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An artificial productive ecosystem based on a fish/bacteria/plant association. 1. Design and management

I. Quilleré[a,*], D. Marie[b], L. Roux[a], F. Gosse[a], J.F. Morot-Gaudry[a]

[a]*Laboratoire du Métabolisme, INRA, Route de St-Cyr, 78026 Versailles Cedex, France*
[b]*Installations Piscicoles Expérimentales (IPE), INRA, Domaine de Vilvert, 78352 Jouy-en-Josas Cedex, France*

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Abstract

An artificial ecosystem integrating three biological compartments (fish, bacteria, plants) in a closed system was developed with the aim of associating fish production with a vegetable crop purifying the fish water. The nitrogenous compounds excreted in dissolved form by the fish, and transformed by the bacteria, provide nitrogen nutrition for the plants. This association has the double advantage of savings in water for fish culture and the recycling of fish excretion as the main source of minerals for producing edible plants.

A pilot system of 2 m^3 was set up for intensive animal and plant production and installed in a greenhouse to enable continuous production throughout all seasons. The fish chosen were tilapias (*Oreochromis niloticus*) and the plants were tomatoes (*Lycopersicum esculentum*) grown according to the nutrient film technique in recirculating hydroponics. A granular filter bearing the nitrifying bacteria was inserted between the fish tank and the plants. The system's design was aimed at optimizing the functioning of the ecosystem, by the size of the different elements (fish tank, bacterial filter, hydroponic troughs) as well as by the choice of the recirculating water flow rate. During the first production cycle, we followed the evolution of the physico–chemical characteristics of the water and of the plant tissues, especially the nitrogen (NH_3, NO_2, NO_3) and mineral compounds (K, Ca, Mg, SO_4, PO_4), in order to evaluate the functioning of the three compartments and to progressively develop the management of the plant compartment. The latter determined the overall equilibrium of the ecosystem by its capacity to absorb NO_3 and NH_4 in the recirculating water. The results were satisfactory as there was a stabilization of the nitrogenous compounds, in particular NO_3, at a low level and a large plant production; in this first trial no attempt was made to improve the animal production.

This trial highlighted the main conditions to ensure the equilibrium of the ecosystem: size relationship between the three interacting compartments, dynamic management of the plant compartment (staggered crops) and the application of a mineral complement to obtain optimum plant growth. The nature of the mineral complement will depend on the composition of the water available on the production site.

* Corresponding author.

Introduction

Fish, an important source of proteins (and minerals), have high growth rates and a protein yield higher than for most farmed animals. Intensive fish production can help reduce the deficit in animal proteins observed in numerous developing countries. However, the digestive metabolism of fish results in the excretion of a toxic compound, ammonia, this involving large-scale water input.

Production in closed systems enables considerable savings in water using nitrifying biofilm reactors to transform ammonia dissolved in the water into nitrate, a compound with low toxicity for fish. But with time nitrate accumulates in the water. The conventional techniques adopted to limit nitrate concentration are partial fresh water flushing or microbial denitrification.

Over the last 15 years, research has been carried out on integrating plant crops into fish farming in closed circuits in order to reduce the water nitrate content (Lewis et al., 1981; Rakocy and Allison, 1981). Combining a plant culture with fish production enables significant savings in water and valorization of the fish farming wastes through an edible plant production.

The designing of an artificial ecosystem, transposable in developing countries where water and fertilizers are limiting factors, was proposed. It is based on a continuous trophic chain between a primary consumer (fish) and secondary consumers which transform (bacteria) and assimilate (plants) nitrogen excreted by fish. The aim is to obtain intensive production of the fish and plant compartments in a closed system both water and nitrogen efficient.

This paper deals with the design of the ecosystem and the management of the plant compartment in order to achieve a state of equilibrium. In a second paper (Quilleré et al., 1993b), we will report the results of its functioning in varied conditions during a 2-year period.

The design of the system

The ecosystem set up at Versailles included three biological compartments, fish, bacteria and plants, interconnected through pumping systems (Fig. 1). Solid nitrogen waste (faeces, food residues) was not directly recycled in this system.

Biological requirements of the different compartments

Animal compartment

The animal compartment contained tilapias, tropical fish of the Cichlidae family (genus Oreochromis, species *Oreochromis niloticus*). They were chosen on the basis of their high growth rate and their high tolerance with regard to water quality. They can live indifferently in fresh or brackish water (Stick-

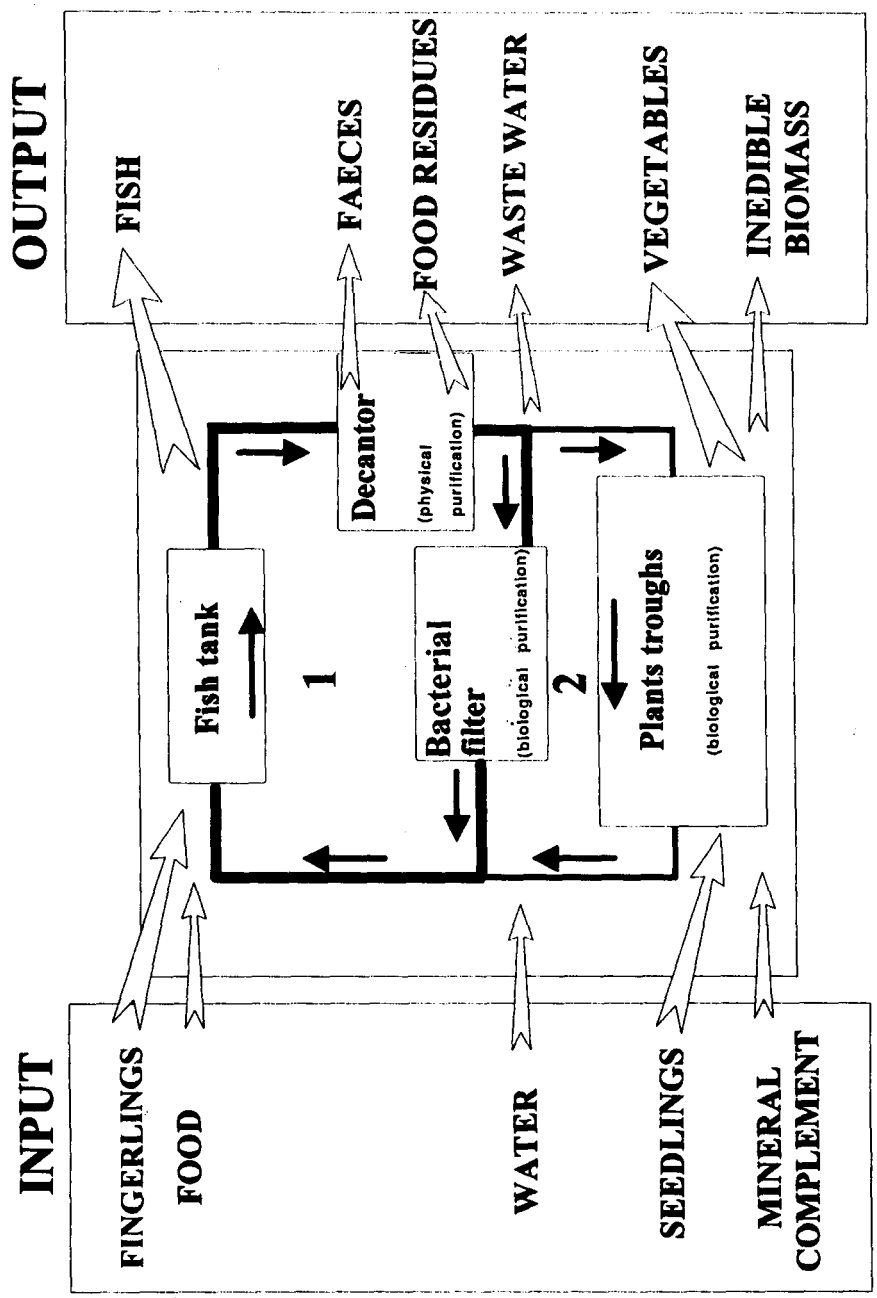

Fig. 1. General scheme of the artificial ecosystem (matter flow).

ney, 1986) and are marketable after 6–7 months with an average weight of 300–400 g.

This compartment is the critical one which imposes physical and chemical water limitations: optimal temperature between 24 and 35°C, the lethal temperature being below 12–13°C (Huet, 1970); pH between 5 and 11 (Balarin and Hatton, 1979), the most favorable for fish farming being generally between 6°C and 8°C (Billard and Marie, 1980); concentration of dissolved oxygen beyond the critical level of 0.1 mg l^{-1} (Chervinsky, 1982).

All nitrogenous compounds issuing from the digestive metabolism of fish are toxic to it. According to temperature, pH and salinity a variable equilibrium establishes between the unionized ammonia and the NH_4^+ ion. Un-ionized ammonia is particularly toxic to the fish: the 48-h median lethal concentration (LC50) is 2.9 mg l^{-1} NH_3 (0.17 mmol l^{-1}) for *Tilapia aurea* (Redner and Stickney, 1979) corresponding to a concentration of NH_4^+ ions between 24 and 2 mmol l^{-1} for a pH varying between 7 and 8, at a temperature of 28°C (Trussell, 1972; Emerson et al., 1975). For nitrites, the 96-h median tolerance limit (TLm) is 24. 8 mg l^{-1} NO_2 (0.5 mmol l^{-1}) for a related species, the channel catfish, *Ictalurus punctatus* (Konikoff, 1975). However, the toxicity threshold is not easy to define for fingerlings; it is necessary to take into account the nitrite–ammonia interaction (D. Marie, unpublished data, 1987). Nitrates have a low toxicity level: 1.2 g l^{-1} NO_3 (19 mmol l^{-1}) have no effect on fish growth (Naegel, 1977), however, Balarin and Haller (1982) pointed out that the high levels attained in recirculating water can limit fish production.

Bacterial compartment

The first step in the mineralization of liquid wastes (ammonization) occurs spontaneously in traditional fish tanks. However, nitrification occurs only partially and at a low rate, and therefore recirculating systems require the enlarged surface of a bacterial filter to increase the potential nitrification.

The nitrifying bacteria (*Nitrosomonas*, *Nitrobacter*) reduced CO_2 by chemosynthesis. The yield of the reduction is low: *Nitrobacter* has to oxidize at least 13 mol of NO_2 per mole of reduced CO_2 (Aleem, 1970). Optimal conditions for nitrification are a supply of O_2 and CO_2, a high temperature (optimum at 37°C) and especially a pH near 8.5. At a pH lower than 6, bacterial growth dramatically decreases so the biofilter activity fall down (Antoniou et al., 1990).

Plant compartment

The plant compartment contained vegetables (tomatoes, lettuce, etc.) hydroponically grown i.e. without substrate and with mineral nutrition supplied as a nutrient solution. It is well documented (Chaillou et al., 1986) that exclusively ammoniacal nitrogen nutrition impairs plant growth. This high-

Table 1
Comparative pH and mineral content (mmol l^{-1}) of the recirculating water (at Day 22) and of a standard nutrient solution (Lesaint-Coïc type, 1983)

	pH	Nitrogen		Cations					Anions	
		NO_3	NH_4	K	Ca	Mg	K Ca	K Mg	H_2PO_4	SO_4
Recirculating water	7–8	4.1	<0.1	0.15	2.75	0.6	0.05	0.25	0.1	1.25
Standard nutrient solution	5.6–6	12	2	5.2	3.1	0.75	1.7	6.9	1.1	0.75

lights the necessity of the bacterial compartment from both points of view (fish and plant).

The basis of the nutrient solution was the fish tank effluent, i.e. tap water enriched in nitrates and phosphates by fish excretion. Although the value of fish wastes as plants fertilizer is known (Lesel and Ifergan, 1975), compared with a standard nutrient solution (Table 1) it does not fit plant requirements because of a high pH, low potassium and phosphate content, high level of sulfate and also unbalance between cations.

Root zone temperature was determined by the temperature of the fish tank (28°C). Generally, nitrate assimilation (absorption and reduction in the root) is enhanced by high temperatures (Mengel and Kirkby, 1987; Sauvesty and Gendron, 1989). However, root respiration increases with temperature and may rapidly lead to root necrosis if the oxygen demand is not satisfied. Optimum temperature for tomato growth lies between 25 and 30°C (Fujishige and Sugiyama, 1968), so this species was chosen for the first experiment. For lettuce, the optimum temperature for production depends on cultivars (for winter culture or spring–summer culture).

Description of the pilot system

The system included three distinct circuits (Fig. 2). The first circuit (Circuit I) was loop-shaped. It functioned permanently and was the first step in purifying the fish effluent. Plastic pipes linked it to the following: a fish tank (2 m^3); a new designed cyclonical decanter (0.15 m^3; D. Marie, unpublished data, 1988) where solid wastes (faeces and non-ingested food) were removed by sedimentation; a tank (P1) connected to the tap water supply in which water loss due to evapotranspiration and cleaning was automatically replaced; a pump sending water under pressure (2.5 bars) from the tank P1 towards the 'fixed aerobic granular bed' (a bacterial filter filled with granular clay (50 kg of BIOGROG, granulometry 10–20 mm) covered by the nitrifying bacteria). A cascade aerator placed at the end of the circuit reoxygenated

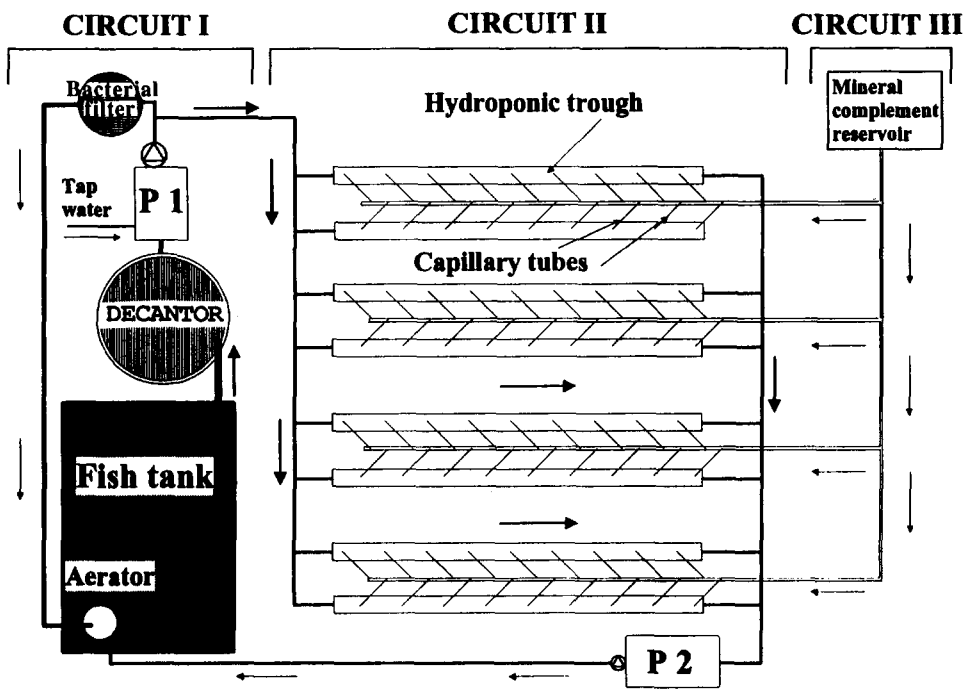

Fig. 2. Schematic view of the pilot system, P1: tank with circulating pump P2: tank with relift pump.

water returning to the fish tank. The fish tank was further oxygenated by a mechanical aerator. The starting period of fish production was critical because the activity of the biofilter, self-loading with natural nitrifying bacteria, had to increase as fish were introduced. To avoid mortality caused by toxicity of nitrogenous compounds, fish were introduced progressively into the system (Marie, 1979) over a period of 45 days beginning on 1 September 1988, until a final charge of 69 adults (total fresh weight of 31.4 kg). We fixed Day 0 of this trial as 1 September. The water temperature was maintained at 28 °C using an electric heater. Fish feeding increased gradually from 100 to 250 g of commercial carp feed daily (92% dry matter, 5.6% N).

The second circuit (Circuit II) operated intermittently. It was a derivation of Circuit I starting just before the biofilter (thus relieving it of some of its activity) and fed the plants set up in the plastic troughs. The water flowed along the troughs by gravity and was collected in the tank P2. From there a pump drove it back to the fish tank. In the troughs, the plants were grown hydroponically in recirculating water according to the nutrient film technique (Cooper, 1973) modified by Lesaint and Coïc (1983). The water flowed intermittently at a height of 2–3 mm to allow root oxygenation. The plants were

initially planted in small perforated pots containing an expanded clay–peat mixture and received a classical nutrient solution. When the plants reached the four to five leaf stage, with roots starting to leave the pot, they were placed in the troughs. Each trough contained ten plants, 50 cm apart. The number of troughs was set at six at the beginning of the experiment, and subsequently increased to eight.

Tomato plants of the Ferline variety (indeterminate growth) were introduced on 15 September (Day 14) at the stage of the first flower cluster. During cultivation, axillary buds were removed. After the fifth flower cluster had appeared (on 10 November, Day 70), growth was stopped by topping the main stem. Fruit picking began on 7 December (Day 97). The whole of the crop was removed on 15 December (Day 105).

This second system completed the effect of the biofilter as the plants absorbed a part of the ammonia remaining in the water and especially the nitrates produced after passing through the biofilter.

Finally, the third circuit (Circuit III) fed each plant through capillary tubes bringing a mineral complement to the plant pots. This was supplied from 26 September (Day 25) to 29 November (Day 89). According to the requirements of the plants, its composition depended on analyses of the recirculating water and of tomato leaves.

Specificity of the Versailles pilot system

Considering the local climate, the system was installed in a greenhouse heated in winter (18°C) and ventilated in summer. The specificity of this system was the association of two optimized intensive biological production circuits, analogous to traditional circuits encountered in agricultural production.

The circulation frequency and flow rate of the water in the different circuits was determined on the basis of traditional circuits. For Circuit I, it was set at 50 l mn^{-1} continuously (depending on fish biomass and on the quantity of oxygen necessary for fish and bacterial activity; Liao, 1981). For Circuit II, it was set at 2.5 l mn^{-1} intermittently for each hydroponic trough (2 l mn^{-1} for a tomato crop according to Cooper, 1973), that is 15 or 20 l mn^{-1} depending on the number of troughs in use. At the beginning of the culture the water ran off during 2 mn every 10 mn, then 3 mn every 15 mn when the root system was well developed.

The frequency and the flow rate in Circuit II determined the size of the tank P1 in Circuit I: during the watering period of the hydroponic troughs the transient level decrease should not allow an input of tap water, otherwise Circuit I will overflow when Circuit II returns to a rest period.

Compared with systems already described, this pilot system presented some particularities:

(1) Although interdependent, the three compartments of the ecosystem

were clearly separated, which provided a flexibility in studying the overall functioning (each compartment could be studied individually) and in optimizing it. It was possible to disconnect one compartment (for example: removing the plant compartment at harvest) without interruption of the general functioning. This is not the case when all compartments are in the same tank (MacMurty et al., 1990) or when the plant growing substrate constitutes the biofilter (Zweig, 1986).

(2) The bacterial filter was small (downsized by 30% compared with the related system described by Watten and Busch, 1984) because part of the NH_4 ions in fish water was directly absorbed by the plants.

(3) The circulation of water was intensive: the system's water flowed through the decanter and the biofilter 29 times per day (Circuit I) and twice a day through the hydroponic circuit (Circuit II).

(4) Its specificity was the supply of a liquid mineral complement at each plant to improve plant growth. In other systems, either no fertilization is applied (Rakocy and Allison, 1981) or, if any, it is applied in solid form (Lewis et al., 1978; Sutton and Lewis, 1982; Watten and Busch, 1984), or by foliar feeding (Lewis et al., 1981).

The management of Circuit I was similar to that of a conventional recirculating fish production system, but as the bacterial population was small-sized maintenance of the biofilter was more delicate (frequent analytical controls and filter cleaning interventions). The management of the hydroponic production circuit required progressive adjustments. The good functioning of this circuit (absorption of NO_3 and NH_4 by the plants) determined the overall equilibrium of the ecosystem.

Setting up management of the plant compartment — preliminary results

To evaluate the functioning of the three compartments and to progressively develop the management of the plant compartment, we followed the evolution of the physico–chemical characteristics of the water and of the plant tissues, especially the nitrogen and mineral compounds.

Measurement of biological parameters

The oxygen content of the water was measured with an oxygen electrode (Clark's method, Clark, 1956). Three times a week, the recirculating water was sampled in the clarifier. After measuring the pH and electrical conductivity, the samples were supplemented with 0.03% of mercuric chloride and stored at 5 °C before mineral analysis. Nitrates were measured by nitration of salicylic acid (Cataldo et al., 1975), nitrites by diazotization of sulphanilamide (Rodier, 1984), phosphorus by formation of the vanadomolybdic com-

plex and sulphates by gravimetry of the baryum sulphate. Ammonia was determined by two methods: colorimetry with Nessler reagent or microdistillation of NH_3 in alkaline medium using the Parnas and Vagner apparatus. Calcium and magnesium analysis was carried out by atomic absorption spectrophotometry and potassium by flame photometry.

The same methods were used for plant tissues after dry matter calcination. Total N was determined by the Kjeldahl method (Kjeltec System 1026 Tecator). Foliar diagnosis on young growing leaves was performed by grinding the leaves in sulfuric acid 0.05 M, centrifuging and determining the mineral content in the supernatant.

Functioning of the system during a production cycle

As the results of this experiment concerned the first production cycle, they integrated special functioning periods, such as setting up the system, for which the system's parameters were not all at their optimum.

Physical characteristics of the water

Water oxygen content varied between 56% of the saturation rate (5.2 mg l^{-1} when leaving the fish tank) and 82% (7.5 mg l^{-1} when leaving the plant troughs). The pH of the recirculating water tended to decrease continuously (Fig. 3). This acidification has already been observed by other authors (MacKay and Van Toever, 1981; Rakocy, 1989). This resulted from the combination of several phenomena: the excretion of acidifying CO_2 originating from fish respiration and plant roots, the functioning of the bacterial filter and, in the present case, the application of an acid mineral complement.

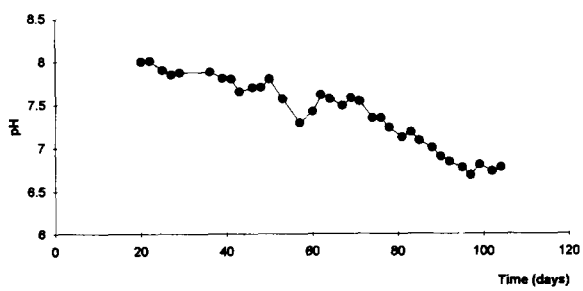

Fig. 3. pH changes in the recirculating water.

Nitrogenous compounds in the water (Fig. 4)

Functioning of the fish compartment: nitrogen excretion. Ammonia excretion increases with the quantity of food ingested by the fish (Savitz, 1971) so it was low during the first feeding stage (before Day 53) then higher. The favourable period for fish growth extended between 24 October (Day 53), when the feed increased beyond 200 g and 28 November (Day 88), when the presence of nitrites became detrimental (higher than 14 μmol l^{-1}).

Functioning of the bacterial compartment: transformation of ammonia. Based on the nature of the nitrogen forms and their levels, this period could be divided into three phases:

(1) From 1 September (Day 0) to 22 October (Day 51) a spontaneous colonization by natural bacteria took place in the filter following the introduction of the fish into the system. After a maximum at Day 27, a large drop in the NH_4 content of the water was observed, while the NH_4 supply remained constant. This meant that after a multiplication phase, the *Nitrosomonas* population had become large enough to oxidize a larger quantity of NH_4 into NO_2 than that produced daily. This NO_2 tended to accumulate, pointing out an insufficient oxidation rate into NO_3 by the *Nitrobacter*, until a sufficient population level was established.

(2) From 22 October (Day 51) to 21 November (Day 81), nitrification occurred in a balanced way so ammonia and nitrites remained at low levels (Petit, 1986) without disturbing effects on fish growth (Colt and Armstrong, 1981).

(3) From 21 November (Day 81) to 7 December (Day 97), NH_4 and NO_2

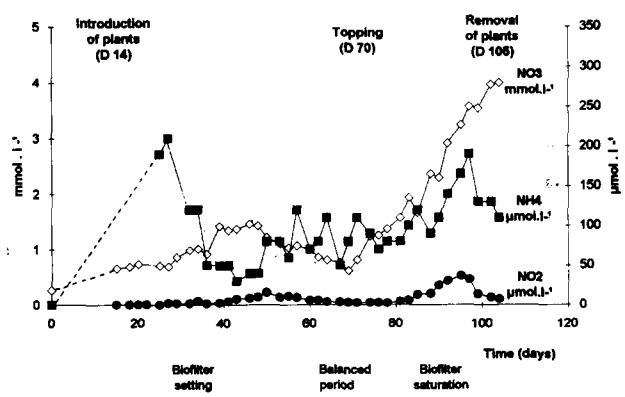

Fig. 4. Nitrogenous compounds in the recirculating water NH_4 μmol l^{-1}, NO_2 μmol l^{-1}, NO_3 mmol l^{-1}.

content increased reflecting a gradual saturation of the bacterial populations. This saturation disappeared when feeding stopped (after Day 97).

Functioning of the plant compartment: nitrogen absorption. The evolution in the NO_3 flow expressed the system's equilibrium. From the introduction of the plants into the system (about 10 days before the beginning of flowering) until their topping (Day 70), the functioning regime was stable: the NO_3 content remained very low and relatively constant (between 0.5 and 1.5 mmol l^{-1}). This indicated that during this period, where the nitrogen requirements of the plants were high, almost all the nitrogen excreted by the fish was absorbed by the roots. It can be presumed that either the nitrogen requirements were satisfied and NO_3 content in the water represents NO_3 in excess, or the nitrogen requirements were not entirely satisfied and the large dilution of NO_3 constituted a check on absorption. Direct NH_4 absorption by plants (Salsac et al., 1987) also contributed to the nitrogen nutrition of the tomato plants.

After the plants were topped, the functioning regime was no longer stable owing to a disequilibrium between nitrogen supply and requirement. Plant growth was stopped and all that remained was the slow swelling of the fruits. This resulted in a decrease of root absorption, so direct NH_4 absorption decreased. Furthermore NH_4 excretion increased as the fish were better fed (250 g after Day 74) so NH_4 content in recirculating water largely increased, leading to the saturation of the bacterial populations not large enough to oxidize all the nitrogen compounds.

Mineral compounds

The mineral balance of the system (NO_3 excepted) was assessed on the basis of the variation in the mineral composition of the circulating water and the nutritional status of the plants (foliar diagnosis). Unlike the nitrogen compounds, the minerals were not balanced: in order to satisfy the plants' requirements a mineral complement has to be provided. Variations in the mineral composition of the circulating water were the result of three factors, mineral excretion of the fish, mineral absorption by plants and addition of tap water to compensate for water loss by evapotranspiration or cleaning of the circuit.

The evolution of the mineral composition of the water could be divided into two phases (Fig. 5):

(1) Day 0 – Day 54, corresponding to low food supply to the fish: K, present in a very small quantity in tap water (0.15 mmol l^{-1}) increased slightly at the beginning because of fish excretion and then disappeared almost completely 10 days after the plants had been introduced; Mg (0.60 mmol l^{-1} in tap water) decreased after 30 days of cultivation (Day 45) and its concentra-

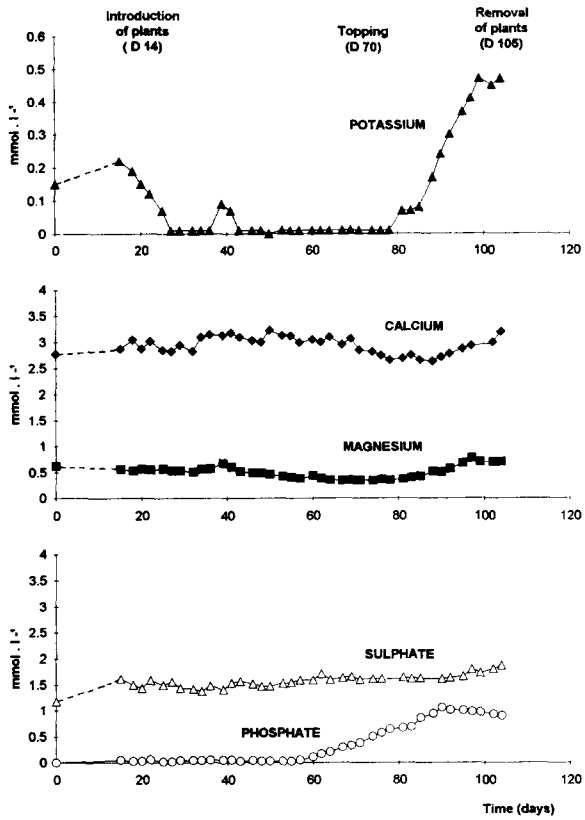

Fig. 5. Mineral content (mmol l^{-1}) of the recirculating water.

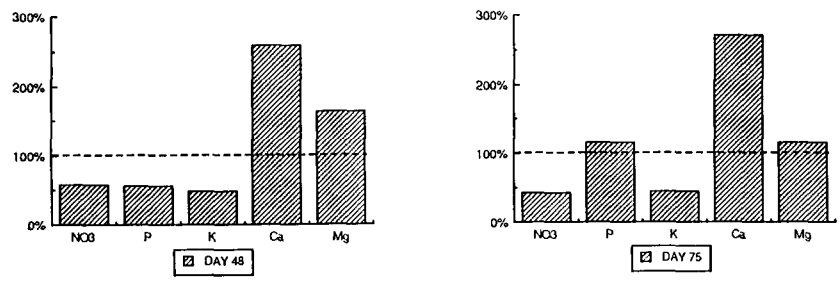

Fig. 6. Mineral content of young leaves of tomato in the pilot system. Results are expressed as percent of mineral content of young leaves issued from conventional hydroponics.

tion subsequently stabilized around 0.35 mmol l^{-1}; P was present in minute quantities; concentration of Ca (2.5–3 mmol l^{-1}) and SO_4 (1.3–1.8 mmol l^{-1}) varied to a slight extent.

Results of the leaf diagnosis (Fig. 6) carried out at Day 48 showed that the

plants had a P and K deficiency equal to 50% of that of the control. Between Day 35 and Day 45 we tested the supply of a complete nutrient solution (Coïc-Lesaint type), which resulted in a sudden increase in K and Mg concentrations (Fig. 5).

(2) From Day 55 onwards the supply of the mineral complement was limited to P and K, as Mg requirements seemed covered by tap water and animal excretion. P and K were applied in the form of KH_2PO_4 acidified by H_3PO_4 to pH 4.5 to avoid the precipitation of calcium phosphate near the point of supply (pH of the recirculating water in the plant troughs was 7–8). This solution (10 l day^{-1}) was able to satisfy three-quarters of theoretical K requirements (10 mmol l^{-1}) while adding four times more phosphate than necessary (14.4 mmol l^{-1}).

A foliar diagnosis made at Day 75 (Fig. 6) showed that P requirements were largely covered whereas the K deficiency persisted. Ten days after topping (Day 80) an increase in K and Mg in the circulating water followed the end of plant growth. The mineral complement was stopped at Day 90 but K and Mg concentrations continued to increase for a few days because of fish excretion (end of feeding: Day 97) and by release from the plant pot in which the mineral complement was delivered.

Plant production

Approximately 10 days after their introduction into the system, the plants exhibited symptoms of chlorosis. Foliar spraying of iron chelate (1.2 mg l^{-1} of metal iron) suppressed chlorosis but, despite subsequent weekly iron applications, the plants maintained throughout cultivation a paler colour than plants grown at the same time in traditional hydroponics. This was related with the fact that during the days following plant introduction, the plants fed at the expense of the reserves accumulated in the plant or the pot. Then with the formation of a dense network of roots in the troughs, they were able to draw mineral elements in the circulating water. But as the pH of the water was close to 7–8, the iron present was hardly soluble (Marschner, 1989) thus, involving plant deficiency. A further application of iron in the mineral complement or by foliar spraying was therefore necessary. The permanent paler appearance of leaves was a result of the low level of potassium (Odet and Musard, 1989).

The 60 tomato plants which reached five clusters produced on average 0.75 kg of fruit per plant. The yield was low compared with yields commonly observed (25 – 30%), both because of a 50% drop in the number of fruit per cluster (lack of pollination in the greenhouse) and a 50% lower average weight of the fruit. Considering the important limiting factor of solar radiation, these results are not surprising for a crop grown late in the season without extra lighting. However, the differences were limited in comparison with a conven-

Table 2
Mineral content (% dry matter) of plants at harvest (Day 105)

	Total N	K	Ca	Mg	P
Leaf blade	3.4	2.3	7.1	0.7	1.1
Fruit	2.7	4.4	0.3	0.2	0.7

tional crop grown at the same time in the greenhouse. The average production per plant represented 82% of the conventional crop with an almost identical number of fruit but lower average weight of fruit (71%). Considering the level of the input, the yield was therefore very satisfactory. The non-edible plant matter represented 6.5 kg of dry matter for 60 plants. It was not recycled in our system, but could be partially recycled by incorporating it in the food for the fish.

The mineral composition of the fruit (Table 2) was similar to that given in literature data (Statistical Analysis Systems, 1969; Musard, 1988), except for the higher total phosphorus (140%) and the slightly lower potassium (90%) contents. The great mobility of potassium in the plant (Mengel and Kirkby, 1987) explains the slight potassium deficiency of the fruit compared with the lowered leaf status (−25%). Potassium feeding had therefore been limiting due to an insufficient supply (Fig. 5). Although the tap water had a high calcium content, this element hardly reaches the fruit (Ferguson and Drobak, 1988) so that its calcium content is normal. The magnesium content of the fruit was low but normal. The high concentration in total phosphorus, both of the fruit and the leaf, proceeds from the supply in excess of phosphate in the mineral complement. The nitrogen content of the plant was not affected by the low nitrate concentration of the circulating water owing to its rapid absorption and migration in a flowing solution (Moorby and Besford, 1983).

Conclusions

Defining the conditions of the ecosystem's equilibrium implies to take in account at any time the proper dynamics of each compartment (fish, bacteria and plants).

In the system's design, we have determined the structural parameters of the fish compartment, size of the tanks and the biofilter, choice of the flow rate of recirculating water through the bacterial compartment. However, optimization also depended on the good management of the fish compartment, nature of the fish population (age and sex), level of feeding and physico–chemical quality of the water. It was essentially the good oxygenation of the bacterial filter which was critical for the fish culture. Maintaining the pilot system therefore involved frequent cleaning of the filter, as the organic particles sus-

pended in the water settled on the gravel and limited the access of oxygen to the bacteria (Martin, 1979).

The intensification of plant production involved determining the size of the plant compartment and optimizing its mineral nutrition. Nitrogen was the critical element of the system as it is both toxic to fish and a limiting factor in the nutritional supply to the plants. On the basis of the results of this experiment, it appears that the nitrogen necessary for plant growth can come totally from fish excretion as we saw that even very diluted, it does not impair plant growth. To establish the ecosystem nitrogen balance, we could determine the size of the plant compartment by drawing a parallel between the theoretical nitrogen requirements of the plants and the quantity of nitrogen supplied by the fish during a production cycle.

We have considered that the quantity of nitrogen excreted by the fish, then transformed by bacteria, was in proportion to the food level (Savitz, 1971). The latter, if it was optimal, depended on the weight of the fish (Melard, 1986). Good management of the fish compartment, that is farming fish of a marketable size starting with fingerlings, will lead to an increasing supply in nitrogen over time in the water. However, the nitrogen requirements of plants depended on phenological stages and growing conditions and they dropped after topping. On account of the toxicity of the nitrogen forms to fish and an optimal effectiveness of nitrogen within the system, we wanted to maintain the nitrogen content of the water at the lowest level. However, beginning the production cycles at the same time as was the case in this trial would lead to a totally unbalanced situation at the end of the cycle, as ammonia production by fish was maximum when plant absorption was decreasing. The solution for this problem is the staggering of plant crops, that is growing at the same time plants which are at different phenological stages in order to create a constantly high demand for nitrogen and thus avoid any rupture in the absorption of nitrates. Managing the fish compartment in the same way is hardly practicable with a single tank and will require a multi-tank system. This latter system could allow a continuous production of fish and vegetables.

During this trial, the flow of nitrogen from the 69 fish was relatively well stabilized and maintained at a low level in the presence of the 60 growing tomato plants. However, fish farming conditions were not satisfying (the fish were old and the feeding rate low), nor were the plant production conditions (autumn). In the follow-up of our work, we studied the functioning of the system in other more or less favourable conditions and established nitrogen balances expressing the state of equilibrium of the system (Quilleré et al., 1993b).

Other parameters are likely to influence the state of equilibrium of the system, both on nitrogen supply (food consumption decreases when environmental conditions are unfavourable) and on its plant absorption (growth limitation via parasites, climatology or mineral nutrition).

Mineral nutrition was optimized by applying a mineral complement. Evolution in the mineral composition of the water and of plant tissues showed that, in our production conditions, only potassium and phosphorus were limiting factors in the mineral feeding of the plants. Magnesium was at the limit of the desirable concentration (Table 1 and Fig. 5): it could have been limiting if the crop season had been more favourable to plant growth.

Mineral fertilisation can be calculated according to the composition of available water on the production site and to plant requirements. The mineral complement will therefore supply the minerals essential to the growth of plants which are not present in sufficient quantity in the water. Its volume will be determined by estimating the system's water loss by evapotranspiration, as the volume of the system's water must remain stable. In the present case, considering the mineral composition of the water (high calcium content) and the pH zone compatible with fish growth (7 – 8), it was not possible to apply this complement in solid form as there would be a precipitation of salts applied in the form of calcium phosphate unavailable to the plants. For these reasons the mineral complement was applied in the form of a concentrated liquid with a relatively low pH (4.5). A complement in microelements does not seem useful as there was no symptom of deficiency in the plants. Only iron posed a specific problem solved by foliar spraying.

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