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United Nations Industrial Development Organization

Regional Seminar on Industrial Applications of Microbiology in Pharmaceutical Industry

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# PRODUCTION OF OXIDATIVE FERMENTATION INCLUDING ACETIC ACID\*

by

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The main subject of the Seminar in Havana, Cuba, from 2-9 July 1979 is the industrial application of microbiology in the pharmaceutical industry. It deals in particular with the production of antibiotics. I have no personal experience in this field and my knowledge on the subject is limited to information taken from textbooks.

The reason that I have been asked to prepare a 'paper' can therefore only be attributed to the fact that I am the inventor of the *submerged vinegar fermentation process* (Annex 1) and have some experience as to how an invention made in a laboratory (Annex 2) was gradually adapted for practical use and the process of submerged fermentation then introduced into the vinegar industry, which formerly only had an empirical knowledge of surface fermentation either by the Orleans-process or the quick vinegar process.

The vinegar industry is chiefly a small-scale industry, a cross between a business and an industry, which lacks the many advantages of trained staff and modern equipment enjoyed by the large-scale pharmaceutical industry. It also deals with the manufacture of a cheap product for every-day use, edible vinegar, to which no development costs can be added as was, and sometimes still is, the case with new antibiotics.

It should also perhaps be mentioned that, shortly after its adaptation for industrial use, the submerged acetous fermentation process was also introduced into the 'developing countries' (Annex 3).

For these reasons the equipment and the development process had to be kept as simple and 'fool-proof' as possible.

Some of the techniques originally developed for the technology of submerged acetous fermentation and some aspects which were considered when factory-works were established throughout the world could also be applied to a discussion on the production of pharmaceuticals by means of submerged fermentation.

Vinegar fermentation differs from all other submerged fermentations in that the alcoholic substrate and the final product, the vinegar as a watery solution of 10–14% acetic acid, show high osmotic pressure such as a 40% glucose solution, a milieu which makes the survival of life and, in particular, the increase of microorganisms, extremely difficult. Our research work also showed that the production of vinegar of a standard concentration by means of the submerged fermentation process could only succeed if the vinegar bacteria were aired thoroughly in all parts of the mash and if there are no areas in the contents of the vessel left unaired. If the aeration is interrupted for even a few seconds, most of the bacterial culture will die.

Sufficient aeration can be easily achieved by means of glass frits at the lower end of the fermenting pipe through which the air is pressed. The aerators formerly used proved to be inadequate for practical use and a new, self-aspirating aerator had to be specially manufactured. This model, a specially constructed hollow agitator surrounded by a stator, was further developed by the machine-factory Heinrich Frings in Germany (Annex 4) and the largest types can now aspirate 1800 and 2400 m<sup>3</sup>/h against a 4 m liquid flow. Driven by a flange-mounted electromotor (Annex 5), the rotor turns at a rate of 1450 or 1750 rpm, aspirates the air after overcoming the hydrostatic pressure of the liquid flow in the fermenter and forms small bubbles of almost equal size.

In this research work it was particularly important to carefully observe the way in which the oxygen was passed to the air bubbles in the liquid. Oxygen is hardly soluble at all in water. With atmospheric pressure and at a temperature of  $30^{\circ}$ C only 4-5 ml O<sub>2</sub>/l are dissolved in nutritive solutions, whereas the oxygen requirements for microbial processes lie between 500 and 5000 ml  $O_2/I$ , h. By measuring the oxygen concentration in the liquid, either by using a simple, chemical sulphite method or by means of the more expensive paramagnetic, polarographic or galvanic measuring devices, it was found that there was often no parallel to metabolism or increase of the microorganisms. Measuring the size of the air bubbles in many areas of the fermenter proved to be very helpful for the method of submerged fermentation and for the construction of a suitable aerator. The method proved by E.K. Todtenhaupt, Chem. Eng. Techn. 43, 336 (1971) consists in aspirating the aired liquid through a capillary tube, sending a ray of light directed onto a phototransistor to a certain point on the capillary tube and converting the different refraction of the light from the air and water through the phototransistor into an electric signal which is proportional after altering the length of the air bubble. The test is made with 'normal bubbles' rising from a nozzle. Dr. Ebner at Frings has taken up this principle and developed a device for measuring bubbles with which the aeration in different areas of the fermentation contents can be measured fairly easily. - H. Ebner in H. Dellwig (publisher): 3. Symposium on Techn. Microbiology, Berlin 1973.

As a result of extensive experiments, special calculations and by controlling the bubblemeasuring device, aerators could be constructed. The largest model is now Type 12000 which, in a giant fermenter containing 80 m<sup>3</sup> of liquid, provides an aeration resulting in the formation of the same size and number of bubbles, calculated at the same pressure and measured at different radii and heights in the fermenter. The average diameter of air bubble is about 1 mm and there is very little variation from this size. No other aeration system today can produce such even and thorough aeration which is a prerequisite for the manufacture of vinegar of up to 14% acetic acid content, although it must be admitted that vinegar of a lower concentration can also be produced using other modern aeration systems.

If the effect of the even distribution of fine air bubbles in the fermenter-substrate is a factor of prime importance, the power requirements for this distribution of air is naturally another point which should be included in discussions about profitability, particularly in the manufacture of a cheap product like vinegar. In modern development methods the figures are 0.8–0.9 kW h for the distribution of 10 m<sup>3</sup> of air under normal conditions. I will later come back to the point that the method of thorough aeration specially developed for the submerged vinegar fermentation processes can be equally well applied to other aerobic submerged fermentation processes and has led to unexpectedly good results.

For the moment, however, we are talking about vinegar fermentation. It was noticed that in some natural raw materials used for the production of vinegar such as malt mash etc. there is a heavy foam formation. This was also the case when parts of the bacterial flora died due to irregularities during the fermentation process, such as sudden changes in concentration or a disturbance in the aeration. The chemical foam-removers formerly used in the manufacture of antibiotics were not allowed in the production of edible vinegar. A mechanical foam-removing device was therefore developed which can also be used for processes other than vinegar fermentation wherever foam problems occur, such as in cellulose decomposition and in numerous other cases.

Vinegar fermentation is a semi-continuous process by which as much ready vinegar is taken from the contents of the fermenter with a remaining alcoholic content of 0.2–0.3% and enough alcoholic mash added to ensure that the amount does not exceed the 5.5% ethanol which is toxic for the bacteria.

The process of removing the finished product and adding new mash can be automised if it is controlled by the 'alcograph', an alcohol-measuring device, which, in two samples of continually refilled boiling liquid measures the difference in temperature between the gas-phase and the non-alcoholic liquid phase with thermoelements and converts them into impulses for the control. The alcograph can naturally not only be used as a measuring and controlling device in the production of vinegar but also in all cases where the concentration of substances with greater volatility than water is to be measured. The example of alcoholic analysis in the production of yeast will be dealt with later on.

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The continuous process of measuring the alcohol content with the alcograph also greatly facilitates the process of continuous submerged vinegar fermentation. It was proved that the increase of the vinegar bacteria in more than 10% acetic acid concentration was not adequate for continuous operation. The continuous process is therefore at present limited to the processing of mashes with a lower alcoholic content (8–10%). The addition of mash is so arranged that an alcohol content of 0.5–0.3 vol.% is maintained in the fermenter. If an alcograph is used, it is still possible to operate at 0.3% (Annex 6).

With regard to the microorganisms themselves used in submerged acetous fermentation, the Genus Acitobacter belonging to the pseudomonoaceae, there is certain scepticism today concerning the classification attempts of past decades — T. Asai, Acetic Acid Bacteria, University of Tokyo Press, Tokyo 1968 and Shimwell, Atonie von Lesswenholk, 7. Microbiol. Serol. 25, 49 (1959). In practice it is not as important to use a particular species as to ensure that the existing acetobacterial cultures are kept in the high-grade mash with few natural substances necessary for practical use, so that they can be re-bred and selected. It is doubtful whether previous mutations are important. At least the application of chemical or physical mutagens have brought no improvement in the adjustment to the necessary milieu. Even a temporary reduction in the concentration of the mashes can permanently impair the qualities of the cultures.

The problem of the initial inoculation in newly-established works, particularly in developing countries where there is a lack of microbiologically trained staff, is a difficult one. We overcame this problem by sending large amounts of the products of a submerged fermentation process containing app. 10% acid and 2% ethanol to be used as an initial inoculum solution in recently established fermentation equipment. Naturally most of the bacteria died due to the long-term lack of oxygen but a small amount survived with the remaining air and, after the aeration had been switched on they could breed within days or weeks.

Another method was to dip large strips of cloth coated with agar in high-grade mash, to inoculate them and to breed a thick surface flora in a sterilised, aired thermostat. These agar plates could then be rolled and packed sterile, but with air holes, and sent by airmail to newly-established factories throughout the world. With the necessary adjustments, this method could also be used for the inoculation of fermenters for the production of pharmaceuticals. The use of lyophilised cultures of acetobacteria is not impossible but usually presents great difficulties because of the deterioration in the practical performance of the culture and this could only be overcome after a long process of selection.

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In comparing submerged vinegar fermentation with other microbiological industries it should be stressed that the main difference lies in the fact that in vinegar fermentation the aeration of the cultures – also that of the inoculum – is of major importance, whereas the infection from outside microorganisms is unlikely considering the high concentration of the mash. In other submerged technical processes the aeration of the inoculum solution is less important but in a neutral milieu infection from outside constitutes a serious problem. The production of yeasts for breeding and for baking which is done in an acidified milieu of pH 3–4, lies roughly between these two extremes.

I do not wish to go into detail about the problem of nutrients, which is not only important in the production of vinegar from ethanol but also in the case of some complex, natural mashes where supplementation through certain, missing components is required. Every submerged fermentation process has this problem. It is ,however, particularly critical in the manufacture of vinegar from pure ethanol, firstly, since only a certain, limited amount of nutrients can be added so as to produce the normal, colourless, crystal-clear spirit vinegar and, secondly, because the low cost of spirit vinegar does not permit the addition of expensive supplementary nutrients. In the submerged production of pharmaceuticals neither factor is of particular importance. However, a scarcity and rise in price of the good, standard nutrient mixture of corn steep liquor and yeast autolysate used up till now could also make a more careful selection of essential organic natural substances and trace quantities of elements necessary.

Under specific economic conditions it also proved an interesting experiment to manufacture *glacial acetic acid* for chemical purposes from alcoholic liquids by means of our newly developed vinegar fermentation process and the ensuing continuous extraction of the vinegar by solvents which boil at a lower temperature such as ethyl acetate and finally fractioned distillation of the extracts. The technology of the extraction and distillation process was already known.

Whereas glacial acetic acid can be produced on a large scale by the catalytic oxidation of alkanes or by the catalytic oxidation of ethanol through acetaldehyde or, eventually, if an outdated process is used, through acetylene or acetaldehyde, the factory costs are too high to justify the production of only 1–5 tons per day of glacial acetic acid. In countries with cheap carbohydrates the production of alcohol by means of acetous fermentation may be interesting from the economic point of view. In conjunction with the firms H. Frings and Zahn & Co., Hamlin, plants with a capacity for 1–5 tons of glacial acetic acid per day were set up in Formose, the Philippines, Turkey and Spain.

The great experience gained by us - and I am referring here not only to the inventors but also to the manufacturer H. Frings - through submerged vinegar fermentation naturally prompted

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us to apply this knowledge to other submerged fermentation processes. Theoretically, we believed it possible to apply this knowledge to every process in which bacteria is used. Even for large concentrations of bacteria which should, technically speaking, be adequate for a quick sequence of reactions, due to its small size the bacterial mash remains of a similar viscosity to water and it could safely be assumed that in such a milieu the thorough ventilation system has the same effect as in the submerged vinegar fermentation process. An earlier known example was the oxidative fermentation of glucose to gluconic acid by means of submerged acetobacter stock cultures.

We were, however, afraid that all the advantages of the thorough ventilation system would be rendered useless by the coalescing of the fine gas bubbles if in the submerged phase of the production of yeast for baking or for fodder purposes such large concentrations of cells much bigger than the bacteria were to appear — such as saccharomyces, candida and torulopsis — that in the final stage of the fermentation, whether in batch or in continuous process, a thick pulp is formed. The same thing could occur with streptomyces, aspillerges and penicillin types and here there was the added danger of mechanical damage to the cells as a result of the shearing action of the rapidly turning rotor and the adjoining stabiliser ring.

But particularly when dealing with biotechnology there should be no preconceived ideas which can hinder progress; it is much better to rely entirely on the experiment itself - in this case for production purposes.

Ventilation experiments in the production of baker's yeast which were carried out in a German yeast factory showed the great advantages of thorough aeration with our self-aspirating aerator. All previously known processes and devices for yeast production had in common the fact that the aeration devices in the fermenters required compressed air. This is either pumped in through distribution pipes (steel tubes) perforated in several places or through rotating bodies of varying constructions. It is difficult to achieve thorough aeration through a steel pipe as the holes cannot be kept very small. In the case of non-aspirating rotating bodies, which let in the air to only a very limited part of the fermenter, violent shaking movements of the water must be made to ensure that the oxygen admitted is distributed as evenly as possible over the whole contents in the fermenter. It is very important to ensure even and thorough ventilation as this has the undisputed advantage of making maximum use of the yeast fermenter. In 1969 a series of tests was therefore made with a Frings-aerator driven by a 90 kW motor and rotating at a rate of 1.450 rpm which continuously aspirated 1000 N m<sup>3</sup> of air through the air passage and a Delbag-filter and distributed it evenly and thoroughly in the contents of a fermenting vat measuring 3.65 m in diameter, 5.60 m in height and with a

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capacity of 59 000. At the end of a charge the container is about 2/3 full. In this particular case a secondary cycle was enough to eliminate the reaction heat and to keep the temperature in the fermenting-room at  $30^{\circ}$ C. Using a self-timing pump, fermenting liquid is taken from the bottom of the fermenter, conducted over a plate-cooler of 55 m<sup>2</sup> and led back into the tank again.

An automatic fermentation oil device keeps the foam at a steady level. The pH is measured constantly and by means of the alcograph the volatile components (mainly ethanol) in the fermenting solution are continuously and automatically registered. Molasses, which had been diluted to a 35-37% concentration, acidified with sulphuric acid to pH 4.5 and sterilised at  $95-97^{\circ}$ C, was used as a raw material. 25% ammonia – or ammonium sulphate – and monoammonium phosphate were used as nutrients.

The molasses intake is programmised and allows for the H-value - a log-phase - the logarithmic increase of the yeast until it is limited by the aeration and the ensuing linear phase.

The opinion of the experts differs over the value of a small sugar surplus which results in the formation of volatile matter, mainly ethanol. Depending on the amount of volatile substances from 0.03-0.05%, the supply of molasses can be controlled by the alcograph in the last 2/3 of the charge and this has the advantage over the programmed method that the individual differences during the fermentation process can be allowed for to a great extent. Four series of tests of at least 75 charges each were carried out under different testing conditions and using different yeasts. 2.2–2.4 Nm<sup>3</sup> of air per kg of yeast was needed. An average of 10-12% of oxygen was found in the waste air expelled from the fermenter.

Annex 7 shows from another series of tests the differences in the aeration system. Since then, as can be seen from Annex 8, 27 industrial plants have been set up which have confirmed the experience gained from the first experimental plant and have served to prove the advantages. It is desirable to aim at maximum cell concentration in microbial breeding, e.g., 35–40 g dry matter per liter of fermenting liquid. This can be achieved by ensuring a thorough passage of air with an optimal use of oxygen. The high cell concentration not only leads to a better utilisation of the fermenter volume. It has the added advantage that only small amounts of sewage appear the removal of which is the main headache of the fermentation industry. The finished yeast product has excellent qualities.

While on the subject of "sewage", it should be mentioned that a self-priming, air-aspirating aerator which has the excellent qualities indispensable for the production of high-grade vinegar, can also naturally be used for many processes apart from that of manufacturing yeast, in which the importance of the passage of matter from the gas phase to the liquid phase is stressed. Such

problems occur all over the world in biological sewage purification systems in which organicchemical pollution can be reduced by aerobic microorganisms. The better the aeration, the greater the cell concentration will be and the purification of the sewage will then also be possible in an even smaller area. As we are dealing here with processes which, in general, operate at a lower concentration and neutral solution, high-grade, acid-proof special steels can be avoided. The so-called "submersible aerators" as shown in Annex 9 are available in the models shown in Annex 10. Annex 11 gives a summary of the submersible aerators which are available today.

By no means all of them are used for sewage treatment in towns etc. Several serve the biological purification of factory-sewage. In such cases the sewage should be kept separate as far as possible as it is then more likely that a microorganism culture can be formed which can reduce certain water impurities or at least similar substances.

Some of them are used in water-conditioning. The introduction of oxygen containing ozone plays a major part in the purification of water for drinking and for bathing purposes.

I hope that my lecture has shown that, based on the necessity for a special kind of fermentation process, we must develop another process for our requirements - that of thorough aeration - and then apply this as far as possible to the problem of the transition from the gas phase to the liquid phase, which is so important both in fermentation and in chemical processes.

I also hope, however - and this seems to me to be more important still - to have proved that we must always aim at simple solutions. Particularly in the case of the desired industrialisation of the developing countries, processes and equipment which are as simple and 'fool-proof' as possible will have to be selected.

As concerns more complicated fermentation processes — and the production of pharmaceuticals by microorganisms belongs to this category — the first step will probably be to set up a special microbiology centre which will be responsible for the procurement and upkeep of cultures and which can sometimes offer assistance regarding the process of cell-increase from the lyophilised or agar-sample of the cell-culture to production fermenter or when outside infections other disturbances occur. Without using too complicated equipment we must ensure smooth operation even with workers who are only semi-skilled and, at least at the beginning, still inexperienced. The introduction of the submerged vinegar fermentation process was often confronted with such problems and it was precisely in the lesser developer' countries that the most modern plants were established, whereas in the fully industrialised countries it is taking much longer to replace the surface fermenting equipment which is still in use today.

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## (1) Production of carboxylic acids,

first need for submerged vinegar fermentation. Prior. from 3.5.1949

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Argentina	Pat. No. 74.847	Australia	Pat. No. 151.162
Austria	Pat. No. 179.283	Belgium	Pat. No. 493.100
Brazil	Pat. No. 42.707	Canada	Pat. No. 510.417
Denmark	Pat. No. 80.147	Finland	Pat. No. 26.761
France	Pat. No. 999.750	Germany	Pat. No. 929.543
England	Pat. No. 686.849	Ireland	Pat. No. 19.543
Italy	Pat. No. 473.916	Norway	Pat. No. 85.666
Portugal	Pat. No. 27.844	South Africa	Pat. No. 9.763
Spain	Pat. No. 191.519	Sweden	Pat. No. 158.002
Switzerland	Pat. No. 279.616	Sweden	Pat. No. 158.002

#### (2) Fermentation process

Prior. from 3.5, 1949

France Pat. No. 1, 004.999

### (3) Combined submerged and surface fermentation Prior. from 13.12.1949

#### (4) Equipment

Prior. from 2.2.1952

Pat. No. 189.148 and other countries Austria

#### (5) Foam-remover

Prior. from 31.12.1952

Pat.No. 189.149 and other countries Austria

# Patents by the firm H. Frings, Fed. Rep. of Germany

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(1)	Germany	Pat. No. 898.134	Prior. from 25.11.1949
(2)	Germany	Pat. No. 949.224	Prior. from 18.8.1950
(3)	Automisation	of vinegar fermentation	
	Germany	Pat. No. 1,063.561	Prior. 1957
(4)	Alcograph		
	Germany	Pat. No. 1,264.108	(1965)
(5)	Semicortin, fe	rmentation through 12% vin	legar
	Germany	Pat. No. 1,517.879	(1966)
(6)	Aerator		
	Germany	Pat. No. 1,667.042	(1967)

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Normal types NT Special types ST	Number	Processing capacity liter alcohol/year	Production capacity liter vinegar 10%/year
75 NT, ST	50	1 611 175	15 847 047
150 NT	89	4 871 850	47 387 932
. 300 NT	1 00	10 948 300	107 677 002
400 ST	-	219 000	2 155 398
600 NT, ST	101	22 074 000	216 766 218
1 200 NT	51	22 27% 000	218 550 024
	392	61 999 825	603 883 621

Exported FRINGS-Acetators - as per 31.12.1978

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ANNEX 3

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<b>J FRINGS-Acetators</b>
Exported

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Construction of the second sec	Population	Exported	Exported F RINGS-Acetators	
	in millions	Number	Processing capacity liter alcohol/year	Production capacity liter vinegar 10%/year
1. USA	212	4.7	11 525 250	113 137 856
2. France	51,5	60	9 0-13 0CO	88 903 990
3. Japan	1 08	19	3 937 500	38 704 266
4. West Germany	62,1	37	3 820 950	37 565 246
5. Mexico	56	10	3 111 750	30 528 625
6. Spain	34	4	2 365 250	23 199 790
7. Italy	55	29	2 673 625	26 313 817
8. Egypt	35	Ŷ	2 628 600	25 864 776
9. Great Britain	56		2 164 575	21 206 529
10. Canada	22	10	2 CB0 500	20 476 201
11. Venezuela	12	0	1 523 675	14 020 200
12. Philippines	41	4	1 204 500	11 853 690

	Population	Exported F	Exported FRINGS-Acetators	
Country	in millions	Number	Processing capacity liter alcohol/year	Production capacity liter vinegar 10%/year
13. Brazil	105	e	1 090 500	10 684 792
14. Algeria	15	Ŷ	1 086 000	10 591 194
15. Switzerland	6,3	10	1 085 875	10 687 181
16. Netherlands	13,5	7	000 186	9 606 393
17. Portugal	9'6	10	926 250	. 9 067 543
18. Turkey	37	7	P76 000	8 621 592
19. Hungary	i 0, 5	*	876 000	8 621 592
20. Australia	, 13,2	ω	848 625	§ 352 168
21. Poland	33,5	4	766 500	7 543 893
22. Austria	7,4	6	738 563	7 262 856
23. Argentina	24,5	4	657 000	6 455 194
24. Southafrican Union	25	4	593 250	5 741 548

Exported FRINGS-Acetators - as per 31.12.1978

Country	Population	Exported F	Exported F RINGS-Acetators	
	in millions	Number	Processing capacity liter alcohol/year	Production capacity liter vinegar 10%/year
25. Belgium	2'6	S	520 125 -	5 119 070
26. Greece	8,8	Ŷ	492 150	4 803 195
27. Finland	4,7	<b></b>	438 000	4 310 796
28. Norway	3,9	5	328 500	3 233 097
29. Yugoslavia	21	5	321, 000	3 140 200
30. Colombia	24	7	300 825	2 940 447
31. Israel	3,2	ŝ	246 375	2 424 822
32. West Malaysia	12	~	219 000	2 155 398
33. Senegal	3,8	7	219 600	2 155 398
34. Uruguay	2,9	-	219 000	2 155 398
35. Jamaica	2	£	191 625	1 335 977
36. Peru	14,5	ĸ	191 625	1 885 977
37. Cuba	8,5	2	1.6.4 250	1 616 548

Exported FRINGS-Acetators - as per 31.12.1978

as per 31.12.1978
rted FRINGS-Acetators
Expor

Country	Population	Exported F	Exported FRINGS-Acetators	
	in millions	Number	Processing capacity liter alcohol/year	Production capacity I vinegar 10%/year
38. Dom. Republic	4,6	2	164 250	1 616 548
39. Angola	6,3	<b>,</b>	1 09 500	1 077 699
40. CSSR	14,7		109 500	1 077 699
41. Iran	32	m	109 500	1 077 699
42. Ireland	c	-	109 500	. 1 077 699
43. Mali	5,2	7	32 125	808 275
44. Cyprus	0,6	<b>м</b>	67 375	663 104
45. Chile	, 9,8	-2	65 700	646 619
46. Guadelupe	0,3	7	54 750	538 849
47. British Guyana	• •		54 750	538 850
48. Burma	29	~	27 375	269 425
49. Reunion	0,4		27 375	269 425
50. Thailand	40		27 375	269 425
51. Tunisia	5,3		27 375	269 425

The FRINGS-Aerator is a specially constructed agitator surrounded by a stator (Fig.1) (46) (47). It consists of a hollow body with six radially arranged air vents open against the direction of rotation. In front of these, vertical blades are arranged in the direction of rotation.

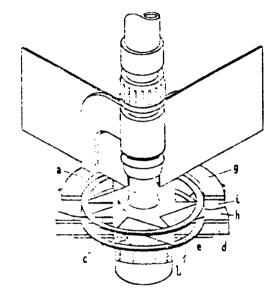


FIG.1. FRINGS-Aerator.

a. Hollow body

e. Upper rotating ring

- f. Lower rotor ring
- b. Air vents
- g. Upper stator ring
- c. Working surfaces
- h. Lower stator ring
- d. Stator
- i. Stator blades

Typ type type tipo	Baureihe model sei série 7 serie de n	1	Gasmenge gas flow <sup>5</sup> quantité de cantidad d	e gaz <sup>s</sup>	Motorie motor moteur moteur	9
			m³/h	cft/min.	kW	HP
25	N		4 10	2 — 6	0,75	1
75	N		8 — 25	4 14	2,2	3
150	N	r	16 — 50	9 — 29	3,0	4
300	N	rs	40 — 100	23 58	5,5	7,5
<b>6</b> 0 <b>0</b>	N	r	80 — 200	47 — 117	11	15
1 200	NI	r s	150 — <b>350</b>	<b>88</b> — 206	22	30
2 000	N	r	250 — 600	147 — 353	40	55
3 000	N	r [	400 — 800	235 - 470	60	80
5 C <b>O</b> O	N		800 — 1 500	470 — 880	100	130
7 000	N		1 100 — 2 000	<b>64</b> 7 — 1 180	140	208
<b>9 0</b> 00	N		1 400 — 2 400	820 - 1 400	180	240
12 000	N		1 800 — 3 000	1 060 — 1 760	240	320

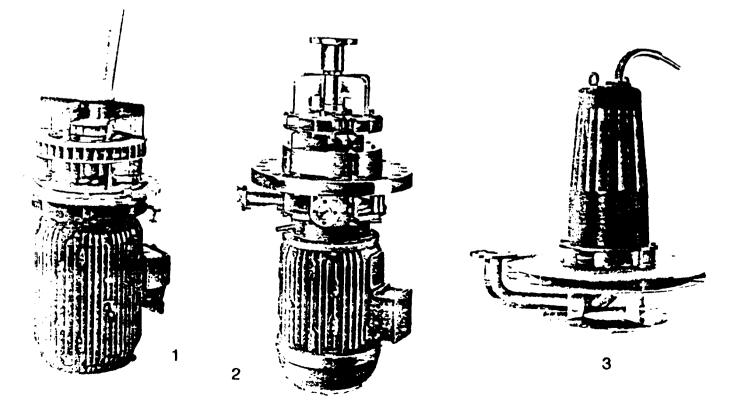
**ANNEX 5** 

1 Werkstoff 4571 oder Kunststoff für alle mit der Flüssigkeit in Berührung kommenden Teile. 2 Angesaugte Gasmenge abhängig von Füllhöhe, Oberflächenspannung und Viskosität der Flüssigkeit. Regelung der Gasmenge über einen größeren Bereich durch Verwendung polumschaltbarer Motoren mögl.cn. 3 Effektiver Energiebedarf 0,4 bis 0,8 kWh je 10 m<sup>3</sup> Gas bei Tauchtiefen von 2 bis 4 mWS.

<sup>4</sup> All parts in contact with liquid in stainless steel or plastic <sup>5</sup> Quantity of gas aspirated dependent on filling height, surface tension and viscosity of liquid. Control of quantity over a wide range possible using multispeed motors. <sup>6</sup> Effective power requirements 0.4 to 0.8 kWh for every 10 m<sup>3</sup> of gas at 2-4 meters submergence, or 1 HP - hr for every 700-350 cft.

7 Matière 4571 ou plastique pour les éléments en contact avec le liquide. <sup>8</sup> Quantité aspirée de gaz en fonction de la hauteur de remplissage, tension superficielle et viscosité du liquide. L'utilisation de moteurs à nombre de pôles variable facilite le réglage de la quantité de gaz pour un rayon de grande dimension. <sup>9</sup> Demande d'énergie effective 0.4 à 0.9 kWh par 10 m<sup>3</sup> de gaz à des profundeurs de 2 à 4 m de CE.

10 Materia 4571 o plástico para todas las piezas que llegan en contacto con el líquido. <sup>11</sup> Cantidad de gas aspirada en función de la altura de relleno, tensión superficial y viscosidad del líquido. Por la utilización de motoras de polos invertibles se puede regular la cantidad de gas sobre un alcance más grande. <sup>12</sup> Energía necesaria efectivamente 0.4 a 0.8 kWh por 10 m<sup>3</sup> de gas con profundidades de inmersión de 2 a 4 m de CA.



#### Continuous process fermenter

## Table 1. Yield (average values)

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Acetator	Alcohol processing per day (liter)	converted to ready vinegar with 5% acid per day (liter)
Continuo 150	150	2750- 3250
Continuo 300	300	5500- 6500
Continuo 600	600	11000-13000
Continuo 1200	1200	22000-26000

#### Table 2. Consumption figures (average values)

Acetator	Power consumption per day (kW/h)	Consumption of cooling temperature of 30°C at a 12°C (I/h)	water at a fermenting I water temperature of 24°C (I/h)
Continuo 150	70	1 000	C 729
Continuo 300	130	2 000	7 000
Continuo 600	240	4 000	14 000
Continuo 1200	460	8 000	28 000

 Table 1.
 Characteristic data of two batches of baker's yeast production obtained in two fermenters equipped with different aeration systems

1

	Fermenter 1 (non aspirating)	Fermenter 2 (Fring <b>s-</b> Aerator)
Total fermenter capacity in gallons	15,000	15,000
Diameter of fermenter in fee	t 12	12
Filled to % of capacity at time of measurement	60	67
Air flow in cbm/hr	1,400	1,0 <b>50</b>
Aeration rate in v/v/min	0.69	0.41
Duration of batch in hrs	15	14
Dry cell matter in kg produced per batch	1,540	1,930
Yield expressed in g dry cell matter per kg of molasse	s 252	266
Cell concentration at time of measurement in g/ltr of dry cell matter	26	43
C in atmospheres	0.003	0.001
C <sup>-</sup> in atmospheres	0.163	0.127
Rate of oxygen absorption in ml 02/100 ml/hr	192	221
Oxygen absorption coefficien in ml 0 <sub>2</sub> /100 ml/hr/atm	it 1,200	1,740
Oxygen utilization efficiency in %	22	39
Aeration efficiency Er in liter 02/watthr	1.0	1.0
Power input in watt/liter fermenting vol.	2.1	2.3
Power consumption expressed as watthours/g dry cells	d 0.6	0.58

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# LIST OF REFERENCES FOR YEAST AERATORS

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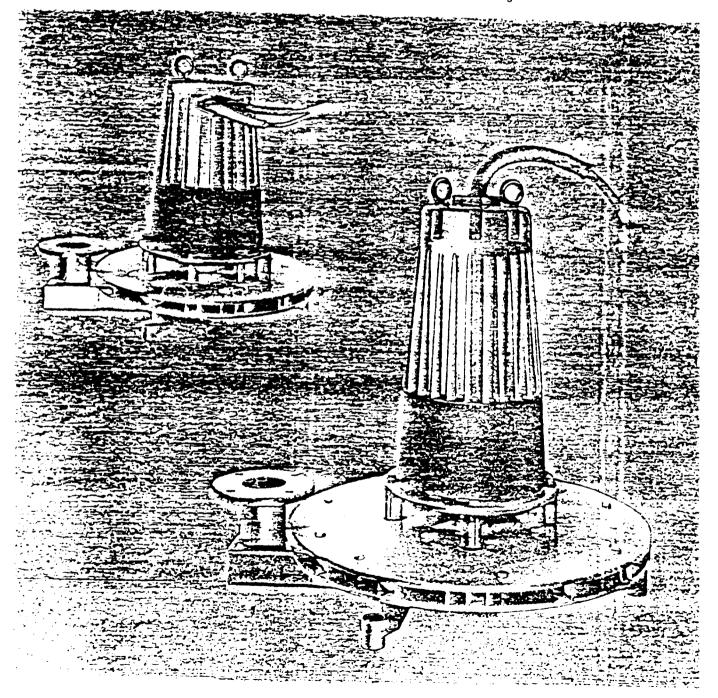
CLIENT	TYPE	REU MATERIAL	PRODUCT
Lindenmøyer, Germany	5000	Beet Molasses	Baker's Yeast
Standa <b>rd Brands, Inc.,</b> USA	4 × 3000	Cane Molasses	Baker's Yeast
Fernentation Industries Pty, Australia	5000	Cane Molesses	Baker's Yeast
Anchor Yeast, Rhodesia	7000	Cane Molasses	Baker's Yeast
Propan, Portugal	2 × 9000 3000	Beet/Cane Molasses	Baker's Yeast
Fáprica de Levadura,			
Spain	12000	Beet Molasses	Baker's Yeast
Anheuser-Busch, USA	Pilot Plant	Cane Molassea	Feed Yeast
Salico, CSSR	2 x 9000 5000	Enet Molasses	Baker's Yeast
Budapester Alkohol- Induștrie, Hungary	3 × 1200 9000, 2000	Baet Molasses	Baker's Yeast
Uhde, Höchst, Germany	Pilot Plant	Hydrocarbon	Feed Yeast
Pleser, Germany	12000	8eet Molasses	Bakar's Yeast
Mossul, Iraq	2 × 9000 3000	Cane	Baker's Yeast
Trabisov, CSSR	<b>2</b> × 1200 5000	Beet	Baker's Yeast
Polimax, Warschau	<b>20</b> 00	Various	Baker's, Feed Yeast



# Tauchbelüfter

# für Abwasserreinigung und Wasserkonditionierung

SUBMERSIBLE AERATOR for sewage purification and water-conditioning



						- OXYgen	Oxygen addition	
					after sulphit	after sulphite exidation	after pure water test	water test
Type	Motor	Air	6	<b>6</b>	in basins with © g	in basins with $O_A$	in basins with Øa	in basins witn ⊘A
	κ	たいのこと	E	E	kg/h	kg/h	kg/h	kg/n
150 T	n	ŝ	2,6	9	ស	ດ	2,5	S
303 T	5,5	C) S	3.5	80	10	15	S	10
600 T	1	130	0, •†	10	20	32	10	8
900 T	18,5	230	4,0	÷	30	50	15	30
1200 T	22	383	10	12	4	65	22	\$
18C0 T	37	550	ີດ	1	ß	06	35	60
2400 T	55	750	ω	0	80	125	50	80

Table 2

Fig.4				Ţ		 		
Weight	kp	110	225	315	370	585	700	830
MN	EC	50	SO	001	125	150	150	200
14	Ē	500	230	<u>.</u>	630	0 C C C C C C	639	1050
w	[: [:	uos	405	02+	425	575	575	550
٥	E	180	190	230	230	245	515	270
υ	E	755	740	061	920	099	1270	1250
,	Abe	150 T	300 T	600 T	- COS	1200 T	13CO T	2-00 T

Changes in construction, measures and weights reserved.

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ANNEX 10

Table 1

- 24 -

HEINRICH FRINGS + 53 BONN 1 + Jon	cs-Cahn-Str. 9 · Te	. (0 22 21) 63 15 71
	Stück Units	Туре
Brennereigenossenschaft Schönsee	1	300 T - VG
Messis. Sayer, Leverkusen	10	1200 T - VA
Messrs. Alliance, Singapore	1	150 T - VG
Gemeinde Hinte	2	500 T - VG
Scennereigenossenschaft Klessing	1	300 T - VG
Stadt Burg e.F.	- 3	600 T - VG
Gemeinde Hochdarf	1	300 T - VG
Geneinde Moormerland	•	-1507 - VG
Pessie. Dahmen	2	1200 T - VG
Gemeinde Jade	3	600 T - VG
Toray Engineering, Tokyo		1200 T - VG
Kurita Industries, Tokyo	1	300 T - VG
Mitsubishi Kakoki, Tokyo	2	300 T - VG
Leybold, Takyo	1	330 T - VG
LFE Corporation, Handen	1.	150 T - VG
Gebr. Herrmann, Köln	1.	150 T - VAS
Patterson Candy International	1	150 T- VA5
LFE Corporation	2	. 300 T - VG
LFE Corporation	1	12CJ T - VG
Fuji Seita, Shimizu	1	300 T - Vg
Bonai Regulator (Shizubka	1	300 T - 13
Messis. Montenari, Italia	1	300 T - VG
Stadt Norderstert	4	300 T - VG
Messis. Lippe-Veser-Zucker, Lage	6 -	960 T - VG
Messis. Pfeifer & Langen, Titz-Ameln	_ 1	1200 T - VA - SO
Messis. Hoephst, Frankfuct	6	1200 T - VG
Messrs. Unilever, Neederlande	. 4	6C0 T - VG
Messos. Montanari, Italia	- 1	300 T - VA - <b>SO</b>
Messis. France A., France	1	1200 T - VG
Geneinde Vestoverledingen	, 2	600 T - VG
Dereinde Vangerland	. 1	6CO T - VG
Schlesuig-Holsteinische Zucker AG, Sch	leswig 2	600 T - VG

HEINRICH FRINGS - 53 BONN 1 - Jonas-Cahn-S Client Kunde Stück	Str. 9 · Tel. Units	(0 22 21) 63 15 71 Type
Ressis. P. Dubislav, Neederlande	6	1200 T - VG
Ressrs. P. Dubislav, Neederlande	б	1200 T - VG
Messzs. Feldmühle, Düsseldorf	1 ·	9CO T - VG
Messrs. Leng, Itelia	2	900 T - VG
Messrs. Dechesne, Belgium	2	1200 T - VG
Messis. Steinmann & Ittig, Minden	2	600 T - VG
Zuckerfabrik Franken, Ochsenfurt	· 2	1200 T - VG
Messis. Monteneii, Itelia	2	900 T - VG
Cessos. Montaneci, Italia	2	660 T - YG
Messrs. Montanari, Italia	· 2	300 T - VG
Massis. Montanazi, Italia	4	150 T - VG
Messrs. GIC-Export-Import, Sugaslavia	5	306 T - VG
Messrs. Leybold Japan	1	300 T - VG
Nessrs. Toyo Gosei Co., Japan	2	300 T - VG
Messis. Toyo Gosei Co., Japan	1	600 T - VG
Messrs. Sanei Regulator, Shizucka	1	15C T - VG
Messis. Sanei Regulator, Shizucka	2	300 T - VG
Messrs. Mitsubishi Kakoki Cox, Ltd., Japan	1	300 T - VG
Messis Mitsukan-Su Nagoya Japan	1	300 T - VG
Messos. Toyo Gas Kegaku Do. Lid. Niigata Pre	f.i ,	600 T - VG
Messrs. Mitsubishi Kokeki Kaisma Ltd., Japan	2 1	600 T - VG 900 T - VG
Masses. Dai Nishon Plastics Co.	1	300 T - VG
Messos. Leybold, Jecan	1 .	300 T - VG .
Messis. Toyo Ges Kegeku Co., Jepan	3 ໌	300 T - VG
Messrs. Toyo Gas Kagaku Co., Japan	1	600 T - VG
Messrs. Eing, Gescher	. 2	900 TVG
Messrs. Rasselstein, Neuwied, Verk Andernach	6	600 T - VG
Nessrs. Dubislev, Niederlande	1	900 T - VG
Messrs. Uiltmann, Versmold	3	300 T – VG
Messrs. Nichalowski, Vechta	1	900 T - VG
Grt Vinterstettenstadt	1	300 T - VG
Gemeinde Wallenhorst	1	900 T – VG
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HEINRICH FRINGS - 53 BONN 1 · Jonas-(	Cahn-Si	tr. 9 •	Tel. (O	22 21)	63 15 71	l
Messrs. Montanari, Italien		1			- VG	
		1 2			- VS	
Messrs. Purator, Österreich	•	2		1200 T		<b>'</b> .
Massrs. Montanari, Italien		2		150 T		
	•	2 <sup>-</sup> 1	· · ·	300 T	- VG	
•		1		600 T 900 T		
Messrs. Rima, Schueden		1		900 T	- VG	
Messrs. Meredith, Grossbritannien		1		.600 T	- VS	
Messrs. Südd. Zucker AG, Offstein		i 3	•	900 T	- VG	
Messis. Praiffer & Langen, Titz-Ameln .		2	נ	200 T	– VG	
Messos. Jaegeo GmoH, Wuppertal		่ 3		305 T	– VG	•
Messrs. Lippe-Weser-Zucker, Lage		2		900 7	- VG	
Messis. Zuckezfabrik Franken, Ochsenfurt	•	2		30 <b>0 T</b>	– VG	
Messrs. Steinbeis & Co., Gemmrigheim		3 1			- VG/N - VG/N	
Messes. Rich.Baune/Jul.Spacht Borgholzhausan	. •	2	·	300 T		21
Messrs. Greviga, Grevenbroich	•	6 3		600 т	- VG/U - VG/V	12A 12A
Messrs. Allgäuer Alpenmilch, München 🐳 🐰	•	4		600 T	•	
Messrs. Boehringer Mannheim, Werk Penzberg		1			- VA -	. <b>5</b> 0
Messrs. Merck, Gerasheim		12	•	500 T		50
Messrs. Rütschi, Schwaiz		1		300 T		
Messrs. Purator, Österreich		1		300 T		
Gemeinde Ellerau		1	•	600 т	- VG	
Messos. Zuckerfeorik Franken Verk Ochsenfurt		4	. 1	200 T 300 T	– VG	•
Messrs. Condee, Hamburg	•	6		900 T		
Messrs. Mono-Pumps, Neuseeland	•	1		500 T		
Messrs. Rima, Schueden		2		500 T		. •
Landkreis Neppen		1		7 D D	-	
Nessrs. Zuckerfebrik Brühl	• •	1		200 T		•
Nessrs. Montanari, Italian		3	-	500 T		
Gemeinde Ganderkesee		4		500 T		
	•	•				

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HEINRICH FRINGS · 53 BONN 1 · Jonas-Cahn-S	Str. 9 · Tel.	(0 22 21) 63 15 71
Messis. Claus Holzer KG, Lockweiler	· 4 ·	900 T - VG
Messrs. Rima, Schweden	1	600 T - VG
Messrs. Hoechst, Frankfurt-Hoechst	6	1200 T - VG
Messrs. Degrement, Frankreich	1	600 T - VG
Ruhrverband, Essen	2.	900 T - VG - SO
Messrs. Gebr. Sthmidt, Mainbernheim	2	900 T - VG
Friedrich Ebert Stiftung	<sup>1</sup> 1	150 T - VG - 50
Geneinde Edewecht	4	900 T - VG -
ARV Untere Radolfzeller Aach	2	900 T – VG
Messrs. Rime, Schweden	2	300 T - VG
Stadt Aalen	1	300 T – VG
Messrs. Sihi, Frankreich	1	600 T - VG
Stadt Bramstedt	2	900 T - VG
Kreisstadt Siegburg	1.	600 T – VG
Gemeinde Hilter	2	600 T - VG - SO
Messis. Duro-Penta, Südafrika	1	300 T - VG
Messrs. Montanari, Italian	2	1200.T VG
Nessrs. Smedegaard, Dänemark	2	1200 T - VG
Gemeinde Ilshofen	1	600 T - VG
Messis. Schweitzer, Murchard	1	300 T - VG
Messis. Soebringer, Tutzing	1	300 T - VG
Messrs. Rime, Schweden	2	1200 T - VG
Messis. Pfeiffer & Langen, Vevelinghoven	2	1200 T - VG
Messrs. France Assaninissent, Frankreich	1 .	900 T - VG
Messrs. Dubislav, Niederlande	4	1200 T - VG
Messrs. Speyer, ven der Vijer & Zwanenburg, Niederlande	3′	1200 T - VG/V2A
Ruhrverband, Essen	4	900 T - VG - SO
Messrs. Zander, BergGladbach	8	18CO T - VG/V2A
Messrs. Duro-Pente, Südefrika	1	300 T - VG
Messrs. Ueltrade AG, Schueiz	2	600 T - VG
Messrs. Degremont, Frankreich	1 1	1200 T - VG 150 T - VC
Messrs. Lehrter Zucker, Lehrte	3	1200 T - VG

HEINRICH FRINGS · 53 BONN 1 · Jonas-Cahn-	Str. 9 • Tel	. (0 22 21) 63 15 71
Messrs. Hornbach Klärenlagen, Hagenbach	1	300 T - VG
Stadt Gemünden	1	300 T - V3
Messrs. Purator, Österreich	1 .	600 T — VG
Aggerverband, Gummersbach	2	12CO T - VG
Abwassertechnische Ges.m.b.H. Gummersbach	3 3	150 T - VG/V2A 300 T - VG/V2A
Messis. Hornbach Kläranlagen, Hagenbach	1	300 T - VG
Messrs. Montanati. Italien	4 3 1 1	150 T - VG 300 T - VG 600 T - VG 1200 T - VG
Lippe-Weser-Zucker, Lage	3	900 T - VG
Messes. Dynamit Novel, Lülsdorf	4	900 T - VG/V2A
Messrs. Assaninissement, Frenkreich	1	300 T - VG
Messrs. Daimler Benz, Stuttgart	1	300 T - VG - SO
Messrs. Rütschi, Sonweiz	1	630 T - VG
Mennesm <b>annröhren</b> Werke, Düsseldorf	1	500 T - V4A - S
Mesars. H. Veicht, Arnstorf	. 2	650 T - VG
Zuckerfzbrik Nordstemmen	2.	600 T - VG
Messos. Meredith, England	1	300 T - VG
Messes. France Asseinissement, Frankreich	2	150 T - VG
Mesars. Callens, Balgien	3	300 T — VG
Geneinde Nüdlingen	2	$\epsilon$ CO T - VG
Landkreis Meppen	<b>1</b>	600 T - VG
Mecors. Mono Pumpes, New Zeeland	1	30J T - VG
Messrs. Durc-Penza, Südafrike	б	150 T - VG
Messes. Rima, Schweden	. 3 -	600 T - VG
Messas. Purator, Österreich	2	300 T - VG
Messrs. Dubislav, Niederlande	2	600 T - VG
Nessos. Smedegaard, Dänemark	4	1200 T - VG
Messrs. Linde AG, Höllriegelskreuth	1 ·	300 T - VG - SO
Messrs. Schrage, Hanau	2	600 T - VG
Stadt Bielefeld	18	90C T - VG
Aggerverband, Gunmersbach	1	300 T - VS

HEINRICH FRINGS - 53 BONN 1 - Jonas-Cah	n-Str. 9 · Te	I. (0 22 21) 63 15 71
Messrs. Chemilite, Johannesburg	1	150 T - VG
Messes. LFE Corporation, Hamden	9	150 T - VG
Messes. Saha Kim Motors, Bangkok	1 .	150 T - VG
Meesrs. US Czoneir, San Francisco	1 .	150 T - VG
Massas. Leybold, Tokyo	5	150 T - VG
Messis. Paterson Candy Int.	3	300 T - VAS
Massas. Leyadld, Tokyo	19	300 T - VG
Messis. LFE Corporation, Hamden	7	300 T - VG
Mess <b>rs. Le</b> ybold, Tokyo	2	600 T - VA
Messes. Leybold, Tokyo	13	600 T - VG
Messis. Paterson Candy Int.	3	600 T - VAS
Ressrs. Leybold, Tokyo	6	900 T - VG
Messos. Elektroschmelzuerk, Frechen	1	900 T - VG
Messos. Chemilite, Johanneaburg	• 1	1200 T'- VAS
Resars. Trailigaz, Paris	4	1200 T - VA
Messrs. LFE Corporation, Handen.	1	1200 T - VG
Mesars. Trailigaz, Paris	1	1200 T - VAS
Messrs. Paterson Candy Int.	3	1200 T - VAS
Geneinde Kirchberg	. 1	300 T MG-SD
Gemeinde Bobenheim-Roxheim	· 1	1200 - VG
Niersverband, Viersen	t 4 -	1200 T - VG
Messrs. Sihi, Frankreich	1	600 T - VG
Messis. Smedegaaid, Dënemaik	3	600 T - VG
Messos, Rima, Schweden	2	300 T - VS 600 T - VG
Messis. Pfeifer & Langan, Vevelinghoven	3	900 T - VG
Zuckerfabrik Brihl	1	1200 T - VG
Messis. Meredith, England	ĺ	· 1200 T - VG
Messes. Mono Pumps, Neusseland	1	300 T - VG
Massis. Mon-tanari, Italia	1.	900 T - VG
Messis. Seibart, Ruppertswailer	1	- 150 T - VG
Messrs. Wabeq, Kulmbach	4	300 T - VG
Messrs. Rine, Schweden	3	600 T - VG
Messrs. Dubislev, Neederlande	6	900 T - VG
Messrs. Artland-Dörffler, Badbergen	1	150 T - VG
Messrs. Mon-tanari, Italia	1	<b>900 T -</b> VG

HEINRICH FRINGS . 53 BONN 1 . JÄGERSTRASSE 9 . TEL. (0 22 21) 65 17 51

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Messrs. Sihi, Frankreich	1	150 T - VG
Messrs. Feldmühle, Düsseldorf VKW, Oberhausen		,
Werk Baienfurth	6	900 T - VG/V2A
Messrs. Fichtel & Sachs, Schweinfurt	3	150 T - VG
Gemeinde Oberer Kraichbach Züblin, Stuttgar	t 4	900 T - VG
Messrs. Sihi, Frankreich	1	900 T _ VG
Messrs. Mannesmannröhren Werke, Düsseldorf	3	600 T - V4A-S
Stadt Freiburg im Breisgau	2	·300 T - VG
Messrs. VEBA-Chemie, Brunsbüttel Sulzer,	4	1200 T - VG/V2A
Mess <b>rs. Ma</b> urer & Söhne, Münch <b>e</b> n	1	600 T - VG
Messrs. ?Kugelfischer, Wuppertal	1	300 T - VG
Messrs. Neynhaber-Chemie GmbH, Loxstedt	2	900 T - VG
Messrs. Montanari, Italia	1	600 T - VG
Messrs. Stahlwerke Südwestfalen, Hagen	1	300 T - VAS
Messrs. Dt. Zündholzfabrik, Baiersbronn	3	900 T - VG
	1 :	· 1200 T - VG
Messrs. Bleihütte Zerzelius, Stollberg	3 :	25 T – VG-F
Messrs. US-Ozonair, San Francisco	2 .	25 T - VG
Messrs. LFE, Hamden	1	1200 T - VG
Məssrs. Gəbr. Herrmann, Köln	2	300 T - VAS

# B = 87

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