A STUDY OF OXYGEN ABSORPTION AND CATALASE PRODUCTION DURING GROWTH OF CHAETOMIUM GLOBOSUM ON COTTON FIBER AND YARN

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Damage to cotton by micro-organisms causes a serious loss to the cotton farmer, manufacturer, and consumer. For this reason studies are in progress in the Department of Agriculture on the growth and effect of various micro-organisms which cause deterioration of cotton.

In an earlier investigation in this laboratory by Rogers, Wheeler, and Humfeld (1940) the growth of Chaetomium globosum Kunze on cotton duck was followed by measuring the evolution of carbon dioxide by absorption in potassium hydroxide and subsequent titration. The present paper discusses the rate of absorption of oxygen by C. globosum growing on cotton fiber and on cotton yarn as measured by means of the Warburg apparatus (1934). This apparatus, which was developed for studying the respiration of tumor growths and used for measuring the rate of respiration of micro-organisms, has not been employed previously for measuring the rate of growth of fungi or other organisms on a cotton substrate.

The enzyme, catalase, is produced by the chaetomium during its growth. The amount of catalase formed may be considered a measure of the growth of the fungus. In this paper the quantity of catalase produced by the organism on the cotton fiber and cotton yarn is also reported.

MATERIALS AND METHODS

_C. globosum_ was selected for the present study because it was found on nearly all samples of awnings, tarpaulins, shock covers,
tents, etc. examined in this laboratory. The various cultures of this fungus isolated from these outdoor fabrics were found to cause an almost complete loss of strength of cotton duck when incubated for two weeks on a mineral salts agar (1940). The strain of *C. globosum* used was isolated from mildewed canvas. The identification of the organism was made from Chivers' monograph (1915) and its identification was later confirmed by Chivers.

Sea Island cotton fiber, after it was ginned, and yarn manufactured from this fiber were used. The cotton was classed by the Appeal Board of Review Examiners who found that its staple was 1½ inches and its grade, Middling Fair. It was spun into 90/2, combed yarn and was not gassed or mercerized.

Six samples each of cotton fiber and cotton yarn were used for the oxygen absorption measurements. Catalase was determined on three of the fiber and three of the yarn samples after the absorption experiments were completed. The samples of cotton fiber were selected at random from a representative pound lot of the raw cotton which had been removed for grading purposes from the bale of cotton used for manufacturing this yarn. The samples of yarn were picked at random from a group of 48 spindles, each of which contained approximately 2 ounces of yarn. Each yarn sample was withdrawn from a single spindle.

The fiber and the yarn were conditioned overnight at 70°F. and 65 per cent relative humidity, and a 0.25 gram portion was weighed from each sample. The weighed samples were placed in separate Petri dishes and sterilized in an autoclave at 20 pounds pressure for 30 minutes. Aseptic technique was employed in handling the materials throughout the remainder of the experiment. The samples were next inoculated with a suspension of *C. globosum* spores in sterile water by using an atomizer. The moisture added to the samples during sterilization and inoculation was removed by placing the samples in desiccators containing phosphorus pentoxide. This procedure was followed so that the moisture content of the samples would approach the moisture equilibrium in the Warburg flasks from the dry side.
OXYGEN ABSORPTION BY C. GLOBOSUM ON COTTON

The Warburg apparatus used was the seven-manometer, refrigerated type. During the first experiment, or run, three of the flasks and corresponding manometers of the apparatus were used for fiber, three for yarn, and the seventh as control to correct for the variation in barometric pressure. The same arrangement was repeated during the second run. Each run extended over a period of 28 days. The Warburg flasks used had volumes varying from 42 to 50 ml. and were fitted with short, removable test tubes which had capacities of approximately 2.5 ml. These inner tubes served as containers for the potassium hydroxide solution used to absorb the carbon dioxide liberated by the micro-organism.

The dried samples of fiber and yarn were placed in the flasks, which had been sterilized and dried, and 2 ml. of 1 per cent potassium hydroxide solution were added to each sterilized, dried test tube to absorb the carbon dioxide evolved. The flasks were connected with the manometers and placed in a water bath, the temperature of which was maintained at 26.8° ± 0.03°C. One per cent potassium hydroxide, which gives a humidity of 99.5 per cent in a closed system, was used, since a humidity as close to 100 per cent as practicable was desired. More carbon dioxide was evolved during the experiment than could be absorbed by the amount of alkali present and therefore the potassium hydroxide was changed every few days. The frequency of this change was gauged by the amount of oxygen taken up by the chaetomium. The oxygen consumption of the fungus was measured for a six-hour period each day, and the amount for 24 hours was calculated from this measurement, because the daily rate of oxygen consumption was so great during the period of heavy growth that it could not be read with one setting of the manometers.

Catalase was determined by the procedure described by Humfeld and Rogers.1 In this method the decomposition of 0.01 N hydrogen peroxide by the catalase was measured at 15-minute intervals for a period of 2 hours.

1 This paper is in manuscript.
RESULTS

The growth of *C. globosum* on the fiber and on the yarn is illustrated in figure 1. The dark colored perithecia are readily seen on both materials although the white hyphae of the fungus which grows on and in the fibers of the cotton are not distinguishable. The photomicrographs in figure 2 show the perithecia more clearly than the photographs in figure 1. In the photomicrograph of the cotton yarn the individual spores scattering from the various perithecia are apparent.

![Fig. 1. Cotton Fiber and Yarn after Incubation with Chaetomium globosum for 28 Days](http://jb.asm.org/)

The rate of growth of the chaetomium on the fiber and on the yarn is shown in figure 3 in which the milli-equivalents of oxygen absorbed each day per gram of cotton are plotted against days of incubation. The daily oxygen absorption for both materials reaches a maximum at approximately the eleventh day, after which the rate decreases. From about the eighteenth to the twenty-eighth day this decrease is so slight that during this period the rate of oxygen consumption tends to become constant.

The rate of oxygen absorption on yarn shows a slight decline from approximately the fifth to the eighth day, after which the rate again increases until the maximum is reached.
FIG. 2. PHOTOMICROGRAPHS OF COTTON AFTER INCUBATION WITH \textit{CHAETOMIUM GLOBOSUM} FOR 28 DAYS
\(A\), fiber; \(B\), yarn. \(\times25\)
ous publication by Rogers, Wheeler, and Humfeld (1940) a similar reduction was reported in the carbon dioxide evolution for the chaetomium from about the third to the seventh day. The decrease in both studies was found to coincide with perithecium formation. The average value for the fiber in figure 3 does not show a corresponding decrease in oxygen absorption before the maximum is reached, although the results for the six individual samples indicated decreases during the period of perithecium formation. Since these reductions occurred at somewhat differ-

Figure 3 also illustrates the greater growth of the chaetomium on the fiber than on the yarn. This difference in growth is apparent from the sixth day to the end of the experiment. The removal of non-cellulosic material from the fiber during the various stages of yarn manufacture probably accounts for this difference, as these substances may act as nutrients for the organism. It is also possible that the difference in physical structure between the fiber and the yarn may have some influence on the rate of growth of the fungus.
Fig. 4. The accumulated amount of oxygen consumed per gram of cotton during incubation with Chaetomium globosum

A, fiber; B, yarn. The lines represent calculated values and the points, experimental values.
The variation in oxygen consumption at the different periods of incubation and the variation for the two kinds of material were both shown to be statistically significant by an analysis of variance.

The difference in the amount of oxygen absorbed by the organism growing on the fiber and the organism growing on the yarn is shown also in figure 4, in which accumulated values are graphed. The amount of oxygen absorbed each day was added to the amount absorbed on the previous days. The average experimental values obtained are shown by individual points in this figure. The total amount of oxygen absorbed by the fungus growing on the fiber for 28 days is 0.32 milli-equivalents and on one gram of yarn, 0.21.

Equations were fitted to these accumulated data by the method of least squares and are plotted in figure 4 as solid lines. These equations are fourth degree polynomials of the general form $y = a + bt + ct^2 + dt^3 + et^4$. In this equation $y$ equals the milli-equivalents of oxygen absorbed, $t$, the time of incubation in days, and $a$, $b$, $c$, $d$ and $e$ are constants, the values of which were found from the data. For the fiber the equation was found to be $y = 10.4733 - 9.3158t + 3.0622t^2 - 0.1422t^3 + 0.0021t^4$ and for the yarn, $y = -3.9399 - 0.1476t + 1.6625t^2 - 0.0858t^3 + 0.0013t^4$.

In a preliminary study it was found that the organisms growing on unsterilized, uninoculated samples of Acala cotton fiber consumed a significantly greater amount of oxygen than those growing on unbleached cotton fabric. This difference, as well as that between fiber and yarn, probably is mainly due to the fact that there are more materials in the fiber to act as additional nutrients for the organism than are present in the fabric. These results indicate that in general raw cotton will deteriorate more readily in a moist atmosphere than will yarn or fabric. Even greater care, therefore, should be taken in storing fiber than in storing products manufactured from cotton unless, of course, these products contain sizing or finishing materials which may act as nutrients for micro-organisms.

A comparison of the oxygen consumption in this study with the carbon dioxide evolution in the previous investigation shows the
influence of the addition of mineral salts on the growth of the chaetomium. During the first 15 days of incubation in this study, in which no nutrient was added to the cotton, an average of 0.17 milli-equivalents of oxygen was absorbed by the fungus growing on one gram of cotton. In the previous investigation in which the cotton was incubated on a mineral salts agar, an average of 8.88 milli-equivalents of carbon dioxide was evolved per gram of cotton during the same length of time.

In the determination of catalase as measured by the amount of hydrogen peroxide decomposed, it was found that the fungus produced much more of the enzyme on the fiber than on the yarn. Figure 5 shows the average percentage of hydrogen peroxide decomposed by the catalase plotted against time in minutes. No statistical analysis was made on the data from the catalase determinations since inspection showed that the difference in the amount of enzyme produced on the two materials was significant. This variation in catalase production agrees with the difference in oxygen consumption and shows that the fiber is a better substrate than the yarn for the chaetomium.
SUMMARY AND CONCLUSIONS

Sea Island cotton fiber and yarn manufactured from this fiber were sterilized, inoculated with *Chaetomium globosum*, and incubated for 28 days in a Warburg apparatus.

In general, the daily rate of oxygen consumption increased during the first eleven days for both substrates and then decreased until the end of the experiment. The amount of oxygen absorbed by the fungus growing on the fiber was significantly greater than that absorbed by the fungus growing on the yarn. By the method of least squares a fourth degree polynomial equation was found for the accumulated absorption values for the fiber and another for the yarn.

A modified Warburg technique was developed for studying the growth of micro-organisms on cotton.

A much greater amount of catalase was produced by the fungus on the fiber than on the yarn. This confirms the results for total oxygen consumption. The determination of catalase furnished another measure of the growth of the chaetomium.

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REFERENCES

