ChemistrySelect

Research Article
doi.org/10.1002/slct.202202105

Chemistry Europe
European Chemical Societies Publishing

www.chemistryselect.org

Biological Chemistry & Chemical Biology

The Bioremediation Capacity of *Sphingomonas melonis* for Methomyl-Contaminated Soil Media: RSM Optimization and Biochemical Assessment by *Dreissena polymorpha*

Gokhan Önder Erguven,*[a] Osman Serdar,[b] Mehtap Tanyol,[c] Nuran Cikcikoglu Yildirim,[d] Numan Yildirim,[e] and Barbaros Durmus[f]

The bioremediation capacity of *S. melonis* for methomyl investigated with RSM. Oxidative stress and neurotoxic response determined in *D. polymorpha*. COD and TOC levels were measured. GSH, MDA levels and CAT, SOD, AChE activities in *D. polymorpha* exposed to before (group A) and after (group B) bioremediated media during 24 and 96 h were tested by using ELISA kit. AchE activity was decreased 24th h but increased during 96th h in group B compared with group A ($p < 0.05$). MDA levels and CAT activities were decreased during 24 h and 96 h in group B compared with group A ($p < 0.05$). GSH levels were increased during 24 h and 96 h in group B compared with group A ($p < 0.05$). SOD activity did not show a significant change in group B ($p < 0.05$). Methomyl could be effectively remediated by *S. melonis* and CAT, AChE activities and GSH, TBARS levels of *D. polymorpha* are useful biomarkers for evaluating the bioremediation capacity.

Introduction

One of the carbamata incesticide methomyl [S-methyl N-((methylcarbamoyl)oxy) thioacetimidate] has highly toxic properties and this pesticide is very dangeours for aquatic living organisms.[1] Microbial bioremediation, which is used as an alternative to the relatively expensive physico-chemical remediation methods, has started to be preferred in recent years because it is cheaper and can provide almost complete degradation of many toxic organic pollutants.[2] Response-surface-methodology (RSM) is an effective tool used in recent years to create a model between variables, to design experiments and variables statistically, to search and evaluate optimum conditions according to the results of this design, and to evaluate the factors affecting different experimental parameters. The use of this method is preferred in many fields such as environmental engineering, food technology and biotechnology.[3] RSM studies are recommended to researchers focused on optimizing the parameters required for the microbial remediation of pesticides, especially from chemical organic-based pollutants.[4–8]

Pesticides, which are persistent organic pollutants, have the potential to cause oxidative stress in organisms living in receiving aquatic environments through the activation of reactive oxygen species (ROS) mechanisms.[9] Oxidative stress is caused by the proliferation of free radicals and ROS production, as well as the decrease in the antioxidant defense system, which causes damage to biological macromolecules and as a result, causes disruption of normal metabolism and physiology.[10] One of the molecular mechanisms of carbamate-induced toxicity is lipid peroxidation (LPO).[11] Antioxidant defense systems arise from a defense mechanism against attacks from exogenous (xenobiotic) or endogenous (physiological) sources of ROS.[12] Antioxidant enzymes can be sensitive to pollutants, and the way of stimulation of antioxidant enzymes is rapid, and this is also sensitive indicators of damage to the environment according to other toxicity parameters.[13]

SOD catalyzes the dismutation of superoxide to oxygen and hydrogen peroxide, which is reduced by CAT to oxygen and water.[14] CAT is also a ubiquitous type of protein that reduces hydrogen peroxide (H2O2) to oxygen and water and participates in the Haber-Weiss reaction.[15] GSH is the most abundant low molecular weight thiol-containing compound in living cells.

[a] *Assoc. Prof. Dr. G. Önder Erguven*
Department of Urbanization and Environmental Issues
Munzur University
Turkey
E-mail: goerguven@munzur.edu.tr
[b] *Assoc. Prof. Dr. O. Serdar*
Department of Basic Sciences of Aquatic Products
Munzur University
E-mail: oserdar@munzur.edu.tr
[c] *Assoc. Prof. Dr. M. Tanyol*
Department of Urbanization and Environmental Issues
Munzur University
Turkey
E-mail: mtanyol@munzur.edu.tr
[d] *Prof. Dr. N. Cikcikoglu Yildirim*
Department of Veterinary Medicine,
Laboratory and Veterinarian Health Program
Munzur University
Turkey
E-mail: nurancyildirim@munzur.edu.tr
[e] *Prof. Dr. N. Yildirim*
Department of Plant and Animal Production
Munzur University
Turkey
E-mail: numanyildirim@munzur.edu.tr
[f] *Dr. B. Durmus*
Department of Environmental Engineering
Firat University
Turkey
E-mail: barbarosdurmus@firat.edu.tr

Since its reduced form (GSH) is a reducing agent for hydroperoxides and free radicals, the defense systems it secretes protect cells against oxidative damage.[16]

Carbamate insecticides mediate neurotoxicities through AChE inactivation.[17] AChE activity is commonly expressed as a biomarker of exposure to various classes of organophosphates and carbamate pesticides.[18]

The zebra mussel, *D. polymorpha*, is a reference species for ecotoxicological studies in aquatic ecosystems. These mussels are predominantly distributed in lakes and reservoirs in Turkey.[19] It is easier to sample as a species that is not endangered and can be encountered continuously in nature, has a stable behavior and sufficient body size compared to other species.[20]

In this study, some answers will be revealed out about the bioremediation capacity of some isolated agricultural soil bacteria like *S. melonis* at methomyl contaminated soil media via the reduction of COD and TOC parameters in filtrates. The neurotoxic and antioxidant response of *D. polymorpha* exposed to before and after bioremediated media were investigated. The activities SOD, CAT, AChE, and the levels of GSH, TBARS in *D. polymorpha* are the biomarkers for revealing out the efficiency of bioremediation capactity of *S. melonis* polluted with methomyl.

Results and Discussion

RSM analysis of COD and TOC removal

According to the RSM analysis, the best TOC and COD removal rates determined in media 1. This media includes 7 cm of soil (Approximately 700 gr) and different concentrations of *S. melonis* (between 20–100 ml) and 180 ppm methomyl. The results showed that; TOC and COD of the methomyl decreases to 63.24 and 86.85 ppm from 527 and 965 ppm in 10.5 days respectively (Table 1). This situation can be explained with; while *S. melonis* concentration given to the system increases, the residual decomposed metabolites of the pesticide and media occurred, so these metabolites increase the TOC and COD value of the filtrated water taken from the medium.

Statistical analysis with CCD

The 13 experiments were designed and conducted using various combinations of *S. melonis* and application time. The experimental responses from CCD experiments are presented in Table 2. An experimental relationship was established to predict subtraction of COD and TOC for all factors. This relationship can be utilized to reveal out the removal rates of COD and TOC in soil when using the bioremediation procedure for methomyl. The quadratic model equations used for this purpose are given below:

$$\text{COD removal } (\%) = +74.00 + 7.18X_1 - 7.73X_2 + 4.50X_1X_2 - 0.94X_1^2 - 1.19X_2^2 \quad (3)$$

$$\text{TOC removal } (\%) = +78.00 + 6.02X_1 - 7.23X_2 + 7.50X_1X_2 - 1.31X_1^2 - 3.56X_2^2 \quad (4)$$

Table 1. Last values of TOC and COD obtained from the filtrated water from the media 1.

Medium number	Time (Day)	TOC (ppm)	COD (ppm)	*S. melonis* (ml)
1	10.5	63.24 ± 1,91	86.85 ± 1,67	21

Table 2. CCD matrix and for bioremediation of COD and TOC.

Run	Factor 1	Factor 2	Response (% removal)	
	X_1: Application time (day)	X_2: *S. melonis* (ml)	COD	TOC
1	0.000	0.000	78	74
2	1.000	1.000	84	78
3	0.000	0.000	78	74
4	−1.000	1.000	52	53
5	−1.414	0.000	72	65
6	−1.000	-1.000	74	71
7	0.000	0.000	78	74
8	0.000	1.414	57	58
9	0.000	0.000	78	74
10	0.000	−1.414	88	89
11	0.000	0.000	78	74
12	1.414	0.000	82	83
13	1.000	−1.000	76	78

Table 3 represents the analysis of variance (ANOVA) of regression variables of the quadratic models of surface response and other statistical parameters for COD and TOC removal. The models are significant (P-value < 0.0026 for COD and P-value < 0.0085 for TOC). R-squared values of 0.8940 and 0.8496 for COD and TOC, respectively, indicate that the models are well fitted. Also, adjusted R-squared values were relatively high in models (0.8182 for COD and 0.7421 for TOC), showing well agreement between predicted and experimental values, suggesting the importance and high predictability of the models. The calculated adequate precisions in both models were greater than 4 (10.838 for COD and 9.103 for TOC), and CV was less than 10% for both cases (5.62 for COD and 6.79 for TOC). According to the ANOVA test, X_1 and X_2 factors were found to be significant for COD removal, while X_1, X_2 and X_1X_2 factors were found to be significant for TOC removal. Other terms were found to be insignificant.

Figure 1a-b shows the relationship between expected and actual model values. Most of the values are close to the diagonal axis, so the predicted values of the models follow the actual (experimental) values. The observed residuals (difference between predicted and experimental responses) are plotted against the appropriate values in Figure 2a–b, also showing the random normal distribution of residuals.

Table 3. ANOVA results for removal of COD and TOC.

Source	Sum of squares	Degree of freedom	Mean square	*F*-Value	*P*-value Prob > F
COD removal (%)					
Model	985.82	5	197.16	11.80	0.0026
X_1	412.65	1	412.65	24.70	0.0016
X_2	478.03	1	478.03	28.61	0.0011
$X_1 X_2$	81.00	1	81.00	4.85	0.0636
X_1^2	6.11	1	6.11	0.37	0.5643
X_2^2	9.81	1	9.81	0.59	0.4686
Residual	116.95	7	16.71		
Model statistics					
R-squared	0.8940				
Adjusted R-squared	0.8182				
Adequate precision	10.838				
CV %	5.62				
TOC removal (%)					
Model	1026.28	5	205.26	7.91	0.0085
X_1	289.71	1	289.71	11.16	0.0124
X_2	418.19	1	418.19	16.11	0.0051
$X_1 X_2$	225.00	1	225.00	8.67	0.0216
X_1^2	11.98	1	11.98	0.46	0.5187
X_2^2	88.29	1	88.29	3.40	0.1077
Residual	181.72	7	25.96		
Model statistics					
R-squared	0.8496				
Adjusted *R*-squared	0.7421				
Adequate precision	9.103				
CV %	6.79				

CV: coefficient of variance.

Response surface plotting

The 3D surface plots and 2D contour plots were used to analyze the combined effect of factors on methomyl bioremediation using *S. melonis* (Figure 3a–b).

The desirability function of Design-Expert was used to determine the medium conditions that give maximum COD and TOC removal in methomyl bioremediation using *S. melonis*. Optimum conditions were found to be 10.50 days of application time and 21 ml of *S. melonis* culture. By considering these conditions, the highest COD and TOC removal efficiency was estimated as 91% and 88%, respectively, according to the proposed model.

Biochemical Response

The biochemical parameters of *D. polymorpha* exposed to this optimized medium before and after the bioremediation by *S. melonis* on methomyl insecticide are shown in Table 4.

AchE Activity

AchE activity was increased in the group A (*D. polymorpha* exposed to Methomyl synthetic solutions before bioremediation) during 24 h but it was decrased during 96 h compared to the control group ($p < 0.05$). AchE activity in the group B was decreased during 24 h but it was increased during 96 h compared to the control group ($p < 0.05$). AchE activity was decreased during 24 h but it increased during 96 h after bioremediation ($p < 0.05$) (Table 4). When the exposure times are compared; statistically significant differences were found in the groups A and B ($p < 0.05$) (Table 4).

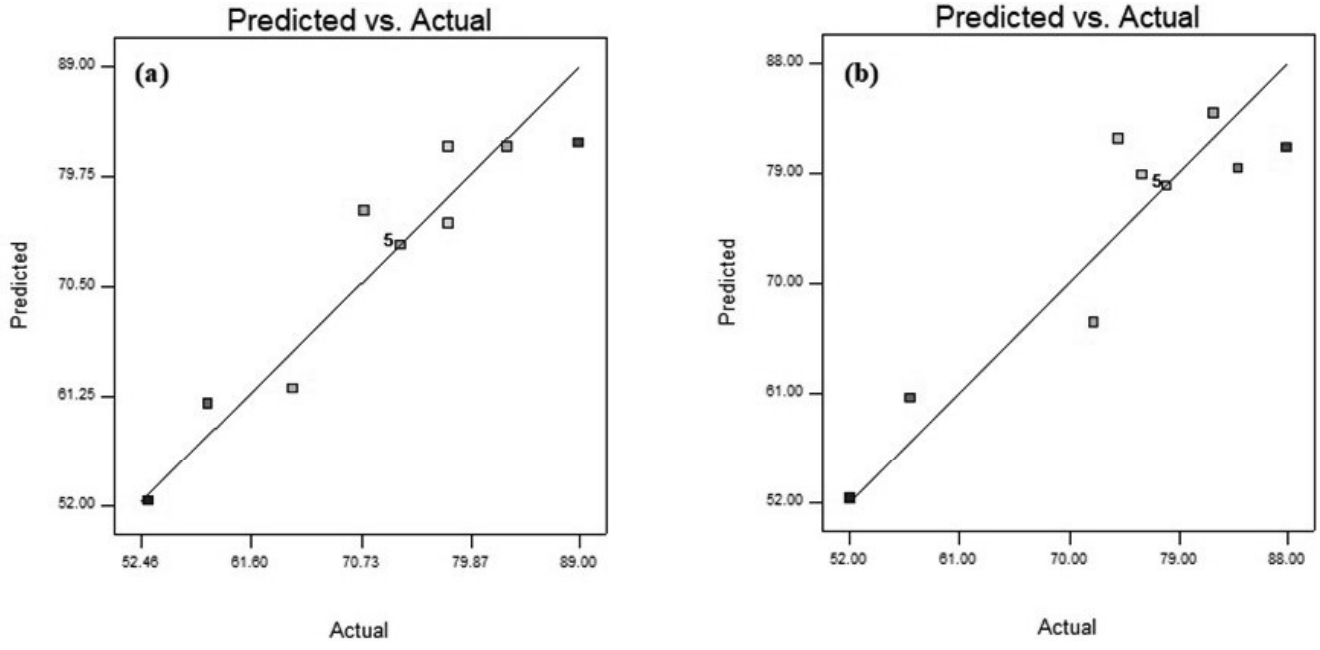

Figure 1. Predicted vs. actual values plot for (a) COD and (b) TOC removal (%).

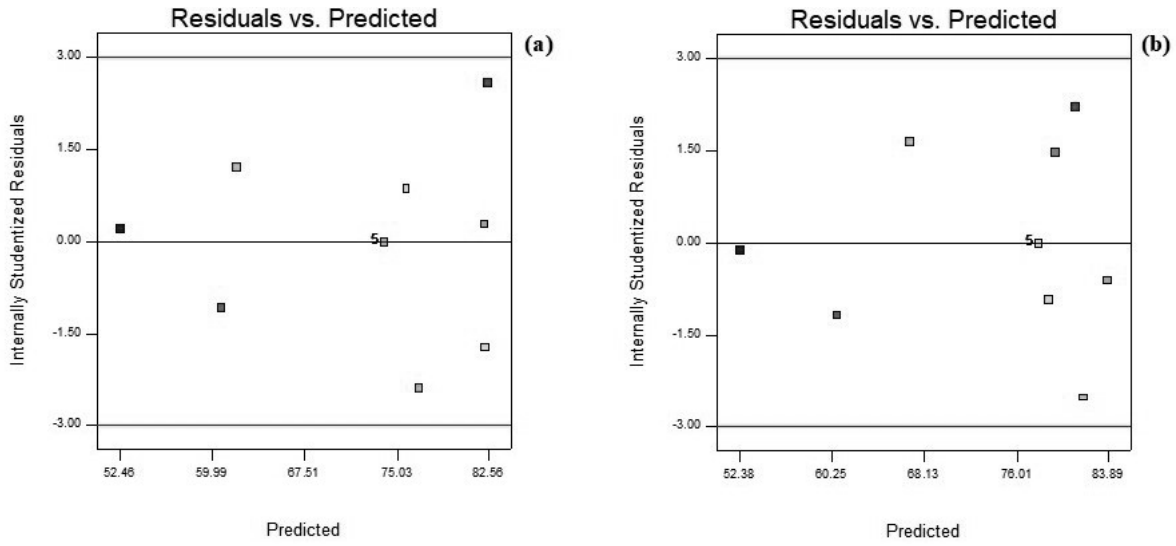

Figure 2. Residual plot of for (a) COD and (b) TOC removal (%).

Table 4. Biochemcal parameters of *D. polymorpha.*

AChE (ng/L)	24th hour	96th hour
Control	7,86 ± 0,88[b]	5,21 ± 1,35[ab]
A	11,62 ± 0,57a	2,79 ± 0,11[b*]
B	2,6 ± 0,05c	6,18 ± 0,63[a*]
TBARS nmol/mg	**24th hour**	**96th hour**
Control	7,29 ± 1,92[b]	8,5 ± 1,39[ab]
A	17,09 ± 0,54[a]	13,1 ± 1,47[a]
B	6,28 ± 0,38[b]	3,99 ± 0,29[b]
GSH μM	**24th hour**	**96th hour**
Control	8,79 ± 0,49[a]	4,64 ± 0,84[b*]
A	2,28 ± 0,08[b]	1,14 ± 0,10[c*]
B	6,91 ± 0,97[a]	8,27 ± 0,49[a]
SOD U/mL	**24th hour**	**96th hour**
Control	2,64 ± 0,026[a]	2,51 ± 0,1[a]
A	2,35 ± 0,23[a]	1,25 ± 0,006[b*]
B	2,93 ± 0,29a[a]	2,51 ± 0,11[a]
CAT nmol/min/mL	**24th hour**	**96th hour**
Control	84,31 ± 0,55[c]	85,11 ± 0,64[c]
A	172,32 ± 3,75[a]	153,43 ± 2,49[a*]
B	95,59 ± 1,66[b]	95,36 ± 1,83[b]

The differences in letters (a, b, and c) on average values show the statistical difference among application groups (control, A, B) at the same application times (24 or 96 h) according to the Duncan's multiple range test. * Shows the statistical differences between the exposure times (24 and 96 h) in the same application group according to the independent t-test.

TBARS Levels

TBARS levels were increased in the group A (*D. polymorpha* exposed to methomyl synthetic solutions before bioremediation) during 24 and 96 h compared to the control group ($p < 0.05$). TBARS levels in the Group B were decreased during 24 h and 96 h compared to the control group ($p < 0.05$). TBARS levels were decreased during 24 and 96 h after bioremediation ($p < 0.05$) (Table 4). When the exposure times are compared; no statistically significant difference was found ($p > 0.05$) (Table 4).

GSH Levels

GSH levels were decreased in the group A (*D. polymorpha* exposed to methomyl synthetic solutions before bioremediation) during 24 and 96 h compared to the Control group ($p < 0.05$). GSH levels in the group B were decreased during 24 h ($p > 0.05$) but it was increased during 96 h compared to the control group ($p < 0,05$). GSH levels were increased during 24 h and 96 h after bioremediation ($p < 0.05$) (Table 4).

When the exposure times are compared; statistically significant differences were found in the control and group A ($p < 0.05$) (Table 4).

SOD Activity

There was no statistically significant change in SOD activity in the group A (*D. polymorpha* exposed to methomyl synthetic solutions before bioremediation) during 24 h but it was decreased during 96 h compared to the Control group ($p < 0.05$). There was no statistically significant change in the group B during 24 and 96 h compared to the control group ($p < 0.05$). SOD activity did not show a statistically significant change after bioremediation ($p > 0.05$) (Table 4).

When the exposure times are compared; statistically significant differences were found in the groups A ($p < 0.05$) (Table 4).

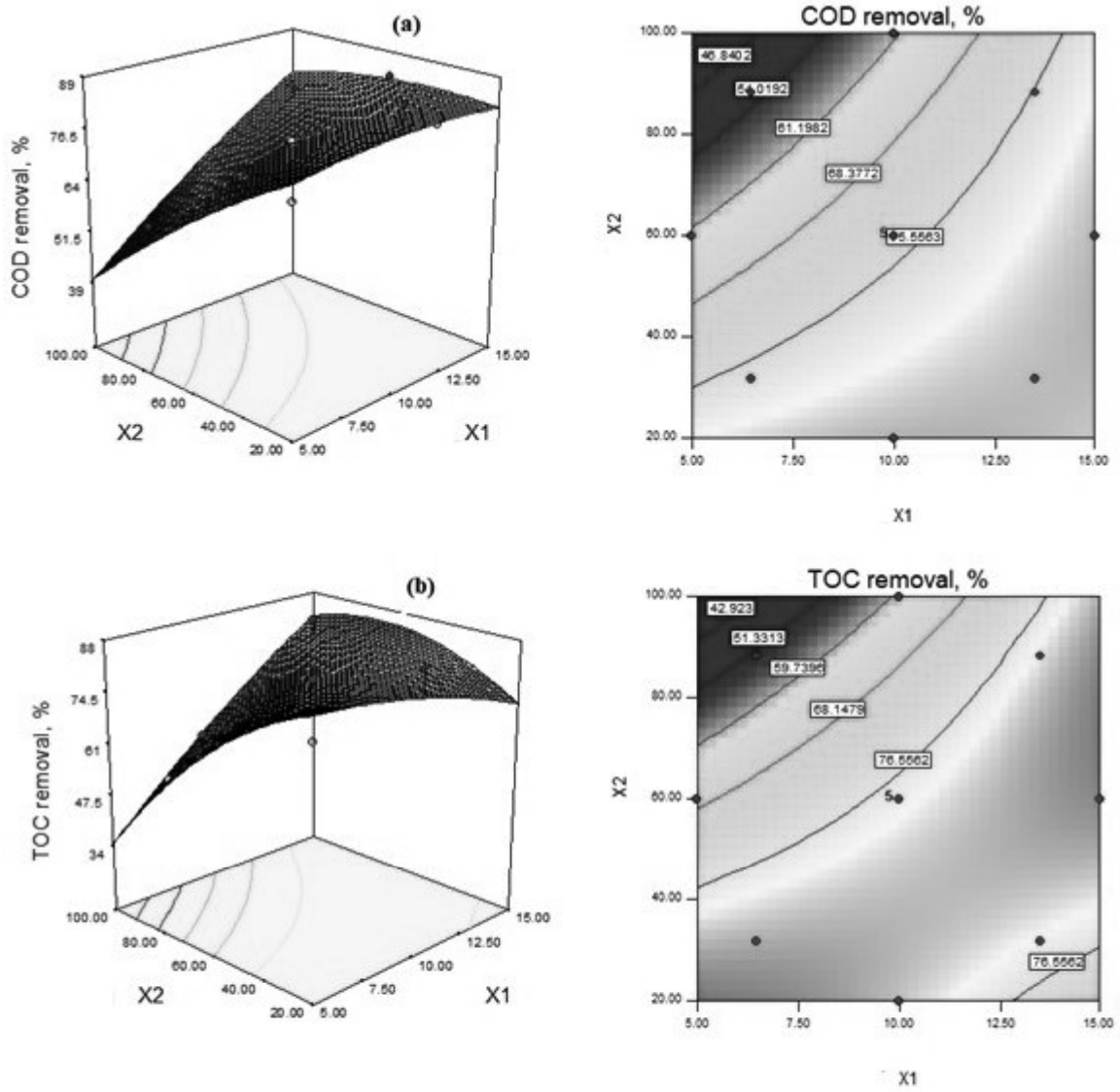

Figure 3. Response surface and contour plots for (a) COD and (b) TOC removal (%) (X_1: application time (day); X_2: *S. melonis* culture (ml)).

CAT Activity

CAT activity was increased in the group A (*D. polymorpha* exposed to methomyl synthetic solutions before bioremediation) during 24 and 96 h compared to the control group ($p < 0.05$). CAT activity in the Group B was increased during 24 h and 96 h compared to the control group ($p < 0.05$). CAT activity was decreased during 24 and 96 h after bioremediation ($p < 0.05$) (Table 4).

When the exposure times are compared; statistically significant differences were found in the groups A ($p < 0.05$) (Table 4).

In this laboratory-scale research, it was aimed to evaluate the toxic effects of mehtomyl insecticide, which is frequently seen in the receiving environments where pesticides are used, in *D. polymorpha* treated according to some biochemical parameters (AChE, SOD, CAT, GSH and TBARS) and the bioremediation efficiency of *S. melonis*. Bioremediation activities were followed for the removal of tested methomyl residues in the receptors.

It has also been proven in previous studies that microorganisms play an important role in the removal and detoxification of these toxic substances.[21] Many species of microorganisms capable of degrading carbamate insecticides have been isolated and identified from agricultural areas around the world[22,23] found that 77% of the initial concentration of a pesticide was removed with the (EB20) isolate and suggested Pseudomonas sp as an active species for bioremediation. (EB20) can be considered as a suitable remediation method/medium for methomyl in drinking water. Mohamed et al.,[24] investigated the isolation of a new bacterial strain (*Stenotrophomonas maltophilia*) from several water samples contaminated with methomyl, which can degrade methomyl pesticide (1000 ppm) in the presence of 0.05% glucose Erguven et al.,[25]

found the bioremediation performance of a soil bacterium, *S. melonis*, on indaziflam, an herbicide at different concentrations, by reducing important environmental parameters. They found that the most effective biodegradation rates were 83% and 73% for COD and BOD_5 at 150 ppm in 6 days, respectively, and the TOC removal efficiency was 70%. The bioremediation efficiencies of *Bacillus aryabhattai*, *Pseudomonas azotoformans* and *Sphingomonas pseudosanguinis* and consortia consisting of them were investigated by reducing the COD and TOC parameters in the permeate water obtained from a bioremediation setup adapted to laboratory conditions and a glyphosate added soil medium. At the end of 11 days, the highest COD reduction rate was found to be 92.1% in environments with *S. pseudosanguinis*, while TOC rate was found to be 69.13% in consortium environments.[25] *S. melonis* was also used in the study by Erguven,[26] for the bioremediation of imidacloprid, another insecticide species. They determined that this bacterial isolate could biodegrade the imidacloprid insecticide with an effective efficiency of over 90%. Similarly, Khatoon et al.[27] used Bacillus bacteria species for the biodegradation of atrazine and optimization of the parameters used during these processes by RSM. pH (7.05), temperature (30.4 °C), agitation speed (145.7 rpm), and atrazine concentration (200.9 ppm) were found to be the best conditions to achieve maximum atrazine removal using the bacterial isolate.

According to the results of this study, it has been argued that metomyl insecticide can be effectively removed by *S. melonis* and given to receiving environments under optimum conditions. Methomyl belongs to a class of pesticides included in the group of oxime carbamates and is often preferred in the fight against pests and nematode species by inhibiting the AChE enzyme that hydrolyzes the neurotransmitter acetylcholine.[28] AChE activity decreased after 24 hours or 21 days amelioration with mettomyl at the 10 mg/kg dose level relative to control treatment[29,30] also found that methomyl decreased AChE. Methomyl caused a net decrease of approximately 48% in the *Pseudorasbora parvain* specific activity of brain AChE at concentrations between 0.043 and 0.213 mg/l.[31] D. polymorpha was exposed to non-lethal concentrations of beta-cyfluthrin pesticide for 24 and 96 hours. AChE activity was inhibited with increasing concentration compared to control,[1,32] investigated the effect of the pesticide methomyl on different enzymatic activities in *Cyprinus carpio* L. Brain AChE activity was significantly reduced after 96 hours of exposure compared to controls throughout the experimental periods. Khalil et al.[33] investigated the acute and non-lethal effects of methomyl lanate on the land snail *Eobania vermiculata*. They suggested that AChE activity of *E. vermiculata* was dose-dependently inhibited by carbamate lannate. In this study, our results were like those obtained by other authors. AchE activity decreased 96 hours after bioremediation in group X1 (*D. polymorpha* exposed to methomyl synthetic solutions before bioremediation). In Group X2, AchE activity increased again for 96 hours compared to the control group. Metomyl can induce inhibition of AChE enzyme activity in accordance with previously published literature which clearly demonstrates the inhibitory effect of carbamate insecticides

Meng et al.[34] found a significant decrease in GSH following 2, 20 and 200 ppm in a study on methomyl exposure, suggesting the presence of oxidative stress. Tilapia was exposed to non-lethal concentrations of 0.2, 2, 20 and 200 ppb methomyl for 30 days. They found that GSH levels dropped significantly. Like the results obtained, methomyl was found to induce glutathione reduction in vitro.[35] Ergüven et al.[36] also interpreted SOD and CAT activity in *G. pulex* exposed to chlorpyrifos-ethyl insecticide before and after biodegradation/bioremediation with *Methylobacterium radiotolerans* and *Microbacterium arthrosphaerae*. According to the results, it was determined that SOD activity increased after exposure to chlorpyrifos-ethyl for 24 and 96 hours. After bioremediation, SOD enzyme activity decreased again for 24 hours ($p > 0.05$) but increased continuously for 96 hours ($p < 0.05$). After bioremediation, CAT activity decreased with CPF exposure, and enzyme activity was increased again. In another study conducted by Tatar et al.,[37] the biochemical response of *G. pulex* to methomyl pesticide was investigated before and after bioremediation by *Ochrobactrum thiophenivorans* and *Sphingomonas melonis*, isolated and identified from two soils. *G. pulex* exposed to methomyl had decreased CAT enzyme after the entire exposure time. CAT activities were converted to control results after bioremediation experiments. CAT, SOD increased at low methomyl concentrations but decreased at high methomyl concentrations.[37] In this study, no statistically significant change was found in SOD activity after bioremediation. In Group B (*D. polymorpha* exposed to methomyl synthetic solutions before bioremediation) CAT activity increased for 24 and 96 h. CAT activity decreased for 24 and 96 hours after bioremediation.

According to Monsour et al.,[38] LPO products formed by free radical-mediated attack on membrane lipids can spread the autocatalytic reaction chain of LPO processes if there is oxygen in the environment, and this may cause membrane destruction. In *D. polymorpha* exposed to beta-cyfluthrin pesticide, MDA levels increased with increasing concentration, while GSH levels decreased.[32] Methomyl significantly increased lipid peroxidation in kidneys of CD-1 mice. It was determined that SOD and CAT activities decreased. In a study by El-Demerdash et al.,[39] *D. polymorpha* bioremediated with methomyl was evident with a significant decrease in GSH content, but with an increase in TBARS levels. While GSH levels increased after bioremediation, MDA levels decreased at 24 and 96 hours after bioremediation (Table 4). The findings are like previous studies. According to Ozden et al.,[40] the elevation in LPO is a result of depleted GSH stores that are otherwise capable of regulating LPO levels.

Erguven and Yıldırım (2019) examined imidacloprid remediation with *Methylobacterium radiotolerans* and *Microbacterium arthrosphaerae* strains. At the end of the 18th day, removal efficiencies for the COD parameter were determined as 52, 96 and 99% for 20, 40 and 80 ml bacteria consortium, respectively, while BOD_5 removal rates were determined as 88, 79% and 50% in the same volumes.[41] Erguven and Yildirim (2016) investigated the bioremediation rate of chlorsulfuron herbicide to reduce the COD parameter. According to the results they

obtained, they determined that the chlorsulfuron removal rates of *B. simplex*, *B. muralis*, *M. luteus*, *M. yunnanensis* and *C. tetani* were between 70–93% at the end of the 120th hour.[42] In a laboratory-scale study of chlorpyrifos, which is biodegraded by soil bacteria, Tatar et al. (2020) investigated the toxic effects of an insecticide with an active ingredient methomyl. As a result of their studies, *Ochrobactrum thiophenivorans* and *Sphingomonas melonis* showed a decrease of 94.7% and 96.8% in COD parameters at the end of 8 days.[37] Góngora-Echeverría et al. (2020) described the degradation of glyphosate in pure strains and microbial consortium. Glyphosate removal was studied with a bacterial consortium of *Pseudomonas nitroreducens* and Ochrobactrum sp., and they demonstrated bioremediation efficiency by inoculation, even in remediation of agricultural soil exposed to herbicides.[43]

Conclusion

This is the first study about the bioremediation capacity of *S. melonis* for methomyl-contaminated soil media. The soil media is prepared in laboratory scale and represents an agricultural area. The concentrations choosen for pesticide is related with the real pesticide concentration that farmers use according to the manufacturers instrductions. In this study, RSM Optimization also used and the biochemical assessment of *Dreissena polymorpha* performed that differs from other bioremediation studies. Although it has been determined that metomyl insecticide can be effectively removed by *S. melonis*; also, the levels of GSH, MDA and CAT, AChE activities in *D. Polymorpha* demonstrate the ability of *S. melonis* in RSM-optimized methomyl bioremediation. Methomyl was found to stimulate oxidative stress and neurotoxic response at the same time. Exposure time also affected the biochemical biomarkers of *D. Polymorpha* at different levels.

Experimental section

Bacteria

The *S. melonis* was already present in the culture collection of the Environmental Research Laboratory of Munzur University. This bacterial strain was kept at +4 °C until use for bioremediation studies. For enrichment of the bacterial strain, approximately 1 cm diameter *S. melonis* culture from agar medium was added to a 250 ml Erlenmeyer flask containing sabouraud dextrose broth (SDB) medium and placed in an orbital shaker incubator at 27 °C with continuous shaking at 140 rpm for 5 days.

Chemicals and medium

Methomyl pesticide was supplied from sigma-aldrich (Germany) Turkey distributor with CAS number 16752-77-5. SDB was purchased from Sigma Aldrich (Turkey).

Design of experiments

Central composite design (CCD) under RSM was used to better understand the bioremediation of methomyl using *S. melonis* and to minimize the number of experiments required. The bioremediation experiments were designed using Design-Expert 7 software version. Two factors including application time (X_1) in the range of 5–15 days and *S. melonis* culture (X_2) in the range of 20–100 ml were intended to achieve the optimum COD and TOC removal efficiency and were coded with five different levels of the CCD model (Table 5), whereas, percent removal of COD and TOC were defined as responses.

Independent factors used in this work were coded based on Eq. (1):

$$X_i = \frac{X_i - X_0}{\Delta X} \quad (1)$$

where X_i represents the dimensionless independent factor value, X_0 denotes the value of X_i at the center point and ΔX is the step change.

Each response can be given as a quadratic model equation as follows.[44]

$$R\ (\%) = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta_{ij} X X_j + \quad (2)$$

where, R is the response (COD or TOC removal (%)), β_0, β_i, β_{ii}, and β_{ij} are the constant, linear coefficient, quadratic coefficient, and interaction coefficient, respectively, k is the number of chosen independent factors, and e is the error of the model.

Bioremediation studies

Five sterilized plastic bottles were cut from their bottoms and approximately 1 ml of holes was open to these bottoms as a filtrate part. These parts were placed to the bottles again for filtrate the liquid phase from the soil (Pesticide + enriched bacteria). The soil samples were sterilized at 70 °C in Pasteur oven about 2 days. The sterilized soil was filled to these bottles to 7 cm level above filtrate part (Figure 4). The reason for choosing this soil level is that methomyl affects the 7 cm root part of the plant. After that, 180 ppm methomyl (suggested concentration for farmers) added to these bottles. The amount design of *S. melonis* was choosen between 20–100 mL according to the literature about bioremediation activities of some soil bacteria isolated from agricultural field and optimized the amount of bacterial culture by RSM studies as 20, 31.72, 60, 88.28 and 100 mL. According to the results taken from the RSM studies, 20, 31.72, 60, 88.28 and 100 mL of enriched bacterial samples (contains approximately 10^9 CFU/mL) transferred to these bottles at the beginning of the bioremediation step and filtrated water taken from these units for COD and TOC monitoring.

COD and TOC measurements

To monitor the bioremediation capacity, the COD and TOC values of the filtered water were measured at each sampling time. These

Table 5. Levels of the two independent factors.

Coded factor	Factor	Levels				
		−α	−1	0	+1	+α
X_1	Application time (day)	5	6.46	10	13.54	15
X_2	*S. melonis* culture (ml)	20	31.72	60	88.28	100

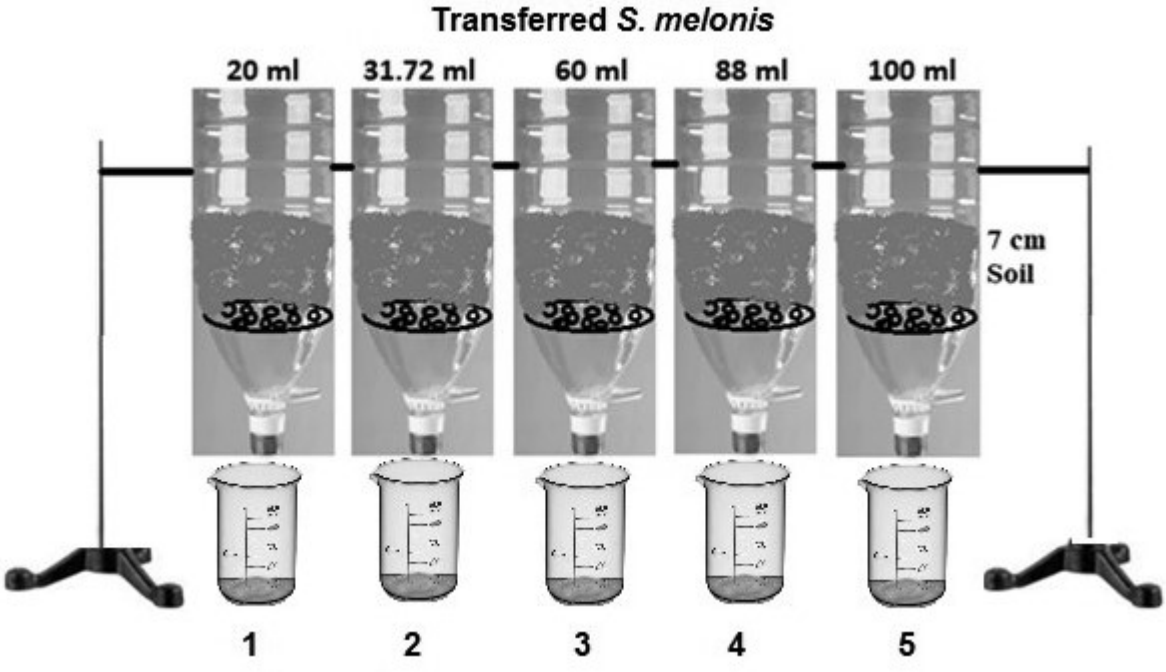

Figure 4. Bioremediation setup.

sampling periods were determined according to CCD as 5, 6, 10, 14 and 15th days. Experiments were carried out with 3 replications. In the COD experiments, the closed reflux titrimetric method specified in standard method 5220 C was chosen. These experiments were done with the HACH DRB 200 model thermoreactor adapted with Hach DR 890 colorimeter device with 23459-52 model COD kits. In TOC experiments, TEKMAR-DOHRMANN Apollo 9000 device used in the light of standard method 5310A high temperature combustion method.[45]

Test organism

D. polymorpha individuals were collected by hand from the Keban Dam Lake, Elazig Turkey and brought alive to the Munzur University Environmental Engineering Department Research Laboratory in air-added plastic boxes (Figure 5).

D. polymorpha individuals brought alive to the laboratory were added to the previously prepared aquariums. 14 hours of light and 10 hours of dark conditions were used as photoperiod, laboratory illumination. For the adaptation of living things and test steps, the temperature given by the thermostat air conditioner to the environment was kept constant by adjusting it to 18 °C. Cultured phytoplanktons were used to feed *D. polymorpha*.

To meet the oxygen, need in stock aquariums, the air motor and external filter was used in the aquarium. Each aquarium consists of 10 individuals with replication of 3 experiments. No nutrient was added to the organisms during the experiments. Organisms were checked every 24 h and then dead individuals were counted and then removed from the aquarium. Immobility was accepted as a criterion for death.

According to the results taken from the bioremediation setup, best removal efficiency seen in bottle that includes 20 ml *S. melonis*, at the end of the 10th day. This treated sample and untreated sample taken for biochemical assessment. The Individuals of *D. polymorpha* (n: 10 for each group) were exposed to these groups for 24 and 96 h.

Three experimental groups were designed for biochemical evaluation as following.

Control: 20 mL Tap water

Group A: Before bioremediation of 180 ppm 20 mL methomyl

Group B: After bioremediation of 180 ppm 20 mL methomyl

Biochemical Response

After 24 and 96 h exposure periods, test organism individuals were cut with a scalpel and dissection was performed. 0.5 g of this organism was weighed and homogenized with the help of ice, and PBS buffer (phosphate buffered saline solution) was added at a rate of 1/5 w/v. The supernatants obtained from these samples, which were homogenized in a cooled centrifuge at 17.000 rpm for 15 minutes, were stored in a deep freezer at −86 °C until the measurement process was completed. The SOD, CAT, GSH and TBARS kits used in this study purchased from CAYMAN, AChE kit was purchased from CUSABIO. Catalog numbers are respectively (CAT: 707002, SOD: 706002, GSH: 703002, TBARS: 10009055, AChE: CSB-E17001Fh). The results were determined and analyzed according to the manufacturer's instructions.

The activities of SOD, CAT, AChE activities and GSH, MDA levels were determined by ELISA reader (Thermo Scientific™ Multiskan™ FC Microplate Photometer).

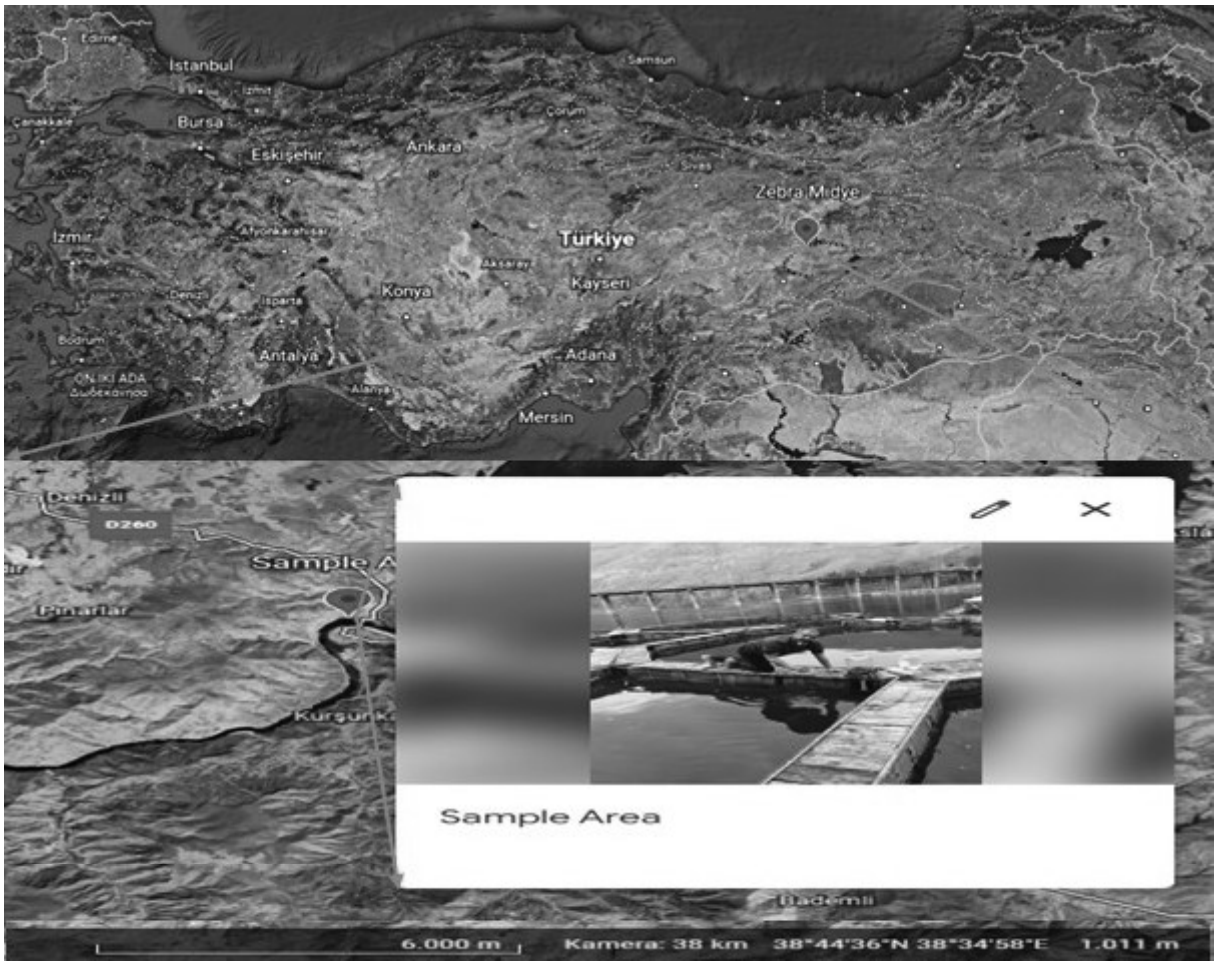

Figure 5. Sampling area.

Statistical analyses for biochemical parameters

Obtained biochemical results were analyzed using the PASW 18 software program (SPSS Inc, Chicago, Illinois, USA). Duncan's multiple range test was used to evaluate the differences amoung the control, A and B groups. Two-tailed independent T test was used to determine the differences of each biochemical parameter between 24 and 96 hours.

Conflict of Interest

The authors declare no conflict of interests

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Keywords: methomyl · bioremediation · *S. melonis* · biochemical assessment · *D. Polymorpha*

[1] D. Hernández-Moreno, I. de la Casa-Resino, J. M. Flores, M. J. González-Gómez, C. M. Neila, F. Soler, M. Pérez-López, *Arh. Hig. Rada Toksikol.* **2014**, *65*, 311–317.
[2] V. Korkmaz, N. Yildirim, G. O. Erguven, B. Durmus, Y. Nuhoglu, *Environ. Technol. Innov.* **202**1, 22, 101535.
[3] M. Dilipkumar, M. Rajasimman, N. Rajamohan, *Chemical Industry a Chemical Engineering Quarterly* **2010**, *16*, 319–327.
[4] V. Sridevi, M. V. V. C. Lakshmi, A. V. N. Swamy, M. N. Rao, *J. bioremediat-biodegrad.* **2011**, *2*.
[5] N. Mansouriieh, M. Khosravi, *Arab. J. Chem.* **2015**, *9*.
[6] N. Debasmita, M. Rajasimman, *Alex. Eng. J.* **2013**, *52*,499–505.
[7] M. Lakshmi, V. Sridevi, M. N. Rao, A. V. N. Swamy, *Int. J. Res. Pharm. Chem.* **2011**, *1*, 925–935.
[8] B. Durmus, E. Aydın, G. O. Erguven, N. Yildirim, E. Kiyan, *IJIAAR* **2022**, *6*, 1, 57–67.
[9] V. I. Lushchak, *Aquat. Toxicol.* **2011**, *101*, 13–30.
[10] M. Trevisan, R. Browne, M. Ram, P. Muti, J. Freudenheim, A. M. Carosella, D. Armstrong, *Am. J. Epidemiol.* **2001**, *154*, 348–356.
[11] B. D. Banerjee, V. Seth, A. Bhattacharya, S. T. Pasha, A. K. Chakraborty, *Toxicol. Lett.* **1999**, *107*, 33–47.
[12] D. R. Livingstone, *Mar. Pollut. Bull.* **2001**, *42*, 656–666.
[13] A. Stara, J. Kristan, E. Zuskova, J. Velisek, *Pestic. Biochem. Physiol.* **2013**, *105*, 18–23.
[14] V. Contardo-Jara, A. Krueger, H.-J. Exner, C. Wiegand, *Environ. Monit. Assess.* **2009**, *11*, 1147–1156.
[15] K. B. Storey, *Braz. J. Med. Biol.* **1996**, *29*, 1715–1733.
[16] F. H. El-Rashidy, W. A. Al-Turk, S. J. Stohs, *Res. Commun. Chem. Pathol. Pharmacol.* **1984**, *44*, 423–430.

[17] N. S. Gupta, R. Michels, D. E. G. Briggs, M. E. Collinson, R. P. Evershed, R. D. Pancost, *Org. Geochem.* **2007**, *38*, 28–36.
[18] C. E. Grue, P. L. Gibert, M. E. Seeley, *Am. Zool.* **1997**, *37*, 369–388.
[19] H. Kusku, *Aquat. Res.* **2022**, *5*, 11–19.
[20] A. Binelli, C. della Torre, S. Magni, M. Parolini, *Environ. Pollut.* **2015**, *196*, 386–403.
[21] A. G. Kulkarni, B. B. Kaliwal, Springer, **2018**, 75–86.
[22] S. Desaint, A. Hartmann, N. R. Parekh, J.-C. Fournier, *FEMS Microbiol. Ecol.* **2000**, *34*, 173–180.
[23] I. I. El-Fakharany, A. H. Massoud, A. S. Derbalah, M. S. S. Allah, *JECE* **2011**, *3*, 332–339.
[24] M. S. Mohamed, *Electron. J. Biotechnol.* **2009**, *12*, 6–7.
[25] G. O. Erguven, K. Gurdal, *JAES* **2020**, *53*, 318–324.
[26] G. O. Erguven, U. Demirci, *Environ. Technol. Innov.* **2021**, *21*, 101236.
[27] H. Khatoon, J. P. N. Rai, *Biotechnol. Rep.* **2020**, *26*, 00459, 30.
[28] A. G. Kulkarni, B. B. Kaliwal, *J. bioremediat. biodegrad.* **2015**, Corpus ID: 38847514.
[29] I. I. El-Fakharany, A. H. Massoud, A. S. Derbalah, M. S. S. Allah, *JECE* **2011**, *3*, 332–339.
[30] S. A. Mansour, A.-T. H. Mossa, T. M. Heikal, *Toxicol. Ind. Health* **2009**, *25*, 557–563.
[31] Z. Ren, Z. Li, M. Ma, Z. Wang, R. Fu, *Bull. Environ. Contam. Toxicol.* **2009**, *82*, 310.
[32] H. Soylemez, O. Serdar, R. Aydin, *Int. J. Pure Appl. Sci.* **2021**, *7*, 462–471.
[33] A. M. Khalil, *JOBAZ* **2016**, *74*, 1–7.
[34] S. Meng, J. Qu, L. Fan, L. Qiu, J. Chen, P. Xu, *Environ. Toxicol.* **2015**, *30*, 483–489.
[35] O. Lohitnavy, P. Sinhaseni, *Arh. Hig. Rada Toksikol.* **1998**, *49*, 231–238.
[36] G. O. Erguven, S. Tatar, O. Serdar, N. C. Yildirim, *ESPR* **2021**, *28*, 2871–2879.
[37] S. Tatar, N. C. Yildirim, O. Serdar, G. O. Erguven, *Curr. Microbiol.* **2020**, *77*, 1301–1307.
[38] S. A. Mansour, A.-T. H. Mossa, T. M. Heikal, *Toxicol. Ind. Health* **2009**, *25*, 557–563.
[39] F. El-Demerdash, Y. Dewer, R. H. ElMazoudy, A. A. Attia, *Exp. Toxicol. Pathol.* **2013**, *65*, 897–901.
[40] S. Ozden, B. Catalgol, S. Gezginci-Oktayoglu, P. Arda-Pirincci, S. Bolkent, B. Alpertunga, *FCT* **2009**, *47*, 1676–1684.
[41] G. O. Erguven, N. Yildirim, *Curr. Microbiol.* **2019**, *76*, 1461–1466.
[42] G. O. Erguven, N. Yildirim, *Cell. Mol. Biol.* **2016**, *62*, 92–96.
[43] V. R. Góngora-Echeverría, R. García-Escalante, R. Rojas-Herrera, G. Giácoman-Vallejos, C. Ponce-Caballero, *Ecotoxicol. Environ. Saf.* **2020**, *200*, 110734.
[44] S. M. Beyan, S. V. Prabhu, T. T. Sissay, A. A. Getahun, *Bioresour. Technol. Rep.* **2021**, *14*, 100664.
[45] A. P. H. Association, A. W. W. Association, W. P. C. Federation, W. E. Federation, *Standard methods for the examination of water and wastewater*, American Public Health Association **1912**.

Submitted: May 31, 2022
Accepted: July 6, 2022