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MINI REVIEW

Development and implementation of microbial sensors for efficient process control in wastewater treatment plants

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Abstract This paper demonstrates the functionality, laboratory testing and field application of a microbial sensor, which can be modified to monitor organic pollution extent, toxicity and over-(under)load of wastewaters both under anaerobic and aerobic conditions. Since nitrification is related to protons formation and the addition of alkaline is necessary for pH control, an aerobic biosensor monitoring Na_2CO_3 consumption was developed and practically implemented to control the nitrification process. As CO_2 is the respiration product from aerobic degradation which can be correlated to the organic pollution extent, the previous biosensor was modified to monitor and measure the online toxicity and BOD/COD. Under anaerobic conditions, the online measurement of NaOH consumption and biogas production allowed the detection of toxicity incidents and over-(under)load in the influent. Such toximeters get in contact with the wastewater the earliest possible, providing sufficient time for protection of sensitive biological wastewater treatment processes and for the implementation of control and management strategies.

Keywords Biosensors · Aerobic/anaerobic · WWTP control · Toxicity

Introduction

Biological treatment of organic pollutants (carbon, nitrogen) in wastewater plants is accomplished by microbial activities. Its effectiveness depends on a series of biological and physical parameters such as temperature, pH, qualitative and quantitative composition of wastewater, including nutrients and inhibiting or toxic components, as well as hydraulic and organic loading rates. Those parameters may strongly vary making performance control more difficult [1–3]. Thus, effectiveness and reliable control of the biological units are strongly related to the availability and analysis of real-time information.

Online monitoring of microbial activity and potential toxicity in wastewaters could be achieved by biosensors. Biosensors are self-contained integrated devices and consist of a biological sensing component (e.g. receptor, enzyme, microbe, antibody), which is placed in contact with a chemical or physical transducer (electrode, electrochemical, optical, mass or thermal) [4, 5]. There are a number of biosensors classified by the transducers into electrochemical, optical and other types of microbial sensors [6]. Various microbial sensors have been developed for indirect online biochemical oxygen demand determination that are generally based on respirographic methods [4, 7–12]. Respirographic methods are also frequently used for online toxicity detection on the nitrifying population [13], and such a device is called the NITROX [14]. Alternatively, the pH effect of the nitrification can be used for quantification of the corresponding activity [1, 14]. As far as anaerobic biosensors are concerned, the most well known is an upstream anaerobic biosensor, known as RANTOX, which monitors overload and toxicity by measuring maximum biogas production [15]. External addition of acetic acid is necessary, whereas, sinter glass carriers are used for maximizing biomass concentration.

This paper demonstrates the functionality, laboratory testing and field application of a microbial sensor, which can be modified to monitor organic pollution extent,

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toxicity and over-(under)load of wastewaters, both under anaerobic and aerobic conditions, aiming to control the biological processes in wastewater treatment.

Materials and methods

Aerobic biosensor

The major part of the system consists of a small conical fluidized bed reactor (50 ml) and a cylindrical oxygen saturation chamber (50 ml). Both cells made of Plexiglas are placed separate from each other, however, they communicate hydraulically in a parallel mode. External biomass feeding for the microbial sensor was unnecessary, because microbial population is immobilized on a reticulated sinter glass (Siran®) placed in the small fluidized bed reactor, which resulted in a high biomass concentration. Thus, the biologically sensing component of the biosensor is the immobilized biomass. Figure 1 depicts the general layout of the biosensor used under anaerobic and aerobic conditions for nitrification control, toxicity and over-(under)load detection. In particular, when the biosensor was tested under aerobic conditions, temperature was kept constant at 25°C (room temperature). The pH in the fluidized bed reactor was controlled at 6.4 by the addition of an alkaline solution (NaOH or Na_2CO_3), using a pH controller and a peristaltic pump. Before its introduction to the central part of the microbial sensor, physically diluted CO_2 in the influent was initially stripped with air. A pH-controller was used to keep the pH at 4 with 0.2 N H_2SO_4. Online data acquisition was accomplished using a microcomputer, supported with specific software (Labtech) running on Microsoft Windows. A data interface device converted analogue output signals of a pH-controller in the fluidized bed reactor and CO_2 analyzer into compatible for the computer digital input signals.

Nitrification control aerobic biosensor

The aerobic biosensor was implemented as a toxicity detector and nitrification controller. The working principle of this modification of the biosensor is based on the nitrification reaction. According to the reaction stoichiometry, 1.98 mol of protons is produced per mole of oxidized ammonium (NH_4^+-N), causing a significant pH decrease, which needs to be continuously compensated by an alkaline solution in order to maintain optimum microbial activity. This is realized in the biosensor by a pH controller at 6.4. The consumption rate of a defined alkaline solution is closely related with the rate of proton formation and thus, it can be used for the online monitoring of nitrification activity. In an acidic environment, carbon dioxide is removed. Since the nitrifying species need inorganic carbon for their growth, its reduction would constitute restriction of their growth. In order to avoid any carbon limitation for these species in the case of ammonium-rich

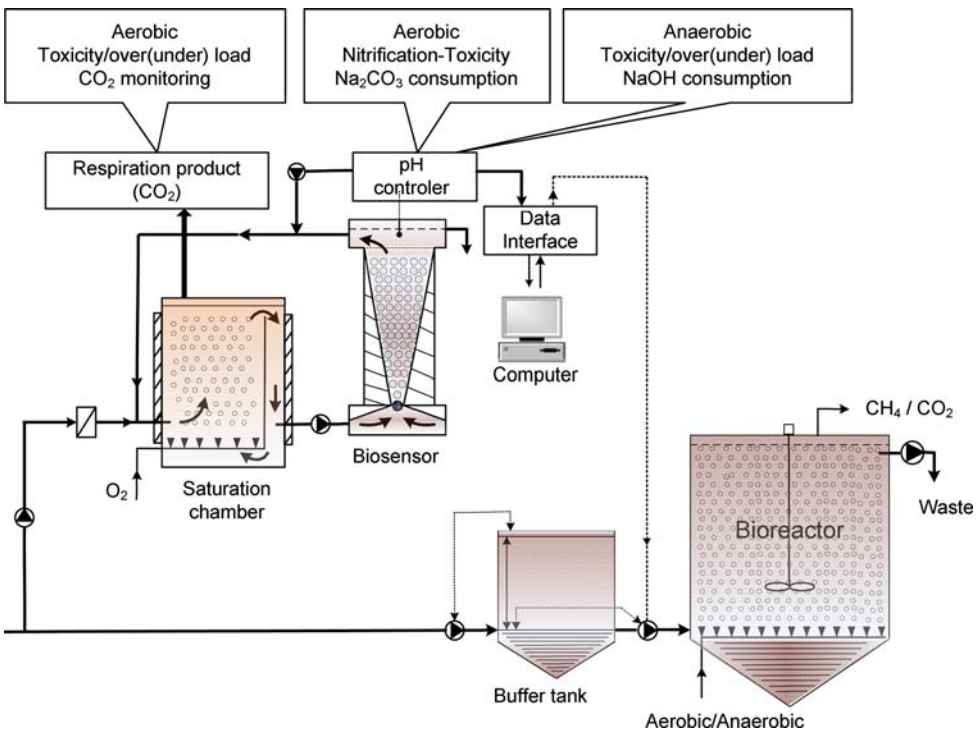

Fig. 1 Basic working principle of the biosensor applications

wastewaters with low carbon content, sodium carbonate Na_2CO_3 is preferentially used for neutralization. Using a calibrated pump for the base addition, the consumption rate of Na_2CO_3 can be easily calculated by a computer, and thus, at a given Na_2CO_3 molarity, the rate of ammonium oxidation results in kg NH_4^+-N/(m^3 day) (nitrification rate). Toxicity can be determined by a decrease of alkaline consumption, which implies microbial activity inhibition. Further details can be found in [2].

Oxygen demand (OD) and toxicity monitoring aerobic biosensor

A wide as well as highly active microbial spectrum is available for the biooxidization of organic matter entering the biosensor. The inoculum is the biomass coming from the bioreactor that is to be controlled. The immobilized biomass of the biosensor as well as the bacterial population of the monitored wastewater treatment plant perform alike biodegrading the same types of organic waste components with CO_2 as a final product, and allowing direct correlation between them.

The basic idea of the microbial aerobic biosensor for biological OD and/or chemical OD determination, as well as toxicity detection depends on online measurement of the CO_2 concentration in the off gas. Under constant operating conditions (oxygen flow rate, pH and residence time), CO_2 concentration closely relates to the extent of organic pollution. Toxicity is detected by a decrease in CO_2 concentration in the off gas. Further details can be found in [3, 16, 17].

Anaerobic biosensor

The anaerobic biosensor is developed for toxicity determination and over-(under)load monitoring. The major part of the system is actually the same and consists of an anaerobic fluidized bed reactor and a cylindrical control chamber (Fig. 2).

The anaerobic bioreactor is thermostated at 37°C and pH is controlled at 6.8. The alkali consumption is calculated by a PC, using the signal from the NaOH feeding pump. Biogas was measured by a volumetric gas meter. The anaerobic biosensor can be fed with either an acidic wastewater or with wastewater produced after acidogenesis. The experimental procedure includes the establishment of a baseline under undisturbed operation, both for biogas production from anaerobic microbial respiration and for the alkali consumption, which is used for pH correction. The presence of a toxicant is detected by fluctuations in the biogas production and alkali consumption, which stand for microbial activity.

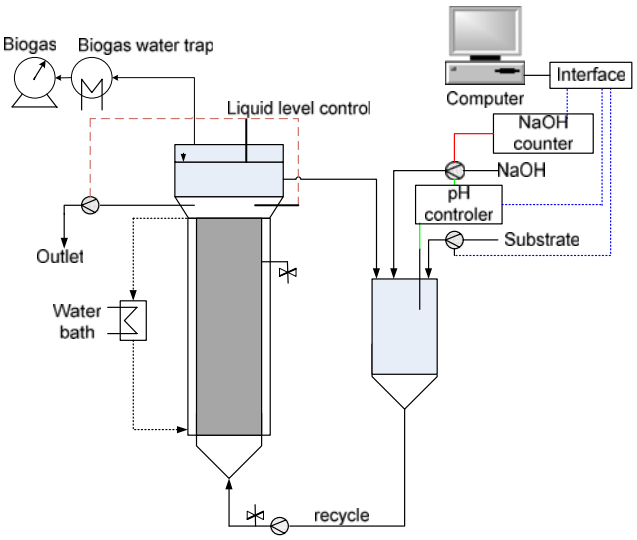

Fig. 2 Schematic layout of the anaerobic biosensor

Results and discussion

Nitrification control aerobic biosensor

For the verification of the practical suitability of the above-described process control concept, programmed intoxication experiments were performed using phenol as a nitrification inhibitor to an aerobic pilot bioreactor of 45 L. The concentration of phenol in the influent was set at 500 mg/L. At a flow rate of 1.5 L/h, corresponding to a residence time of 30 h in the pilot plant, phenol enriched wastewater was introduced over a period of 4 h. The system was set up so that the wastewater supply from the storage tank to the bioreactor was interrupted automatically by the process computer. Actually, the computer initiates the shutdown of the corresponding pump (after 1.5 h) when microbial activity is inhibited by 50% (reduction of the space–time yield) in the biosensor. Within 1 h, the biosensor recognizes the toxic influent, indicated by a sharp decrease of the microbial activity, whereas in the pilot plant the activity inhibition (deterioration) just begins after 4 h. In particular the ammonium conversion in the pilot plant drops in the first 8 h by only 6% and in 12 h after the addition of phenol by 20% (Fig. 3). The wastewater was stored in a buffer tank over a period of 8 h, before the process control unit activated the influent pump. This provides an opportunity for using off-line set points different from the design value, and enables the plant to operate for a certain period of time at a flow rate below the design value, to avoid overloading by the increased dilution of the spillage.

In an additional experiment, the toxicity was monitored by the biosensor, however, the process control unit did not interfere (curve D, Fig. 4). This experiment was repeated with the differences that the buffer tank was used for

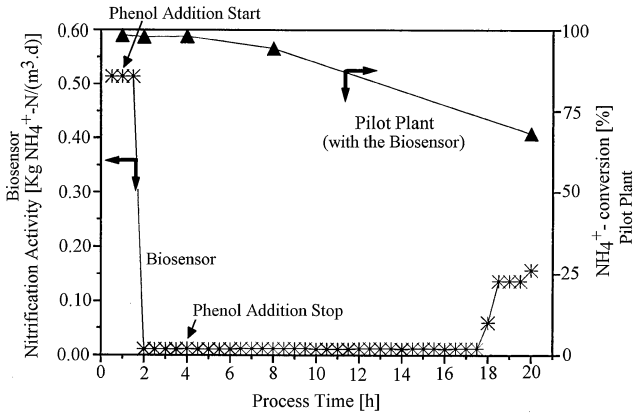

Fig. 3 Nitrification activity in the aerobic biosensor (*) and ammonia conversion in the pilot plant (*filled triangle*) after the toxic impose of phenol (0.5 g/L) at zero time in the influent wastewater

equalization, which was operated at the maximum liquid level (about 50 L) (curve E, Fig. 4). The pilot plant's treatment performance, expressed as ammonia nitrogen conversion over the process time, for three experimental sets of decreasing the influent flow rate using the biosensor in closed-loop (curves A, B, C), is depicted in Fig. 4. These pilot-scale results indicate that process operation with the integrated biosensor in a closed-loop control strategy is superior compared to the uncontrolled operation. Specifically, in the former case, the plant's performance recovers after a period of approximately 1 week, whereas, in the uncontrolled case the ammonium conversion deteriorates strongly, and even after 7 days of operation the plant does not significantly recover.

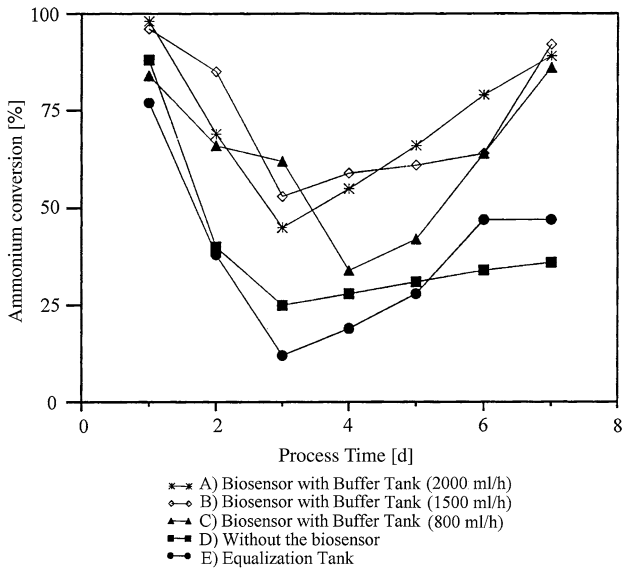

Fig. 4 NH_4^+-N conversion in the nitrification reactor at different operation modes. Curves A, B, C: toxicity monitoring and closed-loop process control. Curves D, E: no process control system

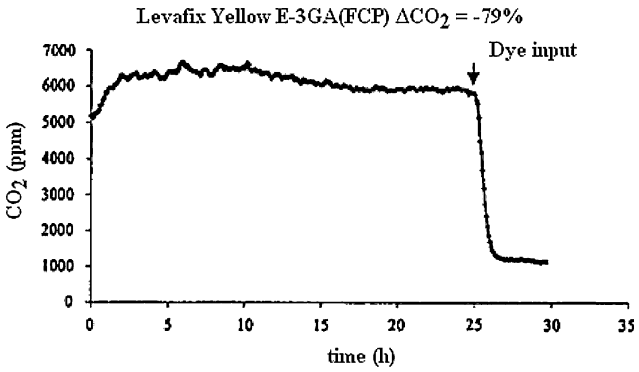

Fig. 5 Inhibition effect of azo-reactive dyes

The aerobic biosensor was also implemented as an early warning system in a wastewater treatment plant of a petrochemic industry proving to successfully predict upset in the full-scale plant [2].

Oxygen demand and toxicity monitoring aerobic biosensor

The efficiency of the aerobic biosensor applied as a toximeter was also tested using azo-reactive dyes (Levafix and Remazol). After the CO_2 baseline was obtained and stabilized, the toxicants were dissolved in the nutritious solution and the microbial activity was monitored. The inhibitory effect of the dyes on the activated-sludge microbes is clearly shown in Fig. 5 [17].

Furthermore, the biosensor was used as an online BOD and COD meter by monitoring CO_2 concentration and correlating it with BOD or COD extent in the wastewater (Fig. 6). In particular, CO_2 concentration under constant operation conditions is closely related to the extent of organic pollution allowing continuous online monitoring of the organic load within a wastewater treatment plant. Thus, real time process control and operation become feasible.

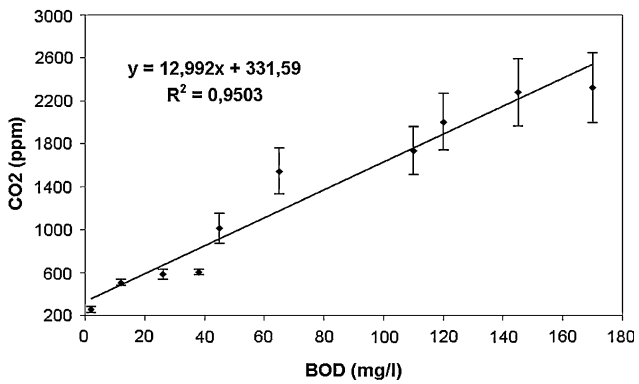

Fig. 6 Correlation between CO_2 respiratory product and BOD concentration

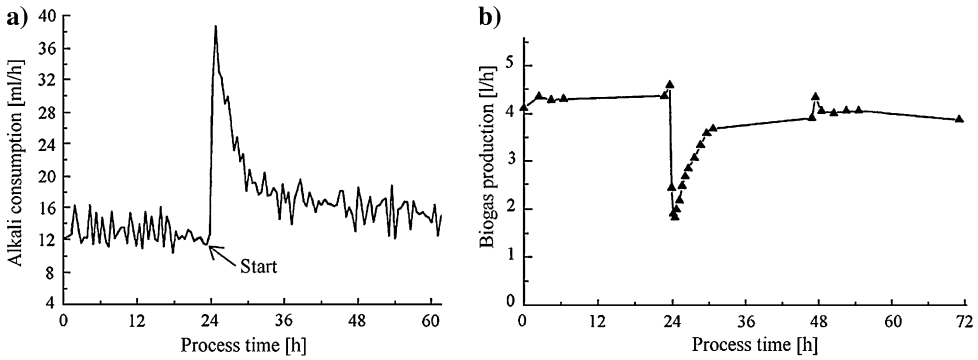

Fig. 7 Crotonaldeyde addition test: **a** alkali consumption and **b** biogas production before and after the inhibition

The BOD and COD meter was applied in the field for online monitoring of a municipal wastewater treatment plant effluent [3].

Anaerobic biosensor

Results show that inhibition in the respiration of the anaerobic microorganisms is evident due to an increase of the alkali consumption and a decrease of the biogas production (Fig. 7). After the inhibition, the microbes get adapted to toxic impose and the system returns to equilibrium, which is observed as a baseline, both for the alkali consumption as well as for the biogas production up to the same levels, as it was before the inhibition. The anaerobic biosensor was integrated in an anaerobic treatment pilot plant [18]. Wastewater supply to the biological stage was immediately interrupted by the control system, after the early diagnosis of upset. Influent wastewater was stored in the buffer tank for a certain period of time.

Conclusions

The functionality, laboratory testing and field application of a microbial sensor, which can be modified to an online monitor organic pollution extent, toxicity and over-(under)load of wastewaters, both under anaerobic and aerobic conditions, have been presented. Field application of the microbial sensor in an activated sludge treatment plant facilitates the unit's real-time reliable control and operation. Furthermore, implementation of the biosensor satisfies the necessity of continuous monitoring of potential toxicity in the influent wastewater to the treatment plant, which is the basic precondition for an appropriate flow pattern control. The main advantage of the biosensor placement, upstream the process, is the significant margin of time between detection of potential toxicity in the influent wastewater and the actual entry of the toxic flow into the full-scale plant.

References

1. Aivasidis A, Hochscherf H, Rottman G, Hagen T, Mertens M, Reiners G, Wandrey C (1992) Neuere konzepte zur prozessüberwachung und regelung bei der biologischen stickstoffelimination. Abwassertechnik 42(5):48–55
2. Melidis P, Aivasidis A (2005) Biosensor for toxic detection and process control in nitrification plants. J Environ Eng 131(4):658–663
3. Vaiopoulou E, Melidis P, Kampragou E, Aivasidis A (2005) Online load monitoring of wastewaters with a respirographic microbial sensor. Biosens Bioelectron 21:365–371
4. Nakamura H, Karube I (2003) Current research activity in biosensors. Analyt Bioanalyt Chem 377(3):446–468
5. Thévenot DR, Toth K, Durst RA, Wilson GS (2001) Electrochemical biosensors: recommended definitions and classification. Bios Bioelectr 16(1–2):121–131
6. Lei Y, Chen W, Mulchandani A (2006) Microbial biosensors. Anal Chim Acta 568(1–2):200–210
7. Guwya AJ, Buckland H, Hawkes FR, Hawkes DL (1998) Active biomass in activated sludge: Comparison of respirometry with catalase activity measured using an online monitor. Water Res 32(12):3705–3709
8. Gernaey K, Vanderhasselta A, Bogaerta H, Vanrolleghem P, Verstraete W (1998) Sensors to monitor biological oxygen removal and activated sludge settling. J Microbiol Methods 32(12):193–204
9. Heim S, Schnieder I, Binz D, Vogel A, Bilitewski U (1999) Development of an automated microbial sensor system. Biosens Bioelectron 14:187–193
10. Liu J, Bjornsson L, Mattiasson B (2000) Immobilized activated sludge based biosensor for biochemical oxygen demand measurement. Biosens Bioelectron 14(12):883–893
11. Rastogi S, Kumar A, Mehra NK, Makhijani SD, Manoharan A, Gangal V, Kumar R (2003) Development and characterization of a novel immobilized microbial membrane for rapid determination of biochemical oxygen demand load in industrial waste-waters. Biosens Bioelectron 18(1):23–29
12. Temmink H, Vanrolleghem P, Klapwijk A, Verstraete W (1993) Biological early warning systems for toxicity based on activated sludge respirometry. Water Sci Technol 28(11–12):415–425
13. Surmacz-Gorzka J, Gernaey K, Demuynck C, Vanrolleghem P, Verstraete W (1996) Nitrification monitoring in activated sludge by oxygen uptake rate (OUR) measurements. Water Res 30:1228–1236
14. Gernaey K, Bogaert H, Massone A, Varolleghem P, Verstraete W (1997) On line nitrification monitoring in activated sludge with a titrimetric sensor. Environ Sci Technol 31(8):2350–2355
15. Rozzi A, Tomei MC, Di Pinto AC, Limoni N (1997) Monitoring toxicity in anaerobic digesters by the Rantox biosensor: theoretical background. Biotechnol Bioeng 55(1):33–40

16. Aivasidis A, Melidis P, Georgiou D (2002) Use of a microbial sensor: a new approach to the measurement of inhibitory effects on the microbial activity to activated sludge. Bioprocess Biosyst Eng 25:29–33
17. Georgiou D, Melidis P, Aivasidis A (2002) Use of a microbial sensor: inhibition effect of azo-reactive dyes on activated sludge. Bioprocess Biosyst Eng 25:79–83
18. Melidis P, Athanasoulia E, Vaiopoulou E, Aivasidis A (2007) Anaerobic biosensor for toxicity and overload monitoring in anaerobic digesters. Proceedings of the International Conference on Environmental Management, Engineering, Planning and Economics (CEMEPE), June 24–28, Skiathos, Greece, pp 1225–1230