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Electrochemical detection of the microbial activity of heterotrophic and autotrophic activated sludge organisms

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Abstract

The microbial activity of microorganisms in biological waste water treatment was determined by means of an electrochemical bioactivity sensor (BAS). The signal of the sensor system is proportional to the substrate degradation and thus to the decisive target value in sewage treatment. By using physiologically active substances the aptitude of the sensor for detecting inhibition of microorganisms in active sludge was investigated. The signals of the BAS were correlated with an established method for determining microbial activity. For this purpose the total dehydrogenase activity as the key enzyme of metabolism was investigated. A good agreement was found between the measuring results. The BAS delivers values online and thus these values are available for automation and control tasks.

The electrochemical bioactivity sensor provides a novel measuring system for determining the activity of both heterotrophic and autotrophic microorganisms in biological waste water treatment.

Introduction

The degradation of the bulk of organic pollution in waste water treatment takes place by various aerobic, anoxic and anaerobic processes of heterotrophic and autotrophic microorganisms. In practice problems keep cropping up with regard to converting this microbial efficiency stably and setting optimal conditions for the microorganisms. Both the degradation efficiency and the long-term stability of the biological degradation processes in sewage treatment plants is determined by the activity of the organisms and the concentration of the heterotrophic and autotrophic biomass which is involved in these processes. However, these fundamental process parameters cannot be determined by present-day methods [1]. In this respect the automised characterisation of the state of the cell can improve process control in terms of stability and reveal the potential for optimisation. Even preventive measures, such as separate pre-treatment or the interception of partial waste water flows, would then be possible.

Of late the necessity of developing a measuring technique for the determination of microbial activity has, therefore, featured more prominently in the literature. The objective of such a bioactivity sensor is the qualitative and quantitative characterisation of the physiological state of the microorganisms. By means of activity sensors it is possible to establish the current condition of cells and, thus, the

degradation potential of microorganism cultures [2]. The aim of the present project is to set up such a measuring technique with practical features. If the online determination of metabolism is successful it will open up interesting perspectives for optimising biological waste water treatment.

The objective of biological waste water treatment is to eliminate waste water ingredients by microbial activity. By this means the results of a method for determining microbial activity should be correlated with this degradation of ingredients or substrate. A scheme of substrate degradation is given in Figure 1.

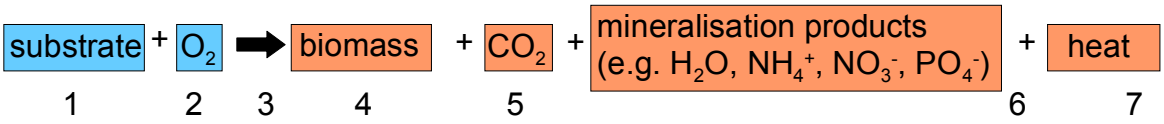

Figure 1: Scheme of microbial processes during substrate degradation (1 = substrate decrease, 2 = oxygen consumption, 3 = enzyme activity, 4 = biomass growth, 5 = CO_2 development, 6 = pH value, redox potential and changes in conductivity, 7 = heat development)

These processes generate a multitude of possible measuring methods which are proportional to the substrate degradation and thus the microbial activity. Substrate degradation, oxygen consumption, biomass formation, carbon dioxide formation and also the changes in conditions in the medium (pH value, redox potential, conductivity) can be established by measuring techniques. However, conclusions cannot necessarily be drawn on microbial activity from the results of a single parameter. The change in pH value of the culture medium can indicate a certain metabolic activity; for example, many fungi and bacteria excrete acids when metabolising glucose, the cause may, however, be a change in pH value in the waste water inlet. With inhomogeneous composition of waste water the determination of a specific enzyme activity is not a suitable indicator of microbial activity. The possible causes of a change in enzyme activity are, for instance, even microorganisms with different metabolic pathways or the absence of inducing substrates. Not even the measurement of biomass content permits a reliable statement on the concentration of active biomass in the reactor, thus in waste water treatment it is assumed that only approximately 50% of biomass is metabolically active. In the past very varied methods were applied to detect the microbial activity of active sludge. A brief survey is given in the following list.

Parameters for the detection of the microbial activity of microorganisms:

> turbidity measurement, biomass, enzyme activities (e.g. dehydrogenase activity, DMSO reduction, catalase activity), DNA content, ATP content, total protein content, waste gas analysis, respiration measurement, NADH fluorescence, calorimetric methods, acoustic resonance density (ARD), impedance measurements, ultrasound techniques.

The practical application of these methods, however, can cause considerable problems. Some of these methods are too expensive in terms of time and personnel, they cannot be automated or they do not provide test results online.

Only the determination of oxygen consumption is relatively widespread. Table 1 gives several examples of measuring instruments and procedures based on the principle of oxygen consumption measurement to detect microbial activity (see also [3] and [32]).

Table 1: Examples of measuring instruments and procedures for microbial activity detection based on oxygen consumption

Measuring instrument and procedure	Literature
Short-time respiratory test	[4]
Bacteria toximeter	[5]
BASF toximeter	[6]
Bio monitor	[7]
BSB sensor	[8]
Determination of inhibition of oxygen consumption of active sludge	[9]
BSB and toxicity test	[10]
Measurement of oxygen consumption	[11]
Hybrid respirometric method	[12]
Parallel measurement of O_2 and CO_2	[13]
STIPTOX	[14]
ToxAlarm pro	[15]
RodTox	[16], [17]
Toxicity monitor	[18]
Consumption / BSB analysis	[19], [20]
Nitrification biosensor	[21], [22]
Determination of oxygen transfer rate in a shaking flask	[23]
Toxicity monitor	[24]
CSTR toximeter	[25]

However, even the application of consumption measurement is subject to some limitations: besides respiration during the breakdown of dissolved substrate, other chemical and biochemical processes take place which may contribute to oxygen consumption. When measuring oxygen consumption it is not possible to distinguish between these processes. For example, during the degradation of storage substances no measuring technique can distinguish between intrinsic respiration and respiration during the consumption of waste water ingredients.

Uncoupling substances, for example, cause measuring problems (e.g. 2,4-dinitrophenol). Even if such substances eventually cause damage, resulting in a decrease in respiration, the acute effect cannot be demonstrated. Moreover, the respiratory measurement cannot be applied when highly oxygen-consuming test substances or waste waters are tested [26]. Even ingredients with coalescence stimulating properties (in technical fermentations, for instance, anti-foaming agents) can cause problems when determining oxygen consumption. The problems that can arise when using respirometers are discussed in [25]. The use of consumption measuring instruments for control purposes, such as control of oxygenation or excess sludge removal, has only rarely been undertaken hitherto [3].

When evaluating biological activity in waste water treatment plants, active sludge from the sewage treatment plant is not necessarily applied. Methods are used which apply adapted or normed organisms, such as luminous bacteria. The results, however, cannot be unreservedly transferred to

real conditions in the sewage treatment plant. For this reason a basic requirement of a system for detecting the activity of sewage sludges is that the culture from the activated sludge tank be used. This takes into account the dynamic changes in bacteria population when determining activity.

The operators of waste water treatment plants should aim to assess the potentially toxic effect of the waste water on the process organisms continuously. Depending on local conditions, this preventive measure makes it possible to intercept, accumulate or pre-treat critical batches. Effective protection is, however, only possible when appropriate methods are available to detect inhibiting influences immediately, biospecifically and reliably. An abundance of static and dynamic standardised laboratory tests do make it possible to assess waste water with regard to degradability and toxicity; generally, however, there is only a limited possibility of applying them for process control due to their lack of biospecificity and the length of time they require. Our objective is to develop online analysers to determine the degree of toxicity of waste water flows at any given time [24]. Here, too, online bioactivity measurement would be a suitable basis for such a measuring method.

One of the main tasks of modern waste water treatment plants is, and will increasingly become so, the most efficient possible removal of nitrogen compounds as they contribute to the euthrophisation of natural waters and, above pH 8.5, the ammonia released from ammonium is highly toxic to fish and amphibians. In waste water treatment plants the elimination of nitrogen compounds generally occurs microbiologically by the combination of nitrification and denitrification, whereby nitrification is the Achilles heel of nitrogen removal. Autotrophic nitrifying bacteria are particularly sensitive, for instance, to toxic substances, low temperatures, low oxygen concentration and unfavourable pH values [27]. Another effect which explains the relatively high sensitivity of nitrifying bacteria, is the comparatively low diversity of species compared with heterotrophic organisms. This provides favourable conditions for causing lasting damage to this partial biocenosis [28]. For this reason breakdowns in nitrification performance are a fairly regular occurrence in water treatment plants. This sensitive, dynamic system calls for a measuring technique to determine the microbial activity in order to protect the plant.

With respect to flexible process control, special demands should be made of online measuring for water treatment plants. The main priority does not always have to be improving control, but even novel measurement methods can trigger off considerable savings of energy and auxiliary agents [29].

Materials and Methods

The investigations were carried out in a laboratory water treatment plant in accordance with DIN 38412 part 24 [30]. This plant consisted of an aerobic reactor and a sedimentation tank. The operating data of the plant are summarised in Table 2.

Table 2: Operating data of the laboratory water treatment plant

Description	Value	Unit
Filling volume reactor	6.2	L
Filing volume sedimentation tank	2.4	L
Waste water feed	10	L d^{-1}
Flow of return sludge	10	L d^{-1}
Ratio of return sludge	1	-

The laboratory water treatment plant was run on communal sewage sludge and synthetic waste water (complex medium with carbon and nitrogen source modified according to [30]: 160 mg/L pepton, 110 mg/L meat extract, 120 mg/L ammonium chloride, 28 mg/L K_2HPO_4, 7 mg/L NaCl, 4 mg/L $CaCl_2 \cdot 2 H_2O$, 2 mg/L $MgSO_4 \cdot 7 H_2O$) or a medium containing ammonium as the sole nutrient (5.04 g monosodium carbonate ($NaHCO_3$) and 2.65 g ammonium sulfate $(NH_4)_2SO_4$ are dissolved in one litre of bidistilled H_2O). When this substrate is diluted 1 : 10 it contains 56 mg nitrogen per L and has a pH value of approximately 7.6. With this substrate the production of 25 mg/L of oxidised nitrogen compounds can be buffered without the pH value changing. The degradation of the waste water ingredients was followed up by analysis of CSB, BSB, DOC and also of the nitrogen products ammonium, nitrite and nitrate.

The detection of microbial activity is carried out with a bioactivity sensor (BAS). The measurement is based on the recording of an electron flow which is induced by microbial energy metabolism. The microorganisms oxidise the degradable waste water ingredients for energy production. In the framework of these intracellular processes reduced metabolites are released. They are oxidised at the anode of the sensor, while electrons are transferred with the release of hydrogen ions. These electrons wander through an external circuit to the cathode where the potential drop is recorded over a resistance. The electrons react at the cathode with the oxygen and the hydrogen ions produced at the anode to water. These reactions are possible as long as sufficient usable substrate is available in the medium and no inhibitions occur. Fig. 2 shows the principle of the sensor system and Fig. 3 a photo of the components of the BAS.

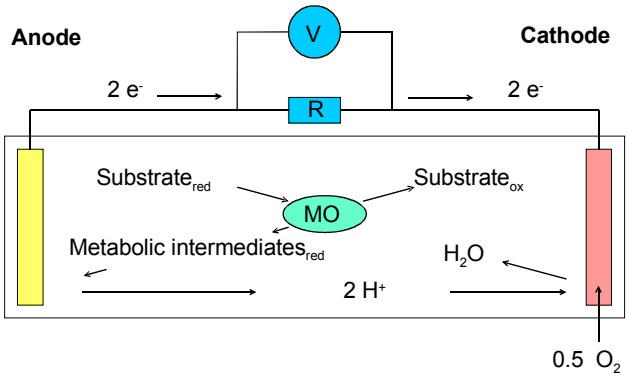

Fig. 2 Scheme of the BAS (MO = microorganism)

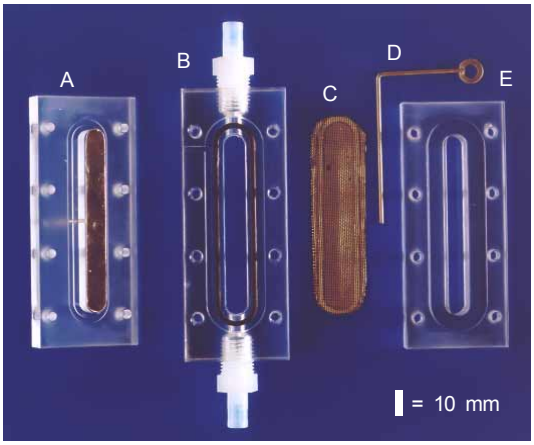

Fig. 3 Photo of the components of the BAS (A = anode part with affixed anode, B = middle part, C = cathode, D = cathode contact, E = cathode part)

The BAS consists of an anode and a cathode enclosed in a poly(methyl methacrylate) case. Platinum foil or platinised titanium (anode surface = 530 mm²) is used as the anode, an oxygen diffusion electrode as the cathode. The cathode consists of a support net to which a catalyst mixture is attached and a PTFE foil which serves as the electrolyte barrier. The BAS casing has three parts, the middle part forming the flow channel. The corresponding tubes are screwed to the interior of the BAS. The platinum anode is stuck to the corresponding side part and the oxygen diffusion cathode is placed in the second side part. The external dimensions of the BAS are 105 x 46 x 40 mm.

The BAS was applied in the bypass of the laboratory treatment plant; a volume flow of 250 mL/min was pumped through the sensor cell. As soon as the plant was in a steady state the operating and feed conditions were varied and the resulting signal changes recorded.

The signals of the BAS were correlated with various established methods of activity determination; for this purpose the dehydrogenase activity was determined by the TTC test, oxygen consumption, protein content according to Bradford, optical density, biomass formation and substrate consumption.

Results

- **Detection of the activity of heterotrophic microorganisms**

Fig. 4 shows the dosing of different volumes of substrate into the laboratory treatment plant; the substrate used was a highly concentrated synthetic waste water according to DIN. The activated sludge biocenosis was adapted to this substrate, thus all the enzymes necessary for the degradation of the waste water ingredients were synthesised by the microorganisms.

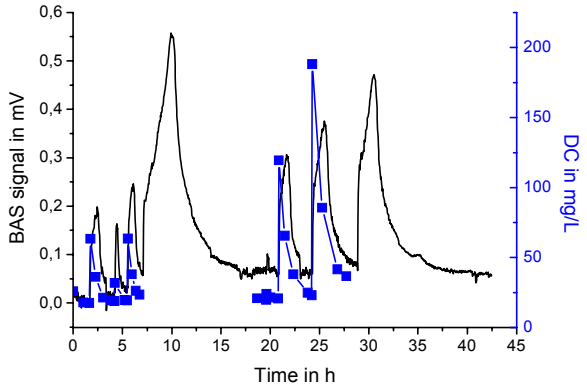

Fig. 4 Signal of the BAS and the course of the DC (dissolved carbon) after repeated substrate dosing

The BAS signal always rose immediately after substrate dosing. The additional supply of substrate stimulated the energy metabolism of the microorganisms. This leads to enhanced leaching of the reduced metabolic substrates which are converted in the BAS. A comparison of the retention times measured by DC with theoretically calculated values for the retention times clearly reveals the elimination of microbes; only a small part of the substrate is washed out. The signals of the BAS show dependencies on the supply of nutrients with regard to peak maximum, peak area and the time until the BAS signal has once again reached its starting level. Figure 5 shows the peak area as a function of the corresponding substrate concentrations.

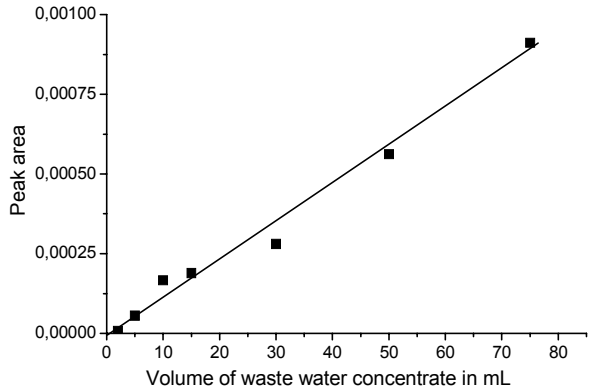

Fig. 5 Peak area as a function of substrate dosing

The result is a linear relation between the signal of the BAS and the available substrate doses. This shows that the substrate present can be deduced from the BAS signal. For control tasks, such as control of the oxygen concentration in the reactor, the immediate rise of the BAS signal would suffice to take corresponding measures (increase the air flow). Given a corresponding control concept, the further course of the signal would bring about further tracking of the corresponding parameter.

In addition these investigations also served to show that even the slightest changes in pollution and the related change in microbial activity could be demonstrated with the BAS. A dosage of 2 mL of waste water concentration can be clearly detected; this is equivalent to a COD load of 21 mg O_2/L in the reactor. This detection limit changes according to the microbial degradability of the substrates: a scarcely degradable substance can only be detected in significantly higher concentrations. The BAS, therefore, gives information on the biological degradability of various substrates.

Figure 6 shows the course of the BAS signals after dosing different substrates into the reactor of the model water treatment plant. In order to ensure comparable starting conditions the doses of substrates were calculated. The fact that with pure substances the CSB can be calculated was fully utilised. For substances which merely contain carbon, hydrogen and oxygen (molecular formula $C_cH_hO_o$) this yields a simplified equation [31]:

$$COD = \frac{16(2c + \tfrac{1}{2}h - o)}{M}$$

Substrates with varying degrees of good degradability were used (maltose, methanol, ethylene glycol and iso-propanol). In each case 20 g CSB were dosed into the reactor.

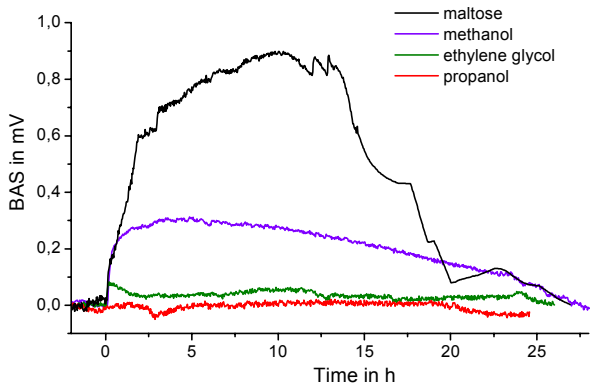

Fig. 6 BAS signal after dosing various substrates

After dosing substrate the different effects on the signal of the BAS were registered. Maltose and methanol brought about a distinct change, ethylene glycol only made a slight impact, and after dosing propanol no change to the BAS signal was registered. This had been anticipated. Maltose and methanol are easily degradable, by contrast of all the microorganisms found in waste water treatment plants ethylene glycol and iso-propanol are either not at all, or only with difficulty, degradable. The

application of different concentrations of these substrates for each substance shows a linear relation between the signal of the BAS and the concentration used according to Figure 5. The following progression of microbial degradability can be made for the available activated sludge population:

maltose > methanol > ethylene glycol > propanol

To verify the results of the BAS the signals were correlated with established methods of microbial activity determination. Figure 7 shows the course of the BAS signal and dehydrogenase activity after dosing glucose into the reactor of the laboratory waste water treatment plant.

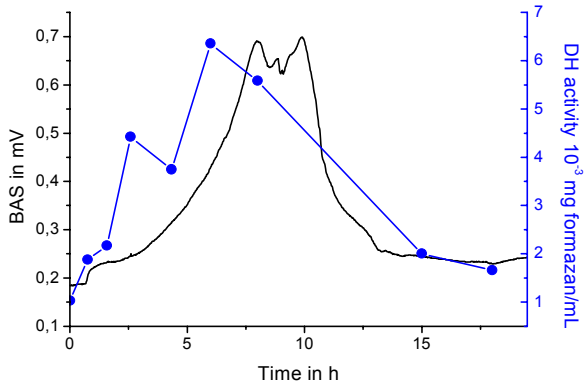

Fig. 7 Correlation of the BAS signal with dehydrogenase activity

After the additional substrate doses both the signal of the activity sensor and the measurable enzyme activity rose. Even the subsequent time course of the measuring signals were comparable, although the signal of the BAS is, of course, available online. By contrast, to determine dehydrogenase activity it takes 2 hours to obtain a result.

In further investigations, batch tests with the model organism *E. coli* also revealed correlations between the signal of the BAS and oxygen consumption, protein content, optical density, biomass formation and substrate consumption. The signal of the BAS can, therefore, be regarded as a novel method of activity determination whose signals correlate with the established methods of determining microbial activity.

Two BAS were operated in parallel at a reactor in order to compare various sensors and to obtain data unvarying production of sensors. During the batch trial the BAS signals ran almost parallel. The mean deviation in current signals between the sensor cells was 7%. Concurrently a batch test with *E. coli* was run five times, the deviations between the resulting BAS signal courses being in the region of 15% from the mean. This demonstrates the fact that the measurement method provides reproducible results.

- **Determination of the activity of nitrifying bacteria**

These investigations were carried out in medium containing ammonium. The laboratory waste water treatment plant was continually fed with substrate and when the system was in a steady state the operating conditions were varied. Figure 8 shows the course of the BAS signal and the concentrations of ammonium and nitrite after a shock dose of ammonium into the reactor.

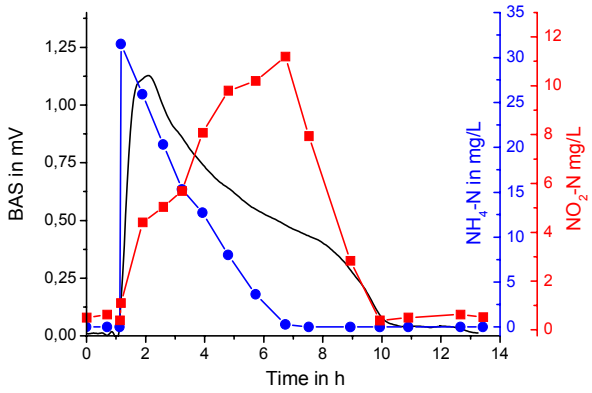

Fig. 8 Signal of the BAS and concentrations of ammonium and nitrite after dosing ammonium into the laboratory waste water treatment plant

After dosing, the BAS signal rose immediately; this can be attributed to the activity of the ammonium-oxidising organisms. Their metabolism produces nitrite which is converted into nitrate by the metabolism of the nitrite-oxidising organisms. The activity measured reaches the starting value simultaneously with the complete degradation of both the ammonium and the nitrite. Therefore the activity both of the ammonium and of the nitrite oxidisers is detected by the BAS. Figure 9 shows the course of the BAS signals after dosing various amounts of ammonium into the reactor.

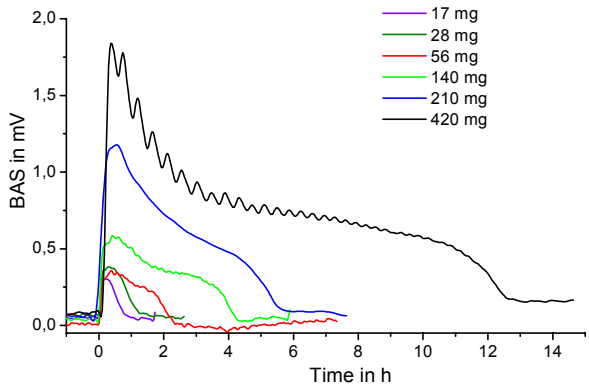

Fig. 9 Signal of the BAS after dosing various amounts of ammonium into the reactor

A dose always caused an immediate increase in activity. This can be ascribed to the substrate-limited operation mode of the waste water treatment plant. Peak height and peak area are dependent on

ammonium content. Thus in this case, too, the sensor signal is dependent on the degradation of the substrate. The 17 mg ammonium added correspond to a concentration of 2.7 mg ammonium nitrogen/L. This added substrate brings about a distinct change in the activity signal and lies far above a detection limit.

Figure 10 presents the inhibiting effect of the nitrification inhibitor allyl thiourea (ATU). The first experiment was carried out without any inhibiting impact. In the second ATU was dosed into the reactor shortly after substrate dosing. The nitrifying bacteria were inhibited and accordingly the signal of the BAS dropped immediately to the base signal. Consequently substrate was again dosed but as no more active microorganisms were present there was no change in the BAS signal.

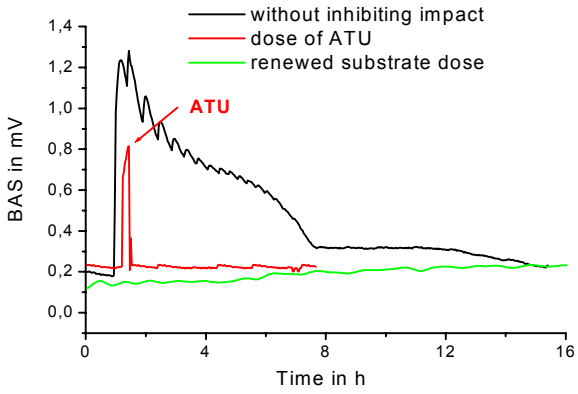

Fig. 10 Evidence of inhibiting effects with the BAS – use of the nitrification inhibitor ATU

- **Simultaneous determination of the activities of hetero- and autotrophic microorganisms**

Figure 11 shows the starting phase of an experiment in the laboratory waste water treatment plant. In the first 24 hours a steep increase in the BAS signal can be observed; this correlates with a marked DOC elimination at this time. At the beginning of the experiment a 120 mg/L DOC was measured; after 24 hours over 90 % of the substrate had been degraded.

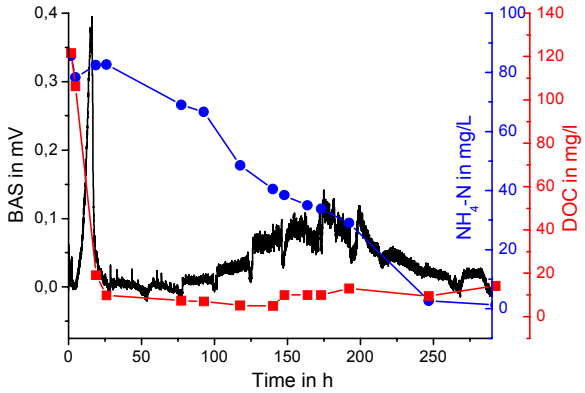

Fig. 11 Determination of the microbial activity of heterotrophic and autotrophic microorganisms in parallel

The concentration of ammonium did not change significantly during this period. Only in the course of the process did it come to a significant decrease in the substrate of the nitrifying bacteria. This corresponds to the frequently described phenomenon in the literature that, compared with autotrophic microorganisms, heterotrophic carbon-degrading organisms have a more effective metabolism. In the further course of the process there was a rise in the BAS signal after approximately 100 hours on account of the increased activity of the autotrophic microorganisms. The peak correlated with an enhanced breakdown of ammonium. The BAS signal reached a constant low level when the ammonium was almost completely consumed. The tests with the complex medium showed that the activities of the heterotrophic and autotrophic biomass can be determined in parallel. As was to be expected, in doing so the total signal was determined by the heterotrophic carbon-degrading organisms.

Discussion

The online recording of metabolic activity is successful using the bioactivity sensor described. The method can account for the dynamic process of biological waste water cleaning at any given time. This opens up interesting perspectives for optimising waste water treatment. For instance, based on microbial activity it is possible to optimise the oxygen feed in the aerobic steps, the dosing of easily degradable carbon sources in the denitrification or the control of cycle time in sequencing batch reactors.

The bioactivity sensor uses biomass directly from the reactor. For this reason by means of this determination method information on the physiological state of the corresponding biomass can be derived and the findings can be directly transferred to the plant. With methods which apply specific test organisms this is only possible to a limited extent.

Since inhibiting effects and a change in nutrient supply are recorded, the treatment process can be designed more flexibly. This means that batches fluctuating between daily minimum and maximum values can be compensated for by optimal control processes. Particularly for industry the indication of inhibiting substances is of great importance. Breakdowns incur not only higher costs, but a loss of image, the price of which is incalculable.

In contrast to respiratory measurement processes, this method is independent of oxygen consumption. This means that on the one hand the chemical processes, which influence oxygen solubility, are not taken into account and on the other hand the dependencies of microbial activity on oxygen concentration can be indicated. The BAS should be regarded less as a competitor to the respiratory methods than as a complement; the measurement principles should permit further improvements in the automation of waste water treatment plants. Even their use in substitution concepts should be the subject of further investigations.

A further important demand made of online devices, such as the BAS, is the convenience of the system. This means that maintenance costs have to be low and the equipment solidly constructed so that down time is kept to a minimum. In order to integrate it into an automated concept dead time during measurements should be short. Since long transportation distances also involve long dead

times for the sample, an online unit should be set up as close as possible to the place of measurement. The BAS fulfils these requirements.

The BAS is designed in a way that makes fine filtration superfluous. Only in the inlet area is the use of a coarse filter optimal. The BAS can be operated without auxiliary chemicals which means that, from this point of view, long-term operation is not limited. The sensor system has been designed to keep maintenance to a minimum, only the oxygen diffusion cathode requires changing from time to time. By integrating the system into a weatherproof case it can even be set up on the spot outdoors, enabling the sensor to be installed directly at the measurement location.

Abbreviations

ATP	adenosine 5'-triphosphate
ATU	allyl thiourea
BAS	bioactivity sensor
BOD	biological oxygen demand
c	stoichiometric factor for carbon
CFU	colony-forming units
COD	chemical oxygen demand
DC	dissolved carbon
DH	dehydrogenases
DMSO	dimethyl suloxide
DNA	deoxyribonucleic acid
DOC	dissolved organic carbon
h	stoichiometric factor for hydrogen
M	molecular weight
MO	microorganism
NADH	nicotinamide adenine dinucleotide, reduced form
NH_4N	ammonium nitrogen
NO_3N	nitrate nitrogen
o	stoichiometric factor for oxygen
PTFE	polytetrafluoroethylene
TTC	triphenyl tetrazolium choloride

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