Effect of Oil in Pilot Plant Fermentations

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Research was undertaken to determine the optimum conditions for stimulation of penicillin production by lard oil and to determine the fate of the added oil. Yields of penicillin in pilot plant fermentations were increased from 1400 to over 2000 units per ml. by intermittent addition of lard oil. The added oil lengthened the productive phase of the fermentation and maintained the pH at a more favorable level. The oil was utilized at a fairly constant rate and clearly served as a nutrient as well as a defoaming agent. The unsaturated fatty acids were used at a more rapid rate than the saturated fatty acids. The increased yields obtained in the pilot plant could undoubtedly be duplicated in large tanks, such as are used in the industrial production of penicillin.

Animal and vegetable oils from various sources have been used routinely as antifoam agents in penicillin fermentations. Because foaming tendencies vary considerably from run to run, the amount of defoamer added to individual fermentations also varies widely. In the course of studies on the penicillin fermentation it became evident that high penicillin yields were frequently associated with fermentations to which a relatively large amount of antifoam had been added.

A considerable number of papers and patents have appeared on the role of oils in penicillin production. Stefaniak, Gailey, Jarvis, and Johnson (*18*) suggested that oils acted primarily as foam-breaking substances, thereby enhancing the aeration and penicillin production. Goldschmidt and Koffler (*8*) reported that small amounts of unsaturated oils greatly increased the penicillin production in shaken flasks inoculated with spores. Colingsworth (*6*) claimed in a patent that an increase in penicillin yield of more than 50% could be obtained if 0.1 to 1% of a suitable oil was added to the fermentation shortly after inoculation. Lard oil and corn oil were claimed to be most suitable for the stimulation of penicillin production. Perlman and Langlykke (*14*) found that oils could be used instead of carbohydrates as the energy source in penicillin fermentations. The yields were about the same as those obtained on a lactose medium (700 to 900 units per ml. for oil *vs.* about 800 units per ml. for lactose with culture W48-749), provided the oil was added in the same amount as lactose. Several Japanese workers (*9–13, 19*) have studied the influence of oil on penicillin fermentations and found that oil could both increase and decrease the penicillin production, depending upon when it was added, and the quantity, acidity, and kind of oil.

Rolinson and Lumb (*16*) reported that at high aeration rates lard oil was used in preference to carbohydrate, while at low aeration rates carbohydrate served as the principal carbon source. The effect of high aeration on lactose utilization appeared, however, to be peculiar to fermentations inoculated with spores. In fermentations started with a vegetative inoculum, lactose was used rapidly and completely even at the highest rate of aeration. The latter results are in agreement with those obtained earlier by Bartholomew and others (*2*) and Brown and Peterson (*3*).

Although the published papers show clearly that under certain conditions penicillin yields are increased by oil additions, not much information is available as to the fate of the oil and the effect of its utilization on mycelial growth and chemical changes in the medium. The present paper deals with these and other aspects of penicillin fermentations in pilot plant fermentors.

Table I. Composition of Media Used in Pilot Plant Experiments

Components	% in Medium: 30-liter seed tank	% in Medium: 50-gallon tank
Corn-steep liquor (solids basis)	2.5	3.0
Lactose	3.0	4.0
Calcium carbonate	0.1	0.4
Sodium sulfate	...	0.1
Cerelose	0.2	...
Potassium monobasic phosphate	0.025	...
Magnesium sulfate heptahydrate	0.01	...
Lard oil containing 6% Alkaterge C	0.1	0.05

Source of raw materials.
Corn-steep liquor. A. E. Staley & Co., Decatur, Ill.
Lactose. Western Condensing Co., Appleton, Wis.
Alkaterge C. Commercial Solvents Corp., Terre Haute, Ind.
Cerelose (commercial grade glucose). Corn Products Refining Co., Argo, Ill.

Experimental Procedures, Equipment and Analyses

Lard oil was used as the antifoam agent in 50-gallon, glass-lined fermentors. The tanks have been previously used for submerged production of citric acid and have been fully described by Buelow and Johnson (*5*). The volume of the medium was 100 liters, and the temperature of fermentation was held at 25° C. Aeration was done at a rate of 1.0 volume of air per volume of medium per minute (superficial air velocity 1.13 feet per minute). Agitation at 200 r.p.m. was obtained by means of a two-bladed, anchor-type impeller which conformed to the "dish" bottom of the tank. Vegetative inoculum for these experiments was propagated in a 30-liter fermentor (*4, 15*) and used at a level of 5% of the tank medium. Inoculum for the 30-liter seed tank was grown in 500-ml. conical flasks (100 ml. per flask), which were incubated on a rotary shaker. Cultures were maintained in the spore state as described by Gailey and others (*7*). The organism was *Penicillium chrysogenum* Wis. 49-133.

The composition of the medium used for the seed and tank fermentations is given in Table I. The corn steep solids

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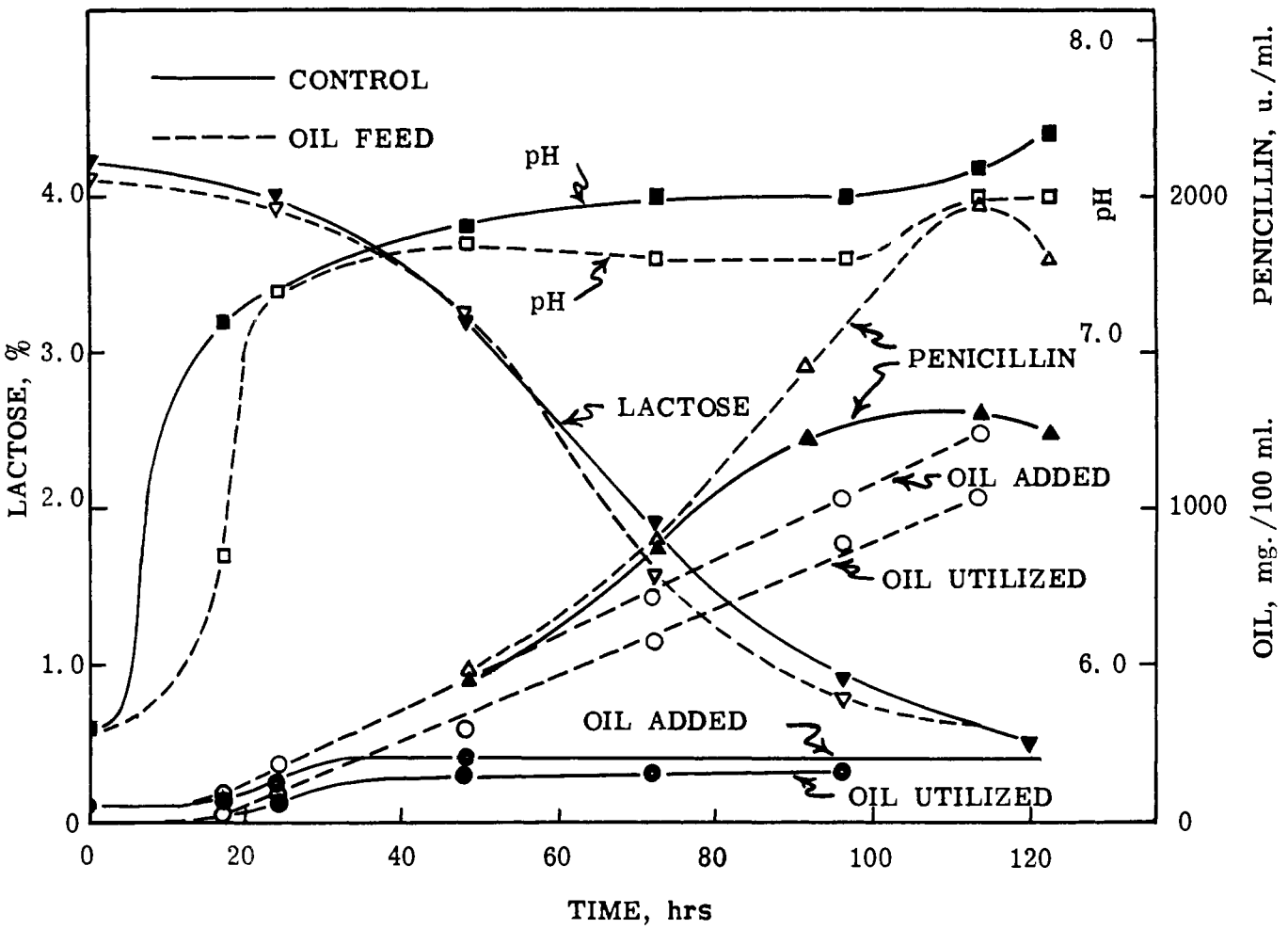

Figure 1. Chemical changes in an oil-feed fermentation and its control

Run 1, Table II

supply the nitrogen and some carbon; lactose is the chief energy source; calcium carbonate neutralizes any acids produced; and sodium sulfate ensures an adequate supply of sulfur for the biosynthesis of penicillin. A precursor of penicillin G, phenylacetic acid, was added as a solution of its potassium salt at a 0.05% level at 24 hours and every 24 hours thereafter. It was necessary to have lard oil containing 6% Alkaterge C (Commercial Solvents Corp., Terre Haute, Ind.) present initially in the 50-gallon tank to prevent foaming during the sterilization and cooling cycle. All equipment and medium were sterilized at 121° C. for 15 minutes.

Sterile lard oil for addition during the run was contained in a stainless steel reservoir under 30 pound pressure. The passage of antifoam to the fermentor was regulated by a solenoid valve which was controlled by an electric timing

Table II. Summary of Data from Oil-Feed and Control Fermentations in 50-Gallon Tanks

Run No.[a]	Type of Fermentation	Oil Feed Started, Hours after Inoculation	Oil Added, Mg./100 Ml.	Penicillin Maximum U./Ml.	Time to Maximum Hours	pH at Pen. Max.
1	Oil for foam control		210	1335	113	7.5
	Oil feed	21	1250	1980	114	7.4
2	Oil for foam control		500	1450	114	7.7
	Oil feed	20	1220	1960	120	7.4
3	Oil for foam control		800	1325	112	7.4
4	Oil for foam control		350	1320	112	7.7
5	Oil for foam control		450	1560	112	7.9
6	Oil for foam control		270	1230	96	7.8
7	Oil for foam control		200	1630	120	7.7
8	Oil for foam control		200	1260	113	7.9
12	Oil for foam control		560	1500	139	7.7
9	Oil feed	19	1090	1990	114	7.2
10	Oil feed	21	1450	2100	120	7.2
11	Oil feed	21	1080	2200	120	7.5
12	Oil feed	22	1900	1960	139	7.1
Av. 9 expts.	Oil for foam control		393	1401	115	7.7
Av. 6 expts.	Oil feed	21	1332	2022	121	7.3

[a] Runs 1 and 2 are pair runs with same inoculum. Others are individual runs.

motor (*7*), so as to be open only 0.5 to 1.0 second each minute, even though the foam was in contact with the foam-detecting electrode. An electronic relay actuated the timer, which was so adjusted that the solenoid valve delivered only 8 to 15 ml. of antifoam each time it opened. In fermentations to which oil was added at regular intervals, the timing motor was driven by another timing device which turned on the 1-minute timer for 1 minute each hour. Thus, from 8 to 15 ml. of lard oil could be added to the fermentor during each hour of oil feeding.

Residual fat in the broth was extracted by ether from a slightly acidified sample (50 to 100 ml.) in a 250-ml. separatory funnel. The extract was transferred to a weighed 100-ml. Erlenmeyer flask and the amount of fat determined after the ether had been evaporated on a steam bath and the solids dried to constant weight in an oven at 105° C. For determination of fat in the mycelium, the finely ground, dried mycelium was first digested with boiling methanol and then extracted with ether in a Goldfish extractor in a conventional manner. The iodine number of residual and mycelial fat was determined according to Rosenmund and Kuhnhenn (*77*). Weight of dry mycelium was obtained by acidifying the broth to dissolve insoluble calcium salts, filtering, washing, and drying to constant weight at 105° C. Penicillin, lactose, and pH were determined by methods given in a previous paper (*7*).

Results

The highest penicillin yields were obtained (in tank fermentations) when lard oil was added at regular intervals beginning about 20 hours after inoculation. If the oil feed was started later, lower yields were obtained, and when the additions were begun appreciably before 20 hours, the pH was depressed below the optimum range.

The yields of penicillin realized when lard oil was added in optimal amounts were 50% higher than those obtained in the control fermentations—i.e., antifoam only as necessary. Summary data from these experiments are given in Table II. The penicillin yields were more constant in the oil-supplemented fermentations, varying only from 1960 to 2200 units per ml. The potencies obtained in the control runs varied from 1230 to 1630 units per ml. The rather wide variations were probably due to differences in the amount of lard oil required to control the foam—viz., 200 to 800 mg. per 100 ml. There was no direct correlation between the amount of oil added and the penicillin yield in the control experiments. This was to be expected, because the oil additions were made only to suppress foam, and hence were very irregular and never continued beyond 40 hours.

The time necessary to reach maximum pencillin yield averaged 6 hours longer in the supplemented fermentations than in the control runs. This difference may not be significant, however, as samples were assayed arbitrarily at 8-hour intervals. The difference in the pH values is probably of more significance. The pH of the broth at the time of maximum yield in control runs averaged 7.7, whereas the pH in the oil-supplemented fermentations was 7.3. A pH of 7.3 is undoubtedly near the optimum for penicillin production under these conditions, while a pH of 7.7 is near the upper limit of the desired range.

Chemical changes representative of some typical control and lard oil–supplemented fermentations are shown in Figure 1. Addition of oil in the control run stopped at 40 hours, after 200 mg. per 100 ml. had been added. At this time residual oil had accumulated to the extent of 60 mg. per 100 ml. of medium. This residual oil then decreased gradually until only 25 mg. per 100 ml. remained at 96 hours. In other control fermentations, the oil content dropped to 18 mg. per 100 ml. at 113 hours. This indicates that the mold could probably metabolize more oil than was supplied.

In an oil-fed fermentation to which 1250 mg. of oil per 100 ml. was added over a period of 120 hours, 80% of the oil was metabolized. The optimal rate of oil utilization in this case and in similar runs was about 12 mg. per 100 ml. per hour.

The rate of lactose utilization in the two types of fermentation was nearly the same. This indicates that the lard oil was used concomitantly with the lactose. A comparison of the penicillin curves in the two fermentations shows that the rate of production was nearly equal until 80 hours. Then penicillin production began to slacken in the control run and reached only 1360 units per ml. at 113 hours. In the oil-supplemented run, the formation of antibiotic continued until 120 hours, at which time there were 1950 units per ml. A comparison of the pH curves shows that the pH remained nearly constant and somewhat lower in the oil-fed fermentations than in the unsupplemented runs.

The strong lipolytic action of the mold is indicated by the fact that the oil recovered after 40 hours was 98 to 100% hydrolyzed. The residual oil at 20

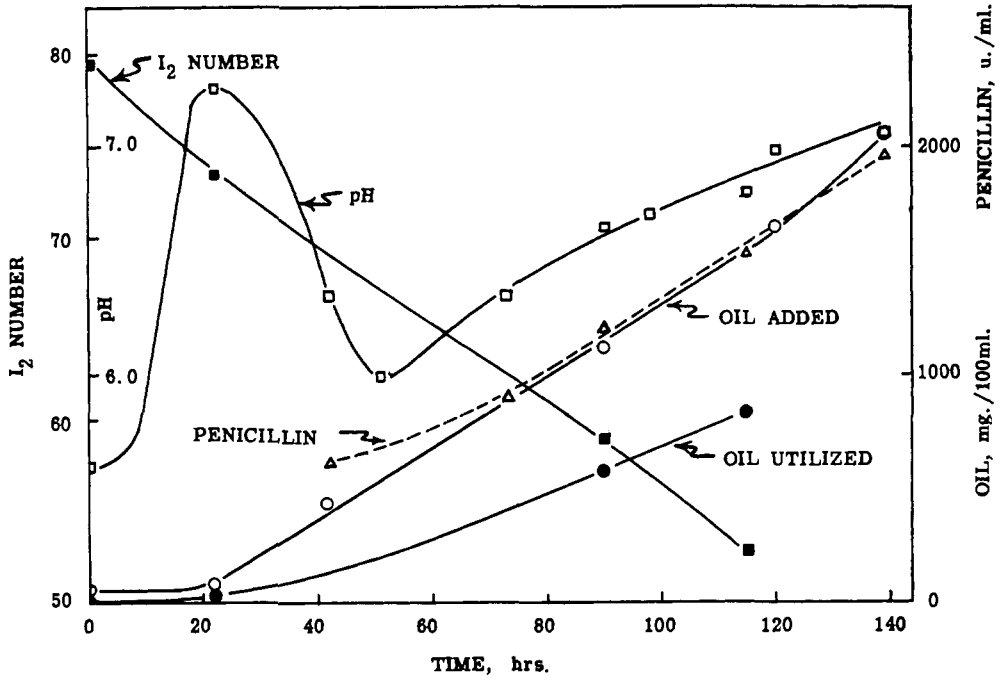

Figure 2. Effect of excess oil on pH, penicillin production, oil utilization, and iodine number of residual oil

Run 12, Table II

Table III. Effect of Oil on Amount and Composition of Mycelium

	Run 2						Run 12					
	Control, Hours			Oil Feed, Hours			Control, Hours			Oil Feed, Hours		
Oil utilized, mg./100 ml.	20	65	97	20	65	97	22	90	115	22	90	115
Mycelium, g./100 ml.	26.1	425	460	21.0	445	690	43	375	480	36	582	840
	0.82	1.63	2.00	00.82	1.64	2.31	0.81	2.12	2.51	0.85	2.31	2.92
Fat in mycelium, %	2.8	2.9	3.0	2.8	2.8	3.6	2.96	2.75	3.34	2.90	2.79	5.65
Iodine number of fat in mycelium	...	...	...	...	...	...	95.3	98.7	101.3	94.3	96.0	99.5

hours was also completely hydrolyzed, except when quantities exceeding 500 mg. per 100 ml. had been added. This rapid liberation of fatty acids caused a considerable drop in the pH, if too much lard oil was added early in the fermentation.

Figure 2 shows the results obtained when the oil-feed rate was too high. The excessive amount of oil allowed fatty acids to accumulate to such an extent that there was a decided drop in pH between 25 and 50 hours. The buffer capacity of the medium at this stage of the fermentation was only about 0.3 mmole. per 100 ml. between pH 6 and 7. The production of penicillin was somewhat slow, but 1950 units per ml. was reached at 140 hours. Retarded penicillin production could perhaps be attributed to the slightly toxic effect of phenylacetic acid at the lower pH.

The iodine number of the oil recovered from oil-supplemented fermentations always decreased during the run (Figure 2). The iodine number of the first sample was 79, whereas the added lard oil had an iodine number of 63.1. The reason for the high initial value was the presence of 0.15 to 0.20% of oily material in the corn steep of the medium. The iodine value of this material was 95 to 100 and, despite the relatively small amount present, contributed considerably to the iodine value of the early samples. The later decrease in iodine number was very marked and reached 52.8 at 115 hours. This value was definitely lower than the iodine number, 66.5, of the free fatty acids obtained from the oil. Evidently the unsaturated fatty acids were utilized somewhat more rapidly than the saturated acids. The degree of unsaturation, however, did not appear to be related to penicillin production, as corn oil, which is more unsaturated than lard oil (iodine number 95 *vs.* 65 for lard oil), gave no higher penicillin yields.

The weight of mycelium was 15 to 20% higher at 115 hours in the supplemented runs than in the fermentations which received lard oil only for foam control. Table III shows data related to the mycelium in paired fermentations. A difference in fat content of the mycelium from the two fermentations is evident. The mycelium from the runs to which the larger amount of lard oil was added had a fat content of about 2.90% at 22 hours. This increased to 5.65% at 115 hours, whereas the fat in the mycelium of the control remained nearly constant throughout the run. There was a slight but consistent increase in the iodine number of the mycelial fat. This increase in unsaturation occurred in both types of fermentation and does not appear to be significant.

Discussion

The natural oils generally added to penicillin fermentations as a means of controlling foam also enhance penicillin production. The reasons for this stimulatory effect may be partly physical—e.g., changes in surface tension due to the lyophobic character of the fat—or related to physiological effects of the defoamer. Undoubtedly the energy content of the oils serves to increase the metabolic rate of the mold, since there is no diminution in the lactose utilization rate when oil is present. It is also possible that the lard oil supplies fatty acids that are incorporated into the lipides of the cell and thus facilitates its development and activity. Although no known fatty acids appear indispensable for the growth of *P. chrysogenum*, it is possible that it cannot synthesize lipides from carbohydrate at a sufficiently rapid rate to meet the requirements for optimal growth and metabolism.

Summary

Penicillin yields in 50-gallon fermentors were increased from 1400 to over 2000 units per ml. by adding lard to the fermentation at intervals throughout the run. The added oil maintained the pH at the desired level and lengthened the productive phase of the fermentation.

Nearly all the added oil was catabolized by the mold if the additions were kept at a level of 0.1% or less. The unsaturated fatty acids disappeared from the medium at a faster rate than the saturated acids, but there was no significant increase in the iodine number of the fat stored in the mycelium.

Added oil had no effect on lactose utilization but increased the growth of mycelium about 15%.

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