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Removal of Carbon and Nitrogen Compounds in Hybrid Bioreactors

Małgorzata Makowska, Marcin Spychała and Robert Mazur

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1. Introduction

Biological wastewater treatment methods allow to remove pollutants at high efficiency but they require application of modern knowledge and technology. In bioreactors used for carbon and nutrients removal from wastewater two forms of biomass are utilized: a suspended biomass (dispersed flocs) and an attached biomass (biofilm). The latter needs a carrier on surface which it can grow.

Both types of biomass, despite some similarities, show also many differences. Probably as a result of complex relations (competition, migration, physical factors like flow velocity and biochemical factors like oxygen supply) the flocs and attached biomass can demonstrate many differences, e.g. texture, active surface, heterotrophs and autotrophs ratio, and especially biomass age. A compilation of these two technologies in one hybrid reactor allows to utilize advantages of these technologies and to achieve high carbon and nitrogen removal efficiency. The additional advantages of this new technology (moving bed biological reactor – MBBR; other similar terms: Integrated Fixed Film/Activated Sludge - IFAS, Mixed-Culture Biofilm - MCB) are cost savings and reactor volume reduction. Simultaneous processes maintenance (SND reactor) and specific parameters preservation enable treatment of specific wastewater.

2. Bioreactors' characteristics

2.1. Suspended biomass reactors

Reactors with suspended biomass (activated sludge), commonly used in wastewater treatment, utilize a biocenose of various heterotrophs and autotrophs which are able in certain conditions to remove efficiently pollutants from wastewater. They use dissolved and suspended matter (after hydrolyzing) for biosynthesis and assimilation. One of the basic activated sludge process

parameter is the biomass age. This parameter indicates the time of biomass retention in the system and is calculated from biomass balance. The biomass age (sludge retention time, SRT) has an impact on the substrates removal and can be maintained using recirculation, independently on the hydraulic retention time (HRT). On the other hand the pollutions' loads on biomass have a direct impact on the nitrogen and phosphorus removal.

The basic kinetic equation of substrate removal, used in many mathematical models of biological wastewater treatment, is that of Monod type: it describes the substrate utilization rate as a function of specific rate of microorganisms growth [1]:

$$\frac{dS}{dt} = \frac{\mu_{max}}{Y_{max}} \cdot \frac{S}{S + K_S} \cdot X \tag{1}$$

where:
μ_{max} – maximum growth rate, 1/d,
Y_{max} – substrate utilization yield, g_{sm}/g_{sub},
K_S – saturation coefficient, g/m^3,
S – substrate concentration, g/m^3.

In the activated sludge technology three types of reactors are used: continuous stirred tank reactor (fully mixed flow reactor), plug flow reactor and sequencing batch reactor (SBR). The other differentiating factor is aeration of fluid in the reactor, so there are in general three types of reactors: aerated, non-aerated, and intermittently aerated.

Activated sludge flocs have an irregular structure. The disperse rate is related to accessibility of substrate and oxygen for the inert layers of flocs. Li and Bishop [2] prepared microprofiles of the oxygen and substrates concentration in the floc using clark-type microelectrode (figure 1). Redox potential (ORP) changes and oxygen concentrations indicate conditions inside the floc and concentrations of different nitrogen forms describe nitrification process performance (mainly in the top layers of floc) and denitrification (inert layers of floc). The diameter of flocs was in range from 1.0 to 1.4 mm, and oxygen uptake rate was equal to approximately 1.25 mg O_2/dm^3 min.
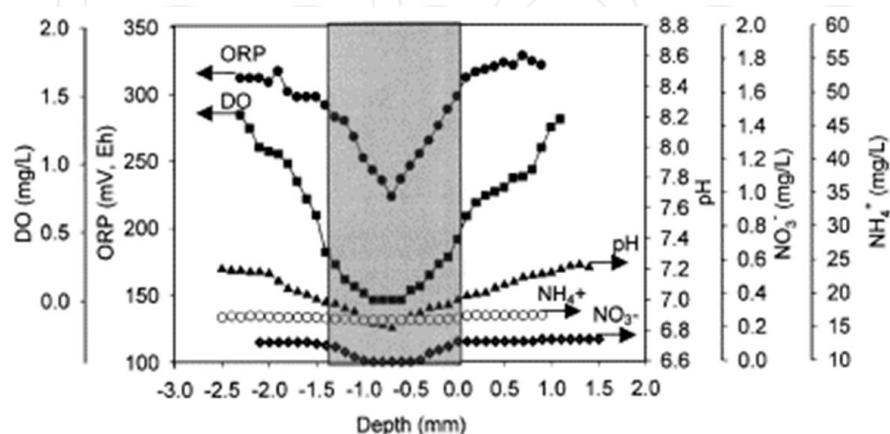
Figure 1. Microprofiles of dissolved oxygen redox potential, pH, nitrates and ammonium concentrations in a floc of activated sludge [2]

2.2. Bioreactors with attached biomass

Attached biomass reactors operate as moving beds, packed (fixed) beds and membranes. The attached biomass (biofilm) has a thickness up to 1.5 mm. The substratum can be fixed (trickling filters and submerged beds) or moving (moving bed biofilm reactors). A main factor affecting access of biomass to the substrate is the effective surface area. The biofilm volume concentration can be even 10 times higher than concentration of activated sludge floc biomass and commonly is in range of 10 to 60 kg/m³. The biomass age is much longer in biofilm than in flocs and ranges from several to even more than 100 days. The other important factors are: organic substrate loading on substratum surface area and (what is often correlated) the organic substrate loading of the biomass.

The substrate utilization rate in biofilm can be expressed by equation:

$$\frac{dS}{dt} = \frac{\mu_{max}}{Y_{max}} \cdot \frac{S}{K_S + S} \cdot d \cdot X_b \cdot A_b \tag{2}$$

where: d – biofilm thickness, m,
X_b – biofilm density, g/m³,
A_b – biofilm specific surface area, m²/m³.

The substrate penetration into the biofilm depth is affected by SUR (substrate utilization rate) and diffusion coefficient. The relative depth of substrate penetration into the biofilm can be expressed by penetration coefficient (β) assuming that the rate of reaction is zero-order [1]:

$$\beta = \sqrt{\frac{2 \cdot D \cdot S}{k_0 \cdot z^2}} \tag{3}$$

where: D – substrate diffusion coefficient, m²/s,
k_0 – zero-order reaction constant for biofilm, kg/m³s,
z – biofilm thickness, m.

When β > 1, the substrate penetrates trough the whole depth of biofilm, when β < 1 – substrate penetrates the biofilm only up to the certain depth. When two substrates are considered e.g. oxygen and organic compounds, one of them can be limiting. If the condition: $\beta_{O2} < \beta_{BZT5} < 1$ is satisfied, the conditions in the biofilm will be anaerobic. Conditions and processes in biofilm can be indicated by microprofiles [3]. A dramatic peak of redox potential (figure 2) indicates a change in oxygen conditions – from aerobic near the biofilm surface – to anaerobic – near the substratum. Nitrogen compounds changes indicate the nitrification process caused by oxygen penetration into the subsurface layers of biofilm depth.

2.3. Hybrid bioreactors

A practically useful solution is compilation of the described above two technologies in one reactor named a hybrid reactor. Both activated sludge and biofilm technologies advantages are utilized in this system.
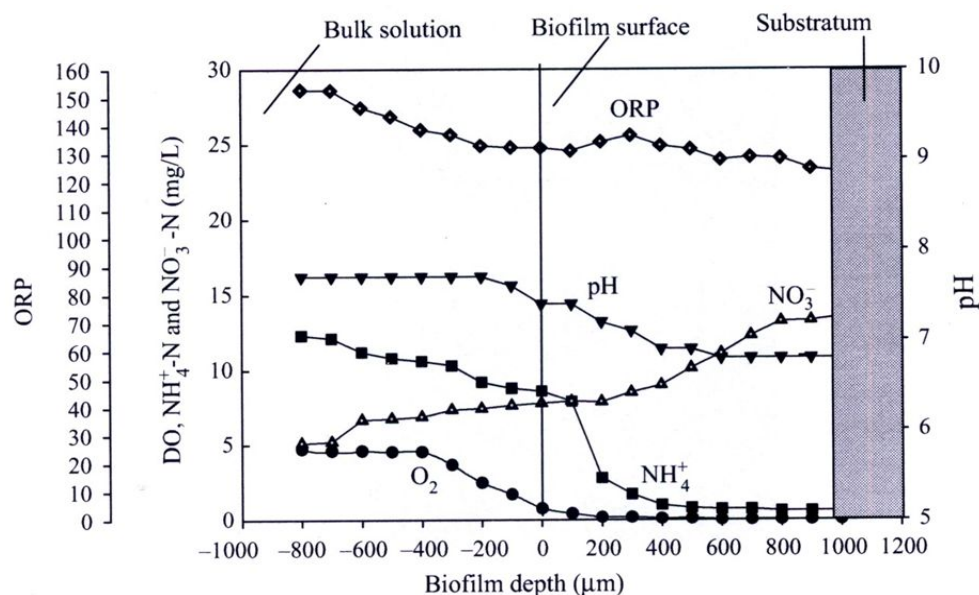
Figure 2. Microprofiles of biofilm [3]

In this type of reactors (Integrated Fixed Film/Activated Sludge - IFAS, Mixed-Culture Biofilm – MCB, hybrid bioreactors) a secondary settler is used and suspended biomass is returned to the bioreactor, so certain suspended biomass concentration can be maintained. However, when suspended biomass flocs are relatively large (up to 1500 μm of diameter) they can clog the small pores of carriers [4] resulting in attached biomass growth interruptions and oxygen access limitations.

Additional modifications can reduce energy consumption. The biofilm substratum may consist of various plastic carriers with effective surface area up to several hundred square meters per cubic meter. The volumetric density of carriers with biomass in fluidized beds should be similar to the wastewater density or slightly higher. There are many market-available types of carriers.

Hybrid reactors with moving carriers were firstly developed in Norway in nineties of XXth century. Characteristics of this technology were given firstly by Odegaard et al. [6]. They proposed the carriers filling rate of 70% of volume and obtained the removal efficiency of 91-94% for organic compounds and of 73-85% for nitrogen compounds. The impact of the substrate loading of reactor on the treatment performance was studied by Orantes and Gonzales-Martinez [7] and Andreottola et al. [8]. These researchers applied this technology to the specific conditions – for resorts in Alps. Andreottola et al. [9] and Daude and Stephenson [10] designed such reactor as a small WWTP for 85 p.e.

The hybrid reactor can be designed basing on organic loading of biomass and knowing the geometry of carriers. The number of carriers (N) can be calculated as [11]:

$$N = \frac{L_S}{A_{k1} \cdot A_b^* \cdot G_b} \tag{4}$$

where: L_s – removed organic load, kg/d,

A_{k1} - one carrier effective surface area , m²,

A_b^* - organic loading of biomass, g/g$_{dm}$d,

G_b – biofilm surface density, g$_{dm}$/m².

The simultaneous application of activated sludge and moving bed technologies has a positive influence on the nitrification process. Paul et al. [12] found that 90% of autotrophs in hybrid bioreactor is a component of biofilm (autotrophs are 40% of total number of microorganisms). Despite relatively low kinetic constants of autotrophs growth and substrate utilisation rate comparing to the heterotrophs (Y_H = 0.61 g$_{dm}$/g$_{COD}$, Y_A = 0.24 g$_{dm}$/g$_{COD}$, μ_{Hmax} = 4.55 d^{-1}, μ_{Amax} = 0.31 d^{-1}), the high (over 90%) nitrification efficiency in hybrid reactor can be achieved, even in terms of high hydraulic loading rates.

The nitrifying bacteria in the biofilm on the carriers are able to reach the nitrification rate up to 0.8 gN/m²d at 10°C [13] and even up to 1.0 gN/m²d at 15°C [14].

2.4. Spatial and ecological forms of biomass

There is a little research related to the interactions between activated sludge flocs and biofilm e.g. migration of the organisms. These interactions are complex, and both relations: between flocs and biofilm, and between heterotrophs and autotrophs in the biofilm should be considered in modeling [15] and operation. Albizuri et al. [15] assumed that these interactions could act with mediation of colloidal components. It is well know fact that there are many grazing species (e.g. *Ciliata*) which creep on the flocs/biofilm surface or swim near the flocs and biofilm surface. On the other hand there is some number of species existing in deeper layers of biofilm, which probably can not migrate. It is worth to note that the structure of activated sludge flocs is heterogeneous and deep layers of biomass in flocs are anaerobic, what results in different species composition (anaerobic bacteria).

The biological composition of flocs in hybrid bioreactors is similar to typical biological content of activated sludge flocs. Similarly the size of hybrid bioreactor flocs in hybrid reactors is close to typical activated sludge flocs - diameter in the range of 150-500 µm [16].

In hybrid bioreactors various nitrogen removal processes pathways are possible, including autotrophic processes, e.g. anammox [17]. A sufficiently thick layer of biofilm or flocs is needed for complex nitrogen process transformations including denitrification. On the other hand a relatively thin biofilm (due to shearing stress) results in high activity of biomass [18]. Some authors [19,18] found that in sequencing batch biofilm reactors (SBBR) the biofilm is fully penetrated by substrates and electrons acceptors can be released.

Similarly as in case of other attached biomass systems, e.g. trickling filters in MBBR design procedure, surface area loading rate should be the design parameter [5,11]. This approach is based on some typical range of biofilm thickness (in this case surface area can be the indicator of the biomass concentration). From this point of view the size and shape of carriers seem to be less important. The substrate to biomass loading rate is base but not sole design criterion. Important but poorly recognised factors are: access to total biofilm surface area and access to

aerobic biofilm surface area. Some authors indicated that carriers of high total surface area thanks to micropores should have some amount of macropores, enabling fluid reach in oxygen contact with deeper inner spaces of carriers, e.g foamed cellulose carriers [20]. The macropores are important for nitrifying biomass, which needs contact with dissolved oxygen. Micropores are often filled completely with biofilm preventing the oxygen penetration. Oxygen access factor is crucial for biofilm thickness, porosity and surface roughness.

The ratio of the suspended to the attached biomass can vary accordingly to many factors and conditions. The amount of attached biomass can reach over 90%. Plattes et al. [21] indicated 93% of biomass in form of biofilm attached to the carrier elements and only 7% of biomass – as suspended in the bulk liquid. Detachment (or sloughing) of biomass is variable in time [5]; probably this phenomenon is similar to sloughing of excess biomass from biofilm growing in others attached biomass systems, e.g. trickling filters. Some authors [22] suggested that in such systems detachment process occurs periodically.

Due to mechanical contact with others carriers and shear stress, the biomass grows mainly on the internal area of carriers, what was reported by several authors [16, 23], excepting carriers having outgrowths on the outside walls surface.

The common forms in typical activated sludge system are aggregated flocs and planktonic free-swimming cells, and bacterial communities are dominated by: *Betaproteobacteria, Alphaproteobacteria, Gammaproteobacteia* and more less frequent: *Bacteroidetes and Firmicutes* [24]. Some authors [24] observed in biofilms in MBBR limited bacterial diversity and *Firmicutes* domination. The research of Biswas and Turner [24] indicated that MBBR communities differ from communities existing in conventional activated sludge reactors. The characteristic feature of MBBR bacteria community was a presence of two distinct communities: suspended biomass with fast-growing aerobic bacteria and biofilm biomass, which was dominated by anaerobic bacteria [24]. In biofilms of WWTP which were studied by these authors the prevailing forms were *Clostridia* (38% of clones) and sulfate-reducing bacteria (*Deltaproteobacteria* members). The another forms were less abundant: *Desulfobacterales* (11-19%), *Syntrophobacterales* (8-10%), *Desulfovibrionales* (0.5-1.5%). The other groups were also observed: *Bacteroidetes, Synergistes, Planctomycetes, Verrucomicrobia* and *Acidobacteria*.

The suspended biomass observed in two MBBR reactors by Biswas and Turner [24] was consisted mainly of aerobic microorganisms: *Alphaproteobacteria* (*Rhizobiales, Rhodobacterales*), Gammaproteobacteria (*Pseudomonadales, Aeromonadales*), *Betaproteobacteria* (*Burkholderiales, Rhodocyclales*). Majority of *Firmicutes* was represented by *Clostridia* and one MBBR reactor suspended biomass was reach in *Campylobacteraceae* (54% of clones).

The differences in microbial composition can appear not only between biofilm and activated sludge in MBBR reactor but also between MBBR bioreactors themselves. Biswas and Turner [24] observed the biomass, both black with sulfurous odour in one MBBR reactor and grayish-brown without obvious odour - in other MBBR reactor. Some authors indicated that in continuous-flow MBBR in which SND process was established, the microbial community structures of biofilm are related to C/N ratios [25]. In MBBRs the volume concentration ratio

of biofilm to the activated sludge flocs cab be even higher: 5-13 [26] than for separated attached biomass and suspended biomass systems. Some important differences between biofilm and flocs features in MBBRs were found by Xiao end Garnczarczyk [26]. They observed 3 - 5 times higher geometric porosity in biofilm than in activated sludge flocs. Biofilm boundary fractal dimension was higher than activated flocs one. These authors observed also some similarities: two different space populations both in biofilm and in flocs were indicated and both attached and suspended biomass shifted some of their structural properties to larger values (thickness, density) with the increased hydraulic loading.

2.5. Carriers material characteristics and impact on attachment conditions and biomass structure

For the MBBR pollutants removal efficiency and biomass concentration the crucial role plays the material of which carriers were made (table 1). The basic features are as follow: material type, specific surface area, shape and size of carriers and other features: porous surface, e.g. polyurethane or not porous material surface e.g. polyethylene [27,28].

Material	Density, g/cm³	Specific surface area, m²/m³	Type of carrier	References
Cellulose (foamed)	-	-	continuous macro-porous, Aquacel, 1-5 mm	[20]
Polyvinylformal (PVF)	-	-	cubic, 3 mm	[20]
PVA-gel beads	-	-	-	[23]
Polyethylene (PE)	-	-	K1, EvU-Perl	[55]
Nonwoven fabric	-	900	-	[56]
Reticulated polyester urethane sponge (foam)	0.028 (volumetric density)	-	S45R, S60R, S90R, Joyce Foam Products	[57]
Polypropylene (PP)	1.001	230-1400	cylindrical rings 4 mm	[58]
Polyethylene (PE)	0.95	230-1400	cylinders 7 mm/10 mm	[59]
Polyethylene (PE)	-	-	Kaldnes, Natrix, Biofilm-Chip	[60]
Polyurethane coated with activated carbon	-	35,000	cubes (1.3 cm), Samsung Engineering Co	[61]

Table 1. Selected types of carriers and material of which carriers were made

2.6. Carbon and nitrogen compounds removal

The basic role in biochemical transformations have three types of processes:

(*i*) hydrolysis (slow decomposition of polymeric substances to easy biodegradable substances, (*ii*) substrates assimilation by microorganisms correspondingly with Monod equation and (*iii*) growth and decay of microorganisms, what can be written in form:

$$\frac{dX}{dt} = \mu_{max} \cdot f(S) \cdot X - K_d \cdot X \qquad (5)$$

where: *f(S)* – substrate concentration related function,
X – biomass concentration, g_{dm}/m^3,
K_d – biomass decay constant, 1/d.

Organic substrates in the wastewater are in the form of: suspended solids, colloids and soluble matter. In overall form it can be described as $C_{18}H_{19}O_9N$. Due to their different forms (easy degradable, slowly degradable and not biodegradable fractions) they can be oxidized, assimilated or not biologically decomposed.

The organic substrate fractions, relating to the form and decomposition pathways can be identified correspondingly to commonly used methodology [29-31]. Typical wastewater consists 10-27% of soluble easy biodegradable substances (S_S), 1-10% of not biodegradable soluble substances (S_I), 37-60% of slowly biodegradable suspended solids (X_S) and 5-15% of very slowly biodegradable suspended solids (X_I) [31-34].

Easy biodegradable organic substances are an energy sources for denitrifying and phosphorus accumulating bacteria (PAB) and its concentrations have direct impact on the nitrogen and phosphorus removal.

Nitrogen in wastewater appear usually in form of soluble non-organic forms (mainly ammonium nitrogen, seldom nitrites and nitrates), organic soluble (degradable and not degradable) and as the suspended solids (slowly degradable, not degradable and as a biomass). Nitrogen compounds transformations are carried by autotrophic and heterotrophic bacteria and elementary processes need to preserve adequate technological conditions for these microorganisms.

The polymeric substances hydrolysis is catalysed by extracellular proteolytic enzymes to transform into simple monomers, which can be assimilated by microorganisms.

This process and ammonia nitrogen assimilation is related to fraction of nitrogen in biomass (5-12%). The nitrogen assimilation rate depends on the C/N ratio.

Another process of nitrogen transformation is nitrification – oxidation of ammonium nitrogen to nitrites and nitrates by chemolithotrophs: *Nitrosomonas, Nitrosococcus* and *Nitrosospira* in first phase (hydroxylamine is the intermediate product) and *Nitrobacter, Nitrospira, Nitrococcus* in the second phase, what can be described in form [35]:

$$NH_4^+ + 1,5O_2 \rightarrow NO_2^- + H_2O + 2H^+ - 278\,kJ\,/\,mol$$

$$NO_2^- + 0,5O_2 \rightarrow NO_3^- - 73\,kJ\,/\,mol$$
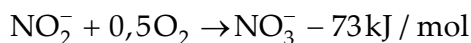
Nitrifying bacteria, as autotrophs, take the energy from carbon dioxide and carbonates. The utilisation rate for *Nitrosomonas* is equal to 0.10 g_{dmo}/g_{N-NH4}, and for *Nitrobacter* – 0.06 g_{dmo}/g_{N-NO2} [1]. The important process parameters are: oxygen supply (4.6 g $O_2/1_gN-NH_4$), temperature (5 – 30⁰C), sludge/biomass age (more than 6 days is recommended), biomass organic compounds loading (over 0.2 g BZT_5/g_{dm} is recommended) and BOD/N ratio: when the value is more than five – organic compounds removal dominates, when is lower than three – the nitrification is a prevailing process. The decrease in alcanity is the result of nitrification (theoretically: 7.14 g $CaCO_3/1$ g $N-NH_4$) and it causes decrease in pH from 7.5 – 8.5 to 6.5.

Nitrates are transformed in the dissimilation reduction process (*Pseudomonas*, *Achromobacter*, *Bacillus* and others) to the nitrogen oxides and gaseous nitrogen correspondingly to the path:

$$2\,NO_3^- \xrightarrow{-4e} 2\,NO_2^- \xrightarrow{-2e+2H^+} 2\,NO \xrightarrow{-2e} N_2O \xrightarrow{-2e} N_2$$
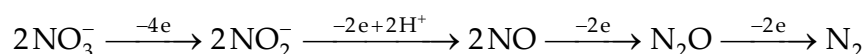
In the terms of dissolved oxygen deficiency (anaerobic or anoxic conditions) those organisms use nitrates as H^+ protons acceptors.

Denitrifying bacteria, as the heterotrophs, need organic carbon for their existence. The source of organic carbon can be: organic compounds in wastewater (internal source), easy assimilated external source of organic carbon, e.g. methanol/ethanol, or intracellular compounds as an energetic source. This ratio of organic carbon should be in range of 5 – 10 g COD/g $N-NO_3$ [35].
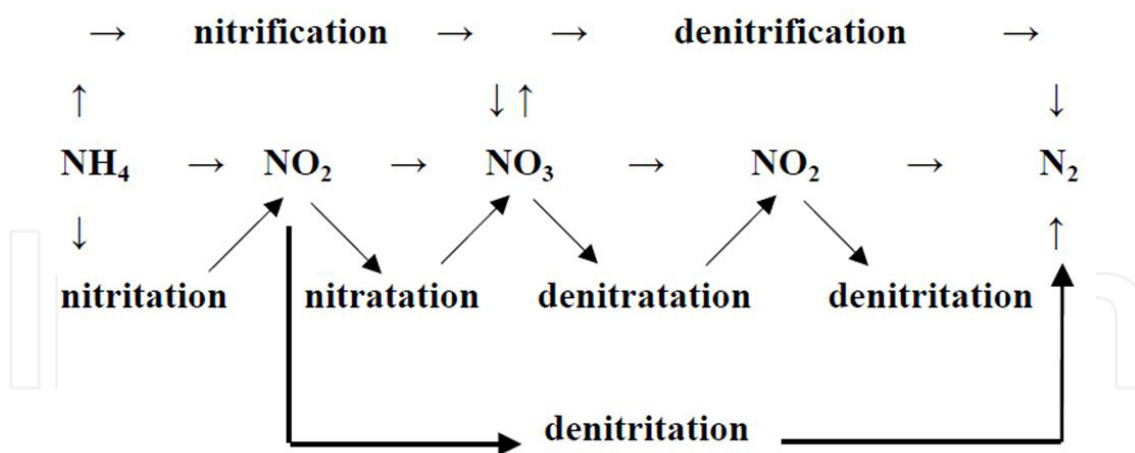

Figure 3. Elementary processes of nitrification and denitrification in nitrogen treatment

In the figure 3 the elementary processes of nitrification and denitrification in nitrogen removal are shown [11]. It is easy to recognise that denitrification is partly the reverse process to the nitrification. Due to the fact that some nitrifying bacteria can live without oxygen and some denitrifying bacteria can survive in oxygen conditions there is the possibility to carry out the simultaneous nitrification and denitrification (SND) in one reactor. As the SND reactor both continuous and sequencing batch reactors can be used. In

the SBR-SND reactor high removal efficiency for organic and nitrogen compounds can be achieved – 79% and 96% respectively [36]. The biomass growth rate can be in range of 0.3 – 0.75 g$_{SDM}$/g$_{sub\ rem}$ [37]. Similarly high nitrogen removal efficiency in SND process in continuous reactor (about 90%) can be achieved at certain pH and N-NH$_4$ concentration [38]. The nitrite concentration rise indicates that the nitrification process has stopped after the first phase. The process can run at low C/N ratio.

The short version of SND process needs preservation of certain technological conditions. Important conditions are aerobic and anoxic conditions, what in one reactor system can be achieved by intermittent aeration and non aeration. It causes the characteristic variability of parameters such: pH, redox potential or nitrogen compounds concentration [39]. Examples of changes of some variables in bioreactor with intermittent aeration are presented in figure 4 [40].
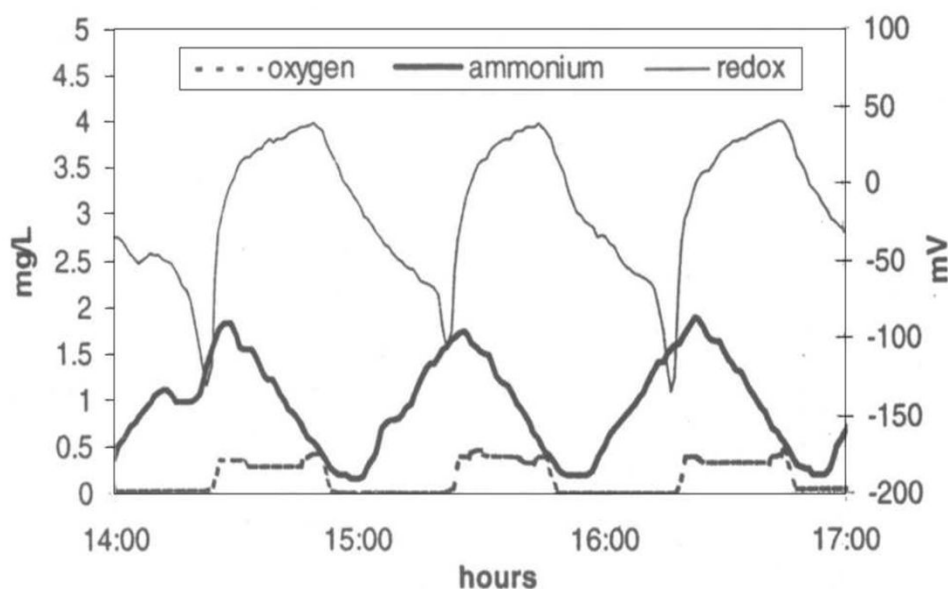
Figure 4. Change of sewage parameters for oxic and aerobic phase of biological reactor [40]

The blockage or limitation of second phase of nitrification can be achieved by limitation of oxygen availability up to approximately 0.7 mg O$_2$/dm^3 [41] or by free ammonia inhibition, which concentration is impacted by the temperature and pH of wastewater. Anthonisen et al. [42] identified the limiting values of partial and full inhibition of second phase of nitrification: 0.1 g N-NH$_4$/m^3 and 1.0 g N-NH$_4$/m^3 respectively. They proposed the description of free ammonia concentration in the reactor in form [42]:

$$S_{NH_3} = \frac{17}{14} \cdot \frac{S_{N-NH_4} \cdot 10^{pH}}{\exp(6344/T) + 10^{pH}} \tag{6}$$

where: S_{N-NH4} – ammonium nitrogen concentration, mg/dm^3,
         T – temperature, K.

The free ammonia concentration has an impact on the ammonium nitrogen removal velocity, what is described by substrate inhibition model presented by Haldane [43]:

$$r_{NH} = r_{NH\,max} \cdot \frac{S_{NH_3}}{K_{sNH3} + S_{NH_3} + S^2_{NH_3} / K_{iNH3}} \qquad (7)$$

where: r_{NH} – ammonia nitrogen and ammonium removal velocity, mgN/mg$_{sm}$h,

r_{NHmax} – ammonia nitrogen and ammonium removal maximum velocity, mgN/mg$_{sm}$,

K_{sNH3} – saturation constant, mgN-NH$_3$/dm^3,

K_{iNH3} –inhibition constant, mgN-NH$_3$/dm^3.

For the mathematical description of organic and nitrogen compounds removal many existing models are used with certain modifications, e.g.: substrate inhibition in Brigs–Haldane model [44] or ASM1 model [45], intermittent aeration or oxygen limitation inhibition in ASM1 [46-48], or two stages process of nitrification and denitrification in ASM3 model [49].

3. Laboratory research

The aim of this study was to determine elementary processes related to the organic and nitrogen compounds removal in hybrid reactors with intermittent aeration, to assess removal efficiency under various organic and hydraulic loadings and organic and nitrogen compounds' utilization rates. The utilitarian aim was to determine technological conditions which could make the process shorter and more economically efficient. The attempts to modeling using various technical parameters (together or separately) were conducted.

3.1. Laboratory model and methods

Carriers used in the research were corrugated cylindrical rings made of PP diameter and length of 13 mm, 0.98 g/m^3 density and 0.86 porosity (figure 5).

The research studies were conducted in four stages of 10 months duration. Each stage was consisted of three or four series. In each stage three reactors worked simultaneously as continuous flow system in stage I and III and as a sequencing batch reactor in stage II. The volume of reactors was equal to 75 dm^3 and volume of settler for the continuous flow was equal to 20 dm^3.

The studies were focused on an intermittent aeration. The most attention was put on the last stage – with increased wastewater pH value using lime (Ca(OH)$_2$). The aim of higher pH maintaining was to inhibit the second phase of nitrification by ammonia. The wastewater originated from one family household. The retention time before the sewage discharging into the reactors was relatively high – about 6 days (septic tank and retention tank). The activated sludge originated from Poznań Central WWTP and was inoculated to each reactor at the same amount in each stage beginning. Mixing and aeration of the reactors was made using large-bubble diffusers. The air was supplied by compressor of 0.1-2.0 m^3/h capacity. The sludge recirculation was made using an air-lift cooperating with a membrane pump. Characteristic research parameters are shown in table 2.
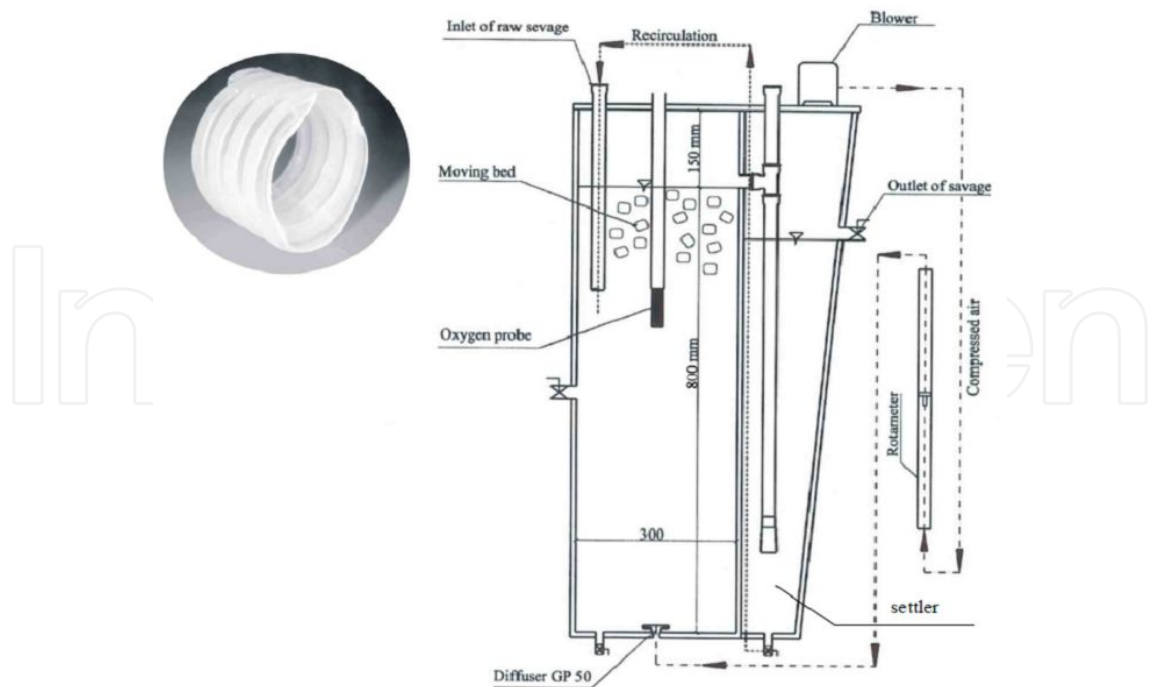
Figure 5. Scheme of laboratory model

	Continuous flow reactor (CFR)	Batch reactor (SBR)	Continuous flow reactor (CFR) with increased pH
Sewage volume per day, dm³/d	70 - 290	45 - 270	140
Number of series	4	3	3
Variable factor for series	time of aeration	length of reactor cycle	number of carriers
Variable factor for reactors	hydraulic load pollution load	active volume of reactor	pH value
C/N	1.31 ± 0.09	1.67 ± 0.10	1.39 ± 0.07
Total solids, g/m³	34.95 ± 6.65	62.30 ± 3.30	37.00 ± 5.20
Organic compounds as COD, g O_2/m³	188.25 ± 1.62	172.80 ± 3.7	184.00 ± 8.00
Nitrogen compounds as N_{tot}, g N/m³	46.56 ± 1.35	41.65 ± 1.09	52.92 ± 2.43

Table 2. Technological characteristics of model investigation and average concentrations of pollutants in sewage

The characteristic feature of the used sewage was a low C/N ratio caused by pretreatment in a septic tank. The pollutants in sewage and suspended biomass concentrations were measured according to the standard methods. The attached biomass concentration was identified via the Kjeldahl nitrogen measurement: 1 g N_{TKN} corresponds to 0.11 g_{dm} [50]; pH and oxygen were measured using calibrated electrodes.

The detailed description of research results of all experimental stages is included in the Makowska's monography [11]. In this chapter only the most important processes and parameters related to the carbon and nitrogen removal efficiency are presented. Results related to the parameters like: biomass loading, pollutants' removal efficiency and substrates utilization rates were analyzed statistically.

3.2. Carbon and nitrogen compounds removal efficiency in continuous and sequencing flow MBBR reactors

The oxygen accessibility play a very important role in bioreactor performance. Four aeration/nonaeration time intervals were tested: 75/45, 45/45, 30/30 and 15/15 minutes. The most effective was the last interval in which oxygen deficit lasted 10 min was observed in nonaeration phase and maximum for SND process oxygen concentration (0.8 mg O_2/dm^3) was achieved. In these conditions for continuous flow, the maximum removal efficiencies for carbon and nitrogen compounds were equal to 98% and 85% respectively [16,11]. The optimal hydraulic retention time was equal to 12 hours.

The outflow pollutants concentrations were related mainly to biomass loading: the higher loading – the lower the removal efficiency, especially for medium and high loaded reactors (the most evident for sequencing batch reactor). This relationship was more evident for nitrogen compounds removal (figure 6) excepting total nitrogen removal in SBR.
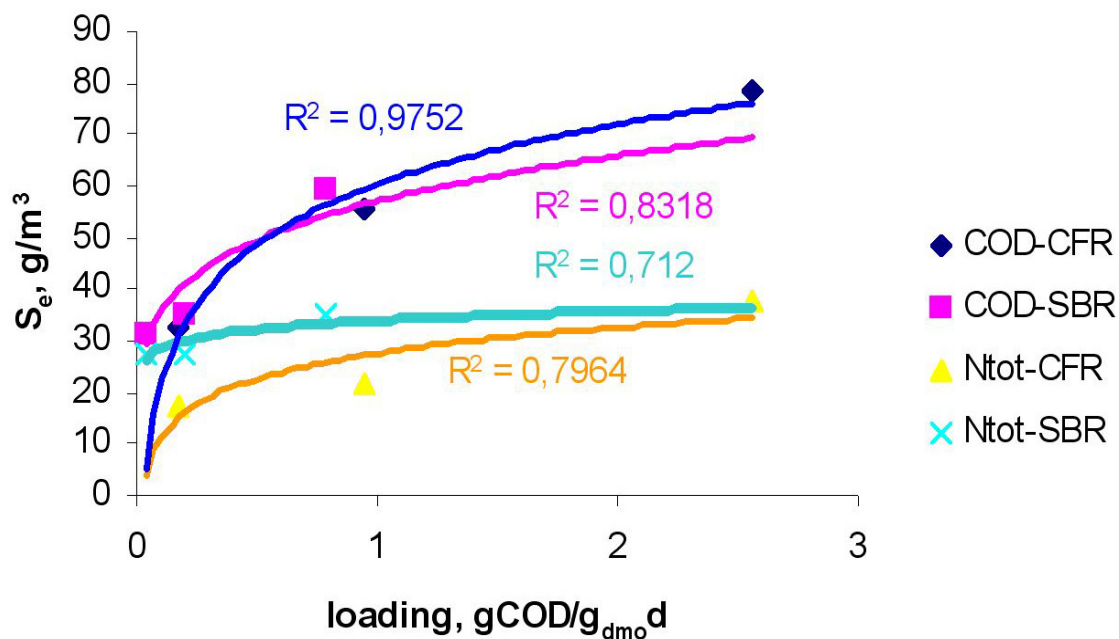
**Figure 6.** Relationship between pollutants' concentration in purified sewage and biomass loading

The increase in loading up to 2.5 g COD/g_{dm}d caused the rise of contaminants removal rate (figure 7), although it was partly related to biomass concentration decrease as a result of lading rise. The removal efficiency in SBR was related to the volumetric exchange ratio (0.2-0.5 range); the higher ratio – the lower removal efficiency.
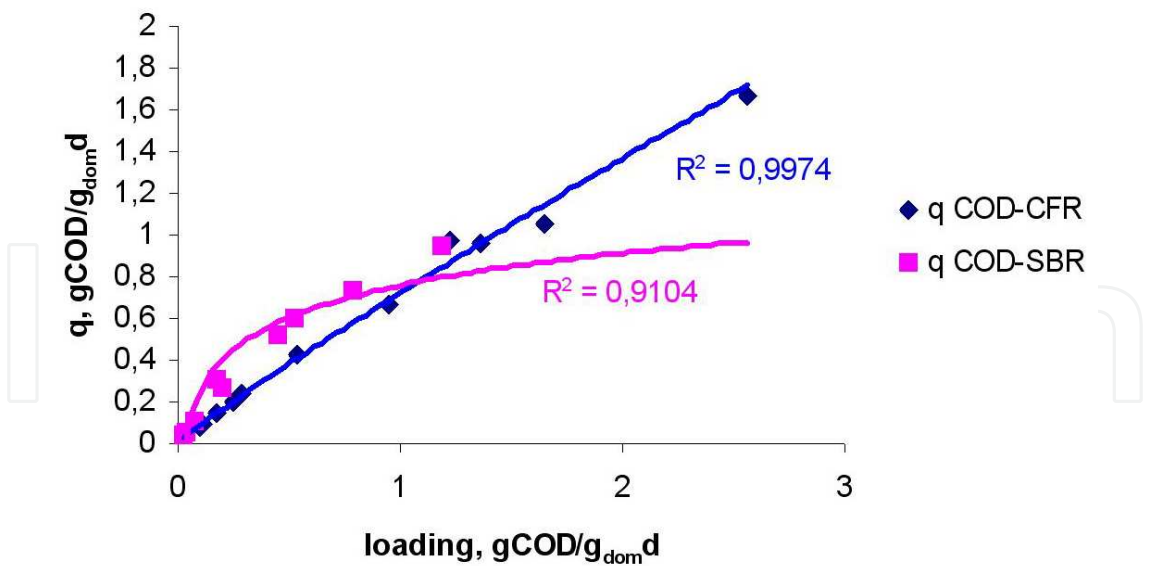
Figure 7. Relationship between pollution removal rate and biomass loading

Some authors stated the higher resistance for hydraulic overloading and more stable nitrification in hybrid reactors than in conventional activated sludge reactors [12]. These research showed that suspended/attached biomass ratio was related to the biomass organic compounds loading (figure 8). The higher biomass loading – the higher attached biomass concentration (the same - lower suspended biomass concentration). This phenomenon was also observed by other authors [26].
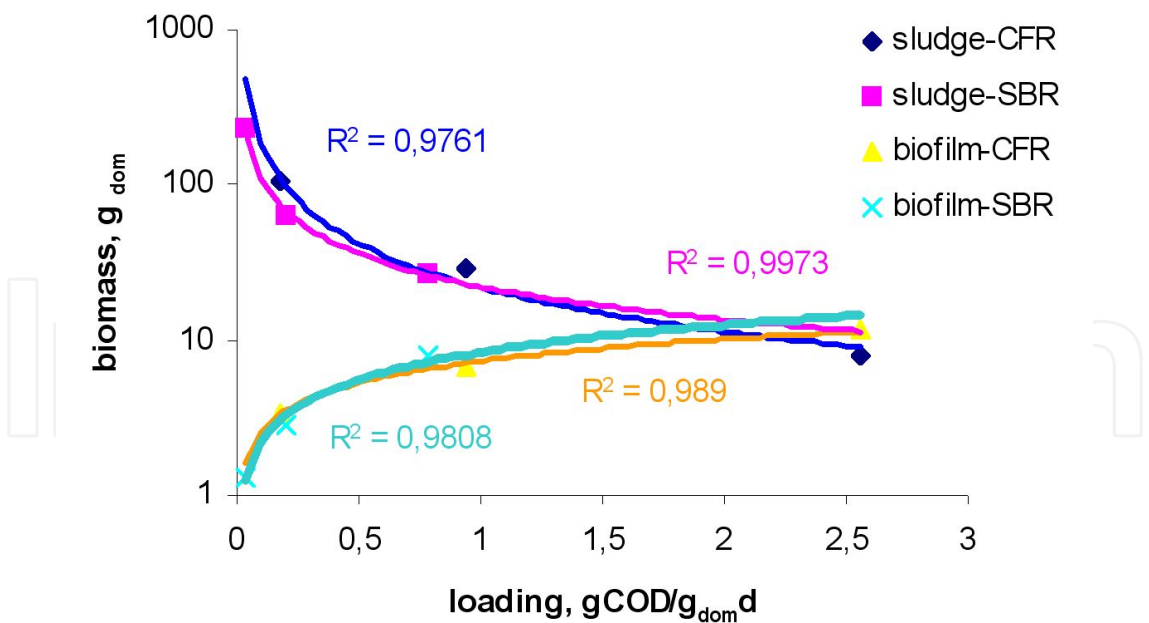
Figure 8. Relationship between biomass in reactors and organic load

So the conclusion can be drown that highly loaded reactors (especially SBR) do not need excess sludge removal, although biomass growth yield can reach values in range 0.31 - 0.50 $g_{dm}/g_{sub\ rem}$.

3.3. Carbon and nitrogen compounds removal efficiency in continuous flow reactor with elevated pH of sewage

Continuous flow reactor with elevated wastewater pH was maintained at 12 hours HRT and 15/15 minutes aeration/nonaeration intervals. Values of pH were in the range of 8.0-8.5. Three rates of volume reactor filling by carriers were investigated: 60%, 40% and 20%.

The elevated pH caused the ammonia release and inhibition of the second phase of nitrification and this way the total nitrogen removal process was shorten by elimination of two elementary processes: nitratation and denitratation (figure 8).

The free ammonia concentration value, calculated accordingly to equation 6 was equal to appr. 1 mg N/dm^3, what is known as a limiting value for inhibition of nitrification second phase [42]. The part of free ammonia could be released as the result of amino acids denaturization during alkalinity process, but due to relatively low concentration of organic nitrogen (mainly amino acids) in inlet wastewater (maximum 10% of total nitrogen) this factor can be neglected. In these conditions the SND process was achieved (shortened nitrogen removal process) what had been indicated by temporary nitrites accumulation. It is known and was stated by several authors [51] that as a result of ammonia inhibition of second phase of nitrification, mediate and final products are released simultaneously.

The higher removal efficiency was achieved at higher volume carriers filling of reactor (figure 9). The rise in pH value versus the rise of nitrogen compounds removal rate, what was observed by other authors [52].
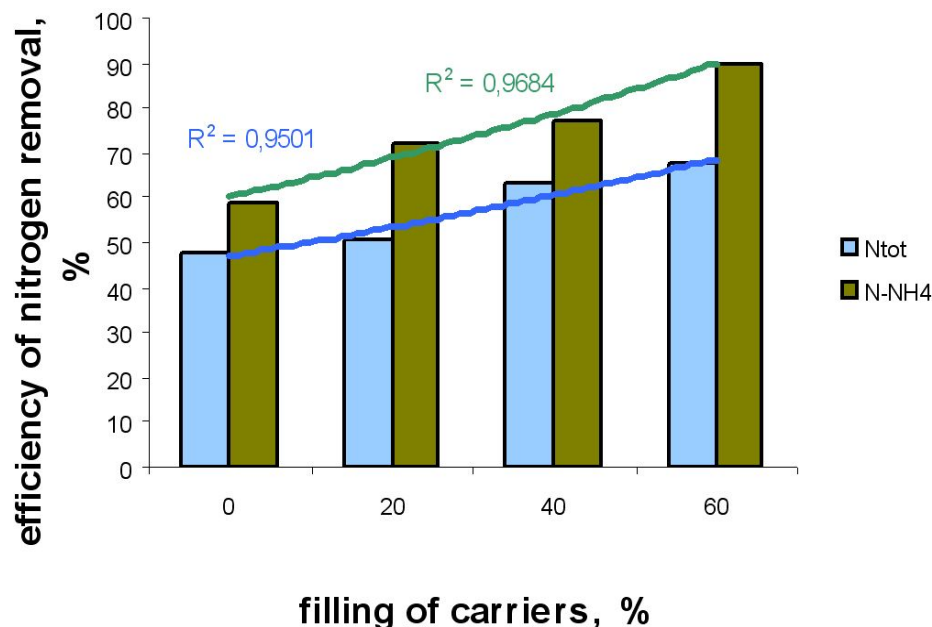
Figure 9. Relationship between effect of nitrogen removal efficiency and filling by carriers

The lime addition resulted in some changes in biomass, e.g. reducing organic fraction rate in biomass of 25% comparing to the process without lime addition. The lime in the liquid phase and on the carriers surface were some kind of "condensation centers" and caused the

higher concentration of both biomass form. The smaller amount of carriers enabled more undisturbed carriers movement (figure 10). The lime addition caused also the less susceptibility of carriers pores for clogging by biomass.
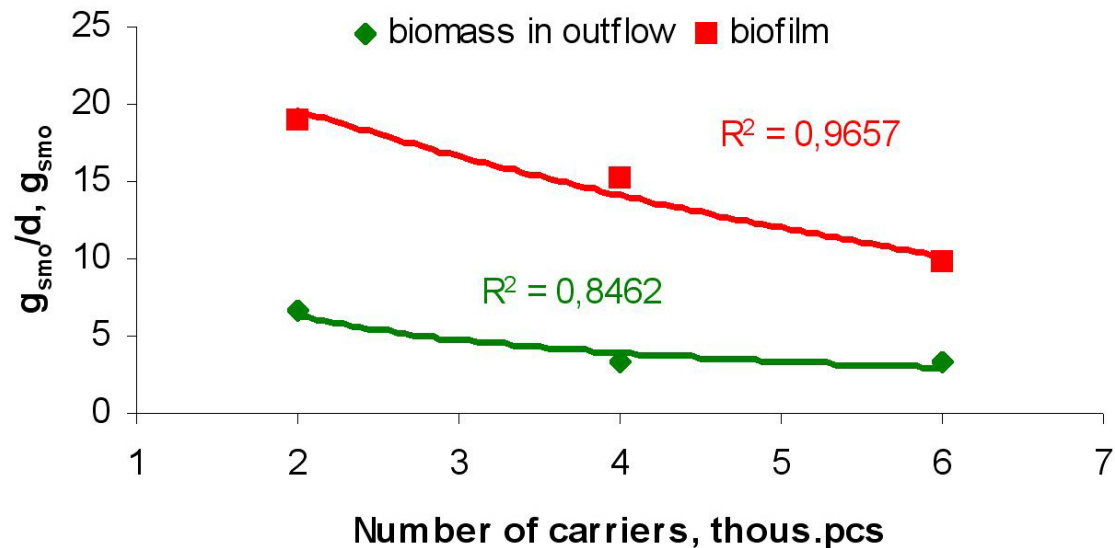
Figure 10. Biomass removed from reactor and amount of biofilm with quantity of carriers

3.4. Hybrid bioreactors biomass characteristic

In this research relatively wide (but typical for activated sludge reactors) range of activated sludge flocs size was observed: 150-500 μm in all reactors.

The biomass attached to the moving bed carriers surfaces was poorly developed. Attached biomass did not cover the outside carriers surface and existed only on the inert surface not as continuous film but as separate small mushroom shape colonies. Small colonies of stalked ciliates (figure 11) were observed on the inner surface of carriers. There were observed some differences between biofilm and activated sludge flocks groups of organisms, in both biomass forms. Stalked, creeping and free-swimming ciliates, filamentous microorganisms, rotifers and nematodes were observed.

Epistylis and *Vorticella* were dominating genera of ciliates in both suspended and attached biomass. Stalked ciliates were observed in relatively high number both in attached and suspended biomass. The domination of this form of *Ciliata* was probably related to good pollutant removal efficiency, what was reported by other authors [1, 54].

The observed number of rotifers in the attached biomass was much higher than in suspended biomass (t-Student statistics; t calculated: 2.83, critical value: 2.78, α: 0.05, replications no.: 5, df: 4), what was the most evident in period 3 in SBRs, and was probably related to the long time of growth of rotifers. Also the differences in concentrations of filamentous microorganisms were observed - in continuous flow reactors the number of filamentous microorganisms was often lower in attached biomass than in suspended biomass (figure 12). Filamentous microorganisms concentration was the highest in the
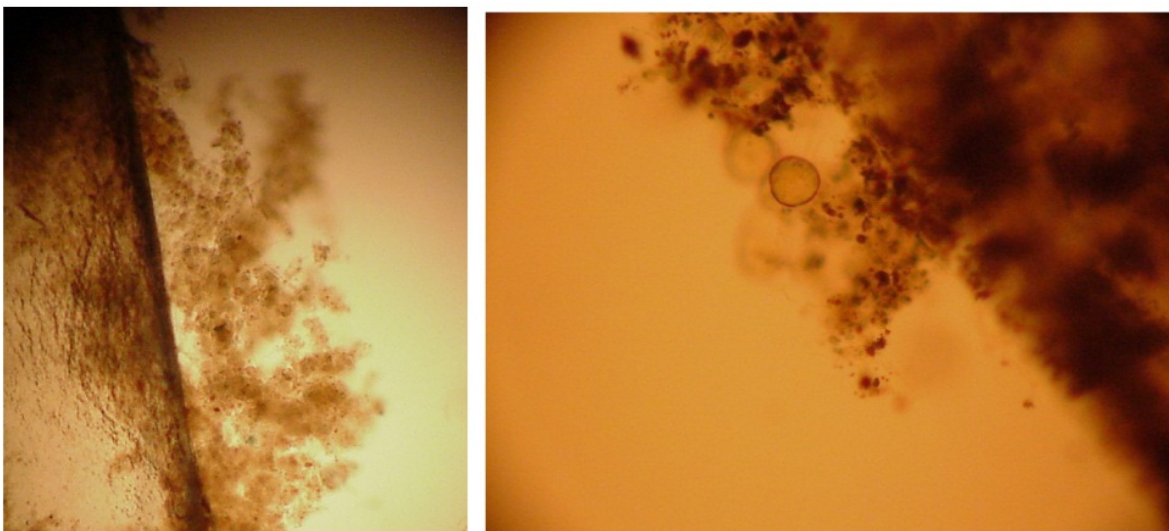
highest COD loaded reactor: R3 (t-Student statistics; t calculated: 8.98, critical value: 2.2, α: 0.05, replications no.: 13, df: 11).

Figure 11. The inner surface of a carrier covered by a small stalked ciliates colony [16]
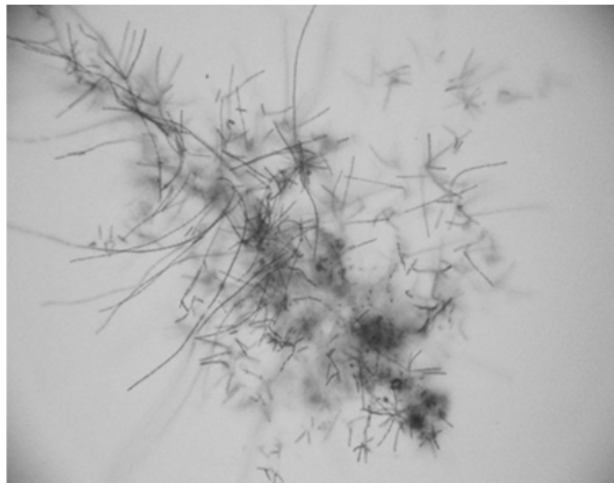
**Figure 12.** Filamentous organisms in reactor R2 during stage II [16]

Figure 13. Biofilm on the MBBR carrier

3.5. Hybrid bioreactor mathematical modeling

The mathematical modeling is a very useful method of process simulation, because there is no need time and costs consuming experimental methods using. However, there are many problems in adequate mathematical description of complex biochemical processes and parameters' estimation.

For hybrid bioreactor modeling the ASMH1 model [11] can be applied. It is based on the ASM1 model, but significantly modified: nitrogen removal processes are more completely treated by implementation of two stages nitrification and denitrification (figure 14). The intermittent aeration and oxygen accessibility to the second phase of nitrification was considered. The free ammonia inhibition was also implemented.
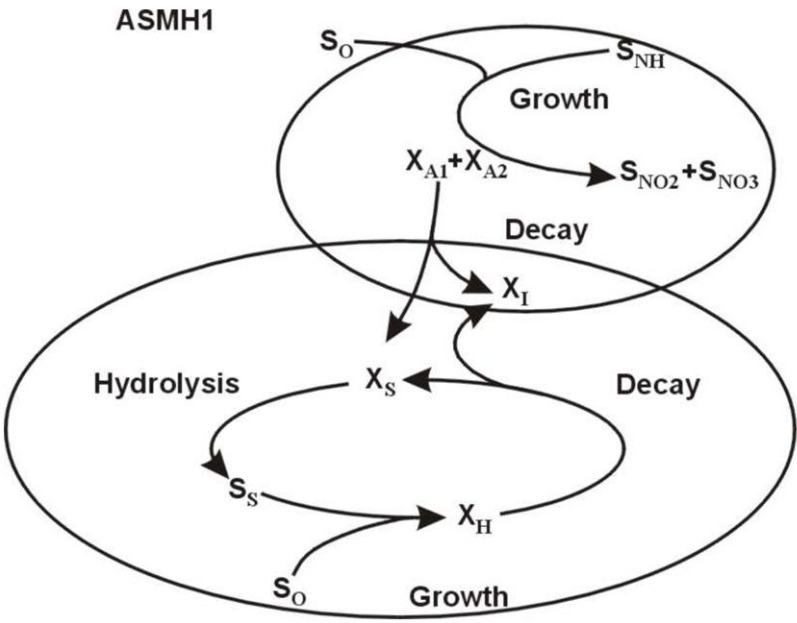
Figure 14. Scheme of process in the ASMH1 model

The model calibration using laboratory data allowed to identify the kinetic and stechiometric parameters values. The model was implemented in to the POLYMATH program and relatively good agreement with experimental results was achieved (st. dev. 10%).

4. Conclusions

The basic technological parameters related to removal efficiency of pollutants from septic tank in hybrid MBBR, at intermittent aeration, continuous/sequencing flow and elevated pH were presented in table 3. The parameters' values concerning the purified sewage fulfill the Polish law requirements for 2000 p.e. WWTPs.

The hybrid MBBR has occurred an effective system for carbon and nitrogen compounds removal from septic tank effluent. The carbon and nitrogen compounds can be removed

with at least 80% and 50% removal efficiency respectively. It can be achieved even at loading of 2 g COD/g_{dm}d and 12 hours HRT. Similar results were reported by [53] for partial nitrification-denitrification process in combination of aerobic and anoxic reactors with Kaldnes carriers. The system needed internal recirculation. Thanks to attached biomass the nitrification process in the hybrid MBBR was effective at low and high loaded reactors. The remaining of ammonium nitrogen in treated wastewater appeared at high loadings only. The intermittent aeration and dissolved oxygen limitation enabled simultaneous nitrification-denitrification process (SND) in one reactor. The inhibition of second phase of nitrification by free ammonia has intensified nitrogen removal and resulted in energy savings and internal source of carbon using as a sole source. The shortened process of carbon compounds removal was confirmed by medium products appearance. The long time aeration cycles and long time operating cycles can result in denitrification disturbances due to the organic substances oxidation and limitation of that energy source for denitrifying bacteria.

Parameter	Continuous flow reactor CFR	Batch reactor	CFR with increased pH
Pollution load -organic compounds, g COD/d -nitrogen compounds, g N_{tot}/d	19.29 – 29.25 5.93 – 6.42	15.56 – 31.57 2.77 – 7.87	26.51 – 27.89 7.30 – 8.25
Hydraulic load, dm^3/dm^3d	1.53 – 1.90	1.65 – 3.05	1.85 – 2.04
Concentration in purified sewage -organic compounds, g COD/m^3 -nitrogen compounds, g N_{tot}/m^3	26.91 – 56.22 21.57 – 31.07	31.31 – 59.46 27.49 – 34.94	23.00 – 37.00 17.10 – 25.28
Biomass: -activated sludge density, g/dm^3 -biofilm mass, g/m^2	0.62 – 1.18 2.58 – 5.46	0.48 – 4.03 0.62 – 3.70	5.58 – 6.52 1.55 – 3.58
Biomass loading of -organic compounds, g COD/g_{dm}d -nitrogen compounds, g N_{tot}/g_{dm}d	0.247 – 0.945 0.067 – 0.201	0.081 – 1.080 0.011 – 0.155	0.075 – 0.082 0.011 – 0.021
Efficiency of removal,% -organic compounds -nitrogen compounds	75 – 84 37 – 54	69 – 82 20 – 35	80 – 87 51 - 68
Pollution removal rate -organic compounds, g COD/g_{dm}d -nitrogen compounds, g N_{tot}/g_{dm}d	0.206 – 0.663 0.030 – 0.113	0.110 – 0.620 0.004 – 0.032	0.064 – 0.124 0.011 – 0.021
Yield coefficient, g_{dm}/g_{CODrem}	0.31 – 0.43	0.42 – 0.50	0.34 – 0.47

Table 3. Technological parameters and treatment efficiency in hybrid reactors

The pH elevation brought about higher treatment efficiency. The higher volumetric fraction of moving media (carriers) – the better performance. The continuous flow reactor was more effective in treatment and more stable than the sequencing batch reactor.

It was stated and statistically confirmed that: aeration regime, biomass loading and media volume fraction have an impact on the pollutants (especially organic compounds) removal efficiency.

Advantages of hybrid MBBR reactors operating in the modified conditions are as follows: lower energy consumption (up to 40%) related to the shorter aeration time, possibility of specific wastewater treatment (low N/C ratio), simultaneous processes maintaining in one reactor (internal recirculation elimination), overloading resistance (stable performance) and reduction in smaller reactors' volume. The mathematical model ASMH1 allows to simulate the reactor performance at the specific conditions.

Author details

Małgorzata Makowska, Marcin Spychała and Robert Mazur
Poznan University of Life Sciences, Department of Hydraulic and Sanitary Engineering, Poznań, Poland

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