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Technical and economical feasibility of a rotifer recirculation system

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Abstract

A feasibility study was performed on the use of a recirculation system for the mass culturing of rotifers at industrial level. Rotifer culture systems with a culture volume of 750 l were operated at three different stocking densities (3000, 5000 and 7000 individuals ml^{-1}) in a completely closed recirculation system. At all operating rotifer densities, a reliable production of 2.2 billion rotifers could be obtained on a daily basis during 3 weeks. Excellent water quality was maintained by the use of protein skimmers, the use of ozone and a submerged biofilter. The microbial counts remained stable during the whole culture period (10^6 CFU ml^{-1} on marine agar and 10^4 CFU ml^{-1} on TCBS after 15 and 23 days, respectively). No difference in HUFA and protein content were obtained between rotifers harvested from the recirculation system or from a conventional batch culture system. Compared to a commercial batch culture system, the use of a recirculation system can contribute to a 43% saving on the capital investment and the annual operation cost. By using this system, capital investment cost is considerably reduced by 46%. Savings are also made on labour cost (65%) and feed cost (21%) during a 1-year production.

In general terms, it can be stated that by using a simple recirculation system, a cost-effective technology and a reliable rotifer culture can be obtained.

Keywords: *Brachionus plicatilis*; Recirculation; Rotifers; Ozone; Protein skimmer; Operational cost

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1. Introduction

The economic profitability of larval rearing in marine finfish hatcheries depends to a large extent on the continuous availability of high quality live food. In this respect, the demand for rotifers has gradually increased over the last years which can be explained by the relatively stagnating *Artemia* supply for an increased aquaculture production. The most extreme need for rotifers can probably be found in Japan where an average hatchery requires 20 billion rotifers day^{-1} (Fushimi, 1989; Fu et al., 1997). The increasing need for rotifers forces hatcheries to re-organize hatchery space and to employ extra personnel or intensify the mass production of rotifers. As a result, these factors have a direct impact on the larval production cost.

The cost of rotifer production depends to a large extent on the rearing technique applied in hatcheries. In some European hatcheries, rotifers are cultured in batch systems using algae and yeast as a food source. This type of rotifer production is labour intensive and results in an average cost of € 0.4 per million rotifers (Candreva et al., 1996; Komis, 1992). In Japan, prefectural hatcheries are characterized by their large size and a preference for more automated large-scale culture systems. Some prefectural hatcheries have invested in the continuous culture of rotifers and the use of concentrated algae (Fu et al., 1997; Yoshimura et al., 1997). In these sophisticated rearing systems, the cost of rotifer production is estimated at € 0.05 per million of which food accounts for 86% (Fu et al., 1997). Labour cost in these hatcheries has been reduced to 13% which is about half the cost of that which is generally accepted for European rotifer production (28%).

In European hatcheries, the biggest problem in rotifer production systems is the unpredictability of such systems (Candreva et al., 1996) which require several duplicate and back up cultures. These extra production units have a direct impact on the labour cost and the final competitiveness of the whole production. In order to solve these problems, new rearing systems need to be developed primarily to increase production stability and productivity and secondly to reduce the labour and investment cost.

Based on this philosophy, an experimental rotifer recirculation system (Suantika et al., 2000, 2001) was adapted for the needs of the industry. A pilot rotifer production system was constructed and a financial study was performed to evaluate the benefits of the system compared to the conventional rotifer batch system.

2. Materials and methods

2.1. Rotifer strain

All experiments were performed with *Brachionus* (lorica length: 185 ± 15 μm) originating from batch cultures reared following the culture procedure described in Sorgeloos and Lavens (1996).

2.2. Experimental set up

The culture rearing tank (1 m^3) was filled with diluted seawater (25 ppt salinity) and maintained at a constant temperature of 25 ± 1 °C.

Fig. 1 gives a schematic outline of the recirculation culture system used for the high-density production of rotifers. The essential parts of the recirculation system prototype are illustrated in Fig. 2. The tank was equipped with a central nylon screen (mesh size of 33 μm) to retain the rotifers in the tank (Fig. 2B). Due to the dimension of the central filter (0.5 m diameter, 0.9 m length), the available water volume to culture rotifers (Fig. 2A) was reduced to 750 l. An aeration collar enclosed on the outer bottom part of the filter created a constant aeration (2.1 ± 0.2 l min^{-1}) ensuring a continuous cleaning of the filter and a homogenous distribution of food and rotifers. A floccule trap (0.3 m diameter, 0.6 m length) based on an air–water-lift principle was built in the culture tank to trap organic waste material from the tank (Fig. 3). The flow rate over the air–water lift was 120 l h^{-1}. The effluent water from the culture tank flowed by gravity to a 100-l settlement tank from where it was pumped to a series of protein skimmers (Aqua Medic, Germany) (Fig. 2C). In the first protein skimmer, ozone was injected at the flow rate of 500 mg l^{-1} h^{-1} to improve the removal of suspended solids and soluble protein (Dhert et al., 2000; Suantika et al., 2001). In order to improve the efficiency of the ozonisation, the incoming air was dried through 1500 g silica gel that was renewed every 24 h. The ozone concentration in the protein skimmer was controlled by measuring the redox potential (SMS510, Milwaukee Instruments, USA). The ozone supply was automatically switched off when a redox potential of 450 mV was exceeded. Residual O_3 was stripped from the effluent water by the action of the second and the third protein skimmer which also removed residual particles and soluble proteins. The recirculation rate over the protein skimmer was set at 1200 l h^{-1} using a closed loop in the circuit. After the physical separation in the protein skimmer, the effluent water was filtered over a submerged biofilter (Fig. 2D) with a capacity of 750 l filled with 350 l (~ 500 kg) gravel (size = 3–8 mm) and 100 l (~ 160 kg) $CaCO_3$ (pH buffer, size = 10–20 mm). The biofilter was inoculated with an enriched culture of nitrifying bacteria (10^5 CFU ml^{-1}, ABIL Aqua, Belgium) 6 days before the inoculation of rotifers in the culture tank (Suantika et al., 2001). After the biological filtration, the treated water was pumped into the fourth protein skimmer in order to remove the bacterial flocs from the biofilter. The O_2 saturated water (7 mg l^{-1}) was then discharged into the culture tank at a daily water renewal rate of 500% (~ 200 l h^{-1}). The experimental set up was used for three rotifer culture trials starting with an initial density of 500 individuals ml^{-1}. In each of the culture trials, the rotifers were allowed to grow until they reached 3000, 5000 and 7000 individuals ml^{-1}. As soon as these densities were reached, the rotifer density was kept constant by daily harvest of the rotifers produced in excess of the set density. During these harvests, new diluted seawater was added to compensate for the loss in water volume (Table 4). Besides this, no other water changes were performed during the culture period. Due to electrical problems during the experiment, the trial with 5000 individuals ml^{-1} stocking density was terminated on day 21.

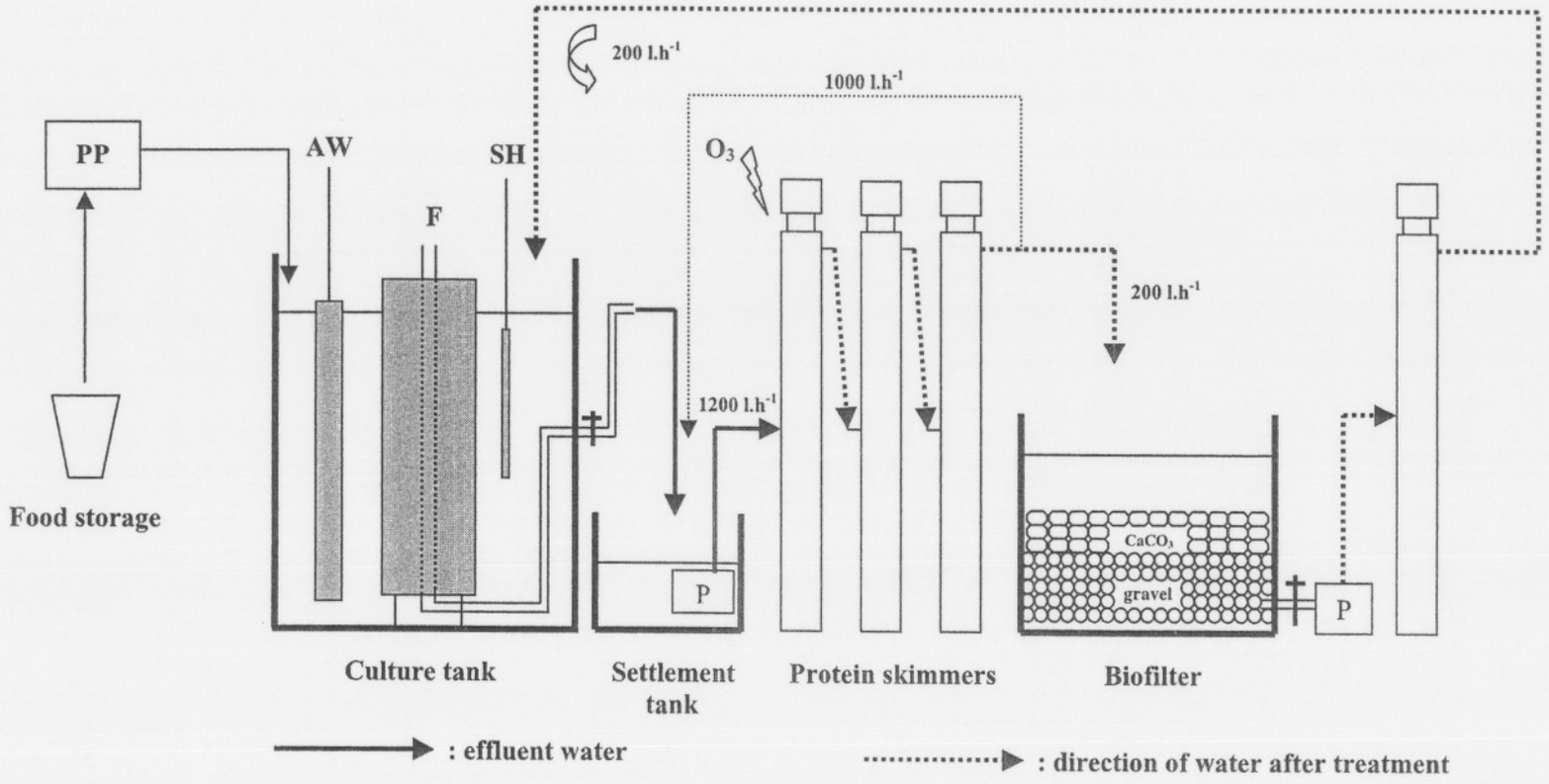

Fig. 1. Schematic overview of the recirculation system used for high-density rotifer production. PP: peristaltic pump, AW: air–water lift, F: nylon filter, SH: submerged heater, P: pump.

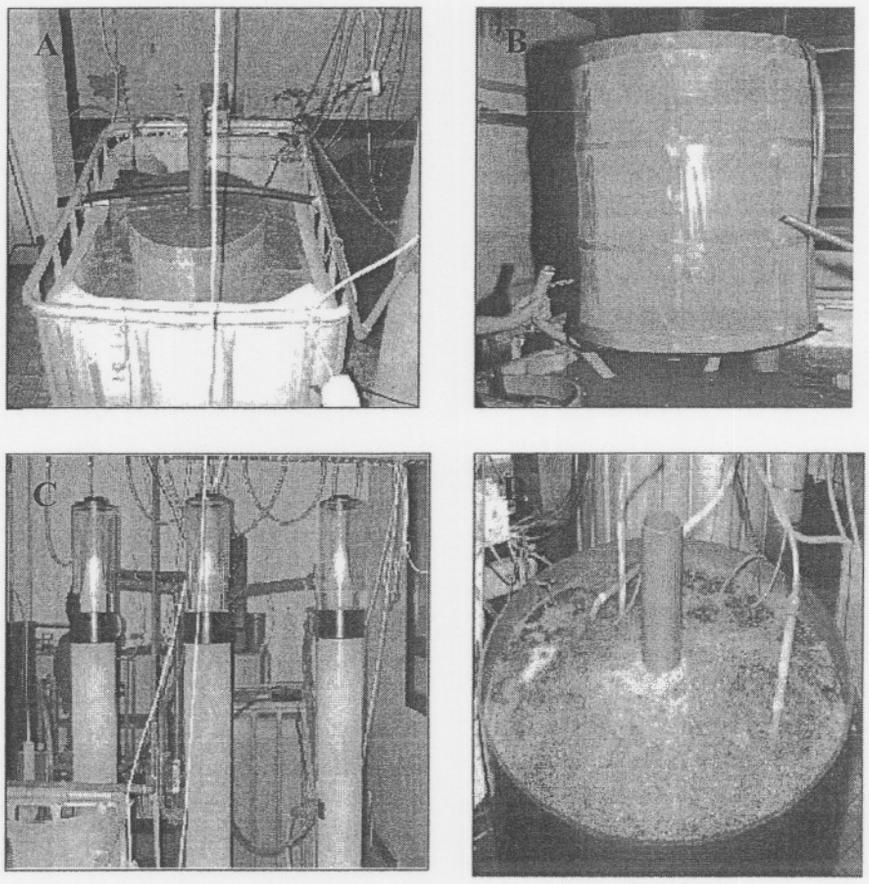

Fig. 2. Overview of main equipment used in the rotifer recirculation system prototype. (A) Rotifer culture tank with the central filter. (B) Filter of 33 μm retaining the rotifers in the system. (C) Set of protein skimmers for particle removal. (D) Submerged biofilter.

2.3. Rotifer diet

The rotifer diet consisted of an experimental Culture Selco, CSH (INVE, Belgium) (De Wolf et al., 1998; Dhert et al., 1998, 2000; Suantika et al., 2000, 2001) suspended in 4.8-l water and mixed vigorously with a kitchen blender. The suspension containing exactly the daily food ratio was kept in suspension by aeration in a 10-l food container at ambient temperature (20 ± 1 °C) for 24 h. Under ideal circumstances, cooling of the food should be foreseen but this is often not available in commercial hatcheries. From the food container, the food was administered automatically by means of a peristaltic pump to the culture tank at 30-min interval (feeding 200 ml h^{-1} or 4.8 l food suspension day^{-1}). The rotifers were fed following a standard feeding regime (Suantika et al., 2000):

$$\mathrm{CSH} = 0.035 D^{0.415} V,$$

where CSH = the weight of experimental diet (g), D = rotifer density (individuals ml^{-1}), V = culture water volume (l).

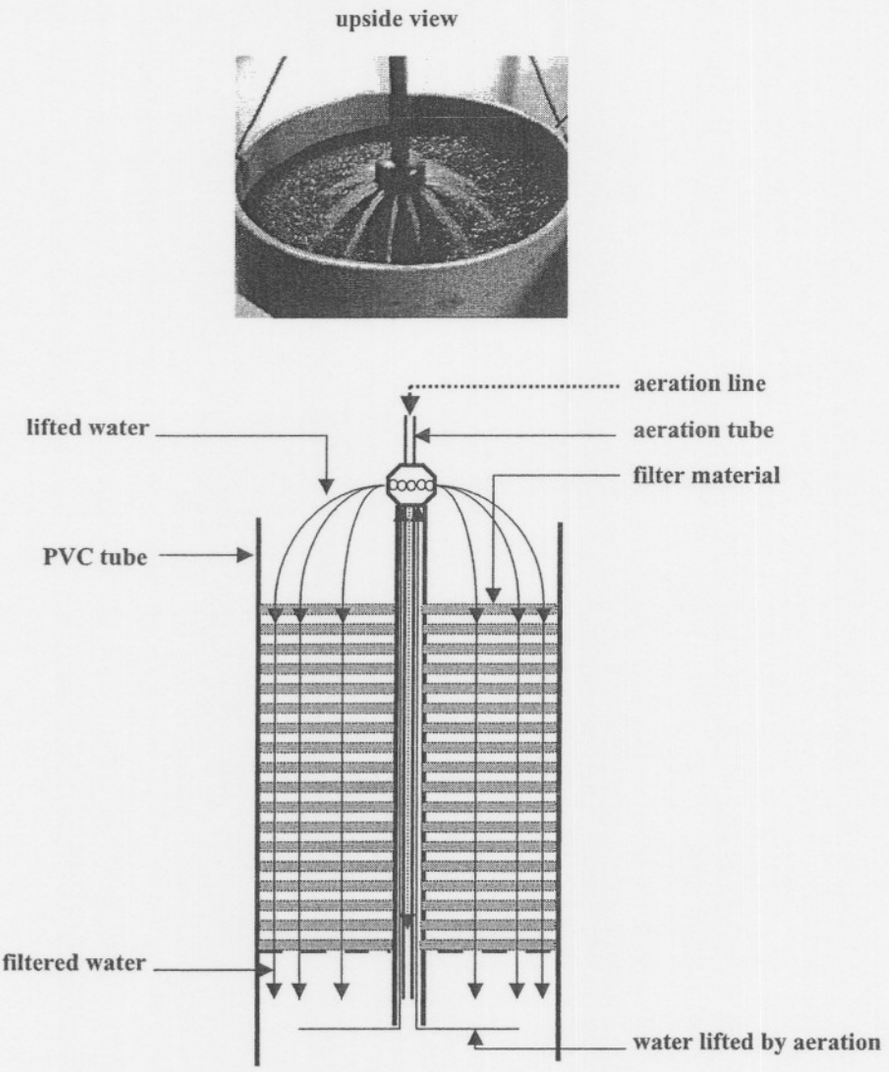

Fig. 3. Schematic overview of the floccule trap used in the intensive rotifer culture tank.

This feeding regime implies a high ration at start feeding to reach an acceptable diet concentration for a small filtering rotifer population and it allows a faster bacterial colonisation.

2.4. Sampling and counting

Rotifer counts were obtained from four subsamples of 400 μl taken from the rotifer culture using an automatic micropipette. The rotifers in each sample were killed by adding three drops of lugol. Empty and transparent loricae belonging to dead rotifers were not counted. The specific growth rate was calculated using the following equation:

$$\mu = (\ln N_t - \ln N_0)/t$$

where μ = specific growth rate, N_t = rotifer density after culture period t (individuals ml^{-1}), N_0 = initial rotifer density (individuals ml^{-1}), t = culture period (day).

2.5. Length of rotifers

In order to document the effect of a long-term culture period on the size characteristics of rotifers, regular samples were taken for lorica length measurement. Fifty rotifers were placed on an objective glass and immobilized by low pressure of a cover glass without addition of killing and staining agents to avoid shrinking of rotifers during measurement.

2.6. Physico-chemical parameters

The pH, NH_4^+, NO_2^-, NO_3^- and the dissolved oxygen (DO) of the water were measured as first daily activities during the experiment. NH_4^+, NO_2^- and NO_3^- were performed on filtered culture water (30 μm) of the rotifer tank using test kits (Aquamerck®, Viscolor Eco®, Germany).

2.7. Bacterial sampling

Microbiological analyses were performed on a weekly basis by sampling 10 ml from (1) the rotifer culture tank, (2) the outlet of the first protein skimmer and (3) the outlet of the biofilter. Serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) were prepared in sterile saline solution (1.5%) from the homogenised water samples and 100 μl was plated in duplicate on MA (marine agar) (Difco, USA) and TCBS (thiosulphate citrate bile sulphate agar). The plates were incubated at 25 °C and bacterial counts performed after 48 h.

2.8. Protein and fatty acid analysis

In order to investigate the oxidative effect of ozone on the nutritional content of the rotifers, their protein and fatty acid content were analysed on days 1, 6, 11 and 16 of the harvesting period from the tank containing 3000 individuals ml^{-1} (trial I). Protein content and fatty acids were measured by Kjeldahl and FAME analysis (Lepage and Roy, 1984), respectively.

2.9. Economic and financial analysis

Capital and operational costs for the recirculation system were compared with those from a commercial batch culture system with a production capacity of 2 billion rotifers day^{-1}.

2.10. Statistical analysis

All data were statistically treated using one-way ANOVA. Significant differences among means ($P<0.05$) were tested by Duncan's multiple range test.

3. Results

3.1. Rotifer culture and production characteristics

The line diagrams in Fig. 4 illustrate the increase in rotifer density as a result of the three different harvesting strategies. The daily harvest is illustrated in the block diagram.

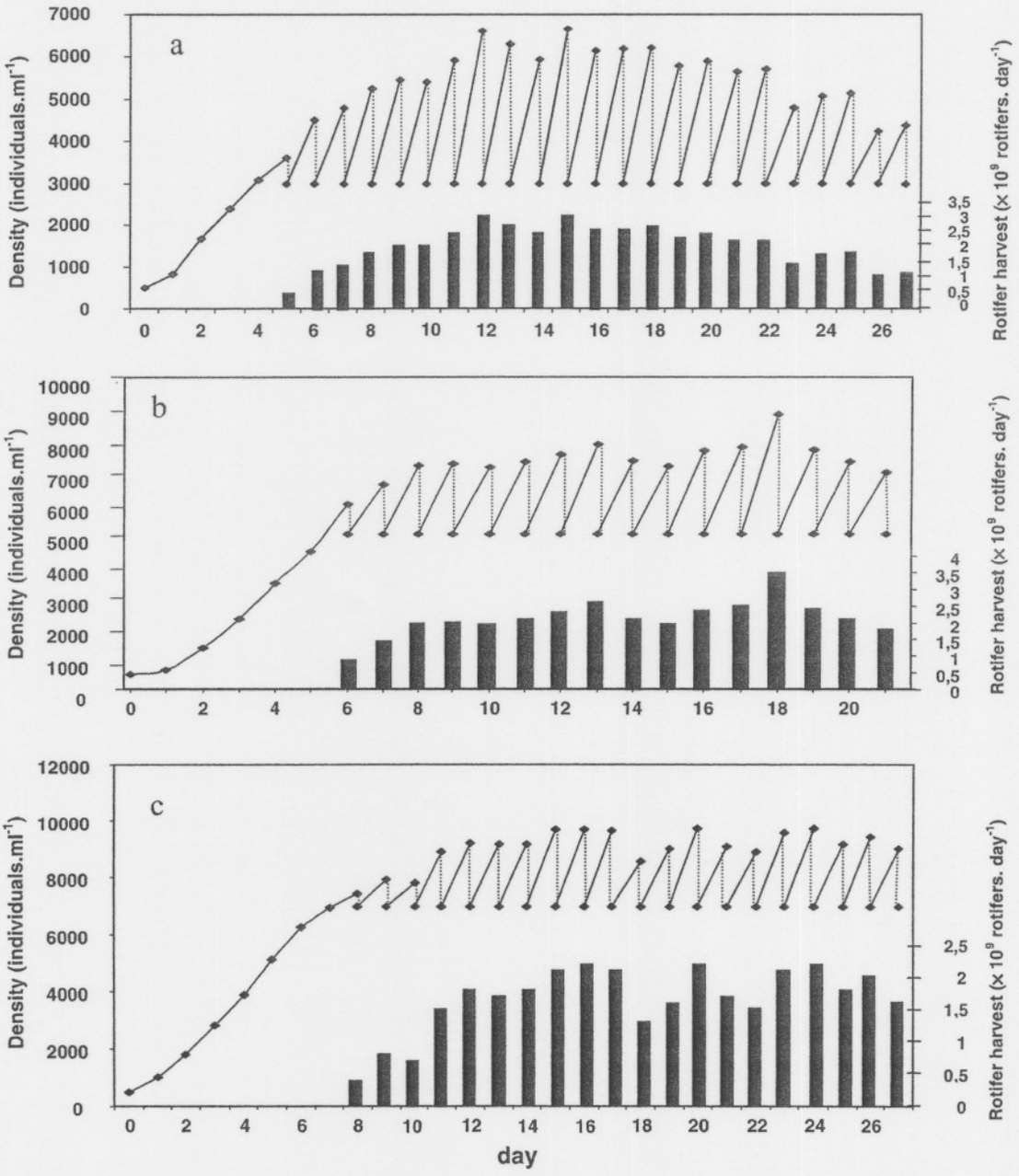

Fig. 4. Rotifer density (solid line) and daily rotifer harvest (solid bar) obtained in recirculation systems kept at 3000 (a), 5000 (b) and 7000 individuals ml^{-1} (c). Dotted lines show the reduction in rotifer density obtained by daily harvesting.

During the first 4 days of the culture, no significant difference ($P<0.05$) was noticed in the specific growth rate of the rotifers of the different treatments. In the three treatments, the specific growth rate of the rotifers was stable (0.50) and a rotifer density of 4000 individuals ml^{-1} was reached after 4 days. Based on the specific growth rate of the rotifers, it could be calculated that densities of, respectively, 3000, 5000 and 7000 individuals ml^{-1} were reached after 3.8, 5.1 and 7.0 days. First daily harvests could thus be performed on the respective tanks on days 5, 6 and 8 when rotifer densities reached, respectively, 3700, 6000 and 7500 individuals ml^{-1} (Fig. 4). In the first system where after each daily harvest the rotifer density was reduced to 3000 individuals ml^{-1} (Fig. 4a), an average daily increase to 5750 rotifers ml^{-1} was noticed corresponding to an average specific growth rate of 0.61. During this period, a total of 51×10^9 rotifers were produced. After 27 days, the cultures were stopped due to fouling and accumulation of dirty particles in the culture tank. In the second system (5000 individuals ml^{-1}), an average increase to 6700 rotifers ml^{-1} was noticed (Fig. 4b) corresponding to an average specific growth rate of 0.40. During this period, a total of 33×10^9 rotifers were produced. In the third system (7000 individuals ml^{-1}), an average increase to 9500 rotifers ml^{-1} was noticed (Fig. 4c) corresponding to an average specific growth rate of 0.26. During this period, a total of 34×10^9 rotifers were produced.

In the rotifer-rearing experiment, where the rotifers were kept at a density of 3000 individuals ml^{-1}, length measurements were performed on a daily basis. The average length of the rotifers obtained during the experiment is shown in Fig. 5. At the start of the experiment, the lorica length of the rotifers, including neonates and adults, was 191 ± 7 μm, it increased significantly during the second week of culture (211 ± 9 μm) after which it decreased to the original size in the third week to measure (182 ± 4 μm) in the fourth week.

3.2. Physico-chemical parameters

Table 1 shows the range in physico-chemical parameters recorded in the recirculation system for the three different operational rotifer densities. For all treatments, the pH level

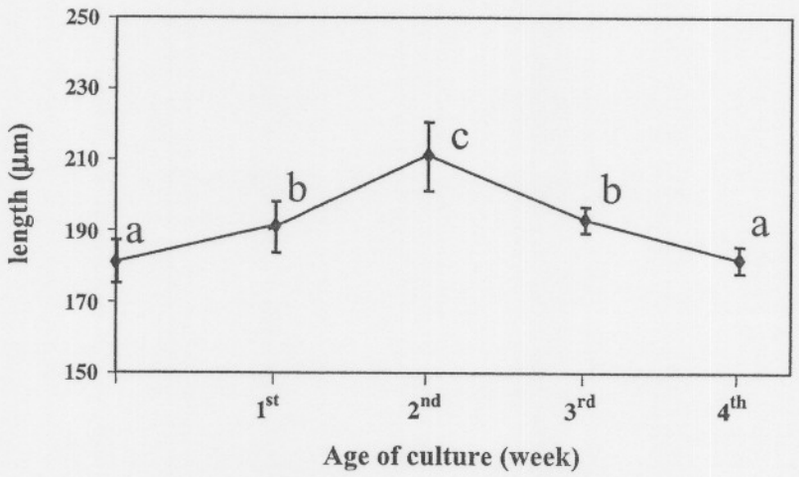

Fig. 5. Changes in rotifer length during the culture experiment at 3000 rotifers ml^{-1}. Data points are average values of daily measurements. Averages with a same letter are not significantly different ($P>0.05$).

Table 1
The physico-chemical parameters measured in the recirculation system

	3000 individuals ml^{-1}				5000 individuals ml^{-1}				7000 individuals ml^{-1}			
	Before harvesting		During harvesting		Before harvesting		During harvesting		Before harvesting		During harvesting	
	day 1	day 5	day 6	day 27	day 1	day 6	day 7	day 21	day 1	day 8	day 9	day 27
pH	7.6	7.3	7.3	7.4	7.8	7.5	7.4	7.5	7.9	7.5	7.5	7.2
NH_4^+ ($mg\ l^{-1}$)	0.4	0.5	0.6	0.8	0.2	0.4	0.6	0.8	0.2	0.4	0.6	1.0
NO_2^- ($mg\ l^{-1}$)	0.7	2.5	3.0	1.3	0.5	1.3	1.5	3.0	0.5	2.5	3.8	0.4
NO_3^- ($mg\ l^{-1}$)	10	70	80	25	15	25	30	150	10	25	100	13
DO ($mg\ l^{-1}$)	6.7	4.4	4.4	3.6	nd	nd	nd	nd	6.5	3.4	2.2	1.2

nd = no data.

decreased slightly before harvesting. A stable pH level was obtained as soon as the daily harvests were performed for the treatments at 3000 and 5000 individuals ml^{-1}. For the treatment where the rotifers were harvested at 7000 individuals ml^{-1}, a slow decrease in pH was noticed.

Low and slowly increasing ammonium levels were observed during the culture period at the three different stocking densities.

Low nitrite levels (NO_2^-) were also obtained in the system during the culture period. In general, the NO_2^- level increased before the harvesting period and decreased during the periodical harvesting. The NO_2^- levels increased from 0.7 to 2.5 and 0.5 to 2.5 mg l^{-1} before harvesting and decreased from 3.0 to 1.3 and 3.8 to 0.4 mg l^{-1} during the periodical harvesting (=addition of fresh seawater) at 3000 and 7000 individuals ml^{-1} stocking density, respectively. For the system where rotifers were kept at 5000 individuals ml^{-1}, an opposite trend was noticed. The NO_2^- level increased continuously from 0.5 to 3.0 mg l^{-1} which was most probably the result of a failure in O_2 supply (electricity failure on days 15 and 19).

Increase of nitrate (NO_3^-) levels were observed in the beginning of the culture period (before harvesting) and decreased slightly during the daily harvests. The NO_3^- levels decreased from 80 to 25 and 100 to 13 mg l^{-1} during the periodical harvesting at 3000 and 7000 individuals ml^{-1} stocking density. However, the NO_3^- level increased drastically from 30 to 150 mg l^{-1} at 5000 individuals ml^{-1} stocking density which is again a result of the electricity failure.

Dissolved oxygen decreased relatively fast during the period before harvesting for 3000 and 7000. It could be maintained around 4 mg l^{-1} by the water supplementation in 3000 individuals ml^{-1} while in 7000 individuals ml^{-1} it was reduced to 1 mg l^{-1}.

3.3. Bacterial counts

The bacterial counts performed on water sampled from the rotifer culture tank, the protein skimmer and the biofilter is given in Table 2. After 7 days of culture, the amount of

Table 2
Bacterial counts obtained from water sampled in the rotifer culture tank (5000 individuals ml^{-1}), after the protein skimmer and after the biofilter

	Sample	Marine Agar (CFU ml^{-1})	TCBS (CFU ml^{-1})
Recirculation			
Day 7	rotifer culture tank	3.4×10^6	1.6×10^5
	after protein skimmer	1.8×10^5	2.2×10^4
	after biofilter	1.4×10^6	3.5×10^3
Day 15	rotifer culture tank	3.4×10^6	3.8×10^4
	after protein skimmer	4.1×10^6	3.5×10^3
	after biofilter	4.9×10^5	0
Day 23	rotifer culture tank	2.3×10^5	3.0×10^4
	after protein skimmer	2.8×10^4	5.5×10^3
	after biofilter	3.5×10^4	0
Batch	rotifer culture tank	3.7×10^7	nd

nd: no data.

bacteria in the rotifer culture water was 3.4×10^6 and 1.6×10^5 CFU ml^{-1} on marine agar and on TCBS, respectively. In the following weeks, no significant difference in bacterial numbers were noticed. Bacterial numbers recorded after the protein skimmer tended to be 1 log unit lower than in the culture water for marine agar and TCBS counts. Vibrio counts were low (3.5×10^3 CFU ml^{-1}) for samples taken after the biofilter at the end of the first week of culture. No vibrios were noticed from the second week onwards.

3.4. Nutritional content of the harvested rotifers

The HUFA and protein content of the harvested rotifers are given in Table 3. The HUFA levels ranged from 10.0 to 11.4 $mg.g^{-1}$ DW during the culture period and the DHA/EPA ratio ranged from 0.52 to 0.66. During the whole culture period, the protein content was higher than 50%.

3.5. Economic and financial analysis

Table 5 gives an idea of the total capital investment required to run a commercial batch system and to run a recirculation system with a daily production output of 2

Table 3
Nutritional content of harvested rotifers cultured in the recirculation system (3000 individuals ml^{-1})

Day	Protein (% DW)	DHA (mg g^{-1} DW)	EPA (mg g^{-1} DW)	DHA/EPA	$\Sigma(n-3)$ HUFA (mg g^{-1} DW)
Recirculation					
1st day harvest	55.6	2.5	4.8	0.52	10.1
6th day harvest	52.3	3.0	4.8	0.63	10.3
11th day harvest	56.8	3.5	5.4	0.64	10.4
16th day harvest	51.2	3.1	4.7	0.66	10.0
Batch	–	3.5	4.9	0.70	11.8

billion rotifers. For the batch system, the total capital cost is about € 52,139, the majority of the cost (77%) is spent for investment on fixed assets while general and scientific equipment accounts for 23%. For a recirculation system, a lower capital investment of € 28,000 is required. In the recirculation system, most of the cost (70%) is spent for investment on fixed assets and 30% is consumed by general and scientific equipment. Using the batch system, the total annual running cost to produce 2 billion rotifers day^{-1} is around € 42,200 made up for 34% in feed cost, 33% labour cost and 25% for depreciation of fixed assets. Total annual running costs for the recirculation system is also lower than for the batch culture system since € 25,800 is needed to produce 2 billion rotifers day^{-1}; i.e. 43% feed cost, 19% labour cost and 28% for depreciation of fixed assets (Table 6).

4. Discussion

A lot of disadvantages inherent to a conventional batch culture system can be solved by the use of a recirculation system, as the latter allows to increase rotifer production, reduce labour cost and water consumption while ensuring stable water quality (Suantika et al., 2000). In the batch culture, maximum rotifer densities of 3000 individuals ml^{-1} were reached after 4 days of culture period (De Wolf et al., 1998). After this, the rotifers need to be rinsed and returned to the system which is a labour intensive process. On the other hand, in the recirculation system a production of nearly 3000 individuals ml^{-1} can also be obtained after a culture period of 4 days but the rotifer culture can be maintained for almost 1 month without rinsing and restocking the rotifers (Fig. 4). During this whole culture period, it was possible to produce 2.2 billion rotifers day^{-1}.

Our experiments (Table 4) show that operating the system at the lowest density (3000 individuals ml^{-1}) resulted in 29% faster growth rate of the rotifers compared to the system operated at the highest density (7000 individuals ml^{-1}). Although the use of a lower operational rotifer density results in an increase in production, it has also some operational disadvantages. Due to the lower density, up to 45% of the standing crop needs to be harvested daily which in a commercial operation may cause problems when the rotifer requirement is not constant. The higher water renewal of 45% at low standing crop is advantageous for water quality and results in better NO_3^- removal and an

Table 4
Rotifer production during a 30-day culture period in a 1-m^3 recirculation system

	Stocking density (mg l^{-1})		
	3000	5000	7000
Average daily production (rotifers day^{-1})	2.2×10^9	2.1×10^9	1.7×10^9
Water renewal (l day^{-1})	382 (45%)	314 (37%)	178 (21%)
Specific growth rate	0.61	0.40	0.26
Σ water consumption (l)	9600	7840	4500
Σ food (kg)	21	25	28

(%): percentage of standing population.

increased dissolved oxygen level (Table 1). On the other hand, this higher water renewal increases the risk for upwelling of sedimentation and destabilisation of the system. Probably the most important advantage of operating at the lowest standing crop resides in the 25% saving on food consumption which could significantly reduce the rotifer production cost which is estimated at 60% of the total production cost in batch systems (Lavens et al., 1994).

By operating the system at the lowest standing crop, acceptable dissolved oxygen levels could be maintained. The DO levels only decreased 2.9 mg l^{-1} (from 6.5 to 4.4 mg l^{-1}) during the whole culture period; a similar or even higher decrease was already measured after 4 days in the batch culture (Suantika et al., 2000). In a continuous culture system, Fu et al. (1997) noticed that stable daily rotifer production can be achieved by stabilising the oxygen levels at around 4.5 mg l^{-1}. In this regard, further optimisation of the system could be envisaged by the use of pure O_2 in the culture tank especially when higher operational rotifer densities need to be maintained.

Acceptable values were measured for all water parameters in the recirculation system at the three different operating densities. For example, pH levels decreased less than 0.5 units during the whole culture period, whereas these changes are already measured in a batch system after 4 days (Suantika et al., 2000). Also low accumulation of ammonium, nitrite and nitrate were obtained in the recirculation system. It can be explained that the daily addition of new seawater to compensate for the losses of culture water contributed to a better control for the physico-chemical parameters and also indirectly reduced the load of the biofilter. Beside daily water renewal, the supplementation of ozone in the recirculation system also contributed to stable and low accumulation of ammonium, nitrite and nitrate (Suantika et al., 2001). Although an opposite trend was noticed for the system ran at 5000 individuals ml^{-1}, it is very likely that this was due to the effect of a total electricity failure and not linked to any density problem at all. It is obvious that the improved water quality had a positive effect on the growth of the rotifer population. Apparently, the better rearing conditions also had an effect on the rotifer lorica size. During the first week, the average lorica size increased and rotifers originating from a batch culture showed an increase of $\pm$ 10% in length. This is in accordance with the observation of Lubzens (1987) who stated that changes in lorica length of up to 15% can be noticed due to changing water quality. The size of rotifers significantly decreased starting day 21 (last week of culture period) when the culture condition started to deteriorate. Starting from this period, fouling of the tank created more dirt particles in the culture water resulting in a less stable bacterial community (Rombaut et al., 2001).

The use of a recirculation system for daily rotifer production obviously also has advantages on the bacterial numbers in the system. Bacterial numbers obtained in rotifers tend to be 1 to 2 log units lower than in a batch system (Rombaut et al., 2001). Moreover, the shift in bacterial communities (elimination of vibrios in the biofilter) could be beneficial when rotifers are used as larval food. From this result, it can be anticipated that a better conditioning of the nitrifying bacteria in the biofilter might also pay off before starting a new culture. Better control of the vibrio community in the rearing system is presently under investigation and could possibly be obtained by a combination of higher filtration rates and active bacterial management (i.e. combination of inoculation and disinfection techniques).

Table 5
Estimated capital investment for a batch system and a recirculation system with a production capacity of 2 billion rotifers day^{-1}

Facility	Item	Batch system		Recirculation system	
		Quantity	Total cost (Euro)	Quantity	Total cost (Euro)
(A) Buildings	Concrete base (inc. channels)	30 m^2	3510	20 m^2	2340
	Building/partitioning	160 m^2	2400	100 m^2	1500
	Rotifer room floor	310 m^2	3100	200 m^2	2000
	Service gantry	30 m	11,160		
(B) Other installations	Fresh water	1	73	1	73
	Pipework installations water	1	729	1	729
	Pipework installations air/O_2	1	729	1	729
	Electrical installations	1	2625	1	2625
(C) Plant and machinery	Live food filter	1	1167		
	Live food blower	1	1896		
	Rotifer culture heating unit	1	2100		
	Air filter	1	1021		
	Peristaltic pump			1	744
	Protein skimmer			4	1280
	Eheim pump			12	888
	Blower			1	1487
	O_3 generator			6	1338
	ORP meter			2	248
(D) Tanks	General purpose tanks	6	1752		
	Rotifer tanks	11	8019		
	1000-l tanks with frame			1	250
	100-l settlement tanks			1	32
(E) General and scientific equipment	Microscope	1 set	3584	1 set	3584
	Haemocytometer	1	66	1	66
	Portable pH meter	1	395	1	395
	Portable O_2 meter	1	467	1	467
	Netting	20	1440	20	1440
	Refrigerator	1	372	1	372
	Airline	1 set	248	1 set	248
	Laboratory balance	1	350	1	350
	Blender	1	321	1	321
	Tank heaters	11	2563	6	2563
	Small lab. equipment	1 set	1095	1 set	1095
	Oxygen stones	11	957		
(F) Filtration	1000-l biofilter tank			1	372
	Stone (substrate biofilter)			3000 kg	87
	Air filter			1	5
	Filter frame			1	87
	Nylon filter mesh			1700 m^2	63
	PVC air–water lift			1	62
	Filter sponge			2 m^2	21
(G) Tubing	PVC tubing			2	248
	Flexible PVC tube			40 m	35
Total capital cost			52,139		28,144

The nutritional content of harvested rotifers from the recirculation system did not differ from this obtained from rotifers originating from batch cultures. The harvested rotifers contained a normal fatty acids and protein content (Table 3), and could, depending on the fish species to be cultured, used as such or further enriched (Dhert et al., 2001).

From an economical point of view, the use of a recirculation system also has a lot of advantages. Investment, labour and feed costs are considerably reduced compared to the conventional batch culture system (Tables 5 and 6). For example, to produce 2 billion rotifers day^{-1}, the investment cost is 46% lower. Savings are mainly made on equipment (especially rotifer tanks and heating systems). Also, the annual operation cost is lower due to savings on labour. One person working 2 h/day (e.g. counting rotifer density, measuring water quality, rotifers harvesting, filter rinsing and feeding) can easily handle two rotifer recirculation systems. Compared to the batch system the gain in managing the system is estimated at € 14,000. The better food conversion rate obtained

Table 6
Estimated annual operation cost to produce 2 billion rotifers day^{-1} in batch system and recirculation system (5000 individuals ml^{-1} stocking density)

Item	Description	Batch system		Recirculation system	
		Quantity	Total cost (Euro)	Quantity	Total cost (Euro)
(A) Labour	Hours per year	14 × 143 h (2000 h/year)	14,000	14 × 50 h (700 h/year)	4900
(B) Electricity	Tank heating, direct heating/cooling, air supply/blower, etc.	60 kW × 12	14	30 kW × 12	7.2
	Pumps, automatic feeding machine			20 kW × 12	4.8
(C) Consumables	Tank heaters replacements	3	699	3	33
	Cleaning materials	12	583	1	24
	Netting for rinsers/concentrators	3	583	3	189
	Miscellaneous material, pipelines, O_2 stones, etc.	5	365	5	365
	Lights—replace once per year	8	16	8	16
	Water quality test kits			6	534
(D) Feed	Culture Selco	360 kg	11,156		
	Protein Selco	5 kg	300		
	DHA Protein Selco	5 kg	325		
	CSH			300 kg	11,154
	Algae	137 m^3	2329		
(E) Miscellaneous	Various		1458		1458
	Total running costs		38,813		26,626
(F) Depreciation	Depreciation on capital costs 20% per annum	20%	10,428	20%	7173
Total running cost including capital depreciation			42,257		25,858

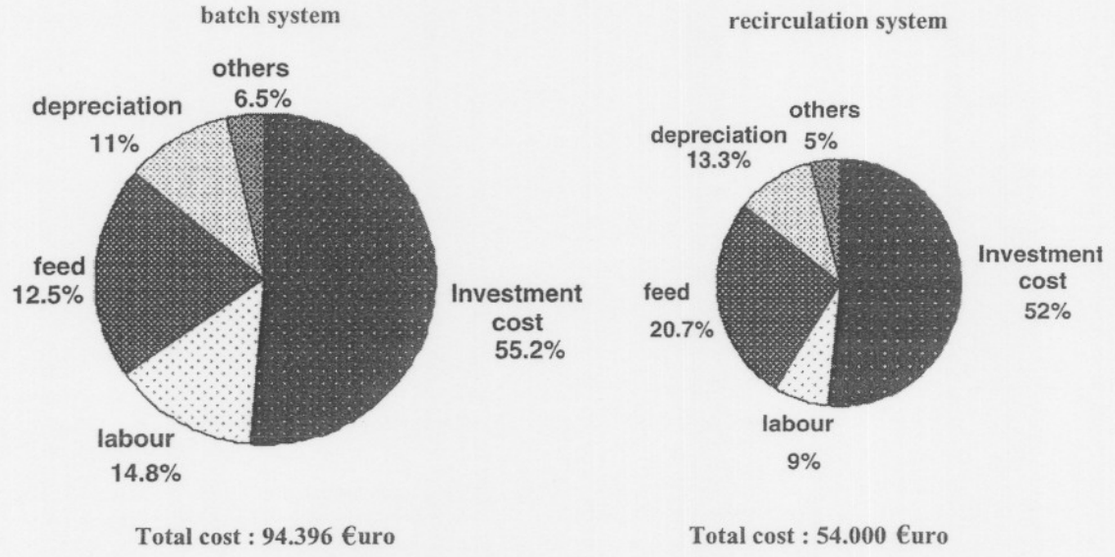

Fig. 6. Estimated capital cost (Euros) and operational cost (%) to produce 2 billion rotifers day^{-1} in a batch system and a recirculation system.

in the recirculation system (Suantika et al., 2000), can also account for 10% savings. In general, the use of the recirculation system can reduce the investment and operational costs for about 43% (Fig. 6). In percentage, there are no differences obtained on investment cost, depreciation cost and other costs between the commercial batch culture system and the recirculation system. By using a recirculation system, saving is mainly made by a reduction of the labour cost from 15% to 9%. When expressed in percentages the cost for feeds is increased using a recirculation system, however, on an absolute basis the cost for feeds remains identical with the batch system. It can be explained that to support optimum growth rate of rotifers in the recirculation system, a 20% higher amount of food is needed to compensate for food losses through the system, but since more efficient food uptake is obtained by denser rotifer cultures this results in a similar food consumption. In conclusion, it can be stated that by using a simple recirculation technology, a cost-effective and above all reliable rotifer culture can be obtained. Moreover, the system opens new perspectives in terms of automated production of clean and healthy rotifers.

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