o$ = The amount extracted in terms of X_o, the amount originally present.

If there is more than one extraction, then after the first extraction the unextracted amount would be

$$X_1 = \frac{KW}{L + KW} X_o$$

After the second extraction, the amount unextracted would be

$$X_2 = \frac{KW}{L + KW} X_1 = \frac{KW}{L + KW} \frac{KW}{L + KW} X_o = \left[\frac{KW}{L + KW}\right]^2 X_o$$

Or

$$X_n = \left[\frac{KW}{L + KW}\right]^n X_o$$

Substituting in these equations the data above, we have

$$K = \frac{\frac{7.3 \times 0.02 \times 0.032}{20}}{\frac{(16.6 \times 0.02 \times 0.032) - (7.3 \times 0.02 \times 0.032)}{60}} = 2.354$$

Or

$$X_1 = \frac{(2.354)(20)}{60 + (2.354)(20)} \times 16.6 \times 0.02 \times 0.032 = 0.004671$$

Tables Numbers XX to XXIII, pages 102, 103, and 104, give results of extractions of SO_2 dissolved in distilled water. Table XXIV, page 104, shows the distribution ratios summarized.

TABLE XX. EXTRACTION OF 20 cc. WATER WITH 20 cc. BENZENE.

% SO_2 originally present.	cc. N/50 I_2 for 10 cc. AQ layer after extraction.	cc. N/50 I_2 for 10 cc. BZ layer after extraction.	C_w / C_b	% SO_2 removed by benzene
0.0259	2.85 - 2.90	1.175	2.44	25.95
0.0259	2.85 - 2.88	1.200	2.38	26.39
0.0316	3.50 - 3.50	1.450	2.42	29.29
0.0531	5.20 - 5.20	3.100	1.68	37.40
0.0512	5.00 - 5.05	3.000	1.66	37.50
0.0505	4.82 - 4.82	3.080	1.67	38.97
0.0998	8.50 - 8.50	7.100	1.20	45.10
0.0992	8.50 - 8.50	7.000	1.21	45.10

TABLE XXI. EXTRACTION OF 20 cc. WATER WITH 40 cc. BENZENE.

% SO_2 originally present	cc. N/50 I_2 for 10 cc. AQ layer after extraction	cc. N/50 I_2 for 10 cc. BZ layer after extraction	C_w / C_b	% SO_2 removed by benzene
0.0259	2.55	0.750	3.40	37.03
0.0259	2.50	0.775	3.23	38.27
0.0316	3.00	0.975	3.08	39.40
0.0531	4.25 - 4.30	2.010	2.12	48.40
0.0505	4.05	1.920	2.10	48.70
0.0998	6.70	4.450	1.50	57.00
0.0992	6.65	4.42	1.50	57.10

TABLE XXII. EXTRACTION OF 20 cc. WITH 60 cc. BENZENE.

% SO_2 originally present	cc. N/50 I_2 for 10 cc. AQ layer after extraction	cc. N/50 I_2 for 10 cc. BZ layer after extraction	C_w / C_b	% SO_2 removed by benzene
0.0259	2.20 - 2.21	0.616	3.57	45.70
0.0259	2.15	0.633	3.40	46.99
0.0316	2.60 - 2.60	0.783	3.32	47.50
0.0531	3.65	1.550	2.354	56.02
0.0512	3.60	1.460	2.462	55.00
0.0505	3.50	1.440	2.45	55.69
0.0998	5.85	3.250	1.80	62.50
0.0992	5.85	3.250	1.80	62.50

TABLE XXIII. EXTRACTION OF 20 cc. WITH 80 cc. BENZENE.

% SO_2 originally present	cc. N/50 I_2 for 10 cc. AQ layer after extraction	cc. N/50 I_2 for 10 cc. BZ layer after extraction	C_w / C_b	% SO_2 removed by benzene
0.0259	1.95	0.525	3.62	51.9
0.0259	1.95	0.525	3.62	51.9
0.0316	2.25	0.675	3.00	53.5
0.0531	3.25 - 3.30	1.280	2.54	60.9
0.0512	3.15	1.180	2.66	61.0
0.0505	3.10	1.200	2.58	61.8
0.0998	5.00	2.650	1.90	68.0
0.0992	5.00 - 5.10	2.610	1.93	67.7

TABLE XXIV. DISTRIBUTION COEFFICIENTS (SO_2 IN WATER AND BENZENE).

cc. benzene	% SO_2 Originally in Water							
	0.0259	0.0259	0.0316	0.0531	0.0512	0.0505	0.0998	0.0992
20	2.44	2.38	2.42	1.68	1.66	1.67	1.20	1.21
40	3.40	3.23	3.08	2.12	—	2.10	1.50	1.50
60	3.57	3.40	3.32	2.35	2.46	2.45	1.80	1.80
80	3.62	3.62	3.00	2.54	2.66	2.54	1.90	1.93

That the distribution ratio of SO_2 between water and benzene is not a constant is plainly shown in the above table. The ratio changes both in regards to the quantity of benzene used and the concentration of SO_2 originally present.

However, inspection of the ratios for low concentrations of SO_2 such as exist in process waters show the distribution coefficient to be fairly constant. Since this fact has been established one can compare sulphured process waters with the same percentage of SO_2 in distilled waters. The comparison will be more valid if the same ratio of water to benzene in each case is employed.

In Plot X, page 106, is shown the amount of SO_2 extracted from different concentrations of SO_2 in distilled water and in starch wash and gluten water.

Plot XI, page 107, shows the pH of different concentrations of SO_2 in distilled waters.

Curves No.1, No.2, and No.3 are for different concentrations of SO_2 in distilled water.

Curves No.4 and No.5 are for gluten and starch wash water. Comparison of the curve for 0.05% SO_2 in distilled water with the gluten water curve (ave. 0.06% SO_2) shows 47.5 and 3% SO_2 extracted by 40 cc. of benzene. A comparison of the curve for 0.025% SO_2 in distilled water with the curve for starch wash water (ave. 0.03% SO_2) shows 37.5% and 7% of SO_2 extracted by

PLOT X
% SO2 REMOVED BY C6H6
10
20
30
40
50
60
70
80
90
1
2
3
4
5
6
20
30 cc. C6H6
40

PLOT XI
CONCENTRATION
SEA WATER
VS
P H
% SEA WATER
P H
45
40
35
30
25
20
15
10
5
16
20
24
28

40 cc. of benzene. It is quite evident the SO_2 in both process waters is combined in some form which is not extracted with benzene.

3. Benzene Extraction of SO_2 from Aqueous HCl.

The amount of SO_2 which is extracted from aqueous HCl has been determined. The results are tabulated in Table XXV and shown graphically in Plot X, page 106, curve No.5A.

TABLE XXV. % SO_2 REMOVED BY BENZENE FROM AQUEOUS HCl.

Mixture H_2O BZ	HCl sp.gr. 1.1875	% SO_2 Removed by Benzene from 0.0246% SO_2	0.04736% SO_2	0.1004% SO_2
20-10	1 cc.	53.76	54.00	52.51
20-10	1 cc.	53.76	54.00	52.51
20-20	1 cc.	67.27	66.66	67.80
20-20	1.cc.	67.27	—	67.80
20-40	1 cc.	78.19	79.46	79.28
20-40	1 cc.	79.61	80.13	79.28
20-60	1 cc.	85.06	85.81	84.61
20-60	1 cc.	85.00	85.81	84.61
20-80	1 cc.	87.74	88.65	88.70
20-80	1 cc.	87.92	88.65	88.70

An inspection of this table shows that in aqueous hydrochloric acid the original concentration of SO_2 does not influ-

ence the amount of SO_2 extracted by given quantities of benzene.

4. Benzene Extraction of SO_2 from Gluten Water.

The same technique for extracting SO_2 from gluten water was used as with SO_2 dissolved in distilled water. In Table XXVI, below, the results are shown.

Curve No.5 on Plot X, page 106, should be inspected for comparison with SO_2 in distilled H_2O.

TABLE XXVI. % SO_2 REMOVED BY BENZENE FROM GLUTEN WATER.

Mixture		% SO_2 Removed by Benzene from					
		0.0743%	0.0640%	0.0640%	0.0601%	0.0630%	0.0560%
H_2O	Benzene	SO_2	SO_2	SO_2	SO_2	SO_2	SO_2
20	20	2.33	2.5	—	—	—	—
20	40	2.33	2.5	3.5	3.72	2.73	3.3
20	60	2.33	3.0	—	—	—	—
20	80	2.33	3.0	—	—	—	—

5. Benzene Extraction of SO_2 from Gluten Water Acidified with Strong HCl.

a. Effect of excess HCl. The same amount of concentrated HCl as with SO_2 dissolved in distilled water was used. The

results are tabulated in Table XXVII, below, and illustrated in Plot X, page 106, curve No.6.

TABLE XXVII. % SO_2 REMOVED BY BENZENE FROM GLUTEN WATER ACIDIFIED WITH HCl.

Mixture H_2O	Mixture Benzene	HCl sp.gr. 1.875	% SO_2 Removed by Benzene from 0.0743% SO_2	0.0640% SO_2	0.0640% SO_2
20	10	1 cc.	54.82	—	—
20	10	1 cc.	—	—	—
20	20	1 cc.	63.80	64.25	67.20
20	20	1 cc.	—	64.58	67.45
20	40	1 cc.	72.90	72.50	74.80
20	40	1 cc.	—	73.05	74.80
20	60	1 cc.	77.40	78.00	79.00
20	60	1 cc.	—	—	79.00
20	80	1 cc.	80.00	80.20	81.10
20	80	1 cc.	—	80.20	81.10

Here as in the case with SO_2 in aqueous HCl the original concentration of the SO_2 does not influence the percentage extracted.

b. Amount of HCl necessary to liberate the SO_2 which is combined in gluten water. Ten per cent C.P. hydrochloric acid was prepared and different amounts of this acid were added to the 20 cc. portions of gluten water after the benzene had been added. A ten per cent concentration was used so as not to have too large dilution of the aqueous phase. The acid was added

by means of a calibrated pipette after the benzene was added. This order of addition was followed to prevent any liberated SO_2 to escape before the bottles were stoppered.

Four samples of gluten water collected at different times from the gluten settler overflow siphons were taken.

In the calculations for per cent SO_2 removed the dilution by the acid was taken into consideration. The results are tabulated in Table XXVIII, below, and plotted on Plot XII, page 112.

TABLE XXVIII. AMOUNT 10% HCl NECESSARY TO LIBERATE COMBINED SO_2 IN GLUTEN WATER. 20 cc. GLUTEN WATER EXTRACTED WITH 40 cc. BENZENE.

cc. HCl added	Ave. pH All four samples	% SO_2 Removed by Benzene from Gluten Water Containing the Following % SO_2			
		0.06336	0.0640	0.0640	0.0627
0.01	4.10	—	—	3.20	3.15
0.05	3.80	—	—	6.48	6.38
0.10	3.50	10.0	9.98	9.97	9.90
0.20	3.00	15.0	17.30	—	—
0.30	2.63	30.0	29.70	29.87	29.79
0.40	2.23	43.0	42.50	—	—
0.50	1.93	52.0	52.40	52.47	52.45
0.55	—	—	58.56	—	—
0.60	1.72	62.5	62.50	62.60	62.60
0.65	—	—	65.00	—	64.90
0.70	1.50	66.0	65.50	—	—
0.80	1.32	66.5	66.50	—	—
0.90	1.30	67.0	67.50	—	—
1.00	1.25	70.0	68.00	69.00	69.00

PLOT XI
#A - pH vs Ml 1/10 N HCl
#B - % SO2 Removed vs Ml 1/10 N HCl
Equal Volumes Water
C.C. 1/10 N HCl in 20 C.C. Solution
% SO2 Removed By 40 C.C. C6H6
#A
#B
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
10
20
30
40
50
60

The table and graph show that as the solution becomes more acid more SO_2 is liberated from the combined form and becomes soluble in benzene.

It has been shown from a bacteriological analysis that the microorganisms are inhibited to a greater extent in gluten water to which had been added a small amount of hydrochloric acid. It was also shown that the lowering of the pH was not alone sufficient to explain the inhibition. Thus, between pH 2.5 and 4.0 in the bacteriological work a very marked inhibition on growth occurred. One can see from Plot XII, page 112, that at pH 2.5 there is over 30% SO_2 extracted by benzene. In the earlier bacteriological work it has been shown that concentrations of SO_2 (fresh SO_2 added) as low as 0.01% were quite inhibitory.

6. Benzene Extraction of SO_2 from Starch Wash Water.

Four samples of starch wash water were collected at different times and the amount of SO_2 extracted with benzene was determined as shown in Table XXIX, page 114, and plotted on Plot X, page 106.

TABLE XXIX. AMOUNT OF SO_2 EXTRACTED BY BENZENE FROM STARCH WASH WATER.

Mixture		% SO_2 Removed by Benzene from Starch Wash Water Containing the Following % SO_2			
H_2O	Benzene	0.0352	0.0317	0.0300	0.0198
20	10	—	6.6	—	—
20	20	—	6.6	—	7.0
20	40	7.30	6.6	7.4	—
20	60	—	6.6	—	—
20	80	7.32	—	7.3	7.0

7. Benzene Extraction of SO_2 from Starch Wash Water Acidified with HCl.

a. Effect of excess HCl. The same amount of concentrated acid was added to starch wash water as with SO_2 in distilled water and gluten water. The data will be found in Table XXX, page 115, and illustrated on Plot X, page 106.

b. Amount of 10% HCl necessary to liberate combined SO_2 in starch wash water. Various amounts of 10% HCl were added to starch wash water and extracted with benzene. The same procedure as with gluten water was followed. Table XXXI and Plot XIII, pages 115 and 116, respectively, contain the data of this series of experiments.

TABLE XXX. % SO_2 REMOVED BY BENZENE FROM ACIDIFIED STARCH WASH WATER.

Mixture H_2O	Mixture Benzene	HCl sp. gr. 1.1875	% SO_2 Removed by Benzene from Starch Wash Water Containing 0.0317% SO_2	% SO_2 Removed by Benzene from Starch Wash Water Containing 0.0300% SO_2
20	10	1 cc.	55.23	53.10
20	20	1 cc.	63.90	62.90
20	40	1 cc.	73.40	73.70
20	60	1 cc.	78.70	77.80
20	80	1 cc.	80.00	80.10

TABLE XXXI. AMOUNT 10% HCl NECESSARY TO LIBERATE COMBINED SO_2 IN STARCH WASH WATER. 20 cc. STARCH WATER EXTRACTED WITH 40 cc. BENZENE.

cc. HCl added	Ave. pH	% SO_2 Removed by Benzene from Starch Wash Water Containing following % SO_2 0.0317%	0.0300%	0.0292%
0.01	3.65	8.0	7.6	8.0
0.05	2.86	24.6	23.8	25.3
0.08	2.43	40.0	39.6	39.8
0.15	2.08	—	—	—
0.20	1.86	60.0	59.9	60.1
0.30	1.60	68.0	68.1	68.3
0.40	1.42	70.0	69.2	69.9
0.50	1.34	71.0	71.2	70.8
0.60	1.28	71.8	71.6	72.0
0.70	1.23	72.5	—	72.6
0.80	1.14	73.0	—	73.0
0.90	1.08	74.0	73.6	—
1.00	1.03	74.5	75.0	74.3

PLOT XIII
#1 PH vs HCL STARCH WASH H_2O
#2 % SO_2 REMOVED vs HCl
% SO_2 REMOVED BY 40 C.C. C_6H_6
c.c. 10% HCL IN 20 c.c. SOLN.
PH
#1
#2
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
2.0
3.0

As shown on Plot XIII, page 116, at pH 3.65 - 3.86 there is from 8 to 25% SO_2 extracted by 40 cc. of benzene. That this corresponds to more than the above percentages of SO_2 liberated can be deduced from the fact that one extraction with 40 cc. of benzene in a strongly acid solution does not extract all the free SO_2 as in the case with SO_2 dissolved in distilled water.

DISCUSSION AND RESULTS

The several phases of this problem will be briefly discussed and joined together.

It has been found that microorganisms which abound in starch process waters are of three general types. They are practically all yeast or yeast-like organisms. It is true that some spore forming, starch digesting types are occasionally encountered. Due to the high acidity of the liquors the condition for growth of bacteria is unfavorable.

These yeast and yeast-like forms will grow luxuriantly if the usual methods of preventing their growth are not followed. Serious losses will be entailed if their growth is unchecked. It has been seen that a count of a few thousand organisms per cubic centimeter will increase to almost 80 million in two to three days.

It has been shown that heat and SO_2 will inhibit growth of many organisms and also actually kill a large percentage of them.

The fact that total SO_2 is not a measure of the germicidal or inhibiting effectiveness of this chemical has been established. It has further been found that the SO_2 in starch wash water is more effective than that in gluten water. The addition of small percentages of fresh uncombined SO_2 very materially reduced the contamination due to microorganisms.

It has been shown from a chemical standpoint that all of the SO_2 in starch process waters does not exist as free or uncombined SO_2. It has been possible to correlate the amount of SO_2 which is extracted by benzene with the inhibitory effect of SO_2 in process waters.

Small additions of a highly ionized acid such as hydrochloric acid liberate a large percentage of the combined SO_2. This is manifest both by its extraction with benzene and by the increased inhibiting or germicidal effect.

In early days it was general practice to send the process waters, especially gluten water, to the sewer. During these times it was not unusual for the various corn starch processors to burn from 125 - 175 pounds of sulphur per 1000 bushels of corn ground.

Most corn starch plants are reusing their process waters today. It is general practice to burn from 75 - 100 pounds sulphur per 1000 bushels of corn ground.

Should it be found that a slight decrease in the pH of the process waters has no operating disadvantages, a still greater reduction in sulphur will be possible.

The smallest amount of SO_2 (on a plant size scale) which inhibits growth in a given range of pH should be investigated. The maintenance cost of equipment in a starch plant is quite large. It may be found by the starch processors that more acidic process liquors would increase the damage due to cor-

rosion. On the other hand, it may be found that a much smaller amount of SO_2 than is normal practice may actually be advantageous.

Concentrations of 0.02 - 0.03% in gluten water at pH 3.9 were found to be inhibitory to growth of microorganisms. In starch wash water 0.02% SO_2 at pH 3.4 was found to be germicidal.

SUMMARY AND CONCLUSIONS

Microorganisms which are found in starch process waters are mainly yeasts or yeast-like forms.

They are facultative aerobes and the continuous pumping and recirculation furnishes ideal conditions, so far as air is concerned, for growth.

Heating the process waters at crucial points to temperatures ranging from 105°F. and upwards has a decided inhibiting effect on growth of microorganisms in starch process waters.

Sulphur dioxide, whether combined or free, has an inhibiting effect.

A combination of heat and SO_2 is quite effective in preventing excessive growths.

Sulphur dioxide in starch process waters is not all of the same germicidal potency.

The combined SO_2 may be liberated by additions of small amounts of hydrochloric acid.

A measure of the liberated SO_2 is obtained by extracting the acidified waters with benzene.

There exists a correlation between the amount of SO_2 dissolving in benzene and the inhibiting or germicidal effect of this chemical.

A large saving , both in regards to costs of maintainence and sulphur, appears quite possible.

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