Uluslararası Tarım Araştırmalarında Yenilikçi Yaklaşımlar Dergisi
International Journal of Innovative Approaches in Agricultural Research 2022, Vol. 6 (1), 57-67
https://doi.org/10.29329/ijiaar.2022.434.5

Original article

Bioremediation Performance of *Sphingomonas melonis* and *Bacillus muralis* on Herbicide Diquot Dipremide-(ethylene-d4)

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Abstract

Bioremediation is a process that utilizes the degradation potential of microorganism to provide a cost-effective and reliable approach for pesticide biodegradation. For this purpose, chosen bacteria *Sphingomonas melonis* and *Bacillus muralis* were isolated from an agricultural soil sample. The biodegradation performance of these isolated bacteria at different Diquot Dipremide-(ethylene-d4) (DDE4) herbicide concentrations (250, 500 and 1000 ppm) was investigated under submerged culture conditions. Biodegradation performance of isolated bacteria was monitored with COD, TOC and, BOD5 reduction rates in culture medium at different incubation periods. According to the results; S. melonis has the highest bioremediation capacity for COD removal (91% at 250 ppm). For TOC, B. muralis has the highest removal rate as 82% at 250 ppm. On the other hand, For BOD5 at 250 ppm 85% S. melonis showed the best removal performance. Most effective removal rate at 250 ppm concentrations was obtained as 91% and 88 by S. melonis and B. muralis respectively at the end of the 216th hour for COD. Additionally, the increase in turbidity related with population dynamics at the end of the 216 th hour positively effected the bioremediation parameters included COD, TOC and BOD5 reductions. These results showed that it can be used for effective COD, TOC and BOD5 removal in S. melonis and B. muralis on DDE4 remediation.

Keywords: Biodegradation, Diquot Dipremide -(ethylene-d4), Sphingomonas melonis, Bacillus muralis, agitated culture conditions, turbidity.

Received: 26 January 2022 * **Accepted:** 30 March 2022 * **DOI:** https://doi.org/10.29329/ijiaar.2022.434.5

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INTRODUCTION

Organic pesticides are widely used in modern agriculture around the world to control weeds on fruits and vegetables. In addition to being effective against a wide variety of harmful species, they are inexpensive and water-soluble on the market (Montuori et al., 2015). When such herbicides are applied above a certain dose, they pose a risk to the receiving environment, in addition to their benefits. In addition, excessive use poses a potential risk to humans in physiological, oxidative and nitrosative terms, resulting in disruption of homeostasis and normal metabolism (Montuori et al., 2016). The extensive use of herbicides or persistent organic pollutants for controlling harmful objects has been widely used in agriculture. However, the extensive use of the persistent organic pollutants has inflicted serious harmful problems to humankind in the ecosystem (Erguven and Yildirim, 2019).

Since organic pesticides have high toxic effects, it is necessary to develop cost-effective and efficient methods for the removal and detoxification of pesticide residues in the environment where they are exposed (Cycoń et al., 2013). Bioremediation is a promising, low-cost, high-yield, simpler and more environmentally friendly approach to pesticide removal and detoxification. Bioremediation can be an efficient and alternative method to remediating environment pollution with methomyl insecticide. Bioremediation of different strains of bacteria was positively enhanced in receiving environments. This means that there were suitable microorganisms to reduce the opposite effects of pesticides in agricultural areas (Tatar et al., 2020). Given the health risks and environmental impacts of pesticide-exposed soils, microbial bioremediation approaches and related technologies should be widely developed and applied in the field in the future. Currently, bioremediation of recipient environments is carried out using non-genetically modified microorganisms isolated from contaminated sources and supplied from the natural habitat, which exhibit the ability to degrade the target pollutant (Dubinsky et al., 2013). The degradation mechanism of herbicides is usually carried out by many microorganisms.

Each microorganism contributes to the bioremediation reactions on herbicides, but there is insufficient literature for examples of mineralization with a single strain. For adequate bioremediation, different microorganisms must be present in the environment (Erguven and Yildirim, 2019).

The DDE4 herbicide is stable binds irreversibly to the soil and causes accumulation in the soil. This herbicide exceeds leaching levels for acute and chronic effects in aquatic and estuarine organisms: however, as it tends to bind rapidly to suspended substances in the water column and these effects are minimal in actual practice, as they become biologically unavailable (EPA, 1994).

The aim of this study is to compare the bioremediation performances of *S. melonis* and *B. muralis* at various DDE4 concentrations. For this purpose, biodegradation performances of these two bacteria were monitored with COD, BOD_5, TOC reduction, which offer alternative ideas for pesticide active ingredient reduction.

MATERIALS and METHODS

Bacteria

S. melonis and *B. muralis* used in study were already available in the culture collection of Munzur University, Environmental Microbiology Laboratuary. The bacterial cultures were kept in refrigator at 4 ºC until used for bioremediation studies.

Chemicals and medium

DDE4 (C12D4H8Br2N2) active ingredient is supplied from sigma-aldrich (Germany) Turkey distributor with CAS number 6385-62-2. Sabouraud dextrose broth (SDB) was purchased from Sigma Aldrich (Turkey).

Bioremediation studies

For bacterial enrichment, *S. melonis* and *B. muralis* were inoculated in a 250 ml Erlenmeyer flask containing SDB medium and placed in orbital shaker incubator at 28 °C with constant shaking at 160 rpm for 5 d.

1 ml bacterial samples taken from the each enriched bacterial cultures (contains approximately 109 CFU/ml) were inoculated into 250 ml erlenmeyer flasks containing 250, 500 and 1000 ppm of DDE4. These bioremediation mediums were incubated in an orbital shaker incubator, at 28 ºC and 160 rpm for 216 h.

COD, BOD_5, TOC and turbidity measurements

For monitoring the the bioremediation process, COD, BOD_5 and TOC levels of bioremediation mediums were measured at each sampling periods (12, 24, 36, 48, 60, 72, 84, 96, 120, 144, 168, 192 and 216). Also, turbidity of culture mediums was analysed for the each smpling times. Experiments were carried out with 3 replications. In the COD experiments, in the light of the closed reflux titrimetric method specified in Standard Method 522 oC, with the HACH DRB 200 model thermoreactor and with the Hach DR 890 Colorimeter device, Cat. While 23459-52 model COD kits were used, the Standard Method 5210B method was used in the BOD_5 test. In TOC experiments, TEKMAR - DOHRMANN - Apollo 9000 device and Standard method 5310A High temperature combustion method was used. (Association et al., 1912). In addition to these, for monitoring the population dynamics of these bacteria, according to the method described in (Harry et al., 1990), 650 nm (Photolab 6600 UV-VIS Spectrophotometer) devices used with approximately 5 ml samples taken from the media. All experiments were carried out at room temperature.

Statistical Analysis

The statistical analysis of the data obtained in this study was made in SPSS 24.0 package programs. The data presented in the manuscript are expressed as the standard errors of the mean of the experiments performed in three times. The independent sample t test was used to compare the differences in the COD, BOD_5 and TOC between the *S. melonis* and *B. muralis.*

RESULTS and DISCUSSION

The COD, TOC and BOD_5 parameters to determine the bioremediation performance of *S. melonis* and *B. muralis* on 250, 500 and 1000 ppm concentrations of DDE4 herbicide were monitored with 12 hour periods.

These concentrations are the recommended levels for farmers that uses this herbicide. In this study, population dynamics was monitored at intervals where the best removal efficiency gainedIt was determined that there is a correlation between COD, TOC and BOD_5 removal and turbidity for each microorganism (Figure 1, 2 and 3). This increase in Turbidity depending on time proven that microorganisms use the pesticide as a carbon source. This decreasing of carbon source results in COD, TOC and BOD_5 reduction (Erguven and Yildirim, 2016). *S. melonis* showed 91% COD removal efficiency, while *B. muralis* performed 88% removal rate at the end of the 216 h at 250 ppm DDE4 (Figure 1 and 2).

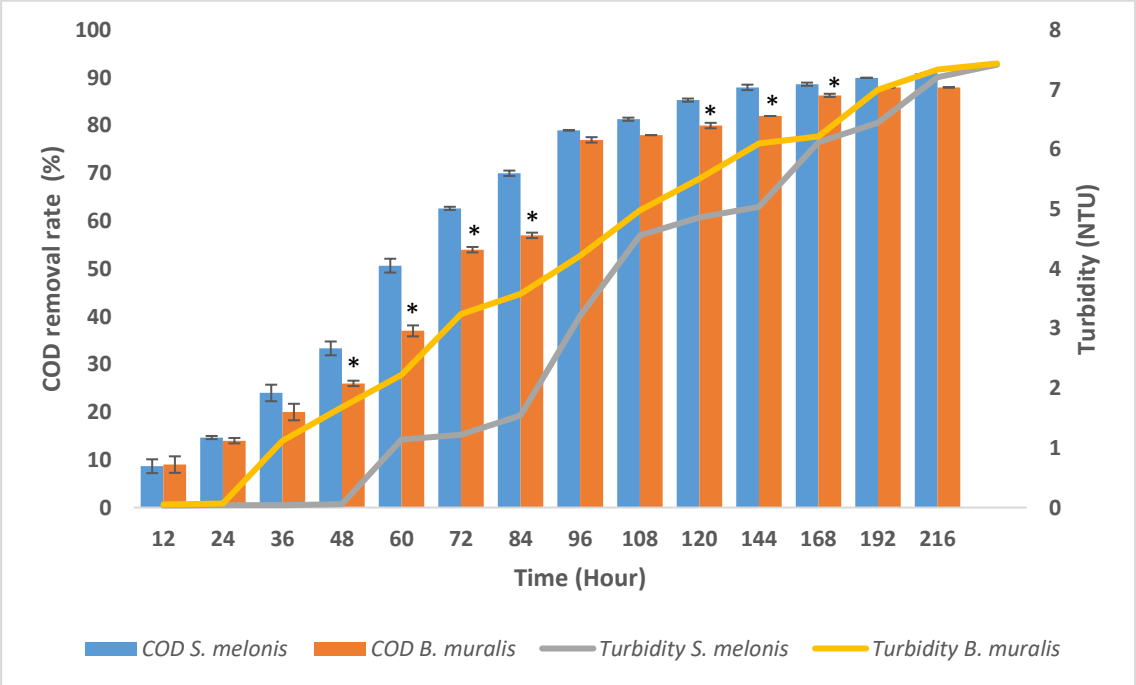

Figure 1. Bioremediation performance of *S. melonis* and *B. muralis* under 250 ppm DDE4 conditions via COD and Turbidity. The * symbol on the bars shows the statistical differences between between the *S. melonis* and *B. muralis* at the same time according to independent t-tests ($p<0.05$).

S. melonis showed 79% TOC removal efficiency, while *B. muralis* performed 82% removal rate at the end of the same timeperiod in same concentrations (Figure 2).

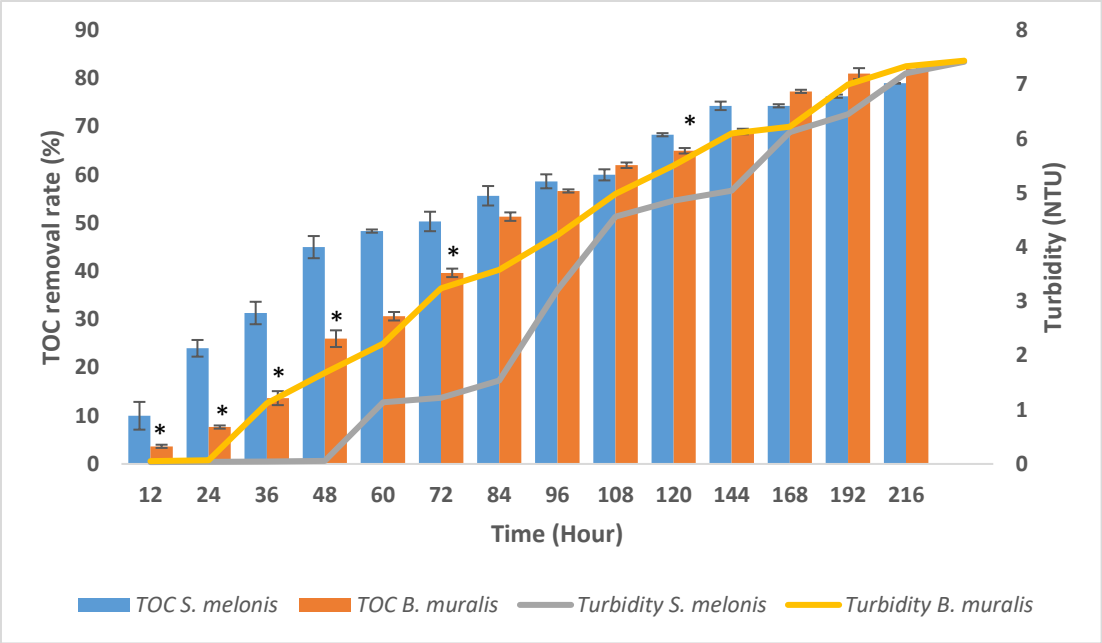

Figure 2. Bioremediation performance of *S. Melonis* and *B. muralis* under 250 ppm DDE4 conditions via TOC and Turbidity. The * symbol on the bars shows the statistical differences between between the *S. melonis* and *B. muralis* in the same time according to independent t-tests ($p<0.05$).

In BOD_5 parameter, the removal efficiencies of *S. melonis* and *B. muralis* were as 85 and 79% respectively at the end of the 216 h in 250 ppm concentrations (Figure 3).

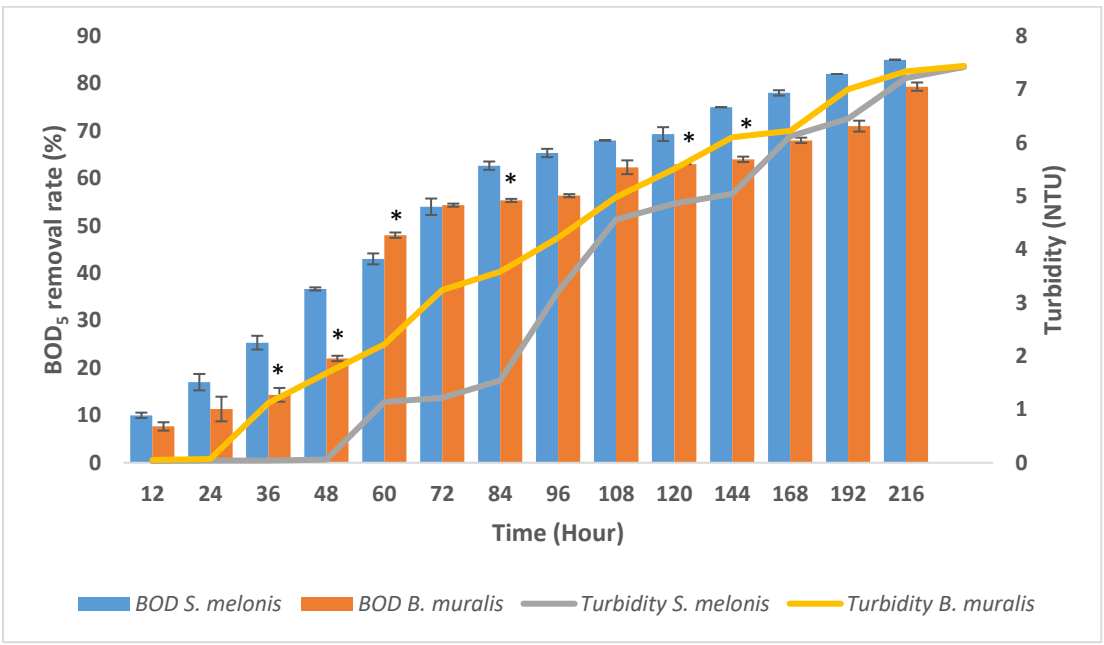

Figure 3. Bioremediation performance of *S. Melonis* and *B. muralis* under 250 ppm DDE4 conditions via BOD_5 and Turbidity. The * symbol on the bars shows the statistical differences between between the *S. melonis* and *B. muralis* at the same time according to independent t-tests ($p<0.05$).

In 500 ppm concentrations of DDE4, the removal performances of *S. melonis* and *B. muralis* were as 91 and 88% at the end of the 216 h on COD (Figure 4).

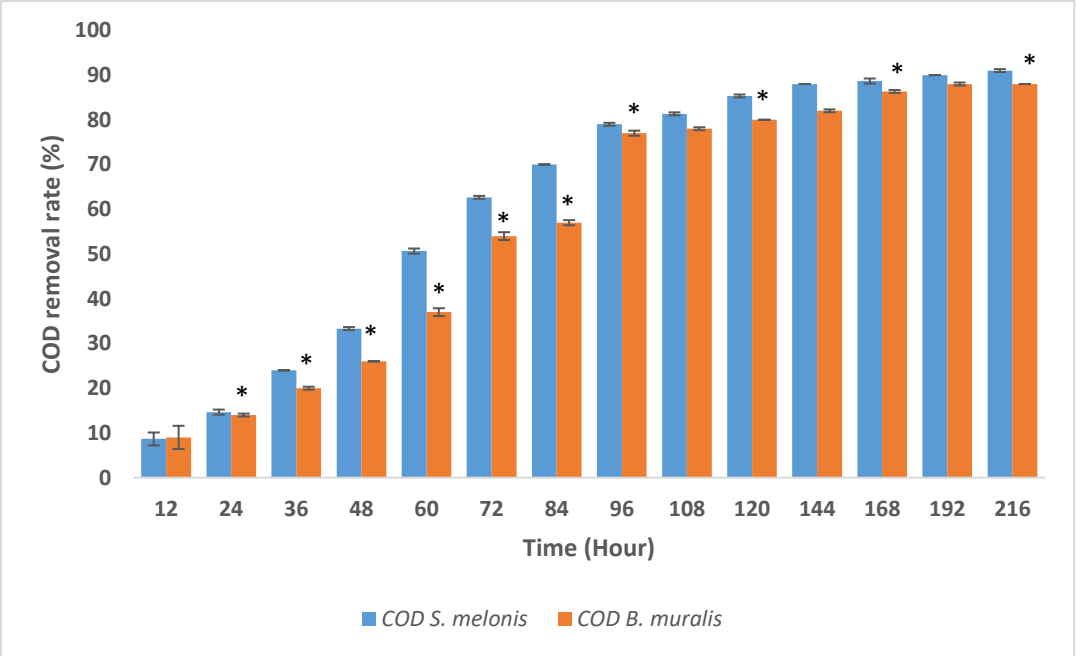

Figure 4. Bioremediation performance of *S. Melonis* and *B. muralis* under 500 ppm DDE4 conditions via COD. The * symbol on the bars shows the statistical differences between between the *S. melonis* and *B. muralis* at the same time according to independent t-tests ($p<0.05$).

In Figure 5, both *S. melonis* and *B. muralis* were achieved 80% TOC removal at the end of the 216 h for 500 ppm concentrations.

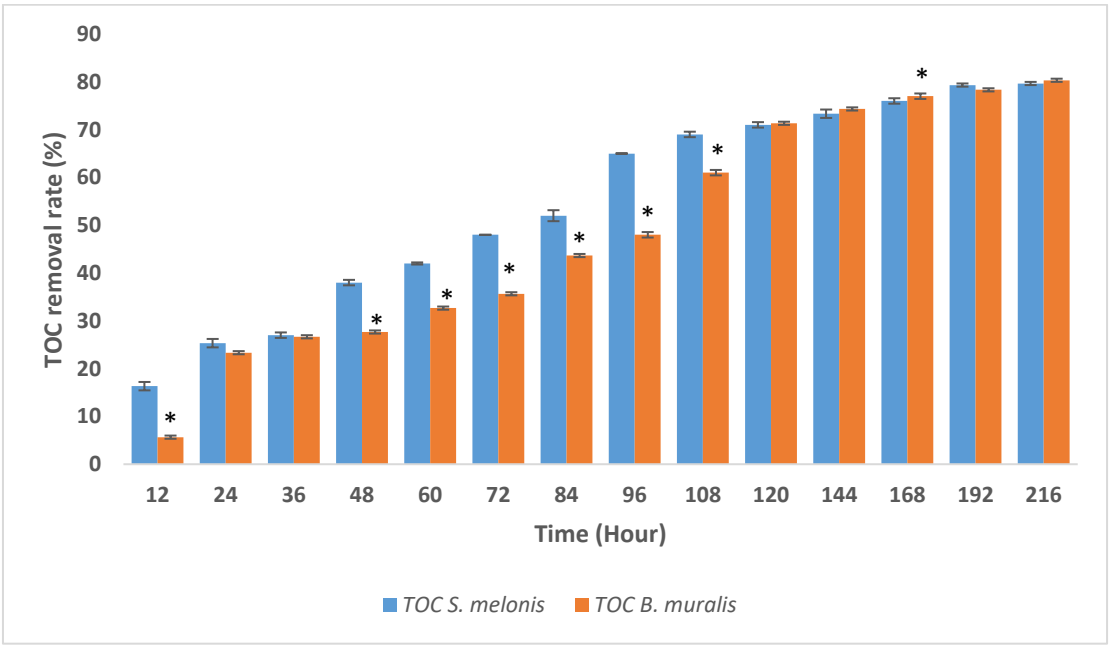

Figure 5. Bioremediation performance of *S. Melonis* and *B. muralis* under 500 ppm DDE4 conditions via TOC. The * symbol on the bars shows the statistical differences between between the *S. melonis* and *B. muralis* at the same time according to independent t-tests ($p<0.05$).

In BOD_5 the removal efficiency of *S. melonis* and *B. muralis* were as 84 and 76% respectively at the end of the 216 h for 500 ppm DDE4 concentrations (Figure 6).

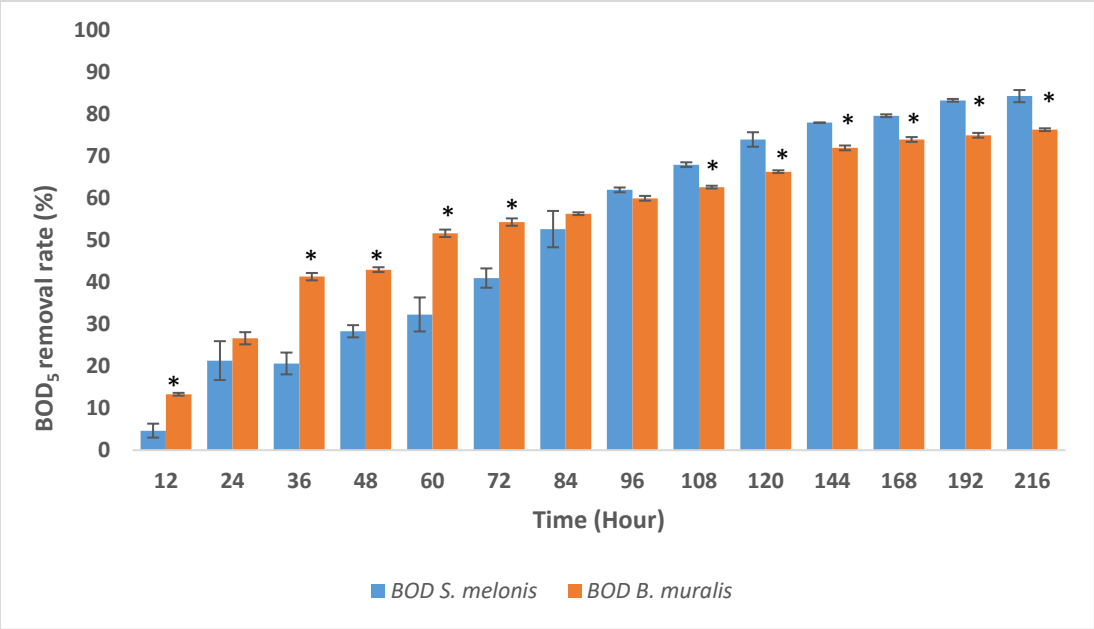

Figure 6. Bioremediation performance of *S. Melonis* and *B. muralis* under 500 ppm DDE4 conditions via BOD_5. The * symbol on the bars shows the statistical differences between between the *S. melonis* and *B. muralis* at the same time according to independent t-tests ($p<0.05$).

In Figure 7, the COD removal efficiency of *S. melonis* was seen as 89% while *B. muralis* was 84% in 1000 ppm concentrations at the end of the 216 h (Figure 7).

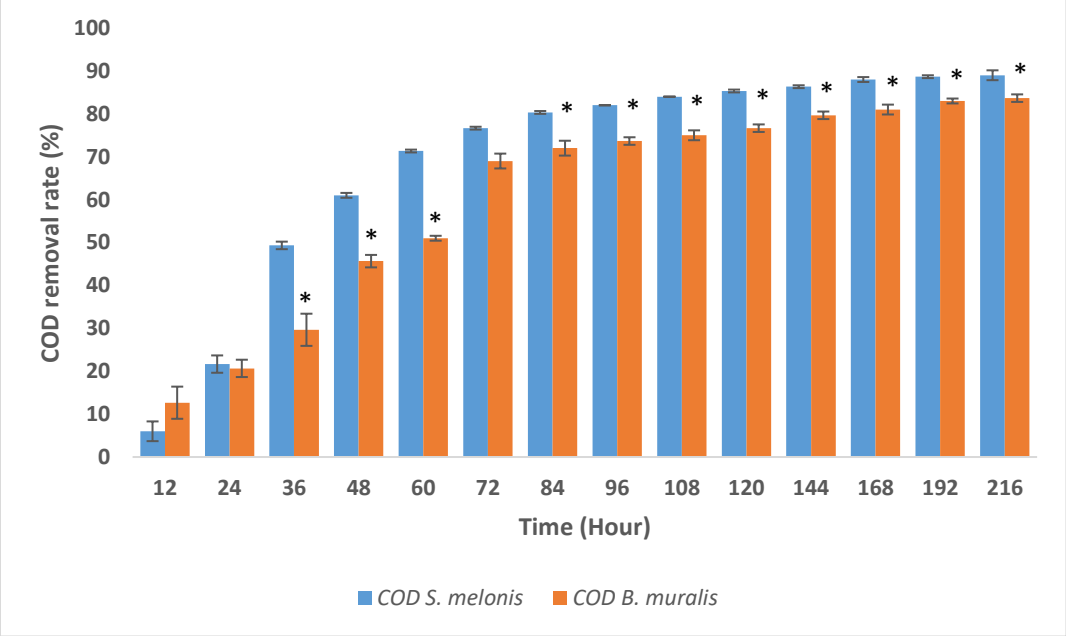

Figure 7. Bioremediation performance of *S. Melonis* and *B. muralis* under 1000 ppm DDE4 conditions via COD. The * symbol on the bars shows the statistical differences between between the *S. melonis* and *B. muralis* at the same time according to independent t-tests ($p<0.05$).

At the end of the 216 h, the same TOC removal efficiency was determined by each bacterium as 78% in 1000 ppm concentrations of DDE4 (Figure 8)

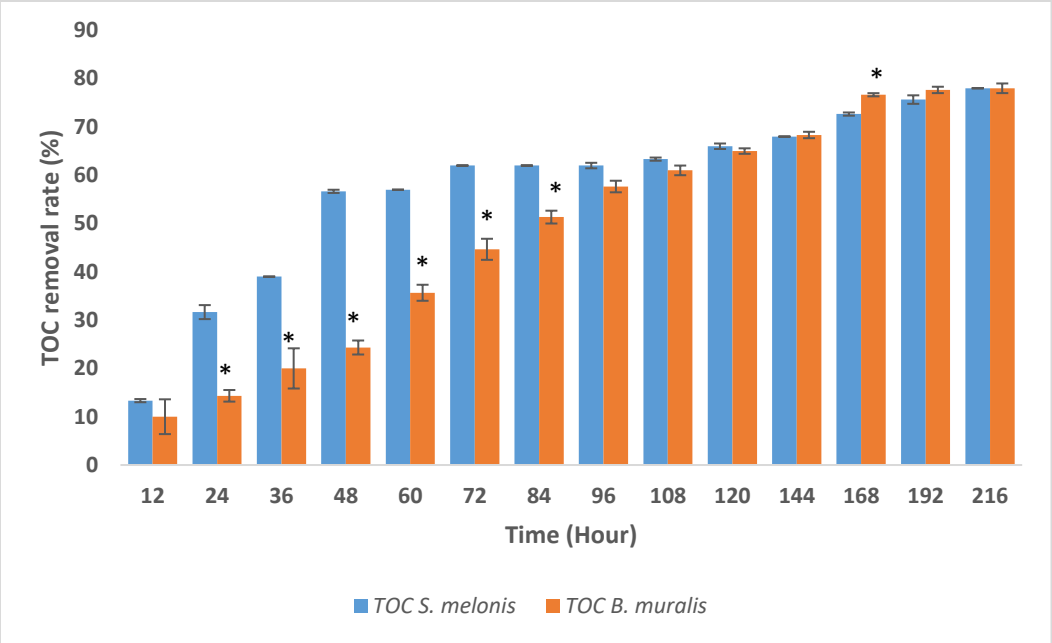

Figure 8. Bioremediation performance of *S. Melonis* and *B. muralis* under 1000 ppm DDE4 conditions via TOC. The * symbol on the bars shows the statistical differences between between the *S. melonis* and *B. muralis* at the same time according to independent t-tests ($p<0.05$).

There is a significant differencies on removal efficiency of these two bacteria on BOD_5 parameter at the end of the 216 h. The efficiency of *S. melonis* was 84% while *B. muralis* was 74% at 1000 ppm concentrations (Figure 9).

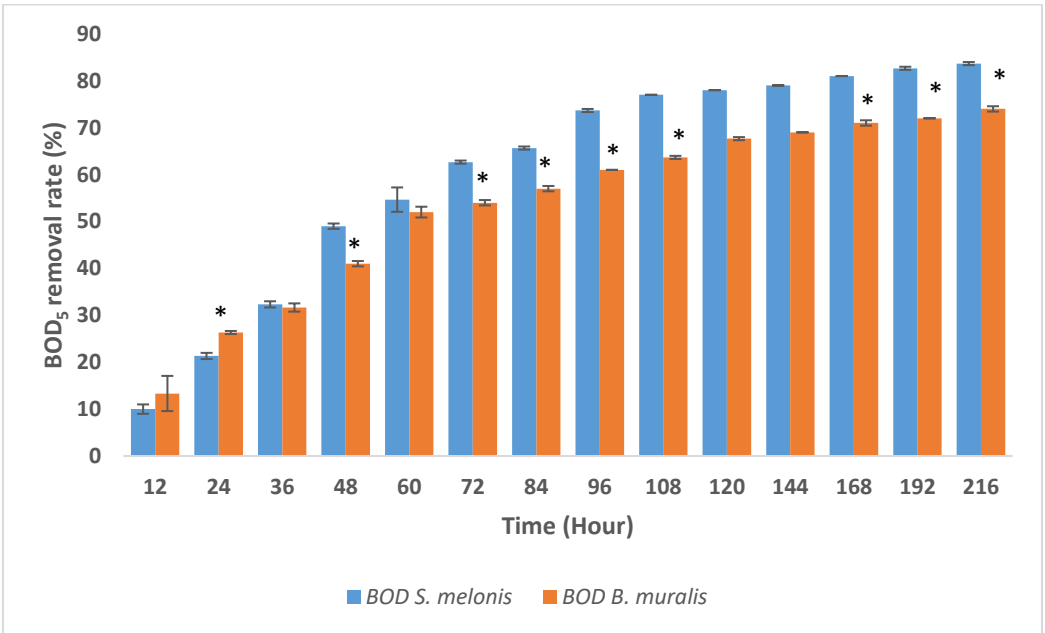

Figure 9. Bioremediation performance of *S. Melonis* and *B. muralis* under 1000 ppm DDE4 conditions via BOD_5. The * symbol on the bars shows the statistical differences between between the *S. melonis* and *B. muralis* at the same time according to independent t-tests ($p<0.05$).

The findings obtained because of the study are compatible with the results of similar studies in the literature. Previously, many researchers have conducted research to determine the bioremediation capacities of microorganisms isolated from the activated sludge of pesticide producing plants or from discharge channels. The difference of this study is that the bioremediation of the herbicide DDE4, the use of which has increased in recent years, with two new species isolated from the agricultural field is monitored with other important environmental pollution parameters other than the active substance. Nevertheless, further studies with other pesticides and fungi are needed to confirm its clear mechanism of bioremediation by other environmental parameters (Ergüven et al., 2017). Most of the researchers thought some microorganisms have tolerance to the pesticide. In a previous study related with this study, *B. cereus, B. subtilis, B. melitensis, P. aeroginosa, P. fluorescence,* and *S. marcescens* were capable of degrading 46–72% of pesticide chlorpyrifos as a sole carbon source in a sedimentary medium after three weeks of incubation period (Lakshmi et al., 2008). In the bioremediation of *Pseudomonas putida* bacteria isolated from soil contaminated with pendimethalin; (Elsayed and El-Nady, 2013) found that, after 4 weeks, pendimethalin at a concentration of 100 µg/mL was removed by this bacterial species. (Erguven and Demirci, 2020) monitored the bioremediation performance of *Ochrobactrum thiophenivorans* and *S. melonis* bacteria and their consortia to reduce the imidacloprid pesticide in soil media. After two weeks period, they found full reduction rates for imidacloprid active material for each bacterium and their mixtures while COD reduction rates were 97 and 96% for two types of bacteria. Additionally, they investigated TOC and BOD_5 removal rates. 97% reduction seen for both types and their consortia. (Erguven, 2018) studied the removal efficiencies of some fungal species and acetochlor herbicide in terms of active ingredient, COD, BOD_5 and TOC. For this purpose, *T. geodes, C. cicadae, M. owariensis, M. cylindrosporae* and *V. chlamydosporium* species isolated from the agricultural soil obtained from the Thrace region and the bioremediation studies continued with them. According to the results, the acetochlor active substance changed between in the range of 91-55%; the COD 90–52%, the TOC 85-50% while BOD_5 was changed in the range of 80-50%. (Erguven and Yildirim, 2016) also investigated the biological recovery rate of chlorsulfuron herbicide by changing the COD parameter. At the end of the 5 day of study period, they determined the removal rates of *B. simplex, B. muralis, M. luteus, M. yunnanensis* and *C. tetani* based on COD parameter between 70-93%. (Erguven and Yildirim, 2019) studied imidacloprid remediation with strains of *Methylobacterium radiotolerans* and *Microbacterium arthrosphaerae*. After 18 days, remediation was determined for COD parameter as 52, 96, and 99% with 20, 40, and 80 ml of the consortia of bacteria, respectively, while BOD_5 removal rates were 88, 79, and 50% in the same amounts of microorganism. According to results obtained from this study, this herbicide acts as a nutrient and at the same time it causes the number of bacteria in the environment to increase logarithmically. This logarithmic increase also brings about the degradation of the herbicide. These findings reveal that the nutritional properties of pesticides enhance bacterial growth.

Conclusion

The present study has shown that *B. muralis* and *S. melonis* were able to bioremediate DDE4 and use it as the sole carbon source to grow in agitated culture conditions. COD, BOD_5 and TOC are important parameters providing us with ideas on microbial degradation of pesticides. These results contribute to the expansion of knowledge on the competence of isolates of these two bacterial genera in degrading herbicides and biotechnological potential for DDE4 biodegradation and bioremediation. The study showed that careful screening of pesticides before field applications should be performed in the laboratory. With high budget projects, decreases in terms of active substances and even changes in the molecular structure of pesticides may be monitored. Since agricultural fields include microorganisms which decompose pesticides, research may be conducted on different pesticides with more bacteria obtained from different agricultural lands. More research on pesticide-bacteria interaction at the molecular level is needed to determine which enzymes or genes are affected by pesticide-stressed bacteria. These studies can be further extended to measure the stimulation of microbial activity with increasing herbicide concentration. As a result, it was concluded that although *B. muralis* has a considerable bioremediation efficiency, especially *S. melonis* is a promising species for the treatment of soil, water and wastewater contaminated with DDE4.

Conflict of interest: No potential conflict of interest was declared by the authors

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