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Study of 4-chlorophenol biological treatment using yeast and mold isolated from industrial and petroleum wastewaters (Imam Khomeini Port, Mahshahr)

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Phenolic derivatives are some of the most toxic pollutants in the environment. Chlorophenols are the most toxic pollutants of water and wastewater. Since 4-chlorophenol is a highly soluble compound in water, it is abundantly found in water and wastewater. Because of high costs, high energy consumption and in some cases environmental inconsistency of the chemical and biological removal methods, biochemical decomposition of 4-chlorophenol is very important. In the present study, 13 strains of bacteria and 6 strains of yeast and mold were purified and isolated from Shahid Tondgooyan wastewater treatment plant (Imam Khomeini Port, Mahshahr), which lasted about 15 days. Then, the ability of each microorganism isolated in the presence of 100 ppm of 4-chlorophenol was studied and two microbial species suitable for TY_1 and TY_2 were selected for use in a mixed microbial culture. 4-Chlorophenol decomposition was performed in the presence of 100 ppm of 4-chlorophenol. In this research, one of the most important factors affecting 4-chlorophenol degradation by mixed microbial culture including glucose concentration with 2 and 5 g/l was investigated. After examination, the microbial species suitable for TY_1 and TY_2 were able to completely remove 100 ppm of 4-chlorophenol, so that the TY_1 strain was removed completely after 45 h and the TY_2 strain after 21 h. Using a mixture of TY_1 and TY_2 strains (50/50) in the presence of 2 g/l of glucose, 100 ppm of 4-chlorophenol were completely removed after 18 h. Based on the 18SrRNA gene sequence analysis, the molecular identification of the two superior strains was carried out, both belonging to the genus *Trichosporon*. Considering the high potential of *Trichosporon* species, including their potential applications in increasing oil recovery and eliminating pollutants, it would be hoped that the further exploration of strains characteristics and the search for new native strains could play a crucial role in the application of native strains in the oil industry.

Keywords: Chlorophenol wastewater treatment, Shahid Tondgooyan, *Trichosporon*, Eliminating pollutants

INTRODUCTION

With the development of societies and the creation of different technologies and industries, despite the diminution of many human problems, several other problems have been added. One of the most important issues that have arisen with the emergence of new industries is the loss of environmental resources and pollution of the environment, including water and soil, by various chemical pollutants from the output of various industries. One of the most important human goals in recent decades was protecting the environment against the growing trend of pollution. Water resources are some of the most important biological resources and protecting and cleaning them from pollutants are among the most important tasks. Phenol and phenolic derivatives are considered among the most significant pollutants in the environment by the Environmental Protection Agency [1]. These compounds are used in several industrial processes for the production of chemicals such as pesticides, explosives, drugs, textiles, paints and resins. Phenolic compounds are produced not only from human activities, but also naturally by the decomposition of leaves and wood. As a result, these materials are found in soils and sediments, and often lead to contamination of groundwater and wastewater [2, 3].

Due to the high toxicity of these compounds and the pathogenic properties of some of them, various methods have been used to remove and analyze these toxic compounds, among which biodegradation methods are some of the most effective and easy methods for the removal and decomposition of this type of pollutants. In these methods, microorganisms are used as a decomposing agent of toxic compounds.

The origin of environmental pollution with chlorophenols

Chlorophenols are abundant in urban sewage, industrial outlets and waterways, which creates serious health risks for humans due to high toxicity.

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Meanwhile, due to their relatively high solubility in water, these compounds easily move in different water environments and pollute groundwater [3, 4]. Because of the stability and resistance of chlorophenols, these materials are not only found in surface and underground waters, but also in soils and sediments [4]. Also, the stability of these compounds in wastewater treatment systems and their mixtures with other chemicals that can be consumed by microorganisms also disrupt the decomposition and removal of these chemicals [5, 6].

The wastewater from these activities with varying concentrations of chlorophenols inhibits the microorganisms that reduce the chemical oxygen demand (COD) and also prevent the use of nitrogen and phosphorus [5].

Conventional methods for the removal of chlorophenols from the environment

There are various methods for the removal and decomposition of chlorophenols, which can be referred to as physical, chemical and biological methods. There are other methods, such as burning and incineration and landfilling, which are the oldest methods for removing pollutants. Different absorption methods, washing with gas and liquid phase, solvent extraction in solid and liquid phase, chemical oxidation, electrochemical methods, catalytic methods using photocatalytic and sonochemical degradation and enzymatic methods are some physical and chemical methods for removal of chlorophenols in the solid phase like soils and sediments, and in the liquid phase, such as water and wastewater. These methods are subject to serious limitations. High costs, hazardous and harmful by-products, disposal of chemical reagents and solvents in the environment and recontamination of environment are among the limitations of these methods [6].

Biodegradation of chlorophenols and effective microorganisms

Today, the biodegradation of aromatic compounds by microorganisms is of particular interest. Many microbial species, including bacteria and fungi, can remove chlorophenols as the only carbon source. Due to the use of decomposing microorganisms in chlorophenols biodegradation, the microbial cultures are classified into two main groups, which are discussed below.

Pure microbial cultures

There are many microorganisms, including bacteria and fungi that can remove chlorophenols. In Table 1, a list of aerobic chlorophenol biodegrading microorganisms is presented as the only carbon source [7, 8].

Mixed microbial cultures

Mixed microbial culture is a group of different species of microorganisms that act as a population together. Examples of microbial collections are found in active sludge ponds; biofilms such as those found in trickling filters and also in various soil ecosystems. Mixed cultures have been used in fermentation processes, such as food fermentation and alcohol production, production of yogurt and cheese, as well as in wastewater and contaminated water treatment from very old times.

In microbial collections, organisms work together in a complex system, all of which utilize each other's activities in the population. For example, microbial collections are more efficient in complex organic waste degradation than single species or even mixtures mixed with microorganisms and with a greater variety of metabolic capabilities [9, 10].

MATERIALS AND METHODS

General stages of the project

- Sampling
- Stages of phenol degrading microorganism culture
- Isolation of molds and yeast phenol decomposing fungi
- Measuring chlorophenol removal
- Determining the identity of selected fungi
- Molecular identification of superior strains

Nutrient and mineral culture media

The culture media used in this study can be used for the isolation of fungi from bacteria which are sabouraud dextrose agar (SDA), sabouraud dextrose agar with chloramphenicol (SC) and potato dextrose agar (PDA). In the next step, for the final purification of yeast from mold, specific culture media, such as yeast peptone agar (YPG) and malt agar extract (YM) were used.

Mineral salt medium (MSM) and trace elements used in enrichment and biodegradation of pollutants are shown in Tables 2 and 3 [12]. 4-Clorophenol of 98% purity, used in experiments, was purchased from Merck Group in Germany. Table 4 presents the 4-chlorophenol specifications. Due to the growth of microorganisms in 4-chlorophenol mineral environments, glucose was used as a substrate for growth supplement or primary substrate in the enrichment and recovery phases of the wastewater. In cases where glucose supplement substrate was added to the environment, its concentration was considered 2 and 5 g/l [13].

Table 1. List of chlorophenols and their degrading microorganisms [11]

Microorganisms	Chlorophenols
Desulfovibrio dechloracetivorans (ATCC700921), *Alcaligenes sp.*, *Ralstonia sp.*, *Azotobacter sp.*, *P. putida*, *Cystobacteri sp.*, *P.cepacia*	2-Chlorophenol
Desulfomonile tiedjei	3-Chlorophenol
P. putida, *Comamonas testosteroni JH5*, *P.cepacia*, *Rulstonie eutropha*, *Alcaligenes sp.*, *Azotobacter sp.*, *Ralstonia sp.*, *Candida tropicalis*, *Fusarium flocciferium* *Penicillium*, *Aspergillus*, *Graphium*, *Phanerochaete*, *Fusarium sp.*	4-Chlorophenol
Desulfitobacterium dehalogenans, *Desulformonile tiediei*, *Ralstonia sp.*, *Clostridium sp.* *Burkholderia cepacia*, *P. pickettii* (DTP0606).	2,4 Dichlorophenol
Desulfomonile tiedjei, *Desulfovibrio dechloracetivorans*	2,5 Dichlorophenol
Desulfitobacterium dehalogenans(JW/IU-DC1), *Mycobacterium chlophenolicum*, *P.cepacia* *Azotobacter sp.*, *P.pickettii* (DTP0606), *Desulforibrio dechloracetivorans*, *Ralstonia sp.*	2,6 Dichlorophenol

Microbial cultures

Since the main objective of the study was the elimination of 4-chlorophenol by native microorganisms, the wastewater treatment unit was used as a source of microbial isolation. The wastewater and sludge used in these experiments were prepared from different parts of the Shahid Tondgooyan Wastewater Treatment Plant (Fig. 1)

Pre-treatment of microbial source and selection of microorganisms

Due to the increased ability to remove pollutants by microorganisms in wastewater and sludge, a series of 4-chlorophenol pollutant adaptation and enrichment was performed on it as follows.

Adaptation of sludge and wastewater of Shahid Tondgooyan Petrochemical Plant

Since the microorganisms of the wastewater at first had a negligible growth, the adaptation operation was done in presence of auxiliary substrates on the sludge and wastewater to enhance the ability to remove and then isolate the microbial species. Aeration was done each time to work with the wastewater and to activate its microorganisms [14, 15].

Fig. 1. Sampling location

Table 2. Specifications of mineral salt media used in the experiments [16]

Mineral salt	NH_4NO_3	$MgSO_4.7H_2O$	$CaCl_2.2H_2O$	$KHPO_4$	KH_2PO_4
Salt concentration (g/L)	0.5	0.2	0.02	0.5	0.5

Table 3. Specifications of trace elements used in the experiments [16]

Trace elements	$MnSO_4.H_2O$	$FeSO_4.7H_2O$	$CuSO_4.5H_2O$	$ZnSO_4.7H_2O$	$NaMoO_4$	$CoCl_2.7H_2O$
Salt concentration (g/L)	0.025	0.15	0.025	0.02	0.034	0.053

Table 4. Specifications of 4-chlorophenol used in the experiments

Molecular formula	Molecular mass (g/mol)	Density (g/cm^3)	Melting point (°C)	Boiling point (°C)	% Purity
C_6H_5OCl	128.56	306.1	45-43	220	98

To initiate the experiments, salt culture medium and trace elements salt solution was used. To strengthen the wastewater and sludge samples and the necessity of existence of a stimulus substrate for

the growth of microorganisms along with toxic contamination of 4-chlorophenol, 2 and 5 g/l glucose were also used.

Adaptation was performed in 5 stages for 60 days. In each stage, 10% of the previous culture was transferred to the fresh mineral culture medium containing pollutants [16]. Finally, the microorganism was separated from the final habituation stage. The pollutant concentration used at each stage of the various habituation operations varied from low to high and finally was 100 ppm.

Isolation and purification of microorganisms

After performing the habituation and enrichment on sludge and effluent and ensuring the removal of 4-chlorophenol by it at the applied concentration range, purification of microbial culture was done by culturing the microorganisms obtained from the last enrichment step on plates containing PDA and using streaking technique.

Isolation of phenol-decomposing mold and yeast fungi

After enhancement of microbial strains, sabouraud dextrose agar (SDA), sabouraud dextrose agar with chloramphenicol (SC) and potato dextrose agar (PDA) were used to isolate fungi from bacteria. In the next step, the yeast strains from mold from specific culture media, such as yeast peptone agar (YPG), malt agar extract (YM) were used for the final purification. Then, each of the pure colonies was examined macroscopically and microscopically.

4-Chlorophenol biodegradation using isolated microorganisms

To perform 4-chlorophenol biodegradation tests, all decomposition tests were first performed on pure microbial species and then, after obtaining the desired results, the main experiments were carried out on a mixed microbial culture. All experiments in this section were carried out under operating conditions of 30 °C, 200 rpm in 100 ml Erlenmeyer flasks in replicate [17].

Biodegradation using single microbial species

After purification and separation of the species obtained from the habituation and the enrichment of wastewater and sludge, biodegradation was first done on single species.

The 4-chlorophenol biodegradation experiments were performed using microorganisms isolated from the enrichment phase in the presence of 100 ppm of 4-chlorophenol. Also, the effects of glucose concentration as growth supplement substrate were investigated at 2 and 5 g/l on 4-chlorophenol biodegradation [17, 18].

4-Chlorophenol biodegradation using mixed microbial culture

At this stage, a mixture of isolated microorganisms from the enrichment phases on sludge and wastewater was used as a mixed microbial culture, which is described below.

In this part of the experiments, a mixture of isolated microorganisms was used as a mixed microbial culture. The experiments were conducted as follows: after ensuring the 4-chlorophenol decomposition by isolated species from the enrichment stages, subsequent experiments were carried out using a mixed culture of isolated microbial species. All experiments were carried out at a concentration of 100 ppm of 4-chlorophenol and 2g/l of glucose [18-20].

Molecular identification of superior strains

A. Extracting genomic DNA from isolated strains: Direct extraction of DNA of isolated strains was carried out using a phenol-chloroform method from a solid and liquid medium.

B. Quantitative analysis of DNA: A quantitative analysis is performed on each sample using a biophotometer. Each sample was studied at a dilution of 1:50 in water and in the absorption of 230/260, 280/260.

C. Identification of fungal strains by direct PCR method based on the sequence of ITS area.

D. Universal primers: Most fungi were identified using the ITS area. In this study, universal strains primers (ITS1 and ITS4) were used to identify the selected strains [21, 22].

Microbial source pre-treatment experiments

After performing a series of adaptation experiments (5 stages for 60 days) and enrichment (5 stages for 15 days), on the sludge and effluent, the selected microorganisms were purified and isolated for 4-chlorophenol biodegradation.

RESULTS

Isolation of microorganisms

In this study, sabouraud dextrose agar (SDA), sabouraud dextrose agar with chloramphenicol (SC) and potato dextrose agar (PDA) were used to isolate the fungi from bacteria. In the next step, for the final purification of yeast strains from mold, specific culture media such as yeast peptone agar (YPG) and malt agar extract (YM) were used.

After adaptation and enrichment of the collected samples and purification of strains, a total of 19

bacterial species (13 strains of bacteria and 6 strains of yeast and mold) were isolated and purified, which were morphologically examined (Table 5). Fig. 2 shows the macroscopic forms (observation) and Fig.3 shows the microscopic forms of the isolated strains.

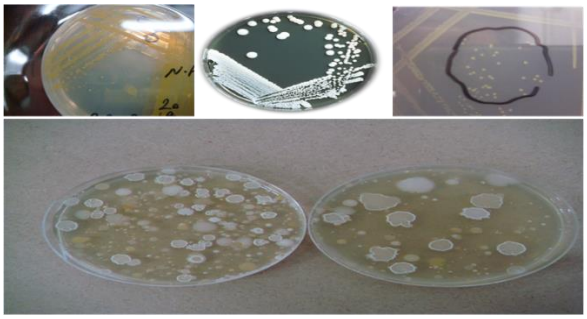

Fig. 2. Macroscopic observations of the isolated microorganisms.

Finally, 2 microbial strains in screening showed the best results in 4-chlorophenol biodegradation. Using microscopic and macroscopic observations and biochemical tests, it was shown that TY_1 and TY_2 strains isolated from the reversible wastewaters are a yeast species.

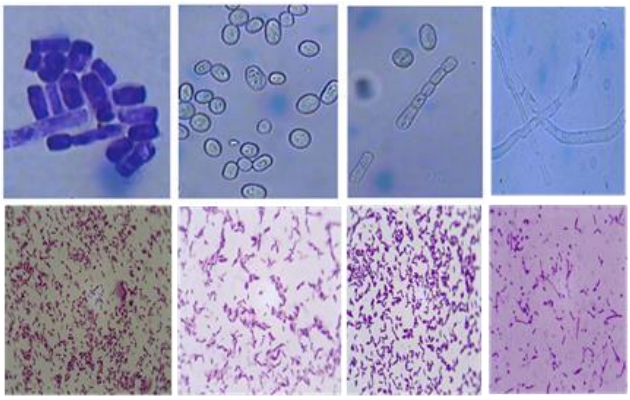

Fig. 3. Microscopic observations of isolated microorganisms

4-Chlorophenol degradation using TY_1 isolated microorganism

After examining the apparent form of the TY_1 isolated microorganism, the microorganism growth curve was examined and drawn up. In Fig. 4 the cellular growth curve of the TY_1 isolated microorganism is shown.

After examining the growth conditions and selecting the end of the logarithmic phase of growth as an appropriate age for microbial inoculation, the 4-chlorophenol 100 ppm decomposition was performed by TY_1 microbial species. In Fig. 5 the 4-chlorophenol biodegradation process is shown in terms of time by TY_1 isolated microorganism. The TY_1 microorganism is capable of completely degrading 100 ppm of 4-chlorophenol in about 45 h.

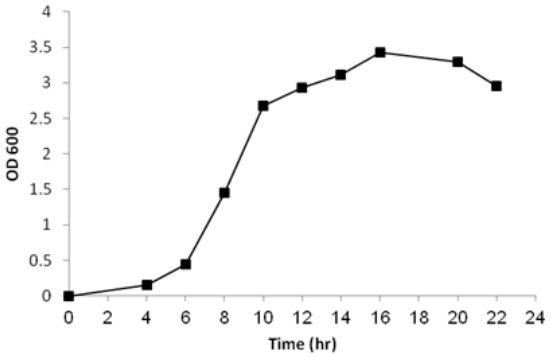

Fig. 4. TY_1 isolated microorganism growth curve

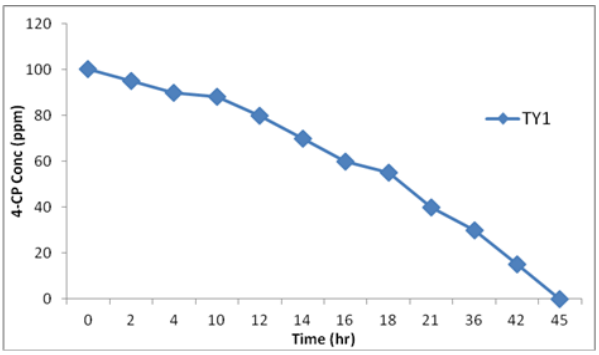

Fig. 5. Changes in 4-chlorophenol concentration with time by TY_1 isolated microorganism.

4-Chlorophenol degradation using TY_2 isolated microorganism

After examining the appearance of the TY_2 species, the microorganism growth curve was examined and drawn up. Fig. 6 shows the TY_2 isolated microorganism growth curve. After examining the growth conditions and selecting the end of the growth logarithmic phase as an appropriate age for microbial inoculant, the 100 ppm 4-chlorophenol degradation was performed by TY_2 microbial species. Fig. 7 shows the 4-chlorophenol biodegradation process in TY_2 isolated microorganism as a function of time. The TY_2 microorganism is capable of completely degrading 100 ppm of 4-chlorophenol in about 21 h. This microbial species is a superior microorganism among microorganisms isolated from wastewater, which is able to remove 4-chlorophenol in the shortest time compared with other isolated microbial species.

Table 5. Microscopic form and isolation site of isolated strains

Isolation site	Microscope form	Sample
Storage tank (interstitial water)	Gram negative bacilli	TB_1
Location of oil drainage of reservoirs to return to the refinery system	Gram positive bacilli	TB_2
Oil storage tank	Gram positive bacilli	TB_3
Oil storage tank	Gram positive coco-bacillus	TB_4
Storage tank	Gram positive bacilli	TB_5
Accumulated oil sludge	Gram positive coco-bacillus	TB_6
Accumulated oil sludge	Gram positive bacilli	TB_7
Total refinery effluent flow	Gram negative bacilli	TB_8
Total refinery effluent flow	Gram positive bacilli	TB_9
Total refinery effluent flow	Gram negative bacilli	TB_{10}
Total refinery effluent flow	Gram negative bacilli	TB_{11}
Total refinery effluent flow	Gram positive bacilli	TB_{12}
Total refinery effluent flow	Gram positive bacilli	TB_{13}
Total refinery effluent flow	Yeast	TY_1
Total refinery effluent flow	Yeast	TY_2
Total refinery effluent flow	Yeast	TY_3
Total refinery effluent flow	Mold	TY_4
Total refinery effluent flow	Mold	TY_5
Total refinery effluent flow	Yeast	TY_6

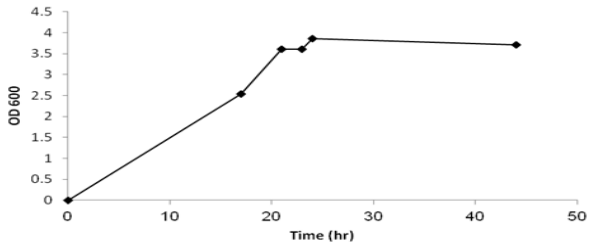

Fig. 6. Growth curve of TY_2 isolated microorganism

4-Chlorophenol degradation using TY_1TY_2 mixed microbial culture

After isolation of wastewater microorganisms, purification and performing removal tests, two microbial species TY_1TY_2 were available for examination of 4-chlorophenyl elimination experiments.

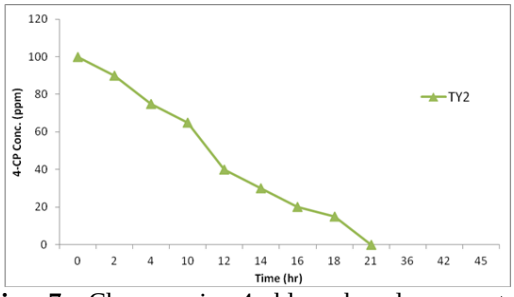

Fig. 7. Changes in 4-chlorophenol concentration *versus* time by isolated microorganism TY_2

Biodegradation experiments were done using mixed culture with two TY_1TY_2 microorganisms (50/50 TY_1TY_2) in the presence of 100 ppm of 4-chlorophenol and 2g/l of glucose as supplementary substrate. In these experiments, 4-chlorophenol degradation by the mixed culture and by the single species was compared. Fig. 8 shows the changes in the biomass growth of pure cultures and mixed microbial culture in the presence of 100 ppm of 4-chlorophenol and 2 g/l of glucose as supplementary substrate. The two TY_1TY_2 microbial species were able to completely degrade 100 ppm of 4-chlorophenol in approximately 18 h.

Fig. 9 shows the changes in the concentration of 4-chlorophenol during the process of degradation by single and mixed microbial culture.

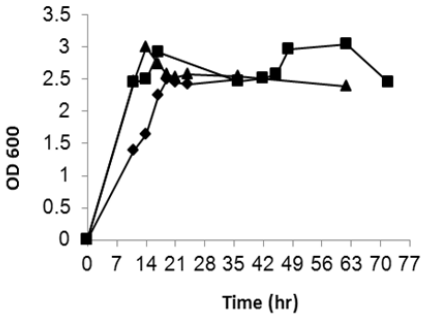

Fig. 8. Comparison of cell mass growth in the 4-chlorophenol degradation process using mixed and pure culture of two microbial species (TY_2:♦, TY_1:■, TY_1TY_2:▲)

Effect of carbon source concentration in pollutant degradation

Considering that the enrichment operation was done in presence of 2 g/l of glucose and the microbial species were isolated at this concentration, additional examination of the degradation was performed with glucose concentration of 5 g/l, because the high concentration of the primary substrate can be a deterrent to 4-chlorophenol degradation and one of the important factors in the removal of pollutants. The primary effect of glucose as the primary substrate is on the growth of existing microorganisms, so at higher concentrations, we will necessarily have further growth of the biomass. In Fig. 10, 4-chlorophenol degradation at two glucose concentrations of 2 and 5 g/l at different times is shown. It is concluded that the concentration of 2 g/l of glucose was sooner consumed and resulted in the elimination of 4-chlorophenol in a shorter time.

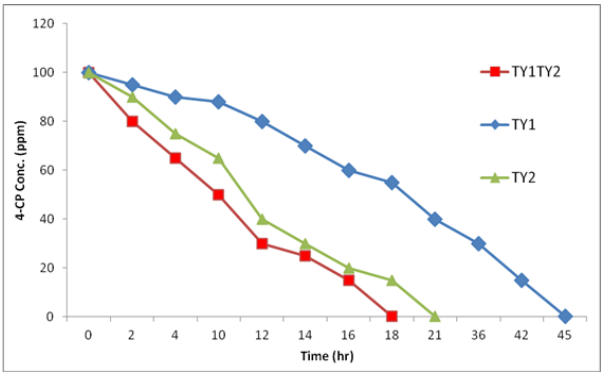

Fig. 9. Comparison of changes in 4-chlorophenol concentration in the 4-chlorophenol degradation process using mixed and pure culture of the two species TY_1 and TY_2 (TY_2:▲, TY_1:♦, TY_1TY_2:■)

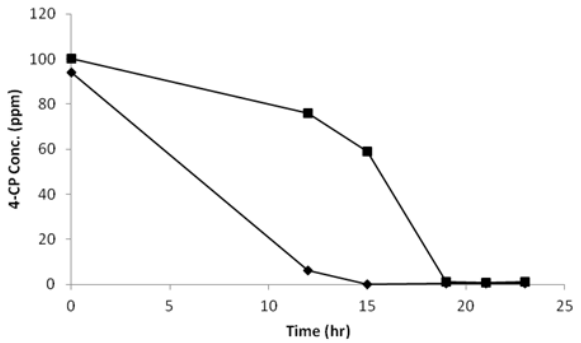

Fig. 10. 4-Chlorophenol changes over time in different concentrations of glucose by TY_1TY_2 (50:50) mixed culture in presence of 100 ppm of 4-chlorophenol (♦: 2 g/l glucose concentration, ■: 5 g/l of glucose concentration)

Molecular identification of selected strains

At first, 2 genomic DNA were extracted from selected yeasts (TY_1 and TY_2), which provided the best results in 4-chlorophenol biodegradation, and then part of their 18srRNA gene sequence was reproduced using universal primers and PCR method. In the next step, the PCR product, 2 selected strains, with a size of 1000-100, were taken on a 1% agarose gel and a specific strip of about 530 bp was observed from the 18SrRNA gene on the gel (Fig. 11).

Results of the molecular identification of selected strains

The next step was to determine the identity and molecular identification of isolated strains. Based on the sequences obtained, using the BLAST software on the NCBI website, they were compared with the NCBI reported sequences, and the closest microbial species was extracted from the NCBI gene data bank. The results show that strains TY_1 and TY_2 also exhibited the highest similarity (99%) to genus *Trichosporon.*

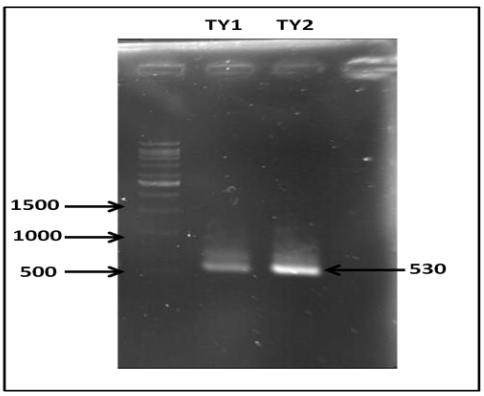

Fig. 11. Electrophoresis of PCR products of 18S rRNA gene of selected strains

DISCUSSION

Comparison of biodegradation performance by mixed culture and pure microbial culture

From the obtained results of the experiments, it seems that the mixed microbial culture acts as an isolated single species, TY_2, so that the elimination time in both TY_2 microbial culture and TY_1TY_2 mixed culture is approximately the same. The complete removal time of 100 ppm 4-chlorophenol by TY_2 strain was 21 h, and the complete removal time of 100 ppm 4-chlorophenol by mixed culture TY_1TY_2 in presence of 2 g/l glucose was about 18 h.

In a study by Puhakka *et al*. (1995), mixed culture performance composed of three isolated microbial species was the same as the function of each of them alone [23]. In another study by Sahinkaya and Dilek (2007), the data (specific rate of removal of 2,4-dichlorophenol) showed that the yield of pure cultures isolated from mixed

cultivation in 30 ppm of 2,4-dichlorophenol was much better than that of mixed habituated culture [24].

In any case, the results of mixed microbial culture should be examined on a more detailed scale. In Table 6, the effect of mixed culture and pure microorganisms in 4-chlorophenol degradation is compared.

CONCLUSIONS

In the present study, microorganisms capable of biodegradation of 4-chlorophenol were first isolated and purified using adaptation and enrichment operations on wastewater and sludge. Due to the increased ability of wastewater and sludge microorganisms in using 4-chlorophenol as the only source of energy and carbon in all experiments, 5 g/l of glucose was used as supplementary substrate. The enrichment and habituation operations lasted for about 60 days, and the microbial species obtained from the last stages of adaptation and enrichment operations were purified and isolated by doing several linear cultures. The results showed that the adaptation and enrichment of the microorganisms in the petrochemical sludge and wastewater of the Tondgooyan Unit was very effective in increasing 4-chlorophenol biodegradation.

After examining the growth process of all isolated species, by examining the elimination by them in the mineral microbial culture, isolated microbial species, suitable microorganisms for the 4-chlorophenol biodegradation were selected. In the following, 4-chlorophenol biodegradation tests were performed by selected microorganisms and a mixed culture, and the behavior of single and mixed microbial culture was compared. The results showed that the mixed microbial culture was not significantly different from pure microbial species, but the mixed culture would probably show this difference in the metabolic path of 4-chlorophenol degradation.

In the final stage of the experiments, the effect of the initial concentration of glucose with two concentrations of 2 and 5 g/l was considered as a supplementary substrate. The results indicated that with increasing 4-chlorophenol concentration, its degradation time would be longer, but higher glucose concentration, despite the higher growth of microorganisms in its presence, had an inhibitory effect on the microbial culture in 4-chlorophenol degradation.

Table 6. Comparison of biodegradation performance by mixed culture of the present study with other researchers

Scholar	Microbial culture	Concentration of 4-chlorophenol (ppm)	Substrate of growth supplement	Removal time (h)	Removal return (%)
Sahinkaya *et al*., 2005 [24]	Enriched mixed culture	300	Peptone	50	100
Yang *et al*., 2008 [13]	Isolated microorganism	100	Glucose or sodium acetate	96	100
Lee *et al*., 2007 [12]	Enriched mixed culture	50	Glucose	40	100
Wang *et al*., 1999 [25]	*P. putida*	200	Glucose	30	100
Lima *et al*., 2004 [26]	Algae collection	50	-	120	100
This research	Microbial mixed culture	100	Glucose (2g/l)	18	100
This research	Microbial mixed culture	100	Glucose (5g/l)	20	80

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ИЗСЛЕДВАНЕ НА БИОЛОГИЧНАТА ОБРАБОТКА НА 4-ХЛОРОФЕНОЛ С МАЯ И ПЛЕСЕН ОТ ИНДУСТРИАЛНИ И ПЕТРОЛНИ ОТПАДНИ ВОДИ (ПРИСТАНИЩЕ „ИМАМ ХОМЕЙНИ“ МАХШАХР)

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(Резюме)

Фенолните производни са едни от най-токсичните замърсители на околната среда, а хлорофенолите са най.токсичните замърсители във води и отпадни води. 4-Хлорофенолът е много добре разтворим във вода и поради това е широко разпространен в природни и отпадни води. Тъй-като химичните и биологичните методи за очистване на водите са с висока стойност и енергоемкост, а в някои случаи са вредни за околната среда, в настоящата работа е изследвано биохимичното разлагане на 4-хлорфенол. 13 бактериални щама и 6 щама от мая и плесен са пречистени и изолирани от завода за обработка на отпадна вода „Шахид Тондгоян“ (пристанище „Имам Хомейни“ Махшахр) в продължение на 15 дни. Изучени са свойствата на всеки микроорганизъм, изолиран в присъствие на 100 ppm 4-хлорофенол и два микробиални вида, TY_1 и TY_2, са избрани за използване като смесена микробиална култура. Изучено е влиянието на един от най-важните фактори върху разлагането на 4-хлорофенола със смесена микробиална култура - концентрацията на глюкоза (2 и 5 g/l). Установено е, че микробиалните видове TY_1 и TY_2, отстраняват напълно 100 ppm 4-хлорофенол, като щамът TY_1 отстранява напълно след 45 h, а щамът TY_2 след 21 h. С използване на смес от TY_1 и TY_2 щамове (50/50) в присъствие на 2 g/l глюкоза, 100 ppm 4-хлорофенол се отстранява напълно за 18 h. На основата на анализа на 18SrRNA генна секвенция е проведена молекулна идентификация на двата най-добри щама, които принадлежат към вида *Trichosporon*. Имайки пред вид високия потенциал на вида *Trichosporon*, включително за повишаване на добива на петрол и отстраняване на замърсителите се очаква, че по-нататъшните изследвания на подходящи щамове и търсенето на нови природни щамове може да играе решаваща роля за прилагането на природни щамове в петролната индустрия.