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Evolution of an activated sludge system under starvation conditions

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Abstract

In this work, the evolution of several physical-chemical and microbiological variables in starvation conditions of an activated sludge are studied. An activated sludge pilot plant was used to study starvation condition effect on biomass. The experiment was studied over a 21-day period, during which no organic material was added to the system. Suspended solids, volatile suspended solids (VSS), pH, dissolved chemical oxygen demand (CODd), dissolved organic carbon (DOC), oxygen uptake rate (OUR), specific oxygen uptake rate (SOUR), identification of microorganisms and total and active cells counting were all monitored. At last of the experiment, an important decrease in the reactor biomass was observed, which may be related both to: the intracellular component degradation from endogenous metabolism, and to the solid depletion occurred, specially, over the first 4 days. Sludge degradation throughout the experiment led to a decrease in the respiratory and enzymatic activity of the microorganisms of the system. During identification tests, some of the typical microbial groups, usually found in an activated sludge, had disappeared and others opportunists appeared. Sludge flocculation capacity decreased with microbial activity diminution and/or microorganism death. From the results obtained, it may be deduced that significant biomass reduction is achieved in first days, specifically, the first one. The highest solid decrease is produced during the first day. Thus, may be interesting to use with an activated sludge system a starvation period of 24 h before stabilization. This can suppose operation and economic benefits.

Keywords: Activated sludge; Activity microbial; Starvation; Reduction biomass

1. Introduction

Wastewater biological treatments involves transformation of dissolved and suspended organic pollutants to biomass and evolved gases (CO_2, CH_4, N_2 and SO_2) which can be separated from treated waters. Microorganisms use organic matter as a carbon source for providing energy and cellular material. Catabolism transforms the organic pollutants into useful energy and metabolites. The energy is consumed in satisfying maintenance functions and for fueling the anabolism of new biomass from the metabolites.

Different authors have shown that bacteria have the capacity to survive a lack of nutrients. This capacity seems to be controlled by different factors, such as bacterial species, the presence of polymers, the structure of the cellular wall, the pre-adaptation period or environmental conditions such as temperature, etc. This is because bacteria starved of substrates either die, provoke lysis of their cells or else adapt to the given conditions.

The sludge biomass is composed of a complex mixture of microorganisms, which are separated in the secondary clarifier and are subsequently eliminated through different technologies. Given the high volume of sludge produced in this type of system, there is both an economic and ecological need to reduce its production due to the costly and complicated treatment which it implies.

Bearing this in mind, and with the intention of decreasing sludge production, some researchers [1,2] have investigated the possibility of incorporating a starvation period prior to sending the sludge to the digester. Another authors [3,4] have also reported that storing the sludge in aerobic conditions before recycling in the aeration tank had no effect on the efficiency of the system (80–90% dissolved chemical oxygen (COD) removal) and held sludge production at zero. This leads to a decrease in sludge dry mass, a significant decrease in enzymatic activity and a release into the medium of cellular material.

The main goal of this research is to compare the behavior of physical-chemical variables, population sequences, and microbial activity indicators in an activated sludge system in starved conditions. Due to the fact that there is none available reference in the scientific literature about

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mixed population of activated sludge system; published papers only study independent variables of these systems [5,6], and about the effect of the starvation on pure culture of bacteria.

2. Material and methods

2.1. Experimental conditions

The experiments were performed in a 6 l reactor filled with 5 l of activated sludge from the aeration tank of the wastewater treatment plant at Jerez de la Frontera (Cadiz, Spain). Aeration was provided by a Tagus 200 air compressor at the rate of 4 l/min. Air transfer to the medium was effected through stainless steel porous diffusers, which produce suitably fractionated bubbling of the output stream, and facilitate the transfer of oxygen to the medium, thus preventing limited oxygen conditions. The reactor was kept at room temperature ($24 \pm 2\,^{\circ}C$), and the pH and dissolved oxygen concentration levels were measured daily. A 100 ml sample was taken each day throughout the experiment, for analysis of the variables under study.

2.1.1. Determination of suspended solids and chemical oxygen demand

Determination of both these variables was in accordance with the guidelines established in APHA-AWWA-WPCF (1989) [7].

2.2. Determination of the biochemical activity of the sludge

2.2.1. Active and total cell counting

Bacterial population (active and total cells) present in the reactor was determined using the method proposed by Griebe et al. [8] based on the combined use of two fluorochromes: 5-cyano-2-3-ditolyltetrazolium chloride (CTC), for the active microorganism determination, and 4,6-diamido-2-phenylindole (DAPI), for total microorganism determination. In Fig. 1, a flow diagram of the assay for total and active cell determination is showed [8]. Activated sludge samples were incubated with CTC (4 mM) at room temperature in darkness. The CTC and DAPI reagents were provided by Polyscience Inc. (Epplheim, Germany). Controls were prepared from incubation samples, previously inactivated by formaldehyde (final concentration 2%). After incubation (2 h), samples were stained with DAPI-lac (10 μg/ml) for total cell determination. Activated sludge samples were homogenized and diluted prior to filtration onto a black polycarbonate membrane filter (0.2 μm pore-size, Millipore, Madrid, Spain). An ultrasound bath for 15 min was achieved for appropriate breaking up of the activated sludge matrix. Microscopic examination and counting of triplicate samples were done according to Schaule et al. [9].

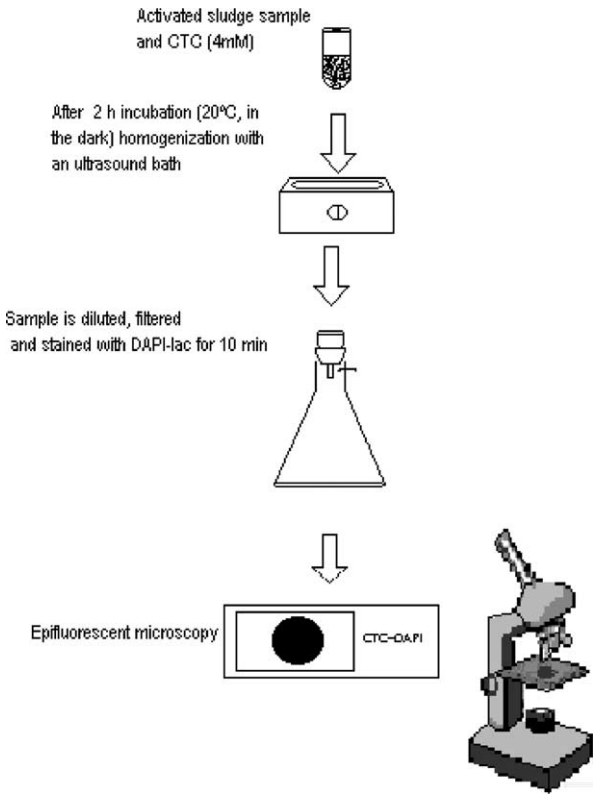

Fig. 1. Flow diagram for total and active cell determination in activated sludge [8].

2.2.2. Specific oxygen uptake rate (SOUR)

SOUR measurements were performed in triplicate using a membrane electrode, and employing 50 ml of sample placed in a stirred bottle with a magnetic mixer to produce an entirely homogenous sample. The membrane oxygen electrode (Mod CRISON OXI330) was inserted, protecting the bottle contents from the atmosphere with a screw top sealed with silicone, to prevent oxygen transfer to the sample. SOUR was calculated in accordance with the equation used by Awong et al. [10], as follows:

$$\mathrm{SOUR} = \frac{1440R}{C} \quad (1)$$

where SOUR is the specific oxygen uptake rate (mg O_2/g VSS per day), R the oxygen consumption rate (mg O_2/(l min)), C is the biomass concentration of the sample (g VSS/l).

3. Results and discussion

3.1. Initial composition of the sludge

Tables 1–4 show the initial composition of the sludge. Volatile suspended solids (VSS) value of 1.97 g/l represents 75% of total solids present in the reactor, reflecting typical VSS levels in an activated sludge. The values corresponding to the respiration rate, OUR (0.32 mg O_2/(l min)) and to

Table 1
Initial composition of the activated sludge

Parameters	Value
SST (g/l)	2.56
SSV (g/l)	1.97
No. of total cells (cells/ml)	3×10^9
No. of active cells (cells/ml)	1.3×10^9
OUR (mg O_2/(l min))	0.4
SOUR (mg O_2/g SVS per day)	292.4
DO (mg O_2/l)	3

the specific respiration rate, SOUR (292.4 mg O_2/g VSS per day) also indicate the healthy state of the microbial population in the system. Of the total population existing in the reactor at the start of the experiment (3×10^9 cells/ml), 43.4% corresponds to the active population.

3.2. Response of the control variables

A continuous decrease in volatile suspended solids was recorded throughout the 21 days of the experiment, reaching 75% of the initial value, as shown in Fig. 2. This figure reveals three clearly distinct phases. The first, lasting until days 3–4, produces the largest decrease in VSS, possibly as a result of the death of higher microorganisms due to the lack of organic material in the reactor. From days 5 to 10, a further decrease occurs, but is not so marked. In the final phase, spanning the last 10 days, the rate of decrease is substantially reduced, producing a much gentler incline than in the previous phases.

This decrease in volatile suspended solids may be attributed to the total suspended solids, since the values of non-volatile solids remain more or less unchanged, as may be observed in Fig. 2.

Table 4
Exponential equations obtained on the parameter fitting

Parameter	Line of best fit	r^2
TSS	$TSS = 2.56e^{-0.0667t}$	0.8745
OUR	$OUR = 0.32e^{-0.175t}$	0.9232
Total cells	Total cells $= 3E{+}9e^{-0.1229t}$	0.9759

Table 2
Results of the control variables obtained in the experiment

Time (days)	TSS (g/l)	VSS (g/l)	pH	DOC (mg C/l)	CODd (mg O_2/l)
0	2.56 ± 0.01	1.97 ± 0.01	7.53 ± 0.01	14.6 ± 0.04	60 ± 0.02
1	1.77 ± 0.01	1.43 ± 0.01	8.09 ± 0.01	15.84 ± 0.04	57 ± 0.02
2	1.79 ± 0.01	1.01 ± 0.01	8.43 ± 0.01	18.37 ± 0.03	63 ± 0.02
3	1.52 ± 0.01	1.17 ± 0.01	7.83 ± 0.01	14.8 ± 0.03	39 ± 0.01
4	1.51 ± 0.01	1.13 ± 0.01	7.81 ± 0.01	12.73 ± 0.04	44 ± 0.01
5	1.63 ± 0.01	1.24 ± 0.01	7.67 ± 0.03	40.42 ± 0.04	170 ± 0.01
7	1.48 ± 0.02	1.08 ± 0.02	7.92 ± 0.01	63.68 ± 0.04	293 ± 0.01
8	1.1 ± 0.01	0.72 ± 0.01	7.45 ± 0.01	29.89 ± 0.04	87 ± 0.01
9	1.12 ± 0.02	0.76 ± 0.02	7.72 ± 001	40.03 ± 0.02	141 ± 0.02
10	1.15 ± 0.01	0.74 ± 0.01	7.52 ± 0.01	19.67 ± 0.02	76 ± 0.02
12	1.2 ± 0.01	0.84 ± 0.01	7.3 ± 0.01	13.29 ± 0.04	131 ± 0.02
14	1.2 ± 0.01	0.84 ± 0.01	7.05 ± 0.01	18.12 ± 0.04	156 ± 0.02
16	0.93 ± 0.01	0.56 ± 0.01	6.9 ± 0.03	19.31 ± 0.02	130 ± 0.01
18	0.72 ± 0.03	0.45 ± 0.03	6.89 ± 0.01	22.01 ± 0.04	98 ± 0.01
21	0.7 ± 0.03	0.42 ± 0.03	6.89 ± 0.02	24.54 ± 0.04	77 ± 0.02

Table 3
Results obtained in the microbial activity parameter under starvation conditions

Time (days)	OUR (mg O_2/(l min))	SOUR (mg O_2/g VSS per day)	No. of total cells (cells/ml)	No. of active cells (cells/ml)	Active cells (%)
0	0.35 ± 0.01	292.38 ± 0.05	3.0E+9 ± 0.05	1.3E+9 ± 0.05	43.37 ± 0.05
1	0.24 ± 0.04	201.39 ± 0.05	2.2E+9 ± 0.05	1.0E+9 ± 0.05	45.93 ± 0.05
2	0.20 ± 0.04	213.33 ± 0.05	2.1E+9 ± 0.05	8.0E+8 ± 0.05	38.58 ± 0.05
3	0.13 ± 0.007	123.08 ± 0.05	2.0E+9 ± 0.05	6.0E+8 ± 0.05	30.75 ± 0.05
4	0.075 ± 0.002	127.43 ± 0.05	1.6E+9 ± 0.06	4.2E+8 ± 0.06	25.72 ± 0.05
5	0.07 ± 0.003	116.13 ± 0.05	1.4E+9 ± 0.05	3.4E+8 ± 0.05	24.93 ± 0.05
7	0.068 ± 0.004	133.33 ± 0.05	8.7E+8 ± 0.05	4.1E+7 ± 0.05	4.64 ± 0.05
8	0.06 ± 0.004	200.84 ± 0.05	7.9E+8 ± 0.05	3.8E+7 ± 0.05	4.79 ± 0.05
9	0.097 ± 0.002	189.47 ± 0.05	7.9E+8 ± 0.05	3.8E+7 ± 0.05	4.78 ± 0.05
10	0.09 ± 0.004	194.59 ± 0.05	7.7E+8 ± 0.05	3.6E+7 ± 0.05	4.66 ± 0.05
12	0.09 ± 0.004	171.42 ± 0.05	6.8E+8 ± 0.06	3.7E+7 ± 0.06	5.41 ± 0.05
14	0.07 ± 0.005	170.21 ± 0.05	4.5E+8 ± 0.06	2.4E+7 ± 0.06	5.25 ± 0.05
16	0.07 ± 0.005	120.85 ± 0.05	2.8E+8 ± 0.06	1.3E+7 ± 0.06	4.76 ± 0.05
18	0.05 ± 0.003	144.00 ± 0.05	2.5E+8 ± 0.05	1.0E+7 ± 0.05	4.00 ± 0.05
21	0.04 ± 0.003	117.55 ± 0.05	2.5E+8 ± 0.05	9.0E+6 ± 0.05	3.67 ± 0.05

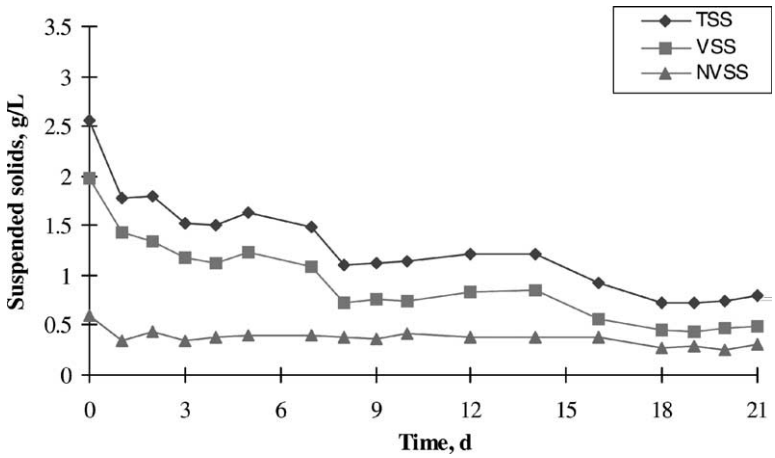

Fig. 2. Evolution of suspended solids in the starved sludge.

The rate at which the total suspended solids disappear from the system corresponds to a first order equation with a rate constant of 0.081 per day. However, if only the first 4 days are taken into account, when the decrease is sharpest, the decay rate constant is 0.14 per day, higher than other published reports: 0.073 per day for Urbain et al. [5], 0.057 per day for Benedek et al. [6] and 0.048 per day for Kim and Hao [11].

The development of COD values in the aeration tank is analogous to that of the total suspended solids, registering a 65% decrease from an initial value of 1993 mg O_2/l, down to 686 mg O_2/l (Fig. 3).

The evolution of the level of soluble organic material in the system was monitored through determination of dissolved chemical oxygen demand (CODd) and dissolved organic carbon (DOC) (Fig. 3). The maximum values recorded for each variable, between days 4 and 9 of the experiment, happens at the same time with the release into the medium of the organic material contained in the cellular protoplasm resulting from the death of the higher microorganisms. These organic polymers are composed primarily of proteins and polysaccharides, and those most susceptible to degradation are eliminated, leading to a subsequent reduction in the CODd and DOC levels in the system. The amounts of these variables still remaining at the end of the experiment are due to organic molecules which may be considered refractory in these experimental conditions.

The pH of the reactor contents underwent significant change over the course of the experiment. During degradation of the sludge constituents, from days 0 to 2, pH increased from 7.53 to 8.43 and thereafter decreased to 6.80 by day 21 of the experiment (Fig. 4). The initial pH increase may be due to the release of ammonium into the medium, as a result of protein hydrolysis. The acidification observed in the system after day 2 of the experiment has been reported by various authors [1,5], and constitutes the principal drawback of the aerobic digestion process; it may be caused by hydrolytic metabolism (volatile fatty acids production), or by nitrification reactions which take place in the system.

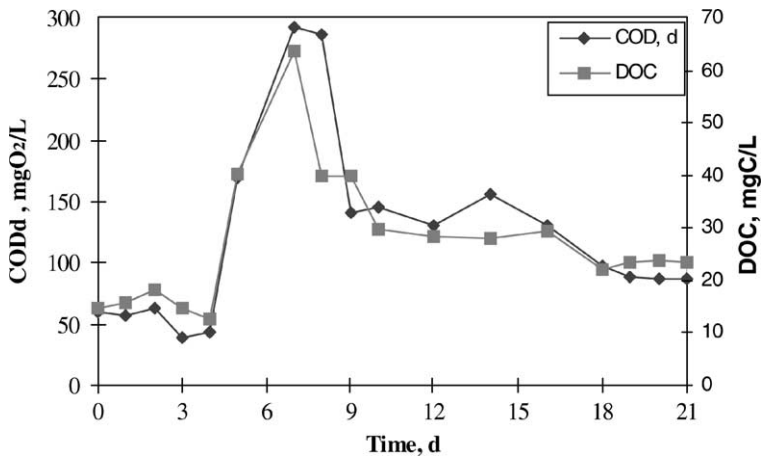

Fig. 3. Organic carbon and chemical oxygen demand in the liquid phase.

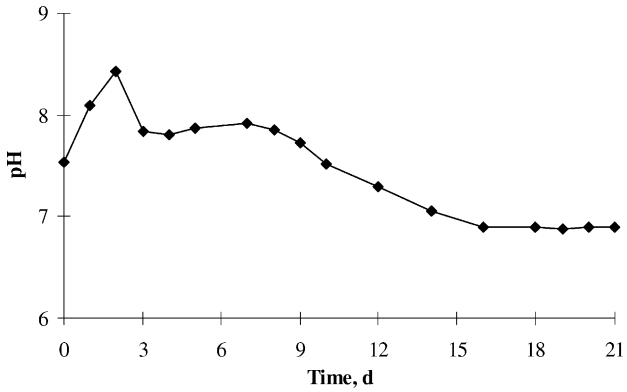

Fig. 4. Evolution of the pH in the sludge.

3.3. Microbial activity in the activated sludge system

Microscopic examination was useful in assessing the state of the population and the changes undergone by the microbiota in the activated sludge sample employed in the experiment. It also helped to explain the evolution of the aforementioned parameters (suspended solids, COD, etc.).

The population existing in the system at the start of the experiment was typical of an activated sludge sample, with the presence of all the functional groups typical to these systems (Fig. 5a). During the early days of the experiment, the sludge in the reactor appears gelatinous and does not settle well in the clarifier. Examination under the microscope revealed a marked reduction in the number of individuals belonging to groups of higher organisms (Fig. 5b). Only sessile ciliates, of the Vorticellidae family, were present, whilst other forms of colonial sessile ciliates, such as *Epistilys* or *Zoothamium*, had disappeared. The process of reduction in the size of organisms present in the reactor is one of the adaptation responses to starvation conditions which has already been described by a number of authors [12]. As time passes, the sludge grows darker in color and the turbid supernatant observable in the clarifier is the result of a marked drop in the sessile ciliate population, which gives way to predominantly flagellate or crawling ciliate, in particular the *Aspidisca cicada*. Around day 10 of the experiment, the individuals established in the system were free-swimming carnivorous ciliates, predominantly *Coleps hirtus*, and small flagellates, as well as some individuals of the *Suctoria* species (Fig. 5c). These organisms are typically opportunist and develop as a consequence of the conditions created in the reactor.

This response in the evolution of the microbial population has been reported widely in the literature, since existing populations depend on their capacity to adapt to the new substrates in the medium. By the end of the experiment, the microbial population existing in the system had almost completely disappeared, replaced by low activity and dispersed flocs (Fig. 5d). Sludge flocculation capacity decreased with microbial activity diminution and/or microorganism death (Fig. 5e).

The evolution of the respiration rate, illustrated in Fig. 6, is similar to that of the suspended solids (Fig. 2), sharply decreased during the first days of starvation and then continuously up to the end of the experiment. Consequently, in the first days of the experiment, there is a sharp decrease in respiratory activity as a result of the elimination of higher organisms.

From day 3 onwards, the respiration rate remains virtually unchanged until the end of the experiment: an indication of the low activity of those organisms remaining in the system. This response is similar to that obtained by Teuber and Brodish [13] who recorded a decrease of up to 88% of respiratory activity in the first 3 days of their test. It differs, however, from the results obtained by Horan and Shanmugan [14], who noted a 60% decrease in respiratory activity at day 7.5 and an 80% decrease at day 12.5 of the test.

The decrease in respiratory activity (OUR) may be considered a first order process with a rate constant of 0.175 per day and its value may be calculated by employing the following equation:

$$\mathrm{OUR} = 0.32\mathrm{e}^{-0.175t} \quad (2)$$

Fig. 7 shows the values obtained for the specific respiration rate. The tendency is irregular and differs from that observed in the previous variables. Thus, after an initial decrease, the system recuperates its activity due to the appearance of opportunist individuals who take advantage of the conditions created in the reactor to develop. The decrease recorded between days 7 and 21 is due to cannibalistic processes, and the microorganisms remaining in the reactor feed on the cellular products resulting from the lysis of previously eliminated individuals.

Fig. 8 illustrates the results obtained in the total and active cell counts. Both develop along similar lines: a sharp decrease between days 0 and 7 of the experiment, coinciding with the decrease experienced by the solids, possibly due to the degradation and death of the microorganisms in the reactor. The decline is more marked in the active than in the total population. The decrease is exponential, with a rate constant of 0.123 per day for the total population existing in the reactor, and of 0.261 per day for the active population. These values are similar to those reported by Urbain et al. [5], but markedly different from those of Mason et al. [15] who obtained a cellular lysis value of $0.05\,\mathrm{h}^{-1}$.

During counting tests, using CTC fluorochrome, a size diminution of surviving cells during the experiment could be observed. This has been confirmed in some published works with purified cultures.

Fig. 9 shows the percentage of the active population remaining in the reactor over the course of the experiment. Initially large (43.4% of the total population), as corresponds to a typical activated sludge system, the population begins to fall from day 1 of the experiment, due to the scarcity of organic material in the unit. On day 5, it experiences another sharp drop, falling below 5%, and this value remains unchanged until the end of the experiment. Only

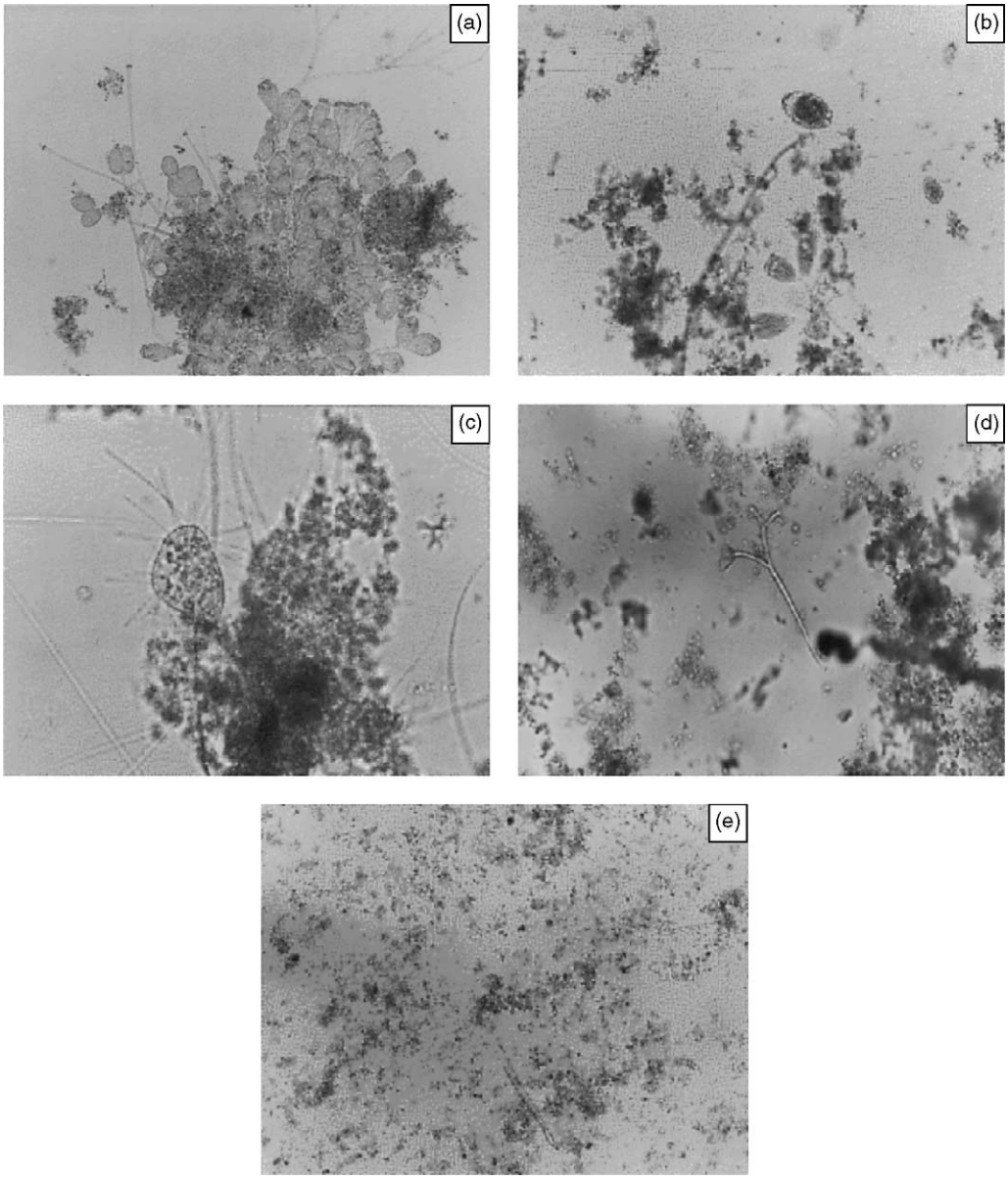

Fig. 5. (a) Microbial composition at the start of the experiment; (b) with starvation conditions the microorganisms number decrease in the system; (c) opportunist microorganisms appear in such conditions as *Suctoria* groups; (d and e) starvation conditions lead to death of the most of the cells and a dispersed growing of surviving cells.

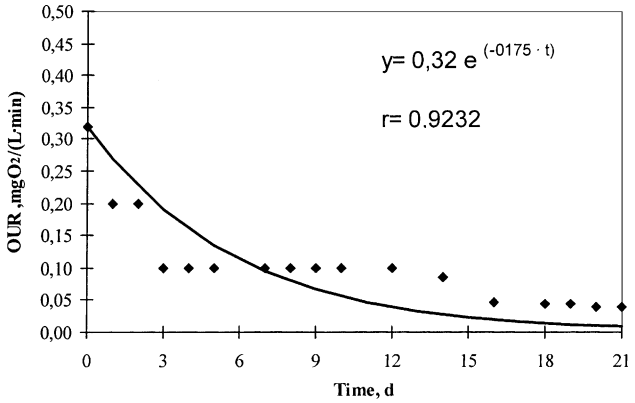

Fig. 6. Evolution of oxygen uptake rate in the starved sludge.

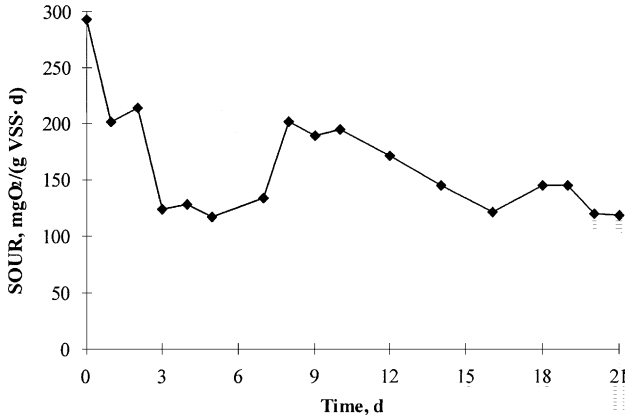

Fig. 7. Effect of starvation on the specific oxygen uptake rate of activated sludge.

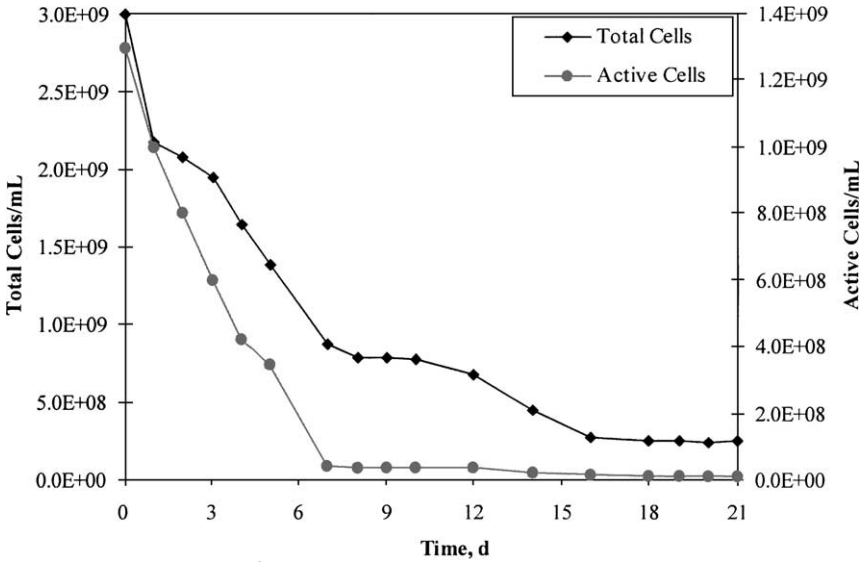

Fig. 8. Changes in the total cell and the active cell number in the starved sludge.

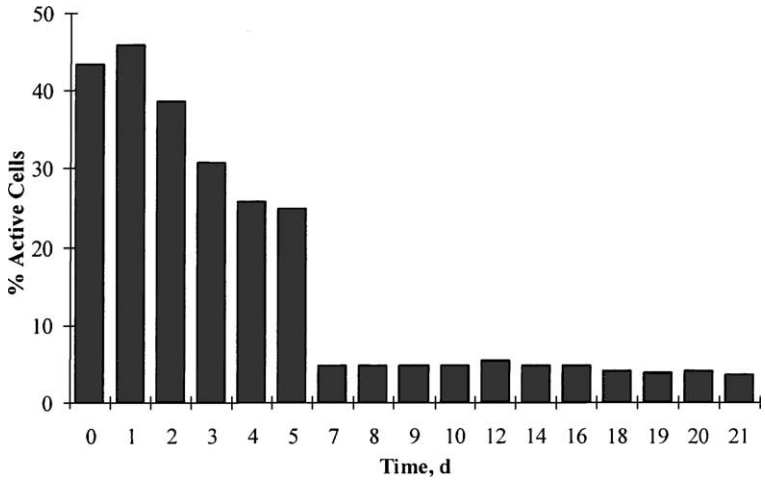

Fig. 9. Degradation of the active population in the starved sludge.

those organisms capable of metabolizing the substrates to gain energy for maintenance are left in the reactor.

4. Conclusions

From this experiment, performed under starvation conditions, it may be concluded that:

- There is a significant reduction in the amount of solids present in the activated sludge system. This reduction is due to cellular lysis resulting from starvation of the microorganisms. Thus, surviving microorganisms use as food proteins and polysaccharides excreted into the medium.
- As a result of the conditions created by the lack of nutrients, the microbiote present in the reactor undergoes changes with the presence of opportunist organisms. These are very different to the types usually found in activated sludge.
- Activity levels are markedly diminished; by the end of the experiment (21 days) the specific respiration rate is very low (147 mg O_2/g VSS per day), and only 3.67% of the population in the system are active.
- The highest solid decrease is produced during the first day. Thus, may be interesting to use with an activated sludge system a starvation period of 24 h before stabilization. This can suppose operation and economic benefits.

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