Environmental biotechnology: a tandem of biocatalytic and engineering approaches

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The review gives examples of integrated approaches based on a tandem of biocatalytic and engineering methods to problems in the biotechnological protection of three basic constituents of the environment, namely, soil, water, and atmosphere, from dangerous pollution. The current situation associated with oil pollution of soils and water surfaces in Russia is analyzed. The results of the field tests of the biopreparation "Roder" recently developed for bioremediation of these surfaces are presented. New solutions aiming at extending the area of application of high-rate anaerobic wastewater treatment at low temperatures (4—10 °C) are discussed. The efficiency of a novel anaerobic-aerobic hybrid reactor for mineralization of recalcitrant wastewater containing azo dyes was estimated. The last two lines relate to a traditional function of the environmental biotechnology (so-called "end of pipe treatment"). A biocatalytic technology for the removal of hydrogen sulfide and the recovery of elementary sulfur from contaminated gases was proposed. This highlights a new phase in the development of environmental biotechnology allowing waste recycling.

Key words: environmental biotechnology, biocatalysis, bioremediation, oil pollution, low-temperature anaerobic sewage treatment, azo dyes, sulfur recovery.

Introduction

The term "environment" is differently interpreted by each of us. A disarmingly simple definition of this notion was proposed by A. Einstein: "The environment is anything which is not me". This laconic definition explains ingeniously why humanity at the threshold of the third millennium is faced with a great deal of ecological problems. In fact, the environment is a typical example of a "tragedy of common things", because it belongs both to everyone and to nobody. As a result, we all (both in the West and the East), following the behavioral criteria of a "second wave society" (as defined by the economist A. Toffler) in the past century, exploited the environment up to a catastrophic limit.

Ecological problems are mainly solved using biocatalytic methods because of their relative cheapness and acceptable capacitance. Taking into account the amounts of treated substances (for instance, ca. 7 million m^3 of sewage is treated daily only at the Moscow biological aeration stations),[1] one can now state that the biotechnology of environmental protection[2] accounts for the largest part of industrial biocatalysis. Obviously, the component "bio" in the biotechnology of environmental protection should be emphasized, at least, to the same extent as "technology", though, in historical prospect, the solution of environmental problems was almost completely monopolized by sanitary engineering. As a consequence, the component "bio" has largely been ignored until recently, and its potential has been underestimated. However, we are now faced with a number of global ecological problems such as a greenhouse effect, acid rains, depletion of the ozone layer, increased content of nutritive components and xenobiotics resistant to mineralization in groundwaters and surface waters, disposal and recycling of urban and animal solid wastes, *etc.* These problems cannot be solved by using a limited set of conventional straightforward engineering approaches (sedimentation, filtration, blowing-out, *etc.*), which often give a classic illustration of the Murphy's law, *i.e.*, they convert a problem into another, more serious one. For example, during the current water treatment contaminants are either emitted into the atmosphere or buried in the ground, thus only masking the problems. Therefore, the strategy of treatment a particular type of waste must take into account all long-term aftereffects on the environment. Thus, modern systems for sewage treatment with activated sludge should not only be oriented to water treatment as such, but also solve problems associated with the recovery of an excess of activated sludge and suppression of volatile organic compounds and odors emitted by these structures. Hence, the modern biotechnology of environmental protection implies a unified philosophy for all of its constituents. For this purpose, integration of many scientific disciplines is required, including, first of all, comprehensive knowledge of the mechanisms of biocatalytic processes

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and its efficient engineering applications. Naturally, the aspects of human psychology and international policy should also be taken into consideration while developing new or updating current methods of environmental protection.

This paper reviews examples of integrated approaches based on an aggregate of biocatalytic and engineering solutions to the protection of three basic components of the environment, namely, ground, water, and atmosphere. In the first section, a situation with oil pollution of grounds and water surfaces in Russia is analyzed, and the results of the field tests of the recently developed biopreparation "Roder" for cleaning (bioremediation) of these surfaces are presented.[3—5] New solutions aiming to extend the area of application of high-rate anaerobic wastewater treatment at low temperatures (4—10 °C) are discussed in the second section.[6—7] The efficiency of a novel anaerobic-aerobic hybrid reactor for mineralization of recalcitrant wastewater containing azo dyes is considered in the third section.[8] The closing section is devoted to a virtually reagentless biocatalytic technology of hydrogen sulfide removal and the recovery of elementary sulfur from polluted gases.[9—10]

1. Bioremediation of oil spills over open water areas, swamps and soils with the use of the biopreparation "Roder"

Because of intensive petroleum transportation from oil-field plants to consumers and its continual leakage from pipelines and storage tanks, some 35 million tons of oil per year enters the sea.[11] One ton of oil pollutes 12 km^2 of the water surface; aerospace photography revealed that *ca.* 30% of the ocean surface is already covered with an oil film.[2] At the same time, 1 L of oil removes oxygen from 40 m^3 of water, thus causing the death of 100 million fish eggs. Even in low concentrations in water (0.1 mg L^{-1}), petroleum kills young marine animals in a few days and significantly inhibits the growth of microscopic algae.[3] The toxic effect of hydrocarbons on all forms of life has long been known and is usually explained by liquefaction of the lipid layer of the cytoplasmic membrane in the presence of oil.[12] Based on the above facts, petroleum should be regarded as one of the most dangerous environmental contaminants because of its high toxicity and large-scale penetration into the biosphere.

Russia ranks third in the world (below Saudi Arabia and Iraq) in explored oil reserves (62.7 billion tons) and extracted 295 million tons in 1999.[2] Because of continual spills in Russia, the environment is yearly polluted by *ca.* 25 million tons of oil (according to apparently overestimated assessment of "Greenpeace").[3]

Despite a variety of approaches proposed for elimination of these spills,[11] three basic methods (mechanical, physicochemical, and microbiological) used both alone and, more often, in different combinations are currently believed to be most promising for the Russian conditions.[4] Each of these methods has its advantages and drawbacks.[2] Mechanical collection and removal of oil and oil products from the polluted surface are usually employed in an emergency of oil spillage. However, oil spill cannot be eliminated completely in such a way. Physicochemical methods can effectively concentrate oil contaminants by using special reagents (surfactants, sorbents, dispersants, hardeners, *etc.*), but they are not always perfect from the ecological standpoint, being often as environmentally dangerous as petroleum itself. Moreover, the question arises as to how an oil-saturated reagent should be collected and recovered. Microbiological methods using both bioaugmentation (*via* addition of biopreparations with high concentration of *ex situ* prepared hydrocarbon-oxidizing (HCO) bacteria) and stimulation of native microflora are effective at the stage of final cleaning of the water surface from the residual oil film and at a medium and low level of ground pollution.[4] However, their effects are not so noticeable for strong oil pollution. An additional factor that hinders the use of these methods in Russia is low average annual temperatures in most of its regions, especially in those containing basic oil fields, since the activity of HCO bacteria dramatically decreases at temperatures below 10 °C. In spite of the aforementioned limitations, microbiological methods become increasingly attractive for Russia, especially for the final ("finishing") stage of elimination of oil spills, because of their relative cheapness and ability to reach very stringent Russian standards for the maximum permissible level of oil pollution. In this section, we aggregated the experience of using the new biopreparation "Roder" in 1994—1999 for bioremediation of open water areas, swamps, and grounds polluted by oil spills.

Biopreparation "Roder". The biopreparation named "Roder" has been developed at the All-Russia Research Institute of Oil and Gas in association with the Moscow State University.[13] "Roder" is composed of two species of bacteria (*Rhodococcus ruber* and *Rhodococcus erythropolis*) that have a synergistic effect on hydrocarbon degradation. The individual strains were isolated from the stratal water of the Bondyuzhskii oil field (Tatarstan), and the corresponding pure cultures were deposited with the All-Russia collection of microorganisms (ARCM Index Nos. 1513-D and 1514-D, respectively) and patented.[14,15] The biopreparation "Roder" is certified and licensed for production and application in Russia (Hygienic Certificate No. 77.99.11.515.P.4865.8.99 was given on 17.08.99 by the Ministry of Health of the Russian Federation). It is a concentrate of living cells with a titre of 10^9—10^{10} HCO bacteria in 1 mL. A working solution of "Roder" is prepared before use by diluting a concentrated suspension with water containing some nutritive substances and biostimulators.

Polluted objects and methods of remediation. Field tests of "Roder" were carried out in 1995—1999 at the following oil-polluted objects: the oxbow of the Chernaya

Table 1. Field tests of the biopreparation "Roder"[5]

Object	S/m^2	C_0 /g L^{-1}	Treatment method	I (%)
The Chernaya river	100	0.44	"Roder" (2 times)	>99.9
Vyngayakha:				
lake 1	5000	15.1	"Roder" (3 times)	>99.9
lake 2	5000	19.1	"Roder" (3 times)	99
swamp	10000	24.3	"Roder" (3 times)	65
Urai:				
lake	1900	11.0	PMC + + "Roder" (2 times)	96
swamp	2000	10.5	PMC + + "Roder" (3 times)	94
Nizhnevartovsk:				
peat bog	1000	758—828 (g kg^{-1})	Plowing + + "Roder" (3 times)	14—24

Note. S is the area, C_0 is the starting level of oil pollution in the upper 10-cm layer, I is the treatment effect, and PMC is preliminary mechanical collection of free oil.

river (Lukhovitsy, Moscow region) and lakes and waterlogged soils in the vicinity of the village Vyngayakha and the towns Urai and Nizhnevartovsk (West Siberia). Some characteristics of these objects are given in Table 1. Initially, when the occasion required and allowed, the spilled oil was collected mechanically with oil-gathering machines (skimmers). Then bioremediation technology was applied, *i.e.*, a working solution of "Roder" was spread over polluted surfaces with the use of fire engines or irrigating machines. Usually, the surface was treated with "Roder" twice or three times at time intervals of two weeks. To estimate the contribution from native microflora to bioremediation, a working solution without "Roder" was spread over areas that have a close level of oil pollution. The summarized results of the field tests are presented in Table 1.

Bioremediation of open water areas. It can be seen from Table 1 that "Roder" is highly effective in treatment of open water areas, especially at a low starting level of oil pollution (SLOP), as is the case of the Chernaya river. It should be noted that the content of oil in water four weeks after the treatment was only 0.04 mg L^{-1}, which is lower than its maximum permissible concentration for Russia (0.05 mg L^{-1}). The concentrations of HCO and heterotrophic bacteria increased one to two orders of magnitude by the middle of the treatment and then decreased to a standard level for river water, because concentrations of carbon-contributing organic compounds in it become lower. Thus, treatment with "Roder" significantly did not change the composition of the microbial community existing in river water. By analogy, the dosage of nutritive substances was chosen so that the residual phosphate and nitrate concentrations at the end of the treatment were rather low to prevent this water body from being overgrown with algae.

Both lakes in Vyngayakha had high SLOP (Table 1) and were visibly covered with oil films. In spite of rather drastic starting conditions, oil pollution was eliminated almost completely upon threefold treatment with "Roder", which was fortunately carried out in unusually warm weather; the residual oil concentrations were 5 and 190 mg L^{-1} in lakes 1 and 2, respectively (Table 1). A larger part of petroleum (90%) in an Urai lake was removed mechanically (Table 1), *i.e.*, the pollution level was reduced from 11 to 1.01 g L^{-1}. Subsequent twofold treatment with "Roder" reduced the oil content in this object to 0.43 g L^{-1}, with an overall remediation effect of 96% (Table 1). A relatively high residual level of pollution was mainly due to oil-impregnated deposits accumulated in the lake, which were permanent sources of oil supply to the water phase.

Bioremediation of swamps. Relatively poor bioremediation of a swamp in Vyngayakha (Table 1) is explained by local geological conditions, which made preliminary mechanical collection of spilled oil impossible. However, taking into account a high SLOP (>24 g L^{-1}) and the spillage age (4 years), the results may be estimated as satisfactory. Oil pollution was eliminated upon threefold treatment with "Roder" by 65% (Table 1). The overall remediation effect in a polluted swamp near Urai was 94%, where mechanical collection (75%) was followed by threefold treatment with "Roder" (Table 1).

Bioremediation of peat bogs. Small remediation effects of "Roder" in the field tests carried out in peat bogs near Nizhnevartovsk (Table 1) are not surprising, considering an extremely high SLOP (>750 g kg^{-1} of dry substance) and the spillage age (6 years). Because the great majority of the spilled oil was adsorbed by peat, mechanical cleaning was impossible. Preliminary treatment included only plowing of the upper layer of the polluted area, liming (to adjust pH), and the use of nitrogen and phosphate fertilizers. The average (over three areas) bioremediation effect in the presence of "Roder" was 19% (Table 1). Natural bioremediation effect (13%) indicates a high HCO activity of native microflora developed in this polluted object over six years, which was substantially stimulated by pH adjustment and fertilization. This conclusion finds support in an increased (from 10^3 at the beginning to nearly 10^6 cells mL^{-1} at the end of the tests) number of HCO bacteria in the areas where "Roder" was not applied.

Thus, the field tests showed that "Roder" is very highly effective for bioremediation of open water areas moderately polluted by oil (< 20 g L^{-1}). When the oil concentration in water areas is higher (oil film thickness > 3 mm), as well as when oil is spilled over swamps and soils, the optimum strategy of their remediation should include either preliminary mechanical collection of free petroleum, or application of adsorbents, or other pretreatment methods followed by microbiological cleaning. In the case that such a pretreatment is impossible because of some reasons, polluted swamps and soils should be repeatedly treated by biopreparations with periodical plowing, pH adjustment, and fertilization. In old oil spills (>5 years), the native HCO microflora is usually so active that extrinsic biopreparations may be excluded. To be economical, the remediation of such spills can simply include stimulation of the native HCO microflora that is already adapted to the polluted object.

2. Low-temperature (4—10 °C) anaerobic wastewater treatment

Anaerobic biological treatment of sewage offers some obvious advantages over conventional aerobic treatment applied in Russia, especially for treatment of wastewater with high concentrations of organic contaminants. Among them are no power requirements for aeration (conversely, energy is generated in the form of biogas); a tenfold reduction in the amount of excess sludge; substantially lower consumption of nitrogen, phosphorus, and other nutritive components; increased organic loading rates, *etc.*[16] However, conventional anaerobic wastewater treatment cannot often be introduced (especially in countries with cold climate such as Russia) because a working temperature must be maintained in the range of mesophilic (30—37 °C) or thermophilic (55—60 °C) regimes, which is significantly higher than ambient temperatures and the temperature of most wastewater. This section deals with the recently obtained results,[6,7] which allow unprecedented low-temperature anaerobic wastewater treatment (at 4—10 °C).

As a rule, the biocatalytic activity of methanogenic microorganisms involved in anaerobic degradation of organic contaminants of wastewater is sharply reduced at low temperatures. For this reason, the strategy of development of the corresponding technology should include a maximum possible increase in the concentration of a biocatalyst (biomass) inside the reactor or/and gradual adaptation of mesophilic microorganisms to low temperatures.[2] Both these approaches were applied[6,7] in an upflow anaerobic sludge bed (UASB) reactor that uses mesophilic seed sludge with high methanogenic activity and promotes self-immobilization (and hence accumulation inside the reactor) of the biomass of methanogenic consortium of microorganisms *via* granular formation. Dilute distillery grains (vinasse) obtained by distillation of low-quality wines were used as a simulated wastewater. The total concentration of organic contaminants was expressed in chemical oxygen demand (COD) units. Other experimental details have been described in Refs. 6 and 7. The results of the prolonged operation of laboratory UASB reactors under psychrophilic conditions are summarized in Table 2.

Low-temperature treatment in one step. In run *1a* (Table 2, 10 °C), the raw wastewater was not pretreated, and the organic loading rate in the reactor was gradually increased to 4 to 5 g of COD L^{-1} day. Organic contaminants were removed with an average effect of 70% (in COD units). Significant amounts of propionate (major component) and acetate were detected in the treated wastewater. However, the reactor liquid contained only traces of sugars, ethanol, and butyrate, while the resulting biogas contained traces of hydrogen. Clearly, low temperatures differently influence various stages of

Table 2. Summarized results of the prolonged low-temperature operation of laboratory UASB reactors fermenting wine distillery grains[6—7]

Run (object)	T /°C	τ /day	Reactor type	Recycling factor	F	t_{ret} /day	COD/g L^{-1}		N_{COD} (%)
							influent	sewage	
1a (A)	9—10	0—67	R	1 : 2.6	0.3—5.1 (2.7)	0.8—5.1 (1.9)	3.6—5.2 (4.0)	0.3—2.7 (1.0)	48—92 (72)
1b (B)	9—10	68—158	R	1 : 2.6	1.4—7.3 (4.7)	0.5—1.6 (0.9)	1.2—9.9 (4.2)	0.5—3.6 (1.8)	48—92 (60)
2a	7—8	159—185	R	1 : 2.6	3.2—4.6 (3.7)	0.85—0.87 (0.86)	2.7—4.0 (3.2)	0.8—1.9 (1.0)	52—79 (68)
2b	7—8	197—236	R	1 : 11.6	2.3—3.5 (3.0)	0.9—1.3 (1.1)	3.0—3.6 (3.2)	0.9—1.5 (1.2)	48—70 (60)
3	3—5	251—273	R	1 : 11.6	1.1—2.7 (1.7)	1.14—1.17 (1.15)	1.3—3.1 (2.0)	0.6—1.5 (0.8)	15—72 (57)
4	9—10	0—63	R1	1 : 1	3.2—5.5 (4.4)	0.8—1.3 (1.0)	3.1—5.4 (4.3)	1.0—3.9 (2.4)	16—76 (46)
			R2	1 : 18	0.8—4.0 (2.5)	0.8—1.2 (1.0)	1.6—3.9 (2.5)	0.4—2.8 (1.2)	24—80 (58)
			R1+R2		1.6—2.8 (2.2)	1.6—2.5 (2.0)			36—91 (78)
5	7—8	82—107	R1	1 : 1	2.3—4.2 (3.5)	1.0—1.1 (1.0)	2.5—4.2 (3.5)	1.3—3.1 (2.3)	19—52 (37)
			R2	1 : 18	1.2—3.0 (2.2)	1.0—1.1 (1.0)	1.4—3.1 (2.3)	0.4—1.9 (1.0)	29—78 (61)
			R1+R2		1.2—2.1 (1.8)	2.0—2.2 (2.0)			42—89 (76)
6	3—5	122—147	R1	1 : 1	2.0—2.7 (2.5)	0.8—1.0 (0.9)	1.9—2.6 (2.4)	1.1—1.9 (1.5)	25—52 (37)
			R2	1 : 18	1.5—2.2 (1.7)	0.8—1.0 (0.9)	1.1—1.9 (1.5)	0.3—1.2 (0.7)	43—74 (53)
			R1+R2		1.0—1.4 (1.3)	1.6—2.0 (1.8)			60—86 (71)

Note. R is a single reactor, R1 and R2 are two reactors connected in series; τ is the test duration; F is the organic rate/g of COD L^{-1} day^{-1}; t_{ret} is hydraulic retention time (the period of time for which the wastewater is retained in the reactor); N_{COD} is the amount of removed COD; A is the raw wastewater, and B is the preacidified wastewater. Average values are given in parentheses.

anaerobic fermentation, while the conversion of propionate becomes the main limiting step.[17] It is also noteworthy that the sludge layer in the reactor expanded by some 20% by the end of the experiment, first of all, because of a significant growth of the biomass of acidogenic bacteria (an amorphous layer covering a more compact central part of biomass aggregates was well seen under a microscope). Such types of aggregations can cause sludge flotation and diffusional limitations (mass transfer of substrates) for propionate-degrading and aceticlastic microorganisms usually concentrated in the central part of aggregates; for this reason, wastewater in subsequent tests was preliminarily fermented (preacidified) by keeping it in an air-open vessel for one day. However, the use of preacidified vinasse did not enhance the cleaning effect, and the concentration of propionate in the reactor often exceeded 1.5 g of COD L^{-1} (run *1b*, Table 2). To understood the processes occurring in the low-temperature UASB reactor, the kinetic characteristic of sludge were determined *in situ*, *i.e.*, under the reactor conditions. It was found that the apparent half saturation (affinity) constants of ethanol and volatile fatty acids are rather high (>1.0 g of COD L^{-1}) when an upflow liquid velocity in the reactor (V_{up}) is equal to 0.1 m h^{-1} (under our conditions); this supports the hypothesis about diffusion limitations inside the sludge layer.

A decrease in the working temperature to 7 °C (run *2a*) did not deteriorate the cleaning effect, though the organic loading rates were somewhat lower (~4 g of COD L^{-1} day) than in run *1b* (Table 2). To attenuate the problems in mass transfer, the recycling factor was increased in run *2b* (Table 2). As was expected, a fourfold increase in V_{up} enhanced the conversion of volatile fatty acids, though the cleaning effect in total COD was slightly lower than in run *2a*. This was attributed to an increased sludge wash-out at high V_{up} (small sludge aggregates were continually detected in the treated wastewater at the reactor outlet). Further decrease in the working temperature to 4 °C was accompanied by a reduction of the organic loading rate during the experiment (Table 2, run *3*). The reactor′s overall efficiency under these conditions (*i.e.*, at a temperature of a home refrigerator!) was the same as in run *2b*. Sludge removal was slight, because small sludge aggregates were already mostly removed from the reactor during run *2b*. Microscopic examination of the sludge after run *3* revealed immense domination of large amorphous irregular aggregates (4—5 mm). Such an evolution of sludge can be due to incomplete preacidification of the influent during its one-day exposure to air. For example, ethanol and sugars sometimes entered into the reactor in very high concentrations (up to 2 and 0.6 g of COD L^{-1}, respectively) together with the influent, stimulating the propagation of voluminous acidogenic biomass that deteriorates the sludge quality. Thus, control of preacidification seems to be an important factor for the development of a stable process of low-temperature anaerobic wastewater treatment.

Low-temperature treatment in two steps. To control the preacidification of vinassa wastewater for the purpose of enhancing the cleaning effect, two UASB reactors were connected in series. Reactor R1 mainly served as a preacidifier, generating volatile fatty acids to be transferred to reactor R2. High recycling factor (1 : 18) was used in reactor R2 to facilitate diffusion, while a low recycling factor (1 : 1) in reactor R1 was retained since mass exchange problems are not so important at a rapid acidogenic stage. The sludge accumulated in run *3* and composed of voluminous biomass aggregations (see above) was used for inoculation of both reactors. While analyzing the results obtained in the two-step UASB treatment of distillery grains (see Table 2), one should emphasize the following. The two-reactor system was more effective and steadier in operation at 4—10 °C (no sludge flotation or its excessive wash out) than a single UASB reactor. However, the latter is superior in achieved organic loading rates (with consideration of a total volume of both reactors in two-step treatment). Thus, capital investments and operating costs are higher for the two-reactor technology, which should be taken into account regarding possible introduction of anaerobic wastewater treatment. At the same time, a low-temperature single UASB reactor needs at least partial preacidification of wastewater to ensure its stable operation.

Hence, anaerobic treatment of wastewater contaminated with soluble organic substances is quite possible in high-rate UASB reactors even at such low temperatures as 4—10 °C. However, since such intermediates of anaerobic fermentation as propionate and acetate experience significant mass-exchange problems under these conditions, the application of high recycle rates can substantially enhance the efficiency of anaerobic treatment. It was shown in a specific experiment that the wastewater pretreated under anaerobic conditions can effectively be brought to the accepted standards using aerobic biological methods.[7] All this opens certain prospects for introduction of high-rate anaerobic wastewater treatment at ambient temperatures in countries with cold climate.

3. Biomineralization of azo dyes in a novel anaerobic-aerobic hybrid reactor

Azo dyes constitute the main part of all dyes produced in the world.[18] Approximately 10 to 15% of their total production enters into the environment, mostly through wastewater.[19] Insofar as azo dyes themselves or their decomposition products (*e.g.*, aromatic amines) have strong toxic, mutagenic, or carcinogenic effects on living organisms, the corresponding wastewater must be treated prior to discharge. A great number of azo dyes are not biodegradable under aerobic conditions and thus remain intact while passing through conventional aerobic systems of wastewater treatment. At the same time,

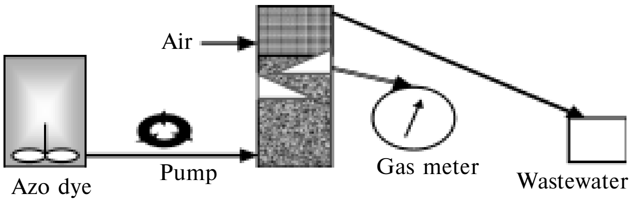
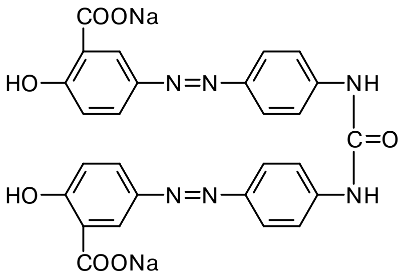


Fig. 1. Scheme of an experimental setup for biomineralization of azo dyes in AnAHR.

azo dyes are decolorized relatively quickly *via* cleavage of the azo bond in anaerobic media. In turn, the products of anaerobic azo dye decomposition are more susceptible to biodegradation under aerobic rather than anaerobic conditions. Because of this, biomineralization of azo dyes should follow an anaerobic-aerobic sequence, for which purpose two separate reactors are usually used.

To optimize the treatment process and enhance its efficiency (by reducing aeration costs and using more compact equipment), we combined the anaerobic and aerobic phases in a single reactor named anaerobic-aerobic hybrid reactor (AnAHR) (Fig. 1). The efficiency of AnAHR was estimated in biomineralization of the azo dye Siriusgelb with the following structure:



The results of these tests are summarized in Table 3.[8] The average biomineralization effect on the azo dye reached 71% during the first eighteen days of the experiment when the azo dye loading rate was ~0.09 g of COD L^{-1} day. With an increase in the azo dye loading rate to 0.18 g (the 19—32th days) and then to 0.3 g of COD L^{-1} day^{-1} (beginning with the 33th day), biomineralization effect was reduced, on the average, to 64 and 56%, respectively (Table 3). Nevertheless, low optical densities of the wastewater at 375 nm (absorption maximum of Siriusgelb) during the experiment indicate almost complete decomposition of the azo dye in the reactor. However, the treated wastewater was not decolorized completely, remaining slightly rose compared to an intense brownish-yellow color of the initial wastewater. 5-Aminosalicylic acid (5-ASA), an intermediate of Siriusgelb decomposition, accumulated in the anaerobic zone of AnAHR rather than in the effluent. The concentration of 5-ASA was maximum (up to 0.06 g of COD L^{-1}) immediately after increases in the azo dye loading rates, gradually decreasing at a constant rate. This suggests gradual adaptation of anaerobic biomass to the decomposition of 5-ASA.[20—21] The great part of the azo dye (36—41%) was mineralized in the anaerobic zone, while the aerobic zone of the reactor accounted for a decrease in COD by only 20—30%.[8] Such a low contribution from the aerobic stage, as well as the residual color of the effluent, may be associated with easy autooxidation of the products of anaerobic Siriusgelb decomposition (5-ASA and 1,4-phenylenediamine) into colored polymeric compounds in the presence of air.[21] These polymeric products have complicated humin structure and environmentally safe, but they are usually only slightly biodegradable.[21]

Table 3. Summarized results[8] of biomineralization of the azo dye Siriusgelb* in AnAHR at 30 °C**

τ/day	ALR	t_{ret}/day	COD_{sew}	ΔCOD_{ad}
0—18	0.08—0.10 (0.09)	2.95—3.97 (3.38)	0.03—0.13 (0.09)	57—90 (70)
19—32	0.14—0.23 (0.18)	1.30—2.13 (1.71)	0.07—0.14 (0.11)	53—78 (64)
33—53	0.20—0.40 (0.30)	0.74—1.49 (1.05)	0.05—0.19 (0.13)	37—84 (56)

* Ethanol was used as a donor of reducing equivalents (concentrations 0.3 (Siriusgelb) and 0.82 g of COD L^{-1} (EtOH)).

** ALR/g of COD L^{-1} day^{-1} is the azo dye loading rate; t_{ret} is hydraulic retention time. Average values are given in parentheses.

Thus, the tests of the new reactor AnAHR with synthetic wastewater containing the azo dye Siriusgelb showed that it ensures almost complete biodegradation of this xenobiotic and substantially decolorizes the treated effluent. However, organic contaminants were not completely mineralized because the autooxidation of anaerobic decomposition intermediates of this azo dye results in nonbiodegradable polymeric compounds. To assess the applicability of this novel reactor design for treatment of industrial waste waters polluted with azo dyes and other xenobiotics (for example, halogenated compounds), further investigations are required.

4. Biocatalytic technology of hydrogen sulfide elimination and sulfur recovery from polluted gases

Biogas usually contains ~1 vol % of H_2S, while natural gas can contain up to 15 vol % of H_2S.[9] Conventional methods for purification of such gases are based on chemical binding in the liquid phase, physical absorption, and direct chemical conversion. They have some obvious drawbacks, namely, high reagent consumption, corrosion of equipment, high operating temperature and pressure, *etc.*, which makes the process rather expensive (a ton of removed sulfur costs $250—750).[22] An alternative and substantially cheaper method is a biocatalytic purification H_2S-polluted gases.[9—10] The technological scheme of the proposed process is displayed in Fig. 2. According to the scheme,

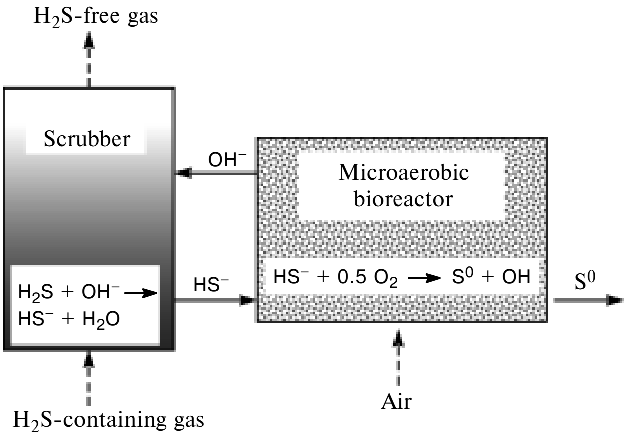


Fig. 2. Technological scheme of a biocatalytic removal of H_2S from contaminated gases.

a polluted gas is passed through a scrubber in which hydrogen sulfide is absorbed by a weakly alkaline solution (pH 8.0—8.5) of an aqueous phase. A nearly neutral (pH ~7.0) solution of H_2S is transferred to a microaerobic bioreactor with strict control of the concentration of dissolved oxygen. The bioreactor contains bacteria of the *Thiobacillus* genus, which can oxidize H_2S to elementary sulfur and transport it to an extracellular medium. The resulting sulfur is removed from the reactor by sedimentation and can be a marketable product (*e.g.*, for manufacturing of sulfuric acid). The weakly alkaline (pH 8.0—8.5) supernatant liquid is returned to the scrubber for absorption of another portion of H_2S from natural gas. Thus, this technology has to offer an almost completely closed cycle of the aqueous phase and elementary sulfur as, ideally, the sole product in the solid state, which is very important for its subsequent recovery.

Insofar as the success of this very elegant technological scheme is largely determined by the operating parameters of the microaerobic bioreactor, our efforts were mainly aimed at optimizing its design and efficiency.[9—10] The problem was that the *Thiobacillus* bacteria used as biocatalysts in this process can oxidize sulfide not only to elementary sulfur, but also further to sulfate:

$$HS^- + 0.5\ O_2 \longrightarrow S^0 + OH^-, \tag{1}$$

$$HS^- + 2\ O_2 \longrightarrow SO_4^{2-} + H^+. \tag{2}$$

Naturally, reaction (2) is undesirable because it decreases an alkaline potential of the medium and yields sulfate, which is difficult to remove from the aqueous phase. It was shown[9] that reaction (2) cannot be excluded completely from this system, but it can be strongly inhibited provided that the amount of oxygen is limited and/or the sulfide rate of the bioreactor is increased. Different reactor designs (conventional reactor with a stirrer, reactor with external aeration, gas-lift reactor) were manufactured and tested under laboratory conditions to reach the maximum rate of sulfide oxidation according to reaction (1) along with minimization of conversion (2). At present, the most optimum design among those developed by us is a sealed gas-lift reactor (with a closed cycle of the gas phase) equipped with sensors for direct measurement of the concentrations of dissolved oxygen and sulfide and pH. Control of the reactor was automated by connecting the sensor output terminals to a computer's analog-to-digital converter. Using this highly controlled reactor and pure oxygen (instead of air), we increased the efficiency of sulfide-to-sulfur conversion to 94—98% for sulfide rates of ~15 g of S L^{-1} day.[9—10]

To sum up, let us note the following evident advantages of the developed biocatalytic method for purification of H_2S-containing gases relative to conventional techniques:

1) almost reagentless character (small amounts of mineral salts are required for bacteria growth, and alkali is necessary for pH adjustment in the scrubber);

2) cheapness;

3) highly efficient removal of hydrogen sulfide;

4) nearly complete recycling and a minimum amount of wastewater;

5) elementary sulfur as the sole product of the process, which can be easily recovered;

6) process is carried out at ambient pressure and temperature, which makes it safe;

7) compact design of the reactor.

Conclusion

The above examples demonstrated that the rates of the target processes in waste treatment can be significantly increased under optimum conditions for microorganisms growth in properly designed engineering systems. This facilitates the solution of many problems of biotechnological protection of the environment. Moreover, this discipline is gradually transforming now: the usual function (so-called "end of pipe treatment") gives way to a new one characterized by the possibility of recovering resources contained in wastes. A typical example is the aforesaid biocatalytic technology of sulfur recovery from H_2S-polluted gases.

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