7. D. GHOSH [1] and S. J. PIRT [2]. — Antifoam agents in aerobic fermentations. Evaluation of the activities of antifoam preparations.

Summary. — 1) A simple laboratory method for evaluating qualitatively and quantitatively the activities of antifoam agents has been described, using the vortex system (aeration by free agitation) with corn steep lactose medium as the foaming liquid.

2) Antifoam preparations for fermentation usually consist of an antifoam agent dispersed in a carrier. The importance of good dispersion of the antifoam agent has been shown.

3 The speed of foam suppression and duration of action of antifoam have been found to be influenced greatly by the nature of the carrier.

4) The activities of various antifoam preparations containing Alkaterge C, Siotol AF, silicone Antifoam A, octadecanol, grape seed oil and lard oil, have been studied. Suitable concentrations of these preparations have been established for an effective control of foaming in corn steep medium for a period of at least 6 hours in both baffled and vortex systems of aeration and agitation.

5) The differences beteween the baffled and free agitation systems of aeration from the point of view of foam inhibition have been discussed.

Riassunto. — 1) Si descrive un semplice metodo di laboratorio per valutare qualitativamente e quantitativamente l'attività di agenti che inibiscono la formazione di schiuma in terreni culturali contenenti corn steep-lattosio, aereati con il sistema vortice (agitazione libera).

2) I preparati antischiuma per la fermentazione consistono ordinariamente in un agente antischiuma disperso in un supporto. Si è dimostrata l'importanza di una buona dispersione dell'agente antischiuma.

3) Si è trovato che la rapidità della soppressione della schiuma e la durata dell'azione di un agente antischiuma dipendono molto dalla natura del supporto.

4) Sono state studiate le attività dei vari preparati antischiuma contenenti Alkaterge C, Siotol AF, antischiuma silicone A, ottadecanolo, olio di vinaccioli e olio di lardo. Si sono stabilite le concentrazioni adatte per un efficace controllo della formazione di schiuma per un periodo di alme-

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no 6 ore in un terreno di corn steep agitato sia con il sistema vortice che con quello ad antivortici.

5) Si sono discusse le differenze fra il sistema di aerazione ad antivortici e quello ad agitazione libera dal punto di vista della inibizione della schiuma.

Résumé. — 1) Les auteurs déscrivent une méthode simple de laboratoire pour l'estimation qualitative et quantitative des activités d'agents anti-mousse; les expériences ont été faites en milieux lactosés et additionnés de corn steep en présence du système vortex d'agitation (aération par agitation libre).

2) Les préparations d'agents anti-mousse destinées aux fermentations consistent en agents anti-mousse dispersés sur un support. On a démontré l'importance d'une bonne dispersion de l'agent anti-mousse.

3) On a constaté que la vitesse de suppression de la mousse et la durée de l'efficacité de l'agent anti-mousse dépendent pour large mesure, de la nature du support.

4) On a étudié divers agents: l'Alkaterge C, le Siotol AF, la Silicone anti-mousse A, l'octodecanol, l'huile de grains de raisin et l'huile de lard. On a déterminé les concentrations efficaces au contrôle de la formation de mousse, valables pour une période de 6 heures au moins, tant en présence d'agitation libre (vortex) qu'en système turbulent (panneaux de turbulence).

5) On discute les différences du point de vue de l'inhibition de la formation de mousse, entre le système vortex et le système à panneaux de turbulence.

Zusammenfassung. — 1) Eine einfache Laboratoriumsmethode für die qualitative und quantitative Auswertung von Antischaummitteln wird beschrieben, in der als Belüftungsmethode das Vortexsystem und als schaumbildene Flüssigkeit das gevöhnlich für die Penicillinproduktion benutzte Cornsteep-Laktosemedium verwendet wird.

2) Antischaummittel bestehen im allgemeinen aus einem Antischaumwirkstoff und einer Trägerlösung. Die Bedeutung einer guten Dispersion des Antischaummittels in der schäumenden Flüssigkeit für seine Antischaumwirksamkeit wird gezeigt.

3) Die Geschwindigkeit und Dauer der Antischaumwirkung ist weitgehend von der Natur der Trägerlösung abhängig.

4) Die relative Wirksamkeit verschiedener Antischaummittel, wie

Alkaterge, Siotol A F, Silicone Antifoam A, Octadecanol, Traubensamenoil und Specköl, wird untersucht. Die Konzentrationen, in denen diese Antischaummittel ihre Antischaumwirkung zum mindersten 6 Stunden lang in Cornsteep-Laktose-Kulturflüssigkeit bei Belüftung durch das Vortexsystem und durch Luftverteiler ausüben, werden bestimmt.

5) Die Unterschiede zwischen Vortex- und Luftverteilerbelüftung vom Standpunkte der Schaumverhinderung werden erörtert.

Introduction

Adequate control of foaming is of utmost importance in aerobic fermentation processes particularly when employing high rates of agitation and aeration. Excessive foam formation may (a) cause the culture to leak out through the propeller stuffing box or outlet filters and thus become contaminated, (b) cause a large part of the organism to be transported out of the liquid on the foam and deposited on the walls above the liquid level; and last but not least (c) reduce the effective aeration (Chain, Paladino, Callow, Ugolini and Van der Sluis, 1952; Chain and Gualandi, 1954).

In the industrial production of antibiotics like penicillin involving aerobic fermentation processes, antifoam agents such as Alkaterge C and octadecanol are commonly employed with animal, vegetable or mineral oil carriers for combating foams. The usual practice is to add to the foaming system an empirically determined amount of these antifoams either by manual operations at regular intervals or by automatic devices. In so far as foam control is concerned such methods are generally considered efficient as long as the foam does not rise high enough to threaten contamination by overflowing out of the propeller stuffing box and other possible outlets.

As yet no systematic data are available as to the relative merits, qualitative and quantitative, of chemical antifoams with particular reference to the degree of foam inhibition throughout the entire period of fermentation. To study this problem it was of primary importance to develop a method of evaluating the foam inhibiting actions of antifoam agents under conditions simulating as far as possible the actual fermentation system. In this paper a simple laboratory method for this purpose is described. With necessary modifications as to the nature of the medium and conditions of aeration and agitation the method could be applied to any other type of aerobic fermentation.

Materials and Methods

Materials.

The foaming liquid consisted of an aqueous solution of corn steep (about 3.5% solids) and lactose (3%) adjusted to pH 7 with sodium hydroxide.

The materials used in the antifoam preparation were as follows: Alkaterge C (Commercial Solvents Corp., U.S.A.), Siotol AF (Imperial Chemical Industries, England), silicone Antifoam A (Dow Chemicals, U.S.A.), octadecanol, grape seed oil, lard oil, paraffin (white medicinal grade) and petroleum ether (b.p. above 120° C)

Vortex system.

(a) *Apparatus.* — Two different foam producing aeration systems were used and compared (i) a free agitation or vortex system in which aeration was effected without an air sparger by sucking air into the solution by means of a fast rotating propeller blade and (ii) a fully baffled agitation system with air sparger (Chain *et al.*, 1952; Chain and Gualandi, 1954). The free agitation system shown in plate 1 consisted of a transparent cylindrical jar A of 18 cm ϕ and capacity about 10 litres. It was provided with a motor driven propeller B (8-blade-disc-turbine), blade height 15 mm, ϕ 75 mm. A cm scale was drawn on the jar. (The vessel shown in the figure has slightly different dimensions to that actually used for experiments). The motor C was provided with a tachometer D to indicate the rate of agitation, which was controlled by a Variac transformer E.

(b) *Determination of minimum amount of defoamer to prevent foaming.* — Five litres of the medium were placed in the vessel and subjected to an agitation of 900 rpm. The liquid foamed rapidly and the level of foaming liquid quickly rose up to a maximum height where it remained for hours if no antifoam was added. The foaming power of the solution was proportional to the height reached either by the agitated liquid or by the foam layer with liquid at rest (plates 2 and 3). The maximum height reached and hence the foaming capacity of the liquid varied to some extent from batch to batch of the cornsteep.

As soon as the maximum level i.e. the maximum foaming condition was reached (in about 10 to 15 minutes) the height was noted.

With the addition of antifoam agents this level dropped down and when complete foam control was achieved the height of the liquid was practically the same as that obtained with water only, instead of the medium, under identical conditions of aeration and agitation.

A typical determination of the minimum amount of an antifoam, e.g. Alkaterge C, required to inhibit foaming is shown graphically in fig. 1. The broken line (curve B) below indicates the height of the foaming liquid at rest, which was determined by stopping agitation momentarily and reading the level of foam after 20 seconds when the liquid came to rest. This level because of absence of movement could be read more precisely than the height of the foaming liquid during agitation (curve A). However, the two curves ran practically parallel and either one could be used to evaluate antifoam activity.

(c) *Duration of antifoam action.* — From practical considerations it was necessary to determine not only the minimum amount of an antifoam agent which would effect a complete inhibition of foaming, but also the minimum amount which would maintain the foam inhibition for a sufficient length of time, taken to be 6 to 8 hours.

Once foaming was completely suppressed, the agitation was continued for at least 6 hours and further additions of the antifoam agent were made if the system started foaming again (see fig. 1). The total quantity of antifoam thus required was noted and the experiment was repeated by adding this amount in one stroke right at the beginning and the duration of inhibition was checked for at least 6 hours. The amount of an antifoam agent thus determined was considered to be the minimum quantity which could be recommended for complete suppression of foam for at least 6 hours under the specified conditions of aeration, agitation and composition of medium.

(d) *Determination of rate of antifoam action.* — The rate at which foaming was suppressed was determined in the vortex system by plotting the height of liquid level against time after addition of antifoam agent to the foaming liquid. For comparative purposes the same batch of foaming liquid was used.

Baffled system.

The behaviour of the more common antifoam agents was also studied in the baffled system (plate 4), by providing four equally spaced baffle plates F (20.5 cm high; 1.5 cm wide) within the cylindrical vessel and passing air through a single orifice glass air sparger G. The vo-

lume of air passed through the medium per minute was regulated by means of a flow meter H.

An agitator speed of 500 rpm with 0.5 vol. air/min was used; this provided about the same degree of diffusion of oxygen into the liquid as was obtained with 900 rpm in the vortex system (Chain and Gualandi, 1954).

The minimum amount of antifoam determined previously in the vortex system was tried in the baffled system for duration of foam inbibition under comparable oxygen diffusion rates. Further additions were made if the system resumed foaming within six hours. The duration was checked as before by a second experiment, if necessary.

Results

Effects of carrier oil.

(a) *Augmenting and inhibiting effects of carriers.* — Antifoam agents are usually diluted with some oil before use. The carrier oil used was found to influence greatly the activity of the agent.

Paraffin oil, itself with no antifoam action at all, had a remarkable synergistic effect on the activity of Alkaterge C. This is clearly demonstrated in fig. 2 where it is shown that the addition of paraffin prolongs the action of Alkaterge.

Paraffin oil not only prolonged the action of a given amount of Alkaterge, it also increased greatly the speed of action of the antifoam. The reverse effect was observed with grape seed oil. The augmenting and retarding effect which paraffin oil and grape seed oil respectively have on foam suppression by Alkaterge C, will be apparent from the experiments shown graphically in figs. 3 and 4. Using the vortex system (fig. 3), when the level of foaming liquid reached the maximum height (33 cm) additions were made at zero, 2 and 5 minutes, of comparable quantities of Alkaterge alone and 50% Alkaterge in grape seed oil and paraffin oil. Whereas Alkaterge dispersed in paraffin oil acted almost instantaneously and effected complete inhibition of foaming within 5 minutes, at comparable levels of Alkaterge dispersed in grape seed oil there was no action at all in the first 5 minutes and it took over 20 minutes before foaming was completely suppressed. Alkaterge alone, as expected, showed intermediate behaviour in speed of action.

Grape seed oil by itself was a very slow acting defoamer. When it was used as antifoam agent in the vortex system in a concentration

of 0.1% it completely inhibited foaming but it required approximately 25 minutes to reach maximum effect. Paraffin oil did not accelerate the action of grape seed oil.

In the baffled system (fig. 4) the differences in the speed of antifoam action were even more pronounced and it deserved particular

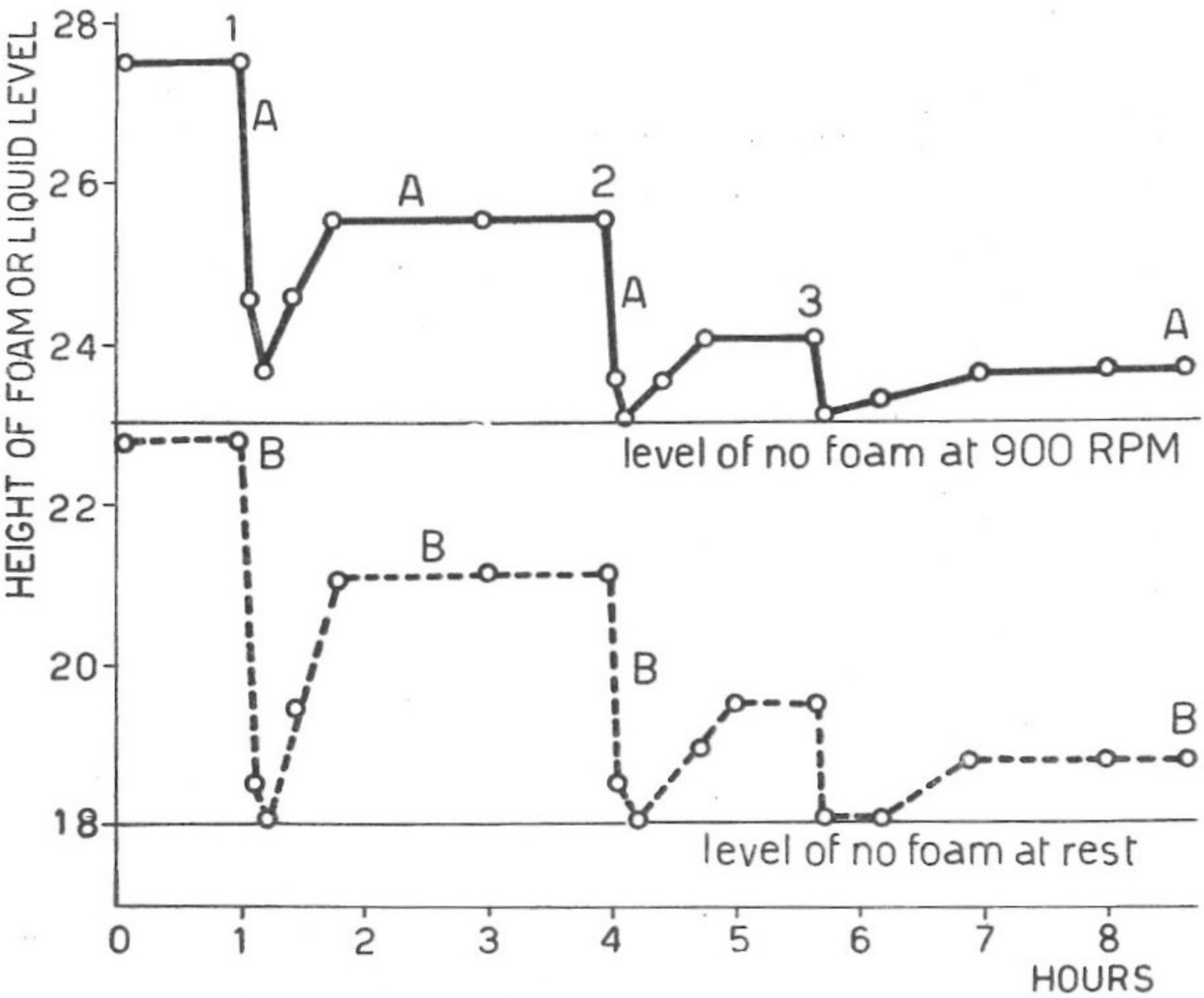

Fig. 1. - Inhibition of foaming by graded amounts of Alkaterge C (Determination of minimum amount of antifoam for complete foam inhibition).
Aeration and agitation: vortex system; 900 R.P.M.
Foaming liquid: Corn steep medium (pH 7), 5 litres.
Addition of Alkaterge C (after maximum foaming state was reached):
at position 1, 0.1 ml.
at position 2, 0.2 ml.
at position 3, 0.2 ml.
A = levels of foaming liquid recorded during agitation.
B = levels of foam with liquid at rest (after momentarily stopping agitation).

attention because of its practical importance. Comparable quantities of the three antifoam preparations of Alkaterge (0.4 ml Alkaterge) were added to the medium before agitation and aeration were started. With 50% Alkaterge in grape seed oil (curve B), the antifoam action was very slow to set in and within 4 minutes the excessively foaming liquid

was almost on the point of being expelled out of the vessel. The foaming could only be brought under control by intermittently interrupting the air flow. The dotted portion of the curve indicates the foam level with

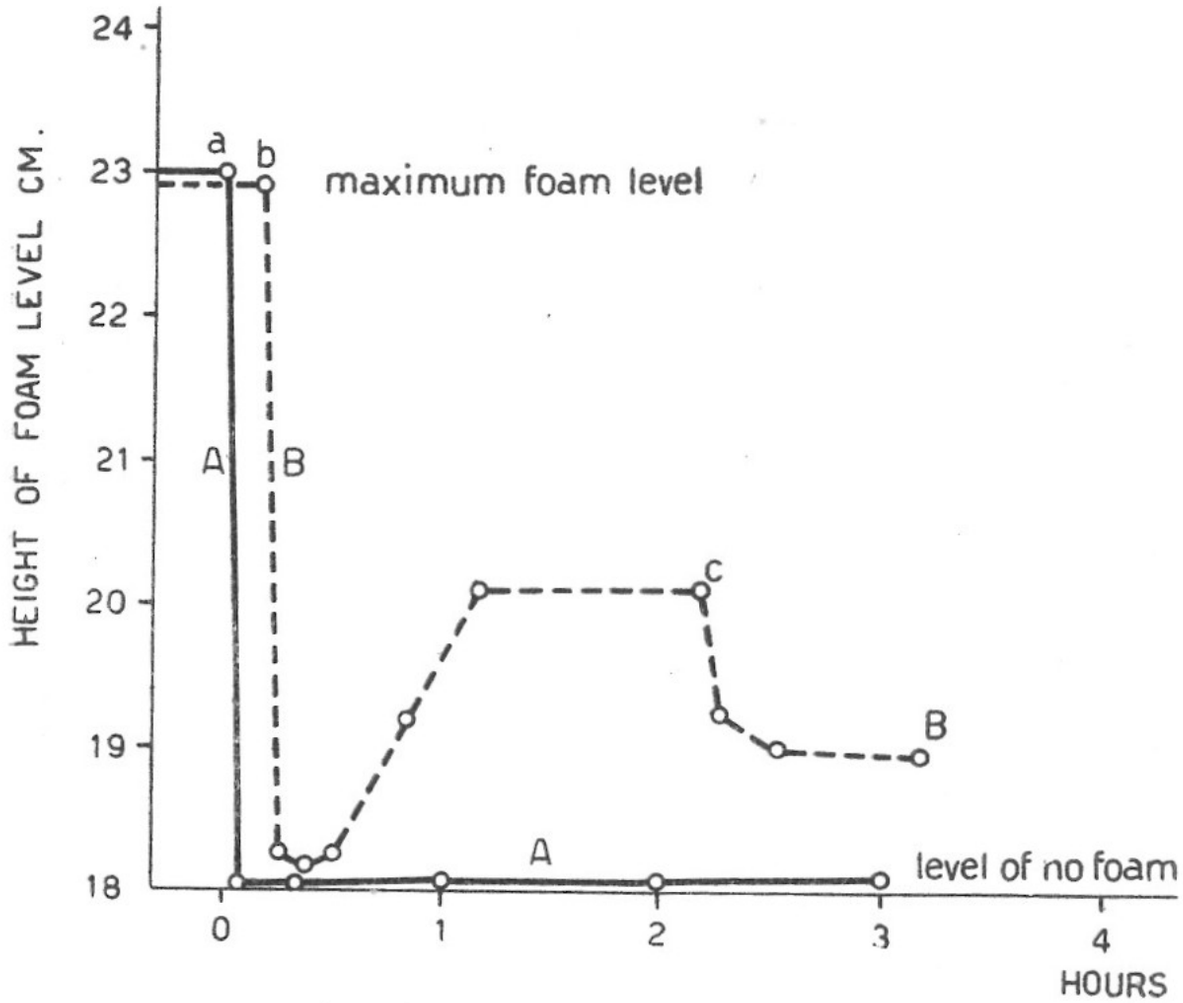

Fig. 2. - Effect of paraffin oil on the antifoam action of Alkaterge C.
Aeration agitation: Vortex system, 900 R.P.M.
Foaming liquid: Corn steep medium (pH 7), 5 litres.
Foam level recorded when the liquid came to rest (in about 20 seconds) after momentarily stopping agitation.
A = levels with 0.2 ml. Alkaterge + 1 ml. paraffin oil added at position a.
B = levels with 0.2 mil. Alkaterge added at position b and 1 ml. paraffin oil at position c.
Additions were begun after the foam level reached the maximum height.

air supply shut off. It took about 30 minutes before complete foam control was achieved.

With Alkaterge dispersed in paraffin oil the initial foaming was very little and was completely inhibited within only 5 minutes (curve A). Alkaterge alone, as in the vortex system, showed intermediate speed of action (curve C).

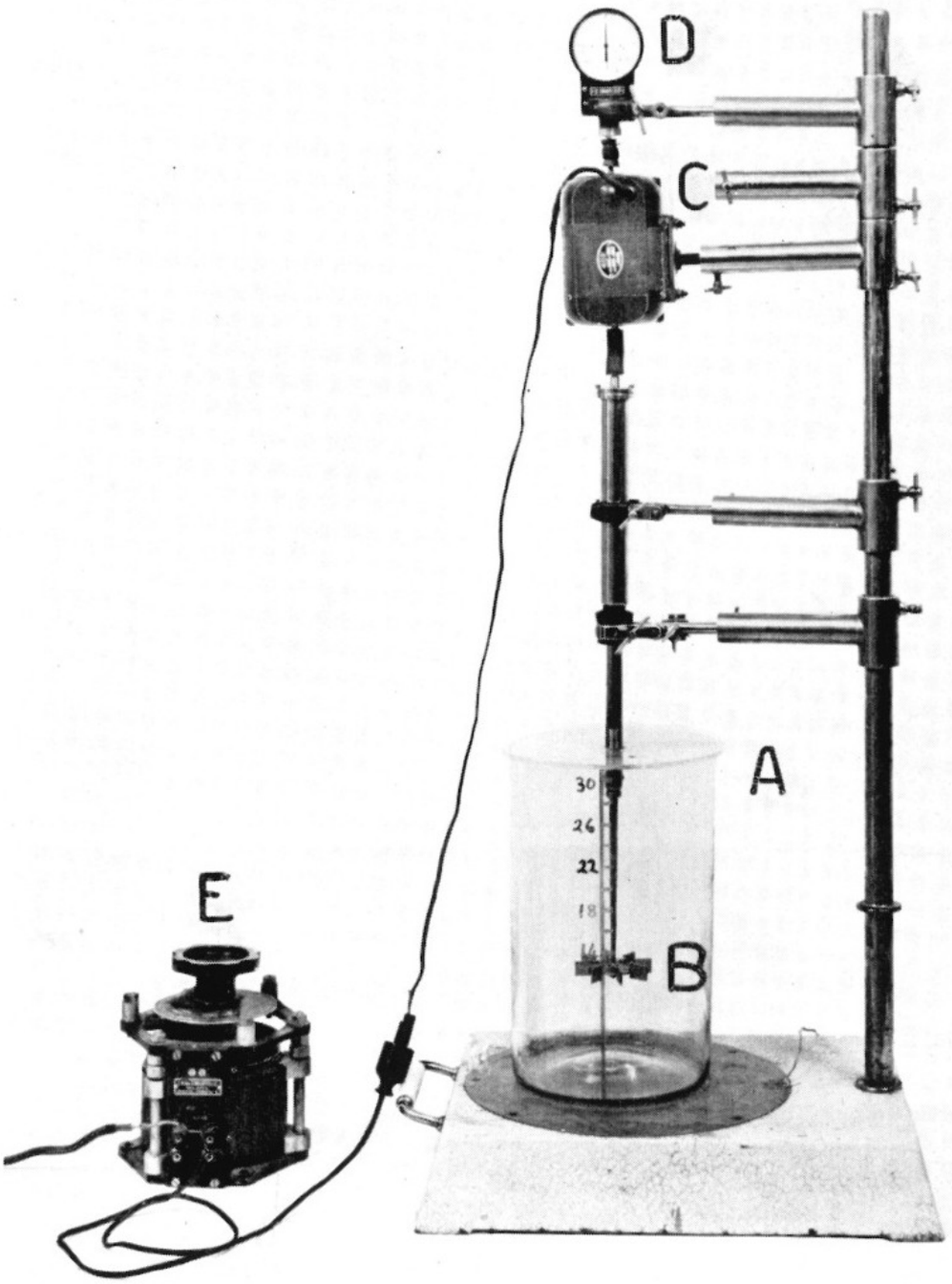

Plate 1. - Apparatus employing aeration by free agitation (vortex system) for evaluating activities of antifoam agents.

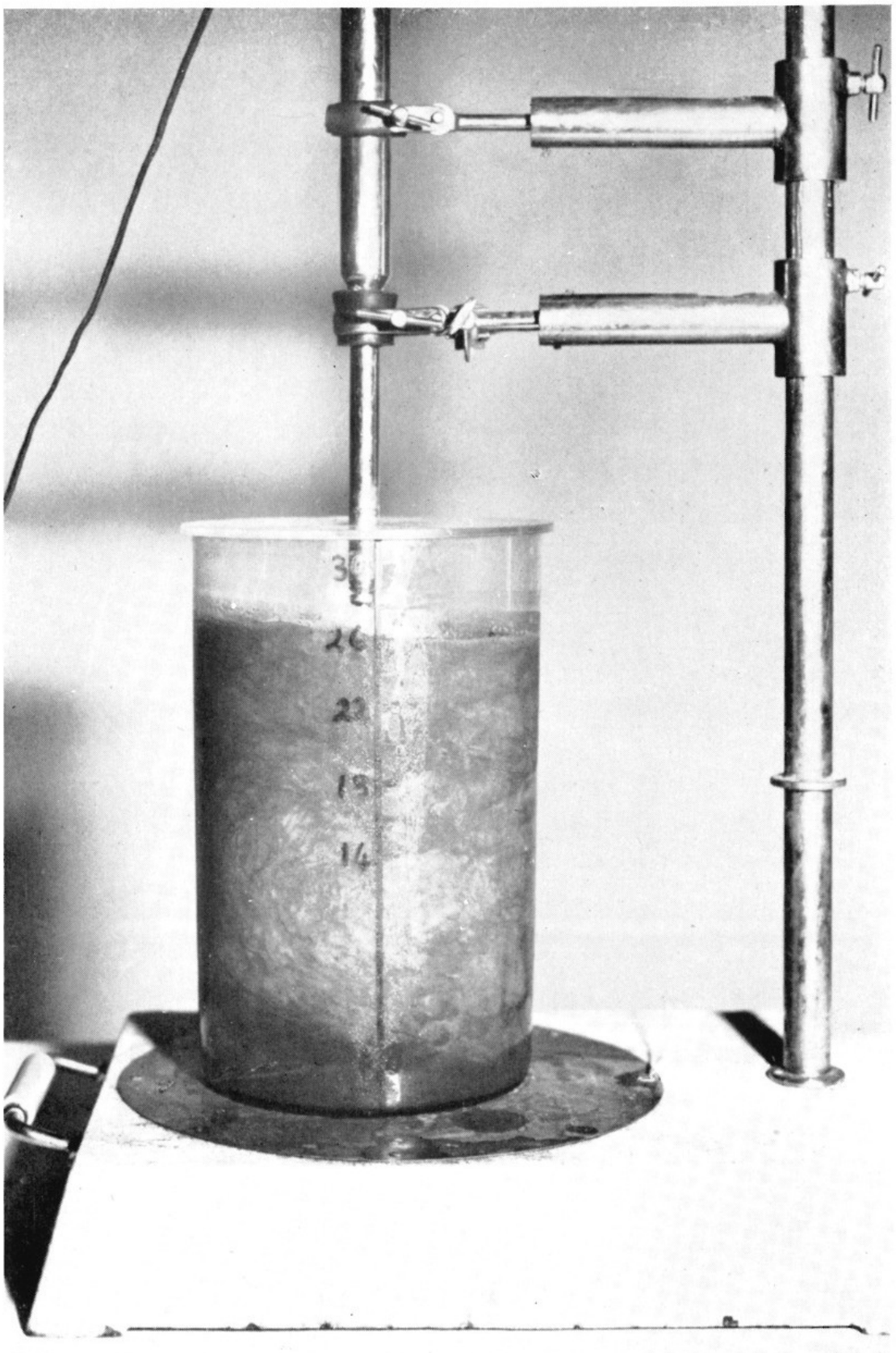

Plate 2. - Foaming liquid during agitation in vortex system.

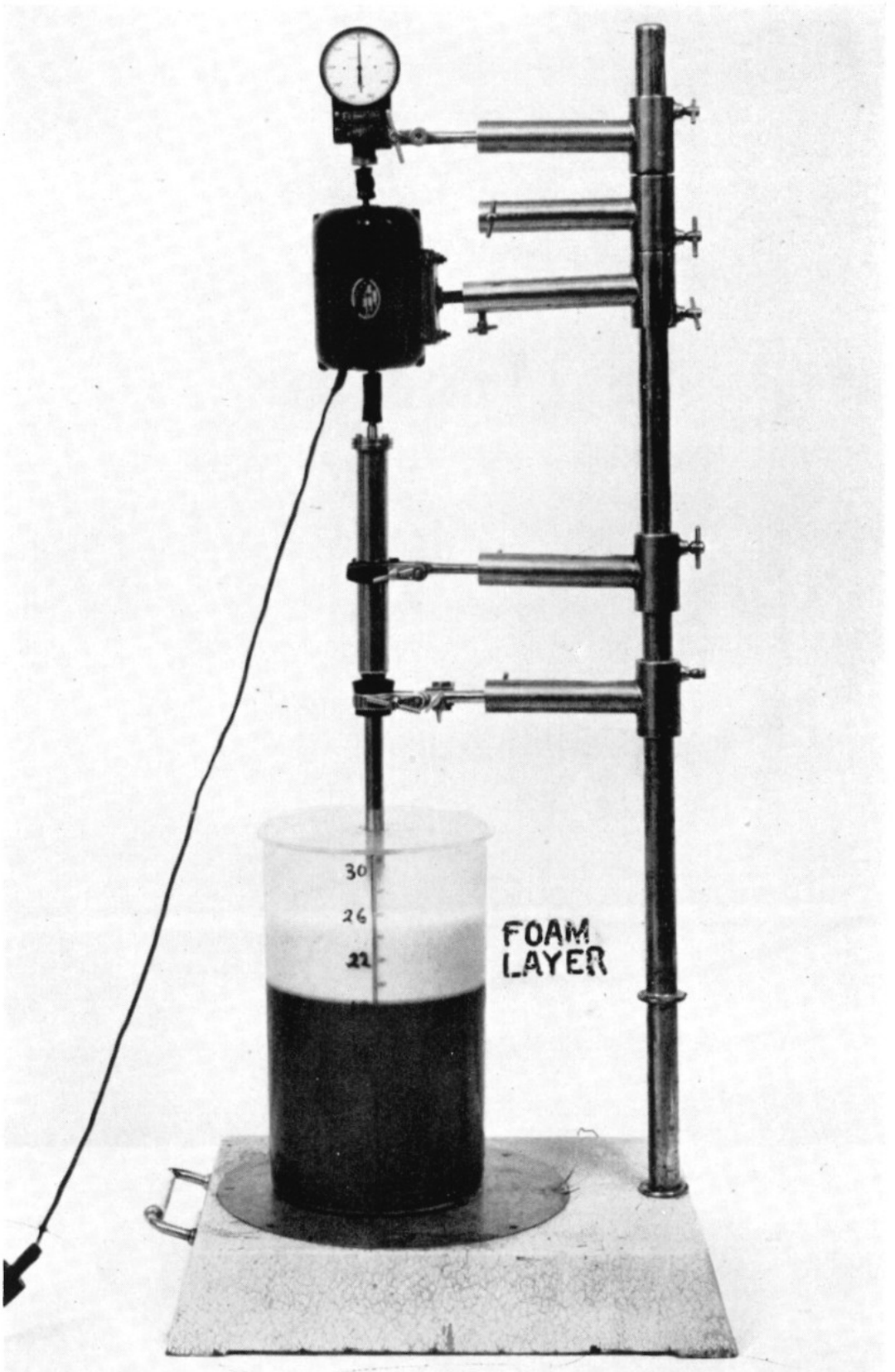

Plate 3. - Foaming liquid in vortex system with agitation stopped, showing separate foam layer.

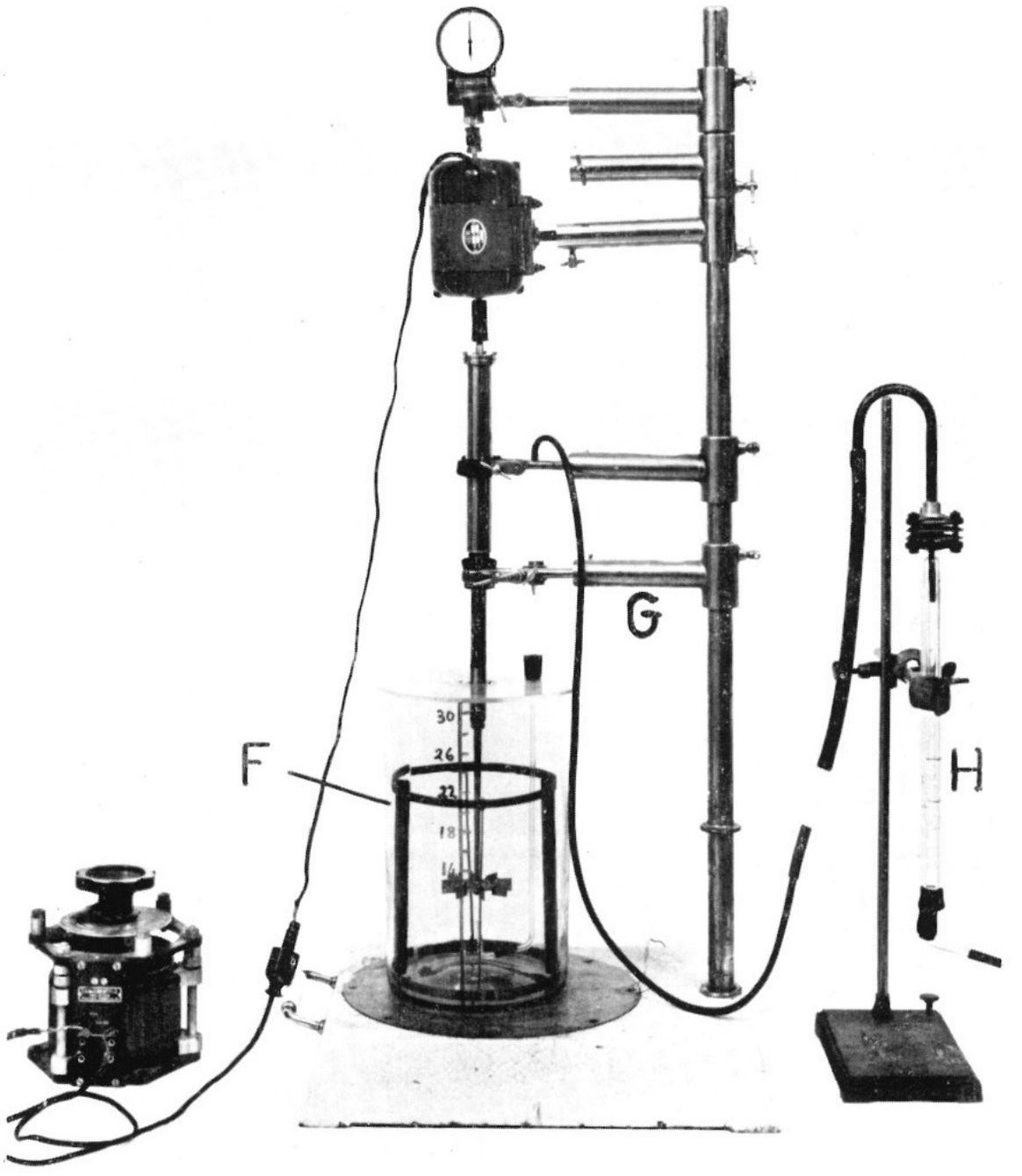

Plate 4. - Apparatus employing baffled system for evaluating activities of antifoam agents.

(b) *Dispersing action of carrier.* — Difficulties in obtaining effective dispersion of antifoam agent were experienced with both octadecanol and silicone Antifoam A.

Antifoam preparations containing up to 6% octadecanol in some

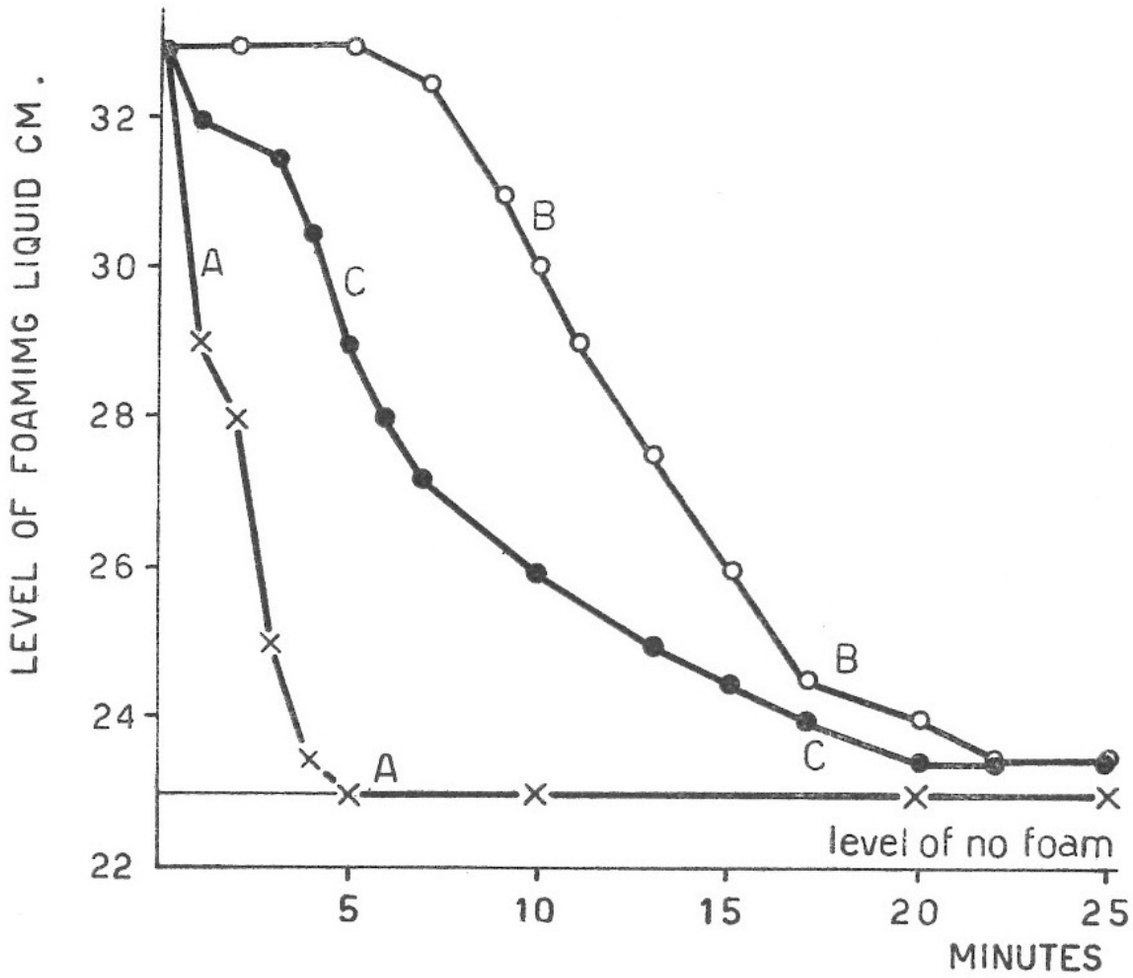

Fig. 3. - Effect of dispersing agent on the speed of antifoam action of Alkaterge C (in vortex system).
Aeration and agitation: vortex system, 900 R.P.M.
Foaming liquid: Corn steep medium (pH 7), 5 litres.
Levels recorded during agitation. Additions of antifoams were begun after maximum foaming state was reached.
A = Levels with 50% Alkaterge in paraffin oil added in aliquots of 0.2 ml., 0.2 ml. and 0.4 ml. at zero, 2 and 5 minutes respectively.
B = Levels with 50% Alkaterge in grape seed oil added in aliquots as above.
C = Levels with Alkaterge only added as 0.1 ml., 0.1 ml. and 0.2 ml. at zero, 2 and 5 minutes respectively.

oil such as lard oil are often employed in penicillin fermentations. The suitability of three carriers, paraffin oil, grape seed and lard oils, was examined. Octadecanol was sparingly soluble in these oils at room

temperature. It dissolved readily on warming but precipitated out as soon as the warm solution was added to the aqueous medium. Com-

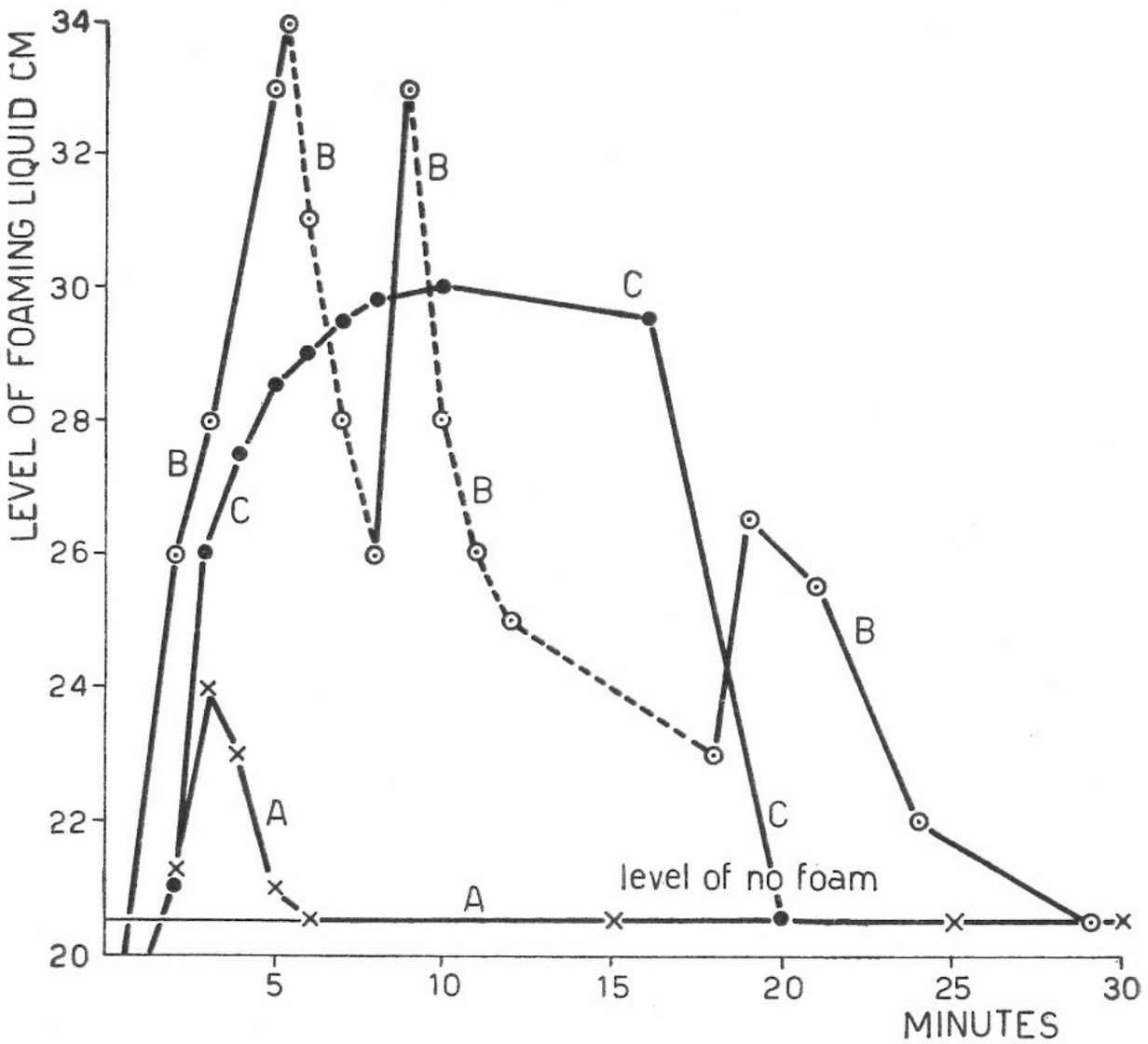

Fig. 4. - Effect of dispersing agent on the speed of antifoam action of Alkaterge C (in baffled system with air sparger).
Aeration and agitation: baffled system, 550 R.P.M. 0.5 vol. air/vol. liq./min.
Foaming liquid: Corn steep medium (pH 7), 5 litres.
Levels recorded during agitation. Antifoams were added to the medium before starting agitation and aeration.
A = Levels with 0.8 ml. of 50% Alkaterge in paraffin oil;
B = Levels with 0.8 ml. of 50% Alkaterge in grape seed oil;
C = Levels with 0.4 ml. of Alkaterge only.
(The dotted portion of curve B indicates the periods when the air flow was stopped)

parative effects of these three dispersing agents on the antifoam action of octadecanol are clearly shown in fig. 5.

Silicone Antifoam A readily disperses in petroleum ether but not at all in paraffin oil. Best foam inhibition was obtained using a mixture

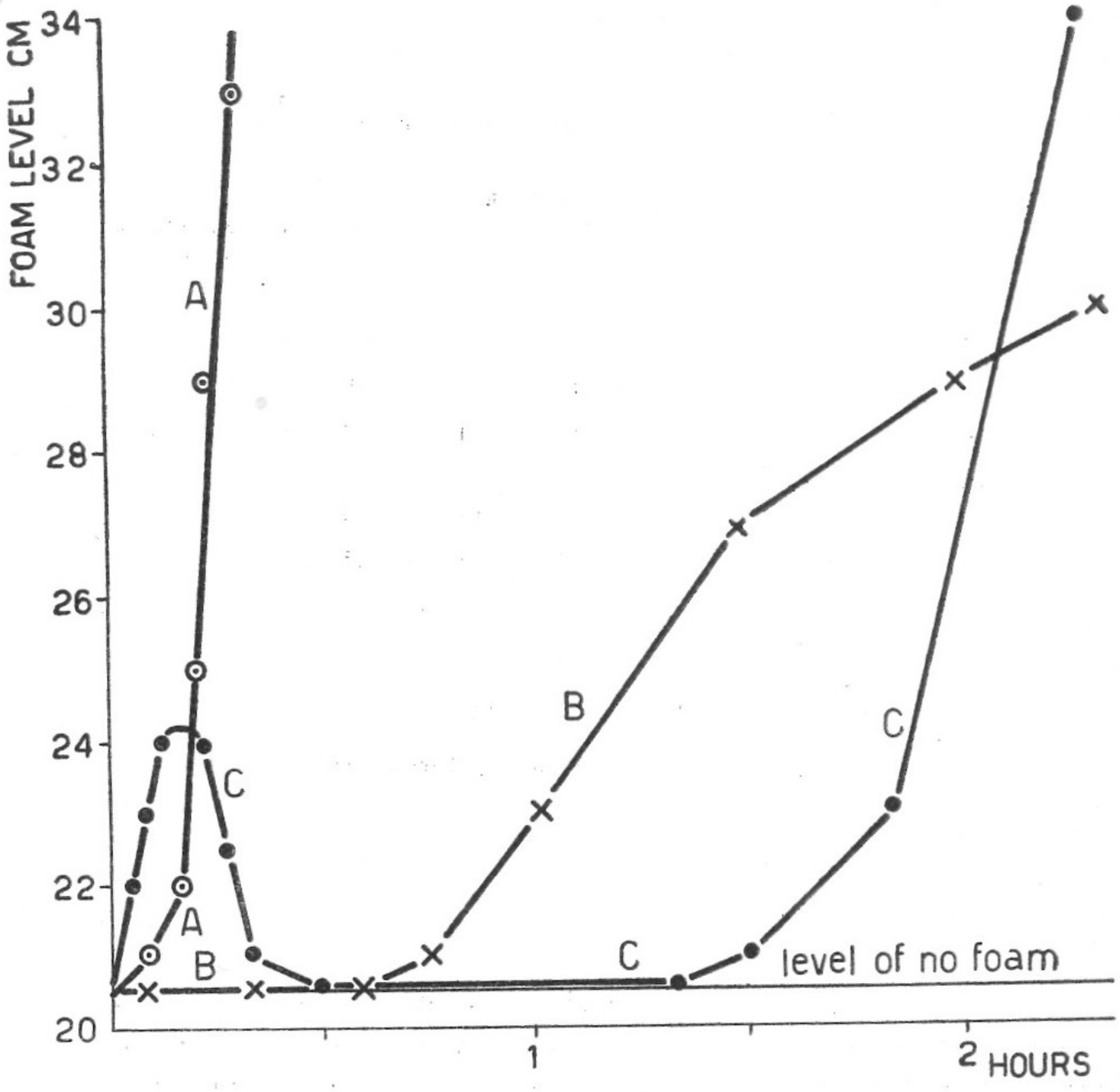

Fig. 5. - Effect of dispersing agent on the antifoam activity of octadecanol.
Aeration and agitation: baffled system, 550 R.P.M., 0.5 vol. air. vol. liq./min.
Foaming liquid: Corn steep medium (pH 7), 5 litres.
Levels recorded during agitation. Antifoams were added before starting aeration and agitation.
A = Levels with 2.5 ml. of 6% octadecanol in paraffin oil.
B = Levels with 2.5 ml. of 6% octadecanol in grape seed oil.
C = Levels with 2.5 ml. of 6% octadecanol in lard oil.

of paraffin oil and petroleum ether (in the proportions 1:1) as the dispersing agents. The influence of the dispersion type on foam inhibition by Antifoam A is shown in Table I.

TABLE I

Effect of dispersing agent on the antifoam action of silicone.

Dispersing agent	Concentration of Silicone		Time for complete destruction of a 6.5 cm depth of foam in vortex system (4).
	In dispersing agent (%)	In medium (ppm)	
Petroleum ether (1)	50	5	No action
Paraffin oil (2)	20	20	10 minutes
Petroleum ether-paraffin mixture (1:1) (3)	20	10	30 seconds
Id. Id. Id.	2.5	5	30 seconds

(1) Dispersion good, very viscous.
(2) Dispersion poor, silicone settled down on standing.
(3) Dispersion excellent, of low viscosity.
(4) Volume of foam with liquid at rest, approximately 1.65 litres.

Comparison of different antifoam agents.

Using the technique described, evaluations have been made of the activities of defoaming preparations containing the following antifoam agents: Alkaterge C, Siotol AF, silicone Antifoam A, octadecanol, grape seed oil and lard oil. The results obtained are set out in Tables II and III.

Effect of mycelium and soaps.

Addition of dried acetone washed mycelium of *P. chrysogenum* to the extent of 1% did not affect the antifoam action, at least in the few cases studied.

The use of oil of plant or animal origin in the penicillin fermentation makes possible the formation of soaps as a result of hydrolysis of the oil by lipase action. Some experiments were therefore carried out in which sodium oleate was added to the medium in order to see what effect such a soap would have on foaming. In the vortex system using corn steep-lactose medium at pH 7 sodium oleate itself had some

antifoam action but it enhanced foaming considerably in the baffled system. The foaming could however be controlled by increasing the amount of antifoam. In an actual experiment, in the baffled system, the enhanced foaming due to addition of a solution of sodium oleate

TABLE II

Suitable concentrations of antifoam preparations for complete foam control in corn steep lactose medium for a period of at least six hours.

Antifoam preparation	Vortex system 900 rpm.			Baffled system, 550 rpm, 0.5 vol. air min.		
	Conc. %	Anti-foam (ppm)	Remarks (*)	Conc. %	Anti-foam (ppm)	Remarks (*)
I. ALKATERGE C						
Alkaterge only	0.01	100	I	0.02	200	S
33% Alkaterge in paraffin oil .	0.024	80	I	0.024	80	I
50% Alkaterge in paraffin oil .	0.016	80	I	0.02	100	I
50% Alkaterge in grape seed oil	0.02	100	S	—	—	U
II. SIOTOL AF						
Siotol only	0.02	200	I, F	—	—	U
50% Siotol in paraffin oil . . .	0.04	200	I, F	0.16	800	I, F
III. SILICONE						
2.5% silicone in petroleum . . ether-paraffin mixture (1:1)	0.16	40	I, F	0.16	40	I, F
IV. OCTADECANOL						
6% octadecanol in grape seed oil	0.05	30	I	—	—	U
6% octadecanol in lard oil . .	0.05	30	I	—	—	U
6% octadecanol in paraffin oil .	0.33	200	I, F	—	—	U
V. GRAPE SEED OIL	0.10	1000	VS	—	—	U

(*) I = Antifoam action instantanious.
S = Antifoam action slow.
VS = Antifoam action very slow.
U = Unsuitable for use (see text for explanation).
F = Slight persistent foaming throughout the period but negligible from the practical viewpoint.

equivalent to 1.0 ml of oleic acid per 5 litres of the medium was completely brought under control by 160 ppm level of Alkaterge in paraffin oil. Extensive investigation of this soap effect was beyond the scope of this paper.

TABLE III

Comparative activities of antifoam preparations in the vortex and baffled systems of aeration and agitation with corn steep-lactose solution as foaming liquid.

Antifoam preparation	Quantity of preparation per 5 litres of medium	Antifoam agent	Complete inhibition of foaming maintained	
			Vortex 900 rpm	Baffled 500 rpm, 0.5 vol. §
	ml.	ppm. (*)	Hrs.	Hrs.
I. ALKATERGE C				
Alkaterge only	0.4	80	> 6.5	2
20% Alkaterge in paraffin oil .	1.0	40	> 2.5	1
50% Alkaterge in paraffin oil .	0.8	80	> 7.5	7.5
50% Alkaterge in grape seed oil	1.0	100	> 8.5	0
II. SIOTOL AF (I.C.I.)				
Siotol only	1.0	200	> 7	0.5
Siotol only	4.0	800	—	1.75
50% Siotol in paraffin oil . .	8.8	800	—	> 6
III. SILICONE				
2.5% Silicone in petroleum ether	8.0	40	> 6	> 6
IV. OCTADECANOL				
6% octadecanol in grape seed oil	2.5	30	> 6	0.75
6% octadecanol in lard oil . .	2.5	30	> 6	1.5
6% octadecanol in paraffin oil .	2.5	30	0.5	0
6% octadecanol in paraffin oil .	33.0	200	> 6	1.5
6% octadecanol in paraffin oil .	66.0	400	—	2
V. GRAPE SEED OIL	5.0	1000	> 9	0

(*) Parts per million by weight octadecanol and silicone; by volume for others.

§ With an air flow of 1 vol./min. in the baffled system times of duration were: Alkaterge C (0.01%) alone, 40 min.; 50% Alkaterge C in paraffin oil (0.02%), at least 8 hours.

Loss of antifoam action. — It has been observed that in the absence of paraffin oil the antifoam action of Alkaterge rapidly diminishes, possibly as a result of the antifoam agent being deposited on the walls of the vessel above the liquid level and thus removed from the system. In fact in the baffled system, after cessation of foam inhibition by an initially effective amount of Alkaterge the foam inhibiting action could be restored by scraping the material deposited on the walls of the vessel back into the liquid, but only when a little paraffin oil (1 ml/5 l of medium) was also added along with the scrapings.

Fermentation trials.

In pilot plant fermentations on a 50 l scale it has been found that Alkaterge C (0.3 ml/l) together with paraffin (2 ml/l) completely inhibited foaming for about 2 days in corn steep medium using the baffled system of aeration. In a similar fermentation Antifoam A at a concentration of 50 ppm also inhibited foaming for about two days. More extensive trials are being made to determine the minimum quantities of antifoam agent necessary in pilot plant fermentations. Octadecanol and Alkaterge C with grape seed oil as the vehicle were quite ineffective in the baffled system with high aeration rates.

Discussion

Nature of the problem of foam control.

Most biological media form stable emulsions with dispersed gas resulting in foam formation on the surface. Mechanical and chemical methods of foam destruction have been suggested. By the chemical method it is possible to cause complete collapse of large masses of air bubbles as soon as they reach the surface. To the authors' knowledge no really successful mechanical device for foam destruction has been described.

Previous work in this laboratory by Chain *et al.*, 1952, has shown that mechanical foam breaking of the type used in the Waldhof fermenter is not very effective for the control of foaming. Both the Waldhof fermenter and that described by Humfeld and Feustel (1945) with mechanical foam breaking elements are really modified vortex systems and it has been shown above that in the vortex system the foam does

not overflow out of the vessel but reaches a steady state, hence in these systems the mechanical foam breaking devices have no real function.

The problem of the mechanism and mode of action of antifoam agents has been attacked in various ways [see Ross (1950) and Pattle (1950)]. It is necessary to stress however, what has recently been said by Matalon (1953) that fundamental knowledge about defoaming is very limited and that there is a considerable need for more work in this field.

Ideal antifoam agent.

The properties of the ideal antifoam agent for a fermentation are as follows; (a) ability to suppress foam instantaneously; (b) long duration of action; (c) absence of undesirable physiological effect.

Instantaneous action by the antifoam is of great importance in the conventional system of aeration and agitation using air spargers and baffle plates. In this system, the level of foaming liquid, unlike in the vortex system, may rise up continuously with agitation and aeration and overflow out of the vessel very rapidly. To combat excessive foaming in an emergency in such baffled systems, the antifoam action of the agent has got to be rapid otherwise foaming could reach the danger point before the antifoam action set in. Because of slow initiation of defoaming action, certain antifoam preparations, e.g. Alkaterge C in grape seed oil, are not of general applicability.

The choice of an antifoam preparation may be conditioned by its physiological activity. It may be objectionable because of certain types of surface action which are known to be toxic for micro-organisms. The antifoam may also have a physiological effect through being metabolized by the culture organism. For instance, the vegetable or animal oils often used in antifoam preparations for the penicillin fermentation are metabolized by the mould. As a result, the pH of the medium is affected presumably through the release of fatty acids into the medium by lipase action, and the constituents of the oil are utilized as carbon sources and have a marked effect on the metabolism of the mould. [See Goldschmidt and Koffler (1950), Rolinson and Lumb (1953)].

The ideal combination of properties, in general, can only be realized by dispersing the antifoam agent in a suitable carrier. A carrier may either accelerate defoaming by an antifoam agent and prolong its duration of action as, for example, paraffin oil acting on Alkaterge

C, or the carrier may show the opposite effect, as observed with Alkaterge C dispersed in grape seed oil.

The mechanism of action of paraffin is not fully understood and needs much further physico-chemical investigations. Dispersion is probably one of the factors involved but not the only one.

Comparison between baffled and vortex systems.

In the vortex system foam formation is much less pronounced than in the baffled system with the same oxygen diffusion. Under the conditions used in the vortex system it was always found possible to achieve a steady foaming state in which foam breaking balanced foam formation even in the absence of any antifoam agent. Such a condition was not realized in the baffled system. The difference is no doubt due to the fact that for a given oxygen diffusion, the volume of air passing through the vortex system is much smaller than it is in the baffled system. Foam breaking in the free agitation system is facilitated by the fact that the foam and liquid are uniformly mixed together in contrast to the baffled system where the foam forms a separate, unagitated layer above the liquid.

In general, with antifoam preparations suitable for use in both aeration systems, the minimum quantity of antifoam agent which suppressed foaming completely in the vortex system was also found to be sufficient in the baffled system but the duration of inhibition was much shorter in the latter. Certain slow acting antifoams such as grape seed oil which successfully controlled foaming in the vortex system were found to be quite ineffective in the baffled system.

From the point of view of comparing antifoam activities, the vortex system, because of the steady foaming state and uniform mixing, has advantages over the baffled system. In fermentation work, when other considerations do not influence the choice, the vortex system is preferable to the baffled system because of the much lower intensity of foam formation and the consequently smaller quantities of antifoam agent required.

Comparison between different antifoam agents.

In Table III the duration of action of given amounts of different antifoam preparations are compared in both vortex and baffled systems of aeration. In Table II are given the amounts of antifoam agents which

will inhibit foaming for at least six hours under the conditions described.

Alkaterge C, Siotol and silicone Antifoam A will all completely inhibit foaming in both baffled and vortex systems provided that the carrier is suitable. Alkaterge and Siotol gave optimal results with paraffin oil as dispersing agent; relatively more Siotol was required to obtain results equal to those with Alkaterge. Alkaterge could be used by itself in both aeration systems, but its action was greatly improved by the addition of paraffin. Alkaterge dispersed in grape seed oil is unsuitable for use in the baffled system though it may be used in the vortex system. One of the advantages of Alkaterge dispersed in paraffin is that, unlike vegetable or animal oils, it does not affect the pH of the medium since it is an oxazoline and cannot be hydrolyzed by lipase to give free fatty acid.

Octadecanol was found to be a very poor antifoam agent. Fig. 5 shows the extent of foam control in the baffled system which could be achieved with this agent dispersed in various carriers. In the baffled system using 6% octadecanol in lard oil foaming could not be controlled more than 90 minutes no matter how much of the antifoam was added. With each new addition foaming was suppressed only momentarily but it resumed immediately after and became uncontrollable in a short time. With grape seed oil as dispersing agent the resumption of foaming was slow and gradual, and foaming could be controlled by repeated additions of the antifoam. In so far as speed of action is concerned, lard oil appeared to be superior to grape seed oil as dispersing agent. When paraffin was substituted for the lard or grape seed oils, foam control for a short period could be achieved in the baffled system only, by using an excessive quantity of the preparation (Table III) Good foam control could be achieved in a vortex system with octadecanol in lard oil or grape seed oil and also with a relatively much larger amount of octadecanol in paraffin. In the two former cases the oils themselves must augment the antifoam action

Consideration of the behaviour of these preparations containing octadecanol coupled with the observation that it precipitates out in the carrier, all point to the fact that the poor activity is due to lack of adequate dispersion. Problably, octadecanol could be made into a useful antifoam if a much more effective dispersing agent were found.

It has been established than an oil like that of grape seeds can be a good antifoam agent in the vortex system. The oil is very slow-acting;

it is conceivable, however, that a suitable carrier could be found which would speed up its defoaming action as paraffin does that of Alkaterge and thus give a preparation effective in the baffled system.

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