Original Research

Biodegradation of Used Metalworking Fluids in Wastewater Treatment

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Abstract

A biotechnological method for metalworking fluids (MWF) wastewater treatment was developed. The research, conducted in a lab-scale bioreactor, proved that the process of eliminating pollutants from MWF wastewater proceeds effectively when the following are applied: multiple reinoculating of biomass by adding active microorganisms into the bioreactor, immobilizing microorganisms on the PVC foam carrier, and carrying out the process in the anoxic/aerobic conditions at phase durations of 0.5 and 5.5 hours, respectively.

The above parameters allowed us to obtain the following eliminations: 87% of chemical oxygen demand (COD), 97% of biochemical oxygen demand (BOD_5), 98% of petroleum ether extractable organic (PEEO) and more than 96% of total content of hydrocarbons determined by infrared (IR) spectrophotometry. The chromatographic analyses showed almost complete reduction of oily hydrocarbons contained in the used MWF.

Keywords: metalworking fluids, wastewater treatment, biodegradation

Introduction

Metalworking fluids (MWF) are used to optimize the process of machining operations such as turning, drilling, boring, grinding and milling. They flush away chips and metal fines from tools and workpieces, as well as acting as coolants and lubricants [1]. The majority of machining operations are carried out using oil-in-water (O/W) emulsions (80% of the total consumption) [2]. The addition of water has made MWF a favourable environment for a variety of microorganisms [3, 4, 5]. Microbial growth results in biodeterioration of MWF due to breakdown of its components such as emulsifiers, oils and additives [6]. Spent MWF must be disposed, which causes a major problem.

All the methods for MWF disposal can be divided into 4 main groups: chemical, electrochemical, physical-mechanical and biological. In the chemical splitting electrolyte (mineral salt, acid or organic demulsifier) is added to neutralize the emulsifier – the emulsion breaks down and water and oil phases are separated in a settling tank [7, 8]. The electrochemical method is based on the destabilization of Stern ionic layer surrounding oil droplets in the emulsion. The electrodes immersed in the emulsion generate the electrostatic field. Oil droplets are moving towards the electrodes and collect into bigger groups. The emulsion disintegrates into water and oil phases. In this method the adsorption and electro-coagulation phenomena occur as well. Aluminium electrodes dissolve; ions passing into solution become hydroxides and adsorb the oil droplets on their surface. Ultrafiltration [9, 10], thermal splitting, evaporation [7, 11] and incineration [12] are the most common physical-mechanical methods of used MWF disposal.

Almost all mentioned classical techniques (except for the incineration) do not solve ultimately the problem of MWF treatment – the emulsion is split into water and oil phases which must both be utilized. Disposal of the

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Table 1. The characteristic of MWF wastewater.

Parameters	Average concentration	Range
COD [mg O_2/dm^3]	20,200	5,300-35,200
BOD_5 [mg O_2/dm^3]	6,500	1,500-11,400
PEEO[1] [O_2/dm^3]	4,800	1,100-8,500
TN[2] [mg N/dm^3]	300	160-440
TP[3] [mg P/dm^3]	53	28-77

[1]PEEO - petroleum ether extractable organics; [2]TN - total nitrogen; [3]TP - total phosphorus

residual sludge phase, which is produced in the process, has become increasingly more difficult and expensive in recent years. Changes in MWF formulas have resulted in increased amounts of organics in MWF wastewater treated by classical methods [7]. As a result of it various biological treatment, schemes have been tested to remove the organics from the water phase obtained during physical-chemical treatment [13, 14, 15, 16].

Hardly any attempts have been undertaken to imply biological methods to MWF wastewater treatment without prior physical-chemical splitting. The aim of this research was to develop a biotechnological method for used MWF full-strength wastewater treatment.

Materials and Methods

Bioreactor Configuration

The studies were carried out at room temperature in a 10 dm^3 (6 dm^3 working volume) laboratory bioreactor made of a polymethyl methacrylate (Plexiglas) tube. 48 PVC foam stripes (150 x 20 x 2 mm) were attached to an aluminium rack inside the bioreactor as carriers for the bacteria that were immobilized at the beginning of the process as described below. The bioreactor was equipped with an aeration system and a mechanical stirrer. Fill and draw were provided by peristaltic pumps.

The bioreactor was operated for 84 days as a Sequencing Batch Reactor (SBR) in 5 cycles. The duration of the first as well as the fifth cycle was 21 days while the duration of each of the second, third and fourth cycles was 14 days. Each cycle consisted of an anoxic phase (0.5 hours in duration) and an aerobic phase (5.5 hours in duration), which repeated continuously one after the other throughout each of the cycles (21 or 14 days). During the first anoxic phase of each cycle (within the first 0.5 hours), 5.4 l MWF wastewater was pumped into the bioreactor. At the end of each cycle (within the last 0.5 hours of each cycle after the 1-hour settling), 5.4 l supernatant was removed from the bioreactor which resulted in 9/10 exchange volume ratio.

The bioreactor operated as a hybrid reactor containing both suspended and immobilized microorganisms. The biomass in the bioreactor had multiple reinoculations every 3 days by adding active microorganisms prepared as written below. pH was maintained at 6.5-7.5 by adding concentrated HCl. A reactor like the reactor described above, operating in the same cycles regime but without immobilized biomass and multiple reinoculating, was the control reactor.

Wastewater

Both the reactors were supplied with water-based metalworking emulsion wastewater obtained from the works producing rolling bearings in Poland. The characteristic of the wastewater is shown in Table 1.

Microorganisms and Immobilisation

Microorganisms were isolated from used real MWF and identified using routine microbiological procedures and the standardized API identification system [17, 18]. The most active strains were selected on a base of the growth intensity at 26˚C on a solid mineral agar medium [19] with 5% addition of MWF as a sole source of carbon and energy. The same medium was used to store the identified strains in a refrigerator at 4˚C.

The most active in MWF biodegradation bacteria strains (*Chryseomonas luteola, Ochrobactrum anthropi, Pseudomonas vesicularis, Alcaligenes faecalis, CDC Gr IV C-2*) were multiplied in a liquid mineral medium [19] containing MWF (5%), broth (0.1%) and yeast extract (0.001%) and incubated in 250 ml Erlenmayer flasks held in thermostated shakers. After 48 hours incubation at 26˚C bacteria cells were centrifuged (10 min, 10,000 rpm) in Biofuge 17 RS (Heraeus Sepatech), suspended in 0.65% NaCl solution and used as an inoculum of the bioreactor.

The process of the immobilization was carried out after 24 hours of bacteria cultivation in the liquid medium in the aerated bioreactor at room temperature. The PVC stripes, previously sterilized in Koch apparatus, were immersed in the 24-hour culture and immobilization was carried out within the next 24 hours.

Analytical Methods

Petroleum ether extractable organics (PEEO), chemical oxygen demand (COD), total phosphorus (TP) and nitrogen (TN) were determined according to relevant Polish Standard Methods [20, 21, 22, 23]. Biochemical oxygen

Table 2. Elimination of pollutants [%] in the research bioreactor - the fifth cycle.

time	COD	BOD_5	PEEO	IR hydrocarbons		GC/MS hydrcarbons
				Raw extract	Cleaned-up extract	
[days]	[%]	[%]	[%]	[%]	[%]	[%]
3	49	70	57	60	49	87.1
7	77	83	95	84	89	99.9
14	87	97	98	89	89	100.0
21	87	97	98	91	97	100.0

Table 3. Elimination of pollutants [%] in the control reactor - the fifth cycle.

time	COD	BOD_5	PEEO	IR hydrocarbons		GC/MS hydrcarbons
				Raw extract	Cleaned-up extract	
[days]	[%]	[%]	[%]	[%]	[%]	[%]
3	15	30	9	18	14	13
7	15	48	18	25	25	8
14	36	57	35	39	37	27
21	37	55	49	45	51	3

demand (BOD_5) was determined using OXITOP IS 12 (WTW) equipment [24, 25]. All the colorimetric analyses were carried out using a “Marcel” S330 PRO spectrophotometer at a relevant wavelength.

Total amount of hydrocarbons were determined by means of infrared (IR) spectrophotometric and chromatographic analyses (GC/MS). IR analyses were carried out using UR-20 (Carl Zeiss Jena) equipment according to Polish Standard Methods [26] before and after the cleaning-up procedure (hydrocarbons determined in raw and cleaned-up extracts respectively) using a column containing florisil [27]. GC/MS analyses were carried out using a gas chromatograph GC (Model 5890 Hewlett Packard) equipped with a mass spectrometer MS (Model 5971) and an HP5 column (30 m). Samples were extracted with three 100 ml portions of methylene chloride. All the extracts were combined, passed through a column containing silica gel and concentrated to 2 ml using a rotary evaporator prior to 1 µl split injection. The standard solution was prepared by diluting 1 mg diesel fuel in 1 ml methylene chloride.

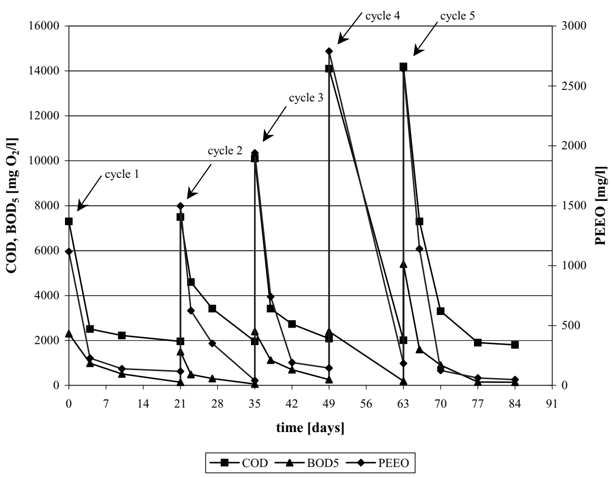

Fig. 1. Changes in concentrations of COD, BOD_5 and PEEO in the research bioreactor during the experiment. Arrows indicate subsequent refillings of the bioreactor.

Results and Discussion

Both the research and control reactors were operated for a period of 84 days in 5 cycles. The performance of the research reactor is summarized in Fig. 1, which shows changes in concentrations of COD, BOD_5 and PEEO in the bioreactor during the cycles. The bioreactor was fed with the MWF wastewater in which concentrations of pollutants were gradually increased. The addition of phosphorus and nitrogen was found to be unnecessary due to their sufficient amounts in the wastewater – all nitrogen and phosphorus came from the used MWF.

It was shown that the process of biodegradation ran most intensively during the first 3 days of each cycle – about 70% BOD_5 and 50% of COD and PEEO were removed.

Tables 2 and 3 present the elimination of pollutants during the fifth cycle in the research and control reactors. The research reactor performed well, removing 98%, 97%

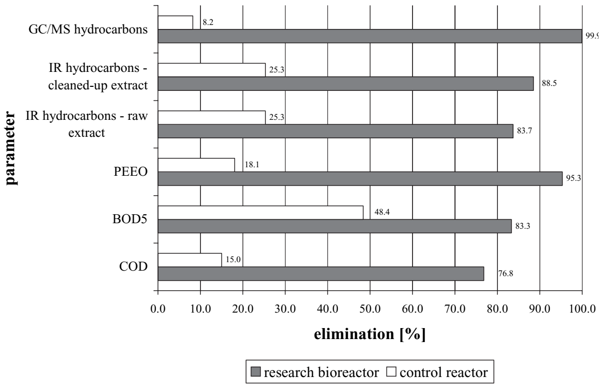

Fig. 2. Comparison of pollutant elimination in the research and control reactors after 7 days of the fifth cycle.

and 87% of PEEO, BOD_5 and COD, respectively.

Similar results were obtained by Gray *et al*. [8], who operated SBR at 3 days hydraulic retention time. The authors attained 95% removal of COD, treating the water phase (influent COD 4 800 mg O_2/dm^3) which was obtained as a result of chemical splitting of MWF simulated wastewater.

The present research showed 95% elimination of PEEO within the first 7 days of the fifth cycle. The removal of those pollutants in the control reactor was significantly lower, which is shown in Fig 2.

IR and GC/MS analyses proved that hydrocarbons are almost completely removed during the biodegradation process, while the elimination of those pollutants in the control reactor was much lower. Fig. 3 presents results of GC/MS analyses, which show nearly 100% reduction of oily hydrocarbons in the research bioreactor.

The COD removal in the research bioreactor was relatively lower than the biodegradation of oily pollutants; about 10-15% of organics represented as COD were resistant to the biodegradation. The presence of residual organics were also reported by Kim *et al*., who studied fluidized bed reactors [13, 14, 15]. The authors stated that 12% of organics in the simulated wastewater containing MWF, were neither aerobically nor anaerobically biodegradable, which is in accordance with the present research. Additional studies carried out by Kim *et al*. [28] confirm that MWF contain some refractory organics, probably branched fatty acids or esters, which are extremely difficult to identify. The identification of the residual organics in the course of the present studies also failed due to lack of suitable standards.

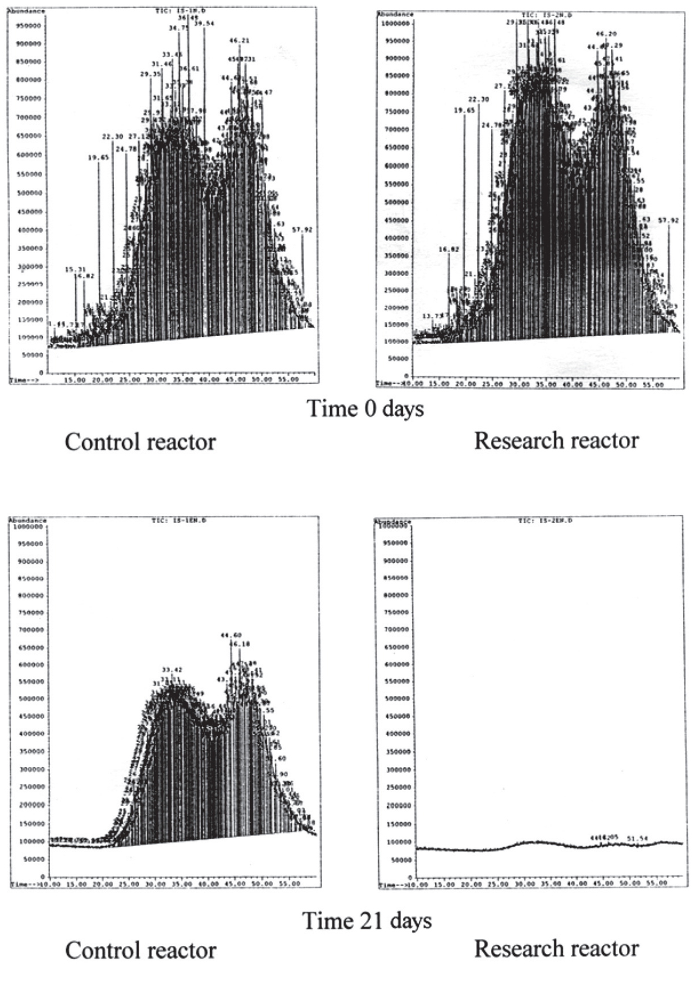

Fig. 3. Chromatograms obtained in the result of GC/MS analyses during the fifth cycle.

Töws *et al*. [29] proved that recalcitrant hydrocarbons detected in the effluent of the bioreactor treating lubricating refinery wastewater represented only a small part of COD (about 2%) and the major part of COD was obviously caused by humic substances. Also, Gulyas and Reich [30] stated that the main part of COD in the effluent was caused by humic substances which are normally formed during aerobic biological treatment.

In the course of the present research the process of MWF biodegradation was intensified by the multiple reinoculating of biomass. Active microorganisms isolated from used MWF were added to the bioreactor. It is known [31] that introducing specific microorganisms gives the chance to recruit useful reaction sequences and enhances the biodegradation rate of recalcintrant substances. There is a variety of situations where bioaugmentation with microorganisms possessing unique and specialized metabolic capabilities significantly improves the process of bioremediation. Inoculation with specific microorganisms is highly recommended when the bioremediation process depends on co-metabolism [32]. On the other hand, there are many limitations to the use of bioaugmentation and the most important is an inability to support the growth and activity of introduced microorganisms in the environment because of competition from indigenous microflora or poor survival characteristics of the inoculum. Non-homogenous environments, like soil or O/W emulsions, may bring about low bioavailability of specific recalcitrant substrates, with the results that microbial degradation cannot take place or proceed slowly. Furthermore, low bioavailability of specific substrates may cause introduced microorganisms to lose specialized metabolic capabilities coded on plasmids [33]. Systems, which are sequentially aerobic and anaerobic, may cause inactivation or even death of aerobes or anaerobes due to the periodic absence or presence of oxygen, respectively [31]. The success, however, can be realized through the implementation of some techniques, e.g. of multiple reinoculation and immobilization of biomass, to reduce the competition from indigenous microflora and improve survival characteristics [32, 34, 35]. According to Rossmoore [6], the same bacteria associated with the biodeterioration of MWF should be dominant species in the biodegradation process. Gray et al. [8], using SBR for treating water phase obtained from chemical splitting of MWF wastewater, decided that the system should be reinoculated with the microorganisms that had been developed in separate shake flasks. The introduction (into the SBR) of these microorganisms, originally collected from diverse environments in a petroleum refining area, produced a culture which would effectively degrade oily organics and produce a good settling sludge. The method of multiple reinoculating in technical conditions has been successfully applied to bioremediate soil contaminated with petroleum derivatives [36].

The present research proved that immobilizing bacteria on the PVC foam carrier can be advantageous in MWF wastewater treatment. It is known that immobilized microorganisms are effective at purifying of refinery wastewater that contains hydrocarbons. The process of immobilization allows: lower operating costs, obtaining high reactor biomass concentration, operating with mixed populations of microorganisms and minimizing the toxic impact of refractory substances on microorganisms [29, 30, 37].

Research shows that SBR can be useful in pretreatment of full-strength MWF wastewater. Because of its flexibility and high efficiency, SBR is widely used in treating hazardous wastewater containing hydrocarbons and their derivatives [8, 38].

Conclusions

The research proves that the process of eliminating pollutants from metalworking fluid wastewater proceeds effectively when the following are applied: multiple reinoculating of biomass by adding active microorganisms into the reactor, immobilizing microorganisms on the PVC foam carrier, and carrying out the process in the anoxic/aerobic conditions at phase durations of 0.5 and 5.5 hours respectively.

The above parameters allowed us to obtain the following eliminations: 87% of COD, 97% of BOD_5, 98% of petroleum ether extractable organic and more than 96% of total content of hydrocarbons determined by infrared spectrophotometry. The chromatographic analyses showed almost complete reduction of oily hydrocarbons contained in the MWF wastewater.

A relatively high value of the COD index of the purified wastewater requires additional analytical research in order to identify possible biodegradation-resistant substances. The optimization of the working conditions of the SBR still remains an open issue.

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