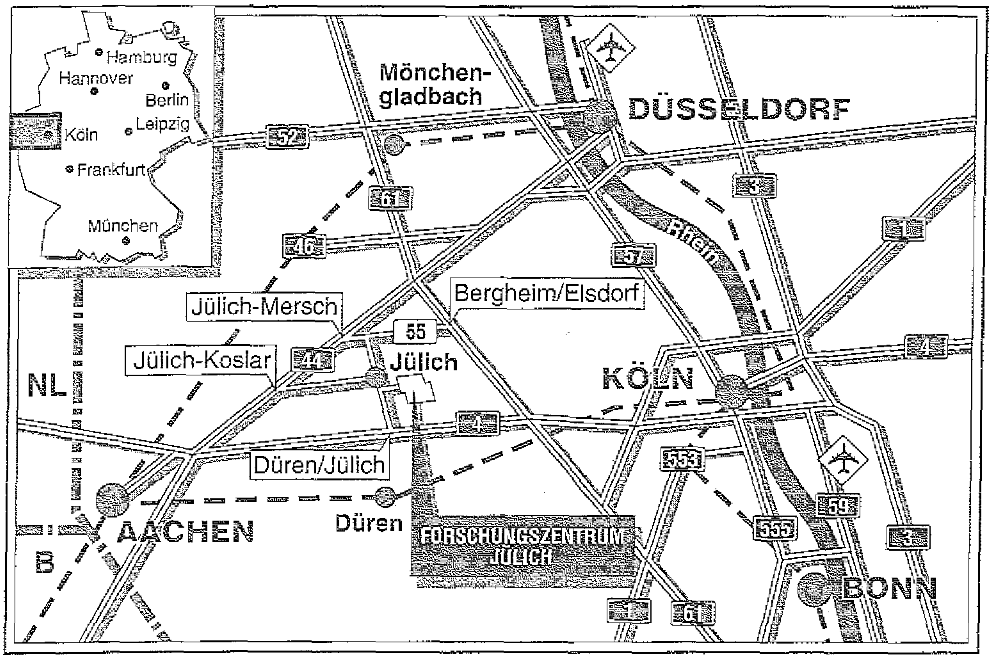
FORSCHUNGSZENTRUM JÜLICH GmbH

Institut für Biotechnologie

Harvesting of the Alga Dunaliella some Considerations Concerning its Cultivation and Impact on the Production Costs of β-Carotene

Friedrich-Helmuth Mohn
Oriana Cordero Contreras



Berichte des Forschungszentrums Jülich ; 2438
ISSN 0366-0885
Institut für Biotechnologie Jül-2438

Zu beziehen durch: Forschungszentrum Jülich GmbH · Zentralbibliothek
Postfach 1913 · D-5170 Jülich · Bundesrepublik Deutschland
Telefon: 02461/61-6102 · Telefax: 02461/61-6103 · Telex: 833556-70 kfa d

Harvesting of the Alga Dunaliella – some Considerations Concerning its Cultivation and Impact on the Production Costs of β-Carotene

Report on centrifugation and flocculation/flotation experiments, carried out at Pesquera Marazul Ltda., Antofagasta, Chile

Project of UNDP No. CHI/87/009
United Nations Development Programme, Santiago, Chile

Friedrich-Helmuth Mohn
Oriana Cordero Contreras*

*Pesquera Marazul Ltda., Antofagasta, Chile

Preface

Information concerning harvesting of the salt water alga Dunaliella on a large scale is seldom found and then difficult to transform into practise. Under leadership of Universidad Católica, UNDP is supporting a small project in the Northern part of Chile, in Antofagasta at the Pesquera Marazul Ltda. fishing company to test the production of Dunaliella and to investigate harvesting processes in practical dimensions.

Due to the initiative of the Escuela de Ingeniería Bioquímica of the Universidad Católica de Valparaíso the two authors were brought together and the UNDP in Santiago met the expenses for Dr. Mohn's excursion to Chile. The purpose was to find options for economical harvesting of Dunaliella as an important step in the ß-carotene production and make them available to any interested parties. The results are published in this report.

Antofagasta, July / August 1990
Jülich, October / November 1990

Acknowledgement

The authors wish to express their gratitude to the UNDP, who kindly supported this investigation by paying the necessary expenses.

Thanks are also due to Prof. Samuel Navarrete from Universidad Católica, Valparaiso, who used all his influence to make the project and this report possible.

We want to thank Prof. Andres Markovits for his fruitful suggestions about harvesting experience in Chile and discussions regarding possible locations of Dunaliella plants.

Prof. Patricio Proust gave valuable hints concerning such projects and this work. He kindly organized the technical scheduling of the experiments. We wish thank him very sincerely.

We are very much obliged to Pesquera Marazul Ltda. in Antofagasta, which supported the whole project by generously placing excellent assistants, rooms and equipment at our disposal.

To the collaborators of Pesquera Marazul, Yanni, Francesco and Robinson we wish to express our particular appreciation. Without their extraordinary personal efforts in the laboratory, at the harvesting equipments and the ponds this investigation could not have been carried out. They interested great efforts, precise workmanship and their personal time to put ideas and considerations into practice and supported the whole project by correct measuring during the experiments and in the laboratory.

Finally, our sincere thanks are due to Prof. C.J. Soeder, head of the Institut für Biotechnologie 3 and to the board of directors of the FORSCHUNGSZENTRUM in Jülich who supported the first author's visit and continuously promoted scientific research in this field.

Summary

Two techniques for harvesting the salt water alga Dunaliella with regard to the production costs of ß-carotene were investigated and evaluated in Antofagasta, Chile.

Centrifugation by means of a self- cleaning plate separator running as a clarifier with closed rising channels can be recommended in smaller projects if a reliable supply of electric energy is available and if the whole of Dunaliella is to be used for human consumption as in Japan. The harvesting *costs are US $ 21.33* per kg ß-carotene.

Flocculation / flotation by using 100 - 150 ppm $Al_2(SO_3)_4$ as a flocculation aid and 5 - 15 ppm of an anionic polymer from Stockhausen, Praestol 2540, as a flocculant, is recommended for bigger plants or if not insufficient electricity is available. The method of stirring or turbulence is very important. The role of the chemicals is still uncertain in the extraction of the ß-carotene and the residues which are to be used as a valuable animal feed and if the whole alga is to be eaten by humans. The harvesting *costs are US $ 17.32 per kg ß-carotene.*

It appeared that the costs should not determine the harvesting technique since it only accounts 22.2% or 18.8% respectively of the total production costs.

Total production costs not including extraction, labour and land are about US $ 96.- and 92.- respectively per kg ß-carotene.

The market price for synthetic ß-carotene is estimated at about US $ 465.- per kg.

Selling of the by products will further increase the economy of ß-carotene production.
The project in Antofagasta should continue with further expertise!

Contents

1. Introduction

Carotenoids are mostly used as a dye or "natural food colour" (e.g. in margarine, butter, yoghurts etc.). Also as additives for animal feed to enrich the colour of their products (egg yolks, poultry and fish meats and others) or to enhance their health or their fertility. Only a few carotenoids, like ß-carotene, are used for the above mentioned purposes commercially. As the demand for ß-carotene is higher than its "natural" production some companies offer a synthetic product. Whereas natural ß-carotene is good for health, it even prevents cancer, the synthetic one is said not to harmless to human health. Therefore attempts are being made to locate natural sources like the salt water algae Dunaliella to bring natural ß-carotene into use. The northern part of Chile seems to be predestinated for the cultivation of Dunaliella. Good climatic conditions, plenty of desert along the shore line and relatively good infrastructures promise success.

Production costs rely very much on the growth rate and the harvesting density (mg dry substance per litre). Unfortunately both important factors have very low values which are difficult to increase for biological reasons. Therefore the harvesting process should be optimized and cheaper pond systems applied.

2. Harvesting Procedures

Little has been published about the economical harvesting of Dunaliella. In laboratory tests the filtration time for 100 ml Dunaliella suspension on acetate 8 Ì membrane filter takes about 20 minutes. This indicates the difficulty for surface filtration of Dunaliella. So most economic harvesting processes described in the literature fail. Only centrifugation or flocculation and sedimentation or flotation remain as possible processes.

Centrifuges are safe and reliable but very expensive instruments to harvest algae. The need for up to about 1 $kWh \cdot m^{-3}$ suspension to be harvested prevents its use at places with an underdeveloped infrastructure.

Flocculation and flotation or sedimentation units are expensive, too. On the one hand they need less energy than centrifuges but on the other they require costly chemicals which are in part harmful. However chemical storage also permits its use at places with an underdeveloped infrastructure. Unfortunately this process is not always reliable. Small changes of the growth rate, caused by the weather for instance, upset a standardized application of flocculant, stirring methods and the use of flotators.

An economic evaluation of the harvesting methods tested by the authors considers, beside, the feasibility, the running cost (kWh and or chemical costs per unit of biomass separated) and the dry substance of the harvested biomass. The centrifuge tested did not allow a clear account with respect to the dry substance. Due to its construction and the desludging technique the laboratory centrifuge always obtains a much lower concentration of the biomass than an industrial one which reaches about 12 - 15%.

2.1. Centrifugation

All tests were carried out with a WESTFALIA Separator SA-1. This type is small enough for extended running tests with small quantities as its maximal throughput is fixed at about 0.650 $m^3 \cdot h^{-1}$. It is large enough to scale up the results directly. However the rated power of its motor is about twice as great as that of industrial machines. Its use as a multipurpose laboratory apparatus and the numerous switchings on and off required should be kept in mind. The SA 1 installed in Antofagasta is equipped with rising channels near the periphery of the plates. Before buying the machine it was ascertained that it provided a highly concentrated biomass. The channels prevents clarified liquid passing the sediment holding space and whirling up the already sedimented biomass. The SA-1 can be run in two ways:

1. as a separator,
   mainly used in liquid liquid separation processes e.g. oil water separation

2. as a clarifier,
   mainly used in liquid solid separation processes e.g starch centrifugation.

Westfalia delivered the SA-1 already assembled. Staff from of Pesquera Marazul Ltda. mounted it. During the first running tests the centrifuge's maximum capacity was only 0.065 $m^3 \cdot h^{-1}$, which is far too small. After dismounting the machine and changing parts of the centripetal pump the capacity was increased up to 0.55 $m^3 \cdot h^{-1}$. Then we tested the following parameters systematically:

1. Separation

   1. open channels
   2. closed channels
   3. pressure of effluent

2. Clarifying

   1. closed channels only

For all parameters different throughputs had been approximately adjusted by means of a valve installed before the inlet: 0.05; 0.10; 0.15; 0.20; 0.25; 0.30; 0.35; 0.40; 0.45; 0.50 $m^3 \cdot h^{-1}$ etc.

We determined the clarifying effect for every throughput by measuring the optical density with a spectrophotometer (SPECTRONIC 20 D, absorbency at 540 nm wavelength) and the energy demand by means of an electric meter.

Tables 1 - 5 show the results of the centrifugation experiments. The legend is explained on page 5. The tables also indicate the electricity consumption for harvesting 1 m^3 suspension and its demand for 1 kg biomass by subtracting from the biomass content of the inlet the biomass loss in the effluent which increases with a rising throughput. It is indicated as ODe in the effluent. Running the machine with closed channels caused a little higher retention of the biomass in the plate stack. The best exploitation of the energy was obtained when running the centrifuge as a clarifier with closed channels (Table 5). Fig 1 includes all results. It also shows that an increasing throughput decreases the production costs.

The unsteady biomass concentration (mg dry substance per litre) in the inlet resulted in a different amount of harvested biomass per volume or per kWh. To compare the energy consumptions of all experiments, we multiplied in an additional calculation the throughput by an assumed dry substance content of 400 mg per litre in the inlet. We called this result Yc (corrected yield).
Due to practical experience, we assumed further that a value of OD 0.001 measured at a wavelength of 540 nm can be set as 1 $mg \cdot l^{-1}$ dry substance (e.g. an OD of 0.250 = 250 $mg \cdot l^{-1}$).

On this basis we calculated the energy demand for harvesting 1 kg product which is named in the tables "1,000 units" (OD 1,000).

Explanation of the legend of Tables 1 - 5:

I	=	throughput ($m^3 \cdot h^{-1}$)
OD	=	optical density of the suspension to be harvested, measured at a wavelength of 540 nm, we found that an OD of 0.001 accords with 1 mg dry substance per l
ODe	=	optical density of effluent (540 nm)
ODi	=	optical density of inlet (540 nm)
% p	=	% throughput of the possible rated capacity of the centrifuge (0,65 $m^3 \cdot h^{-1}$)
Y	=	expected yield ($I \cdot ODi \cdot 1000 \cdot h^{-1}$)
Yc	=	corrected yield postulated to a OD of 400 ($I \cdot OD\ 400 \cdot 1000 \cdot h^{-1}$)
Yr	=	real yield $(Y - L) \cdot h^{-1}$
L	=	loss ($I \cdot ODe \cdot 1000 \cdot h^{-1}$)
eff %	=	efficiency ($Yr—Y \cdot 100$) of clarification (biomass kept back in the centrifuge)
kWh	=	energy consumed for I
kWh p.1000 Yr	=	energy necessary to harvest 1000 units (1 kg) due to the ODi. $(I \cdot 1000 \cdot kWh \cdot Yr^{-1}) \cdot m^{-3}$
kWh p.1000 Bc	=	energy to harvest 1000 units (1 kg) ß-carotene due to the ODi (8% ß-carotene content of Dunaliella).
kWh p.1000 YrC	=	energy to harvest 1000 units on a postulated ODi of 400 to compare all measured data, due to fluctuating inlet concentration. $I \cdot 100.000\ kWh \cdot m^{-3} \cdot (Yc \cdot eff\%)^{-1}$ see Yc.
kWh p. 1000 BcC	=	energy to harvest 1000 units of ß-carotene due to a postulated ODi of 400 (8% ß-carotene content)
Bc	=	ß-carotene
BcC	=	ß-carotene, corrected yield
YrC	=	real yield, corrected

I	ODe	% P	Y	Yc	L	Yr	eff %	kWh	kWh·m^{-3}	kWh p.1000 Yr	kWh p.1000 Bc	kWh p.1000 YrC	Kwh p.1000 BcC	US$ p.1000 YrC	US$ p.1000 BcC	
0,049	0,037	7,5	19,1	19,6	1,8	17,3	90,5	0,328	6,69	19,0	237,0	18,5	231,1	3,14	39,29	
0,115	0,051	17,7	44,9	46,04	5,9	39,0	86,9	0,360	3,13	9,2	115,3	9,0	112,4	1,53	19,12	
0,152	0,054	23,4	59,2	60,72	8,2	51,0	86,2	0,371	2,44	7,3	90,9	7,1	88,6	1,21	15,07	
0,228	0,064	35,1	88,9	91,2	14,6	74,3	83,6	0,447	1,96	6,0	75,2	5,9	73,3	1,00	12,46	
0,294	0,072	45,3	114,8	117,72	21,2	93,6	81,5	0,464	1,58	5,0	62,0	4,8	60,4	0,82	10,27	
0,334	0,084	51,4	130,3	133,6	28,1	102,2	78,5	0,459	1,37	4,5	56,1	4,4	54,7	0,74	9,30	start of overflow
0,411	0,087	63,3	160,4	164,52	35,8	124,6	77,7	0,474	1,15	3,8	47,5	3,7	46,4	0,63	7,88	

Table 1: Experiments with Westfalia centrifuge SA-1

Date : 24.07.90
Setting : as separator 01
Centripetal pump: upper opened 0 52mm
lower closed
Channels : open
ODi : 0.39
For explanation of legend see page 5

I	ODe	% P	Y	Yc	L	Yr	eff %	kWh	$kWh \cdot m^{-3}$	kWh p.1000 Yr	kWh p.1000 Bc	kWh p.1000 YrC	Kwh p.1000 BcC	US$ p.1000 YrC	US$ p.1000 BcC	
0,091	0,053	14,0	29,9	36,48	4,8	25,1	83,8	0,310	3,40	12,4	154,5	10,1	126,7	1,72	21,54	
0,146	0,064	22,4	47,8	58,32	9,3	38,5	80,5	0,328	2,25	8,5	106,5	7,0	87,3	1,19	14,85	
0,226	0,079	34,8	74,1	90,4	17,9	56,3	75,9	0,342	1,51	6,1	76,0	5,0	62,3	0,85	10,59	
0,295	0,066	45,4	96,8	118,04	19,5	77,3	79,9	0,389	1,32	5,0	62,9	4,1	51,6	0,70	8,77	
0,318	0,077	48,9	104,3	127,16	24,5	79,8	76,5	0,409	1,29	5,1	64,1	4,2	52,5	0,71	8,93	
0,386	0,088	59,4	126,7	154,56	34,0	92,7	73,2	0,399	1,03	4,3	53,8	3,5	44,1	0,60	7,50	
0,468	0,105	72,1	153,6	187,36	49,2	104,5	68,0	0,405	0,86	3,9	48,5	3,2	39,7	0,54	6,76	
0,506	0,107	77,8	165,9	202,36	54,1	111,8	67,4	0,451	0,89	4,0	50,4	3,3	41,3	0,56	7,03	start of overflow

Table 2: Experiments with Westfalia centrifuge SA-1

Date : 24.07.90
Setting : as separator 01
Centripetal pump: upper opened 0 52mm
lower closed
Channels : closed
ODi : 0.328
For explanation of legend see page 5

I	ODe	% P	Y	Yc	L	Yr	eff %	kWh	$kWh \cdot m^{-3}$	kWh p.1000 Yr	kWh p.1000 Bc	kWh p.1000 YrC	Kwh p.1000 BcC	US$ p.1000 YrC	US$ p.1000 BcC	
0,056	0,038	8,6	16,7	22,44	2,1	14,5	87,2	0,773	13,78	53,2	665,0	39,5	493,8	6,72	83,94	
0,115	0,049	17,7	34,2	46,08	5,6	28,6	83,5	0,789	6,85	27,6	345,2	20,5	256,3	3,49	43,57	
0,149	0,061	22,8	44,1	59,4	9,1	35,0	79,5	0,779	5,25	22,2	277,8	16,5	206,3	2,81	35,07	
0,190	0,066	29,2	56,3	75,8	12,5	43,8	77,8	0,777	4,10	17,8	221,9	13,2	164,7	2,24	28,01	
0,283	0,079	43,5	83,9	113	22,3	61,6	73,4	0,783	2,77	12,7	158,9	9,4	118,0	1,60	20,06	
0,322	0,085	49,6	95,8	128,98	27,4	68,4	71,4	0,809	2,51	11,8	147,9	8,8	109,8	1,49	18,67	∞
0,359	0,079	55,2	106,5	143,48	28,3	78,2	73,4	0,805	2,24	10,3	128,7	7,6	95,5	1,30	16,24	start of overflow
0,417	0,095	64,1	123,8	166,72	39,6	84,2	68,0	0,815	1,96	9,7	121,0	7,2	89,8	1,22	15,27	
0,439	0,085	67,6	130,5	175,72	37,3	93,1	71,4	0,828	1,88	8,9	111,1	6,6	82,5	1,12	14,03	
0,517	0,092	79,6	153,6	206,84	47,6	106,0	69,0	0,867	1,68	8,2	102,2	6,1	75,9	1,03	12,90	

Table 3: **Experiments with Westfalia centrifuge SA-1**

Date : 25.07.90
Setting : as separator 01
Centripetal pump: upper opened
lower closed
Channels : opened
ODi : 0.30

For explanation of legend see page 5

I	ODe	% P	Y	Yc	L	Yr	eff %	kWh	$kWh \cdot m^{-3}$	kWh p.1000 Yr	kWh p.1000 Bc	kWh p.1000 YrC	Kwh p.1000 BcC	US$ p.1000 YrC	US$ p.1000 BcC	
0,064	0,038	9,8	19,0	25,6	2,4	16,6	87,2	0,427	6,67	25,8	322,0	19,1	239,1	3,25	40,64	
0,100	0,047	15,4	29,6	39,92	4,7	25,0	84,2	0,545	5,46	21,8	273,0	16,2	202,7	2,76	34,47	
0,154	0,050	23,7	45,7	61,52	7,7	38,0	83,2	0,420	2,73	11,1	138,2	8,2	102,6	1,40	17,44	
0,196	0,052	30,1	58,2	78,32	10,2	48,0	82,5	0,417	2,13	8,7	108,7	6,5	80,7	1,10	13,72	
0,293	0,065	45,0	86,9	117,04	19,0	67,9	78,1	0,456	1,56	6,7	84,0	5,0	62,3	0,85	10,60	
0,305	0,062	46,9	90,6	122,04	18,9	71,7	79,1	0,474	1,55	6,6	82,6	4,9	61,4	0,83	10,43	
0,353	0,073	54,2	104,7	141,04	25,7	79,0	75,4	0,504	1,43	6,4	79,8	4,7	59,2	0,81	10,07	
0,390	0,070	60,0	115,8	156,00	27,3	88,5	76,4	0,488	1,25	5,5	68,9	4,1	51,2	0,70	8,70	start of overflow
0,472	0,083	72,7	140,3	188,96	39,2	101,1	72,1	0,509	1,08	5,0	62,9	3,7	46,7	0,64	7,94	
0,537	0,083	82,7	159,6	214,92	44,6	115,0	72,1	0,875	1,63	7,6	95,1	5,7	70,6	0,96	12,01	

Table 4: Experiments with Westfalia centrifuge SA-1

Date : 25.07.90
Setting : as separator 01
Centripetal pump: upper opened
lower closed
Channels : closed
ODi : 0.297
For explanation of legend see page 5

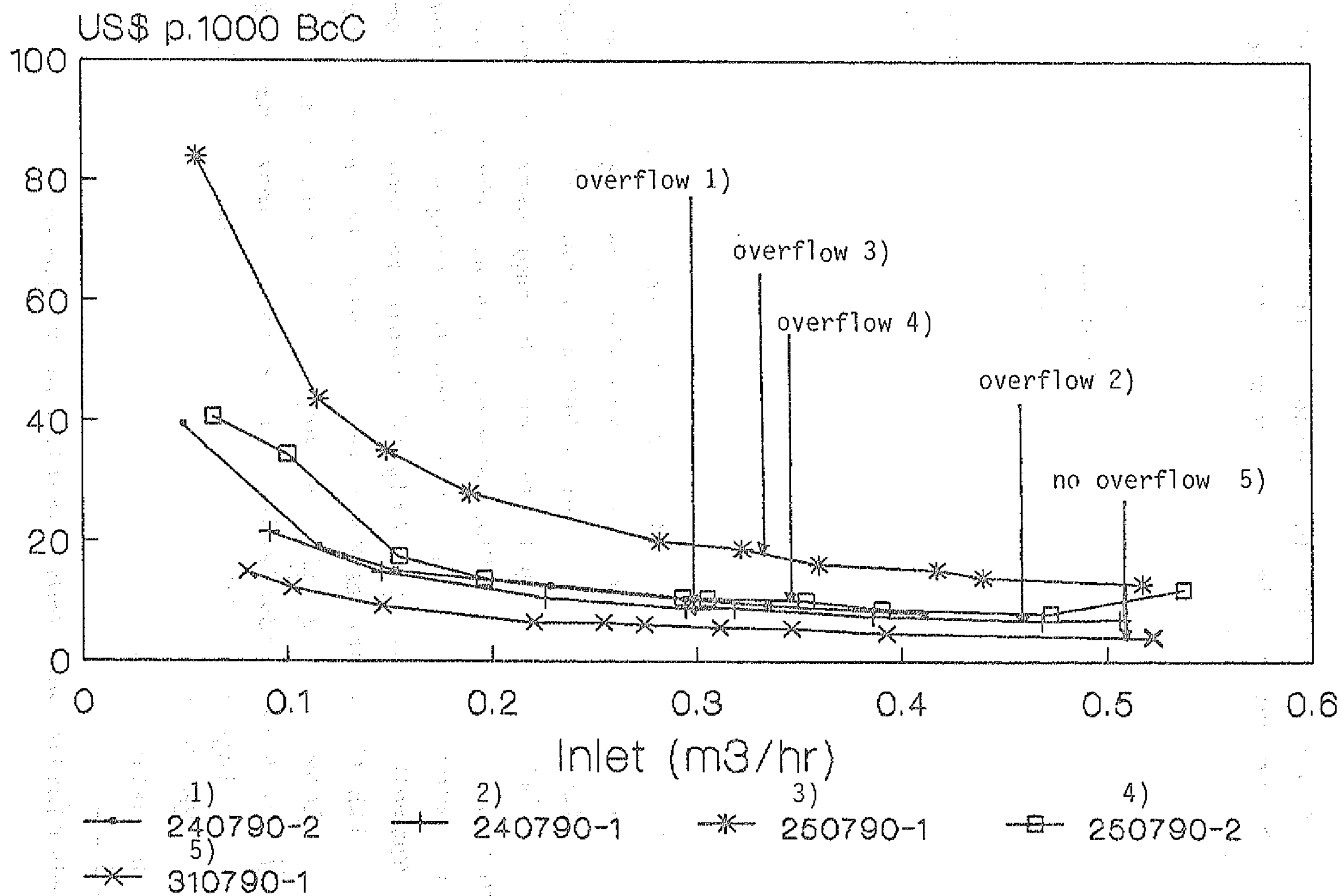
I	ODe	% P	Y	Yc	L	Yr	eff %	kWh	$kWh \cdot m^{-3}$	kWh p.1000 Yr	kWh p.1000 Bc	kWh p.1000 YrC	Kwh p.1000 BcC	US$ p.1000 YrC	US$ p.1000 BcC	
0,080	0,042	12,3	25,1	31,92	3,4	21,7	86,6	0,195	2,44	9,0	112,3	7,1	88,2	1,20	14,99	
0,102	0,048	15,6	31,9	40,68	4,9	27,1	84,7	0,198	1,95	7,3	91,5	5,7	71,8	0,98	12,21	
0,146	0,055	22,5	46,0	58,56	8,1	37,9	82,5	0,208	1,42	5,5	68,6	4,3	53,8	0,73	9,15	
0,220	0,067	33,9	69,1	88,04	14,7	54,4	78,7	0,210	0,95	3,9	48,3	3,0	37,9	0,52	6,44	
0,254	0,073	39,1	79,8	101,6	18,5	61,2	76,8	0,236	0,93	3,9	48,2	3,0	37,8	0,51	6,43	
0,274	0,074	42,2	86,1	109,72	20,3	65,8	76,4	0,240	0,87	3,6	45,6	2,9	35,8	0,49	6,08	
0,311	0,080	47,9	97,7	124,44	24,9	72,8	74,5	0,242	0,78	3,3	41,6	2,6	32,6	0,44	5,55	
0,346	0,095	53,2	108,6	138,32	32,9	75,7	69,7	0,249	0,72	3,3	41,1	2,6	32,3	0,44	5,48	
0,392	0,091	60,3	123,0	156,72	35,7	87,4	71,0	0,244	0,62	2,8	34,9	2,2	27,4	0,37	4,66	
0,522	0,105	80,3	163,8	208,68	54,8	109,0	66,6	0,253	0,48	2,3	29,0	1,8	22,8	0,31	3,87	
0,522	0,115	80,3	163,9	208,8	60,0	103,9	63,4	0,263	0,50	2,5	31,6	2,0	24,8	0,34	4,22	no overflow

Table 5: Experiments with Westfalia centrifuge SA-1

Date : 31.07.90
Setting : as clarifier 02
Centripetal pump: upper closed
lower opened
Channels : closed
ODi : 0.314
For explanation of legend see page 5

Fig. 1:

RUNNING COSTS DEPENDING ON THE THROUGHPUT OF THE CENTRIFUGE



1) = as separator channels closed
2) = as separator channels opened
3) = as separator channels opened
4) = as separator channels closed
5) = as clarifier channels closed

Tables 1 - 4 also show the beginning of the overflow in the centrifuge. The amount of the overflow and its OD was measured and added to the loss (marked as "L").

Thereafter the experiments ended in most cases. Sometimes the overflow ceased again when the throughput was increased further. However these values have not been considered.

Tables 1 - 5 further indicate under "%p" which percentage of the centrifuge's possible throughput (rated or possible capacity) was achieved. Certainly, a high throughput works economically but involves a high wear. Therefore it is advised that the centrifuge should be run at about 70 - 80 % of its rated capacity to avoid too rapid wear and to keep maintenance costs down.

The dry substance of the centrifuged biomass ranged over about 10 %. The algal cells were mostly broken when desludged because the high centrifugal force of about 8,000 g presses the biomass against the bowl wall with 80 bar and decompresses immediately at the moment of desludging. The same effects are observed in decompression chambers which are often used for single- cell homogenization. Therefore it is strictly advisable to cool the biomass and to start with the posttreatment immediately. In the authors' experience, algae spoil quickly after cell disruption due to enzymatic activity. A very advisable process is ultrapasteurization by direct steam injection for about two to four seconds. The authors are not aware whether this is permitted by subsequent steps in ß-carotene extraction.

2.2. Flocculation and Sedimentation or Flotation

Flocculation tests started in the laboratory. It was not possible to calculate the running costs per kg biomass as it had been previously done with the centrifuge, because the flocculant demand depended much more on the physico- chemical condition of the suspension (e.g. pH) than its dry substance. We also observed that the turbulence was a very important factor in the effectiveness of the agents. Economic calculations are based on the quantity of the flocculants. The experiments with the best values were transferred to outdoors experiments. Their results differ very much from those obtained in the laboratory where we used two methods:

1. Jar tests

2. Quick tests in simple graduated 100 ml laboratory cylinders

For jar tests we used clear, slightly conical polystyrene beakers bought in a department store. They were filled with 500 ml Dunaliella culture and the flocculants to be tested. A sample at a depth of 40 mm below the suspension surface was taken 15 and 30 minutes after agitation ended with a pipette and the optical density measured in a photometer at 540 nm. A "Biobloch Scientific" laboratory stirrer with variable speeds ranging from 25 to 550 rpm provided the turbulence (agitation). A sickle-shaped plastic blade with a width of 52 mm operated adequately during the first experiments. We replaced it later by a rectangle tin with a height of 100 mm and a width of 75 mm containing four holes with a diameter of 19 mm each. This blade proved to be more advantageous for certain flocculants such as "Sachtoklar" from "Sachtleben", Duisburg FRG, as it afforded a smooth laminar agitation of the whole content. Turning slowly the sickle-shaped blade did not provide enough laminar flow but destroyed flocks already formed whilst rotating at a higher speed. It must be pointed out that our stirring methods were not recommendable for the jar tests. However, other techniques were not available.

We stopped using the laboratory units later and changed to 100 ml graduated laboratory cylinders. After filling the medium into the cylinders we added the calculated amount of agents. Then they were shaken by turning them slowly 15 times with both hands (one at the top, the other at the bottom) for 180° on the top and back. The result was examined after 15 minutes. The cylinder test could not be used for all flocculants. However the flocculant which had been considered to be useful could be tested more quickly than with the jar test. We eliminated those which proved to be too expensive or required uneconomically high doses and those giving no results.

Name	Type	Manufacturer
Praestol 2540	Anionic not toxic; in FRG permitted for drinking water processes	Stockhausen, Krefeld, FRG
Südflock	cationic not toxic slightly acidic in FRG permitted for drinking water processes	Südchemie München, FRG
Ökoflock A2	non ionic not toxic natural compounds mainly used for sludge thickening in general not used in waterworks	as above
Ökoflock K1	like Ökoflock A2	as above
Sachtoklar (Polyalumchloride, PAC)	neutral, with a slight cationic surplus; acidic non toxic, perm. for drinking water treatment in FRG	Sachtleben, Duisburg, FRG
Magnofloc LT 20	non ionic (LT = Low Toxicity) no further information	Allied Colloids Bradford, UK
Magnofloc LT 24	cationic, as above	as above
Magnofloc LT 25	anionic, as above	as above
LP85/97	non ionic ampholite; non toxic especially development to flocculate solutions	Röhm Darmstadt, FRG
$FeCl_3$	non toxic often used in waterworks	generally available
$Al_2(SO_3)_4$	toxicity controversial	generally available
Chitosan	non-toxic, natural product	mostly produced by user

Table 6: Flocculants under test

2.2.1. Laboratory Jar and Graduated Cylinder Tests

Till now aluminium sulphate has been used as the sole flocculant at Pesquera Marazul. 400 $g \cdot m^{-3}$ (400 ppm) was added to the suspension. Flocks appeared over night and the biomass was taken off the following morning. Disadvantageous is the large amount of aluminium sulphate (1 mg per mg alga) and the long time until the algae were withdrawn. The authors can not say whether this relatively long time causes damages to the algae and losses to the ß-carotene content. The results of the jar test clearly show that the new flocculants, especially if combined, reduce the demand dramatically. However application is difficult and requires a complicated stirring technique.

Table 7 gives an idea of the use of flocculants. We added them in stages using different turbulences, which unfortunately could not be described. Aluminium sulphate was always added first. A high stirring speed guarantueed good mixing and led to recharging of the particle surfaces. If stirred unsatisfactorily the added flocculant failed. In these combinations aluminium sulphate was a flocculation aid, and the polymer a flocculant. As Table 7 shows three stages were generally tested. The calculated aluminium sulphate dose was stirred at a speed ranging from 450 to 530 rpm. After three to five minutes the polymer was added. The speed was kept constant for one minute and then reduced for one or two minutes to about 90 rpm. A minimum dose of 150 - 200 ppm aluminium sulphate, in one case 100 ppm, combined with at least 5 ppm PRAESTOL 2540 was necessary for sufficient clarification. Adding aluminium sulphate to PAC increased the quality of the supernatant, too, though according to the manufacturer's advice it should only be used as a sole flocculant.

$FeCl_3$ as a flocculation aid yielded poor results. An explanation could be that it coloured the suspension yellowish, which the photometer registered as high dry substance content.

Many difficulties occurred during flocculation tests. One day we were very successful with a certain combination which failed completely the following day. Temperature, pH, OD was exactly the same. We can only hazard a guess but perhaps physico-chemical characteristics changed. For instance, the algae may have excreted undefined components such as colloidal substances. Only a combination of aluminium sulphate with PRAESTOL 2540 was satisfactory and tested outdoors.

	Flocc. 1	Amount ppm	rpm 1	t 1 min	Flocc.2	Amount ppm	rpm 2	t 2 min
1)	$Al_2(SO_3)_4$	150	450	6	---	---	---	---
2)	$Al_2(SO_3)_4$	150	450	3	Praestol	0.5	450	1
3)	$Al_2(SO_3)_4$	200	450	2	Praestol	5.0	100	2
4)	$Al_2(SO_3)_4$	200	530	3	Praestol	5.0	530	1
5)	$Al_2(SO_3)_4$	150	530	3	Praestol	5.0	530	1
6)	$Al_2(SO_3)_4$	150	530	3	Chitosan	20.0	530	1
7)	$Al_2(SO_3)_4$	150	530	3	Praestol	2.5	530	1
8)	$Al_2(SO_3)_4$	200	530	3	Praestol	5.0	530	1
9)	$FeCl_3$	200	530	5	Praestol	5.0	530	1
10)	PAC	150	530	1	---	---	90	1
11)	$Al_2(SO_3)_4$	100	530	2	PAC	200	530	1
12)	PAC	100	530	1	---	---	90	1

	rpm 3	t3 min	OD 15′	OD 30′
1)	---	---	0.291	0.270
2)	100	2	0.249	0.230
3)	100	2	0.163	0.135
4)	90	2	0.047	0.049
5)	90	2	0.064	0.057
6)	90	2	0.272	0.253
7)	90	2	0.207	0.207
8)	90	2	0.118	0.132
9)	90	2	0.263	0.230
10)	---	---	0.060	0.059
11)	90	1	0.055	0.060
12)	---	---	0.295	---

Table 7: Results of some jar tests by measuring the OD after 15 and 30 minutes at 4 cm below the surface

Additional we "screened" flocculants in graduated laboratory 100 ml cylinders as described above. These results were very encouraging. The smooth agitation by hand also points to the importance of correct stirring or turbulence input.

Other flocculants such as LP 8597/43 needed to be measured out in such high doses that they were not tested further.

The results of the screening tests which were evaluated by subjective observations are given in Table 8 and are marked as follows:

- – = poor, hardly any difference from the untreated comparison
- + = slight difference from the comparison
- ++ = better difference from the comparison
- +++ = very good difference from the comparison, the clarified phase looks almost like tap water, the OD varies between <10 to 40, however the use of $FeCl_3$ coloured the clarified phase yellowish (see above)

Flocculant 1	Amount ppm	Flocculant 2	Amount ppm	pH	Observation
$Al_2(SO_3)_4$	200	Ökofloc K1	10	7.0	-
$Al_2(SO_3)_4$	200	Ökofloc A2	10	7.0	-
$Al_2(SO_3)_4$	200	Magnafloc LT-20	100	7.0	-
$Al_2(SO_3)_4$	200	Magnafloc LT-20	150	7.0	-
$Al_2(SO_3)_4$	200	Magnafloc LT-20	200	7.0	-
$Al_2(SO_3)_4$	200	Magnafloc LT-20	250	7.0	-
$Al_2(SO_3)_4$	400	---	---	7.0	+
$Al_2(SO_3)_4$	200	Ökofloc K1	20	7.0	+
$Al_2(SO_3)_4$	200	Ökofloc A2	20	7.0	+
$FeCl_3$	100	$Al_2(SO_3)_4$	200	7.0	+
$FeCl_3$	150	$Al_2(SO_3)_4$	200	7.0	++
$FeCl_3$	200	$Al_2(SO_3)_4$	200	7.0	++
$FeCl_3$	250	---	---	7.0	++
$Al_2(SO_3)_4$	500	---	---	7.0	++
Ökofloc K1	10	$FeCl_3$	200	7.0	++
Ökofloc A2	10	$FeCl_3$	200	7.0	++
$Al_2(SO_3)_4$	200	Praestol	5	7.0	-
$Al_2(SO_3)_4$	200	Praestol	10	7.0	-
$Al_2(SO_3)_4$	200	Praestol	15	7.0	-
$FeCl_3$	150	Praestol	5	7.0	+
$FeCl_3$	150	Praestol	10	7.0	+
$FeCl_3$	150	Praestol	15	7.0	+
$Al_2(SO_3)_4$	150	Praestol	20	7.0	+++
$Al_2(SO_3)_4$	200	Magnafloc LT-24	5	7.0	-
$Al_2(SO_3)_4$	200	Magnafloc LT-24	10	7.0	-

Flocculant 1	Amount ppm	Flocculant 2	Amount ppm	pH	Observation
$Al_2(SO_3)_4$	200	Magnafloc LT-24	15	7.0	-
$Al_2(SO_3)_4$	200	Magnafloc LT-24	20	7.0	-
$Al_2(SO_3)_4$	150	Magnafloc LT-24	40	7.0	-
$Al_2(SO_3)_4$	150	Magnafloc LT-24	10	7.0	-
$Al_2(SO_3)_4$	150	Magnafloc LT-24	15	7.0	-
$Al_2(SO_3)_4$	150	Magnafloc LT-24	20	7.0	-
$Al_2(SO_3)_4$	250	Praestol	20	7.0	-
$Al_2(SO_3)_4$	200	Praestol	20	7.0	-
$Al_2(SO_3)_4$	150	Praestol	20	7.0	+++
$Al_2(SO_3)_4$	100	Praestol	20	7.0	+++
$Al_2(SO_3)_4$	75	Praestol	20	7.0	++
$Al_2(SO_3)_4$	50	Praestol	20	7.0	+++
$Al_2(SO_3)_4$	100	Praestol	25	7.0	+++
$Al_2(SO_3)_4$	100	Praestol	20	7.0	+++
$Al_2(SO_3)_4$	100	Praestol	15	7.0	+++
$Al_2(SO_3)_4$	100	Praestol	10	7.0	+++
$Al_2(SO_3)_4$	100	Praestol	5	7.0	-
$Al_2(SO_3)_4$	50	Praestol	10	6.0	+
$Al_2(SO_3)_4$	50	Praestol	20	6.0	-
$Al_2(SO_3)_4$	25	Praestol	20	6.0	-
PAC	50	---	---	7.0	-
PAC	100	---	---	7.0	+++
PAC	50	$Al_2(SO_3)_4$	100	7.0	++
Chitosan	200	---	---	8.0	-
Chitosan	300	---	---	8.0	-
Chitosan	400	---	---	8.0	+
Chitosan	200	Praestol	20	8.0	-
Chitosan	300	Praestol	20	8.0	+
Chitosan	400	Praestol	20	8.0	+
Chitosan	500	---	---	8.0	++

Table 8: Result of the screening test with 100 ml graduated laboratory cylinders by subjective observations

Based on the results of the jar and graduated cylinder tests we compared the flocculants and put them in relation to the costs per kg biomass to be harvested. In this comparison we assumed a biomass content of 400 mg/l, as we did with the centrifuge above. Further more we assumed an efficiency of almost 100 %.

Flocculants	amount necess. ppm or $g \cdot m^{-3}$	Price of of flocc. US $ p.kg	costs p.m^3 susp. US $	costs p.kg biom. US $	costs p.kg ß-car.. US $	evaluation
Alum. sole	400	1.83	0.73	1.83	22.89	+
Sachtoklar	200	0.80 1*)	0,16	0.40	5.00	+
Sachtoklar	400	0.80	0.32	0.80	10.00	++
Sachtoklar	900	0.80	0.72	1.80	22.50	+++
Alum. plus	200	1.83	0.37	0.92	11.43	
Praest.2540	20	5.88 1*)	0.12	0.29	3.63	+++
total			0.49	1.21	15.06	
Alum. plus	150	1.83	0.27	0.69	8.58	
Praest.2540	20	5.88 1*)	0.12	0.29	3.63	+++
total			0.39	0.98	12.21	
Alum. plus	100	1.83	0.18	0.46	5.72	
Praest.2540	15	5.88 1*)	0.09	0.22	2.76	+++
total			0.27	0.68	8.48	
Alum. plus	50	1.83	0.09	0.23	2.81	
Praest.2540	15	5.88 1*)	0.09	0.22	2.76	++
total			0.18	0.45	5.57	
Alum. plus	100	1.83	0.18	0.46	5.72	
Praest.2540	10	5.88 1*)	0.06	0.15	1.81	++
total			0.24	0.61	7.53	
Chitosan 2*)	200	10.00 3*)	2.00	5.00	62.50	+
Chitosan	400	10.00	4.00	10.00	125.00	++

Table 9: Different flocculants and combinations and their impact on the harvesting costs

1*) FOB FRG Bremen harbour
2*) Chitosan should in to our experience not be used. However it can be produced locally, especially at those places with seafood processing. This supports the philosophy of the so-called "adapted technology". It is completely natural and not hazard to health.
3*) This price is still very low. Personally we were informed that chemical costs to produce 1 kg of chitosan were in the range of about US $ 25.00. We already considered the possibility of using raw materials with a much higher chitosan content than in Antofagasta.

The data in Table 9 show that we succeeded in dramatically decreasing flocculation costs by the consistent application of flocculant combination. The problem in practice, however, is to find an adequate stirring system. The authors recommend consulting a reputable company experienced in the use of flocculation units and in the application of stirrer systems. If stirring is optimized and if the Dunaliella are cultivated carefully flocculation works adequately at economic running costs. An in-line stirrer, also called a "static mixer", can be recommended to optimize stirring. It should be installed between the first stirring unit to which aluminium sulphate will be added and the flotation unit. Furthermore it must be pointed out that flotation is the significant subsequent step. Only a few samples half floated and half settled, all others floated.

2.2.2. Practical Flocculation Test on a Small Scale

Based on the results of the laboratory tests we started with outdoor experiments in 0.5 m^3 and 1.0 m^3 tanks.

Schedule of the experiments:

1. pH in ponds 8.5
2. pumping of 1.0 m^3 into the round, 1 m^3 storing tank equipped with a small propeller stirrer at the side
3. decreasing the pH to 7.0 with HCl
4. switching on of the propeller
5. adding 100 ppm ALUM; stirrer runs one minute
6. adding 15 ppm PRAESTOL; stirrer runs another minute
7. switching off of the propeller
8. waiting 15 minutes

Result of this experiment:

poor, small soft not stabilized flocks,

OD of the supernatant 50

Next experiment:

Point 1 to 5 like above. Then however:

6. pumping 0.500 m^3 into a 0.500 m^3 tank
7. adding 15 ppm PRAESTOL at the same time

Result:

foam and big flocks appear immediately, the flotated biomass can be skimmed off by hand easily and it remains even on a wide meshed mosquito net .

Moreover:

8. 15 ppm Praestol was added into the 1.00 m^3 tank with the remaining 0.5 m^3 suspension and stirred for one minute

Result:

as before

In both tanks the supernatant had an OD of only 10. The two experiments were repeated twice with the same results. Poor flotation when the 1 m^3 suspension was flocculated at once, good results with 0.5 m^3 suspension only. This again clearly indicates the importance of the right stirring technique on the impact of the flocculation.

2.2.3. Possible Use of an Air Decompression Unit, an AQUATECTOR

A flotator works satisfactorily only if a good air decompression unit is installed. Electro-flotators in former use have too many disadvantageous due to high electrode wear and can therefore not be considered. We tested a patented unit which advantageously produces extremely fine bubbles of a few μ. The feeding pump needs a pressure of only 3 bar. The necessary air pressure should exceed this by about 0.5 bar. Due to pumping, mixing and dosing hindrances experiments were performed batchwise without flocculants by using tap water instead of recycled supernatant. It provided the correct pressure and simulated the feeding pump well. We observed the expected fine bubbles and flotation. However, it was insufficient due to the missing flocculants. Mostly protein components and soiling flotated. Nevertheless, such a unit supports flotation of Dunaliella in a economical way.

A unit like the AQUATECTOR could also be used to distribute the expensive CO_2, pure or mixed with air, into the culture units with a much higher efficiency than by bubble stones.

3. Harvesting of Dunaliella, Estimate and Costs

There are clear differences between the running costs of a centrifuge operated carefully and optimum flocculant combination. Running costs to produce 1 kg ß-carotene with the best data are:

1. Centrifugation		
Centrifuge rebuilt as clarifier with one small centripetal pump and closed channels	US $	03.87 - 04.66
Centrifuge rebuilt as separator with one large centripetal pump and closed channels	US $	06.76 - 07.50
Centrifuge rebuilt as separator with one large centripetal pump and open channels	US $	10.27 - 12.46
2. Flocculation		
Alum plus Praestol	US $	07.53 - 08.48
Sachtoklar	US $	10.00 - 12.00

Table: 10: Running costs of different centrifugation and flocculation methods per kg ß-carotene

If preference is given to centrifugation, the centrifuge should run as a clarifier. Additionally it must be pointed out that a large industrial appliance will run much more economically than the one we tested in Antofagasta. Its electric installation is oversized as we mentioned above. A centrifuge is easy to run and always provides the same product quality. Its efficiency will not be influenced by differences in the culture media. Changes in weather or failure of the CO_2 supply cause severe variation of the algae culture with respect to harvesting demand. In Germany sometimes certain harvesting processes with easy to filtrate green algae failed, but centrifugal processes never did. It should also be mentioned that a centrifuge, especially when running with a high throughput, does not clarify 100 %. In Antofagasta only 70 - 80% was clarified economically. Higher clarifying efficiency by reducing the throughput would cause higher running costs. Therefore it is necessary to recycle the culture medium. However this should be considered anyway as the pumping costs and possibly additional surfaces to evaporate sea water to a salinity of about 20% and nutrients increase the production costs of Dunaliella. We already mentioned that Dunaliella was some how "homogenized" when desludged. An evaluation of this is difficult. Subsequent steps must follow rapidly to avoid spoiling of the biomass.

In the case of flocculation/flotation a combination of aluminium sulphate and an anionic polymer is considered. Praestol should be preferred. It showed good results and is available in Chile. The Stockhausen Company has a laboratory and technical equipment in Santiago, and provides advice and supports with practical tests. Though the running of flocculation seems to be more costly than centrifugation it has some advantages. In combination with tanks flotation can be used anywhere. The cells are not destroyed and can be stored longer before further processing. It may also be possible to drain the flotate on simple cloths or by means of a decanter. But this must be verified. The efficiency of flocculation/flotation is higher than centrifugation as the supernatant contains less biomass (OD 10 - 40). But flotation is complicated to handle. We mentioned above that the different quality of the culture caused by exterior influences brings many problems. It requires great intuition on the part of the person running the flotation unit. Jar tests are necessary frequently, at least every day, to find optimum flocculant concentration and combination. Another point is the chemical contamination of the biomass. For instance the use of 150 ppm flocculants leads to a chemical share of 35 % if the culture has a dry substance of 400 $mg \cdot l^{-1}$. It has not yet been tested whether washing the flocculated biomass with HCL and water removes aluminium sulphate as reported from Israel. However this demands extra chemicals and equipment.

4. Cultivation of Dunaliella, Estimate and Costs

Many factors effect these expenses. The report evaluates the most important ones from which data are obtained. These are:

4.1. Pond Construction

4.1.1. Material

An easy and relatively cheap method to build ponds is the use of plastic sheets as lining on a prepared underground. Traditional concrete ponds should not be used.

a. They are expensive and need additional earth works. The concrete must be coated with expensive varnishes like epoxy resins to avoid the dissolvation of Ca^{+} by CO_2 which precipitates with the phosphates of the nutrients. Or a special CO_2-resistant concrete is necessary.

b. It is difficult to restrain cracks, particularly if large surfaces are constructed which are very sensitive to smallest alterations of the underground. They also can appear due to large temperature contrasts. Through them the suspension penetrates into the underground and permits the settling of organisms which causes fouling and diseases.

4.1.2. Turbulence

In most algal plants paddle wheels agitate the cultures. They are described as safe and reliable and particularly in small plants easy to build. Bigger paddle wheels demand more sophisticated techniques for the bearing and the drive, which makes them expensive. Additionally their high energy consumption increases the production costs. The literature reports an energy demand of $1 - 6\ W \cdot m^{-2}$ pond surface. If only $2\ W \cdot m^{-2}$ were to be assumed, paddle wheels require $66{,}000\ kWh \cdot a^{-1}$ ($330\ d \cdot a^{-1}$ and $10\ h \cdot d^{-1}$). for a 2 t ß-carotene plant.

The FORSCHUNGSZENTRUM Jülich developed a mixing system similar to the dragboard described by Markovits and used in Antofagasta. It consists of a float carrying turbulence bodies by means of PVC pipes. Their clearance to the pond bottom is about 30 mm. The energy demand was measured with a strain gauge and calculated as 1/30 of that of paddle wheels. In a further step, small electric motors obtaining their energy from photovoltaic cells mounted on the float drive the whole device. In the meantime a raft with a width of 4.00 m and a length of 6.00 m has been constructed and operated satisfactorily. It must be investigated how such big constructions agitate in ponds with a depth of only 20 cm, as is considered for Dunaliella. In Jülich the first float was tested in a 29 cm deep pond. The price of the above mentioned unit was about US $ 6,500.-.

4.1.3. Pipes and Pumping Units

Every pond including the small cultivation units should be connected with each other to transport the cultures, broths, water and supernatants from one place to any other within a plant. This includes pumping to intermediate tanks, to harvesting units, discharges etc. Smaller plants can be equipped with mobile pumps and hoses. Larger plants, however, need installed pumps, if possible in accordance with the medium, pipes and valves for which we calculated about US $ 1.50 - 2.00·m^{-2} pond surface.

4.2 Laboratory Equipment

Minimum laboratory equipment is essential to care for the cultures in the laboratory and outdoors. There should be oxygen and pH meters, if possible with pH regulation, a photometer, a membrane filter unit, a drying chamber, a muffle furnace, a good microscope, a refrigerator with a freezer and an illuminated small room or chamber kept at a moderate temperature, a small shaking unit, a small laboratory centrifuge, laboratory glassware etc. Such equipment does not automatically need to be supplemented if a plant increases in size. Such equipment is estimated to cost about US $ 30,000.-

4.3. Nutrients

These are so- called variable costs.

1. fertilizer and microelements
2. CO_2
3. freshwater to replace the evaporated water

5. Calculation of the Production Costs.

We estimated an expressed amount of 2 to 4 t ß-carotene per year covering demand for certain equipment and installations based on the following data:

<u>Note: costs for labour and land are not included</u>

1.	ß-carotene per year	:	2	t	
1.	ß-carotene content	:	8	%	
2.	growth rate	:	4	$g \cdot m^{-2} \cdot d^{-1}$	
3.	harvesting density	:	4	$g \cdot l^{-1}$	
4.	working period	:	330	$d \cdot a^{-1}$	
5.	working time	:	18	$h \cdot d^{-1}$	
6.	energy costs	:	00.17	US $\$ \cdot kWh^{-1}$	
7.	pumping systems	:	01.50 - 02.00	US $\$ \cdot m^{-2}$	
8.	pond construction:				
	earthwork & lining	:	09.39	US $\$ \cdot m^{-2}$	
	turbulence by floats	:	05.59	US $\$ \cdot m^{-2}$	
9.	fertilizer and microelements	:	00.80	US $\$ \cdot kg^{-1}$	biomass, calculated in Antofagasta
		=	10.00	US $\$ \cdot kg^{-1}$	ß-carotene
10.	CO_2	:	00.40	US $\$ \cdot kg^{-1}$	if delivered in tanks
		=	00.88	US $\$ \cdot kg^{-1}$	biomass
		=	11.00	US $\$ \cdot kg^{-1}$	ß-carotene
11.	tap water	:	00.33	US $\$ \cdot m^{-3}$	
12.	evaporation	:	10.00	$l \cdot m^{-2} \cdot d^{-1}$	
13.	capital interest for investments	:	16.5	% per year	
14.	amortization of installed equipment	:	4 - 12	years depending on the material	
15.	maintenance	:	2	% of investment per year	
16.	total pond surface	:	18,950	m^2	
17.	throughput of the harvesting equipment	:	10.5	$m^3 \cdot h^{-1}$ or 62.370 $m^3 \cdot a^{-1}$	

16 and 17 were calculated by considering 1 - 5 and will change with different annual consideration.

Formula used to calculate the equipment costs per year:

$$Y = \frac{(\frac{1}{2} I + M) C \cdot A + C}{A} + R$$

Y = annual costs of the equipment
I = interest in % per year
M = maintenance in % of capital costs
C = capital or investment costs
A = amortization in years
R = running costs: electric power or chemicals per year

Equipment and/or supplies	Investment US $	Amortization years	Investment Total US $	Costs p.a. US $	Costs p.a. Total US $
(1) Pond construction					
earthwork for the ponds (levelling with bulldozers)	104,000	12		19,326	
plastic lining	74,000	8		17,020	
turbulence as descr.	106,000	4		37,365	
pumping and lining	34,000	10	318,000	6,970	
Energy for pumps (0.085 kW$\cdot$m^{-3})				904	81,585
(2) Laboratory	30,000	12	30,000	5,575	5,575
(3) Nutrients					
fertilizer				20,000	
CO_2				22,000	
water				20,637	62,637

Equipment and/or supplies	Investment US $	Amortization years	Investment Total US $	Costs p.a. US $	Costs p.a. Total US $
(4) Harvesting Equipment					
(4.1) Centrifuge					
pump 20 $m^3 \cdot h^{-1}$	2,000	12		372	
energy for pump				901	
basket filter	3,000	12		565	
storage tank 1.5 m^3	1,000	12		188	
Westfalia centrifuge SA 20	160,000	12		29,733	
energy demand for centrifuge				8,530	
mixer for the harvested biomass	4,000	12		733	
energy demand for mixer				505	
storage tank for the harvested biomass 50 l (cooled)	4,500	12	174,500	825	
energy for tank				300	42,652
Total costs if centrifuge is used (1+2+3+4.1)			522,500		192,449
(4.2) Flocculation Flotation					
(100 ppm $Al_2(SO_3)_4$ & 10 ppm Praestol)					
storage tank for aluminium (80 - 100 days) 2.5 to	2,000	12		376	

Equipment and/or supplies	Investment US $	Amortization years	Investment Total US $	Costs p.a. US $	Costs p.a. Total US $
tank for Praestol 0.5 to	1,500	12		283	
tank for the daily aluminium solution (1%) approx. 2 m^3	7,000	12		1,318	
energy for stirrer				56	
tank for the daily Praestol solution (0,05%) approx. 0.5 m^3	4,000	12		753	
energy for stirrer				56	
tank for HCL (pH regulation)	3,000	12		565	
flotation unit	70,000	12		13,008	
energy for flotation unit (0.14 $kW \cdot m^{-3}$)				1,484	
pump 20 $m^3 \cdot h^{-1}$	2,000			377	
flocculants				15,060	
collecting tanks	2,000	12		372	
dosing pumps	3,000	12		558	
mixing unit	3,000	12	97,500	372	34,638
total costs if flotation is used (1+2+3+4.2)			445,000		184,435

Table 11: The costs of the Dunaliella process, considering the cultivation and the two harvesting techniques

6. Conclusion and Discussion

In the authors' experience centrifugation and flocculation and / or flotation are the only techniques to harvest Dunaliella on a large scale safely and economically. The extreme salinity (20%) of the culture medium did not affect either procedure. The authors feared that the resulting high density (1.16 $kg \cdot dm^{-3}$) of the culture medium might impede centrifugal forces for separation. Crumbling of the long molecules of the polymeric flocculants was expected as well. However, both systems separate effectively and almost reliably if applied accurately.

The most important factors are the cultivation costs which include the ponds, nutrients and laboratory equipment. Others, like land and the further treatment, the extraction, were not estimated and must be considered in another investigation.

6.1. Centrifugation

A self- cleaning plate separator running as a clarifier with closed rising channels worked most economically. Other techniques such as using open channels or running as a separator harvested less effectively and / or not so reliably. Increasing the outlet pressure behind the centripetal pump did not alter the clarifying effect but enlarged the energy consumption.

Centrifugation proved to be a very safe and reliable harvesting technique. The average energy costs to harvest 1 kg of ß-carotene by means of a centrifuge are:
US $ 4.27.

The energy demand of centrifuges for production plants will be lower, as the rated electric power of that tested was nearly twice that of an industrial centrifuge.

Additional costs such as amortization must be calculated per kg ß-carotene:
US $ 21.33

Total harvesting costs by means of centrifugation per kg ß- carotene:
US $ 25.60

6.2. Flocculation and / or Flotation

Flocculation and flotation was proved as a good harvesting method.

A combination of $Al_2(SO_3)_4$ and an anionic polymer from Stockhausen (Praestol 2540) flocculated the biomass best. The reproducibility of the results was good. Other flocculants were not as reliable or too difficult to obtain in Chile. However $FeCl_3$ should replace aluminium sulphate at a later point in time if possible as it is less toxic.

Average flocculants costs to obtain 1 kg of ß-carotene:
US $ 8.00.
This price does not include energy for the flotation unit, such as air decompression, recycling pump, and flotate removal. They are calculated as 0.14 $kWh \cdot m^{-3}$ and amount per kg final product:
US $ 0.74

However, on the basis of our experiments in Antofagasta it is not clear whether these installations will be needed. The fall plump of the algae suspension provided such good soft turbulence and aeration in the flotation tank that the biomass flotated at once without any additional aid. Therefore it must be investigated whether flocculation and flotation can be done batchwise with several specially constructed harvesting tanks.

Equipment costs for flocculation and flotation per kg product:
US $ 9.05

Total harvesting costs by means of flocculation and flotation amount per kg ß-carotene:
US $ 17.79

6.3. Cultivation

The low growth rate of 4 $g \cdot m^{-2} \cdot d^{-1}$ demands a big pond surface. Two-stage cultivation techniques by starting with a low salinity and a high growth rate in the first phase and ending with a high salinity to build up the ß-carotene decreases the pond surfaces required and the costs. However, this cannot be recommended at this stage of the project. It is sophisticated to handle and the low salinity of the first stage enhances diseases. In Antofagasta Dunaliella was cultivated in one stage without any problem for many weeks. Turbulence production accounts for about 46% of the pond costs. This is so high because the authors calculated an amortization of 4 years only for this equipment. Nowadays firms guarantee 10 to 12 years. In later calculations this must be considered accordingly.

Pond costs without turbulence generation per kg ß-carotene:
US $ 22.11

Costs for the turbulence equipment per kg ß-carotene:
US $ 18.68

Also nutrients such as fertilizer including microelements, CO_2 and water to replace evaporation losses cause expenses. Fertilizers and CO_2 are variable costs. Other techniques, like the availability of additional evaporation ponds and a small variation of the salinity can reduce the needs for freshwater. However this should be considered thoroughly as the freshwater costs are about US $ 10.32 per kg final product which comes to 13.8% of the cultivation costs.

Nutrient demand per kg ß-carotene:
US $ 31.32

Laboratory and equipment demand per kg ß-carotene:
US $ 2.79

Total cultivation costs per kg ß-carotene are:
US $ 74.90

6.4. Evaluation

The authors evaluated possible harvesting techniques and their impact on the production costs of ß-carotene. The expenses for harvesting by means of flocculation and flotation gained by practical experiments in Antofagasta, amount to US $ 17.79 per kg ß-carotene. This is US $ 7.81 cheaper than harvesting by centrifugation which requires US $ 25.60 per kg ß-carotene (1.4 times flocculation / flotation or US $ 15,620 per year).

At first glance this difference seems to be high. However, if the total process is evaluated it turns out that harvesting only accounts for a small part of the whole.

Process	Part costs US $	% of part costs	All costs US $	% of all costs
all			96.23	100.0
total harvest			21.33	22.2
energy	4.27	4.4		
centrifuge	14.87	15.5		
pond construction			40.79	42.4
nutrients			31.32	32.5
laboratory			2.79	2.9

Table 12: The relation of the harvesting costs by means of centrifugation to the total costs per kg ß-carotene

Process	Part costs US $	% of part costs	All costs US $	% of all costs
all			92.22	100.0
total harvest			17.32	18.8
running costs	8.27	9.0		
flocculants	7.53	8.2		
energy	0.74	0.8		
flotator	6.50	7.1		
pond construct.			40.79	44.2
nutrients			31.32	34.0
laboratory			2.79	3.0

Table 13: The relation of the harvesting costs by means of flocculation / flotation to the total costs per kg ß-carotene.

Table 12 shows that centrifugation amounts to 22.2 % of the production costs whilst flocculation / flotation requires 18.8% (Table 13). The calculations do not include costs for extraction, land and labour. If added later the proportion of the harvesting expenses will decrease further.

Centrifuges work safely, reliably and can be easily attended. Variations of the culture quality do not influence the result of separation. The product does not contain any chemicals from flocculants. The maximum dry substance content varies by about 12 - 15% and cannot be increased by further treatment. The cells are already broken and must be cooled or extracted immediately after desludging.

The advantage of flocculation / flotation is that at the beginning of a plant one can start with just a few tanks, as a so-called low sophisticated technique. However flocculation demands complicated attention and does not work reliable every day. The high chemical content in the harvested biomass is very doubtful, especially the aluminium sulphate which comes to more than 25%. It is not yet known whether the flocculants still remain in the ß-carotene, whether they disturb the extraction process itself or whether they remain in the residues which are to be sold as a valuable animal feed, for instance to fish hatcheries. Later it should be investigated whether washing of the flotated biomass with HCL and water will decrease the aluminium sulphate content and whether this is economic. Also, whether the less toxic $FeCl_3$ can adequately replace aluminium sulphate as a flocculation aid. In the laboratory, $FeCl_3$ coloured the supernatant yellow which possibly prevents the recycling of the culture medium. This however is necessary to keep nutrient costs down. Otherwise the whole process will be uneconomic.

According to these estimates, the application of an explicit harvesting does not essentially affect the production price. Production costs are envisaged by the above calculated data only and range between US $ 96.- and 92.- per kg ß-carotene. The market price for 1 kg synthetic ß-carotene is estimated at about US $ 465.-.

At the present stage of knowledge, extract extraction costs by means of hypercritical CO_2 cannot be estimated clearly. This depends on the required purity of the product and efficiency of extraction. The retention time of the raw material (harvested Dunaliella) in the extractor influences the efficiency and the size of the apparatus. Machinery with a low efficiency will be cheaper but demands a higher annual production of the raw material as the loss in the extractor must be calculated. The extraction techniques must be studied in an additional investigation.

We have not yet mentioned that Dunaliella also provides other highly valuable products besides ß-carotene, such as glycerol and others. After extraction, the residues still present a worthy animal feed with an excellent biological value of the proteins which does increase not only productivity but also the quality of meat, eggs and fish. Especially fish hatcheries or mollusc industries are very much interested in feeding algal products or such residues. In other words, selling of the by-products makes the process still more economic than it already is.

From the described harvesting procedures the following guidelines are given. They should be understood as a general recommendation only. At all events they must be decided individually for each plant:

6.5. Instructions for Harvesting

1. Smaller units like that in Antofagasta or other small production plants with an annual capacity of 2 or 4 t ß-carotene should harvest with centrifuges if there is sufficient electricity infrastructure (about 11 kWh installed capacity for 2 t ß-carotene per year). It must be mentioned that for certain applications of Dunaliella, for instance if it is to be used as a health food as in Japan, chemicals from flocculants must not be used at any time. In those cases centrifugation is the only safe process.

2. Bigger plants or places with insufficient electricity infrastructure should use flocculation / flotation. This is also recommended if a unit increases its output and the capacity of the centrifuges is not big enough. It is also a reasonable technique to start with during the test phase. Scruples about the application of chemicals, even if they are "not toxic", should be considered at any time if the product will be used as described above.

7. Suggestions for the Project in Antofagasta

The work should be continued in any case. The plant is well equipped and has a highly skilled staff. It has been running continuously for a long time and produces small samples without interruption. The results realized in Antofagasta look very promising. Recommended activities in the near future:

1. An additional plastic-lined pond of about 100 - 200 m^2 should be built as described in this report together with the new photoelectric agitation system.

2. Harvesting shall be done with the centrifuge already installed. Recycling of the overflow.

3. Continuation of harvesting by flocculation / flotation on a pilot scale. If possible tests with air saturation units such as the Aquatector and differently shaped flotation tanks should be added.

4. Sending of biomass samples to Universidad Católica in Valparaiso. If possible the samples should be frozen to avoid premature spoiling and to allow a better quality control of the two harvesting devices. Production of ß-carotene extracts by the technique already installed. Samples should be collected to show or to give to interested buyers.

5. Search for safe extraction systems. The author in Antofagasta should therefore send about 10 - 15 kg harvested deep frozen biomass to the author at the FORSCHUNGSZENTRUM Jülich by direct air freight. If delivered at Santiago airport the author can get the shipment about 30 hours later at Düssel dorf airport from the Lufthansa air freight office and store it again deep frozen. The author has already contacted two firms with laboratory units to study extraction tests with hyper-critical CO_2. Other firms experienced in traditional extraction techniques will be contacted in the near future.

6. Estimation of the market for natural ß-carotene in Europe or Germany. Finding the amount and the price.

7. Delegating a firm for marketing studies.

8. Finding of buyers for the residues in Chile, e.g. fish hatcheries the south of the country.

9. Planning of a production plant for at least 2 t per year together with the Universidad Católica in Valparaiso.

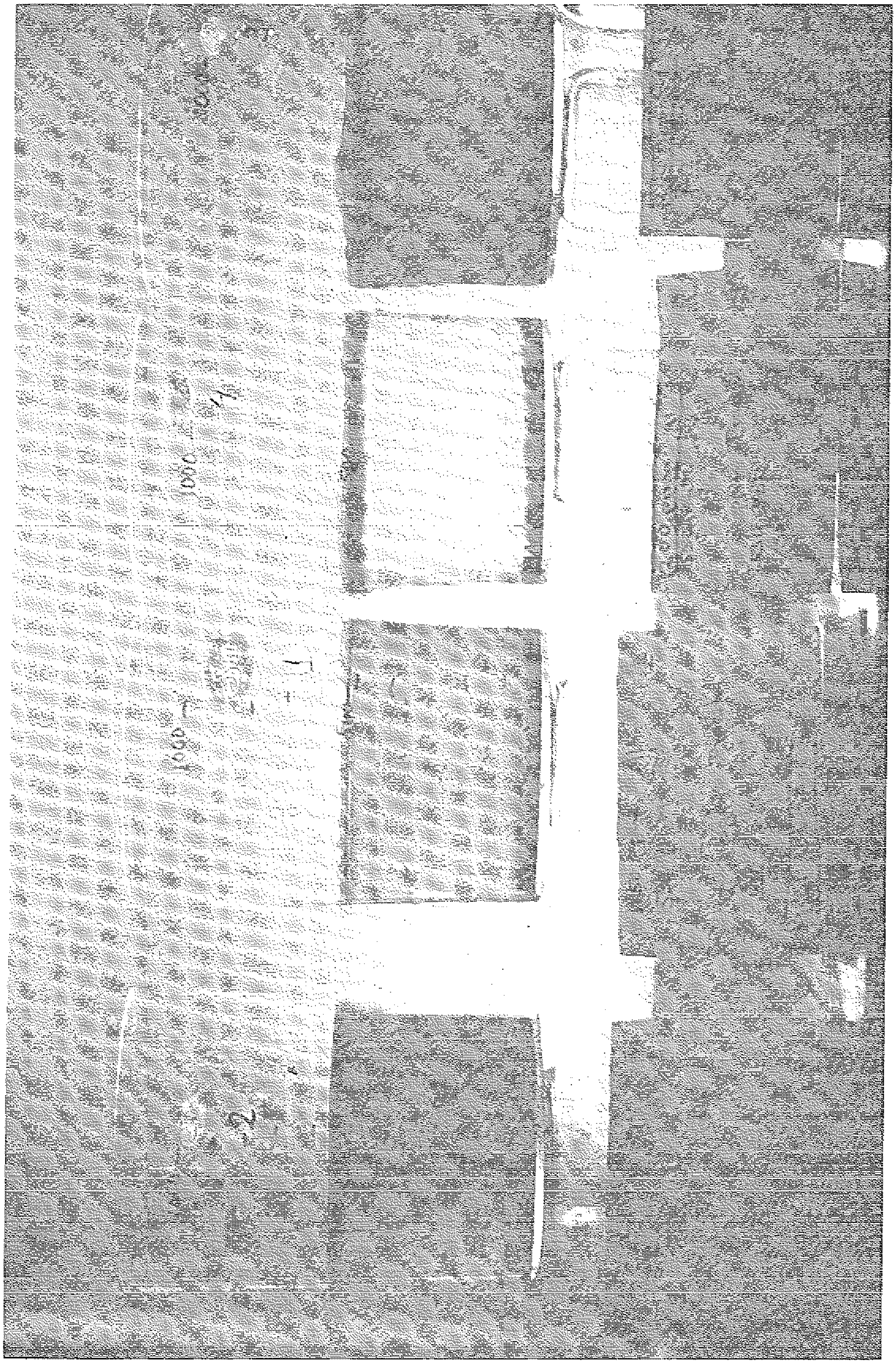


Fig. 2: Jar tests; left old suspension: no sedimentation; 2nd same condition but fresh suspension: little sedimentation; 3rd 200 ppm Al instead of 150 and 5 ppm Praestol instead of 2.5: good sedimentation and flotation; right Fe_3Cl instead of Al: good sedimentation, but the supernatant becomes yellow. (results measured after 30 min.)

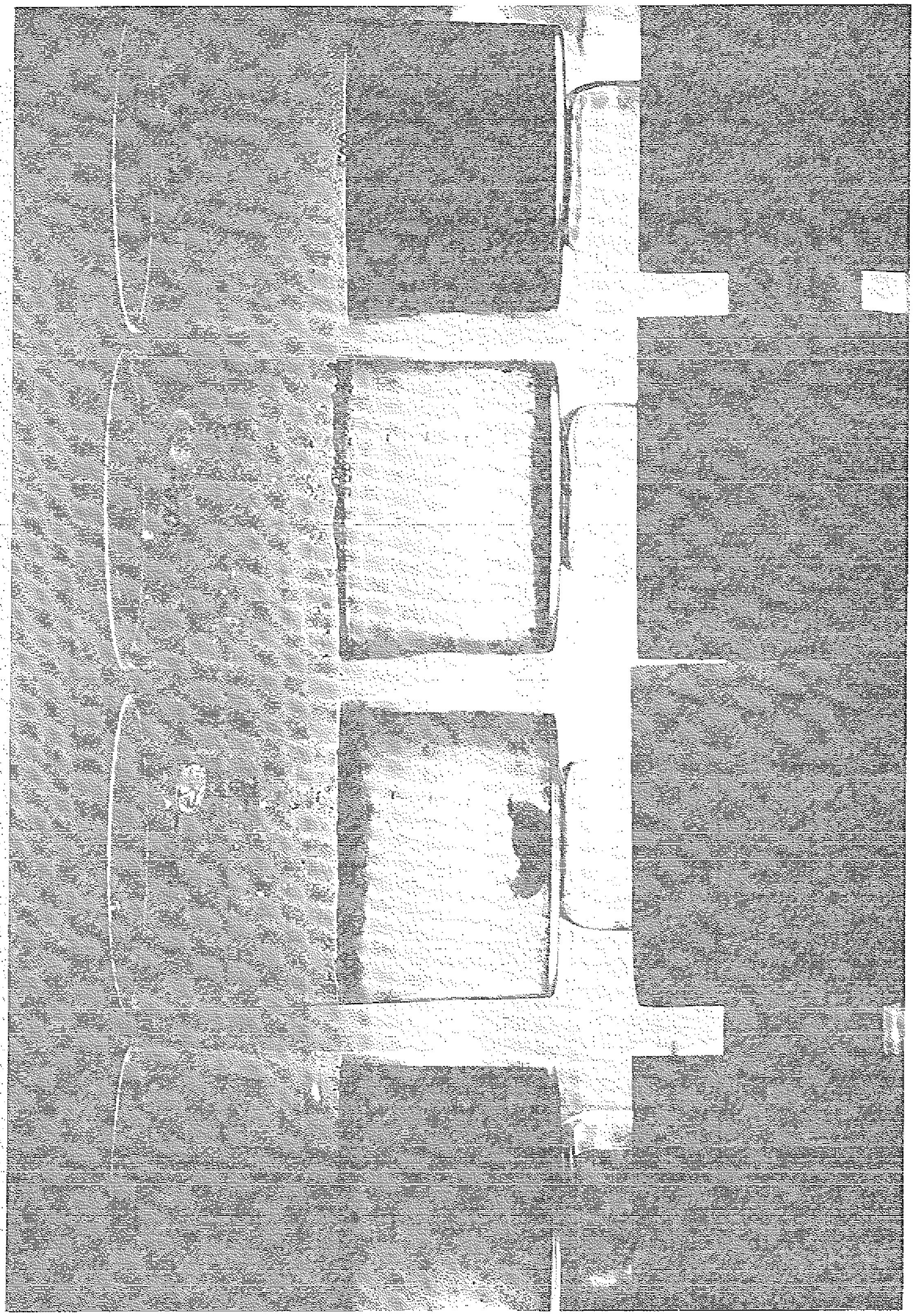


Fig 3: Jar tests; 2nd, the same flocculants as on the left, but different speeds of the stirrer: good sedimentation; 3rd, 150 ppm of Al instead of 200 ppm on the left: good sedimentation; right, Al and Chitosan: poor result (30')

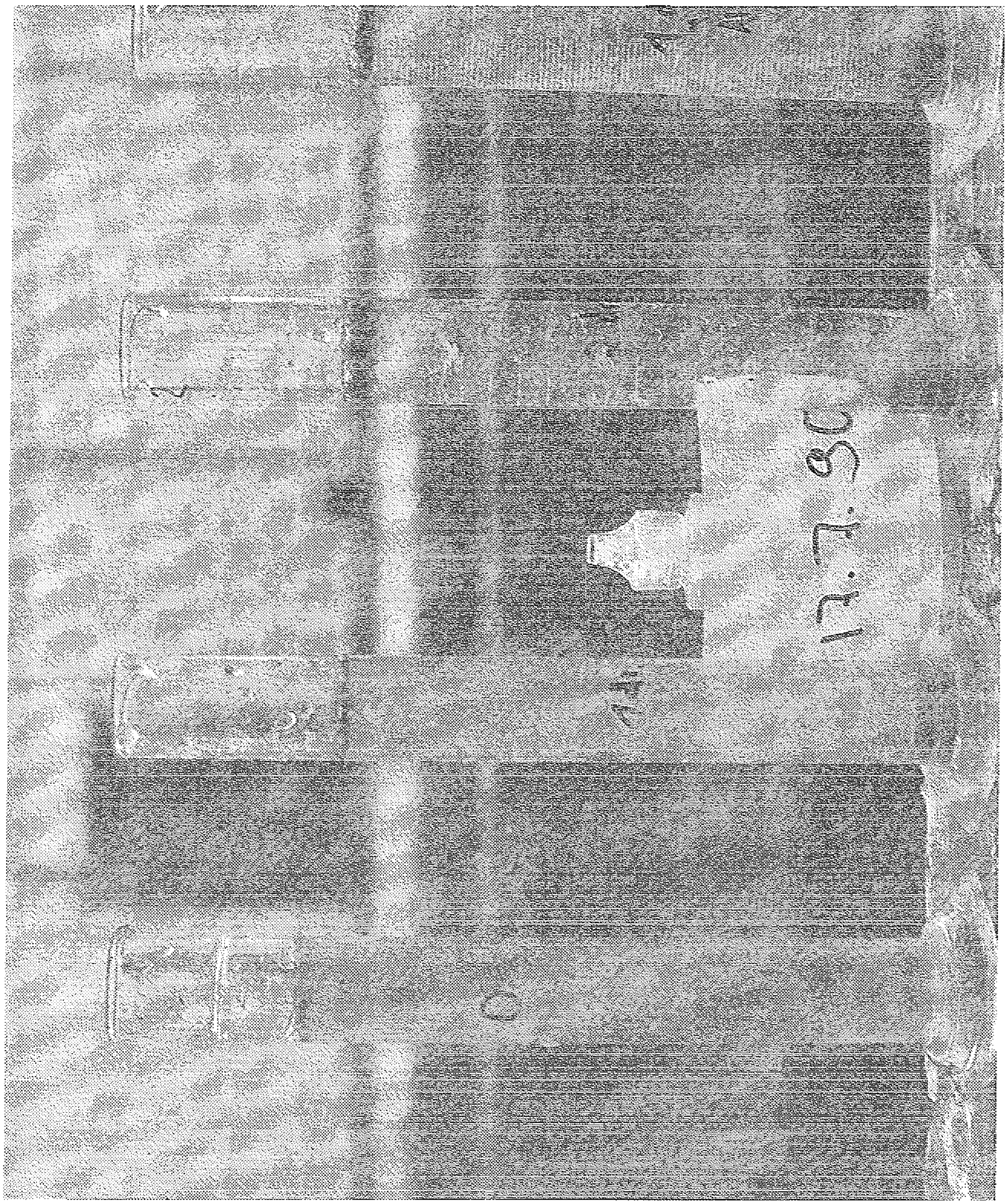


Fig. 4: Graduated cylinder test; different amounts of PAC (Poly-aluminium chloride) 50, 100 ppm and 50 plus 100 ppm Al

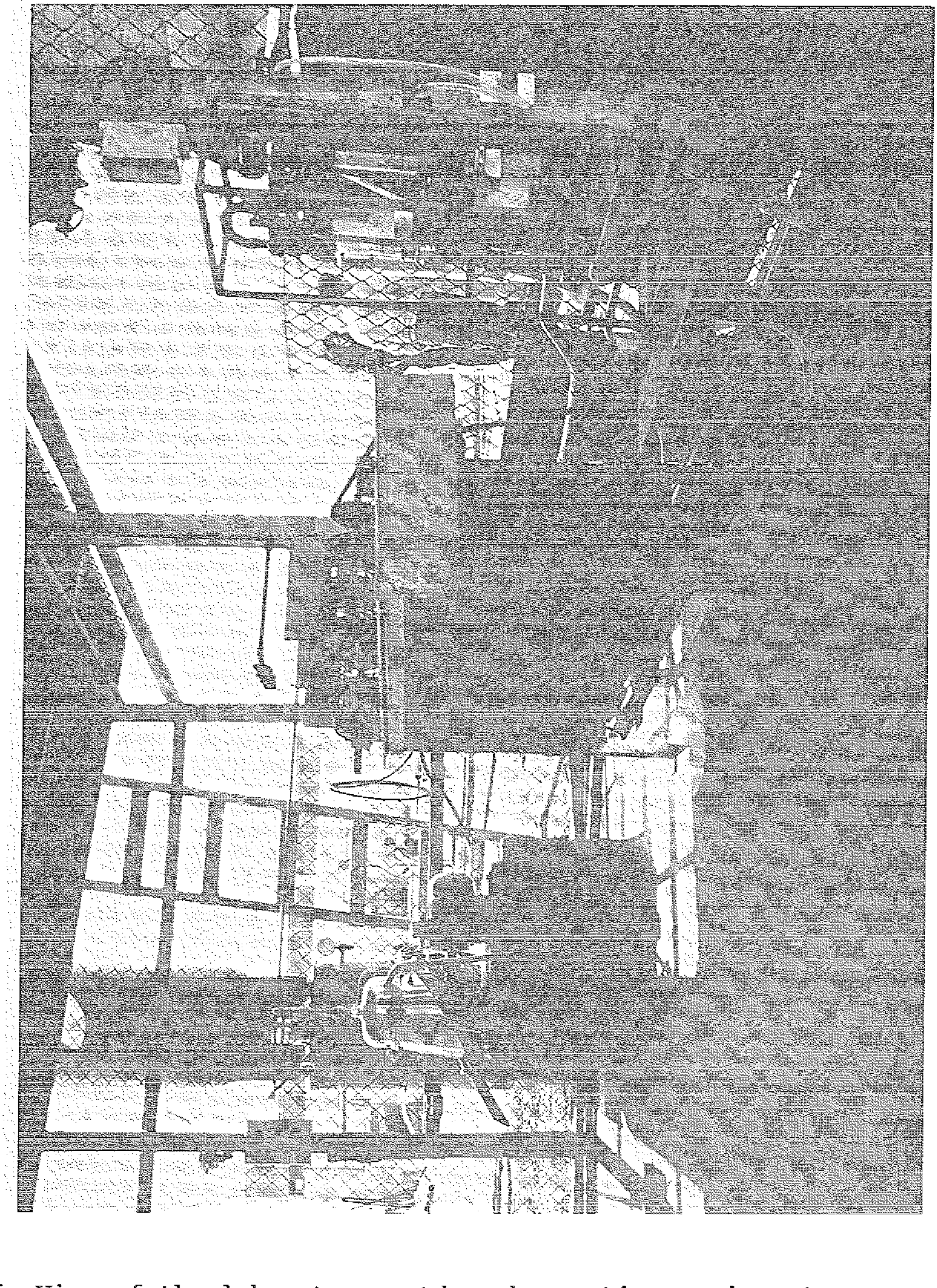


Fig. 5: View of the laboratory outdoor harvesting equipment left: centrifuge; centre: flotation unit; right: air decompression (saturation) unit (Aquatector)

Fig. 6: Flocculation of 1 m^3 Dunaliella suspension; after stirring 5 min. 100 ppm $Al_2(SO_3)_4$, 15 ppm Praestol is just added and then mixed for 1 min. (s. page 20 & 21)

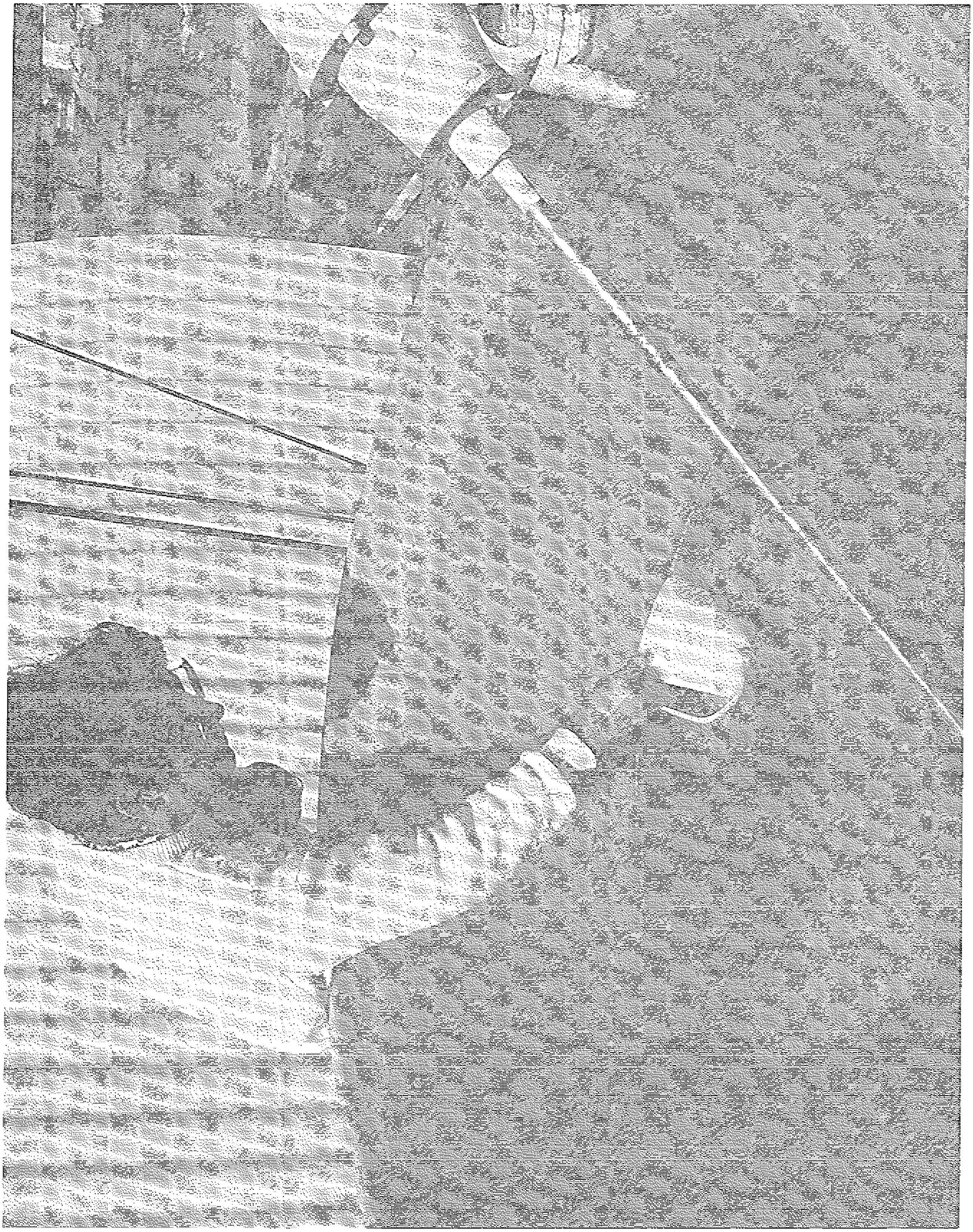


Fig. 7: Flocculation of Dunaliella in a 1 m^3 tank; the flotate is taken off by hand

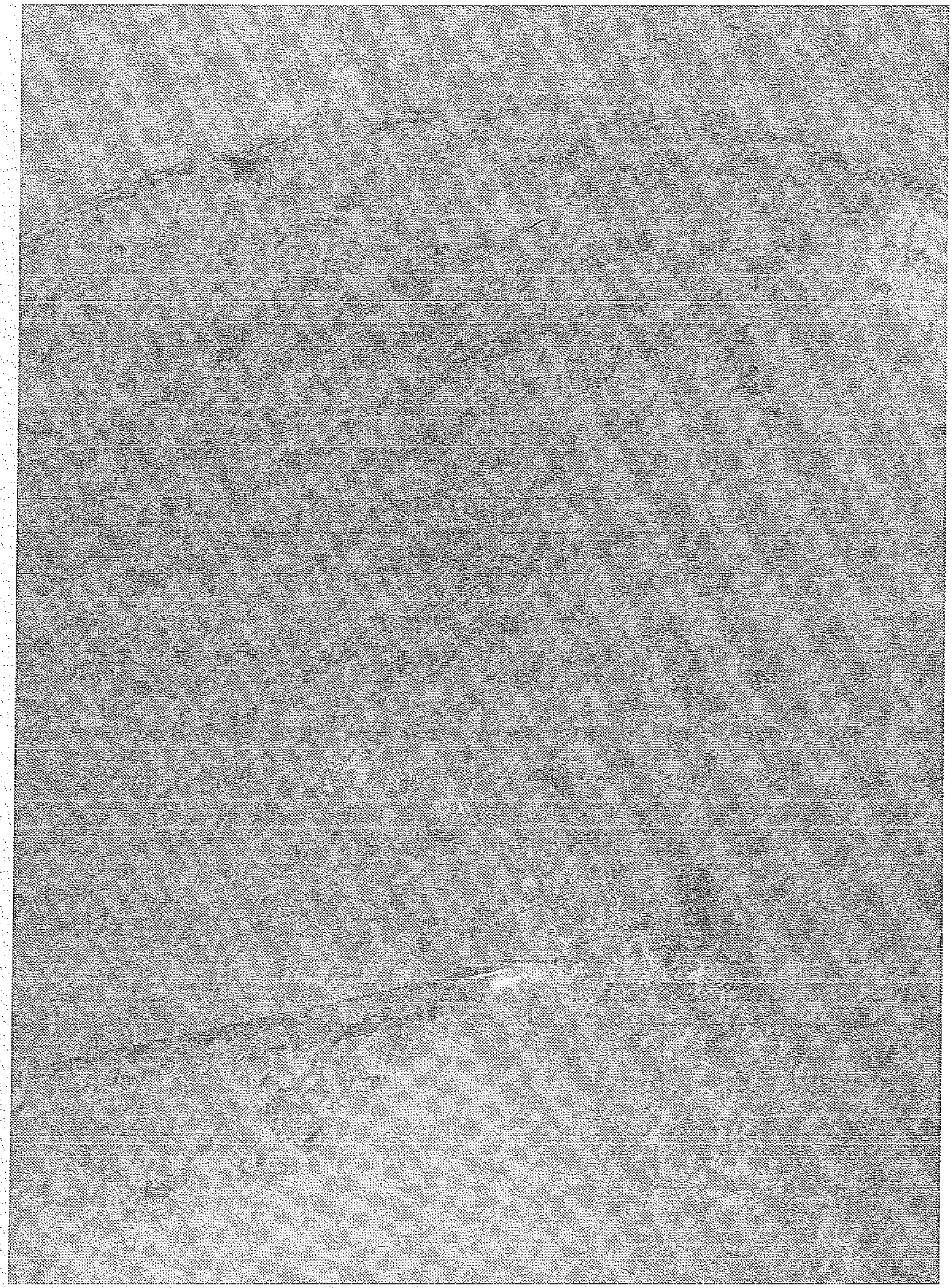


Fig. 8: Flocculation in a 1 m^3 tank; after withdrawing the flotate the supernatant has an OD of about 10,; the propeller reaches to the bottom of the tank and can be seen clearly

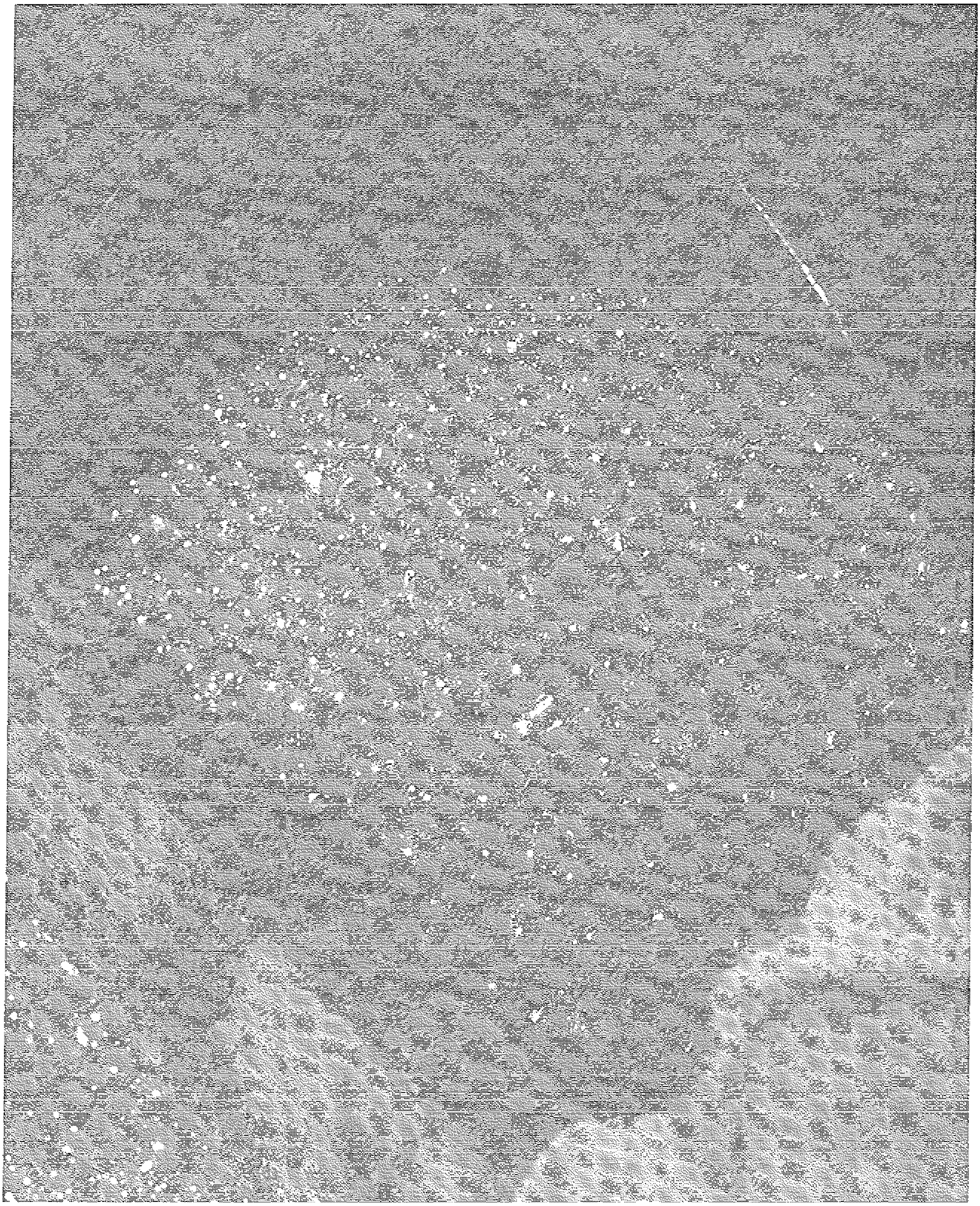


Fig. 9: **Flocculation** in a 0.5 m^3 tank; after $Al_2(SO_3)_{4\,3}$ was added in the 1 m^3 tank, and stirred for 5 min. 0.5 m^3 were pumped into a 0.5 m^3 tank and Praestol added at the same time; the "fall plump" aerated the mixture so strongly that flocks appeared immediately during the process

Fig. 10: Flocculation in a 0.5 m^3 tank; 1 min. after filling the tank a thick stiff biomass was floating on the surface

Fig. 11: Flotation of Dunaliella; after skimming the thick and stabilized biomass was put into a pail lined with a cloth to drain

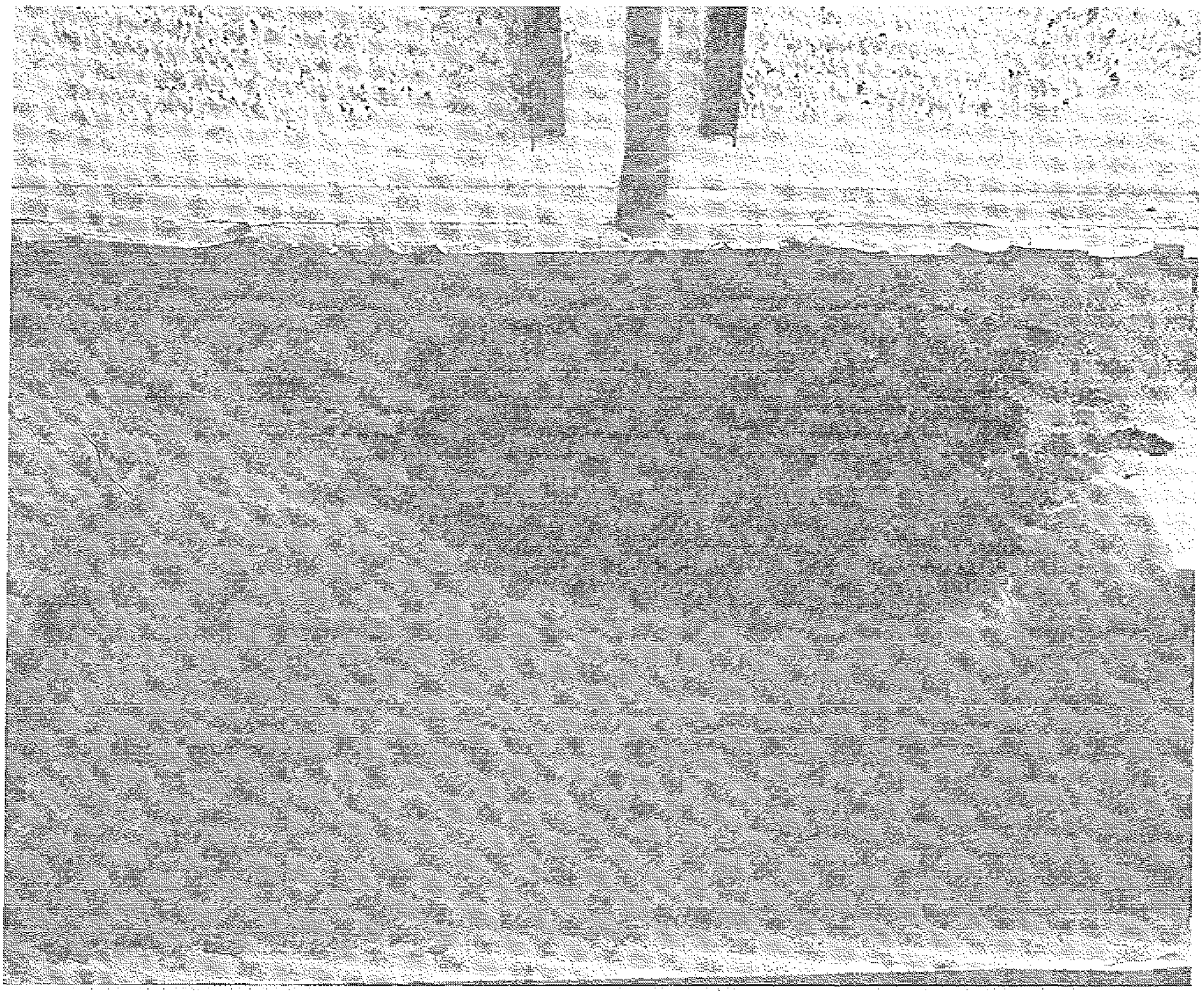


Fig. 12: Flotating Dunaliella; the flotated and skimmed biomass is so stabilized that it remains even on a wide meshed mosquito net

FORSCHUNGSZENTRUM JÜLICH GmbH

KFA

Jül-2438
Februar 1991
ISSN 0366-0885