ENVIRONMENTAL BIOTECHNOLOGY: THE TANDEM OF BIOCATALYTICAL AND ENGINEERING DEVELOPMENTS

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The paper gives several examples of integrated approaches based on the tandem of biocatalytical and engineering developments in environmental biotechnology for treatment of 3 main compartments of the environment—soil, water and gas phase. The first topic analyses the current situation with oil pollution of soils and water surfaces in Russia and presents the results of field bioremediation trials on the basis of recently developed biopreparation "Rhoder". The second topic discusses the recent findings aiming to extend an applicability of anaerobic wastewater treatment at temperatures as low as 4–10 °C. A performance of novel anaerobic-aerobic hybrid reactor is analysed in the third topic with regard to treatment of recalcitrant azo dye wastewater. The latter 2 topics lye within a conventional function of environmental biotechnology (so-called "end of pipe treatment") while the fourth topic dealing with the development of biocatalytical technology of H_2S removal and sulphur recovery from polluted gases highlights a transformation of this discipline into a new phase substantially contributing to resource conservation and sustainable production in the modern society.

Introduction

The disarmingly simple definition originated from Albert Einstein ("The environment is anything, which isn't me") explains succinctly why society has so many environmental problems at the beginning of the 3rd millennium. Indeed, the environment is the "tragedy of the commons"—it belongs to nobody and to everybody. This results in the fact that ample examples of ecological problems or even disasters are encountered. Treatment of environmental problems is mainly based on biocatalytical methods due to their relative cheapness and reasonably high efficiency. The whole subject area is defined as environmental biotechnology, which is currently the biggest area of industrial application of biocatalysis with regard to overall quantity of processed matter. However, with respect to the term "environmental biotechnology" the emphasis should be put on "bio" at least equally as on the "technology" though in the historical perspective, the treatment of environmental problems was monopolised by sanitary engineering. As a consequence, the "bio" component has until recently largely been ignored and dealt with stochastically rather than mechanistically. However, at present, we are facing a number of formidable environmental problems such as greenhouse effect, acid rain, depletion of ozone layer, enrichment of ground and surface waters with nutrients and recalcitrant xenobiotics, disposal of municipal solid and animal wastes etc. These problems can no longer be solved by a limited number of straightforward techniques, which are often a perfect illustration of Murphy's Law, i. e., they transform one problem into another often more intractable problem. Examples: one cleans water by stripping the pollutants into the air or removes organics from water which are then dumped in the soil. Hence, a particular type of waste can not anymore be treated without considering all the consequences for the environment. For instance, activated sludge treatment now not only refers to the water component, but also to the biosolids produced and volatile organic compounds and odour generated. Thus, the philosophy inside of environmental biotechnology should be holistic and this requires both a detailed knowledge about biocatalytic mechanisms involved and well designed engineering systems.

The paper gives several examples of integrated approaches based on the tandem of biocatalytical and engineering developments in environmental biotechnology for treatment of 3 main compartments of the environment - soil, water and gas phase. The first chapter analyses the current situation with oil pollution of soils and water surfaces in Russia and the results of full-scale bioremediation trials on the basis of recently developed biopreparation "Rhoder". The second chapter presents the recent findings aiming to extend an applicability of anaerobic wastewater treatment at temperatures as low as 4–10 °C. A performance of novel anaerobic-aerobic hybrid reactor is discussed in the third chapter with regard to treatment of recalcitrant azo dye wastewater. Finally, biocatalytical technology of H_2S removal and sulphur recovery from polluted gases is highlighted.

Spilled petroleum remediation in open water aquatories, wetlands, and soils: using novel biopreparation "Rhoder"

Due to massive movements of petroleum from the oil-producing countries to the major oil consumers and continuous oil spills and leaks in pipelines and storage tanks followed by runoffs, approximately 35 million tons of oil enters the sea per annum [1]. Since 1 ton of oil contaminates 12 km^2 of water surface, it results in the fact that 30% of the World Ocean surface are already covered by oil film [2]. Meantime, 1 l of oil eliminates oxygen from 40 m^3 of water and kills 100 million of fish larvae. Even low concentrations of oil such as 0.1 mg/l exert the death of juvenile forms of marine animals after several days of exposition and substantially inhibit the growth of microalgae [3]. The toxic effects of hydrocarbons to all forms of life

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was recognised long ago and is usually ascribed to the oil dissolving the lipid portion of the cytoplasmic membrane, thus allowing cell contents to escape [4]. On the basis of the facts presented above, oil should be considered as one of the most dangerous pollutants for the environment taking into account both its high toxicity and enormous scale of invasion into biosphere.

Russia occupies the 3rd place (after Saudi Arabia and Iraq) with regard to oil reserves (62.7 billion tons) and the total oil extraction accounted for 295 million tons in 1999 [5]. Due to systematic accident spills, an annual release of oil into the environment in Russia accounts for 25 millions tonnes according to the estimations (may be a little bit exaggerated) of "Greenpeace" [2]. Among a variety of approaches proposed for elimination of these spills [1], three main methods (mechanical, physico-chemical and microbiological ones being applied both separately and in various combinations) are currently considered as the most perspective methods for Russian conditions [6]. Each of these methods has its advantages and drawbacks. Under fresh and abundant spills, the mechanical methods of oil collection are usually applied as a principal treatment. However, oil pollution is not eliminated completely. The physico-chemical methods using special reagents (detergents, emulsifiers, solidifiers, adsorbents, etc.) can efficiently concentrate oil pollution, but frequently they themselves are not fairly irreproachable from the ecological point of view, e. g., collection of oil-saturated adsorbents as well as their subsequent utilisation becomes sometimes troublesome. The microbiological methods using both external introductions of oil-degraders cultivated *ex situ* and stimulation of indigenous microorganisms (if they are present in necessary concentrations) are usually quite efficient for treatment of low polluted water surfaces and soils. However, their effects are frequently not so pronounced at a high level of oil pollution. Besides, the low average annual temperatures on the overwhelming majority of territory, especially where the principal oil fields are located, is another critical bottleneck for application of these methods in Russia because bacterial oil-degrading activity drops dramatically under temperatures below 10 °C. Despite the above-mentioned limitations, microbiological methods are drawing more and more attention in our country, especially as post-treatment or polishing steps, due to their economic attractiveness and ability to fulfil with the stringent legislation requirements concerning a permissible level of oil pollution (PLOP). In this chapter, the experience accumulated in 1994–1999 with application of recently developed biopreparation "Rhoder" for spilled petroleum bioremediation in open water aquatories, wetlands and soils is summarised.

Biopreparation. The biopreparation known under commercial name "Rhoder" and recently developed in All-Russian Research Institute of Oil and Gas together with Moscow State University [7] consists of two bacteria - Rhodococcus ruber and Rhodococcus erythropolis revealing a synergistic action on hydrocarbon degradation under a joint application. The individual strains were isolated from oil-water mixture originating from Bondyuzhskoye oil field (Tatarstan, Russia), and the corresponding pure cultures were then deposited to the All-Russian Collection of Microorganisms (ARCM indexes are 1513-D and 1514-D, respectively) and patented [8-9]. The biopreparation "Rhoder" was certificated in 1999 for production, delivery and application on territory of Russia (Certificate No. 77.99.11.515.P.4865.8.99 issued 17.08.99 by the Russian Ministry of Health). The delivery form of "Rhoder" usually includes a concentrated wet suspension of cells of both bacteria (1 : 1 w/w) with a titre of hydrocarbon-degrading bacteria of 10^{9}–10^{10} cells/ml. The working solution is prepared on site by dilution of concentrated suspension with water followed by addition of some nutrients and biostimulators.

Sites and remediation methods used. The following oil polluted sites were used for field testing of biopreparation "Rhoder" in 1995–1999: bay of river Chernaya (Lukhovitsy, Moscow region), lakes and wetland (Vyngayakha, Western Siberia), lake and wetland (Ural, Western Siberia), marshy peat soil (Nizhnevartovsk, Western Siberia). Some characteristics of these sites are listen in Table 1. When necessary and possible, preliminary mechanical collection (PMC) of spilled oil on the site was undertaken before application of bioremediation technology. The latter include a spraying of the working solution on the polluted areas using pump equipment. Usually the treatment with biopreparation was repeated twice or triple with a time interval of 2 weeks. The impact of activity of indigenous hydrocarbon-degrading bacteria (HDB) was assessed by a spraying of the working solution lacking "Rhoder" on the control areas having a similar oil pollution level. The generalised results of field tests are presented in Table 1.

Bioremediation of open water aquatories. From Table 1, it is seen that Rhoder has demonstrated a very high efficiency for treatment of aquatories, especially at low initial oil level (IOL) as in the case of bay of river Chernaya. It should be noted that the residual oil level (ROL) after 4 weeks of bioremediation of this site was only 0.04 mg/l, i. e., lower than the Russian PLOP (0.05 mg/l). The concentrations of HDB and heterotrophic bacteria firstly increased by 1–2 orders of magnitude at day 14 and then returned back to the initial level after an exhaustion of organic substrates in the river water. Thus, an addition of the external bacteria seemed not to result in substantial changes of microbial community existing in the river water. Analogously, the initial dosage of nutrients was chosen in such a way that the residual level of phosphate and nitrate after treatment was low enough to prevent a possible eutrophication of this bay. The both lakes in Vyngayakha had a high IOL (Table 1) and the thick (till 1–2 cm) oil film was clearly seen on their surfaces. In spite of rather tough conditions, the triple treatment with Rhoder accompanied by unusual warm weather in that period resulted in an almost complete elimination of oil pollution—the ROLs were 5 and 190 mg/l in lakes 1 and 2, respectively (Table 1). Moreover, after bioremediation both these lakes were certificated by the local ecological authorities as "the objects almost free of oil pollution". During the treatment of the lake in Ural (Table 1), a majority of oil pollution was removed by mechanical collection (90%), i. e., the oil contamination level decreased from 11 to 1.01 g/l after this step. The subsequent treatment by Rhoder (twice) led to the residual oil contamination of 0.43 g/l resulting in an overall treatment efficiency of 96% (Table 1). A relatively high level of residual contamination could be mainly related to the presence of oil polluted sediments accumulated in this

Table 1

Bioremediation results of "Rhoder" field tests [6]

Site	Area, m^2	Initial oil pollution in the upper layer (10cm), g/l	(Pre)-treatment	Treatment efficiency, %
River Chernaya	100	0.44	"Rhoder" (twice)	>99.9
Vyngayakha:				
lake 1	5.000	15.1	"Rhoder" (triple)	>99.9
lake 2	5.000	19.1	"Rhoder" (triple)	99
wetland	10.000	24.3	"Rhoder" (triple)	65
Ural:				
lake	1.900	11.0	PMC* + "Rhoder" (twice)	96
wetland	2.000	10.5	PMC* + "Rhoder" (triple)	94
Nizhnevartovsk:				
marshy peat soil	1.000	758–828 (g/kg)	ploughing + "Rhoder" (triple)	14–24

* PMC is the preliminary mechanical collection of free oil.

lake. These sediments served as a continuous source of oil emission to the lake water.

Bioremediation of wetlands. Relatively inferior results of remediation of the wetland in Vyngayakha (Table 1) can be attributed to the fact that due to specific local geological conditions it was hardly possible to apply a PMC of free oil on this site. However, taking into account a high IOL (> 24 g/l) and age of spill (4 years old), the results look quite satisfactorily - approximately 65% removal of oil contamination (Table 1). On the contrary, application of PMC of spilled oil (75% removal) followed by triple treatment with Rhoder has resulted in much higher overall treatment efficiency (94%) in the case of remediation of the wetland in Ural (Table 1).

Bioremediation of soils. The inferior results of "Rhoder" bioremediation field tests obtained on the marshy peat soils in Nizhnevartovsk (Table 1) were not surprising taking into account an extremely high IOL (> 750 g/kg of dry matter) and age of spill (6 years). Since an overwhelming majority of the spilled oil was adsorbed by peat, it was not economically reasonable to apply a PMC of oil. The pre-treatment used included only a ploughing of upper layer of contaminated area accompanied by addition of lime (to increase pH) and nitrogen and phosphorous fertilisers. An average (for 3 lots) reduction of oil pollution was 19% (Table 1) under application of "Rhoder", while without "Rhoder" addition it was 13% (data not shown). The latter fact manifested about a high activity of indigenous HDB already developed on the contaminated site during 6 years and substantially stimulated by pH adjusting and nutrient addition. This supposition was further confirmed by direct counts of MPN of HDB from the lot without Rhoder addition, which were 10^3 and almost 10^6 cells/ml in the beginning and in the end of experiments, respectively (data not shown).

Summarising the results presented in this chapter, one can say the following. Field tests showed a very high efficiency of biopreparation "Rhoder" for remediation of aquatories moderately contaminated by oil (< 20 g/l). However, for treatment of heavy polluted aquatories (thickness of oil film > 3 mm) as well as oil spills on wetlands and grounds, the best strategy should include a preliminary mechanical collection of free oil, or application of adsorbents, or other pre-treatment methods followed by microbiological polishing step. If for some reasons it is impossible to apply pre-treatment, the possible strategy can include multiple microbiological treatment with ploughing, pH adjusting and supplementing by nutrients throughout several years. On the aged spills (> 5 years), the oil-degrading activity of indigenous microflora is usually high enough to omit an addition of biopreparations produced *ex-situ.* The economically reasonable strategy can include a stimulation of indigenous HDB already adapted to the site environment.

Anaerobic wastewater treatment at cold temperatures (4–10 °C)

Anaerobic treatment has several well known advantages in comparison with aerobic treatment, especially for treatment of high-strength wastewater - no energy needs for aeration (on the contrary, generation of energy in the form of biogas), substantially reduced nutrient requirements, high organic loading rates (OLR) etc. [10]. However, an implementation of conventional anaerobic treatment (especially in the countries with a cold climate such as Russia) is often hindered by the necessity of maintaining an operation temperature—mesophilic (30–37 °C) or thermophilic (55–60 °C) which is significantly higher than ambient temperatures. This chapter discusses the recent findings [11–12] aiming to extend an applicability of anaerobic wastewater treatment at psychrophilic temperatures as low as 4–10 °C.

Since low temperatures usually lead to a sharp decrease of the biocatalytical activity of methanogenic microbial consortium involved in anaerobic digestion, a strategy in maintaining a reasonable efficiency of wastewater treatment should include an increase (as much as possible) of concentration of biocatalysts inside the reactor or/and a gradual adaptation of the consortium to psychrophilic conditions. Both these approaches were combined in the present study by using granular mesophilic sludge having rather high methanogenic activity and up-flow anaerobic sludge bed (UASB) reactor promoting self-immobilisation (and thus accumulation inside the reactor) of the cells of methanogenic consortium in the form of well-settled granules. Raw vinasse obtained by distillation of low quality wines and diluted by tap water was used as feeding influent. The other details of experimental study are presented in works [11–12]. The performance data of long-term treatment of vinasse under psychrophilic conditions are generalised in Table 2.

Table 2

Performance data of long-term treatment of vinasse under psychrophilic conditions (average values are given in brackets) [11–12].

Parameters	Temperature, °C					
	9–10		7–8		3–5	
	Single UASB reactor					
Run	Run 1a*	Run 1b*	Run 2a	Run 2b	Run 3	
Run days	0–67	68–158	159–185	197–236	251–273	
Recycle ratio	1:2.6	1:2.6	1:2.6	1:11.6	1:11.6	
OLR, g COD/l/d	0.3–5.1(2.7)	1.4–7.3(4.7)	3.2–4.6(3.7)	2.3–3.5(3.0)	1.1–2.7(1.7)	
HRT, days	0.8–5.1(1.9)	0.5–1.6(0.9)	0.85–0.87	0.9–1.3(1.1)	1.14–1.17	
Influent COD, g/l	3.6–5.2(4.0)	1.2–9.9(4.2)	2.7–4.0(3.2)	3.0–3.6(3.2)	1.3–3.1(2.0)	
Effluent COD, g/l	0.3–2.7(1.0)	0.5–3.6(1.8)	0.8–1.9(1.0)	0.9–1.5(1.2)	0.6–1.5(0.8)	
COD removal, %	48–92(72)	48–92(60)	52–79(68)	48–70(60)	15–72(57)	
	Two UASB reactors in series					
Run days	0–63		82–107		122–147	
Reactor	R1	R2	R1	R2	R1	R2
Recycle ratio	1:1	1:18	1:1	1:18	1:1	1:18
OLR, g COD/l/d	3.2–5.5(4.4)	0.8–4.0(2.5)	2.3–4.2(3.5)	1.2–3.0(2.2)	2.0–2.7(2.5)	1.5–2.2(1.7)
HRT, days	0.8–1.3(1.0)	0.8–1.2(1.0)	1.0–1.1(1.0)	1.0–1.1(1.0)	0.8–1.0(0.9)	0.8–1.0(0.9)
Influent COD, g/l	3.1–5.4(4.3)	1.6–3.9(2.5)	2.5–4.2(3.5)	1.4–3.1(2.3)	1.9–2.6(2.4)	1.1–1.9(1.5)
Effluent COD, g/l	1.0–3.9(2.4)	0.4–2.8(1.2)	1.3–3.1(2.3)	0.4–1.9(1.0)	1.1–1.9(1.5)	0.3–1.2(0.7)
COD removal, %	16–76(46)	24–80(58)	19–52(37)	29–78(61)	25–52(37)	43–74(53)
	Combined system (R1+R2)					
OLR, g COD/l/d	1.6–2.8(2.2)		1.2–2.1(1.8)		1.0–1.4(1.3)	
HRT, days	1.6-2.5(2.0)		2.0–2.2(2.0)		1.6–2.0(1.8)	
COD removal, %	36–91(78)		42–89(76)		60–86(71)	

* Run 1a—non-preacidified influent; run 1b—preacidified influent.

One stage UASB psychrophilic treatment. During run 1a (10 °C), when non-preacidified influent was treated, an OLR was increased stepwise to 4–5 g COD/l/d with a total chemical oxygen demand (COD) removal of around 70%. (Table 2). A significant presence of propionate (predominant component) and acetate was observed in the effluents. However, only traces of sugars, ethanol and butyrate were detected in the reactor liquor, while the headspace gas hydrogen concentration was negligible. These facts clearly demonstrate that low temperatures affect the various stages of anaerobic digestion differently, with propionate conversion becoming the rate-limiting step [13]. It should be also noted that a substantial increase ($\sim 20\%$) of sludge bed height had occurred over this run that was primarily due to a substantial growth of acidogens in the reactor, because a fluffy outer layer covering the granules was seen under microscopic observations of the sludge aggregates. Since such types of aggregates can provoke sludge flotation and create mass transfer limitations for substrates of propionate-degrading and aceticlastic bacteria which are usually located in the central part of aggregates, it was decided to apply preacidification of wastewater in order to achieve better COD removal. However, feeding with preacidified vinasse (run 1b, Table 2) did not result in any enhancement of COD removal with the effluent propionate concentrations often exceeded 1.5 g COD/l. In order to have a deeper insight into the processes occurring in the psychrophilic UASB reactor, the sludge kinetic characteristics were assessed *in situ*, i. e., under reactor conditions (days 120–138). Apparent half saturation constants K_m for all the substrates tested were found (data not shown) to be greater than 1.0 g COD/l at the imposed up-flow liquid velocity (V_{up}) of 0.1 m/h, which supports the above-mentioned supposition about the existence of mass transfer limitations inside the psychrophilic sludge bed.

Decrease of temperature during run 2a to 7 °C did not result in deterioration of reactor performance though the OLRs were somewhat lower (around 4 g COD/l/d) than those applied during run 1b (Table 2). In order to decrease mass transfer limitations, the recycle ration was increased during run 2b (days 197–236, Table 2). As expected, an almost 4 times increase of V_{up} resulted in a better VFA removal though a total COD removal efficiency slightly decreased compared to run 2a. This was mainly due to an increased sludge washout because small sludge aggregates were continuously accumulated in the effluent recipient vessel during this run. A further decrease of working temperature to 4 °C was accompanied by a decrease of OLR imposed during run 3 (days 251–273, Table 2). In general, the overall reactor performance was similar to that during run 2b. A sludge washout was also observed but it tended to decease during this run. It was because a majority of fine sludge aggregates were already eliminated from the reactor during run 2b. Microscopic observation of the sludge taken at the end of run 3 showed an overwhelming predominance of fluffy large aggregates (4–5 mm) with irregular forms and it looked like flocculent one. Such evolution of the sludge can be attributed to the fact that the reactor influent was not completely acidified by preacidification procedure applied, e. g., sometimes quite noticeable concentrations of ethanol (till 2 g COD/l) and sugars (till 0.6 g COD/l) entered to the reactor stimulating a development of fluffy acidogenic biomass which deteriorated a sludge quality. Thus, a control of preacidification efficiency seems to be essential for a stable pretreatment process of winery wastewater at low temperatures.

Two stage UASB psychrophilic treatment. In order to control preacidification of wastewater with the aim to enhance a COD removal, two UASB reactors were combined in series. Reactor R1 mainly served as preacidificator to generate VFA for feeding reactor R2. High recycle ratio (1 : 18) was applied in reactor R2 in order to decrease mass transfer limitations while recycle ratio in reactor R1 was kept at low level (1 : 1) because diffusional limitations are not very important for fast acidogenic step. The sludge from run 3 consisting predominantly of fluffy large aggregates (see above) was used as a seed for both the reactors. Analysing the results obtained during two-stage UASB pretreatment (Table 2), one can say the following. A combined system with two reactors in series has demonstrated higher removal efficiencies and significantly better operation stability compared to a single UASB treatment at temperatures as low as 4–10 °C. Any difficulties in a combined system performance including sludge lifting or heavy washout have not been observed at all. It should be noted, however, that a single UASB reactor was operated at higher OLRs (but with preacidified wastewater) than the OLRs imposed on a combined system treating non-preacidified wastewater if one takes into account the overall volume of both reactors. Thus, an application of two UASB reactor system implies higher capital and operational costs which should be taken into account under possible implementation of low temperature anaerobic pretreatment. From the other hand, a single UASB reactor operating at psychrophilic temperatures seems to need at least a partial preacidification of wastewater in order to ensure its more-or-less stable operation.

Concluding this chapter, it should be noted that anaerobic treatment in high rate reactors like UASB reactors is feasible at temperatures as low as 4–10 °C. However, substantial mass-transfer limitations for the soluble substrates inside the reactor sludge bed were encountered. Therefore, an application of higher recycle rations is essential for enhancement of UASB treatment under psychrophilic conditions. The produced anaerobic effluents were shown to be efficiently post-treated aerobically—final effluent COD concentrations were around 0.1 g/l [12]. A successful operation of the UASB reactors at quite low temperatures (4–10 °C) opens good perspectives for application of high-rate anaerobic treatment at ambient temperatures, e. g. in south regions of Russia.

Biomineralisation of azo dyes in innovative anaerobic-aerobic hybrid reactor

Azo dyes represent a major group of all the dyes produced world-wide [14]. Approximately 10–15% of overall production is released into the environment mainly *via* wastewater [15]. This is very dangerous because some of the azo dyes or their breakdown products have a strong toxic, mutagenic or carcinogenic influence on the living organisms; therefore, the corresponding wastewaters should be treated before discharge. However, a majority of azo dyes are quite resistant to biodegradation under aerobic conditions and easily pass through conventional aerobic wastewater treatment systems. On the other hand, azo dyes are readily decolourised by splitting the azo bond(s) in anaerobic environments. In turn, the anaerobic breakdown products are more susceptible to biodegradation under aerobic conditions rather than under anaerobic conditions. These properties of azo dyes dictate the anaerobic-aerobic sequence in designing an efficient biomineralisation process. Two separate treatment steps are usually applied for this purpose. In order to optimise the treatment process and overall economics of the corresponding technology, we combined the anaerobic and aerobic phases into one single unit called the anaerobic-aerobic hybrid reactor (AnAHR) in this study (Fig. 1*a*). The advantages of this innovative design include reduced aeration costs and lower space requirements while offering substantial mitigation of a broad spectrum of recalcitrant xenobiotic contaminants (not only azo dyes) found in industrial wastewaters. This chapter discusses the performance of the mesophilic (30 °C) AnAHR using the azo dye Siriusgelb (Fig. 1*b*) and ethanol as donor of reductive equivalents (Fig. 2) [16]. The concentrations of Siriusgelb and ethanol were 0.3 and 0.82 g COD/l, respectively. It should be noted that throughout the entire experimental run, only traces (if any) of ethanol and acetate (very rarely) were detected in the upper part of the anaerobic zone of the AnAHR. This suggests that the conversion of ethanol to methane was already complete in this zone and the measured COD content of the samples taken from the upper part of the anaerobic compartment as well as those of the AnAHR effluent represented only non-degraded azo dye and its breakdown products.

During the first 18 days, when the azo dye loading rate (ADLR) was 0.09 g COD/l/day using a HRT (hydraulic retention time) of approximately 3.4 days (Fig. 2*a*), azo dye treatment efficiency (TE) in the anaerobic compartment was 51% and the overall TE of the AnAHR was 71% (Fig. 2*b*). After an increase of ADLR to an average value of 0.18 g COD/l/day for the period from day 19 to day 32 (Fig. 2*a*), azo dye anaerobic and overall TEs dropped slightly and were on average 50 and 64%, respectively (Fig. 2*b*). In the final stage of this experiment (day 33 onwards), the ADLR was further increased to 0.3 g COD/l/day keeping the HRT around 1 day (Fig. 2*a*). This resulted in a further drop of both TEs—44 and 56% (on average) for the anaerobic compartment and the entire AnAHR, respectively (Fig. 2*b*). Negligible absorbancies at 375 nm (maximum absorbance of Siriusgelb) were observed in the reactor effluent throughout the entire experimental run indicating complete decomposition of this azo dye. However, complete decolouration of the effluent did not occur, rather, it remained slightly rose in colour compared to the intensive brownish-yellow colour

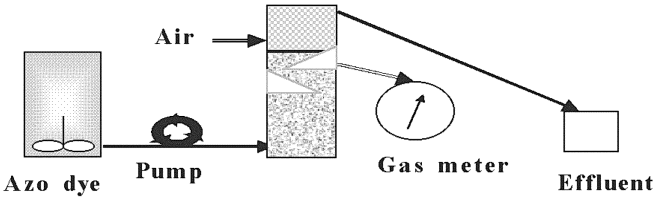

Fig. 1a. The experimental set-up for biomineralisation of azo dyes in the AnAHR.

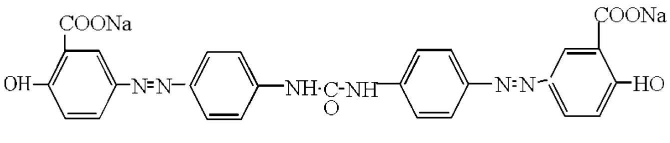

Fig. 1b. The structural formula of azo dye Siriusgelb GG.

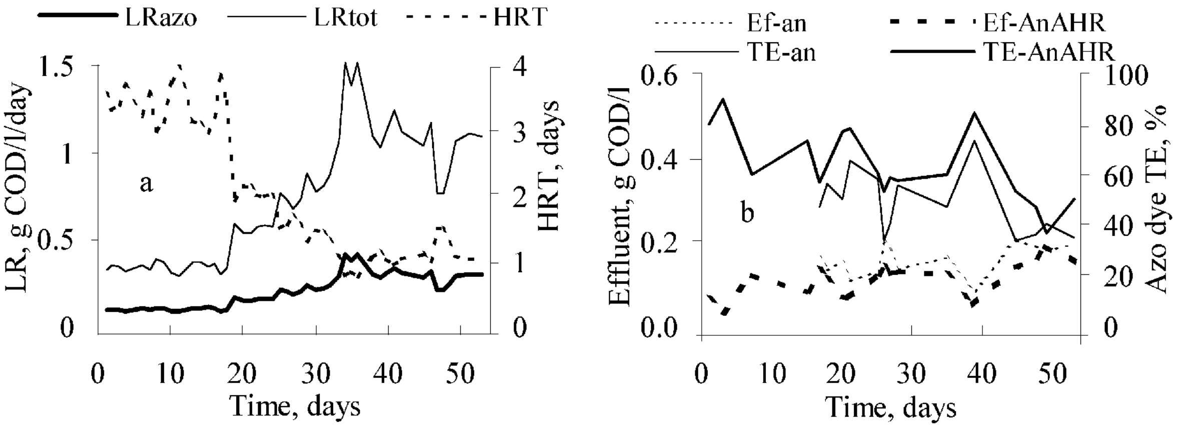

Fig. 2. Operation performance of AnAHR treating a Siriusgelb (0.3 g COD/l) synthetic wastewater supplemented with ethanol (0.82 g COD/l).

of influent. A transient accumulation of intermediate of Siriusgelb decomposition—5-aminosalycilic acid (5-ASA)—was detected in the anaerobic compartment of the AnAHR but not in the effluent. The 5-ASA concentrations peaked (till 0.06 g COD/l) immediately after increases of ADLR and then gradually decreased if the ADLR was kept constant. This observation suggests a stepwise adaptation of anaerobic sludge for decomposition of 5-ASA. As can be seen in Fig. 2*b*, a majority of the azo dye COD was removed in the anaerobic compartment and the aerobic section had a relatively minor impact on the overall TE. The aerobic removal, as a percentage of the influent, varied between 20 and 30%. Such low TEs achieved in the aerobic step as well as effluent colouring can be attributed to the fact that the breakdown products of anaerobic Siriusgelb decomposition (5-ASA and 1,4-phenilenediamine) are readily autooxidised to coloured polymeric products upon exposure to air [17]. These autooxidation products are often complex humic compounds that are non-biodegradable. Incomplete recovery of ammonia (data not shown) also supports the above-mentioned supposition about the inclusion of generated aromatic amines in these persistent polymeric products.

Thus, an innovative reactor construction where the anaerobic and aerobic phases were combined in one single unit called an AnAHR is proposed for the treatment of azo dyes as well as other aerobically persistent xenobiotic contaminants. The performance of the AnAHR was tested with a synthetic wastewater containing Siriusgelb and ethanol as co-substrate at 30 °C. Almost complete decolouration of the influent and 56% removal of the azo dye COD was achieved using a HRT of 1 day and volumetric loading rate of 0.3 g azo dye COD/l/day. The effluent contained no ethanol or acetate and its COD content could be attributed to the presence of non-biodegradable autooxidation products of Siriusgelb breakdown intermediates. Further research is needed to assess the feasibility of this reactor concept for treatment of industrial wastewater containing persistent compounds.

Biocatalytical technology for H_2S removal and sulphur recovery from polluted gases

Biogas usually contains around 1 vol.% of H_2S, while natural gas can contain till 15 vol.% of H_2S [18]. Conventional technologies for treating such gases based on chemical reactions in a liquid phase, physical absorption and direct chemical conversion have several evident drawbacks: high reagent consumption, equipment corrosion, application of high temperature and pressure, etc., resulting in high process costs—250–750$/ton of removed sulphur [19]. This chapter highlights the development of substantially cheaper alternative—biocatalytical technology for treatment of H_2S polluted gases. The schematic representation of the process proposed is shown in Fig. 3 [18]. Briefly, In the scrubber, the H_2S containing gas comes into contact with a slightly alkaline (pH 8.0–8.5) scrubbing solution where absorption of H_2S takes place. Scrubbing liquor then passes to the bioreactor containing immobilised bacteria of genus Thiobacillus where a soft oxidation of sulphide into elemental sulphur accompanied by regeneration of alkalinity proceeds. Solid sulphur is removed and the liquid is returned to the scrubber for absorption of the next portion of H_2S.

Since the success of elegant technological scheme presented in Fig. 3 is determined (in major extent) by efficiency of bioreactor, significant efforts were put on optimisation of its construction and productivity [18]. The crucial point is that the bacteria of genus Thiobacillus used in the process oxidise sulphide not only into sulphur but also into sulphate:

$$HS^- + 0.5O_2 \rightarrow S^0 + OH^-, \qquad (1)$$

$$HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+. \qquad (2)$$

Obviously, the reaction (2) is highly undesirable for the process under development because it leads to expenditure of alkalinity of the liquid phase and formation of hardly removable dissolved product (sulphate). To suppress this reaction, oxygen-limiting conditions and high sulphide loading rates should be imposed on the system [18]. In an engineering context, the various reactor constructions (conventional CSTR, reactor with external aerated loop, gas-lift) were tested on the laboratory level [18]. Currently the best construction consists of an automated close (with respect to gas-phase) gas-lift reactor equipped with on-line sensors for measuring dissolved oxygen, sulphide and pH. The electric signals from these sensors were transferred to a programmable data logger system. A personal computer programmed to function as a terminal emulator was used to communicate with the data logger and to control the feeding pumps. Using this highly controlled reactor and

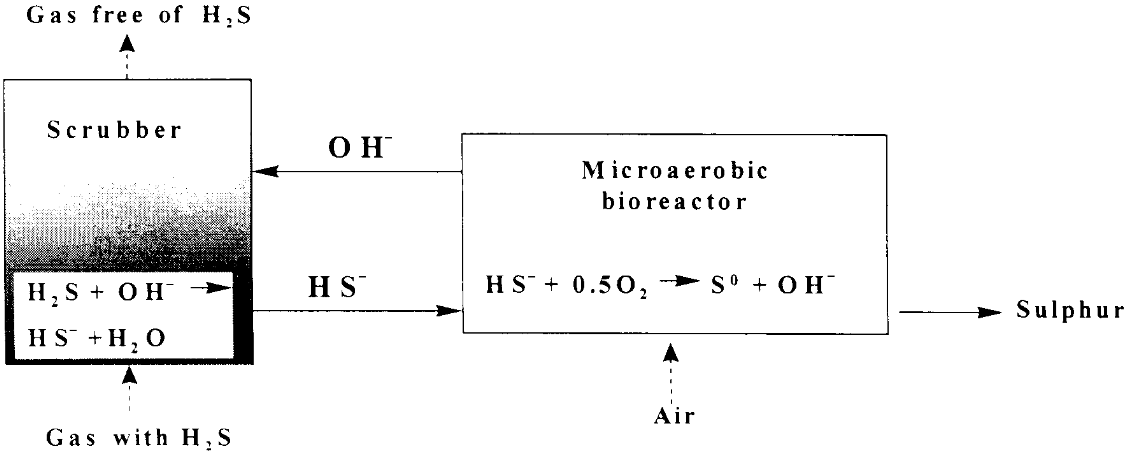

Fig. 3. Schematic representation of biocatalytical reagentless method for H_2S removal from polluted gases.

pure oxygen (instead of air), 94–98% efficiency of sulphide conversion into elemental sulphur under sulphide loading rates as high as 15 g S/l/day was achieved [18].

Finally, the manifest advantages of the proposed technology compared to the conventional methods should be underlined: practically reagentless character (some salts are necessary for bacteria); cheapness; practically closed cycle and minimum of wastewater; sole process product (sulphur) can be readily re-used (for sulphuric acid production); ambient temperature and pressure for the process making it safe.

Concluding remarks

The presented examples clearly demonstrate that by creating optimal growth conditions for microorganisms in proper designed engineering systems, conversion rates can be significantly increased resolving many problems of environmental biotechnology. Moreover, during last decade this discipline has matured from its conventional function (so-called “end of pipe treatment”) to a new phase substantially contributing to resource conservation and sustainable production in the modern society. The discussed above biocatalytical technology for H_2S removal and sulphur recovery from polluted gases is a typical example of this.

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