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Original Research

Efficiency of some soil bacteria for chemical oxygen demand reduction of synthetic chlorsulfuron solutions under agiated culture conditions

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Abstract: This study searches the efficiency of certain soil bacteria on chemical oxygen demand (COD) reduction of synthetic chlorsulfuron solutions under agitated culture conditions. It also aims to determine the turbidity of liquid culture medium with chlorsulfuron during bacterial incubation for 120 hours. As a result the highest and lowest COD removal efficiency of bacteria was determined for *Bacillus simplex* as 94% and for *Micrococcus luteus* as 70%, respectively at the end of the 96th hour. It was found that COD removal efficiency showed certain differences depend on the bacterial species. It was also observed that *B. simplex* had the highest COD removal efficiency and it was a suitable bacterium species for bioremediation of a chlorsulfuron contaminated soils.

Key words: Chlorsulfuron, chemical oxygen demand, bacteria, turbidity.

Introduction

Water pollution by pesticides is considered as a pervasive problem, since these compounds affect the living organisms adversely (1). The environmental contamination caused by the presence and accumulation of pesticide residues in soil, in addition to surface and ground waters should be assessed (2). Some organisms have pesticide remediation ability which is primarily based on their biodegradation activity levels, although bioremediation was initially achieved with bacteria or fungi (3). Any factor that could change growth or metabolism would affect biodegradation as well. Two other factors should also need to be mentioned: co-metabolism and consortia condition. Other substrates are required by certain biodegraders to degrade pollutants (4). This is called co-metabolism and it is particularly required by organochlorine compounds. However, it was demonstrated that organophosphate biodegradation is reduced by the existence of other carbon sources (5). Pesticide characteristics and biological and chemical reactions determine the metabolic fate of pesticides (6).

Other carbon or phosphorous sources reduce the efficiency of organophosphate biodegradation. The application of these biodegraders on bioremediation is considerably limited by this fact. Further research to determine the factors affecting biodegradation efficiency is required to improve their bioremediation (3). Several studies were focused on microbial degradation, which was reported as a primary mechanism of pesticide dissipation in the soil and water media (7). During the composting process, it was reported that the organophosphorus pesticides such as chlorpyrifos-methyl and malathion, as well as the organochlorine pesticide linden were almost fully degraded (over 99%) (8). High organic matter causes reduced degradation (9). It was argued that high organic matter could result in decreased substrate bioavailability for degrading microorganisms (10).

Chlorsulforon is among the most frequently used herbicides in sunflower cultivation in Trakya region, Turkey. This herbicide is degraded under natural conditions via evaporation, transportation into ground water via rain water, and surface runoff intakes through photolysis, chemical and microbiological degradation. The aim of this study is to investigate reduction of synthetic chlorsulfuron on chemical oxygen demand (COD) and to determine the turbidity of liquid culture medium with chlorsulfuron during bacterial incubation.

Materials and Methods

Chemicals

Chlorsulfuron, sold under the trade name "Hammer Extra 75 DC", was supplied from an agricultural products shop. The physical and chemical properties of chlorsulfuron are given in Table 1. This herbicide contains 75% chlorsulfuron active ingredient. All media for isolation and enrichment of the bacteria were purchased from Sigma Aldrich.

Microorganisms

The bacteria used in this study, the codes of identified bacterial species, accession numbers and references are presented in Table 2.

Preparation of culture media

Plate count agar (PCA) and sabouraud dextrose broth (SDB) media were autoclaved at 121 ^{0}C for 15 min to ensure a sterilized solution. After cooling, diluted agricultural soil with no chlorsulfuron background was added to petri dishes with an isotonic NaCl solution. The medium pH was adjusted to 7.0 and temperature was set at 25 ^{0}C to isolate and enrich bacterial species (21).

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Table 1. Physical and chemical properties of chlorsulfuron.

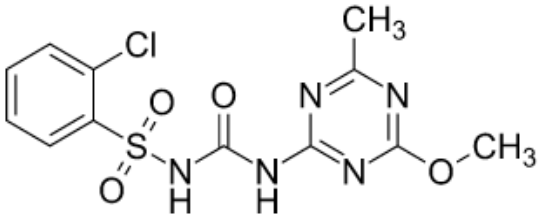
Parameter	Value	Reference
Molecular structure		(11)
Molecular weight	357.77 g/mol	
IUPAC name	1-(2-chlorophenyl)sulfonyl-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)urea	(12)
Molecular formula	$C_{12}H_{12}ClN_5O_4S$	
Physical description	colorless crystals	
Melting point	174-178 ^{0}C	(13)
Vapor pressure	2.2 x 10^{-11} mm Hg at 25 ^{0}C	
Solubility in water	In water, 125 mg/L at 25 ^{0}C	(14)
Half life in water	4 weeks at pH 5.7, 8 weeks at pH 7.0	(15)

Table 2. Identified bacterial codes and their species (16).

Accession Number	Bacterial Code and Approximate Species Identity	Identity	Reference
KF831394.1	*Bacillus simplex* (B1)	99%	(17)
HE646789.1	*Bacillus muralis* (B2)	99%	(18)
KF555623.1	*Micrococcus luteus* (B3)	99%	(19)
KC634108.1	*Micrococcus yunnanensis* (B4)	99%	(20)
HG530135.1	*Clostridium tetani* (B5)	99%	(21)

Isolation and enrichment of bacteria

Bacterial species were isolated from the soil samples using serial dilution (10^{-4}) on plates, which contained cooled plate count agar media. Bacteria incubation took about three days in the incubator at 25 ^{0}C. After bacterial growth, the agar media were screened for any colonies that were visually different than the others. After incubation, the cultures were placed carefully in an enrichment media and kept there for seven days and allowed to grow under the same temperature and shaken at 150 rpm continuously (22).

Identification of studied bacteria

Identification studies were conducted according to Wizard Genomic DNA Purification Kit. "Isolating Genomic DNA from Gram Positive and Gram Negative Bacteria" methods were used (23).

Phire Hot Start II DNA Polymerase was used for PCR, since it does not allow DNA isolation. Then, longer PCR bands of various lengths (1000–3000 bp) were obtained via bacterial 16S ribosomal general primers. The pipette instructions and cycling protocols were:

Heat cycle conditions; 1 cycle: 98^{o}C – 5 min / 40 cycles: 98 ^{o}C – 5 s, 72 ^{o}C – 20 s / 1 cycle 72^{o}C – 4 min/4^{o}C-∞. Final concentrations; (total 20µl reaction volume); 1X Phire Animal Tissue PCR Buffer (includes dNTPs and $MgCl_2$) / 0.5µM forward primer / 0.5µM reverse primer / Phire Hot Start II DNA polimeraz and H_2O.

Bacteria were identified using 16s rRNA Universal Primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′; *Escherichia coli* positions 8–27) (24). 16S rRNA universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′; *E. coli* positions 8–27) (23), 1492R 5' TACGGYTACCTTGTTACGACTT 3' positions 1492–1512) (25, 26).

Microbial Biodegradation Studies

In order to determine the capacity of chlorsulfuron biodegradation (COD reduction) for five different soil bacteria species, *B. simplex, B. muralis, M. luteus, M. yunnanensis* and *C. tetani* (approximately 2 x 10^7 CFU/ml each) were incubated under liquid culture conditions with chlorsulfuron.

To prepare the liquid media, 1 ml of hammer extra 75 DC (including 0,75g/ml of chlorsulfuron) and 1 ml of enriched culture were added to 98 ml 0.8 % isotonic saline water. The chlorsulfuron was prepared from hammer extra 75 DC in the concentration that is actually used in the field (75 mg/l).

In biodegradation studies, solution samples were monitored at 12-hour intervals for COD levels and turbidity. COD was measured by standard 5220C closed reflux titrimetric method (27). According to this method, 1.5 ml of standard potassium dichromate digestion solution ($K_2Cr_2O_7$) and 3.5 ml of 0.0176M Ag_2SO_4 solution were added to a 2.5 ml sample. Later on, these samples were heated in a Velp WTW CR3200 thermoreactor for 2 hours at 150^{0}C. After it was cooled, samples were taken to Erlenmeyer flasks and 3 drops of ferroin indicator ($FeSO_4.7H_2O$) were added to the samples. Then, samples were titrated with 0.25M standard ferrous ammonium sulfate (FAS) titrant and COD results were calculated. Sample turbidity measurements were taken from chlorsulfuron media at 650 nm (Photolab 6600 UV-VIS Spectrophotometer) according to (28).

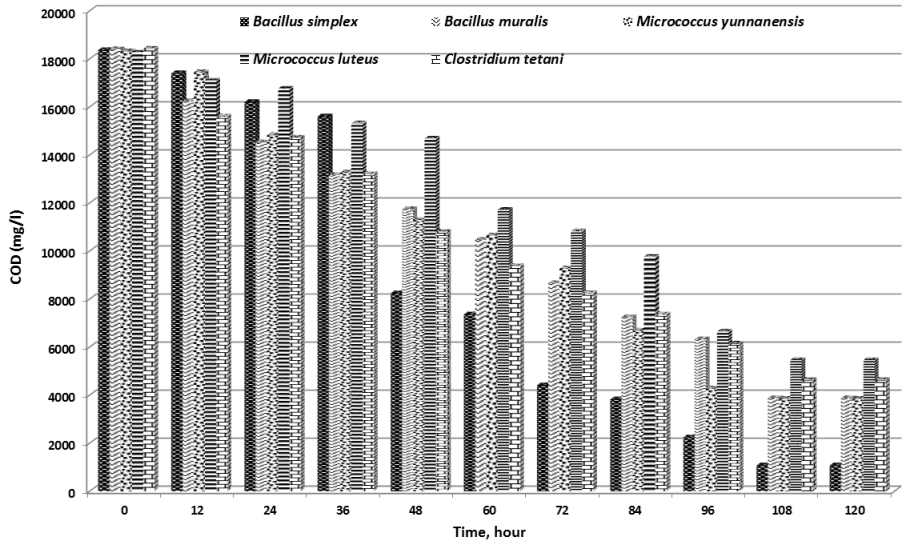


Figure 1. COD reduction in liquid media with chlorsulfuron by isolated bacteria.

Results

COD reduction in liquid media

The results of COD reduction in chlorsulfuron solution by five different bacterial species are presented in Figure 1.

COD reduction rates in the media have showed different results depend on differences in bacterial species in the liquid medium. The COD reduction efficiencies of *B. simplex, B. muralis, M. yunnanensis, M. luteus* and *C. tetani* species were 94, 78, 79, 70 and 74%, respectively (Figure 1). At the end of the 108th hour, there were negligible changes in COD. Previous studies on microbial degradation of certain herbicides revealed that relatively few bacterial species were actually able to degrade these compounds. In another study, bacteria species were isolated in agricultural soil contaminated with trifluralin to decompose the herbicide in a liquid medium (29). In a previous study about biodegradation of aclonifen, COD removal rates were observed between 93% and 70%. According to these results, the highest COD removal level was achieved by *M. yunnanensis.* At the end of 5 days, 15600 mg/l COD of aclonifen was reduced to 1090 mg/l. *M. luteus* displayed the lowest COD removal capacity (from 15600 to 4680 mg/l) (30).

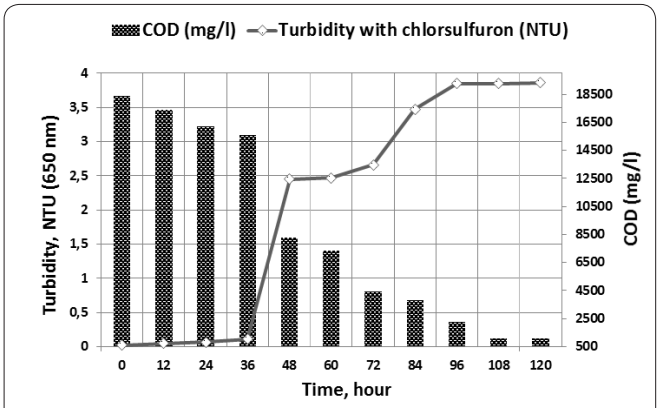


Figure 2. The changes in COD reduction and turbidity by *B. simplex* in media with chlorsulfuron.

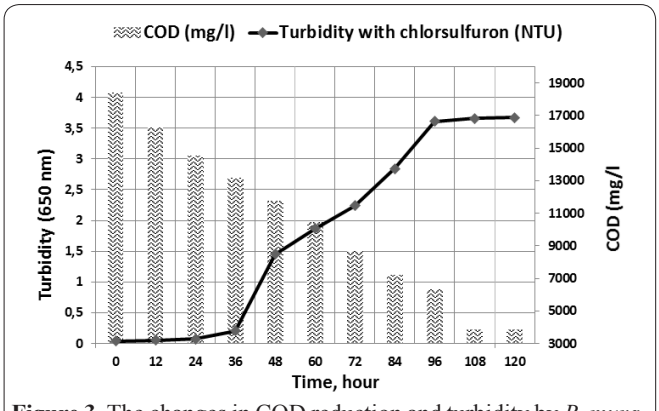


Figure 3. The changes in COD reduction and turbidity by *B. muralis* in media with chlorsulfuron.

Monitoring microbial activity in chlorsulfuron media through turbidity

The results of the experimental study conducted with *B. simplex, B. muralis, M. luteus, M. yunnanensis* and *C. tetani* species in media with chlorsulfuron are illustrated in Figures 2, 3, 4, 5 and 6, respectively.

Discussion

In the present study, it was observed that COD removal rates obtained by *B. simplex, B. muralis, M. yunnanensis, C. tetani* and *M. luteus* were 94, 78, 79, 74 and 70%, respectively. Based on these results, it could be concluded that *B. simplex* had the highest removal rate.

Experimental results on monitoring microbial activity in the medium with chlorsulfuron showed a slight increase in turbidity, particularly after from the 12th hour.

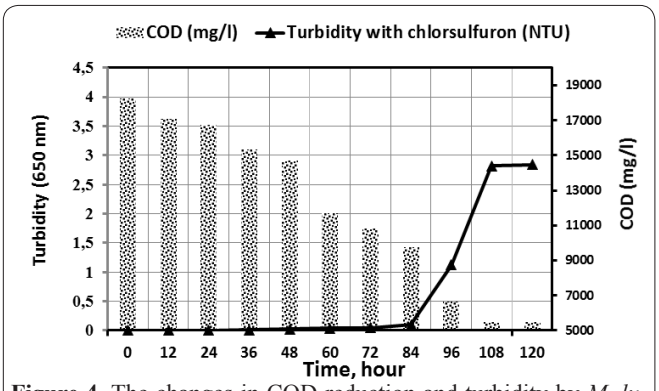


Figure 4. The changes in COD reduction and turbidity by *M. luteus* in media with chlorsulfuron.

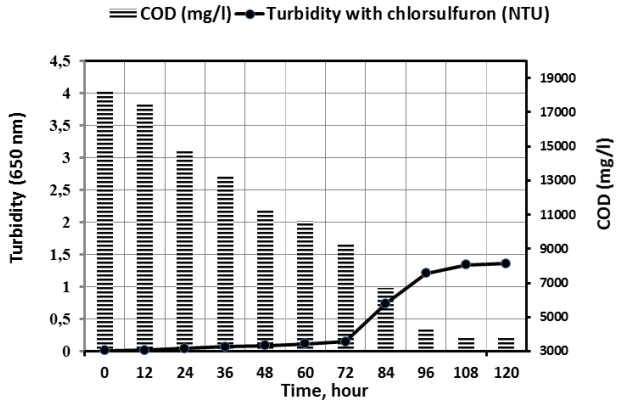


Figure 5. The changes in COD reduction and turbidity by *M. yunnanensis* in media with chlorsulfuron.

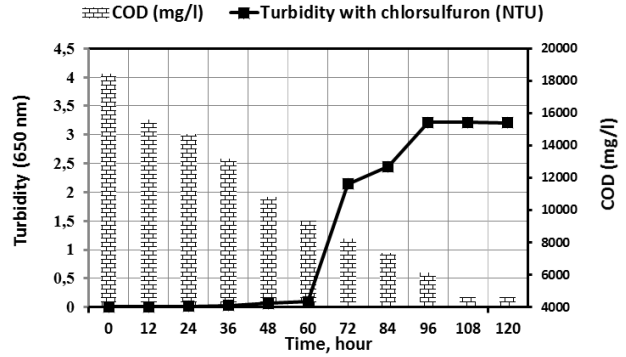


Figure 6. The changes in COD reduction and turbidity by *C. tetani* in media with chlorsulfuron.

The distinct increase in turbidity occurred after from the 36th hour, which demonstrated that the best COD removal in chlorsulfuron medium was observed with *B. simplex* (Figure 2).

As a result of this study, it was observed that *B. simplex* had the highest COD removal efficiency and it was a suitable bacteria species for bioremediation of chlorsulfuron contaminated soil field.

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