DISTRIBUTION AND CHARACTERISTICS OF BIOMASS IN AN UPFLOW BIOLOGICAL AERATED FILTER

by

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[ABSTRACT]

The biomass from a pilot-scale two-stage (carbon oxidation first stage, ammonia oxidation second stage) fixed-film biological aerated filter (BAF) was divided in three fractions depending on their attachment strength to the media: detached, easily detachable and strongly attached. VSS measurement showed that the detached and easily detachable fractions accounted for 25 to 40% of the biomass in the bed and are present even after backwash. Protein was the major constituent of all fractions of the biomass. The ratio of carbohydrate to protein differed between fractions and between type of biofilms, with a larger value for detached and detachable fractions and a lower value for a largely heterotrophic biofilm, implying a difference in the composition of the biomass matrix that could be related to the attachment state of the biomass. The biomass did not appear to be substrate-limited anywhere in the system, although the specific activity of the biomass was dependent upon the position in the column. Activity of the strongly attached biomass was less than 70% of the total activity, even after backwash. A mass balance on VSS showed that the backwash flushed a mass equivalent to less than 35% to 45% of the detached and detachable fractions, which was less than 15% of the total biomass present in the system. Data also suggested that during backwash, part of the strongly attached biomass was sheared off the media and regenerated the mass of biomass in the detached phase. In conclusion, it can be stated that a non-negligible part of the biomass in a BAF is in a detached state. Actual mechanistic BAF models based solely on biofilm modeling may be overlooking the role of that biomass, especially in the performance recovery of BAF systems after backwash.

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To my father,

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LITERATURE REVIEW

Biological aerated filters (BAFs)

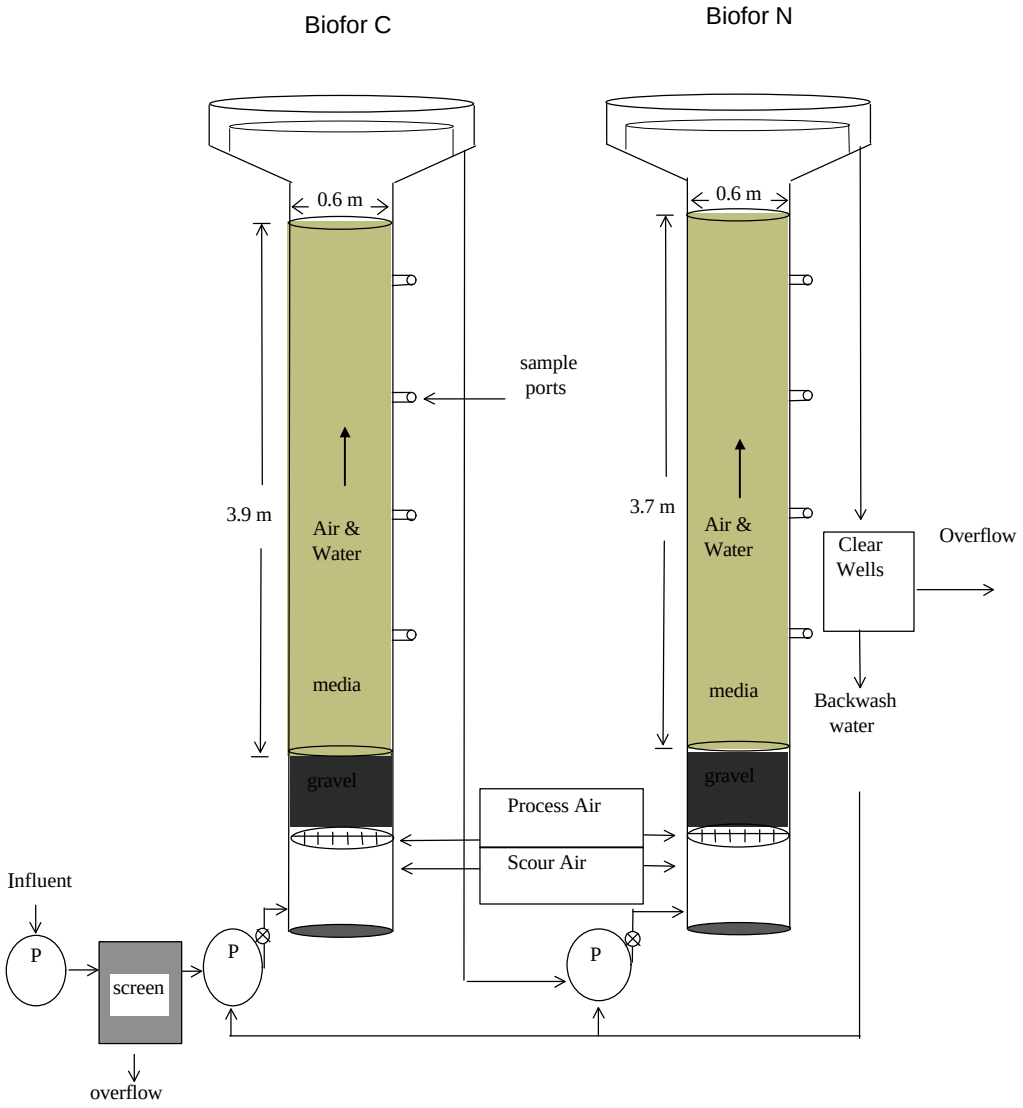
Biological aerated filtration is an alternative to activated sludge for biological wastewater treatment. BAFs are upflow submerged packed-bed reactors. The bed is composed of a granular media that physically captures solids from the influent and that provides a support on which biomass grows. Unlike trickling filters, the media is submerged and influent is fed upflow through nozzles below the bed. Aeration (if needed) is added above the nozzle deck. Excess solids from influent or from biomass growth is regularly wasted by backwash, during which the bed is washed with recycled effluent at high water and air flow. Backwash water is fed upflow in co-current mode, or downflow in counter-current mode. In the former case, the media (expanded clay, for example) has a specific gravity greater than one, where in the latter case, the media (polystyrene beads, for example) is lighter than water (M'Coy, 1997). An additional source of air (air scouring) is used during backwash to increase the effect of the higher flow rates. Sludge wasted during the backwash is either recycled to the primary clarifier or treated for disposal.

BAFs have been used primarily for carbon oxidation (Amar *et al.,* 1986), ammonia oxidation, or for combined carbon-ammonia oxidation. Nitrification with BAFs is a reliable process as washout of nitrifiers is prevented by the fixation of the biomass on the media. BAFs have also been proven efficient over a wide range of temperatures for nitrogen and phosphorus removal (Rogalla *et al.,* 1991). BAF performance depends on three criteria: degree of soluble substrate removal, effluent suspended solids concentration and head-loss build-up.

BAFs have been developed in Europe as a substitute or a supplement to activated sludge. Some cities in the United States (Roanoke, VA and New York, NY) are currently using or have been considering this technology for new facilities or for upgrading existing facilities. The main advantages of these systems are their reduced footprint (low capital investment) and low hydraulic retention time as high loading can be handled by the large biomass concentration on the media. Operation of such systems is also simplified as secondary clarification is not needed, which eliminates the potential for

experiencing typical clarifier problems such as bulking. Additionally, the system can be built in individual cells to handle varying hydraulic loads (M'Coy, 1997). As only the effluent, located on top of the bed, is in contact with the ambient air, the system is relatively odor-free.

Schematic of the Biofor™.



The system used for this study was a pilot scale of a two-stage (carbon and ammonia oxidation in two separate filters in series) biological aerated filter. Influent was fed to the first column (C column) for carbon oxidation; the effluent of that first column was then fed to the second column (N column) for ammonia oxidation. The filter was run in co-

current mode, with effluent from the N column recycled as backwash water. The media was expanded clay (Biolite^{TM}) with a specific gravity of 1.5 g/ml. Some of the effluent from the N column was collected in clear wells and used for backwashing.

Biofilm

Biofilm structure

BAF systems rely on the biomass growing attached to a surface, thus forming a biofilm layer on the media. A biofilm is composed of bacterial cells embedded in a gel-like matrix. This matrix consists of extracellular polymeric substances (EPS), produced by the cells (secreted or released after lysis) or captured from the bulk solution, and inorganic salts. The organic fraction of this matrix can be divided into capsular polymers (CPS), which remain attached to the cells after centrifugation, and extracellular polymers (Bonet *et al.,* 1993). Composition of this EPS matrix varies with type of biofilm system (Nielsen *et al.,* 1997) and is thought to be very similar to the structure embedding the cells in activated sludge flocs. Nielsen *et al.* (1997) reported protein as the major constituent (73-78%) of the organic fraction of biofilm EPS, whereas humic acids (11-14%), carbohydrates (6-7%), uronic acids (less than 2%) and DNA (less than 2%) were found in smaller quantities. Horan and Eccles (1986) investigated carbohydrates from activated sludge and found an additional degree of variation in the monomer contents of the exopolysaccharides in activated sludge from one treatment plant to another.

Biofilm growth

Capdeville and Nguyen (1990) identified five phases in biofilm development:

- a latent phase where single bacteria attached to the bare surface.
- a dynamic phase where attached bacteria grew from pinpoint colonies to form a thin biofilm over the whole surface of the media. At the end of that phase the effluent substrate concentration of their system (rotating plate) tended toward a constant limit value.
- A linear phase during which no change in effluent substrate was observed but the thickness of the biofilm increased as biomass accumulated at a high rate.
- A decelerating transition phase before the biofilm reached a steady state.

- A stabilization phase, where the biofilm reached a steady state (mass and thickness constant). At that point, the biofilm was in equilibrium between growth of new biomass and loss due to decay and sloughing of excess biofilm.

Biofilm macro-properties

Biofilms are described by several important characteristics such as thickness, density, resistance to shear, substrate diffusion rate, attachment strength and protein content. These characteristics vary from biofilm to biofilm and are influenced by the environment of the biofilm. Density and thickness are related (Hoehn and Ray, 1973), as density increases with thickness for thin biofilms, then decreases and reaches a plateau for thicker biofilms (Deront *et al.*, 1998). As substrate loading was increased, Peyton (1996) observed an increase in biofilm density and thickness of a monopopulation of *Pseudomonosa aeruginosa* grown in an annular reactor. By controlling the rotational speed of the reactor axis, he established that sheer stress influenced the roughness (variance in thickness between locations) of the biofilm, but not the average thickness itself. Capdeville and Nguyen (1990) noted a decrease in activity per unit biomass as the biofilm became thicker, which could be caused by an accumulation of inert or inactive biomass as the biofilm grow. Zhang *et al.* (1998) observed that EPS protein and carbohydrate concentration increased with biofilm depth, whereas the ratio of protein to carbohydrate remained almost constant.

EPS composition and biofilm properties

The nature of the extra-cellular polymers directly influences some of the biofilm macroproperties by altering the charge density and/or the hydrophobic/hydrophilic properties of the EPS matrix (Christensen 1989). Bonet *et al.* (1993) studied the distribution of polysaccharides between capsular (CPS) and extracellular polymers and found an increase of cell hydrophilicity and of cell autoagglutination when CPS production was high. Nielsen *et al.* (1996) found that changes in the amount of protein in the EPS matrix of activated sludge flocs could be related to the disintegration behavior of activated sludge flocs. Davies and Geesey (1995) reported that *P. aeruginosa* cells that did not show up-expression of *alg*C (one of the genes involved in the synthesis of alginate, a common exopolysaccharide among bacteria species) were less capable of remaining attached to a glass surface than those cells which were increasing the expression of *alg*C.

Studies of the regulation of carbohydrate synthesis in bacteria capable of forming biofilms have suggested that changes in macroproperties of a biofilm could be the result of an active bacterial response to their surroundings. Bonet *et al.* (1993) showed that although the total amount of polysaccharides excreted by cells did not vary, the distribution of these carbohydrates between extracellular polysaccharides and capsular polysaccharides depended on the yeast concentration in the culture. The total amount of carbohydrate produced was also found to increase with the level of carbon or nitrogen in the culture. In turn, this change in EPS composition was altering the surface properties of the cells and changing the attachment capacity of the cells. Using a gene reporter technology to monitor *Alg*C regulation, Davies and Geesey (1995) found an up-regulation of this gene in *P. aeruginosa* when bacteria remained attached to a surface more than 15 minutes, whereas the gene was down-regulated after detachment.

Modeling

Biofilm modeling

Unlike suspended culture, the main mechanism for supplying substrate to the cells in a biofilm is substrate diffusion within the biofilm as well as transport in the bulk solution. A comprehensive mechanistic biofilm model was developed by Rittmann and McCarty (1980). The model considered:

- mass transfer resistance due to substrate diffusion through an effective diffusion layer surrounding the biofilm
- simultaneous diffusion and reaction of substrate within the biofilm according to Monod-type kinetics.

For a steady-state biofilm, they were able to calculate the biofilm thickness along with the minimum bulk substrate concentration required to develop and support steady state biofilm. Biofilm growth in this model was balanced by loss of biomass due to decay and sloughing, both modeled as first order reactions relative to biomass concentration. Detachment rate was also related to shear stress due to the bulk liquid flow rate (Rittmann 1982). In order to take into account the diminution of specific activity inside thick biofilms, Capdeville and Nguyen (1990) proposed the concept of active / inactive biomass. After the biofilm reached a specific thickness, the active fractions of the

biomass reached a maximum, and inactive biomass which play no role in the substrate removal accumulated in the biofilm.

One of the issues in working with this model was the determination of the biofilm parameters such as decay rate, biomass loss by shearing and diffusion layer length. Wanner (1995) developed a "mixed" model (Aquasim), which coupled mechanistic and empirical modeling: some of the unknown parameters were directly determined by data fitting. Thus, parameters that may vary with time or space (density, mass transfer coefficient and detachment rate) could be estimated using experimental data collected over time and space. In this way, multi-layers or multi-species biofilms could be modeled. Additionally, Nielsen *et al.* (1997) proposed a conceptual model to relate the biofilm growth to EPS composition and, therefore, to macroproperties such as detachment rate, substrate diffusibility and particulate trapping.

The main limitation of these models is that they are "unidimensional" so that biofilm properties such as density, diffusion coefficient and microbial distribution, are assumed to vary only with depth. This is in contradiction with the new insights given by confocal scanning laser microscopic imaging showing the three-dimensional organization of biofilms. A biofilm can have a very heterogeneous structure, with cell clusters separated by interstitial voids. Using nuclear magnetic resonance imaging, Lewandowski *et al.* (1995) proved that convection flow could be very important in these interstitial pores and could modify the exchanges between the bulk solution and inner layers of the biofilm. de Beer *et al.* (1994) found that 50% of the oxygen consumed by the cells in an aerobic biofilm was provided by convection in these pores and that oxygen diffused through all interfaces in cell clusters, not just from the bulk solution. Therefore, the inner layer of biofilms may not be as substrate limited as previously believed, whereas anaerobic zones can exist in dense cell clusters near the surface of the biofilm. Therefore unidimensional models are likely to produce some quantitative errors (underestimating substrate diffusion) and qualitative errors (spatial distribution of microbial community) (Wanner 1996).

Modeling BAFs

Mann and Stephenson (1997) proposed an empirical model for BAFs based on a first order rate of substrate removal along the length of the filter. They defined two parameters

for estimating the performance of BAFs relative to their efficiency in removing soluble COD and their performance with regards to varying substrate loading. Based on experimental data, they concluded that BAFs with light media having a specific gravity lower than 1, were more efficient and more consistent in achieving substrate removal. Deront *et al.* (1998) proposed a relationship between head-loss build-up in an upflow packed-bed reactor and biomass growth in the system: as the thickness of the biofilm increased, the bed macroporosity was reduced, inducing an increase in head-loss.

Mechanistic models have also been developed using biofilm modeling designed for trickling filters (Young and McCarty ,1969) or annular reactors (Wanner and Gujer, 1986) and applying the flow conditions specific to BAFs. Wanner (1995) modeled a packed-bed reactor using their biofilm model Aquasim. The reactor was modeled as a series of compartments composed of a completely mixed bulk fluid coupled with a one-dimensional mixed-population multisubstrate biofilm characterized by a surface area of exchange. Each compartment represented a certain length of the reactor and each one was connected to the contiguous compartments by advective and diffusive flow. Parameters that vary with time such as surface of contact between biofilm and bulk solution, detachment rate and mass transfer coefficient were fitted using experimental time series data. Beg and Hassan (1985) built a model of an upflow packed-bed reactor using the homogeneous steady-state biofilm model developed by Rittmann and McCarty (1980) combined with an axial dispersed plug-flow. Using Peclet number as a parameter to measure how far from ideal plug-flow the system was, they showed that increase dispersion lead to increase in effluent substrate for first order kinetics. For zero order kinetics, the effluent concentration did not depend on the Peclet number as the dispersed flow only changed the profile of substrate concentration in the column and not the kinetics of substrate removal.

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DISTRIBUTION AND CHARACTERISTICS OF BIOMASS IN AN UPFLOW BIOLOGICAL AERATED FILTER

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ABSTRACT

The biomass from a pilot-scale two-stage (carbon oxidation first stage, ammonia oxidation second stage) fixed-film biological aerated filter (BAF) was divided in three fractions depending on their attachment strength to the media: detached, easily detachable and strongly attached. VSS measurement showed that the detached and easily detachable fractions accounted for 25 to 40% of the biomass in the bed and are present even after backwash. Protein was the major constituent of all fractions of the biomass. The ratio of carbohydrate to protein differed between fractions and between type of biofilms, with a larger value for detached and detachable fractions and a lower value for a largely heterotrophic biofilm, implying a difference in the composition of the biomass matrix that could be related to the attachment state of the biomass. The biomass did not appear to be substrate-limited anywhere in the system, although the specific activity of the biomass was dependent upon the position in the column. Activity of the strongly attached biomass was less than 70% of the total activity, even after backwash. A mass balance on VSS showed that the backwash flushed a mass equivalent to less than 35% to 45% of the detached and detachable fractions, which was less than 15% of the total biomass present in the system. Data also suggested that during backwash, part of the strongly attached biomass was sheared off the media and regenerated the mass of biomass in the detached phase. In conclusion, it can be stated that a non-negligible part of the biomass in a BAF is in a detached state. Actual mechanistic BAF models based solely on biofilm modeling may be overlooking the role of that biomass, especially in the performance recovery of BAF systems after backwash.

INTRODUCTION

Biological aerated filtration is an alternative to activated sludge for biological wastewater treatment. BAFs consist of a media bed, which physically captures solids from the influent and which provides a support on which biomass grows. Unlike trickling filters, the media is submerged and influent is fed upflow. The system is regularly backwashed with water and air to get rid of the material accumulating in the bed. A BAF can be operated in co-current mode where backwash water flows upward, or counter-current mode where backwash water flows downward. Compared to activated sludge, the main advantages of BAFs are a reduced footprint, as there is no need for a secondary clarifier, and a reduced hydraulic retention time due to a high concentration of biomass in the system.

Modeling of such systems can be approached from an empirical (Mann and Stephenson, 1997) or mechanistic (Wanner, 1995; Beg and Hassan, 1985) direction. Since these systems are biofilm reactors, the mechanistic models are mainly based on biofilm modeling. They utilize models developed for trickling filters (Rittmann and McCarty, 1980) or models developed for biofilm grown on plates as in rotating drum reactors (Wanner and Gujer, 1986), combined with flow conditions specific to BAFs such as plug flow with axial dispersion (Beg and Hassan, 1985). Although biofilm models can be refined to include attachment-detachment kinetics (Wanner 1995) and multi-substrate or multi-species biofilms (Beg *et al.*, 1995), existing models still fail to account for some specific features of BAFs that influence performance, like the effects of backwash and solids retention.

Actually, most biofilm studies have found that deeper regions of biofilms are less active than outer layers (Capdeville and Nguyen, 1990). Backwashing in BAFs is assumed to shear off mainly the layers of the biofilm that have grown during a cycle, thus reducing the biofilm to its less active deeper layers. Therefore, the system should have a certain lag time during which its performance is not as good as it was before backwash. However, no such lag phase has been found in studies of an upflow BAF (Amar *et al.*, 1986; Love *et al.*, 1999) despite the short hydraulic residence time (20-40 minutes) through the bed.

Moreover, focusing mainly on the biofilm and flow conditions inside a BAF overlooks the fact that BAFs remove suspended solids very efficiently through filtration, as several studies have shown (Amar *et al.* 1986). In our system, effluent suspended solids concentration was routinely well below the target of 10 mg/l despite an influent concentration of 54 (±15) mg/l as VSS (Love *et al.*, 1999). As suspended solids are efficiently retained in the system, biomass sheared during operation or not flushed out during backwash are likely to stay trapped in the filter. This should result in a significant mass of biomass in a non-attached state. Such biomass may have sufficient metabolic activity to affect BAF performance and should be taken into account in developing both conceptual and mathematical models for BAF systems.

The following study was conducted to characterize the biomass in an upflow biological aerated filter. The biomass was believed to exist in two phases, either as part of the biofilm (attached to the media) or as rather loose biomass that was filtered out by the bed (detached biomass). The participation of these phases to the performance of the system was estimated by obtaining the activity and characteristics of these fractions. Last, the residence time of the biomass in this regularly backwashed system was calculated to better understand the fate of these fractions during operation and backwash.

MATERIALS AND METHODS

System set-up.

This study was conducting using a two-stage pilot-scale BAF system provided by Infilco Degremont, Inc (Richmond, VA) and located at the Peppers Ferry Regional Wastewater Treatment Facility (PFRWWTF) in Radford, Virginia. The two columns were operated in series; the first stage (C column) received the effluent from a primary clarifier as influent for carbon oxidation, and the effluent from this column was fed to the second stage (N column) for ammonia oxidation. The C and N columns were 0.61 m in diameter and contained 3.9 m and 3.7 m of a clay-based media, respectively. The media specific area was approximately 1400 m^2/m^3 and had a density of 1.5 g/cm^3. The packed bed porosity was 0.4. Performance of the system is described elsewhere (Love *et al.*, 1999).

During the tests, the water and air flows were 8.2 m/h and 5.4 scfm for the C column, 7.1 m/h and 6.5 scfm for the N column. The total COD concentration to the C column averaged 272 (±-26) mg/l, and carbonaceous BOD concentration averaged 62 mg/l (total) and 25 mg/l (soluble). The ammonia concentration to the N column ranged from 14 to 20 mg/l as nitrogen. The volatile suspended solids (VSS) concentration going into the system was 55 (±5) mg/l, while effluent VSS leaving the C and N column averaged 19 mg/l and 11 mg/l respectively. The C column removed approximately 80% of the total carbonaceous BOD, the N column removed 85% of influent ammonia-nitrogen.

Both columns were fitted with two sets of 4 sampling ports, evenly spaced along the column. One set was used for liquid sampling. The other set was adapted for media sampling: a hollow steel tube (core sampler) could be forced across the whole section of the column using this port.

The system was regularly backwashed with effluent from the N column which was in a clear well until used. The C column was backwashed every 12 hours, and the N column was backwashed every 24 hours. The backwash occurred in 5 phases after partial draining of the column. During the first three phases, water and air were fed at higher flow than during operation, along with air scouring. Water was effectively wasted (column overfilling) only during the last four phases. During the fourth phase, air scour

was stopped along with aeration. The last phase ("water-to-waste") was also a water-only phase but primary effluent (C column) or C Column effluent (N column) was used instead of clear-well water at normal operational flows. Each backwash lasted around one hour.

Sampling of biomass fractions

Media was sampled from the lower port (0.6 m within the media bed) and middle port (2.2 m within the media) of each column after draining . Samples were kept at 4°C and analyzed within 2 hours in the Environmental Laboratory. Liquid was collected at the same level as the media sample for use as "dilution liquid".

An amount of media (around 80 g wet weight) was placed in a 1-liter square bottle, and 500 ml of dilution liquid was gradually added. The bottle was slowly turned upside down once using a specially designed apparatus in order to gently shuffle the media. The resulting liquid was immediately collected as the "detached" fraction. Again, 500 ml of dilution liquid was added and the bottle was shaken more vigorously during a standardized time (60 rev, 1 rev/sec). The resulting liquid was immediately collected as the "detachable" fraction. Some of the remaining media was then used for further analytical procedure and was referred as the "strongly attached" fraction.

Liquid composite samples were taken during backwash during each of the backwash phases when the column was overfilling.

Analytical procedure

Specific Oxygen Uptake Rate (SOUR)

Liquid samples from detached and detachable fractions were used for SOUR measurement in 300 ml BOD bottles without dilution. To measure the SOUR of strongly attached fractions, a known mass of media (15-20 g) was placed at the bottom of a BOD bottle and dilution liquid was added. After oxygenation, the decrease in oxygen concentration was recorded using a DO Probe (DO Probe: YSI 5905; DO Meter: YSI model 58) equipped with a mixer. Two spiking solutions were used: an ammonia chloride solution in order to increase the ammonia-N concentration by 20 mg/l, and a synthetic biogenic wastewater solution (Bailey and Love, 1999) in order to increase the COD

concentration by 330 mg/l. For N column samples, the ammonia spike was analyzed before the COD spike.

SOUR assays were conducted in a constant temperature room and the liquid temperature was recorded. The temperature of the liquid during assays were within 2°C of the temperature in the pilot plant system. The SOUR test could not be conducted on site due to uncontrolled outside temperatures which varied widely from day to day.

Total and Volatile Suspended Solids

Total suspended solids (TSS) and VSS were measured according to Standard Method (APHA, 1995)) using a 1.5 μm glass fiber filter (Whatman Inc, Clifton, NJ).

For VSS measurement of the strongly attached fractions, the media was dried in 105°C overnight, weighted and then burned in a muffle (550°C) for 30 minutes. Dry media tended to be highly hygroscopic and absorbed water rapidly thereby causing a tendency to underestimate VSS.

To standardize some of the measurements to a mass of media basis, the media was weighed as wet weight or dry weight (less than 1% difference between the two measures) and the result was expressed on a per g media basis.

Protein and Carbohydrate determination

Protein was determined using the Bicinchoninic Acid (BCA) Assay (Sigma procedure TPRO-562, Sigma chemicals, St Louis, MO). Liquid samples were processed according to BCA kit procedures after hydrolysis of the whole sample in 1 M NaOH, and absorbances were read at a wavelength of 562nm with a spectrophotometer (Beckman spectrophotometer DU640, Beckman Instrument, Inc, Fullerton, CA). Cells in the detached and detachable fractions were not pelleted before alkaline hydrolysis to be consistent with the treatment of strongly attached samples for which pelleting was not physically possible. Dilution liquid protein concentrations were subtracted from the value obtained for detached and detachable fractions. For protein determination of biomass strongly attached to the media, a known mass of rocks (2-3 g) was placed in a glass tube with 5 ml 1 M NaOH and hydrolyzed at 100°C for 30 minutes. Supernatant was collected, diluted if necessary with 1 M NaOH and BCA procedure was conducted using bovine serum albumin (Sigma Chemicals, St Louis, MO) standards which were also prepared in 1 M NaOH.

Carbohydrates were determined on samples using the phenol-sulfuric acid method (Dubois *et al.* 1956) and glucose standards. For carbohydrate determination of biomass strongly attached to the media, supernatant from the alkaline hydrolysis described above for protein analysis, was collected. The phenol-sulfuric acid method was then conducted as for other biomass fractions except that the glucose standards were prepared in 1 M NaOH.

Blanks and standards were analyzed in the presence of clean media to confirm that the media did not interfere with protein or carbohydrate measurements.

COD, BOD and Ammonia were analyzed according to Standard Methods (APHA, 1995).

RESULTS AND DISCUSSION

Sampling method

RBC-type biofilms are frequently studied in laboratory settings by growing biomass on flat surfaces where the biofilm can be easily characterized without disruption, in a environment close to the prevailing one in the biological reactor (Capdeville and Nguyen, 1990). For BAF system, sampling is more disruptive to the biomass because of the configuration of the media bed. In previous BAF studies, biofilm thickness measurements have often been performed microscopically on biofilms still attached to the media after sampling, whereas biofilm mass is determined by shearing the biomass by agitation (Ohashi *et al.*, 1995). This sort of procedure assumes that no biomass is sheared during sampling and that the whole biomass can be sheared off the media by agitation. In a procedure similar to this study, Deront *et al.* (1998) vigorously mixed media samples collected with a core sampler after draining a BAF reactor. They considered the biomass collected after this manner as being representative of the biomass grown between two cycles. However, part of the newly grown biomass may have remained attached after mixing, or part of the biomass detached during mixing may not be sheared by the backwash.

The method used in this study was designed to separate the biomass collected into three phases which represented different degrees of attachment to the media. The first phase (detached) was collected by gently shuffling the media so that any trapped (non-attached) or very loosely bound material would be freed from the media bed and collected in the liquid phase. The second phase (detachable) represents that biomass that was weakly bound to the media. The third phase (strongly attached) represents the biomass fraction that is more strongly attached to the media. One objective of the study was to estimate the relationship between these fractions obtained with a standardized procedure, and the biomass collected in the backwash water.

The method used to collect media (forcing a steel tube in the column) caused some disruption of the clay media. The concentration of inorganic suspended solids was higher than expected in the detached and detachable fractions with a ratio of VSS to TSS as low

as 20% for the detached fraction versus a ratio of 70% for samples taken during backwash. However, on a mass basis, this additional inorganic fraction represented less than 0.5% of the mass of media collected by the core sampler, indicating that only a minor fraction of the media was crushed during sampling.

Mass of biomass in each fraction

Figure 1 shows the mass collected at each port as VSS per g media for each fraction of biomass and for both columns. The total amount of biomass averaged between the two ports collected was 10.9 and 6.3 mg VSS per g media for the C column and N column respectively. These figures are quite close to 6.9 mg VSS / g media found by Amar *et al.* (1986) with the same type of clay media in a upflow BAF treating primary effluent. This gives a VSS concentration per unit volume of reactor (void space + rocks) of 9810 mg/l for the C column, and 6210 mg/l for the N column, which confirms that BAFs have a higher density of biomass than activated sludge (typically 1300-2500 mg/L MLVSS, WEF and ASCE, 1991).

According to figure 1, the strongly attached biomass represented 60% to 75% of the total biomass. The concentrations of each fraction per g media decreased from lower port to upper port and from C column to N column, but this decrease was only significant for the strongly attached fraction. The concentrations of each fraction at the beginning and end of a cycle were not significantly different. However, 6 samples out of 8 exhibited an increase in biomass concentration from 0.5 to 1.5 mg VSS per g media (less than 18% of the total biomass) in the strongly attached fraction whereas detached and detachable fraction concentrations were unchanged (data not shown).

Ratio protein/VSS – carbohydrate/protein

The ratio of protein (as g BSA) to VSS shown on figure 2 stresses that protein was the main constituent of the biomass in our system. The protein measured by the modified BCA procedure represented the total protein in the biomass including cells and EPS matrix. The ratio of protein to VSS for the strongly attached fraction was greater than one. This surprising value could be attributed to error in the use of BSA as a standard for protein determination when bacterial protein was actually being measured (Frølund *et al.*,

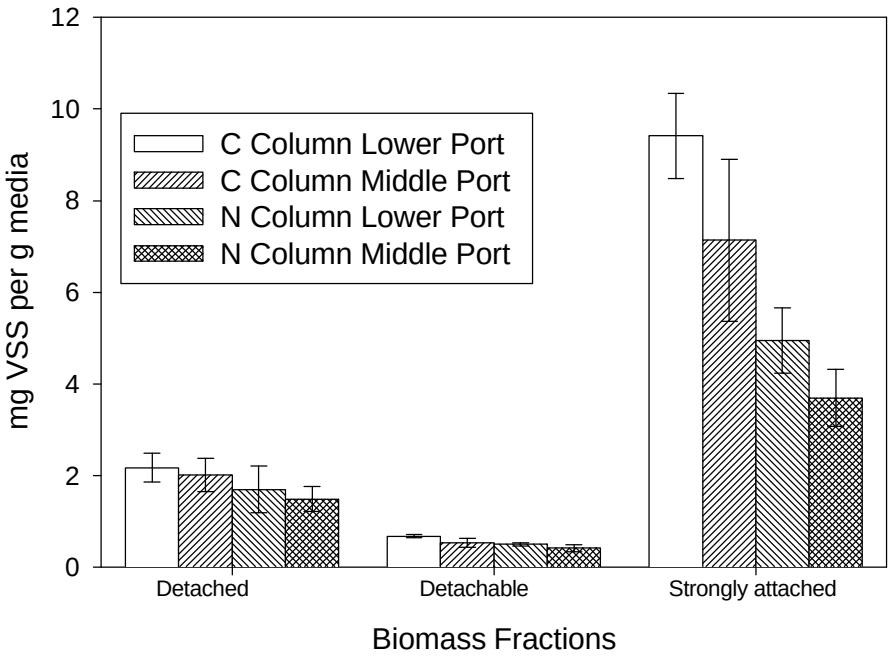

***Figure 1**, Concentration (mg VSS per gram of media) of each biomass fraction, averaged per port and per column. The media density was 1.5 g/ml and the bed porosity was approximately 0.4.*

1995). It may also be due to the tendency to underestimate VSS for strongly attached biomass because of the high hygroscopic property of the media. This tendency to take up water was reproducible between samples as duplicate media samples gave comparable values for VSS per g media (within 10% of error). The protein to VSS ratio was nevertheless expected to be higher for the strongly attached fraction as it represented deeper (and older) parts of the biofilm, where protein tends to accumulate (McKinley and Vestal, 1985; Zhang *et al.*, 1998). For the whole biomass, the protein to VSS ratio was greater than 70% and this high proportion of protein supports the contention that the biofilm is relatively rich in cells.

Figure 3 presents the ratio of carbohydrate (standardized to glucose) to protein. Measured carbohydrate represented the total polysaccharide content of the biomass including cells and EPS matrix. The detached and detachable fractions did not differ in composition, with a ratio of 0.39 ±0.10. The value of this ratio was significantly lower for the strongly attached fraction for which a significant difference could be noted between the C column biomass (primarily heterotrophs) and the N column biomass (primarily nitrifiers) with values of 0.020 (±0.016) and 0.091 (±0.027), respectively. The ratio for the whole biomass was 0.072 (±0.020) for the C column and 0.16 (±0.06) for the N column. In the literature, values for this ratio vary over a wide range, as it depends on sampling and measuring methods and on the type of biomass studied. Frølund *et al.* (1996) reported a ratio for activated sludge (cells + EPS) ranging from 0.35 to 0.55. Nielsen *et al.* (1997) found a ratio of 0.085 for a biofilter (Biostyr™) when focusing on EPS. Lazarova *et al.* (1998) also reported a difference in the ratio of carbohydrate to protein in a three phase reactor between a tertiary nitrification (0.16-0.22) and a combined carbon oxidation and nitrification (0.30-0.71) process. In our study, the ratio was lower for the (mostly) heterotrophic biomass. Although these two studies are difficult to compare since the biological systems, the method of sampling, and the analytical techniques were different, these results confirmed a structural difference in the attachment of the two types of biofilm. This can be related to the findings that nitrifiers tend to form very densely populated aggregates in activated sludge (Wagner *et al.* 1995).

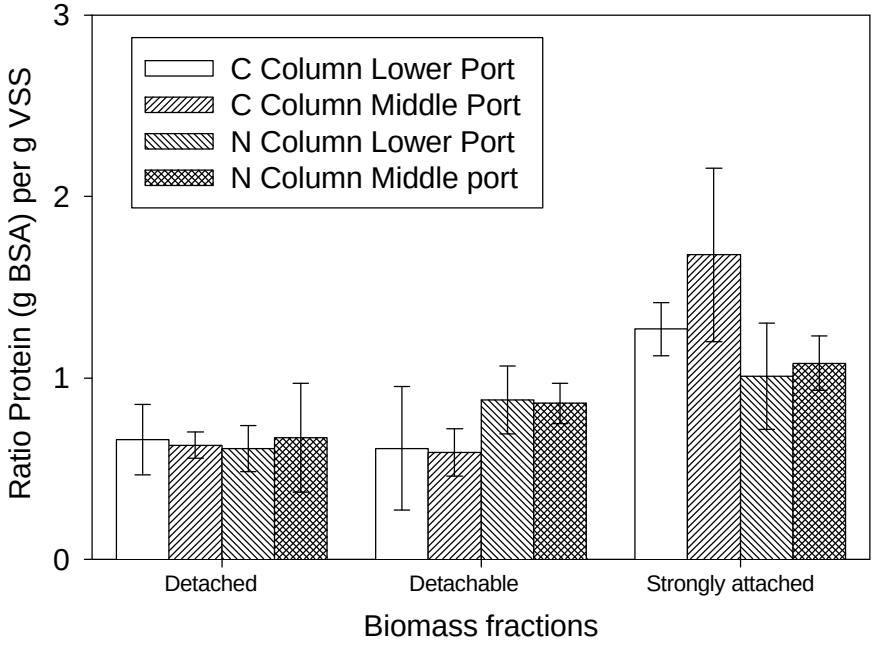

***Figure 2**. Ratio of protein to VSS, for each biomass fraction averaged per port and per column. Protein was measured with the BCA method using BSA standards.*

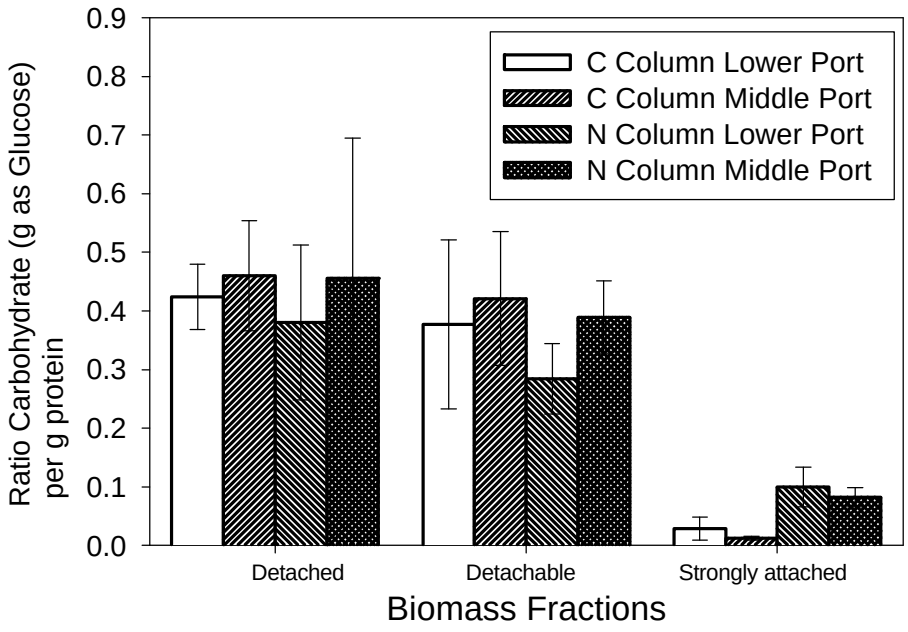

Figure 3. *Ratio of carbohydrate to protein for the three biomass fractions, averaged per port and per column. Carbohydrate was measured with the phenol-sulfuric acid method using glucose standards. Protein was measured with the BCA method using BSA standards.*

The contrast in ratio between the strongly attached fraction and the detached or detachable fractions indicates that from one biomass fraction to another, the polysaccharides are not produced in the same amount or may not be of the same nature, as the response of the phenol-sulfuric acid method may vary from one type of polysaccharide to another (Horan and Eccles, 1986). This difference may originate from larger storage of polysaccharide within cells for the detached and detachable fractions which are not subject to the same substrate diffusion limitation than the strongly attached biomass. It may also indicate a variation in EPS composition due to their distinct environmental surroundings. In fact, it has been shown by others that bacteria can regulate EPS production depending on their attachment state. Using alginate (an exopolysaccharide produced in a wide range of bacteria) reporter gene, Davies and Geesey (1995) showed that alginate up-regulation in *Pseudomonas aeruginosa* was dependent on the attachment state of the cells. Bonet *et al.* (1993) showed that the distribution of polysaccharides between capsular polymers and extracellular polymers was a function of the nutrient level and could modify the surface properties of the cells. As EPS carbohydrate content has been shown to influence the macroproperties of biofilms (Christensen, 1989), both the difference between the two phases and between N biofilm and C biofilm may be indicative of an active bacterial response to changing surrounding conditions. This response could be an increased carbohydrate production in the detached phase, or a different type of polysaccharide produced in any phase, that was not accurately quantified by the carbohydrate analytical method.

Activity of biomass as Specific Oxygen Uptake Rate.

Oxygen uptake rate was recorded for the three fractions. The SOUR of the liquid used for dilution was subtracted, and the result is reported in figure 4. No significant difference was found between beginning of cycle and end of cycle (data not shown) and samples were averaged per port and per column. The liquid used for dilution was the liquid collected at the same level as the biomass in each column so that the unspiked SOUR measured was assumed to be indicative of the activity of the biomass *in situ*.

The strongly attached biomass had a lower specific activity than the detached or detachable fraction, especially for the C column biofilm. The actual value for the overall

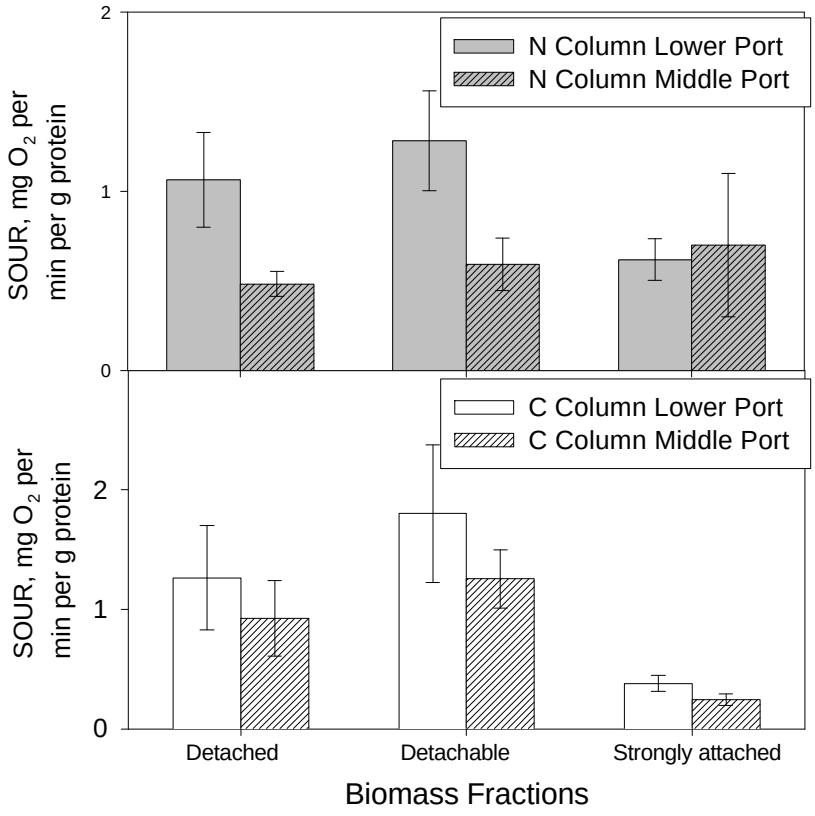

Figure 4. *Specific Oxygen Uptake Rate, for each biomass fraction averaged per port and per column. The activity shown is "unspiked", meaning the liquid used for diluting the samples was the liquid collected at the same level in the reactor as the biomass.*

C biomass (0.43±0.13 mg O2/min/g protein or 0.34±0.10 mg O_2/min/g VSS) is close to the one (0.2 mg O_2/min/g VSS) found by Amar *et al.* (1986) on biomass from a similar reactor system. The respirometric activity of the strongly attached biomass of the N column (primarily nitrifiers) was larger than that of the C column biomass (primarily heterotrophs) by a factor 2.3, a trend comparable to the factor 3 reported by Lazarova *et al.* (1998) in a three phase reactor between a system fed with ammonia only and a system fed with primary effluent.

The detached and detachable fractions did not significantly differ from each other but the activity of these fractions was found to depend on the port whereas the difference was less evident for the strongly attached biomass. The decrease in activity for the detached and detachable fractions from lower port to upper port was sharper in the N column (+110%) than in the C column (+40%). Spiking with ammonia and/or COD did not increase the specific activity for the N column (data not shown). The absence of a response to COD spiked into the N column biomass showed that the heterotrophic activity known to be present, particularly at the lower part of the N column (Gilmore *et al.*, in press), was too low to be detected by respirometry against nitrifying activity. Spiking the C column biomass with COD did increase its specific oxygen uptake rate by 15% to 25% (data not shown), but without attenuating the noticeable decrease of specific activity from lower port to upper port that can be seen on figure 4. Therefore, the difference of activity between ports in the two columns was not the result of substrate limitation as could be expected as the substrate concentration in the dilution liquid decrease from lower port to upper port. Such variation in activity may rather be attributed to a different extant respirometric activity, with the biomass at the lower port being acclimated to an environment richer in substrate and oxygen.

The lower activity of the strongly attached heterotrophic biomass could be caused by to some substrate and oxygen limitation due to diffusion, as this fraction represents a deeper layer of the biofilm. It could also be due to the accumulation of non-cellular protein including exopolymers and cell lysis products. These two explanations are consistent with the fact that the decrease of activity from detached to strongly attached biomass was more obvious in the C column, where the strongly attached biomass was

more concentrated (on a VSS per g media basis) and therefore probably thicker than the biofilm of the N column.

Distribution of biomass in the filter

The previous results showed that the detached and detachable fractions were comparable in terms of the ratio protein/VSS, carbohydrate/protein and specific activity; therefore, in the following discussion, they are grouped into a single detached-detachable fraction.

Models of upflow packed-bed reactors available in the literature (Wanner, 1995; Rittmann and McCarty, 1980; Beg *et al.* 1995) are based on biofilm modeling and some modification of a plug flow reactor, and, therefore, assume that substrate removal and activity in the reactor comes from attached biomass (Beg *et al.* 1995). However, as summarized on figure 5, this study showed that more than one third of the specific activity in a given volume of reactor is due to a biomass fraction which is either detached or loosely bound. The "detached" fraction represented more than two-thirds of that non-strongly attached biomass, and we assumed that most of this biomass is trapped inside the bed rather than attached as part of a biofilm structure. No significant change in the volumic distribution of activity was found during the cycle, indicating that this detached biomass is present and active even right after a backwash. Moreover, the distribution of activity did not vary widely between ports, which indicated that the detached biomass is evenly distributed along the column. This fraction of biomass is more likely to be released during backwash. Comparison of protein to VSS, carbohydrate to protein ratios and of specific activity showed that the backwashed solids were effectively similar to the detached-detachable biomass (data not shown).

Mass balance on biomass during a backwash

The presence of the detached-detachable fractions even after a backwash indicated that these fractions were not totally flushed out of the system during backwash or were partly regenerated during a backwash.

A mass balance on VSS flushed out of the columns during regular backwash is shown on Table 1. The water wasted during backwash was sampled and the total mass of VSS

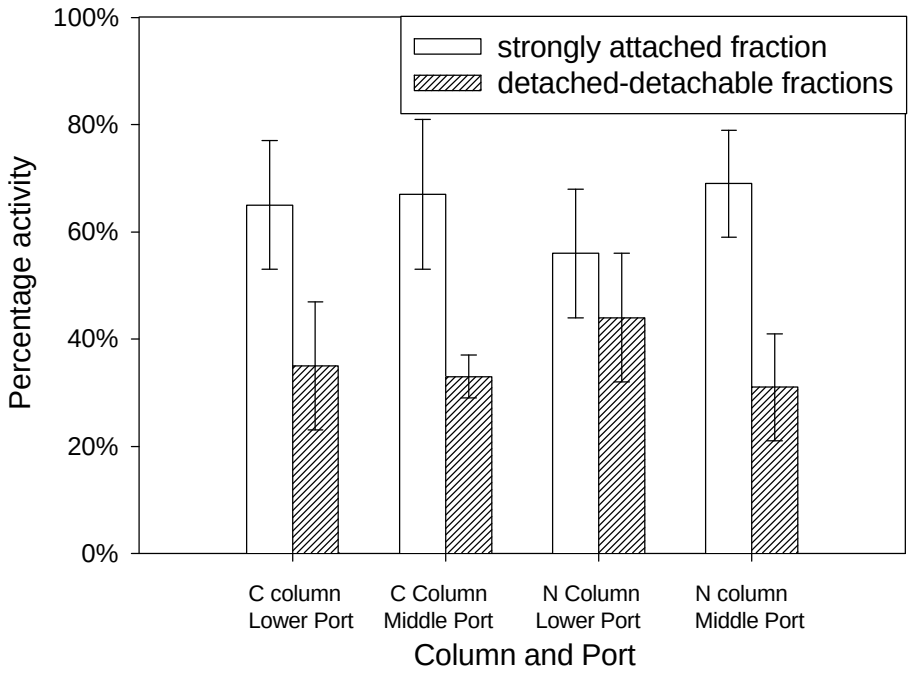

Figure 5. *Distribution of respirometric activity between the detached or detachable biomass fraction and the strongly attached fraction. The respirometric activity was measured for unspiked samples, and is averaged per port and per column.*

flushed out of the columns during the process was calculating for different dates. The total mass was around 1.05 kg VSS for a 12-hour cycle in the C column and 0.85 kg VSS for a 24-hour cycle in the N column. As the mass of media in each column was known, it was possible to compute the average amount of VSS flushed per g of media in each reactor and compare those values to the concentration of the detached-detachable fraction given in figure 1.

Backwash appeared to flush a mass of VSS equivalent to no more than 35% (C column) or 45% (N column) of the detached-detachable biomass. As this non-strongly attached biomass represented less than 40% of the total biomass as shown in figure 1, each backwash was wasting less than 15% of the total biomass in the system. This figure is low compared to other studies : Ohashi *et al.* (1995) reported a 50% decrease in biofilm on their bench scale BAF. However, Ohashi used clay pellets whereas the rocks used in this study were of a very irregular shape and more likely to give more tortuosity to the flow (reducing the impact of backwash). Their methods of sampling also differed as the biomass was measured after stripping it from the pellet by agitation (therefore assuming no attached biomass remained on the pellets). Our study showed that the detached biomass had a mean residence time greater than two cycles. The stabilization of the slowly biodegradable particulate material contained in the influent should be more thorough than what would be expected if the mean residence time was only one cycle (12 hours for the C column).

As backwashing did not flush all the loosely bound material, this material was set free and was redistributed along the column by the backwash. This explains why the concentration of the detached fractions was not significantly lower at the upper port of the column, whereas we would expect solids to accumulate in the lower part of the column where the influent solids enter the filter and would be trapped. Moreover, for 6 samples out of 8, a small decrease (10 – 18%) in biomass as VSS could be detected after a backwash and this decrease was mainly located in the strongly attached fraction (data not shown). This indicated that during a backwash, when high flow rates of water and air are combined with an extended air scouring phase, some of the strongly attached biomass was sloughed off the rocks. This sheared biomass became mixed with the detached and

Table 1. *Mass balance between the biomass flushed out of the reactor during a backwash and the biomass in the detached or detached fractions.*

		Total mass of VSS backwashed		Detached-detachable biomass (mg VSS/g media)		
		in g	in mg/g media	detached	detachable	Total
Backwash C column	Day 1	1176	**1.15**			
	Day 2	972	**0.95**			
	Day 3	1009	**0.98**			
Backwash N column	Day 1	815	**0.84**			
Biomass Fraction (average)	C column			2.09	0.61	**2.70**
	N column			1.59	0.46	**2.05**

detachable fractions, reducing all the more the visible impact of backwash on the detached-detachable biomass.

BAFs have been reported to recover quickly after backwash, without the lag time that could be expected when outer and more active layers are sheared off a biofilm (Amar *et al.*, 1986). Figure 6 shows a typical profile for soluble COD and total COD during and just after a backwash for the C column. The automated backwash was divided in 5 phases, and the system only wasted water during the last four phases (sample BW1, BW2, BW3 and Water to Waste). After phases BW1 and BW2, air scour (additional air added to the water used for backwash) was stopped whereas water flow was still more than 2-fold normal operational flow. During the last phase "water to waste", influent was fed back into the column at the normal flow rate but the effluent was wasted. During the roughest part of the backwash (BW1 ,BW2 and most of BW3), both soluble and total effluent COD were above the composite effluent despite the low COD content of the water used for backwash (N effluent collected during previous cycle). The increase of soluble COD indicated that some soluble materials were released from the biomass during backwash.

However, the soluble effluent COD quickly came back to a level comparable to the composite effluent of the previous cycle, despite a low residence time (20 to 40 minutes) of the influent in the column. Total COD was also back to the pre-backwash level with a slight lag that can be explained by the configuration of the system. The bed was actually topped by a zone without media that approximated a CSTR, and solids in this zone could start to settle and would not move out of the column as fast as the flow.

Such a quick recovery of the system may be linked to the ever-presence of this active detached biomass filling the pores of the media bed. As the outer layer of the strongly attached biomass are removed from the media during backwash, the strongly attached biomass loses part of its activity. However, the biomass in the detached phase adds an important surface of exchange which gives an extra capacity for substrate removal and makes up for the lower activity of the biofilm. This detached biomass might also facilitate effective trapping of particles as it fills the pores of the bed.

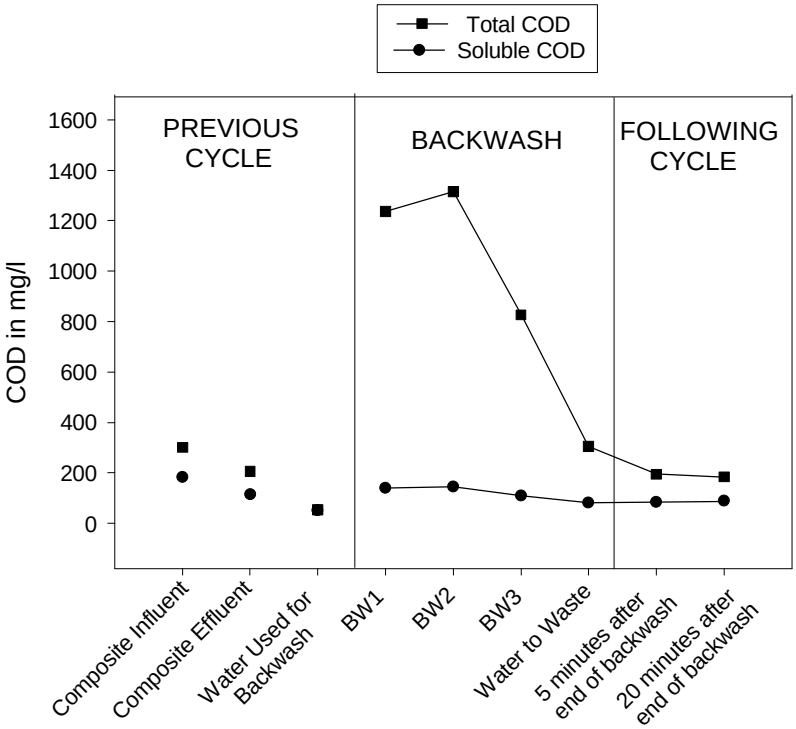

Figure 6. *Evolution of total and soluble effluent CODs during a typical backwash of the C column. The concentration of the composite influent,C-effluent and N-effluent (water that is used to backwash) during the previous cycle are given for comparison with effluent just after the backwash.*

CONCLUSIONS

The biomass of a BAF was categorized into three fractions, depending on its degree of attachment to the media, from detached to strongly attached. A non-negligible portion of the biomass was found to be detached or very weakly bound to the media. This fraction of biomass represented more than 30% of the activity in a given volume of the reactor and was present even right after a backwash, along the whole length of the bed.

The chemical analysis of the biomass showed that protein was the main component, and that the ratio of carbohydrates to protein varied between fractions. A higher ratio for the detached fraction than for the strongly attached suggested some physiological difference in EPS production, probably related to the attachment state of the biomass. A significant difference was also found between the strongly attached fraction of the primarily heterotrophic biofilm and the strongly attached fraction of the primarily nitrifying biofilm.

Study of the backwashed biomass confirmed that the detached fraction was not entirely flushed during a backwash. In this system, the backwash removed a mass equivalent to only 35% to 45% of the detached biomass, or less than 15% of the total biomass present in the system. This means that the detached biomass had a mean residence time greater than two cycles.

Based on these results, the following mechanisms can be proposed to describe the fate of the biomass in a BAF regularly backwashed.

- during a cycle, biomass is active both in the detached phase and in the attached phase and probably exhibit different biodegradation kinetics. EPS composition differs between these two phases as a consequence of their distinct environmental surroundings.
- During a backwash, some of the strongly attached biomass is sheared off the media and mixed with the already detached fractions. Part of this mixture is then flushed out of the filter during subsequent backwashes.
- At the end of a backwash, the bed is no longer expanded and solid filtration occurs again, preventing further wash out of detached biomass.

- The strongly attached biomass loses some of its most active layers during backwash. However, some of this material becomes part of the active detached biomass and is redistributed along the whole column. The continual redistribution of biomass prevents a loss of treatment performance at the beginning of cycles, and participates in the trapping of smaller particles from the influent.

Any modeling of a BAF should therefore take into account the presence of a detached biomass, especially when the time between backwash is short due to high concentrations of suspended solids in the influent. Backwash can also be optimized to insure that enough detached biomass remains in the filter while getting rid of sufficient biomass to reduce headloss in the system.

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ENGINEERING SIGNIFICANCE

Biological aerated filters are an alternative to activated sludge in biological wastewater treatment. The system consists of a media bed that physically captures solids for the influent and that provides a support on which biomass grows. Consequently, the system does not require a secondary clarifier and can retain a high concentration of biomass fixed on the media. As solids from the influent and biomass accumulate in the bed, the filter is regularly backwashed with effluent.

In the literature, models for BAFs are based on biofilm modeling, and backwash is modeled as a process that shears off the newly grown biomass. This biomass is generally the more active part of the biofilm and models tend to predict a lag time for the system before the biofilm recovers. Such a lag time was not found in the upflow packed bed reactor studied here. Moreover, solids removal was also efficient quickly after backwash, indicating that the bed was efficiently filtering solids even after the wash out of the excess biomass.

This study showed that biomass in a BAF is not only in the form of a biofilm attached to the media and regularly sloughed by the backwash. A non-negligible part of the biomass is in a detached or loosely attached state. This fraction of the biomass was present throughout a cycle, even right after a backwash. This detached biomass is significantly active, and its chemical composition is different from the biomass strongly attached to the media. This suggests a difference in physiological state due to a different attachment condition. Based on a mass balance on biomass during a cycle and during backwash, the following mechanism can be proposed:

- during a backwash part of the strongly attached biomass is sheared off and is mixed with the detached biomass.
- Only a part of the biomass in detached phase is washed out during backwash.
- Right after backwash, the media bed is no longer expanded and the remaining detached biomass is trapped in the bed.
- Both soluble removal and solids filtration are efficiently achieved right after backwash due to the detached biomass making up for the loss of efficiency of the strongly attached biomass.

From an engineering perspective, the presence of a detached phase means that the headloss at the beginning of the cycle will be higher than if all the bed voids were cleared. It may also mean that some of that detached phase is more likely to get through the filter during a cycle and to end up in the effluent. However, backwash is a stressful phenomenon in the operating cycle of a BAF. The presence of a detached biomass may improve the recovery of the system by adding a suspended component to the attached biomass, insuring continuity from cycle to cycle. Additionally, it may improve the trapping of particles from the influent as it fills the pores of the bed. The detached biomass may also have a different response to shock loads or toxicity. The intensity and type of backwash is, therefore, critical as both will influence which part of the biomass is sloughed off the media, and what percentage of the detached biomass will be left in the system. It is expected that a carefully designed backwash strategy could reduce the impact of the backwash on the system, while minimizing the amount of effluent water wasted in backwashing the filter.