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IASON – Intelligent Activated Sludge Operated by Nanotechnology – Hydrogel Microcarriers in Wastewater Treatment

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Abstract Performance of biological wastewater treatment depends to a large extent on mechanical strength, size distribution, permeability and other textural properties of the activated sludge flocs. A novel approach was developed in applying synthetic polymer materials to organize floc architecture instead of spontaneously formed activated sludge floc. Developed microcarrier polymer materials were used in our experiments to mitigate technological goals. Preliminary results suggest that the PVA–PAA (polyvinyl alcohol–polyacrylic acid copolymer) is a feasible choice for skeleton material replacing "traditional" activated sludge floc. Use of PVA–PAA hydrogel material as microreactors and methods for biofilm formation of wastewater bacteria on the carrier material are described. Laboratory scale experimental results with microscopic size bioreactors and their potential application for simultaneous nitrification and denitrification are presented.

Keywords Activated sludge · Biofilm development · Hydrogels

Introduction

The driving forces for international efforts during the past few decades in the field of wastewater treatment technologies were the recognition of environmental problems with respect to surface waters (oxygen depletion, eutrophication, etc.) as well as more stringent environmental legislation (such as the 91/271/EEC directive). Apart from biologically degradable organic compounds, nitrogen forms, such as ammonium ion (nitrification), nitrate (denitrification) as well as phosphorus compounds and various micropollutant materials (heavy metals, oil derivates, hormone residues, etc.) have to be removed in biological and/or combined wastewater treatment technologies. As the results of these requirements and consequent technological developments the layout, design and process control of biological wastewater treatment plants have become highly diversified and complex.

The tendency of increasing technological complexity has remained the same during the past few decades. The question remains, however, whether this is the only right tendency to follow. In biological wastewater treatment technologies the majority of systems are based on activated sludge technology. In these systems the population structure and dynamics of the activated sludge microbial community is largely governed by indirect regu-

latory mechanisms, such as aerated/non-aerated ~~reactors~~, reactor configurations and sludge recycling ratios. Yet, despite all the R + D efforts, the build-up of activated sludge flocs (bacterial aggregates) that is the key elements of the wastewater treatment process remains uncontrolled. Their morphology, microscopic architecture and biochemical build-up could only controlled by indirect methods. Furthermore, a design tendency is that for each particular functions (i.e., ammonium oxidation, nitrate reduction, etc.) a separate reactor space is provided, thus making nutrient removal costly.

In framework of the IASON (*Intelligent Activated Sludge Operated by Nanotechnology*) project we introduced a non-traditional concept by utilizing the results of nanotechnological development in biological wastewater treatment process. Artificially constructed microreactors could perform complex tasks within a single aerated basin, as the biochemical processes are regulated by the responses of the microenvironment of IASON structures. These are governed directly by the build-up of the nanostructures constructed within. The new concept of IASON changes the traditional wastewater treatment approach. Hitherto, wastewater treatment plants and technologies were designed on the basis of the spontaneously formed activated sludge floc.

In biological wastewater treatment the biomass consists of multiple species, complex microbial communities either in suspended or immobilized form. The traditional activated sludge flocs, commonly called as suspended biomass in reality are also organized by immobilized bacterial consortia [1]. The heterogeneous multiple layered flocs (or biofilm) consist of several strata having an average size of several hundreds of μm, with porous structure, embedded EPS materials (extracellular slime materials). The knowledge to regulate or furthermore to design the microenvironment of activated sludge community is largely missing. Typical examples are the spontaneously occurring simultaneous nitrification/denitrification phenomena either in bioreactors or in secondary clarifiers or in both. The same bacterial layer within a spontaneously formed activated floc might have aerobic, anoxic or anaerobic microenvironment depending on the prevalent shear stresses, mixing and aeration regime. Should oxygen penetration depth change into the immobilized cell layers (irrespective ~~of~~ floc or biofilm structure) the consortia of microorganisms might switch from anaerobic fermentation pathways to the aerobic TCA cycle or could use the ammonium ion as energy source (i.e., nitrification) or depend on nitrate as final electron acceptor [2]. The heterogeneity of bacteria with respect their biochemical repertoire as well as their microscopic structure expressed in biofilm build up makes the predictions, modeling and process control difficult [3].

Activated sludge flocs used in biological wastewater treatment could be regarded as a sort of self-immobilized system where the carrier material itself consists of living and dead cell biomass. Self carrying biofilms are also used in several full-scale wastewater treatment applications (e.g., UASB – *upflow anaerobic sludge blanket reactors*). Similarly to suspended cell immobilized systems (activated sludge) the mechanisms of biofilm formulation, maturation and final structure formation (density, microchannels, other textural properties) are largely unknown and directly could not be controlled [4]. To overcome these drawbacks a novel concept has been developed (IASON) where biofilm development and final microstructure is directly controlled on the surface and within the inner layer of hydrogel materials in the same particle size dimensions of spontaneously formed activated sludge flocs.

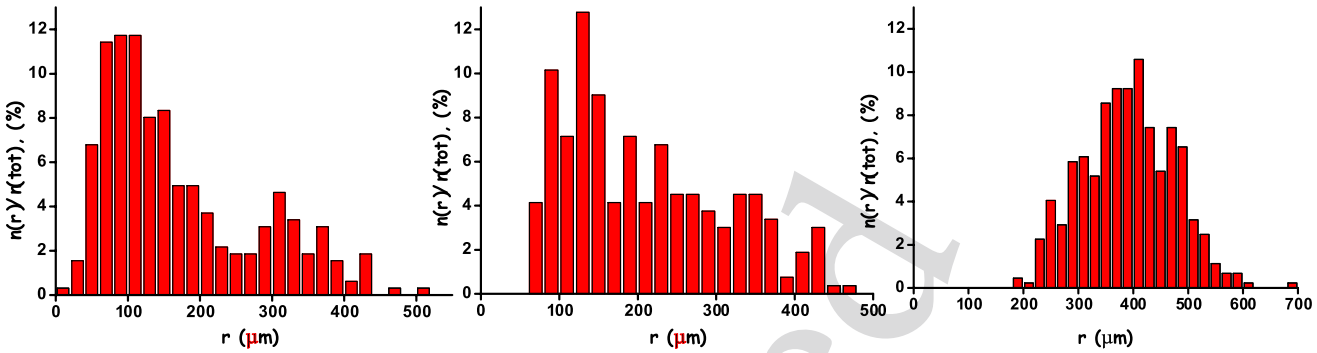
Establishment of heterogeneous layers in biofilm developing on the PVA–PAA carriers was the most difficult step in the multifunctional reactor. The structure of the biofilm depends on many environmental conditions and biotic factors (inoculation of the gels, composition of influent (raw) wastewater, diurnal changes of influent wastewater, flow conditions and apparent shearing forces in the reactor, the continuously changing density and average thickness of the biofilm, texture and 3D structure of the biofilm. Two papers report various effects of presence or absence of microscopic channels and voids, dosing of the methanol, buffer solutions on biofilm structure [6, 7].

Laboratory scale results of artificial microcarriers (IASON) to which the activated sludge bacteria adhere and function in a designed and controlled microenvironment are presented in the paper. The objective of our laboratory scale research is to explore on how the hydrogel carrier materials can be designed and test their environmental reaction with and without wastewater bacteria. Technological results with the newly developed hydrogel materials (PVA–PAA (*polyvinyl alcohol–polyacrylic acid copolymer*) for nitrification and denitrification are detailed in the paper.

Materials and Methods

Synthesis and Treatment of Microcarrier Hydrogel Materials

Several batches of new hydrogel materials were tested and selected on the basis of their durability, size distribution (Fig. 1), density, surface properties regarding bacterial adherence as illustrated by Fig. 2. where the steps of the selection process of the technologically appropriable hydrogels materials are depicted. Some examples of investigated hydrogel beads are: PVA–PAA (polyvinyl alcohol–polyacrylic acid copolymer), modified starch and cellulose materials, ferromagnetic PVA, NIPA (*N*-isopropyl-acrylamide) gels. Technical details were discussed elsewhere [5]. In this paper the results with the PVA–PAA copolymer hydrogel beads are presented. In this carrier the regulation of the porosity (inner structure) of hydrogel pearls was achieved by the addition of fine



a) Non-treated PVA/PAS b) NaOH treated PVA/PAS c) NaOH treated PVA/PAS with fully developed biofilm attached to surface

Fig. 1 Size distribution of non-treated (**a**), NaOH treated (**b**) and biofilm covered (**c**) PVA/PAA gels with starch based on measurements of 350–400 particles (disappearance of small sized fraction is due to separation steps in preparation)

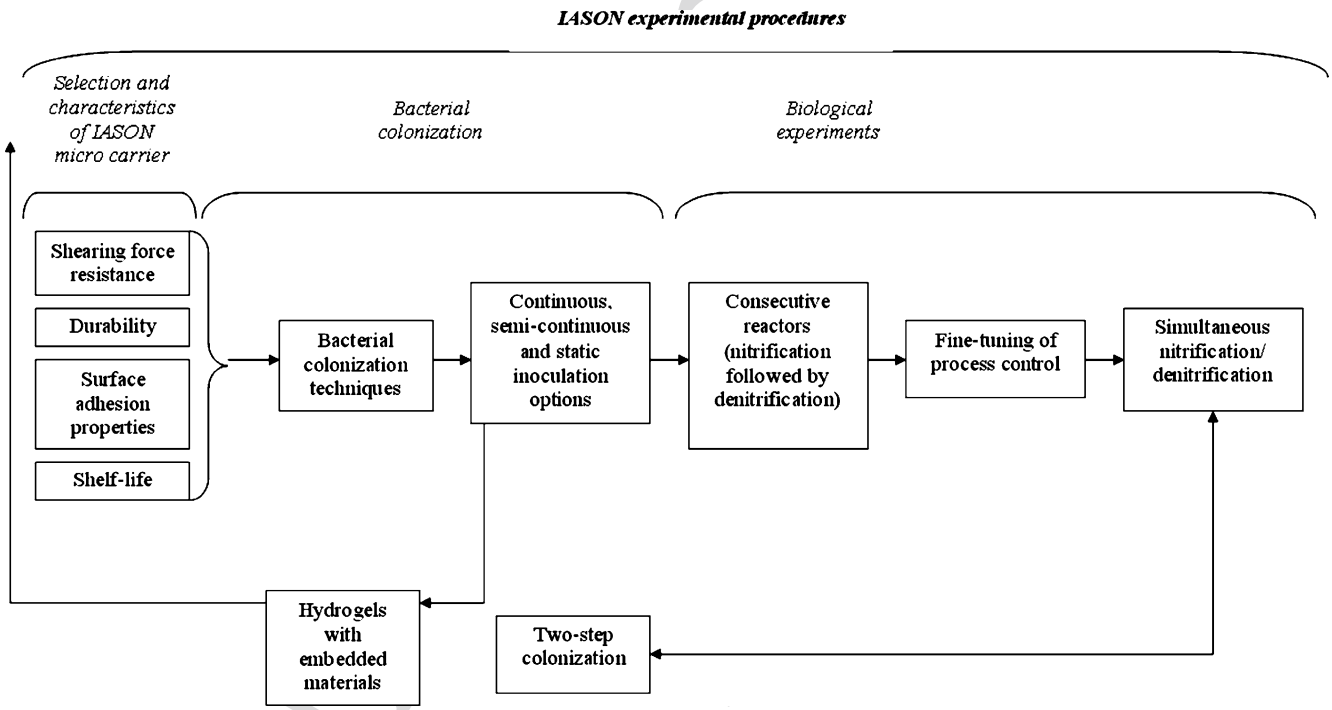


Fig. 2 Flow scheme of experimental procedures in selecting the technologically feasible hydrogel type(s)

starch suspension into the gelifying liquor hence embedding easily biodegradable substrate into the inner structure of the gels.

The starch contents of the gels was partially removed by simple method (heat and chemical treatment for 120 min, at 60 °C in 10 N NaOH solution) prior to bacterial colonization. Heat treatment and partial removal of starch from the polymer matrix did not alter the particle size distribution of the original gels beads (Fig. 1a,b). Presence of residual starch was checked by KI tests (potassium iodine solution staining and visual observation under microscope). KI test showed that starch was removed from the polymer matrix in the depth of 20–30 μm from the surface. Removal of near-to-surface embedded starch granules from the polymer matrix provided surface roughness and microholes for the wastewater bacteria for initial adherence. After the preliminary treatment with NaOH the process of bacterial colonization on the outer surface started. Further biodegradation of the inner starch particles within the gel beads provided additional adhesion surfaces to bacterial cells.

Previous experimental results focusing on the selection of adequate microcarrier for biofilm development [5] revealed that PVA–PAA gels concerning their durability, surface properties, resistance against mechanic strength were found to be the most favorable carrier material. On

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the basis of these preliminary experimental results, various types of breeding and inoculation techniques were conducted on laboratory scale to investigate bacterial colonization processes. Investigations included the rate of biofilm attachment/detachment, as a function of hydraulic properties (turbulence) and loading conditions (i.e., substrate and nutrient loads per volume of gel) for wastewater microbial consortia in batch tests.

Tests with colonized hydrogels were carried out in continuously fed sequential biological reactors: the system was designed for complete nitrogen removal by the implementation of nitrifying (aerated and mixed tank, see Fig. 3.) and a post-denitrifying (mixed only) reactor. Methanol was dosed as external carbon source into the non-aerated tank to fulfill electron donor requirements of denitrification. Adjustment of methanol addition and aeration served for fine control of nitrogen removal processes.

Laboratory Scale Experimental Systems

The outline of the laboratory scale series of biological reactors for full nitrogen removal is depicted on Fig. 3. The system consisted of two consecutive reactors, reactor "A" (hydraulic volume $4.5–6.3 \times 10^{-3}$ m^3) for nitrification, aerated and mixed and consequent reactor "H" (hydraulic volume $6.3–7 \times 10^{-3}$ m^3) for denitrifying bacteria. Methanol dosage was adjusted to the varying nitrate concentration (40–70 g/m^3). The dissolved oxygen concentration was measured at sampling points 2, 3 and 5, on Fig. 3.

Wastewater analyses (at the sampling points on Fig. 3) were focused on traditional wastewater parameters (COD (*chemical oxygen demand*), SS (*suspended solids*), N and P forms) and biomass concentration parameters. pH and DO measurements were conducted at daily intervals.

Wastewater Composition and Origin

During the experimental period (3 months) wastewater of the effluent of secondary clarifiers from the South Budapest Wastewater Treatment Plant was utilized as inflow water to the system. Adjustment of water quality was needed according to the experimental objectives, such as dosing surplus ammonium ion, etc. At sampling point 1 (Fig. 3) ammonium concentration was raised to be around 60 g/m^3 (by dosing NH_4Cl solution into the raw wastewater), and pH was set and maintained by dosing 0.5 kg/m^3 $NaHCO_3$ to provide the necessary buffering capacity to influent wastewater. Influent wastewater composition in experimental system is given in Table 1.

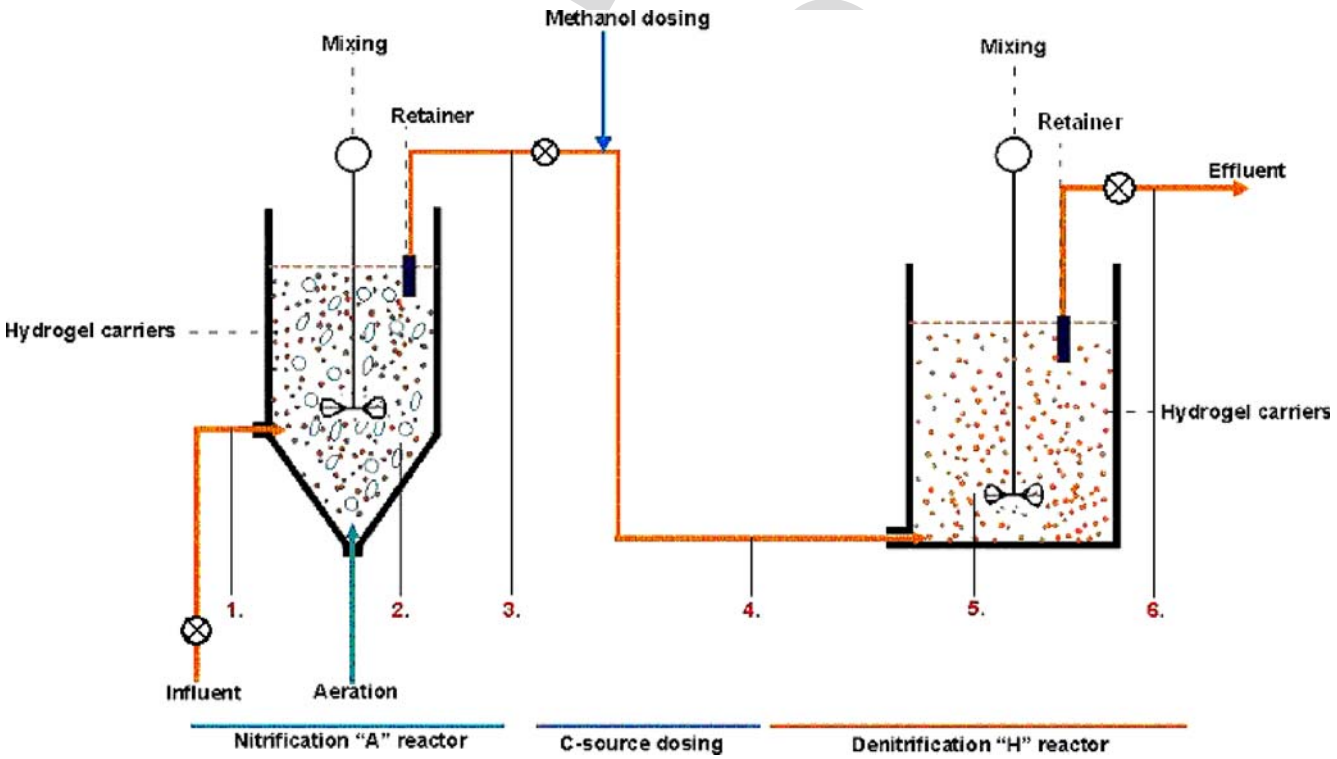

Fig. 3 Lay-out of laboratory scale experimental treatment system for nitrification and denitrification with C-source dosing. (Sampling points are numbered. Flow rates were regulated by PLP 66 peristaltic pumps)

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Table 1 Composition of the influent wastewater used in consecutive reactors

Parameter	Average	Minimum	Maximum
pH	7.89	7.56	8.06
o-PO_4-P g/m^3	1.6	0.16	3.2
NH_4-N g/m^3	57	54	71
NO_2-N g/m^3	3.66	1.27	7.8
NO_3-N g/m^3	4.26	1.2	7.3
Kjeldahl N g/m^3	58	57	60
org N g/m^3	0.75	0.5	1
Total N g/m^3	64	62	66
SS g/m^3	4.1	3.5	5
COD g/m^3	71	55	87

Results and Discussion

Bacterial Colonization and Biofilm Maturation of Autotrophic and Heterotrophic/Denitrifying Bacteria

Biofilm establishment on microcarrier surfaces was achieved by using the natural attachment processes of the suspended wastewater bacteria into hydrogel carriers. Inoculation of the initial 500 ml gel material per reactor (A and H) was conducted by using wastewater bacterial consortia from the same plant from where the influent was originated. Suspended biomass (activated sludge) was ultrasonicated for disaggregating the flocs that was followed by a filtration step on 50 μm glass fiber filter. Initial biomass concentration measured as SS (suspended solids as g/m^3) was in the range of 5–10 g/m^3. Biomass development was monitored by regular microscopic investigations, SS measurement, and dry material contents determination of gels and attached biomass.

Results indicated that biofilm development and establishment of solid biofilm layers on the surface of PVA–PAA gels requires about 10–12 days (for heterotrophic bacteria) and more than 21 days in case of nitrifyers (autotrophic bacteria). The stages of the autotrophic bacterial growth on the carrier material were started initially by protobiofilm patch formation (approx. 3 days) that is followed by the slow appearance (see Fig. 4.) of single and multiple cell Proto- and Metazoa organisms (14–21 days). Upon the completion of the formation of the good textured, solid biofilm surface technological and environmental stress experiments were started. During the process of biofilm formation continuous care was taken for the removal of the spontaneously formed activated sludge flocs (detached biofilm particles in stirred and aerated reactors). Figure 4a–d illustrate the different stages of biofilm development.

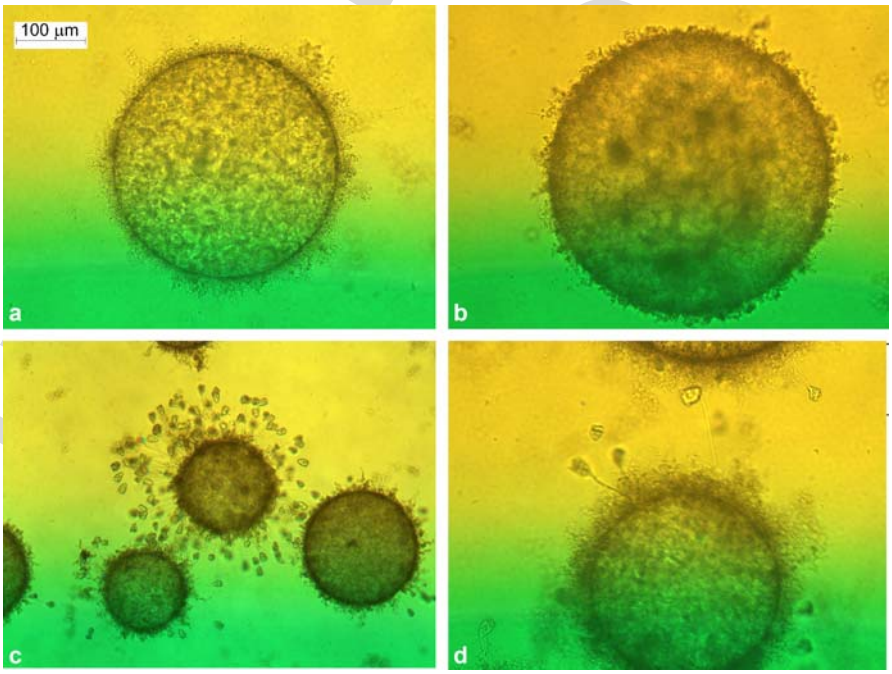

Fig. 4 Biofilm development on NaOH treated starch containing PVA–PAA hydrogel beads. **a** State of heterotrophic biofilm development on the 18th day. At this time the second stage of the inoculation was started. **b** Multiple layered biofilm on the 22nd day. 3 days after the inoculation with autotrophic (nitrifying) bacteria. **c** Fully developed multiple layer mature biofilm on the 25th day. *Epistylis* spp. and *Vorticella* spp. started to grow on the surface of the autotrophic biofilm indicating non-toxic environmental conditions and high sludge retention time. **d** Heterotrophic biomass from deeper biofilm layer starts to overgrow the upper autotrophic biofilm

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Simultaneous Nitrification and Denitrification

Simultaneous nitrification and denitrification was achieved by the establishment of multiple strata protobiofilm formed by wastewater bacterial culture. The biofilm was spontaneously attached to the surface of the PVA–PAA hydrogels beads embedded with starch. Suspended growth activated sludge flocs and in our experiments the biofilm on hydrogel surfaces as well have the average diameter of several hundreds μm (see also Fig. 1 regarding the changes of particle size distribution). On microscopic scale there are steep concentration gradients within such entities, at a given dissolved bulk oxygen concentration in the deeper layers of the biofilm anoxic or anaerobic conditions could develop. The upper layer of the protobiofilm was dominated by the autotrophic nitrifyer bacteria as this layer was well aerated, nearest to bulk solution. The deeper layers of the established biofilm were supposed to be composed predominantly by the heterotrophic bacteria (facultative anaerobic microorganisms) hence denitrification was expected to occur in this zone.

Inoculation Procedures

The multiple layered biofilm was established by using double step inoculation process. At starting suspension of heterotrophic bacterial cells was dosed to the reactor twice at 24 h intervals. Prior to inoculation the suspended biomass was treated by ultrasonication for disaggregating the flocs followed by a filtration step on 50 μm glass fiber filter. In the second step of inoculation autotrophic nitrifying bacteria were dosed into the reactor on the 18th day of the inoculation process. Pretreatment and inoculation technique of the bacterial suspension was the same as described above. Wastewater characteristic during the inoculation period is shown in Table 2.

Indirect Control of Biofilm Composition and Structure by the Wastewater Parameters

The texture of the double layers biofilm could be controlled indirectly by the concentration levels of various electron acceptors and donors in influent wastewater and aeration intensity. Environmental conditions in bulk solution (i.e., within the reactor volume) can be finely tuned by regulating aeration intensity (fine and coarse bubble aeration) and methanol dosing that effects indirectly the dissolved oxygen concentration via the oxygen utilization of microorganisms. Conditions favoring the growth of heterotrophic biomass having higher growth rate than autotrophic counterparts could be altered by organic carbon source (methanol) dosage as evidenced by microscopic observation during the experiments. These observations were supported by regular nitrification and denitrification activity measurements (see also Fig. 5).

Spontaneous Growth of Suspended Flocs in Bioreactor

The presence of spontaneously suspended flocs (truly "planktonic" biomass fraction) in the bioreactor might cause false results as well as structural alterations in biofilm used in these small-scale laboratory experiments. Spontaneously formed suspended planktonic flocs were removed constantly from the bioreactor by thorough washing and withdrawal of the not polymergel-bound biomass at every second day.

Flow Conditions in the Bioreactor and Biofilm Density and Thickness

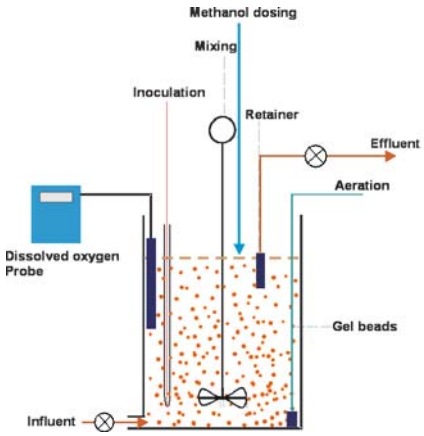
Environmental conditions such as temperature, exposure time, concentration of suspended bacteria (initial inoculum concentration) and most notably reactor hydraulics effect bacterial adhesion and the ultimate structure of formed biofilm. Shearing forces as dominant factors [8] effect the structure and hence technological performance of immobilized cell bioreactors [9]. As a general rule supported by many experimental evidence it is stated that higher shearing forces result in more compact but less tenuous biofilm structures [10].

Control of Dissolved Oxygen Concentration

The efficiency of denitrification in the lower layers of biofilm depends on the bulk level of dissolved oxygen

Table 2 Influent wastewater quality during the multiple step inoculation

Parameter	Unit	Heterotrophic biofilm			Double layered biofilm		
		Minimum	Maximum	Average	Minimum	Maximum	Average
NH_4-N	g/m^3	13.7	24	21	23.8	52.2	30
NO_2-N	g/m^3	0.1	3.9	1.8	0.05	0.7	0.14
NO_3-N	g/m^3	21	63	44	17	52	28.5
pH		7.8	8.2	8.1	7.86	8.26	8.04
Conductivity	μS/cm	1765	1862	1800	2110	2120	2110
Temperature	°C	20	21	20.5	20	21	20.5



Hydraulic reactor volume: 6.3-7 * 10^{-3} m^3
Volume of the PVA-PAA gels: 0.6 * 10^{-3} m^3
Hydraulic loading: 0.5-0.7 * 10^{-3} m^3/hour
Methanol dosing: 0.3 g/m^3 of influent wastewater (corresponding to 415 g/m^3 COD value)

Dissolved oxygen level was measured at every 15 minutes (see also Fig 6a and 6b).
The required DO levels were set by the intensity of aeration.

Fig. 5 Laboratory scale experimental system for simultaneous nitrification and denitrification with additional C-source dosing

and the diffusion through the upper layers of fully grown biofilm. Structural changes of biofilm were monitored microscopically at daily intervals, while technological performance of the biomass was continuously checked. Should nitrification efficiency drop throughout the experimental period dissolved oxygen concentration in the bioreactor was increased to favor autotrophic growth (i.e., stepwise increase of bulk oxygen concentration).

Dosage of the Methanol (Organic Carbon Source for Denitrification)

Growth rate of heterotrophic denitrifying bacteria had to be controlled to maintain the depth and texture of the deeper layers of biofilm. During development period of the biofilm the growth rate of heterotrophic bacteria was limited by the concentration of organic carbon source (methanol). The background COD concentration of methanol in influent wastewater was kept constant throughout the experimental period. The continuously aerated and methanol dosed system containing the biofilm covered PVA–PAS hydrogel beads is shown on Fig. 5. The ~~HRT (~~hydraulic retention time~~)~~ was set to 9.5–10.5 h close to the normal operating range of full-scale wastewater treatment systems.

Technological Results

Influent and effluent wastewater quality of the laboratory system was measured daily to obtain information on the technological capabilities of the system and its temporal changes. Figure 6 depicts the daily removal efficiencies for N compounds upon the development of multiple layered biofilm. In this biofilm the upper layer consisted of mostly autotrophic bacteria able to oxidize ammonium ion in the presence of oxygen (nitrification) and the lower layer consisting of predominantly heterotrophic bacteria able to reduce nitrate to nitrogen gas in the absence of oxygen

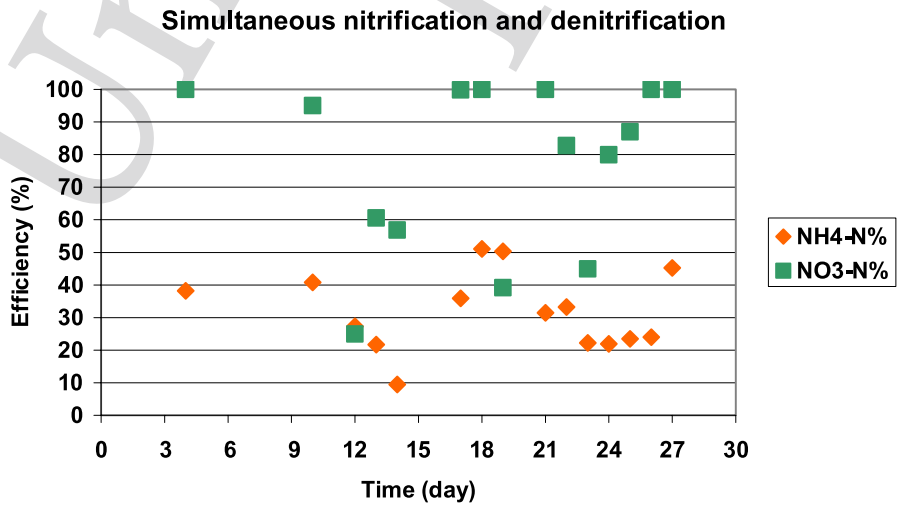


Fig. 6 Removal efficiencies of NH_4-N and NO_3-N in the simultaneous nitrification/denitrification system

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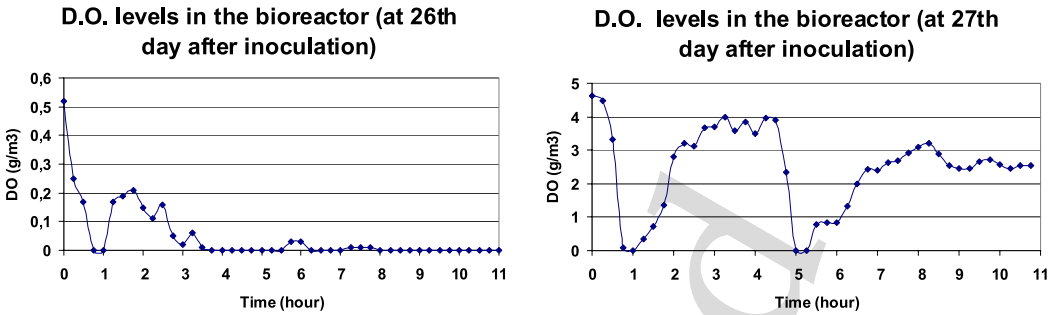

Fig. 7 Dissolved oxygen concentration profiles in the bulk solution during simultaneous nitrification/denitrification experiments

by utilizing methanol as electron donor for the reduction process (denitrification). Figure 6. shows the removal efficiency fluctuations in terms of ammonium ion and nitrate that are largely due to altered oxygen concentrations, inoculations and formation of spontaneous flocs. In the volume of the reactor the mixing was always complete as evidenced by DO levels checked at various points. This might indicate along with the measured ammonium and nitrate removal rates (Fig. 6) that spatial heterogeneity of DO was established within the microscopic layers of the biofilm enabling to form anoxic zones for denitrifier bacteria.

As the oxygen requirement of these microbiologically mediated processes is contradicting it is of interest to monitor closely the bulk DO level across the experiments as depicted on Figs. 7a and 7b. Considering the low dissolved oxygen concentrations on Fig. 7a nitrification would not be expected to occur under these conditions. The corresponding results on Fig. 6 however clearly indicate the opposite (days 26 and 27). Nitrification activity of autotrophic bacteria was observed at DO level below 0.2 g/m^3 on the 26th day (23%), while it was 45% on the 27th day. Removal efficiency of denitrification was close to 100% irrespective to bulk DO levels (in the range of 0.2 g/m^3 and higher than 2 g/m^3). It is noted that efficiency of nitrification increased slightly by elevated DO concentration levels.

Conclusions

In biological wastewater treatment technologies most of the full-scale systems are based on activated sludge technology. In these systems the population dynamics of the activated sludge microbial community cannot be regulated directly. The architecture of activated sludge flocs (bacterial aggregates) that are the key elements of the wastewater treatment process remains uncontrolled. The IASON project aimed to overcome these problems by the introduction of microscopic carrier structures to which the activated sludge bacteria adhere and meet technological objectives. In this paper the results of the preliminary laboratory experiments of simultaneous nitrification and denitrification are presented.

On the basis of the preliminary durability checks we selected the PVA–PAS hydrogel material with embedded starch particles for further testing and to address wastewater technological problems. We developed colonization techniques for the establishment of multiple species biofilm structures on hydrogel surfaces having different biochemical potential (auto- and heterotrophic populations). Application of two steps inoculation techniques (in consecutive manner) made possible the coexistence of ammonia oxidizer (nitrification) and nitrate reducer (denitrification) bacteria in the same bioreactor volume. The layered biofilm structures were able to proceed with the simultaneous removal of nitrogen forms (NH_4^+, NO_2^- and NO_3^-) in a single, aerated biological reactor.

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