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Research Article

Biodegradation of chlorpyrifos by bacterial genus pseudomonas putida

Yakup CUCİ[1,*], Sait ÇELİK

[1] *Kahramanmaras Sutcu Imam University, Environmental Engineering Department, 46100, Kahramanmaras, Turkey*

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*Corresponding author e-mail: cuci@ksu.edu.tr

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ABSTRACT

Chlorpyrifos is an solid organophosphorus pesticide widely used in agriculture. It is relatively stable to hydrolysis in neutral pH and acidic aqueous solutions. Stability decreases with increasing pH. Chlorpyrifos practically insoluble in water, but highly soluble in most organic solvents such as acetone xylene and methyl bromide. It is an effective skin, stomach and respiratory insecticide and is effective against aphids, spider mites, soil bugs and house pests. It is also highly toxic bees and fish. Most pesticides are complex organic molecules and are persistent in the environment, either biota or accumulate in the environment. In pesticide-used areas, the pesticide itself or its residues are transported by rain and irrigation water and mixes into groundwater. The effects caused by the pesticide are closely related to the level of accumulation. During the studies, contamination of chlorpyrifos has been found about 24 km from the site of its application. There are many physico-chemical and biological approaches to remove organophosphorus pesticides from the ecosystem, among them most promising is biodegradation. In this study, the biodegradation potential of chlorpyrifos with *P. Putida* was investigated in the batch stirred reactor. In the optimum conditions, the maximum pesticide removal rate was determined as 1.51 mg g^{-1}. d. m.o.h.

Keywords: Pesticides, Chlorpyrifos, P. Putida, biodegradation.

Pseudomonas putida ile chlorpyrifos'un biyodegradasyonu

ÖZ

Chlorpyrifos tarımda yaygın olarak kullanılan katı bir organofosforlu pestisittir. Chlorpyrifos nötral ve asidik çözeltilerde kararlı olmasına rağmen pH artışı ile kararlılığı azalmaktadır. Chlorpyrifos pratik olarak suda çözünmez fakat aseton ksilen ve metil bromür gibi çoğu organik çözücüde oldukça yüksek miktarda çözünmektedir. Temas, sindirim ve solunum etkili bir insektisit olup yaprak bitleri, kırmızı örümcek, toprak böcekleri ve ev haşerelerine karşı etkilidir. Aynı zamanda arılar ve balıklara karşı da toksiktir. Pestisitler gibi pestisit kalıntıları da çok yönlü ve karmaşık özelliğe sahip olup birikim de yapabilmektedirler. Pestisit kullanılmış alanlarda bu ilacın kendisi veya kalıntıları yağmur ve sulama sularıyla yeraltı sularına karışarak sucul bitki ve hayvanlar için toksik etkiler oluşturmaktadır. Pestisitin neden olduğu etkiler canlıdaki birikim düzeyine ve canlının yağ içeriği ile yakından ilişkilidir. Yapılan çalışmalarda Klorpyrifos kontaminasyonu uygulama alanından yaklaşık 24 km uzaklıkta tespit edilmiştir. Organofosforlu bileşiklerin ekosistem giderilmesinde birçok fiziko kimyasal ve biyolojik proses rol oynamaktadır. Ancak bunlar arasında en umut verici yöntemler biyobozunma yöntemleridir. Bu çalışmada chlorpyrifosun *Pseudomonas putida* ile biyodegradasyon potansiyeli incelenmiştir. Optimum şartlarda chlorpyrifosun *P. putida* ile biyodegraasyonunda maksimum pestisit tüketim hızı 1,51 mg/g. K. mo.sa. olarak bulunmuştur.

Anahtar Kelimeler: Pestisitler, Chlorpyrifos, P. Putida, biyodegradasyon

1. INTRODUCTION

Pesticides (or biocides) are synthetic, organic compounds used to destroy undesirable organisms. The word pesticide is of Latin origin and means a disease-killing substance. These produced substances are completely foreign to nature. These substances, their chemical and biological change products (metabolites) are of interest not only in terms of their biocidal effects, but also in terms of their targets and effects within the overall ecosystem.[1] More than 10,000 insects, 600 weeds, more than 1500 plant diseases and 1500 species of nematodes are known that can harm humans, animals and plants in varying degrees.

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Millions of tons of pesticides have been used in the world, especially in the last 40-45 years. Turkey, which is an agricultural country, has taken its share from this amount. Residues of pesticides have been detected all over the world, including at the polesPesticides also adversely affect non-targeted organisms by mixing with air, water and soil during manufacture, storage, marketing and use. When unconscious and careless use is added to this, it is seen that pesticides accumulate at increasing rates in water, soil, plant and animal foods.[2] Pesticides, pesticides that are thrown in our environment aimlessly, unlimitedly and almost uncontrollably, are found in almost all items.[3]

In order to protect natural resources from pollution, it is important to use chemicals used for agricultural purposes in a controlled manner and to prevent further pollution. Some properties of pesticides such as extreme mobility, durability and evaporation cause negative consequences in terms of environmental pollution. In minimizing the pesticide pollution potential, it is also necessary to take into account the expected benefit from pesticide use.[4-6] There is no doubt that agricultural pesticides, which have wide spread routes in the environment, have negative effects on all living things. Pesticides accumulated in water and soil pass into the bodies of creatures such as fish and insects living in these environments. Therefore, birds that feed on these creatures that carry the drug residue are also poisoned. Beneficial insects such as predators and parasitic insects, which have a more sensitive structure, are also highly affected by unconscious spraying.[7] In addition, the uncontrolled use of pesticides causes soil, air, surface and underground water pollution. Controlled use of chemicals used in agriculture is very important to prevent further pollution of these natural resources. As a result of the use of pesticides indiscriminately and in excessive doses without scientific control, negative effects on beneficial living things and other elements of the environment occur as well as pests. However, the most important, perhaps the most important issue for human health is the environmental pollution of the pesticides used and their undesirable effects on the natural balance. While some of these substances have mutagenic and carcinogenic effects in the living body, some of them both accumulate and cause toxic effects in the living body.[8] Artificial chemicals used in agricultural areas are quite durable as a result of their molecular structure and maintain their structural properties under natural conditions. As a result of this stability, they participate in natural cycles thanks to their structure not deteriorating for years. Due to their structural durability, they cause significant pollution in soil, water, groundwater and surface waters.[9]

The most important feature sought in pesticides is that they are very toxic and effective against harmful animals and organisms. In addition, it is expected to be less toxic or harmless to warm-blooded animals, especially humans. However, among the drugs produced so far, those with these qualities are very few. For this reason, it should be accepted that pesticides are poisonous to humans and all other living things as a general rule. Because it is clear that drugs with biological activity will cause some potential dangers and cause some undesirable problems such as environmental contamination as a result of their use, and this has been well established recently.[7]

Agrochemicals are applied directly to the soil surface or into the soil, on the plant, or on the seedlings in the form of seed spraying. A significant part of the drug thrown on the plant falls into the soil. Drugs that fall into the soil can move in the soil over time, depending on factors such as soil type, solubility, permanence and climate.[10] After the pesticides applied to the plant, soil or seed for agricultural struggle have fulfilled their lethal effects, some of the pesticides are absorbed by the various organs of the plant, and the remaining part is distributed in the application area in the form of removal from the soil, retention in the soil and transformation into other compounds.[11]

The structure, mode of action and properties of the active substances of pesticides are different from each other. Pesticide active substances that contaminate the plant surface stay there for a certain period of time, some of them enter the plant, and some dissolve in water and translocate into the plant through roots, leaves or branches. In this way, the effective substances of pesticides found with the plant gradually decrease over time with removal mechanisms such as being washed with rain, leaking, dripping, being carried by the wind, evaporation, oxidation under the sun, hydrolyzed by high humidity, or decomposing by mixing with plant secretions after completing their activities. The important point here is that the existing pesticide residues are present in all kinds of foodstuffs at a level that will not harm human, animal and environmental health before coming to the market for consumption. For this reason, each drug is subjected to toxicological and pharmacological trials before being put on the market. These trials are carried out on experimental animals in the form of feeding with medicated product for a short period of 2-3 months and for a long period of at least 2 years.[12] The effects caused by pesticides are closely related to the accumulation level and the oil content of the living thing.[13]

Artificial organic pesticides are usually produced as concentrated (technical) substances. The active ingredient content of a formulated commercial pesticide can vary between 1 and 95 percent by weight and is applied as powder, granule, solution, emulsion or wettable fine powder.[10] According to the researches, the way of application of the drug contributes to the environmental pollution as well as the efficiency. It is obligatory to work on the use of other drugs that do not

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form pesticide residues and to continue this struggle with biological methods.[14]

Biological degradation of pesticides is under the influence of factors that affect normal microbial activity in soil. As is well known, these factors are temperature, moisture content, organic matter present, pH, etc. are factors. Most pesticides are new compounds for soil microorganisms. Therefore, biodegradation is slow at first due to the lack of adaptation of microorganisms. Some polar groups such as -OH, -COO, $-NH_2$ and $-NO_2$ contained in pesticide molecules form the action points for organisms. Studies show that the pesticide concentration in the soil decreases with the addition of easily decomposable organic materials.[1] While microorganisms have an effect on pesticide concentrations due to their activities, pesticides also greatly affect the biological activity and microbial composition of the soil. The main purpose of metabolic processes in the world of microorganisms is energy production and cell synthesis. The vast majority of organic materials are capable of meeting these two purposes of heterotrophic bacteria. However, the most striking feature of microorganisms is that they go through an acclimation process. In other words, if substances that are resistant to biological decomposition are applied slowly and in very low concentrations to a certain microorganism species, it is certain that microorganism species that have adapted to them over time and can use these substances as energy and nutrients will develop.[15] Fallmann and co-workers[16] investigated the applicability of the Photo-Fenton method to treat pesticide-containing waters in their study. They successfully applied this Photo-Fenton process including Fe^{+2}/H_2O_2/UV-V to 10 commercial pesticide mixtures. Experiments with a single pesticide yielded notable differences in reaction rates, although each pesticide degraded. Aksu (2005)[17] states that biosorption studies are also carried out for pesticides, and that it is possible to remove some pesticides with a few microorganism species including bacteria and fungi.

Bellinaso and co-workers[18] investigated the biodegradation of trifuralin herbicide with bacteria isolated from soil. They found that five bacteria isolated from the soil could be used in the biodegradation of trifural, and three of these isolated bacteria increased the degradation of trifural by more than 20%. Sanchez and co-workers[19], in their study investigating the effect of sewage sludge on pesticide biodegradation in soil, found that the biodegradation of pesticide residues caused changes in the microorganism population of the soil.

In this study, it was aimed to biodegrade free *P. putida* and chlorpyrifos pesticide in cut cup. Substrate consumption is a result of the activities of microorganisms and reactions in cell metabolism. In the study, pesticide biodegradation occurred in aerobic environment with microorganism species were determined. For this reason, the maximum growth rate and pesticide removal of the microorganism were determined without acclimatization and after acclimatization of the selected pesticide in aerobic environment. In addition, the possibility of acclimatized bacteria to use this pesticide as a single substrate was determined.

2. MATERIALS AND METHODS

After *P. putida* bacteria are grown in media containing chlorpyrifos pesticide, the effects of temperature, initial pH, agitation speed, initial pesticide concentration and different media parameters on microorganism growth and pesticide consumption rates were examined in batch system, and optimum temperature, initial pH, shaking speed were examined. and initial pesticide concentrations were determined. Determining the amount and types of degradation products of chlorpyrifos pesticide, which was exposed to physical and microbiological effects throughout the entire experimental study, was excluded from the scope of this study due to the difficulties encountered in the analysis.

In order to obtain a lower concentration solution from the stock solutions by dilution, the stock solutions were taken from the deep freezer and kept under laboratory conditions enough for their temperature to reach room temperature. Standard solutions were prepared separately at concentrations of 10, 20, 30, 40 and 50 mg L^{-1} by diluting the stock solutions at room temperature.

2.1. Preparation of samples for gas chromatography analysis

It is very important to determine the exact and precise amounts of pesticides in pesticide analysis. (Table 1) Since the pesticide concentrations to be found as a result of the analysis are generally low, pesticide analysis is difficult. Quantitative analysis of pesticides by gas chromatography; It is done by obtaining chromatograms of pesticide standards prepared at known concentrations in gas chromatography and by comparing the peak length (or peak area) of the solution with unknown concentration and the peak length (or peak area) of the solution with known concentration.[20]

For this, standard pesticide solutions prepared at different concentrations were injected into gas chromatography and chromatograms were obtained. Peak areas or peak sizes obtained for pesticides from the chromatograms were plotted against pesticide concentration. Thus, a calibration curve was obtained for each pesticide.

Pesticide concentration in the samples was determined by calculating the pesticide amount corresponding to this peak area or height by substituting the peak area or peak size of the sample in the equation obtained from the calibration curve.

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Table 1. Optimum determination conditions in gas chromatography of Chlorpyrifos insecticide

Detector	Flame Ionization Detector (FID)
Column	Restek Brand Rtx-5MS (Crossbond 5% diphenyl 95% dimethyl polysiloxane) programmable temperature 350 °C, decomposition temperature 330 °C
Column Dimensions	0.25 mm inner diameter - 30 m length
Temperatures	
Injection Unit	300 °C
Column	280 °C
Detector	300 °C
Gas Flow Rates	
Air	330 ml min^{-1}
Hydrogen	33 mL min^{-1}
Carrier Gas (N_2)	1.4 mL min^{-1} (10 lb/in^2)
Carrier Gas + Reference Gas	30 mL min^{-1}
Injection Technique	
Split	1/25
Recorder	0.5
Paper Speed AT	64
Retention Time	5,17 min

2.2. Microorganism production

The *P. putida* used in the study was obtained from the American Type Culture Collection. NRRL B-252 coded *P. putida* from the American Type Culture Collection (A.T.C.C) was produced in the laboratory for use in studies. The composition of the rich broth used in the production of *P. putida* is given in Table 2.

Table 2. Composition of rich broth used in the production of P.putida.

Glucose	3
Yeast extract	2
bacteriological peptone	2
K_2HPO_4	1
KH_2PO_4	1
$(NH_4)_2.SO_4$	1
$MgSO_4.7H_2O$	0.5

Bacterial culture brought in lyophilized form was first produced in petri dishes containing solid nutrient medium with agar and in tubes containing slanted agar in an oven at 30 °C and then transferred from these media to rich liquid nutrient media containing glucose. 100 ml of the medium with the composition given in Table 2 was added to 250 ml flasks, and the mouths of the flasks were closed with cotton plugs and aluminum foil. The media prepared for sterilization in this way were sterilized by keeping them in an autoclave at 121 °C for 45 minutes. Bacteria were inoculated at a ratio of 1/10 to the medium prepared by sterilization. Then, P. putida was grown by keeping it in an orbital incubator operating at 30 °C and 100 rpm stirring speed for 72 hours. In order to demonstrate the effect of substrate inhibition on the microorganism used in the study, the amount of glucose was changed between 3-10 g in a pesticide-free medium. According to the data obtained, it can be seen from Figure 1 that glucose alone does not have an inhibitory effect on the growth of *P. putida*.

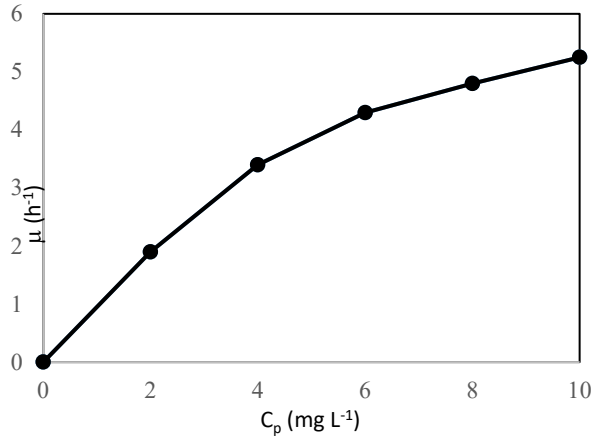

Figure 1. The effect of initial glucose amount on the specific growth rate of P. putida
(T=30 °C, X_o= 10 mL, MS=100 rpm)

2.3. Acclimatization of the microorganism to the pesticide-containing environment

Accustoming microorganisms to toxic organic compounds such as pesticides is an important process that must be done in order to increase microbial activity. The acclimatization time ranges from a few hours to a few weeks, depending on the nature of the vaccine used.[21] Accordingly, a series of experiments were conducted to acclimate *P. putida* to pesticides.

The acclimatization of the microorganism to the pesticide was carried out gradually. First, liquid nutrient media containing 3 g L^{-1} glucose + 0.01 g L^{-1} pesticide were prepared, and cultures grown in 3 g L^{-1} glucose medium were planted in the prepared liquid nutrient medium. After the growth was observed, acclimation of the microorganism was continued. The acclimation process is similar in media containing 1 g L^{-1} glucose + 0.01 g L^{-1} pesticide, 0.5 g L^{-1} glucose + 0.01 g L^{-1} pesticide and 0.0 g L^{-1} glucose + 0.01 g L^{-1} pesticide and then transferred to the biodegradation medium. By determining the amount of increase in the mass of microorganisms in the solution during the acclimatization period, it was decided that the acclimatization process of the microorganism to the pesticide environment was realized.

The composition of the nutrient medium used in biodegradation studies is given in Table 2. Biodegradation experiments were carried out by adding the microorganism produced after the acclimatization process to this medium at a ratio of 1/10. Pesticide was added to the solution prepared according to the

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composition in Table 3, in varying amounts depending on the nature of the experiment.

Table 3. Nutrient media used in biodegradation studies.

Glucose	0.5
K_2HPO_4	1
KH_2PO_4	1
$(NH_4)_2.SO_4$	1
$MgSO_4.7H_2O$	0.5

In order to prevent all kinds of infections that may occur in the working environment, all the studies with living organisms should be carried out under sterile conditions. The flasks, tubes and pipettes containing the nutrient medium were sterilized by keeping them in an autoclave at 121 °C for 45 minutes. The environment where the sowing process was carried out was chemically sterilized with ethyl alcohol before sowing and a UV lamp was used for a certain period of time. The sowings were made in such a way that sterilization would not deteriorate in the presence of the burner flame. Tubes and flasks containing solid and liquid nutrient media produced in active form were stored in a refrigerator at 4 °C. The microorganism stored in the refrigerator was transferred to new nutrient media once every 15 days to maintain the activity of the microorganism.

2.4. Preparation of pesticide solutions

In the biodegradation of Chlorpyrifos pesticide with *P. putida*, a stock pesticide solution was prepared from Sarban 4 E and Folicor WP 25 pesticides at a concentration of 2000 mg L^{-1} on the basis of active substance. Pesticide solution was added to the nutrient medium with the help of a pipette to form the desired concentration from these stock solutions. [22]

2.5. Reproduction Studies in Mixed Pot Working in Batch Order

Reproduction studies in batch order were carried out in 250 mL flasks in which 100 mL of medium was left. Pesticide biodegradation studies were carried out by placing these flasks used in biodegradation in orbital incubators that can operate at constant temperature and mixing speed. In reproductive studies in batch order; After mixing 50 mL of medium with 10 mL of microorganism, the required amount of stock pesticide solution was added to the medium+microorganism solution of this mixture to contain pesticide at the determined concentration. Then the working volume was completed to 100 mL with distilled water. Except for the microorganism concentration, the pH of the prepared experimental setup was adjusted separately and a sample was taken from the mixture to determine the initial pesticide concentration and stored in the refrigerator. Then, the growth medium, whose pH was adjusted, was sterilized in an autoclave at 121 °C for 45 minutes. 10 mL vaccine (*P. putida*) was added to the pesticide-containing medium prepared for production by sterilization, and it was kept in an orbital incubator at constant temperature and stirring speed for 72 hours. After the growth was completed, the microorganisms were separated from the liquid medium by centrifugation at 5000-6000 rpm for 3 minutes. Dry microorganism concentration was determined by keeping the microorganisms in an oven at 60 °C for 12 hours on tared aluminum foil. The liquid part was immediately extracted with dichloromethane for pesticide determination and stored in a deep freezer until analysis.

3. RESULTS AND DISCUSSION

The biodegradation of *P. putida* bacteria and chlorpyrifos was investigated in batch stirred reactors. Glucose, selected as the substrate, was used as an additional carbon source to the pesticide. In the experiments, the effects of parameters such as initial pH, temperature and initial pesticide concentration on substrate consumption and microorganism production rates and productivity were investigated in batch system with free *P. putida*.

3.1. Effect of initial pH

The initial pH is an important parameter for the specific growth rate of *P. putida* and its effect on the pesticide consumption rate in a growth medium containing chlorpyrifos. Hydrogen ion concentration significantly affects the activities and growth of microorganisms. Each microorganism has an optimum pH range where it shows maximum activity. In order to maximize the activities of organisms, the pH of the environment must be kept under control at an optimum value. By keeping the mixing speed, temperature and chlorpyrifos concentration constant, the amount of change in the microorganism concentration in different initial pH values (pH= 3-9) is given in Figure 2.

As a result of the studies carried out in the media containing chlorpyrifos pesticide and prepared at different initial pH values, it was observed that the growth rate of *P. putida* decreased in the media with pH values lower than pH = 8 and with high pH values.

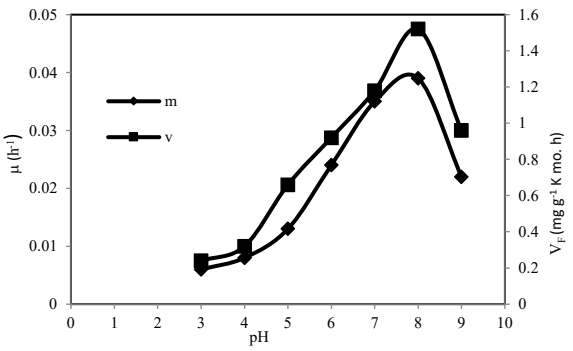

Figure 2. The effect of the initial pH' mm on the specific growth and pesticide consumption rate of the microorganism.

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3.2. Effect of temperature

One of the most important parameters affecting the growth of microorganisms and accordingly the rate of substrate consumption is temperature. The effect of temperature on the growth of *P. putida* in a breeding medium containing Chlorpyrifos pesticide was investigated in the range of 20-35 °C.

In Figure 3, the effect of temperature on the specific growth and chlorpyrifos pesticide consumption rate of *P. putida* is given. From Figure 3, it is seen that the optimum temperature determined for the growth of *P. putida* bacteria and maximum consumption of chlorpyrifos pesticide in the pesticide environment is 30 °C.

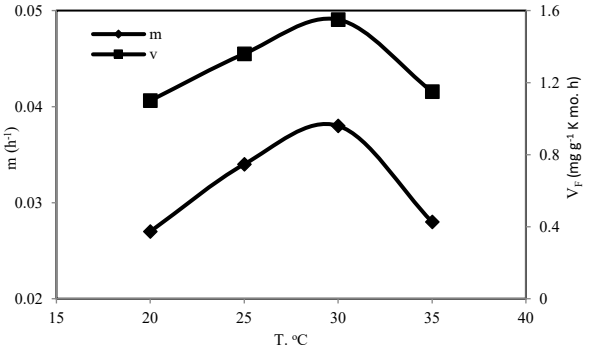

Figure 3. The effect of temperature on the specific growth and pesticide consumption rates of the microorganism. (*P. Putida,* pH = 8,0, C_{Fo} =50 mg L^{-1}, X_o= 10 mL; MS= 100 rpm)

Temperature is especially effective on the enzyme systems of microorganisms. As can be seen from Figure 3, it is seen that low temperatures have an inhibiting effect on the growth of *P. putida* and decrease the growth rate of this microorganism. It is known that the enzyme system of microorganisms deteriorates at high temperatures. Above the optimum temperature, microorganism growth decreases with the increase in temperature, and as a result, microorganism death occurs with excessive increase. At higher temperatures than the optimum temperature (30 °C), lower microbial specific growth rates and pesticide consumption rates were obtained at higher temperatures, since microorganisms lost their metabolic activities due to the deterioration of the enzyme structures in the microorganism.

3.3. Effect of initial chlorpyrifos concentration

One of the most important parameters affecting the growth rate and substrate consumption rate of microorganisms in biodegradation studies is the initial substrate concentration. In order to investigate the effect of the initial concentration of the substrate on the growth of *P. putida* and the biodegradation of chlorpyrifos pesticide, the effect of the initial chlorpyrifos pesticide concentration on the growth and chlorpyrifos consumption rate of *P. putida* bacteria in experimental studies carried out at constant temperature and mixing speed. 25-500 mg L^{-1} initial pesticide concentration and the results are given in Figure 4 and Table 3. It can be seen from Figure 4 that the maximum specific growth rate for P. putida microorganism is obtained when the initial substrate concentration is 50 mg/L at optimum constant temperature and stirring speed. It can be clearly seen from Figure 4 that the growth rate of microorganisms decreases rapidly when the initial pesticide concentration rises above 50 mg L^{-1}. Pesticide concentration above 50 mg/L causes substrate inhibition, and pesticide concentrations greater than 50 mg L^{-1} cause a rapid decrease in the specific growth rate of microorganisms. The reason for this inhibition is that excessive pesticide disrupts the bacterial structure and prevents their cellular functions.

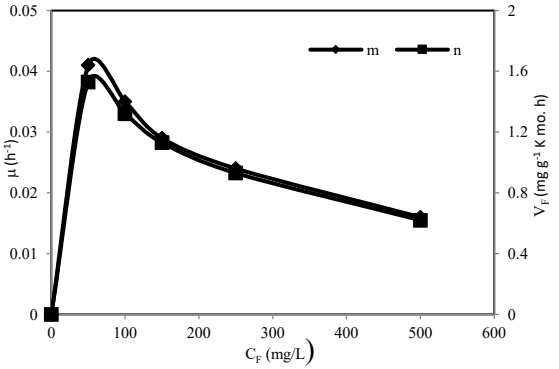

Figure 4. Effect of initial pesticide concentration on microorganism specific growth and pesticide consumption rates. (*P. Putida,* pH= 8, T= 30 °C, X_o= 10 mL, KH= 100 rpm)

Table 3. Maximum microorganism concentrations, % pesticide consumption values and doubling times obtained at different pesticide concentrations (*P. putida*)

C_{Fo} (mg/L)	X_m G k.mo/L)	% Cosuption	t_d(h)
52.31	0.675	68.01	17.5
101.41	0.575	61.61	20.9
146.47	0.554	50.16	24.2
207.84	0.450	41.68	26.3
264.63	0.340	33.85	32.34
533.41	0.176	14.64	53.3

The decomposition of organic substances in the metabolic processes of microorganisms affects the amount of oxygen in the waters. Microorganisms decompose the organic substances in the water. In the presence of toxic substances in the environment, the activities of microorganisms slow down and thus the decomposition of organic substances is prevented. During the decomposition of organic substances, a dynamic balance occurs between the active microorganisms present in the water and the degradable organic matter. Depending on the concentration of organic matter in the water, an oxygen consumption proportional to the microorganism concentration occurs. When Table 3 is examined, it is seen that the percentage of pesticide consumption decreases continuously after 50

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mg L^{-1} pesticide concentration, while the microorganism doubling time (td) increases.

In optimum conditions where the initial pH is 8, the temperature is 30 °C and the initial pesticide concentration is 52.31 mg L^{-1}, the specific growth rate of the microorganism is 0.0395 h^{-1}, the pesticide consumption rate is 1.51 mg pesticide/g k. B.C. h, and the percentage of pesticide consumption was found to be 68.01% at the end of 24.

When the pesticide is used as the sole carbon source, it is the substrate pesticide that inhibits growth. However, when a carbon source other than pesticide is used, both the pesticide and other carbon source may inhibit growth. The data were evaluated in terms of both substrate and toxic compound inhibition, and the compatibility of substrate and toxic compound inhibition with the Monod equation given below was investigated. In this equation, µm represents the maximum growth rate (h^{-1}), and Ks the saturation constant (g L^{-1} or mg L^{-1}).

$$\mu = \frac{\mu_m C}{K_S + C} \quad (1)$$

It was determined that non-competitive substrate inhibition (Halden's equation) best describes the system. It is written as the Halden equation.

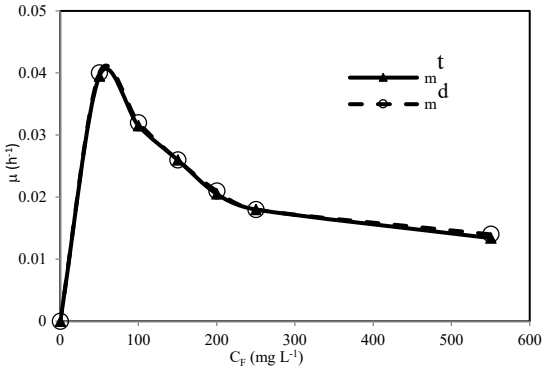

Figure 5. Comparison of microorganism specific growth rate values calculated from the experimental and Monod equations

$$\mu = \frac{\mu_m}{(1+\frac{K_S}{C})(1+\frac{C}{K_I})} \quad (2)$$

Here, K_I= is the inhibition constant (mg/g/L) and $K_I \gg K_s$ is the equation as follows;

$$\mu = \frac{\mu_m . C}{K_S(1+\frac{C}{K_i}+C)} \quad (3)$$

In Figure 4, the microorganism specific growth rate values calculated by experimental and nonlinear regression method were compared. The constants in Equation 2 were calculated as the maximum microorganism growth rate q_{max}= 0.054 h^{-1} the saturation constant K_s=3.62 mg L^{-1} and the inhibition constant K_I = 171.71 mg L^{-1}.

3.4. Effect of mixing speed

Mixing is necessary to increase microbial growth by ensuring that the microorganism is in good contact with the nutrient medium. The mixing speed is also one of the important parameters that affect the growth and substrate consumption rates of the microorganism. The mixing speed in the biodegradation of Chlorpyrifos pesticide was investigated in the range of 50-150 rpm. Microorganism concentration in the medium and chlorpyrifos pesticide concentrations remaining in the medium were determined for each mixing speed value, and the effect of mixing speed on the specific growth and pesticide consumption rates of the microorganism is given in Figure 5 below. As can be seen from Figure 5, optimum microorganism concentration and pesticide consumption were obtained at a stirring speed of 100 rpm. A reduction in both the microorganism concentration and the degraded chlorpyrifos concentration was observed at mixing speeds lower and higher than 100 rpm.

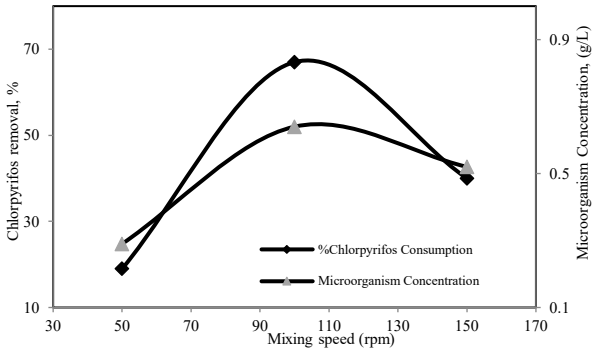

Figure 6. Comparison of microorganism specific growth rate calculated from the experimental and Monod equations

4. CONCLUSIONS

It is aimed to obtain a smooth and as high a peak area as possible for the peaks of the substance analyzed in gas chromatography. For this, while developing a gas chromatography method for the study, different columns and detectors were tried. The optimum injection, column and detector temperatures were investigated for each column and detector tested. As a result of these studies, flame ionization detector (FID) and Rtx-5MS capillary column were used for chlorpyrifos pesticide. Various temperature programs were applied to the column in order to obtain peaks resulting in a higher peak area by ensuring the separation of the pesticide from its solvent in the column. As a result of increasing the column temperatures according to a program, the baseline of the detector increased continuously until the end of the analysis with the injection, so it was decided to work with constant column temperatures.

The sample volume injected into the injection unit is generally between 0.5-1 µL for capillary columns. For this reason, the injection volume was applied as 1 µL in

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the studies. The sample, which turns into steam due to the temperature of the injection unit, is dragged to the column by the nitrogen gas. Pesticide analyzes could not be performed properly because the pesticide could not be separated when the whole injected sample was dragged into the girth (splitless). For this purpose, the method in which only part of the sample injected into the injection unit is used and the other part is thrown out (split) has been tried and very good and clear separation peaks that can be used in the analysis of pesticides have been obtained.

As a result of these studies, a calibration curve was created within the framework of the optimum analysis conditions obtained for chlorpyrifos in gas chromatography. The correlation of the obtained calibration curve did not fall below 99%. Since the sensitivity of the detectors to the determined substances changes over time, calibration charts were reconstructed at the beginning and end of the analysis.

It has been understood that the composition of the biodegradation media greatly affects the consumption of pesticides, and it has been understood that when another carbon source is present in the environment, the bacteria prefer the other carbon source, which is easier to use, instead of consuming the pesticide. In experimental studies, where glucose was used as the sole carbon source and the inhibition effect of glucose on microorganism growth rate was examined, it was observed that glucose did not inhibit microorganism growth (Figure 1).

In the batch system, the effects of system parameters such as pH, temperature and initial pesticide concentration on the specific growth and substrate consumption rate of the microorganism were investigated. 8, optimum temperature was determined as 30 °C and optimum initial pesticide concentration was determined as 50 mg L^{-1}. Under these conditions; The maximum microorganism specific growth rate obtained in the biodegradation of chlorpyrifos with *P. putida* was 0.0395 h^{-1} and the pesticide consumption rate was 1.51 mg g^{-1} k.mo.h.

It was observed that excessive toxic component inhibition was effective at concentrations higher than 50 mg L^{-1} pesticide concentration. Maximum microorganism growth rate (μ_{max}) for Chlorpyrifos, *P. putida*; 0.054 h^{-1}, saturation constant (Ks); 3.62 mg L^{-1} and inhibition constant, (KI); It was found to be 171, 71 mg L^{-1}. In the biodegradation of pesticides in living systems in batch order; By working with mixed cultures, the increasing and decreasing effects of pesticides on the growth rate can be examined. This study, which is done in a batch mixing vessel, can be studied in continuous, filled and semi-batch reaction vessels.

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Conflict of interests

I declares that there is no a conflict of interest with any person, institute, company, etc.

REFERENCES

1. Haktanır, K. *A. Ü. Ziraat Fakültesi, T. no:107*, **1985**, Ankara.
2. Barlas, N. *Tübitak* **1996.** Project: YDABÇAG 217/A.
3. Güler, Ç.; Uz, H.; Sur, H. *TSE Standard Ekonomik ve Teknik Dergi*,**1998**, 440(37), 54-59.
4. Ünlü, K.; Özenirler, G.; Sözüdoğru, S. *Turkish J Eng Env Sci.*, **1997**, 21, 189-202.
5. William, M.D.; Coates, J.A.; Garcia, K.L.; Signorella, L.L.; Delfino, J.J. *J. Chromatogr. A*, 1993, 643, 341-350.
6. Yıldırım, E. *Tarımsal Zararlılarla Mücadele Yöntemleri ve Kullanılan İlaçlar*. Atatürk Üniv. Ziraat Fak. Yayınları, No:219, Erzurum, 350 s. 2008.
7. Baltensweiler, W. Z. Ang. Ent., **1985**, 99:77-85.
8. Güler, Ç.; Uz, H.; Sur, H. *TSE Standard Ekonomik ve Teknik Dergi*,**1998**, 440(37), 54-59.
9. Gürman, A. *Kimya Mühendisliği Dergisi,* **1993**, 138. Sayı.
10. Öztürk, S. *Tarım İlaçları,* Hasat Yayıncılık, İstanbul. 1990.
11. Ecevit, O.; Bayraklı, F. *Pestisit kalıntı sorunu ve Önemi, Çölleşen Dünya ve Türkiye Örneği*, T.C. A.Ü. Çevre Sorunları Araştırma Merkezi, Erzurum. 13-17 Mayıs, 1985.
12. Güvener, A. *Pestisit Kalıntı Sorunları*, I. Ulusal Zirai Mücadele İlaçları Sempozyumu, DİE, Ankara.27-29 Kasım, 1980.
13. Türkiye Çevre Vakfı, 1995, *Türkiye'nin Çevre Sorunları'95*, Altıncı Baskı, Önder Matbaa, Ankara, 1995.
14. Ayas, Z.; Barlas, N.; Kolankaya, D. *Aquat. Toxicol.* **1997**, 39(2)171-181.

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15. İnce, N.; Bekbölet, M. *Türkiye'de Pestisit Tüketimine İlişkin Kirlenme Öncelikleri*, Türkiye'de Çevre Kirlenmesi Öncelikleri Sempozyumu, 21-22 Mayıs, İstanbul, 1991.

16. Falmann, H.; Krutzler, T.; Bauer, R.; Malato, S.; Blanco, J. *Catal. Today*, **1999**, 54, 309-319.

17. Aksu, Z. *Process Biochem* **2005.**, 40, 997-1026.

18. Bellinaso, M. L.; Greeer, C. W.; Peralba, M. C. C.; Henriques, J.A.P; Gaylarde, C.C. *FEMS Microbiol. Ecol.* **2003**, 43, 191-194.

19. Sanchez, M.E.; Estrada, I.B.; Martinez O.; Martin-Villacorta, J.; Aller, A.; Moran, A. *Chemosphere*, **2004**, 57, 673-679.

20. Tutarlı, A. Elazığ'da Tarımsal Mücadele Amacıyla Kullanılan Pestisitlerin Topraktaki Kalıntılarının Araştırılması, Master's Dissertation, F.Ü. Fen Bilimleri Enstitüsü, Elâzığ, 1991.

21. Buitron, G.; Koeffed, A.; Capdeville, B. *Envıron Technol*, **993**, 14, 227-236.

22. Barlas, N. *Tübitak* **1996.** Project: YDABÇAG 217/A.