Growth of Mycoderma Scum Under Oil [1] [2]

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Introduction

THE production of salt stock under conditions not affording direct sun-light is accompanied by heavy scum production, resulting in utilization of acid in the brine. When salting is done outside, little or no scum formation is evidenced on the brine surface; however, dilution by rain and evaporation are factors to be considered.

Rahn (1931) studied the prevention of scum formation by use of ultra-violet light supplied by an Uviac lamp. He found that radiation of one-half hour per day, at a distance of 6 feet from the surface of the brine, would prevent scum from appearing. Laboratory experiments by Fabian and Bryan (1932), using a Cooper-Hewitt mercury vapor lamp for radiating mycoderma cultures, showed that (a) young cultures were more resistant than old cultures, (b) that there was considerable difference in resistance to radiation by different strains, and (c) deep layers of films were difficult to kill.

The purpose of this study was to determine the utilization of acid and dextrose in brine by a pseudo-yeast associated with pickle scum formation when covered with a layer of mineral oil. The use of mineral oil was thought to offer a possible means for controlling scum formation. The experimental work was carried out in the laboratory using media approximating that usually found associated with scum formation on surface brine.

Experimental Procedure

A MYCODERMA culture isolated from fermenting cucumber brine (40° salometer) was employed. This strain gave abundant growth on solid and liquid media prepared from cucumber juice. Luxuriant scum formation was obtained on broth made from enriched fermented brine. The media were prepared as follows:

(a) *Cucumber-juice broth.* Ten lbs. of produce cucumbers were sliced lengthwise and autoclaved 1 hour at 15 lbs. pressure. The juice was filtered through 4 layers of cheese cloth and then through coarse, folded filter papers until clear. Autoclaving and filtering were repeated. The clear juice was then diluted with an equal volume of distilled water, placed in suitable containers (300 cc. amounts), and finally autoclaved for 30 minutes at 15 lbs. pressure. The resulting pH of juice was 5.2 to 5.6 (Solid medium prepared by adding 1.5 per cent agar to the juice.)

(b) *Enriched-brine broth.* One gallon jar of produce cucumbers were covered with 40° salometer brine and allowed to ferment until the titratable acidity had reached 0.2 to 0.3 percent lactic. The initial salometer was not adjusted during the fermentation. The brine was removed, enriched with 0.2 percent peptone and 0.5 percent dextrose, adjusted to 25° salometer, and autoclaved for 30 minutes at 15 lbs. pressure. The brine was filtered twice through coarse, folded filter papers and finally put in suitable containers (300 cc. amounts) and autoclaved for 30 minutes at 15 lbs. pressure.

In a preliminary experiment, inoculations of the mycoderma culture grown for some time previously on solid and liquid media were used. The first set of 125 cc. flasks containing 50 cc. of cucumber-juice broth was inoculated with one loop from a 72-hour-old slant culture. A second set was inoculated with 0.5 cc. from a cucumber-juice broth culture. To duplicate flasks from each source of inoculation 10 cc. of "heavy" mineral oil were added. An identical set was left without added oil. Controls with broth alone and broth with oil were made. All flasks were incubated at room temperature. At intervals of 4, 14, and 28 days plate counts were made using dextrose agar (pH 6.6). The plates were incubated 3 days at 30° C. and counted. An initial plating was made at the time of inoculation.

In the next experiment a series of 125 cc. flasks containing 50 cc. of enriched-brine broth, having a known lactic acid and dextrose content, were inoculated with 0.5 cc. of a 72-hour-old broth culture of the mycoderma. The flasks were treated as follows: Individual flasks in the first set containing 10 cc. of "light" mineral oil (3) were (a) not shaken; (b) shaken when plated; and (c) shaken daily; a second set received the same inoculation and treatments, but had no oil added. Controls with broth only and broth with oil were made. All flasks, at intervals of 3, 7, 15 and 21 days were analyzed for titratable acidity, reducing sugars and plate counts. An initial analysis was made at the time of inoculation.

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[3] Mineral oil (Light) kindly supplied by the L. Sonnenborn Sons Inc., New York, N. Y.

Platings were made using cucumber-juice agar (pH 6.6), incubated 3 days at 30° C. and counted.

Titratable acidities were determined by titrating with standard sodium hydroxide, using phenolphthalein as the indicator. Reducing sugars, calculated as dextrose, were determined by the Shaffer and Hartman micro method, standardized to the conditions under which it was used.

TABLE 1

Growth of mycoderma in cucumber-juice broth with and without mineral oil (Heavy grade)

Time In Days	Plate Count in Millions per CC.* Flasks with oil — Inoculated from Broth	Slant	Control	Flasks without oil — Inoculated from Broth	Slant	Control
Initial	1.11	1.24	0	1.16	1.15	0
4	5.35	5.00	0	58.00	34.00	0
14	10.82†	13.02	0	125.50	101.50	0
28	12.60	37.45	0	22.50	21.50	0

* Figures represent averages from duplicate flasks.
† An additional 10 cc. of oil added.

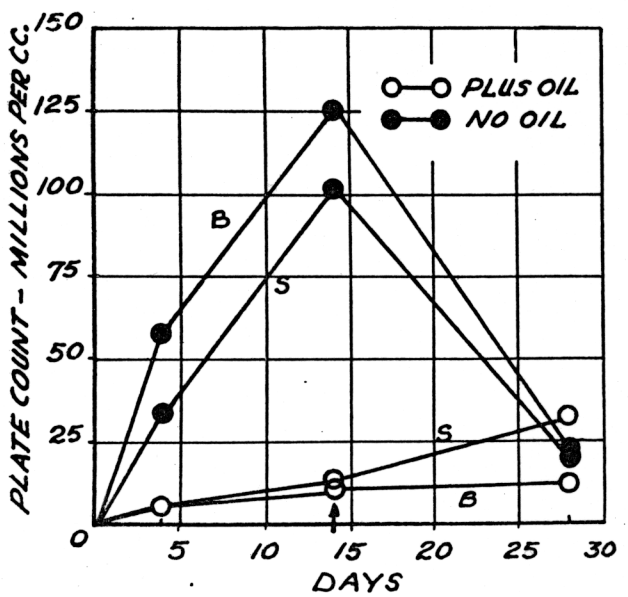

Figure 1. *Growth curves of mycoderma cultures with and without mineral oil (heavy grade); B, broth culture inoculations; S, slant culture inoculations; arrow at 14 days shows the influence of an additional 10 cc. of oil added to set B.*

Two grades of white mineral oil were used to cover the broth media and are designated as "heavy" and "light" in the text. The Saybolt viscosity ranges at 100° F. were as follows: Light grade, 65 to 75; heavy grade, 165 to 175.

Discussion of Results

TABLE 1 and figure 1 show plate counts from flasks with and without added mineral oil (heavy) and receiving inoculations from different media sources. The results show that the mycoderma used was able to grow under a layer of mineral oil, since there was an increase of cells during the incubation period over the initial count made at the time of inoculation. Flasks without oil showed a rapid rise in count, reaching a peak after 14 days, and then a decline. At the 14th day interval, the flasks with oil were noted to have a slight film of growth at the broth and oil interface. This was brought about, in part, by the gentle swirling of the broth necessary to obtain an even suspension for plating. This action broke the solid layer of the oil and reduced the efficiency of the seal. An additional 10 cc. of oil was added to each flask in one set showing the film (table 1, column 1) at the 14th day interval. The results at the 28th day show a marked decrease in cell count, as compared to the flasks left with the original amount of oil. The slant culture inoculations compared favorably with the broth culture inoculations, as indicated by the plate counts made from flasks with and without added oil.

The rates of growth of the mycoderma culture as indicated by plate counts and the utilization of acid and dextrose in flasks of enriched-brine broth, with and without the addition of mineral oil (light), are shown in tables 2, 3, and 4, respectively.

The plate counts in table 2 show that the mycoderma culture grew under oil, although at a decidedly slower rate than when no oil was used; this result was constant, regardless of the treatment given the flasks. With regard to results of flask treatments with oil, (a) those not shaken showed a low count when analyzed at the conclusion of 21 days, (b) those shaken at the plating interval showed a low count, quite constant until the 21st day, then a slight increase, and (c) those shaken daily showed a gradual rise from the time of inoculation up to 15 days, then a decrease. In the case of flask treatments without oil, (a) those not shaken had a moderately high count at the conclusion of 21 days, (b) those shaken at the plating interval showed a rapid rise on the 3rd day, remained constant until the 15th day, and then declined, and (c) those shaken daily grew rapidly, reaching a high peak on the 7th day and then declined.

The utilization of lactic acid is shown in table 3. The rate at which the acid was utilized was faster in cases where no oil was used, as would be expected from observing the growth rates (table 2). In flasks containing oil, the disappearance of acid was slower in those shaken at the plating interval than in those shaken daily. When flasks were not shaken, approximately one-half of the original acid content remained. The acid was utilized rapidly in flasks without oil, being absent after 7 days when shaken.

Table 4 shows the rate of dextrose utilization in flasks with and without oil. In cases where oil was used, the dextrose utilization was practically the same for flasks shaken at the plating interval and those shaken daily, being absent at the 15th day analysis. When the flasks were not shaken, approximately one-fifth of the original dextrose content remained. Where no oil was used, the dextrose disappeared rapidly and there was little significant difference due to treatment which the flasks received, both (shaken daily and shaken at intervals) showing absence of dextrose after 7 days.

In figure 2 the data from tables 2, 3, and 4 are plotted and the corresponding curves are shown.

TABLE 2

Growth of mycoderma in enriched-brine broth with and without mineral oil (Light grade)

Time In Days	Plate Count in Millions per CC. — Flasks with oil: Not shaken	Shaken when plated	Shaken daily	Control	Flasks without oil: Not shaken	Shaken when plated	Shaken daily	Control
Initial	0.10	0.15	0.15	0	0.16	0.15	0.17	0
3	...	3.85	2.50	0	...	58.50	76.50	0
7	...	3.05	13.40	0	...	66.00	106.50	0
15	...	3.90	26.00	0	...	35.00	23.00	0
21	3.10	9.50	20.08	0	34.50	30.50	16.50	0

TABLE 3

Utilization of acid by mycoderma culture when grown in enriched-brine broth with and without mineral oil (Light grade)

Time In Days	Titratable Acidity—Grams per 100 CC. as Lactic — Flasks with oil: Not shaken	Shaken when plated	Shaken daily	Control	Flasks without oil: Not shaken	Shaken when plated	Shaken daily	Control
Initial	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
3	...	0.12	0.12	...	...	0.05	0.08	...
7	...	0.12	0.05	...	...	0.00	0.02	...
15	...	0.06	0.03	...	...	0.00	0.02	...
21	0.09	0.05	0.04	0.15	Basic	0.01	0.00	0.15

TABLE 4

Utilization of dextrose by mycoderma culture when grown in enriched-brine broth with and without mineral oil (Light grade)

Time In Days	Grams Dextrose per 100 CC. — Flasks with oil: Not shaken	Shaken when plated	Shaken daily	Control	Flasks without oil: Not shaken	Shaken when plated	Shaken daily	Control
Initial	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73
3	...	0.63	0.59	...	...	0.27	0.30	...
7	...	0.38	0.32	...	...	0.00	0.00	...
15	...	0.03	0.03	...	...	0.03	0.03	...
21	0.15	0.03	0.03	0.66	0.03	0.03	0.03	0.67

Summary and Conclusion

IN conclusion, it may be said that the mycoderma culture was capable of growth under a layer of mineral oil and utilized normal constituents of brine, namely, acid and dextrose. In comparison with growth without oil, the action upon the above named constituents was somewhat slower.

The plate counts showed that the viable cells per cc. in flasks with oil were more numerous when shaken daily, and that the accompanying destruction of acid and dextrose was more rapid.

When no oil was employed, the plate counts were

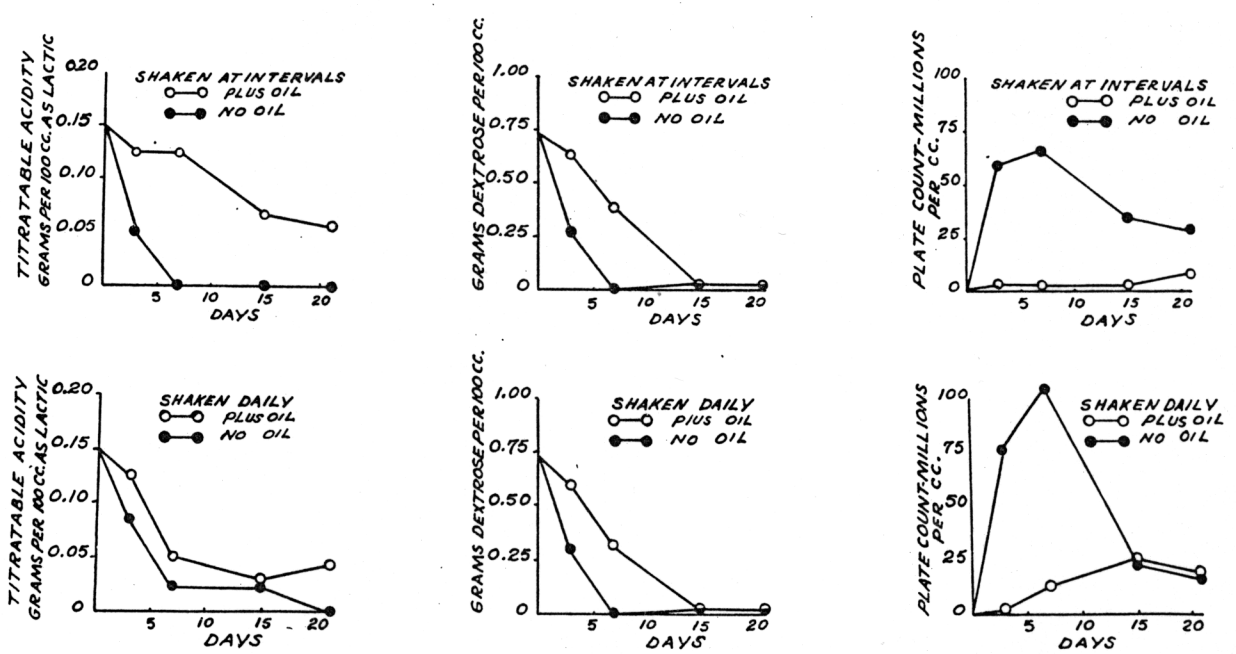

Figure 2. *Curves showing utilization of acid and dextrose by growth of mycoderma cultures with and without mineral oil (light grade). Upper part shows curves when cultures were shaken at intervals of analysis; lower part shows curves when cultures were shaken daily*

decidedly higher than when oil was used. When the scum was shaken down daily, the cells per cc. were higher than when it was shaken down at less frequent intervals for analyses. No detectable amounts of acid and dextrose were present in the shaken flasks after 7 days.

The "light" mineral oil proved more satisfactory than the "heavy" oil for covering the surface of liquid media, as evidenced by reduction in number of cells per cc., and there was no visable film formation at the broth and oil interface when the former oil was used. Also, when the flasks were shaken to obtain an even suspension for plating, the "light" grade oil showed less tendency to emulsify when disturbed, and gave a more even, effective seal.

The data seem to indicate that if a grade of mineral oil comparable to that used in these studies was employed in an attempt to control scum formation, there would be a somewhat slower rate of destruction of the available acid contained in the brine.

References

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