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MOLDS AND BACTERIA THAT DELAMINATE PLYWOOD BONDED WITH CASEIN AND SOYBEAN GLUES

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(MOLDS AND BACTERIA THAT DELAMINATE PLYWOOD
BONDED WITH CASEIN AND SOYBEAN GLUES)

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Wood joints properly made with protein glues should last indefinitely under dry conditions. However, under moist conditions some glues are subject to the action of micro-organisms and chemical hydrolysis.

In previous work^{2/} the action of bacteria has been discounted as the cause of delamination of casein glued plywood in the continuous soak tests. This study was undertaken to increase the information on molds and bacteria concerned in the breakdown of protein glues as it occurs in plywood, and to determine the conditions under which the various organisms attack such materials.

MATERIALS AND METHODS

The tests described in this report were made with the following materials and methods unless otherwise stated: yellow birch sapwood veneer 1/16 inch thick was made into three-ply, 5- by 12-inch panels, the grain of the center ply running lengthwise of the panel and that of the face plies crosswise. The casein glues were mixed as directed by the manufacturers, using, unless otherwise specified, 1 part glue powder to 1.8 parts water. In mixing soybean glue, the following formula was used:

^{1/} In cooperation with the Forest Products Laboratory, maintained by the Forest Service, United States Department of Agriculture, at Madison, Wis., in cooperation with the University of Wisconsin.

^{2/} Brouse, Don. Behavior of casein and blood glue joints under different conditions of exposure. Forest Products Laboratory Report R1050. Sept. 1934.

Soybean meal (parts by weight) - - -	100
Total water - - - - -	340
Ca(OH) ₂ (98 percent hydrated lime)-	3
NaOH (commercial flake) - - - - -	8
Sodium silicate - - - - -	10
CS ₂ + CCl ₄ (1:1)- - - - -	2-1/2

Glue was applied to the core ply with a brush, 30 to 40 grams (usually 32 to 35 grams) being used to cover both sides of the center ply. Closed assembly was used, with an assembly time not exceeding 12 to 15 minutes. The temperature of the glue room was 70° to 75° F. Pressure of 150 pounds per square inch was applied in a jack screw press, the screw being tightened occasionally during the first few hours to maintain the pressure. After 12 to 16 hours in the press, the panels were removed and piled on stickers in a room maintained at a temperature of 80° F. and a relative humidity of 65 percent. After conditioning for 4 to 6 days the panels were cut into standard shear specimens, 1 by 3 inches, with the grain of the face ply running lengthwise of the specimens. In all but the first 2 experiments, 2 specimens were taken from each panel for tests of the original joint strength, one being tested dry and the other wet after soaking in water for 48 to 72 hours.

The remaining 8 specimens from each panel served as test material. Where a number of different organisms or other factors were to be compared, the same number of specimens were selected in random order from each panel for each test factor, the aim being to equalize and distribute the variation within and between panels. Usually 8 specimens were subjected to each test factor for each test period, and each figure given in the tables is an average of shear tests on 8 specimens.

Glass jars 2 inches square and 5-1/2 inches high, with a top 1-1/2 inches in diameter closed with a screw lid, were used to hold the specimens, preliminary tests having indicated that these gave uniform results that agreed closely with those in the standard mold tests described by Kaufert and Richards.^{3/} It was, of course, necessary to surface disinfect the specimens before they were inoculated, to eliminate the spores of molds and other microflora that they had acquired between the time of drying of the veneer and the final treatment of the shear specimens. Several treatments were tried, including brief dips in formaldehyde, alcohol, sodium hypochlorite, and hot water. Of these, a 45-second dip in boiling water best served the dual purpose of killing such molds as were present on the surface and permitting subsequent growth of molds or bacteria inoculated onto the specimens. In no case was there any evidence that molds had penetrated so deeply into the specimens before treatment that they were not killed by such a hot-water dip.

^{3/}Kaufert, F. H., and Richards, C. Audrey. A procedure for measuring the mold resistance of protein glues. Forest Products Laboratory Report 1344 (Rev.), 3 pp., illus. March 1943.

The standard procedure was to dip the 8 specimens of 1 lot into boiling sterile distilled water for 45 to 60 seconds, constantly agitating them, drain them for a few seconds, dip them for 2 to 3 seconds in a suspension of fungus spores or bacteria, and put them quickly into a sterile jar.

Some specimens were autoclaved. These were subsequently dipped in boiling sterile water, then in cold sterile water, to give them a water content similar to those surface disinfected and dipped in inoculum. Moisture contents of 55 to 60 percent were obtained in the specimens by placing a measured amount of distilled water in each of the jars before they were sterilized. Various times and pressures were used in autoclaving the dry shear specimens. Preliminary tests indicated that autoclaving at 15 pounds for 20 to 40 minutes reduced the dry strength of joints made with either casein or soybean meal glue approximately 50 pounds per square inch, and it apparently increased the wet strength. It may have changed other important characteristics of the glue also. It did not eliminate all bacteria from the glue line, although it reduced their number considerably. It is not unusual for some bacteria to survive even more severe autoclaving than this, in dry material.

ORGANISMS IN THE GLUE AND THEIR SIGNIFICANCE

It is well known that a variety of organisms are present in manufactured casein glues. Nine different commercial brands of casein glues were cultured by placing small quantities of the glue powder in hanging drops of sterile water on sterile slides, and by scattering the glue powder over the surface of an agar medium containing 1 percent casein glue powder and 2 percent malt. No attempt was made to determine the number of organisms per gram of glue powder.

A considerable variety of bacteria and yeasts developed in all of these cultures within 24 hours. The same kinds, in general, were present in all of the 9 glues. Relatively few species of molds were found in any of the glues, although Penicillium brevicaulis^{4/}, which appears to be one of the principal causes of delamination of plywood glued with casein glues, was isolated from 5 of the 9 different glues.

Experiment I

This was to determine whether the organisms normally present in the glue powder survived in the glue line of plywood, or whether they were capable of causing delamination when inoculated onto the outside of plywood specimens.

^{4/}A synonym for Scopulariopsis brevicaulis.

Panels were glued with a commercial casein glue and with a glue made from soybean meal. Eighteen panels were made with each glue to which no preservative had been added; 5 percent sodium orthophenylphenate, based on the dry weight of the glue powder, was added to each glue for a second set of 18 panels; and 5 percent sodium trichlorophenate was added to each glue for a third set of 18 panels. Before gluing, the inner faces of both face plies of 6 panels out of each set of 18 were brushed with a suspension of bacteria cultured from the glue powder, 6 panels were similarly inoculated with a suspension of Torula isolated from material in mold tests, and 6 were left uninoculated.

The shear specimens cut from these panels were selected in groups of 9 from each group of 6 panels, surface disinfected, and placed in sterile jars to which sufficient water had been added to bring the moisture content of the specimens up to 55 to 60 percent.

Subsequent isolations from the glue lines of these specimens yielded apparently only one or two kinds of bacteria, and these were obtained from every specimen of every panel, even from those with preservative added to the glue. Time was not available for the detailed studies needed to determine exactly the kinds present. Torula was isolated twice from panels inoculated with it before they were made up, in both cases from panels with no preservative added to the glue. Inoculating the panels, before they were made up, with bacteria, did not seem to increase the number or kinds of bacteria that survived in the glue line. From these isolations, which were repeated at 2-week intervals for 8 weeks, it seems likely that the molds, yeasts, and many of the bacteria present in the glue powder are killed during the gluing process, probably by the high alkalinity of the glue. Isolations from the left-over glue yielded bacteria apparently the same as were obtained from the glue lines of the plywood. That one or more kinds of bacteria not only survive in considerable numbers in plywood but also continue to grow, if the wood is wet enough, was apparent by examining some of the surface-disinfected specimens to which no organisms had been added, either in the ^{gluing} operation or afterwards. After 2 weeks in the jars masses of bacteria began oozing from the glue lines of those glued with both the casein and the soybean glue. One of these was taken out and photographed, and is shown in figure 1. The bacteria appeared identical with those isolated from 9 different casein glues, from soybean meal, and from the glue lines of plywood made up with these glues. Delamination followed in 1 to 3 weeks when these bacteria were inoculated on surface-disinfected specimens having 50 to 65 percent moisture content.

Experiment II

Selected groups of the shear specimens described in the foregoing were surface disinfected and inoculated with a spore suspension of Penicillium brevicaulis and with a mixed culture of bacteria obtained from various glue powders. Both after 7 and after 26 days these were dried to equilibrium at 80° F. and 30 percent relative humidity and shear

tested. The results are summarized in table 1. Each figure is an average of shear tests on 9 specimens. The results indicate that:

- (1) The bacteria normally present in the glue, and surviving in the glue line, are capable of weakening the joints made with soybean glue. They weakened the joints made with casein glue also, but the jars containing these specimens were opened to get cultures and photographs, and the specimens became contaminated with Penicillium brevicaulis and delaminated.
- (2) Joints inoculated with P. brevicaulis were rapidly delaminated; the Penicillium alone would presumably have a similar effect, but since bacteria occurred as contaminants in the Penicillium material the relative effect of the fungus and the bacteria cannot be certainly evaluated.
- (3) The bacteria obtained from the glue and inoculated on the surface-disinfected shear specimens reduced the strength of those made with the casein glue by more than 50 percent in 26 days, and in the same period totally delaminated those made with soybean glue.
- (4) Sodium orthophenylphenate appears more effective against the bacteria than against Penicillium brevicaulis, and sodium trichlorophenolate, during the period of the test, prevented any weakening by either the mold or the bacteria.

ORGANISMS CHIEFLY CONCERNED IN THE DELAMINATION OF
PLYWOOD BONDED WITH CASEIN AND SOYBEAN GLUES

Experiment III

Isolations were made from the glue lines of a number of panels in which the glue or the plywood or both had been treated with sodium orthophenylphenolate and exposed to infection by molds. The specimens were flamed to reduce the danger of contamination from surface-borne spores, the plies separated with a flamed chisel, where such separation was necessary, and scrapings from the glue lines were placed on malt agar, malt-casein agar, and oatmeal-casein agar. Penicillium brevicaulis was isolated from every panel, even those with no obvious delamination, and was easily the most generally present and abundant mold obtained in the isolations. This fungus is known to be common on cheese, and is reported to liquefy gelatin, producing an alkaline reaction and a strong odor of ammonia. Casein glue appears to be an excellent medium for this fungus. Next in abundance was a green Penicillium tentatively identified as P. glaucum, also a common inhabitant of cheese and other casein products. These were the two most common fungi, although a few colonies of Torula and Cladosporium were obtained also. Numerous colonies of bacteria were obtained from every panel.

The ability of these organisms to delaminate joints bonded with various casein and soybean glues was compared. Some lots of specimens were autoclaved at 10 pounds pressure for 40 minutes to reduce the effect of the bacteria in the glue line. Other sets were surface disinfected in boiling water. Of these latter, some were left uninoculated to determine the effect of the bacteria normally present in the glue line. Others were surface disinfected, then dipped for 2 to 3 seconds in a suspension of inoculum. After treatment all specimens were placed in sterile jars containing enough water to bring the moisture content of the specimens up to 55 to 60 percent. One set was not surface disinfected and not inoculated, merely placed in jars containing the same amount of water as the others. These served to show the effect of the normal air-borne microflora upon the strength of the joints. Specimens were taken out after 8 to 10 and 19 to 21 days, dried to equilibrium at 80° F. and 30 percent relative humidity, and shear tested. The results are given in table 2. They indicate that:

(1) The most rapid reduction in joint strength was in those specimens not surface disinfected and not inoculated. These were overgrown chiefly by Penicillium brevicaulis and P. glaucum, the two most common molds isolated from delaminated panels exposed in mold tests.

(2) The two bacteria used as inoculum, both of them isolated from glue lines of panels made in a previous experiment, reduced the joint strength fairly rapidly, and at the end of 19 days one of them had completely delaminated the specimens glued with soybean glue and at the end of 21 days those glued with casein glue. These bacteria originally were isolated from surface-disinfected specimens made with casein glue.

(3) The bacteria present in the original glue and surviving in the glue line had relatively little effect upon the joint strength in this short time. Isolations proved that bacteria were present in the glue lines of all surface-disinfected and autoclaved specimens glued with soybean glue and in many of those glued with casein; and these bacteria appeared identical with those able to cause delamination.

(4) The fungi used were able to weaken the joint strength rapidly, the two species of Penicillium being more efficient than Torula, but here again the presence with them of bacteria makes it uncertain whether the fungi alone would have worked as rapidly.

Experiment IV

A similar experiment was set up using two casein glues, one containing a small amount of preservative added by the manufacturer. The treatments were similar to those in the previous test. The moisture content at the beginning of the test was 55 to 60 percent, and at the end of the test 45 to 50 percent. The specimens were taken out after 17 and 34 days, dried to equilibrium at 80° F. and 30 percent relative humidity, and tested dry. The results are presented in table 3, and support the following conclusions:

(1) The specimens surface disinfected but not inoculated lost significantly in strength, those glued with one unpreserved casein glue being reduced 67 percent in 34 days and those glued with the preserved casein glue 29 percent in 34 days, as compared with the strength of the autoclaved specimens held under the same conditions. If the strengths of the surface-disinfected but not inoculated specimens are compared with the original dry strengths the loss is practically as great.

(2) Of the fungi tested, both Penicillium brevicaulle and Cladosporium reduced the strength of joints rapidly, while Torula and Trichoderma reduced it somewhat more slowly. All of the three bacteria used were fairly effective in reducing the strength of the joints, two of them causing a greater strength loss in the first 17 days than any of the molds. A water content of around 50 to 60 percent probably is almost optimum for the work of bacteria, and might be above the optimum for the molds concerned. The specimens not surface disinfected and not inoculated were delaminated very rapidly, and were covered with such a variety of molds that no attempt was made to identify them. While P. brevicaulle and P. glaucum appear most readily able to digest casein glues, a considerable number of other common air-borne molds apparently share this ability with them.

The writers attribute the strength reduction of the surface-disinfected but not inoculated specimens, as compared with the autoclaved specimens kept under the same conditions, chiefly to the action of bacteria normally present in the glue line. This view is supported by the observed facts that: (a) Masses of bacteria begin to ooze from the glue lines of these surface-disinfected but not inoculated specimens some time before they weaken. These bacteria do not appear uniformly on the edge of the glue lines throughout all the specimens, but first appear here and there, as if a few bacteria located in a favorable spot near the edge of the glue line had begun to multiply, and these gradually spread over the exposed edge of the glue line. Where they were abundant, delamination occurred, and often one end of a specimen was totally delaminated while the center still had a shear strength of 200 to 300 pounds. (b) The weakening proceeds chiefly from the edge inward as indicated by the mode of failure of specimens in the shear test. This is illustrated in figure 2. (c) The delamination is erratic at first, and in tests run only a short time some specimens are totally delaminated, while others in the same jar retain a shear strength of 200 to 400 pounds. The figures in table 4, chosen from the individual specimens that made up the averages in table 3, illustrate this point. They compare adjacent or nearly adjacent specimens from the same panels after 34 days' exposure. These specimens had been surface disinfected at the same time and placed in the same sterile jar, and the erratic delamination is typical of the action of bacteria in the glue line during a short test period. Results of inoculation with these same bacteria prove that when they are uniformly and abundantly distributed over the edges of the glue line by dipping the specimens in a suspension of the bacteria, they are able to cause rapid failure of the joints of shear specimens. Since isolations have indicated that sometimes these bacteria are irregularly distributed in the shear

specimens, this may account for much of the variation from specimen to specimen. In spite of the variation, it still is obvious that the bacteria normally present in the glue line are able to weaken the joints fairly rapidly.

When comparing with one another the effects of the different molds used in this test, it must be remembered that bacteria were active in the mold-inoculated specimens also. In this and other experiments where specimens containing 50 to 65 percent water were inoculated with molds, bacteria soon began to ooze in masses from the glue lines, not just here and there, but wherever the glue line was exposed. (Bacteria appeared erratically on the glue lines of surface-disinfected specimens not inoculated with molds). It may be assumed that once the molds decomposed the exposed glue line enough to release the bacteria contained in it, the bacteria multiplied rapidly. Mold mycelium, covered with droplets as it is, probably serves to spread the bacteria very readily. Certainly this is true of agar cultures. The bacteria concerned in this delamination will form only small colonies on agar free of surface water. However, when growing with molds in agar cultures the bacteria are present even at the advancing edge of large, rapidly growing colonies. They are so intimately associated with Penicillium brevicaulis that the writers have obtained no cultures of this fungus free of the bacteria -- even those caught as air contaminants, supposedly originating from one or a few airborne spores, invariably had the bacteria with them. Some of the molds, such as Trichoderma lignorum and Cladosporium, did not weaken the joints so rapidly as did some of the bacteria inoculated on the specimens, and visual evidence indicated that most of the weakening attributed to these particular species was a result of bacterial action. Probably the principal role of such molds in delamination is the distribution of bacteria uniformly over the specimens.

BACTERIAL DELAMINATION OF WETTED AND SOAKED SPECIMENS

To further elucidate the role of bacteria in the delamination of casein-glued shear specimens, the following experiments were set up, using two casein glues. These were chosen because previous experiments had indicated that one of these glues was comparatively resistant to bacterial deterioration, and the other comparatively susceptible.

Treatments

(1) Shear specimens were autoclaved at 10 pounds pressure for one-half hour. As stated previously, this greatly reduced the number of bacteria in the glue lines, but did not eliminate all the bacteria from the glue lines of all specimens, as indicated by the isolation results presented in tables 5 and 6. (2) Shear specimens were surface disinfected

by means of a 45-second dip in sterile boiling water. This eliminated molds from the outside of the specimens, but did not greatly reduce the number of bacteria in the glue lines.

After autoclaving or surface disinfection, one set of specimens was placed in sterile jars to which enough water had been added before the jars were sterilized to bring the water content of the specimens to approximately 65 percent. This is designated as the "wetted" test. The water content was checked by oven drying specimens that were shear tested after 6 days, and averaged 63 percent for the autoclaved specimens and 67 percent for the surface-disinfected. As soon as the specimens had been placed in jars, the screw caps were tightened and sealed with paraffin to prevent evaporation.

Additional sets of specimens, after having been autoclaved or surface disinfected, were placed in quart fruit jars and soaked as follows: (1) Soaked in sterile water in sealed jars. (2) Soaked in water with 2 percent sodium pentachlorophenate added. (3) Soaked in water with bacteria and a small amount of casein added. These are designated as "soak" tests.

In both wetted and soak tests there was some chance of contamination by air-borne organisms when the specimens were put into the containers. Had such contaminations been at all common they should have shown up in the small jars containing the wetted specimens, where conditions were favorable for mold development. No molds were seen on the specimens in these sealed jars, and it may be assumed therefore that bacterial contaminants also were excluded. In the soak tests, each jar contained enough specimens for four test periods, and it is very probable that when these jars were opened to remove specimens at the end of each test period molds and bacteria entered. Molds, of course, can be practically excluded from consideration in the soak tests, since those molds concerned with the breakdown of glue can grow only very slowly on or in water, and the isolations from these specimens after shear testing (table 6) indicated that molds were not an important factor. Bacterial contaminants could hardly have affected the results in the case of specimens soaked in water containing sodium pentachlorophenate, since they would not have been able to grow; nor could bacterial contaminants have much affected the results of those soaked in water plus casein and inoculated with bacteria, since, as may be seen later, the bacterial population in these jars was so heavy, and the specimens lost strength so rapidly, that it could hardly be attributed to a few chance contaminants. In the case of surface-disinfected specimens soaked in sterile water the strength of the specimens also decreased so rapidly, and bacteria were soon so abundant in the water, that it is unlikely the results were affected much by bacterial contaminants. In the case of autoclaved specimens soaked in sterile water, however, the gradual increase of bacteria in the water, due partly to chance contaminants, but probably more to the multiplication of bacteria not killed by autoclaving, probably was responsible for much of the gradual reduction in strength over the 7-month period of the tests.

Results

Wetted Tests

The results of shear tests on these specimens over a period of 6 months are given in table 5 and figure 3. The autoclaved specimens retained their original strength over this period, while the surface-disinfected specimens did not. In judging the results of isolations from these specimens it must be remembered that at most only a few samples were taken from a small portion of the glue line of each specimen, but these isolations do indicate that bacteria were in general far more abundant in the glue lines of surface-disinfected than in autoclaved specimens.

Soak Tests

(1) Soaked in sterile water in sealed fruit jars.--If bacteria were the main cause of delamination in soaked specimens, the surface-disinfected specimens should lose strength much more rapidly than the autoclaved ones. Table 6 and figures 4 and 5 show that the surface-disinfected specimens were totally delaminated in 3 months, whereas after 7 months the autoclaved specimens retained over 50 percent of their original strength. Isolations indicated that enough bacteria were present in the autoclaved specimens and in the soak water to probably account for most of this gradual loss in strength.

(2) Soaked in water with 2 percent sodium pentachlorophenate added.--If bacteria were the main cause of strength loss in soaked test specimens, there should be relatively little difference between autoclaved and surface-disinfected specimens in this treatment, and both should retain their strength over a considerable period. Table 6 and figures 4 and 5 indicate that there was no significant decrease in strength of either autoclaved or surface-disinfected specimens over a period of 7 months.

(3) Soaked in water with bacteria and a small amount of casein added.--This test aimed to provide an extremely large number of bacteria in the soak water. That it was successful was indicated by the fact that enough pressure developed to blow the tops off the jars when the spring catches were released after only 2 days. The water was definitely cloudy with bacteria after a few days, the stench was abominable, and microscopic examination of the soak water revealed thousands of bacteria per drop. Such a condition probably would seldom or never be approached in normal soak tests. As may be seen in table 6 and figures 4 and 5, all autoclaved and surface-disinfected specimens soaked in this water were totally delaminated in 3 months. Many of them, in fact, were delaminated within a month or 6 weeks.

The results of these two wetted and soak tests indicate that if the bacterial content of the glue line is greatly reduced, by autoclaving, and specimens are held at a moisture content of 60 to 65 percent, there is little reduction in shear strength over a period of 6 months. If autoclaved and soaked in water that was sterile when the specimens were put in, the strength was reduced considerably in 7 months, presumably because some of the bacteria which survived in the glue lines or entered by accident during the several openings of the jars were able to get into the soak water and multiply slowly. Bacteria were isolated in 8 out of 8 attempts from specimens glued with casein glue, autoclaved, and soaked in sterile water for 7 months.

Bacteria were almost totally excluded by soaking in water containing 2 percent sodium pentachlorophenate, and these specimens retained their original strength during the 7-month test period. Adding bacteria to the soak water, plus a small amount of casein to encourage their growth, resulted in rapid and complete delamination of both autoclaved and surface-disinfected specimens.

Under the conditions of these tests bacterial action undoubtedly was the main cause of deterioration. This does not eliminate hydrolysis as a possible factor in the deterioration of casein-glued specimens kept wetted or soaked, but it does prove that bacteria alone can cause weakening and total delamination previously attributed largely to hydrolysis, and suggests that if wetted or soak tests are to furnish a criterion of the properties of a glue, the action of bacteria must be taken into account. Data available at present do not conclusively demonstrate that purely chemical hydrolysis occurs, and experiments in which bacteria are entirely eliminated will be required to settle the question.

SUMMARY AND CONCLUSIONS

(1) Penicillium brevicaulis and P. glaucum made up a major part of the mold flora in casein-glued plywood exposed to molds. Either of these molds, working with the bacteria normally present in the glue and surviving in the glue line, was able rapidly to delaminate plywood shear specimens glued with any of the five casein glues and one soybean glue tested. A number of other common air-borne molds also were able to digest casein glues in plywood. Exposure to the normal microflora of the air resulted in faster delamination than was produced by inoculating specimens with cultures of any of the fungi used.

(2) The nine commercial brands of powdered casein glues and the one soybean meal examined, all contained an abundance of the same kinds of yeasts and bacteria, but comparatively few molds. Of the organisms present in the glue powder, only a few kinds of bacteria survived in the liquid glues and in the glue lines of plywood made with them. These few

kinds, however, survived in considerable numbers. They were able to delaminate surface-disinfected shear specimens held at a moisture content of 50 to 65 percent, or soaked in water.

(3) Bacteria isolated from the glue lines of shear specimens bonded with casein and soybean glue, and inoculated on surface-disinfected specimens bonded with any of the 3 casein glues tested or with soybean glue and kept with a moisture content of 50 to 65 percent, caused complete delamination in 1 to 3 weeks.

(4) Sodium orthophenylphenate appeared more effective against the bacteria than against Penicillium brevicaulis, and sodium trichlorophenate, during the period of the test, prevented any weakening by either the mold or the bacteria.

(5) Specimens glued with two commercial brands of casein glues, surface disinfected, and kept in sterile, sealed jars at a moisture content of 60 to 65 percent, were almost totally delaminated after 6 months, while similar specimens, autoclaved and kept under the same conditions, retained their original strength. The chief difference between these two sets of specimens was in the greater number of bacteria that survived in the glue lines of the surface-disinfected as compared with the autoclaved specimens.

(6) Specimens glued with two commercial brands of casein glues retained their original strength over the 7-month test period when soaked in water plus 2 percent sodium pentachlorophenate, but were delaminated within 3 months when soaked in water to which bacteria and a small amount of casein had been added, or when merely surface-disinfected and soaked in water alone. In these soak tests the rapidity of weakening of the glued joints was roughly proportional to the number of bacteria present. If tests in which shear specimens are kept wet, or are soaked, are to be used as a basis for judging the durability of casein or soybean glues, the action of bacteria, heretofore disregarded, must be taken into account. Data available at present do not conclusively demonstrate that purely chemical hydrolysis occurs, and experiments in which bacteria are entirely eliminated will be required to settle this question.

Table 1.--Joint strength values of birch sapwood plywood made with casein and soybean glues with and without preservatives. The specimens were surface disinfected by a 45-second dip into boiling water, adjusted to a moisture content of 50 to 65 percent, and incubated in jars at room temperature. Specimens were conditioned to equilibrium at 80° F. and 30 percent relative humidity prior to testing.

Glue	Joint strengths and percentages of wood failure ¹						
	Not inoculated		Inoculated with <u>Penicillium</u>		Inoculated with bacteria		
			<u>brevicaule</u> ^{2/}				
	7 days	26 days	7 days	26 days	7 days	26 days	
<u>Casein</u>							
No preservative-----	434-13	(3)	0-0	0-0	469-30	^{4/} 183-3	
5% sodium ortho-phenylphenate-----	557-54	(3)	425-43	0-0	503-50	375-11	
5% sodium trichlorophenate-----	542-48	574-49	427-49	560-33	511-47	555-43	
<u>Soybean</u>							
No preservative-----	341-15	^{4/} 206-0	0-0	0-0	195-11	^{4/} 0-0	
5% sodium ortho-phenylphenate-----	368-5	^{4/} 372-3	120-2	0-0	351-3	319-1	
5% sodium trichlorophenate-----	313-0	390-4	331-4	319-1	338-1	332-0	

^{1/}The first value represents the joint strength in pounds per square inch; the second value represents the percentage of wood failure. Each value is the average for 8 to 10 specimens.

^{2/}Bacteria occurred as contaminants in these specimens.

^{3/}The jars containing these specimens were opened to photograph and culture the bacteria in the glue lines. The specimens became contaminated with Penicillium brevicaule and all were totally delaminated.

^{4/}Molds occurred on these specimens.

Table 2.--Effect of micro-organisms on birch sapwood plywood made with soybean and casein glues. The specimens were adjusted to a moisture content of 55 to 60 percent and incubated in jars at room temperature. Specimens were conditioned to equilibrium at 80° F. and 30 percent relative humidity prior to testing.

Treatment	:Joint strengths and percentages of wood failure ^{1/}			
	: Soybean ^{2/}		: Casein ^{2/}	
	: 8 days : 19 days :		: 10 days : 21 days	
	: : : :		: : : :	
Autoclaved, ^{3/} not inoculated--:	4/ 347-5	: 318-13	: 5.6/ 396-11	: 7/ 466-22
Surface disinfected, ^{8/} not inoculated-----:	4/ 318-13	: 380-1	: 459-54	: 459-48
Surface disinfected, ^{8/} inoculated with:				
<i>Torula</i> (plus bacteria?)-----:	5/ 224-3	: 68-0	: 341-33	: 5/ 232-9
<i>Penicillium glaucum</i> -----:	124-1	: 62-11	: 398-8	: 5/ 96-0
<i>P. brevicaulis</i> (dark strain)--:	239-4	: 0-0	: 407-19	: 5/ 146-0
<i>P. brevicaulis</i> (light strain):	83-0	: 0-0	: 327-23	: 5/ 122-0
Bacteria 82-8-----:	245-3	: 0-0	: 351-13	: 127-0
Bacteria from uninoculated check-----:	183-4	: 0-0	: 322-29	: 0-0
Not surface disinfected, not inoculated-----:	27-0	: 0-0	: 289-11	: 5/ 50-0

^{1/}The first value represents the joint strength in pounds per square inch; the second value represents the percentage of wood failure. Each soybean value is the average of 9 specimens; each casein value is the average of 8 specimens.

^{2/}When tested after 15 days at room temperature (i.e. moisture content 12 to 15 percent) 16 soybean specimens averaged 335-5 and 18 casein specimens averaged 495-34.

^{3/}Casein specimens autoclaved 40 minutes at 10 pounds pressure; soybean specimens autoclaved for 20 minutes at 10 pounds pressure.

^{4/}Bacteria isolated from inner plies of all specimens on casein agar.

^{5/}Bacteria grew out of glue lines of these specimens.

^{6/}Bacteria isolated from 2 of 5 specimens cultured.

^{7/}Some mold contamination.

^{8/}Surface disinfected by 45 to 60 second dip into boiling water, draining, and a 5 to 10 second dip into 95 percent ethyl alcohol.

Table 3.--Effect of micro-organisms on birch heartwood plywood made with casein glues. The specimens were adjusted to a moisture content of 55 to 60 percent and incubated in jars at room temperature. Specimens were conditioned to equilibrium at 80° F. and 30 percent relative humidity prior to testing.

Treatment	:Joint strengths and percentages of wood failure ^{1/}			
	: Casein glue "A" ^{2,3/}		: Casein glue "B" ^{2,3/}	
	: 17 days	: 34 days	: 17 days	: 34 days
Autoclaved, not inoculated----	501-53	478-61	431-37	478-49
Surface disinfected, not inoculated-----	327-16	158-17	^{4/} 343-26	^{5/} 339-27
Surface disinfected, inoculated with:				
<u>Penicillium brevicaula</u> -----	246-2	0-0	296-1	25-0
<u>Cladosporium</u> -----	^{4/} 169-3	^{4/} 0-0	^{4/} 293-18	0-0
<u>Trichoderma</u> -----	385-42	40-0	^{6/} 373-65	84-0
<u>Torula</u> -----	^{4/} 347-19	^{4/} 0-0	321-46	16-0
Bacteria from uninoculated check-----	57-6	0-0	169-6	0-0
Bacteria 82-8-----	126-0	0-0	111-0	0-0
Bacteria 77-A-----	170-3	0-0	317-43	105-0
Not surface disinfected, not inoculated-----	^{7/} 146-0	0-0	273-7	62-7

^{1/}The first value represents the joint strength in pounds per square inch; the second value represents the percentage of wood failure. Each value is the average for 8 specimens.

^{2/}When tested after about 3 weeks at room temperature (i.e. moisture content 12 to 15 percent) 20 specimens glued with casein "A" averaged 481-69 and 20 glued with casein "B" averaged 451-46.

^{3/}When tested after 54 hours' immersion in water at room temperature before autoclaving or surface disinfecting, 20 casein "A" specimens averaged 202-0 and 20 casein "B" specimens averaged 204-0.

^{4/}Bacteria grew out of glue lines of these specimens.

^{5/}These specimens were again surface disinfected after the start of the experiment to eliminate contaminating molds that appeared.

^{6/}Bacteria grew out of the glue lines of 5 of these specimens.

^{7/}Unidentified molds on these specimens.

Table 4.--Joint strength values of individual autoclaved and surface-disinfected casein "A" glued specimens from table 3 after incubation for 34 days

Panel No.	Controls		Joint strengths and percentages of wood failure ^{1/}		Reduction of strength of surface-disinfected over autoclaved specimens
	Dry	Wet	Autoclaved, not inoculated	Surface-disinfected, not inoculated	
					Percent
33	390-100	180-0	445-0	0-0	100
34	555-30	220-0	505-50	315-50	38
36	485-100	200-0	435-100	385-90	11
37	455-100	170-0	505-50	225-0	55
39	630-70	305-0	475-100	0-0	100

^{1/}The first value represents the joint strength in pounds per square inch; the second value represents the percentage of wood failure. Tests were made after the specimens had been conditioned to equilibrium at 80° F. and 30 percent relative humidity.

Table 5.--Joint strength values of casein-glued birch sapwood plywood surface-disinfected or autoclaved (with usual or reduced numbers of bacteria in the glue line and a 65 percent moisture content) tested immediately after incubation

		Joint strengths and percentages of wood failure ^{1/}			
		No. 1 casein glue ^{2,3/} with-		No. 2 casein glue ^{2,3/}	
		out preservative		without preservative	
Incubation at room:					
temperature for:	Dipped into	Autoclaved	Dipped into	Autoclaved	
	boiling water	for 1/2 hour	boiling water	for 1/2 hour	
	for 45 seconds	at 10 pounds	for 45 seconds	at 10 pounds	
	to eliminate	pressure	to eliminate	pressure	
	surface molds		surface molds		
6 days tested dry ^{4/} :	421-24	:	397-3	:	485-68
6 days.....:	131-0	:	236-0	:	219-0
2 weeks.....:	168-0	:	217-0	:	206-0
4 weeks.....:	186-0	:	218-0	:	193-0
6 weeks.....:	5/ 73-0	:	203-0	:	193-0
2 months.....:	4-0	:	179-0	:	191-2
3 months.....:	17-0	:	181-7	:	17-0
4 months.....:	24-0	:	208-1	:	51-0
5 months.....:	6/ 11-0	:	202-0	:	158-0
6 months.....:	6/ 0-0	:	7/ 209-0	:	8/ 23-0

1/ The first value represents the joint strength in pounds per square inch; the second value represents the percentage of wood failure. Each value is the average for 8 specimens contained in a single jar unless otherwise stated.

2/ When tested after 4 days at 80° F., 30 percent relative humidity, 20 specimens glued with No. 1 averaged 404-12 and 20 specimens glued with No. 2 averaged 287-27.

3/ When tested after 72 hours' immersion in water at room temperature, 20 specimens glued with No. 1 averaged 175-0 and 20 specimens glued with No. 2 averaged 220-0.

4/ Tested after 6 days' exposure in test bottles followed by conditioning at 80° F. and 30 percent relative humidity for 16 days. Average of 8 specimens.

5/ Bacteria indicated by foul odor when jar was opened.

6/ Bacteria isolated from the 3 specimens cultured by planting glue-line scrapings from the delaminated faces in beef-peptone broth with pH of 9.25.

7/ No bacteria were isolated from 8 specimens when scrapings from freshly opened glue lines were planted in the beef-peptone broth.

8/ Bacteria were isolated from 1 of 4 of these specimens when scrapings from freshly opened glue lines were planted in the beef-peptone broth.

Table 6.--Joint strength values of casein-glued birch sapwood plywood surface-disinfected or autoclaved (with normal or reduced numbers of bacteria in the glue lines) and kept submerged at room temperature and removed and tested immediately

Exposure	: Joint strengths and percentages of wood failure ^{1/}			
	: No. 1 casein glue ^{2,3/}		: No. 2 casein glue ^{2,3/}	
	: without preservative		: without preservative	
Conditions	: Dipped into : Autoclaved:		: Dipped into : Autoclaved	
	: Incu- :boiling water : for 1/2 :boiling water : for 1/2		: bated :for 45 seconds: hour at :for 45 seconds: hour at	
	: for: : to eliminate :10 pounds : to eliminate :10 pounds		: for: : surface molds: pressure : surface molds: pressure	
Soaked in water.....	1 month :	110-0	202-0	176-0
Do.....	3 months:	0-0	160-0	0-0
Do.....	5 months:	0-0	164-6	0-0
Do.....	7 months:	4/ 0-0	5/ 114-0	4/ 0-0
Soaked in water con-:				
taining 2 percent:				
Santobrite.....	1 month :	232-1	250-3	243-3
Do.....	3 months:	197-3	267-4	300-3
Do.....	5 months:	231-22	266-3	229-21
Do.....	7 months:	6/ 199-3	7/ 251-11	8/ 192-1
Soaked in water con-:				
taining casein :				
and bacteria.....	1 month :	91-0	157-0	127-0
Do.....	3 months:	0-0	3-0	0-0
Do.....	5 months:	9/ 0-0	9/ 0-0	9/ 0-0
Do.....	7 months:	9/ 0-0	9/ 0-0	4/ 0-0

- 1/The first value represents the joint strength in pounds per square inch; the second value represents the percentage of wood failure.
- 2/Value for 32 specimens glued with No. 1 after 14 days at 80° F. and 30 percent relative humidity, 433-13; for specimens glued with No. 2, 389-11.
- 3/Joint strength for 32 specimens glued with No. 1 after 96 hours' immersion in water at room temperature, 199; for specimens glued with No. 2, 209.
- 4/Water cloudy with bacteria.
- 5/Bacteria isolated from all 8 of these specimens by planting scrapings from freshly opened glue line in beef-peptone broth with pH 7.7 or 8.5.
- 6/Bacteria isolated from 3 of the 8 specimens cultured by planting scrapings from freshly opened glue line in beef-peptone broth with pH 7.7 or 8.5.
- 7/No bacteria were isolated from these 8 specimens cultured by planting scrapings from freshly opened glue line in beef-peptone broth with pH 7.7 or 8.5.
- 8/Bacteria isolated from 1 of the 8 specimens cultured by planting scrapings from freshly opened glue line in beef-peptone broth with pH 7.7 or 8.5.
- 9/Water cloudy with bacteria; bacteria isolated from the 2 specimens cultured by planting scrapings from the delaminated faces in beef-peptone broth of pH 7.7 or 8.5.

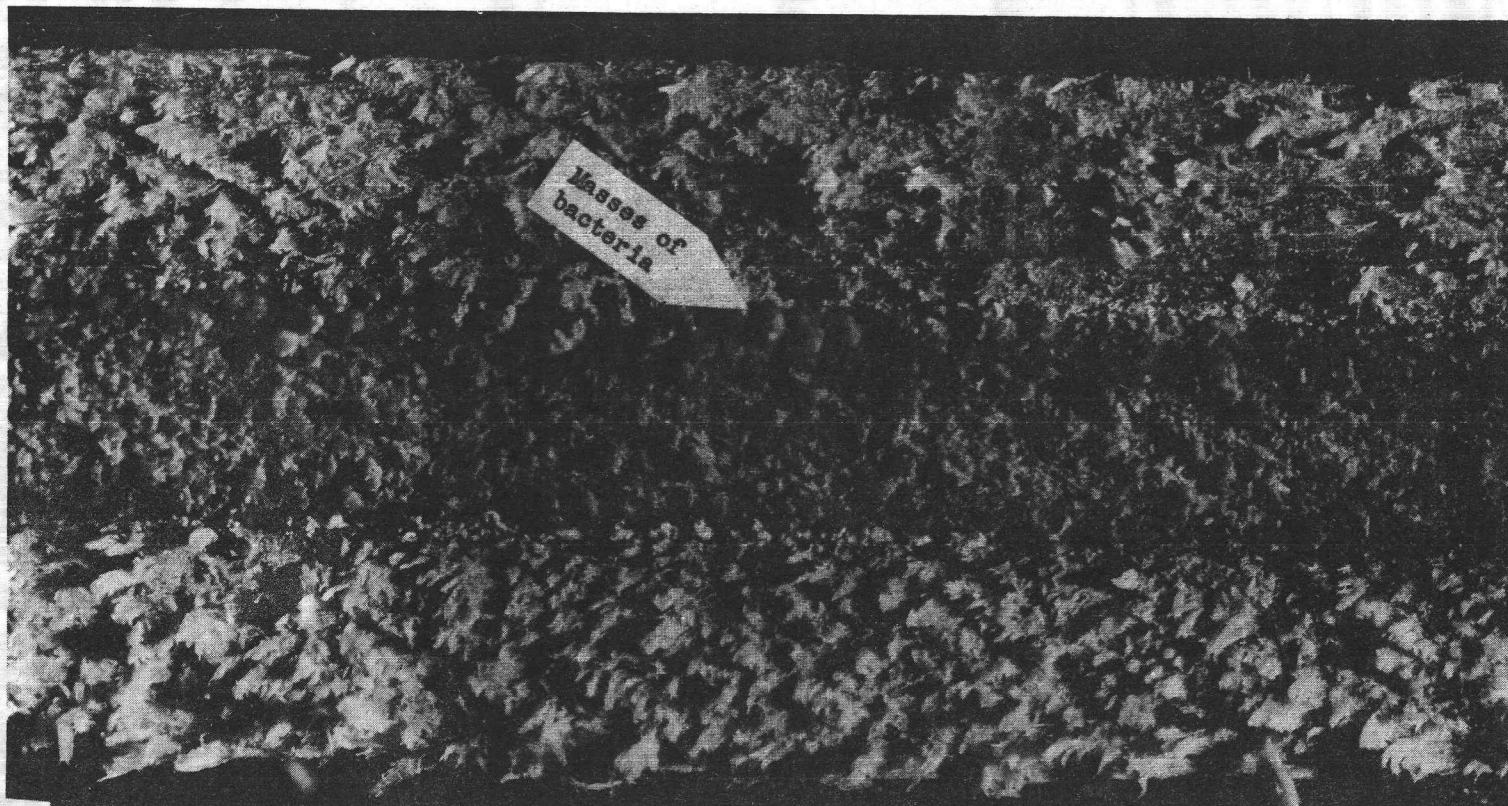


Figure 1.--Masses of bacteria growing from the glue line of plywood bonded with a commercial casein glue.

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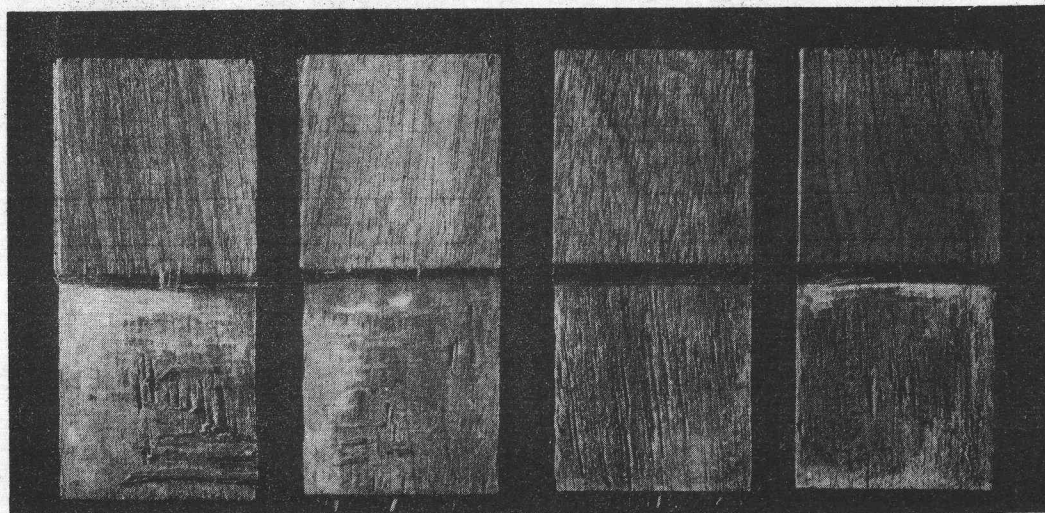


Figure 2.--Three-ply birch shear specimens bonded with casein glues, surface disinfected, and kept in sterile jars for 5 weeks. The joints have been partially delaminated by the bacteria normally present in the glue line. The white areas are chiefly masses of bacteria. These bacteria typically work inward from the edges.

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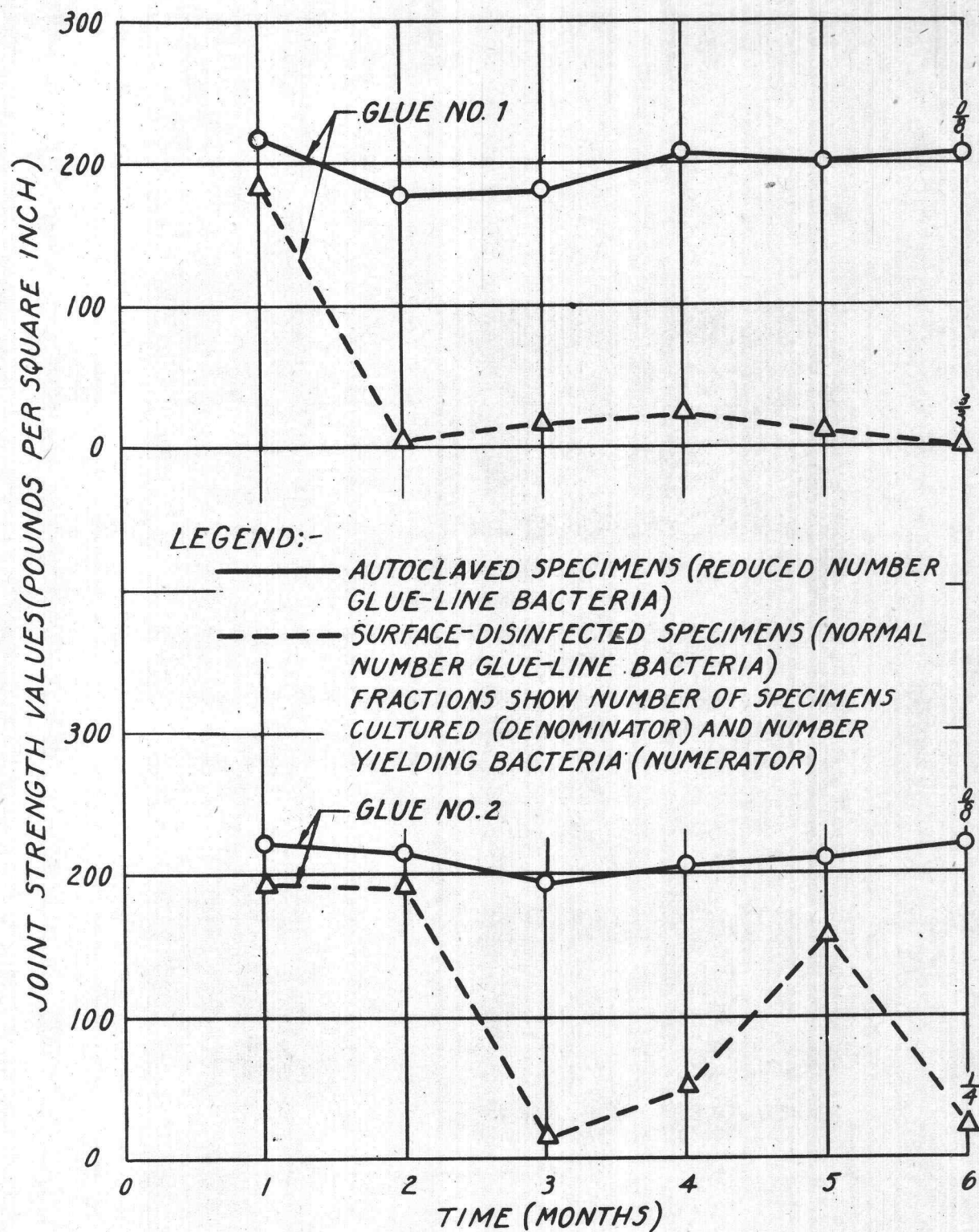


Figure 3.--Relation of bacteria to glue deterioration in wetted plywood made with casein glue.

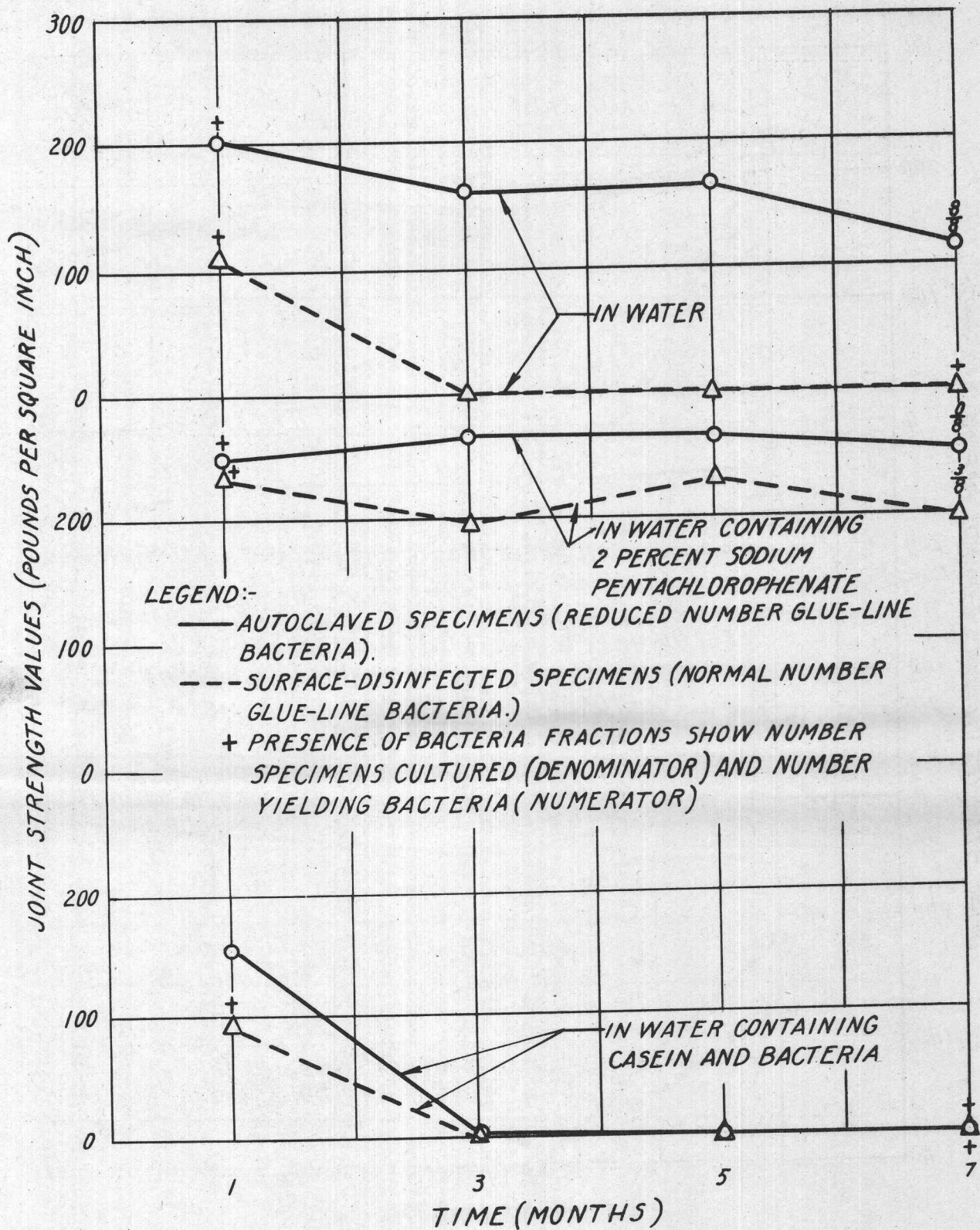


Figure 4.--Relation of bacteria to glue deterioration in submerged plywood made with No. 1 casein glue.

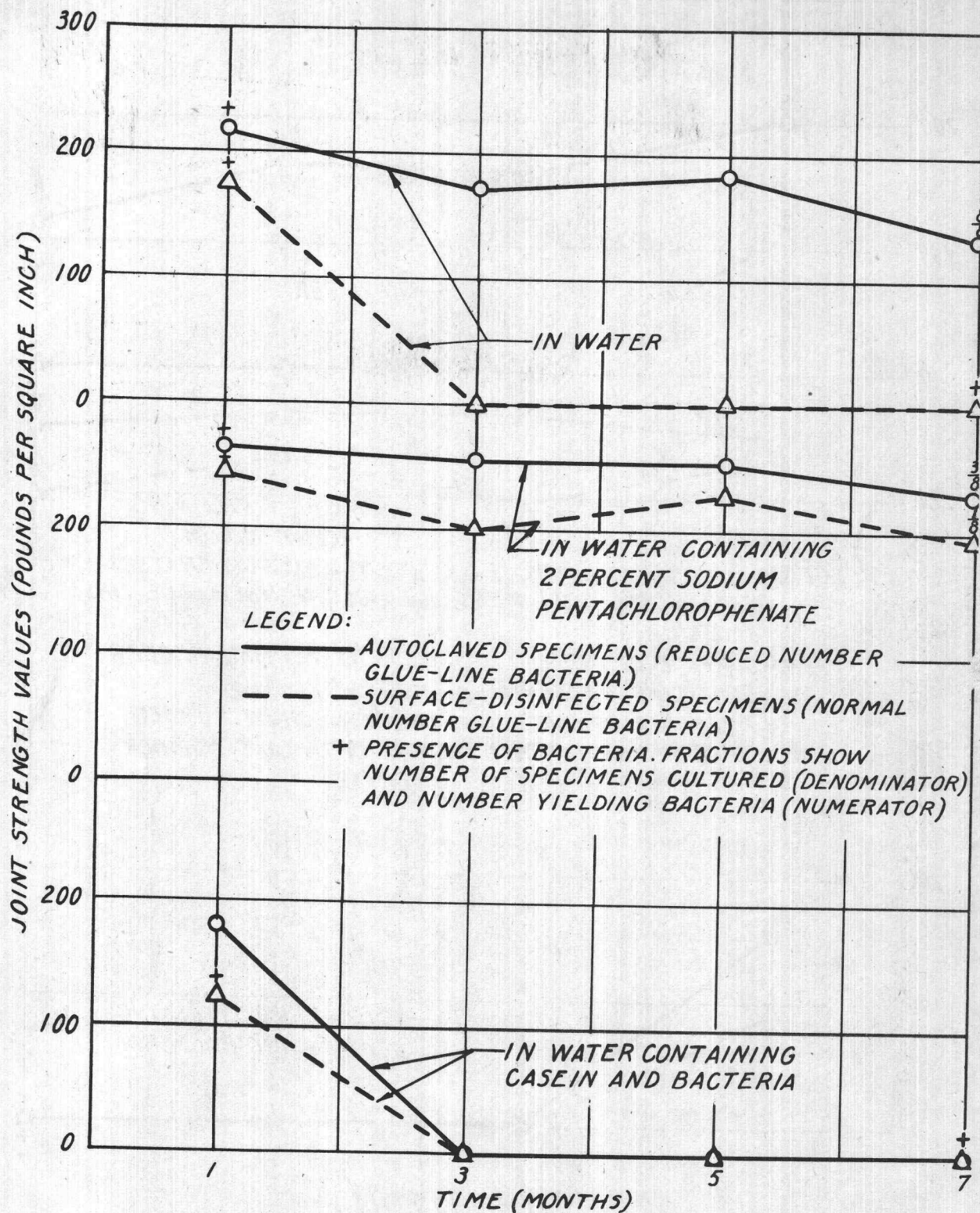


Figure 5.--Relation of bacteria to glue deterioration in submerged plywood made with No. 2 casein glue.