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MIKROBNA DEGRADACIJA EMULZIJA ZA HLAĐENJE I PODMAZIVANJE PRI OBRADBI METALA

Sažetak

Kvarenje materijala je problem koji zaokuplja pozornost stručnjaka oduvijek, jer, prije svega, dovodi do velikih ekonomskih gubitaka. Zato su uzroci kvarenja različitih materijala predmet istraživanja već dugi niz godina. Organizirane grupe istraživača većinom u kemijskoj industriji podržane vladinim organizacijama bave se analizama i istraživanjima u tome području.

Najveću ulogu u kvarenju ima biološko kvarenje koje je po definiciji svaka neželjena promjena svojstava materijala uzrokovana aktivnostima živih organizama. Biorazgradnja pod nazivom kvarenje, nepoželjan je proces kada je u pitanju proizvodnja i čuvanje materijala, ali je vrlo poželjan proces kada taj materijal postaje otpad, a u pitanju su obično često iste vrste mikroorganizama.

Emulzije za hlađenje i podmazivanje pri obradbi metala s velikim udjelom vode do 98% upravo su zato vrlo podložne rastu mikroorganizama. Uljna faza je dobra hranjiva podloga za mikroorganizme kao izvor ugljika. Zbog raspršenih kapljica u vodi dodirna međufazna površina ulje/voda vrlo je velika pa je i površina za rast mikroorganizama dovoljna budući da je voda neophodni medij za razvoj. Pod utjecajem metabolizma mikroorganizama proizvod se mijenja kemijski i funkcionalno zbog čega nastaju sluzave mikrobne nakupine koje uzrokuju stalno čepljenje filtara i uređaja.

Iako su koncentrati emulgirajućih tekućina od kojih se spravljaju radne emulzije obično sterilni, što je ispitano i u našem radu, mikroorganizmi se vrlo brzo nakon namješavanja s vodom razvijaju, jer niti okolina niti voda nisu sterilni. Budući da u *spremnicima već postoje mikrorganizmi, nove emulzije se brzo inficiraju pa broj mikroorganizama vrlo brzo raste, ako nema antimikrobnog agensa i ako se uređaji i spremnici pravilno ne održavaju. Kako kvarenje napreduje, pjena i sluz postaju vidljivi. Suspendirane nakupine na površini upućuju na infekciju gljivama, a često se pojavljuje miris na amonijak, ali češće na sulfide. Emulzija promijeni boju, pH vrijednost progresivno pada, a isto tako i svojstvo zaštite od korozije.*

Naša ispitivanja su pokazala da su spremnici za emulzije u dva pogona bili znatno inficirani s više vrsta mikroorganizama, što je dokazano uzgojem na selektivnim hranjivim podlogama, kao i mikrobiološkom kontrolom u ovlaštenim laboratorijima. Ispitivanja su, nadalje, pokazala da se pravilnim postupanjem i održavanjem postrojenja i spremnika uvelike smanjuje kvarenje emulzija a time i zastoj u pogonu, čega je posljedica znatna financijska ušteda.

UVOD

Štete koje gospodarstvo u svijetu trpi od kvarenja materijala prepoznate su pred više desetaka godina te iznose i do više milijardi dolara godišnje, pa su već od prve polovice prošlog stoljeća razvijene zemlje počele poticati istraživanja u cilju poboljšanja svojstava materijala, odnosno povećanja otpornosti na utjecaj mikroorganizama. Istovremeno, znanost je prepoznala štetnost djelovanja ljudi na okoliš ispuštanjem tisuća kemikalija, odnosno odbacivanjem iskorištenih proizvoda (otpad) pa je s druge strane, biorazgradljivost postala poželjno svojstvo tvari ili sastojaka uporabnih proizvoda.

Kvarenje i biorazgradnja dva su naziva za isti proces ovisno o mjestu i ulozi u okolišu. Dok je materijal sirovina ili proizvod, trajnost i postojanost su poželjne osobine, no u trenutku kada su iskorišteni i postaju otpad, biorazgradnja odnosno kvarenje postaje poželjna osobina.

Stupanj razgradnje, degradacije ili kvarenja ovisi o fizikalno-kemijskim uvjetima okoline: temperaturi, vlazi/vodi, pH vrijednost medija te o fizikalno-kemijskim svojstvima materijala. Razgradnja spojeva koja vodi do krajnjih produkata koji su anorganske prirode naziva se mineralizacija. Kvarenje je najčešće proces djelomične promjene kvalitete ili oštećenja materijala, što je mnogo češća pojava od potpune biorazgradnje. Smatra se da je za biorazgradnju neophodno da se uspostavi povoljna kombinacija tri faktora: kemijski spoj ili tvar, organizam i uvjeti okoliša. Staklo, građevinski materijali, keramika, drvo, metali, koža, tkanine, goriva, petrokemikalije, aditivi, boje samo su neki od materijala koji su podložni mikrobnoj razgradnji.

Razvijene su europske zemlje pedesetih godina prošlog stoljeća prepoznale milijunske štete koje kvarenje nanosi svim granama industrije, a potvrđena je i uloga vlada i državnih tijela u međunarodnoj suradnji i istraživanjima u tom području što ukazuje na nacionalnu politiku podrške istraživanju i razvoju. Kooperacija i konzultacija putem specijaliziranih agencija UN-a, OECD-a i NATO-a te suradnja specijaliziranih laboratorija na multilateralnim projektima u kojima sudjeluju mnoge zainteresirane tvrtke odvija se već dugi niz godina.

Na području dokumentacije i organiziranja specifičnih informacija osnovan je i Biodeterioration Information Centre u Velikoj Britaniji. Zemlje članice OECD-a osnovale su International Directory of Biological Deterioration Research, što svakako ukazuje na prepoznavanje ozbiljnosti problema i gubitaka sa kojima se susreće industrija kao posljedicom kvarenja materijala (1).

Najstariji zapisi iz povijesti čovječanstva svjedoče o kvarenju materijala koji okružuju čovjeka i u svakodnevnoj su uporabi. Plinije u svojim djelima spominje kvarenje drva i tkanina uzrokovano raznim biološkim uzročnicima (2), Plutarh opisuje proces obrastanja na uronjenim oplatama brodova (3), Biblija spominje kvarenje vunenih, lanenih materijala i kože.

Najčešći uzrok promjene svojstava materijala je biološki – glodavci, kukci, a u najvećem opsegu mikroorganizmi kao što su bakterije, plijesni i gljivice koji materijal koriste kao izvor hrane. Procesi kvarenja mogu se klasificirati u tri grupe što je prikazano u tablici 1.

Tablica 1: Procesi kvarenja materijala

PROCES	PRIMJER
1. Materijal je mehanički oštećen	1. Glodavci i insekti
2. Materijal je pod kemijskim djelovanjem a) organizmi koji koriste materijal kao hranu (asimilacija) b) izlučivanjem tvari od organizama (disimilacija)	2. Mikrobiološka razgradnja celuloze a) oštećenje vune (moljac) b) mikrobna korozija željeza oštećenje karbonatnih stijena (lišajevi)
3. Funkcija materijala je narušena prisutnošću organizama	3. Obrastanje na oplati brodova (fouling) algama i drugim organizmima Čepljenje cjevovoda naslagama željeznih bakterija

Mikrobne infekcije proizvoda na osnovi ugljikovodika

Iako je literatura o rastu mikroorganizama na petrokemijskim i naftnim produktima iznimno velika, malo je mikrobiologa koji se sustavno bave tim problemom. Problem kvarenja tih produkata može se klasificirati uglavnom u pet kategorija, koje se iz osnova razlikuju prema supstratu i njegovim svojstvima i to: goriva, hidraulična ulja, tekućine za hlađenje, emulzije ulje-u-vodi i emulzije voda-u-ulju. Ono što im je zajedničko jest prisutnost slobodne vode, iako mikroorganizmi mogu prijeći i u uljnu fazu. Vrlo male količine slobodne vode su potrebne i dovoljne za rast i razvoj mikroorganizama. Budući da je jedan od produkata metabolizma voda, stvara se sustav koji sam sebe uvećava. Velik broj različitih mikroorganizama, bakterija, kvasaca i plijesni, može biti uključen u razgradnju i kvarenje.

Mikrobni rast u emulzijama ulje-u-vodi

Za rast mikroorganizama u ovakvim tekućinama zadovoljeni su svi zahtjevi: prisutni su neophodni elementi za rast stanica – ugljik, dušik, fosfor, sumpor, zatim uvjeti okoliša - temperatura, te aerobni odnosno anaerobni uvjeti, pH i voda.

Emulzije za hlađenje i podmazivanje pri obradbi metala sadrže mineralna ulja, aditive protiv trošenja, površinski aktivne tvari, inhibitore korozije, više alkohole, antipjenušavce, biocide i biostatike. Sastav je vrlo složen kako bi emulzije bile stabilne u uvjetima visokih pritisaka, povišenih temperatura te zaštićene od razgradnje i rasta mikroorganizama. Mineralna ulja sama za sebe sterilna su i mogu sadržavati nešto bakterija u stacionarnoj fazi kada nema proliferacije odnosno rasta. Miješajući se s vodom formiraju stabilne emulzije ulje-u-vodi i potiču rast mikroorganizama (4, 5). Taj rast mikroorganizama ubrzava cijepanje emulzije koja gubi osnovne značajke. Razlog za takvu pojavu je svojstvo mikroorganizama da tijekom rasta na netopljivim uljnatim supstratima proizvode mikrobne metabolite, površinski aktivne tvari odnosno biosurfaktante, kako bi smanjili površinu kapljice i učinili je dostupnom za razgradnju.

Tablica 2: Promjene u emulziji djelovanjem mikroorganizama

POJAVA U EMULZIJI	MIKROORGANIZAM
Smeđe obojenje	bakterije *Pseudomonas sp., Alcaligenes sp. Achromobacter sp.* (7)
Sivkasto obojenje	sulfatoreducirajuće bakterije – stvara se željezo (II) sulfid, ili anaerobi metabolizam kojih rezultira tvorbom željezo (II) hidroksida (8)
Pjena, debele naslage "palačinke"	nakupine mikroorganiazma (fungi, plijesni, kvasci)
Miris po sulfatima (Monday morning odour)	bakterija *Thiobacillus spp.*- anaerobna respiracija oksidacijom tvori sulfate u razdoblju stagnacije (9)
Miris na amonijak	bakterije koje tvore amonijak razgradnjom dušikovih spojeva(10)
Miris na sumporovodik	bakterija *Desulfovibrio* reducira sulfate i razvija se H_2S
Odvaja se uljna faza	produkti metabolizma mikroorganizama – sulfati, sulfatna kiselina, bioemulgatori

Različiti tipovi mikrobnih površinski aktivnih tvari kao što su glikolipidi, lipopeptidi, fosfolipidi, neutralni lipidi i heteropolisaharidi, fizikalnih odlika sličnih sintetičkim površinski aktivnim tvarima, povećavaju topljivost vodonetopljivih supstrata i transport u stanicu olakšavajući razgradnju (6).

U infekciji emulzija uvijek sudjeluje mješovita mikrobna zajednica. Jedan od primjera čestog toka infekcije je kako slijedi: početni pH je 8,5-9,5 i infekcija počinje aerobnim bakterijama koje su uobičajene u okolišu. U spremniku s emulzijom smanjuje se količina kisika, pada redoks potencijal (E_h) što pogoduje bakterijama koje uzrokuju koroziju, pH pada i do pH 4 te se stvaraju uvjeti za rast sulfatoreducirajućih bakterija, željeznih bakterija ili gljiva, te plijesni i kvasaca. Karakteristike i kvalitetu emulzija mijenjaju i ostali produkti metabolizma stanica mikroorganizama ovisno o sastavu emulzije i količini i vrsti izvora ostalih elemenata potrebnih za rast. Posljedica tih biokemijskih i kemijskih procesa su vizualne i olfaktorne promjene kako je prikazano u tablici 2:

UZROCI TROŠENJA RADNE EMULZIJE

Emulzija za obradbu metala mora imati sljedeća radna svojstva:

- dobro hlađenje i podmazivanje dodirnih površina alata i obrađivanog materijala
- efikasno odnošenje strugotina iz područja obradbe
- zaštita od korozije alata, strojeva i obrađivanog metala
- stabilnost prema različitim tvrdoćama vode
- otpornost na razvoj mikroorganizama
- regulacija pjenjenja, lijepljenja i taloženja
- neštetnost
- ugodan miris

Osiguranje ovih zahtjeva za kvalitetom radne emulzije proizvođač koncentrata za pripremanje emulzije postiže pažljivim odabirom sirovina za proizvodnju. Međutim, tijekom primjene dolazi do degradacije radne emulzije i svako odstupanje od početnih radnih svojstava pokazatelj je trošenja radne emulzije. Uzroci trošenja emulzije su u prvom redu potrošak aktivnih komponenti za osiguranje radnih svojstava, oksidacija, čestice metala koji se obrađuje i alata, ulazak različitih zagađivala kao što su: strano ulje, voda za pripremu emulzije, te otvorenost sustava ulasku mikroorganizama iz okoliša.

Strano ulje se skuplja na površini emulzije te sprječava doticaj emulzije sa zrakom i na taj način osigurava bakterijama uvjete za anaerobnu razgradnju emulzije i pojavu neugodnog mirisa. Sloj stranog ulja može poslužiti i kao nosač mikroorganizama, a osobito plijesnima i gljivicama koje mogu biti uzročnici kožnih oboljenja. Vode za spravljanje radnih emulzija mogu biti industrijske ili vodovodne. Industrijske vode mogu sadržavati veće količine mikroorganizama i uzrokovati mikrobiološko zagađenje emulzije. Najčešće se koriste vodovodne vode koje sadrže različite anione i katione i razlikuju se po tvrdoći vode. Tijekom dugotrajnog rada zbog isparavanja dolazi do povećanja koncentracije soli. Porast koncentracije soli utječe na stabilnost emulzije, razvitak mikroorganizama i koroziju.

Osim toga na degradaciju radne emulzije utječe i stupanj industrijske higijene što obuhvaća higijenu ljudi, strojeva i cijelog pogona. Radne emulzije u kojima su se razvili mikroorganizmi mijenjaju svojstva. Sa stajališta primjenskih osobina dolazi da smanjenja stabilnosti emulzija, zatim do smanjenja sposobnosti zaštite od korozije, smanjenja vrijednosti pH, pojave neugodnih mirisa, te skraćenja vijeka korištenja uz moguća štetna djelovanja na osobe koje dolaze s njima u doticaj.

Budući da je emulzija tijekom primjene izložena promjenama izvana, u radionicama korisnik treba osigurati redovitu kontrolu kakvoće. Ako se primjenjuje u centralnom sustavu podmazivanja, kontrolirati ju treba svakodnevno, a u pojedinačnim sustavima primjene barem jedanput tjedno. Osnovna fizikalno-kemijska svojstva koje treba provjeravati su koncentracija i pH-vrijednost, a ostala svojstva se provjeravaju prema potrebi, odnosno mogućnostima. Redovno valja kontrolirati promjenu boje i mirisa emulzije, pojavu korozije, pojavu ulja na površini, te pojavu čestica i taloga. Kontrolu emulzije mogu raditi operateri pri strojevima, služba održavanja, laboratoriji u tvrtki ili proizvodači emulzije i vanjske službe. Za kontrolu svojstava emulzije u pogonu postoje jednostavni testovi kao što su test trake za mjerenje tvrdoće vode s kojom se spravlja radna emulzija, test trake za kontrolu stupnja mikrobiološkog zagađenja, refraktometri za mjerenje koncentracije i sl.

Ako se utvrdi znatno odstupanje od osnovnih svojstava emulzije, potrebno je izvršiti korekcije, jer se pravilnom i pravodobnom intervencijom može riješiti većina problema. Kada se ne da popraviti niti jednom metodom korekcije, možemo reći da emulzija nije više za uporabu. Tada emulziju treba zamijeniti svježom radnom emulzijom, uz odgovarajuće postupke čišćenja sustava, kako bi se spriječila mikrobiološka infekcija svježe tekućine ostatcima prisutnih mikroorganizama.

EKSPERIMENTALNI DIO

Cilj ispitivanja

Svrha ispitivanja bila je utvrditi uzroke degradacije kvalitete emulzije za hlađenje i podmazivanje pri obradbi metala i pritužbe radnika na crvenilo kože i iritaciju očiju.

Materijal i metode

Uzorci koji su sterilno uzorkovani na lokacijama navedenim u tablici 3 nacijepljeni su na Saburaud agar, Rose-Bengal agar, Mc Conkey agar i Malt ekstrakt agar.

Korištene su metode:

- «Dip slide» (EASICULT, ORION DIAGNOSTICA).
- Nacjepljivanje na hranjive podloge 0,1 ml originalnog uzorka.
- Uzgoj kultura na temperaturi 20, 25 i 30^{0}C.

REZULTATI I DISKUSIJA

Ispitivane su biostabilne emulzije iz dva obradna stroja i to tokarilice koje imaju spremnike emulzije od 150 i 300 litara. Uzorke su uzimani s različitih mjesta, a emulzije su bile u radu u pojedinom procesu obradbe kroz različita vremenska razdoblja. Koncentracija radne emulzije bila je oko 3%, a pH vrijednost 9,44 odnosno 9,12. Rezultati ispitivanja rasta mikroorganizama u koncentratu emulgirajuće tekućine za obradbu metala i radnih emulzija uzetih s različitih mjesta na tokarilici prikazani su u tablici 3.

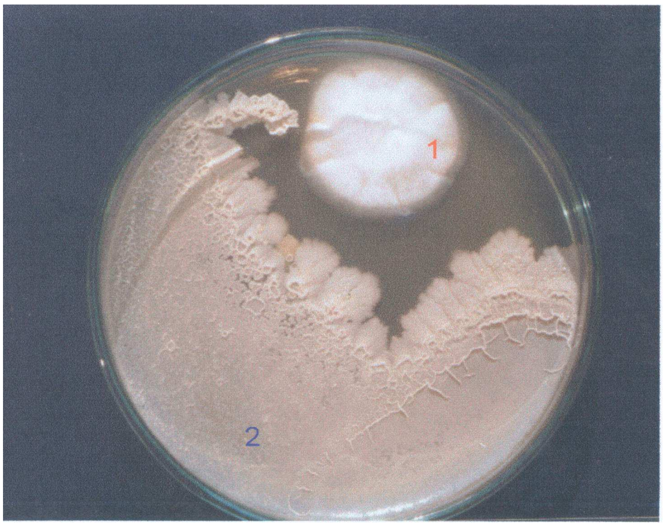
Tablica 3: Rezultati ispitivanja rasta mikroorganizama u uzorcima emulzija

TEMPERATURA UZGOJA VRIJEME UZGOJA	25 °C 30 °C 48 sati	20 °C 72 sata
MJESTA UZORKOVANJA:		
Čisto ulje-novootvorena bačva	nema rasta	nema rasta
Spremnik s uljem iz kojeg se namješava emulzija	nema rasta	nema rasta
Na tokarskom stroju iz slavine (emulzija stara tri mjeseca)	10^7 cfu/ml	++ plijesni
Spremnik za emulziju	10^7 cfu/ml*	+++ plijesni
Na tokarskom stroju iz slavine (emulzija stara tri tjedna)	++ plijesni 10^4 cfu/ml	++ plijesni
Spremnik za emulziju	++ plijesni 10^5cfu/ml	++ plijesni

* cfu/ml (colony forming units) – broj jedinica mikroorganizama koje stvaraju kolonije u mililitru

Izolirani sojevi mikroorganizama na selektivnim podlogama identificirani su u ovlaštenom laboratoriju kao: *Micrococcus sp., Staphylococcus sp., Pseudomonas sp., Sarcina sp., Scopuloriopsis sp., Flavobacterium sp., Penicillium sp., Fusarium sp., Gliocladium sp.*

Slika 1: Kolonija kvasaca i plijesni izolirane zaražene emulzije na hranjivoj podlozi u Petrijevoj zdjelici



Na temelju rezultata analiza i pregleda mjesta uzorkovanja, pogona za obradbu metala i radnih navika zaposlenih zaključeno je:

- Sastav mikrobnih populacija bio je različit ovisno o starosti infekcije i sastavu emulzije.
- U svim pogonima nije zadovoljavajuće održavana čistoća spremnika i cijelog sustava.
- Makroskopskim pregledom utvrđeno je da su na površini emulzije koja je stara tri tjedna već vidljive nakupine mikroorganizama što navodi na zaključak da je svježa emulzija stavljena u nedovoljno čist spremnik i inficirana, odnosno kontaminirana, određenom količinom ranije prisutnih mikroorganizama i na taj način je proces kvarenja ubrzan.
- Radnici u pogonu nisu upućeni u važnost održavanja čistoće sustava i često ubacuju otpatke u spremnike s emulzijama.

Stupanj infekcije i kontrola mogu se provoditi na dva načina:

- Tehnikom uronjenih pločica sa selektivnim podlogama za rast mikroorganizama (11) kojima se mogu razlikovati aerobne bakterije, sulfatoreducirajuće bakterije, gljivice i kvasci, moguće je koristiti priučeno osoblje, a semikvantitativni rezultati mogu se dobiti poslije jednog do pet dana (12).
- Laboratorijsko praćenje mikrobiološke slike stanja emulzije do identifikacije pojedinih mikroorganizama može dati vrlo korisne podatke za što bolji postupak održavanja, budući da je mikrobni sastav inficiranih emulzija krajnje složen, s različitim dominantnim vrstama koje se javljaju tijekom vremena, a svaka opet s različitim mogućim spektrom biokemijskih aktivnosti.

Metode kojima se u svijetu pokušavalo zaustaviti i spriječiti infekcije maziva su fizikalne i kemijske.

Fizikalne metode su zračenja i toplinska djelovanja. Gama zračenje i UV zračenje uz ozon mogu prouzročiti neželjene kemijske oksidacije (13,14). Postupak pasterizacije moguć je samo za termostabilne emulzije. Najefikasnija se do sada pokazala vodena para pod tlakom jer omogućava čišćenje površina cjevovoda i skidanje nakupine stanica sa stijenki spremnika (15).

Kemijski postupci su uporabe biocida (16) koji danas ne predstavljaju pravo rješenje zbog problema zbrinjavanja otpadnih emulzija i njihovog mogućeg utjecaja na okoliš. Postupak treba prilagoditi različitim korisnicima, biocid može gubiti na efikasnosti stajanjem, može, ukoliko je koncentracija previsoka, izazvati iritacije kože i sluznica radnika koji ga koriste, a korištenje vrlo selektivnih biocida može izazvati porast drugih sojeva mikroorganizama.

Utjecaj emulzija za hlađenje i podmazivanje pri obradbi metala na zdravlje

Zbog svoje podložnosti biološkim infekcijama emulzije za hlađenje su i pasivni prenosioci patogenih bakterija pa predstavljaju potencijalnu opasnost za radnike. Na udaljenosti od 60 centimetara od mjesta obradbe metala utvrđeno je 70000 cfu/m^3, a veličina čestica emulzije je takva da lako ulaze u plućne alveole (17). Mikroorganizmi sami ali i njihovi enzimi i toksini mogu iritirati sluznice oka i dišnih puteva. Česti mikroorganizam u procesu kvarenja emulzija je *Pseudomonas aeruginosa,* o čijoj produkciji egzotoksina je objavljen cijeli niz publikacija. Zanimanje izaziva i evidencija o uzrokovanju ekcema (18), iritacije kože (19), oštećenja rožnice i keratolize (20).

Povezanost mikroorganizama uključenih u kvarenje i mogućih profesionalnih oboljenja utemeljena je na prekomjemoj uporabi biocida, te na dopuštanju prihvaćanja iznimno visokih razina bakterija koje mogu pridonijeti akutnom oboljenju dišnih putova. Bakterije koje su se prilagodile tekućinama u uporabi prigodom njihova kvarenja najpogodnije su i za konačnu razgradnju iskorištenih tekućina. S obzirom na mikrobiologiju tekućina za obradbu metala, kvarenje, oboljenja i odlaganje predstavljaju međusobnu trojnu povezanost (22).

I endotoksini gram-negativnih bakterija koje sudjeluju u kvarenju emulzija mogu uzrokovati promjene na koži te manje abrazije kože. Iako dokazanih bakterijskih infekcija kojima je izvor radna emulzija za obradbu metala ima malo, česti su slučajevi dermatitisa te iritacije očiju i pluća, što se s velikom sigurnošću pripisuje i enzimima i toksinima bakterija roda pseudomonas ali i sulfatoreducirajućim bakterijama i produkciji sumporovodika (21).

ZAKLJUČAK

Budući da je emulzija tijekom primjene izložena promjenama izvana, u radionicama korisnik treba osigurati redovitu kontrolu kakvoće. Osnovna fizikalno-kemijska svojstva koje treba provjeravati su koncentracija i pH-vrijednost. Redovno valja kontrolirati promjenu boje i mirisa emulzije, pojavu korozije, pojavu ulja na površini, te pojavu čestica i ostalih taloga.

Najefikasniji način očuvanja kvalitete i trajnosti emulzija za podmazivnje i hlađenje pri obradbi metala pokazalo se čuvanje i čišćenje spremnika s emulzijama te održavanje strojeva i radne discipline. Od iznimne je važnosti temeljito čišćenje i sterilizacija spremnika prije novog punjenja emulzije, jer u protivnom nacjepljivanje nove emulzije već prisutnim mikroorganizmima dramatično ubrzava kvarenje.

Obrazovanje radnika jest svakako jednako važan korak u sprječavanju unosa organskih hranjiva, kao što su otpaci hrane i ostalo smeće, u emulziju.

Održavanje pogona, praćenje i njega proizvoda, obrazovanje zaposlenika i stručnost proizvođača nužni su za unapređenje kvalitete i produljenje vijeka sigurnog rada proizvoda, s ciljem postizanja ekonomske dobiti i ugleda.

Sigurnost i zaštita zdravlja uz smanjivanje utjecaja na okoliš omogućavaju korištenje proizvoda u vremenu povećane osjetljivosti za zdravu životnu sredinu.

MICROBIAL DEGRADATION OF EMULSIONS FOR COOLING AND LUBRICATION AT METALWORKING

Abstract

Material degradation is a problem which has always been occupying the attention of experts, because, first of all, it leads to major economic losses. That is why the causes of deterioration of various materials have been the object of research for a number of years now. Organized groups of researchers, mostly in chemical industry, supported by governmental organizations, are engaged in analyses and research of this particular area.

The biggest role in degradation is that of biodeterioration, defined as any unwanted change of the material properties caused by the activities of living organisms. Biodegradation in the form of deterioration constitutes an unwanted process when it comes to the production and storage of materials, but is in turn a most desirable process when the material in question becomes waste, both of these processes usually involving often the same kinds of microorganisms.

Emulsions for cooling and lubrication at metalworking with a high water content of up to 98%, and are precisely for this reason most prone to microorganisms growth. The oil phase makes a good nutritional basis for microorganisms as a source of carbon. Because of the tiny drops dispersed in water, the contact interphase oil/water surface is very large, which is why the surface for microorganisms growth is sufficient as well, water being the essential medium for their development. Under the impact of the metabolism of microorganisms, the product changes both chemically and functionally, which is why the slimy microbial growths cause constant clogging of filters and devices.

Although the concentrates of emulsifying fluids from which working emulsions are made are usually sterile according also to the results of this paper, microorganisms, after blending with water, develop very soon. That is because neither the environment nor the water are sterile. Since there already are microorganisms in the containers, new emulsions are soon infected and the number of microorganisms grows very fast unless there is an antimicrobial agent present, respectively unless the devices and containers are properly maintained. As deterioration progresses, the foam and the mucilage become *visible. Suspended growths on the surface point to infection by fungi, while the smell of ammonia and even more frequently that of sulphides also frequently appears. Emulsion changes colour, while pH progressively decreases, along with the corrosion protection property.*

Our tests have shown that reservoirs for emulsions in two plants were quite infected with several kinds of microorganisms, which has been proven by cultivation on selective nutritive media, as well as through determination of species in authorized laboratories. The tests have furtherly shown that proper handling and maintenance of the plants and reservoirs considerably reduces emulsion deterioration, and hence also plant standstills, resulting in considerable financial savings.

INTRODUCTION

Damage sustained by global economy due to material deterioration has been identified decades ago, amounting to several billion dollars annualy. That is why since the first half of the past century, developed countries have started encouraging research in view of improving material properties i.e. increasing their resistance to the impact of microorganisms. At the same time, science has recognized the detrimental environmental impact of human activities caused by the discharge of thousands of chemicals, i.e. disposal of used products or waste which is why, on the other hand, biodegradability has become a desirable property of the usable products' substances or ingredients.

Degradation and biodegradability are two names for the same process, depending on the place and role in the environment. While a material is a raw-material or a product, its durability and persistence are desirable properties. However, once they are used and become waste, biodegradability, i.e. degradation becomes a desirable property.

The degree of degradation or deterioration depends on the physico-chemical environmental conditions: temperature, humidity/water, pH of the medium, and on physico-chemical properties of the material. Degradation of compounds leading to end products of inorganic nature is called mineralization. Deterioration is most frequently the process of partial change of quality or material damage, which is much more frequent than complete biodegradation. It is considered that biodegradation requires a favourable combination of the following three factors: chemical compound or substance, organism and environmental conditions. Glass, construction materials, ceramics, wood, metals, leather, fabrics, fuels, petrochemicals, additives, paint – are only some of the materials prone to microbial degradation.

Developed European countries have in the 50s of the past century recognized the millions worth damage caused by degradation to all industrial branches. The role of governments and governmental bodies in international co-operation and research in this area has also been confirmed, indicating a national policy of supporting research and development. Co-operation and consultations through specializied agencies of UN, OECD and NATO, as well as co-operation of specialized laboratories on multilateral projects, involving many interested companies, has been going on for a number of years now.

In the area of documentation and organization of specific information, the Biodeterioration Information Centre has been established in Great Britain. OECD member countries have founded the International Directory of Biological Deterioration Research, which certainly reveals the appreciation of the seriousness of the issue, as well as of the losses faced by industry as a result of material degradation (1).

The oldest records from the history of mankind testify of the degradation of materials surrounding mankind and being the object of his everyday use. Plinius mentions in his work the degradation of wood and fabrics caused by various biological causes (2), Plutarch describes the process of fouling on immersed boat hulls /3/, while the Bible mentions the degradation of woolen and linen materials, as well as of leather.

The most frequent cause of material properties change is biological – rodents, insects, and to the largest extent microorganisms such as bacteria, mould and fungi, using the material as source of food. The biodeterioration of materials or substances is defined as any undesirable change in the properties of material caused by the vital activities of organisms. Degradation processes may be classified in three groups, as shown in Table 1.

Table 1: Material Biodeterioration Processes

PROCESS	EXAMPLE
1. Material which is mechanically damaged	1. Rodents and insects
2. Material is under chemical activity a) organisms using the material as food (assimilation) b) excretion of substances on the part of the organisms (dissimilation)	2. Microbiological degradation of celulosis a) Wool damage (moth) b) Microbial corrosion of iron Damage of carbonate rocks (lichen)
3. The function of the material is impaired by the presence of organisms	3. Immersed hull fouling caused by algae and other organisms Pipeline plugging by layers of iron bacteria

Microbial Infections of Hydrocarbon-Based Products

Although the references on the growth of microorganisms on petrochemical and petroleum products are extremely abundant, there are few microbiologists dealing systermatically with this issue. The problem of degradation of these products may be mostly classified in five categories, which basically differ according to the substrate and its properties, as follows: fuels, hydraulic oils, cooling fluids, oil-in-water emulsions and water-in-oil emulsions. Their common feature is the presence of free water, although microorganisms may also pass to the oil phase. Very low volumes of free water are both necessary and sufficient for the growth and development of microorganisms. Since one among the products of metabolism is water, the system which is multiplying itself is thus created. A large number of various microorganisms – bacteria, fungi and moulds – may be involved in degradation and deterioration.

Microbial Growth in Oil-in-Water Emulsions

All the requirements are met for the growth of microorganisms in such fluids: the necessary elements for the growth of cells are all there – carbon, nitrogen, phosphorus, sulphur, as well as environmental conditions - temperature, along with aerobic i.e. anaerobic conditions, pH and water.

Emulsions for cooling and lubrication at metalworking contain mineral oils, antiwear additives, surface active substances, corrosion inhibitors, higher alcohols, antifoaming agents, biocides and biostatics. The composition is higly complex in order to make the emulsions stable under conditions of high pressures and increased temperatures, as well as protected against corrosion and microorganisms growth. Mineral oils are by themselves sterile and may contain some bacteria in the stationary phase when there is no cell proliferation. Mixed with water, they form stable oil-in-water emulsions and encourage microorganisms growth (4, 5). The said microorganisms growth impairs emulsion stability which is loosing its basic properties. The reason for this is the property of microorganisms to produce microbial metabolites - surface active substances respectively biosurfactants during growth on insoluble oily substrates, in order to decrease droplet surface and make it available for degradation. Different types of microbial surface active substances, such as glykolipides, lipopeptides, phospholipides, neutral lipides and heteropolysaccharides, whose physical properties are similar to syntethetic surface active substances, increase the solubility of water-insoluble substrates and their transport to the cell by facilitating degradation (6).

In the infection of emulsions, there is always a mixed microbial community involved. One among the examples of frequent infection courses is as follows: initial pH is 8.5-9.5 and the infection starts with aerobic bacteria which are common in the environment. In the emulsion reservoir, the amount of oxigen reduces, the redox potential (E_h) decreases, favouring bacteria which cause corrosion, pH drops even down to pH 4 - thus creating conditions for the growth of sulfatoreducing bacteria, iron bacteria or fungi, as well as moulds and yeasts.

The characteristics and quality of emulsions are also changed by other cell metabolism products - depending on emulsion composition, as well as the volume and kind of the sources of other elements needed for growth. The result of these biochemical and chemical processes are visual and olfactory changes, as shown in Table 2.

Table 2: Changes in emulsion through microorganisms activity

APPEARANCE IN EMULSION	MICROORGANISM
Brown colouring	bacteria *Pseudomonas sp., Alcaligenes sp. Achromobacter sp.* (7)
Grayish colouring	sulphate-reducing bacteria – generating iron (II) sulfide, or an anaerobic metabolism resulting in the generation of iron (II) hydroxide (8)
Foam, thick layers “pancakes“	microorganism accumulations (fungi, moulds, yeasts)
Sulfate odour (Monday morning odour)	Bacteria *Thiobacillus spp.*- anaerobic respiration through oxidation generates sulphates in the period of stagnation (9)
Ammonia odour	Bacteria generating ammonia through degradation of nitrogen compounds (10)
Hydrogen sulphide odour	bacteria *Desulfovibrio* reduces sulphates and H_2S is generated
The oil phase is separated	products of microorganisms metabolism – sulphates, sulphate acid, bioemulsifiers

Causes of the metalworking emulsion wear

Metalworking emulsion must have the following performances:

- good cooling and lubrication of the contact surfaces of tools and workpiece
- efficient removal of chips from the metalworking area
- protection of tools, machinery and worked metal against corrosion
- stability to various water hardnesses
- resistance to microorganisms growth
- regulation of foaming, sticking and sedimentation
- non-harmfulness
- pleasant odour

The ensurance of these requirements for the quality of metalworking emulsion is achieved by the manufacturer of the concentrate for preparing the emulsion by a careful selection of ingredients. However, during application, the metalworking emulsion is degraded and any aberration from the initial performances indicates the metalworking emulsion wear. The causes of emulsion wear are primarily the consumption of active components for ensuring performances, oxidation, particles of the metal machined and of the tools, penetration of various contaminants, such as: tramp oil, water for preparing the emulsion, and the system’s openness to the entry of microorganisms from the environment.

Tramp oil accumulates on the surface of the emulsion, thus preventing its contact with air and ensuring the bacteria conditions for anaerobic degradation of the emulsion and appearance of unpleasant odour. The film of tramp oil may also serve as the bearer of microorganisms, and especially of moulds and fungi which may cause skin diseases. Water for preparing metalworking emulsions may be industrial or from waterworks. Industrial waters may contain increased volumes of microorganisms and cause microbiological pollution of the emulsion. Most frequently used is the water from waterworks, containing various anions and kations, differing in hardness. During prolonged operation, evaporation causes an increase in the concentration of salts. Increase in the concentration of salts impacts emulsion stability, microorganisms growth and causes corrosion.

Apart from that, the degradation of metalworking emulsion is also influenced by the degree of industrial hygiene, encompassing the hygiene of people, machinery, and that of the entire plant. Metalworking emulsions in which microorganisms have developed change their properties. From the viewpoint of applicative properties, the emulsion stability is reduced, followed by decreased corrosion protection, lowered pH value, appearance of unpleasant odour, and reduced service life, accompanied by possible harm caused to people handling them.

Since during application the emulsion is exposed to outside changes, the user must ensure regular quality control at workshops. If it is applied in a centralized lubrication system, it must be controlled daily, and, in individual application systems, at least once a week. The basic physico-chemical properties that should be controlled are concentration and pH-value, while other properties are controlled depending on needs i.e. possibilities. Regular control must encompass volume, corrosion appearance, appearance of tramp oil on the surface, appearance of particles and other sediments, as well as change of emulsion colour and odour. Emulsion control may be performed by machinery operators, maintenance service, company labs or emulsion producers, or even outside services. For controlling emulsion properties at the plant, there are simple tests, such as test strips for measuring the hardness of water used to prepare the metalworking emulsion, test strips for determining degree of microbial contamination, refractometers for measuring concentration, and the like.

If a considerable aberration from the basic emulsion properties is established, corrections must be done, because a correct and timely intervention may solve most of the problems. When no correction method works any more, one may say that the emulsion is no longer fit for use. That is when it must be replaced by fresh metalworking emuslion, including proper system cleaning procedures, in order to avoid microbial infection of the fresh emulsion with the remaining microorganisms present.

THE EXPERIMENTAL PART

The Purpose of Investigation

The purpose of the investigation was to establish the causes of degraded quality of the emulsion for cooling and lubrication at metalworking and of the employees' complaints of skinredness and eye irritation.

Material and Methods

The samples taken under sterile conditions from locations listed in Table 3 were inoculated on Saburaud agar, Rose-Bengal agar, Mc Conkey agar and Malt extract agar. The methods used:

- «Dip-slide» (Easicult, Orion Diagnostica)
- Inoculating 0.1 ml of original sample on nutritive media
- Cultivating cultures at the temperature of 20, 25 and 30 ^{0}C

RESULTS AND DISCUSSION

Biostable emulsions were tested from two metalworking machines. They are lathes with emulsion reservoirs of 150 and 300 liters respectively. The samples were taken from various spots, while the emulsions were used in individual metalworking processes during various time periods. The concentration of mealworking emulsions was around 3%, and the pH value 9.44 i.e. 9.12. The results of testing microorganisms growth in the concentrate of emulsifying metalworking fluid and metalworking emulsions taken from various spots on the lathe are shown in Table 3.

Table 3: Results of microorganism cultivation from investigated emulsions

CULTIVATION TEMPERATURE CULTIVATION TIME	25 ^{0}C 30 ^{0}C 48 hrs	20 ^{0}C 72 hrs
SAMPLING SPOTS		
Fresh oil – newly opened can	no growth	no growth
Reservoir with oil from which emulsion is blended	no growth	no growth
On the lathe from the pipe (three months old emulsion)	10^7 cfu/ml*	++ moulds
Emulsion container	10^7 cfu/ml	+++ moulds
On the lathe from the pipe (three weeks old emulsion)	++ moulds 10^4 cfu/ml	++ moulds
Emulsion container	++ moulds 10^5cfu/ml	++ moulds

*cfu=Colony forming units per ml

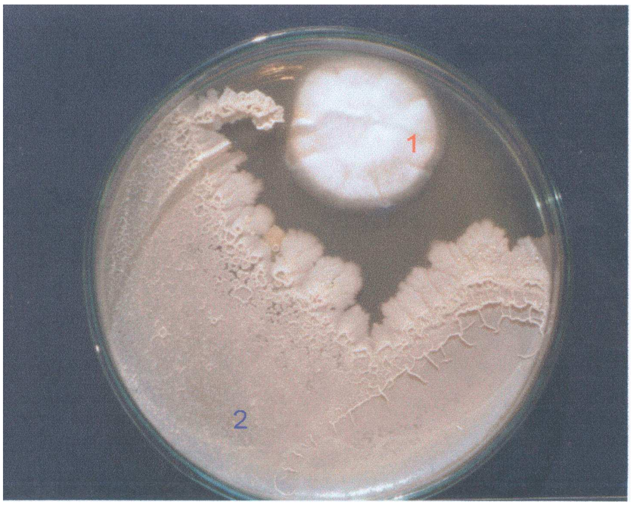
The isolated microorganism compounds on selective supports were at the authorized laboratory identified as follows: *Micrococcus sp., Staphylococcus sp., Pseudomonas sp., Sarcina sp., Scopuloriopsis sp., Flavobacterium sp., Penicillium sp., Fusarium sp., Gliocladium sp.*

Based on the results of analyses and inspection of sampling spot, the metalworking plant, as well as of the employees' occupational habits, the following has been concluded:

The composition of microbial populations was different depending on infection age and emulsion composition. In all the plants, the cleanliness of reservoirs and of the entire system was not properly maintained.

Macroscopic inspection revealed that on the surface of a three weeks old emulsion one may already observe microorganism accumulations, indicating that fresh emulsion was put in an insufficiently clean reservoir and was hence infected with a certain volume of microorganisms, thus speeding up the biodeterioration process.

Figure 1: A colony of yeasts and moulds of the isolated infected emulsion on nutrient agar in Petri's vessel



Workers at the plant are not aware of the importance of maintaining the cleanliness of the system and often throw garbage into emulsion reservoirs.

Infection degree and control may be determined in the following two ways:

- Through the technique of immersed strips (Dip-slide) with selective nutrient media (11), one can distinguish aerobic bacteria, sulphatoreducing bacteria, fungi and yeasts. They can be used by semiskilled staff and will give a semiquantitative answer in one to five days (12).
- Laboratory monitoring of the microbiological emulsion condition identifying individual microorganisms may provide very useful data for improved maintenance procedure, since the microbiology of infected emulsions is highly complex, with different microbes dominant at different times each one with a different spectrum of biochemical activities.

The methods applied worldwide in order to try to stop and prevent lubricant infections are physical and chemical.

Physical methods are radiation and heat treatments. Gamma and UV radiation with ozone may cause unwanted chemical oxidation (13, 14). Pasteurization procedure is possible only for thermostable emulsions. The most efficient so far has proven to be the water steam under pressure, because it enables the cleaning of pipeline surfaces and removal of cell accumulations from container walls (15).

Chemical procedures are the use of biocides (16), which today do not constitute a real solution due to the problem of managing waste emulsions and their possible environmental impact. The procedure must be suited to various users, the biocide may loose its efficiency if it is left standing, or may cause irritation of skin and mucous membranes of the workers using it if the concentration is too high, while the use of highly selective biocides may cause increase of other microbial strains.

Health Impact of Metal Cooling and Lubricating Emulsions

Because they are prone to biological infections, cooling emulsions may also passively transfer patogenic bacteria and hence constitute a potential threat to the employees. At the distance of 60 centimeters from the metalworking operation, 70000 cfu/m^3 was established, while the size of emulsion particles is such that it may easily access pulmonary alveoli (17). The microorganisms themselves, as well as their enzymes and toxins, may irritate the mucous membranes of the eye and the respiratory organs. A frequent microorganism in emulsion deterioration process is *Pseudomonas aeruginosa* whose production of exotoxins has been the subject of a number of papers. Interest is furtherly raised by the records of causing eczema (18), skin irritation (19), cornea damage and keratolysis (20).

The connection of microorganisms involved in biodeterioration with possible occupational diseases is based on excessive use of biocides and permitting the acceptance of extremely high bacteria levels which may contribute to acute diseases of respiratory organs. Microorganisms usually inhabiting infected emulsions are often the same that may be involved in final biodegradation. Given the microbiology of metalworking fluids, biodeterioration, diseases and disposal are mutually triply connected (22).

Even the endotoxines of gramme-negative bacteria taking part in emulsion deterioration may cause skin changes and also small skin abrasions. Although there are not many proven bacterial infections caused by metalworking emulsion, there are frequent cases of dermatitis and eye and lung irritation, which may safely be ascribed to the enzimes and toxins of pseudomonades, but also to sulfatoreducing bacteria and hydrogen sulphide production (21).

CONCLUSION

Since the emulsion is during application exposed to outside changes, the user must ensure regular quality control at workshops. The basic physico-chemical properties that should be controlled are concentration and pH-value. Regular control must encompass volume, corrosion appearance, appearance of tramp oil on the surface, appearance of particles and other sediments, as well as change of emulsion colour and odour.

The most efficient way of keeping the quality and durability of metalworking emulsions for cooling and lubrication turned out to be the keeping and cleaning of containers with emulsions and of the machinery, as well as work discipline. Of exceptional importance is a thorough cleaning and sterilization of containers before introducing fresh emulsion, because otherwise the inoculation of the new emulsion with the already existing microorganisms speeds up biodeterioration dramatically.

Personnel training is by all means an equally important step in preventing the input of food remains and other waste as organic nutrients into emulsion.

Plant maintenance, product monitoring and care, personnel training and manufacturer expertise are necessary for advancing the quality and extending the period of safe product life, with the purpose of achieving economic gain and prestige.

Health protection and safety, along with reduced environmental impact, enable the use of products in these times of increased awareness of the need for a healthy living environment.

Literatura / References:

1. Biodeterioration of Materials, Ed.:A.H. Walters, J.J. Elphick, Elsevier Publ. Co. Ltd., London, 1968.

2. Herfs A.: *Zoologica,* **34**, Heft 90, 1936.

3. Laidlaw F.B.: Marine foulong and prevention,Ed.U.S. Naval Institute, Annapolis, Maryland, 1952.

4. Lee M., Chandler A.C.: J. Bacteriol., **41**, 373-386, 1941.

5. Fabian F.W., Pivnick H.: Appl. Microbiol., **1**,199-293, 1953.

6. Falatko D.M., Novak J.T.: Water Environment Research **64** (2) 163-169, 1992.

7. Duffet N.D., Gold S.H., Weirich C.L.: J.Bact. **45,** 37-38, 1943.

8. Herbert B.N., Gilbert P.D.: Society for Applied Bacteriology, Technical Series,1984.

9. Obuekwe C.O., Westlake D.W.S., Plambeck J.A.: Corrosion **37**,(8) 461-467,1981.

10. Hill E.C.: Monitoring and Maitenance of Aqueous Metalworking Fluids, John Willey and Sons, London, 1983.

11. Hill E.C., Gibbons O., Davies P.: Tribology international, 121-130, June 1976

12.Genner C.: Process Biochemistry **11,** 39, 1976.

13. Mixer R.Y., Cavallo J.J., Hart V.C.: Society of Tools and Manufacturing Engineers, U.S.A., Technical Paper MR, 69-261, 1969.

14. Rossmore H.W., Brazin J.G. U. Proceedings 1st International Biodeterioration Symposium, Southampton, pp. 386-401. Elsevier Publishing, London, 1969.

15. Hill E.C., Genner C.: Proceeding 4th International Biodeterioration Symposium, Berlin, pp. 37-43, Pitman Publishing, London, 1980.

16. Benett E.O.: Progress in Industrial Microbiology, **13**, 121, 1974.
17. Hill E.C., Al-Zubaidy T.S.: Tribology International, **12** ,161, 1979.
18. Singh V., Current Medical Practice, **20**, 214.,1976.
19. Jacobson J.A., Hoadley, A.V.: American Journal of Public Health 66, 1092, 1976.
20. Hodgson G.: Industrial Medicine and Surgery **36**,16.,1979.
21. Al-Zubaidy,T.S., Ph.D.Thesis, University of Wales, Cardiff, 1978.
22. Rossmore H.W., Mikrobiologija tekućina za obradu metala-kvarenje, degradacija, oboljenja, *Goriva i maziva* 33, 3:165-176, 1994.

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628.513 tehnička zaštita od mikroorganizama u industriji	industrial microbial contamination and protection
576.8.07 mikrobiološka izlučna analiza i dijagnostika	microbiological screening and diagnostics
613.6 higijensko tehnička zaštita na radu	occupational hygiene

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