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Enhanced Methyl *Tert*-Butyl Ether Removal by Mixed Consortium: Performance and Adaptability

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Abstract: Methyl *tert*-butyl ether (MTBE) is widely used as a gasoline additive with toxicity and carcinogenicity, and has caused environmental pollution worldwide. Biodegradation is a promising method for the removal of MTBE from contaminated sites. In this paper, three strains with high adaptability and different degrading characteristics to MTBE were cultured. The kinetic models were established to systematically simulate the biodegradation of MTBE by various strains at different concentrations. Moreover, the removal of MTBE in a synergistic system containing a mixture of three pure strains was studied. The mixed consortium enhanced MTBE removal at high concentrations (30–50 mg/L), and the degradation efficiency was increased by about 20% compared to pure strains at a concentration of 30 mg/L. Further, the mixed consortium degraded MTBE nearly three times faster than any of the individual, indicating that the co-cultures of three pure cultures improved both efficiency and the rate of MTBE biodegradation. In addition, it was found that the mixed consortium effectively removed MTBE in the presence of other gasoline components, and exhibited stronger adaptability, especially at low or high temperatures. This study supports the cultivation of a mixed consortium to remediate MTBE-contaminated environments, either as a single substrate or in environments containing other gasoline components.

Keywords: biodegradation; co-contamination; methyl *tert*-butyl ether; microbial mixed culture

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1. Introduction

Methyl *tert*-butyl ether (MTBE) has been commonly used as an oxygenated additive to increase the octane number of gasoline [1]; the global MTBE market is expected to reach $22.9 billion by 2026 [2]. It is the second most frequently detected volatile organic compound in shallow groundwater due to the accidental leakage of gasoline [3]. Moreover, it has a high solubility in water and is hardly adsorbed by soil particles [4,5]. The persistent contamination caused by MTBE is responsible for the irritation to skin and eyes. Further, it has been reported to be genotoxic, and has the possibility to depress the nervous system [3,6]. As a result, the remediation of MTBE contamination is required.

Various techniques including adsorption, air stripping, advanced oxidation, and biological processes have been applied for MTBE treatment. However, there are some drawbacks in chemical and physical methods for MTBE removal, such as spent adsorbent disposal [3]. The low Henry's law constant of MTBE limits the efficiency and increases the cost of air stripping [7]. Moreover, the formation of by-products during MTBE decomposition is a major disadvantage in oxidation process since more toxic compounds are formed [3]. As a result of these challenges, the biological process is considered as a promising technology due to its low cost, environmental friendliness, no secondary pollution, and ease of operation [8]. MTBE can be broken down into carbon dioxide and water as well as harmless metabolites through aerobic biodegradation [8,9]. There are some pure cultures that can degrade MTBE through direct metabolism, such as *Methylibium petroleiphilum* PM1 [10], *Aquincola tertiaricarbonis* L108 [11], *Hydrogenophaga flava* ENV735 [12], *Mycobacterium austroafricanum* IFP2012 [13], *Rhodococcus* sp. EH831 [14], *Enterobacter* sp. NKNU02 [15], and

Achromobacter xylosoxidans MCM1/1 [16]. Among them, NKNU02 isolated from gasoline-contaminated water could grow with MTBE as the sole carbon and energy source, reaching a removal efficiency of 60% at 100 mg/L [15]. At the same concentration, higher removal was obtained by MCM1/1, which biodegraded up to 78% MTBE in a mineral medium in 5 days [16]. Although pure cultures have been shown to degrade MTBE, the degradation rate and concentration range are limited.

At present, the application of the mixed consortium for pollutant degradation has attracted increasing attention because of its advantages over pure cultures, such as metabolic diversity, stronger adaptability, and higher decomposition efficiency [17,18]. The removal of pollutants is enhanced by the synergism between different strains in the mixture [19]. Most studies have focused on the screening and domestication of natural mixed bacteria existing in the environment, but selective mixing of different pure cultures has rarely been studied. On the basis of determining the characteristics of several bacteria, the composition of the mixture can be designed, and the proportion of strains can be controlled. That is, the simultaneous cultivation of different strains facilitates the investigation of biodegradation mechanism. Ghorbannezhad et al. [17] screened four strains from an aged oil-contaminated area and found that a mixture of four bacteria raised the removal efficiency of crude oil by about 11% compared to the best pure isolates. Two strains of *Acinetobacter baumannii* and *Talaromyces* sp. were isolated from oil-contaminated soil and sludge and combined in a ratio of 1:1, and the obtained co-cultures displayed a higher degrading ability to n-alkanes and excellent alkali resistance [20]. In this way, a new idea for MTBE bioremediation was developed.

Furthermore, studies on the impact of co-contaminants on MTBE biodegradation are limited. Considering the composition of gasoline, it is speculated that alkanes, BTEX, and heavy metals may co-exist in MTBE-contaminated soil or water. Iturbe et al. [21] simultaneously detected MTBE, total petroleum hydrocarbons, BTEX (benzene, toluene, ethylbenzene, and p-xylene) as well as metals including Zn, Cr, Pb, and Fe in soil near an out-of-service oil distribution and storage station in Mexico. The bioremediation of sites contaminated with multiple constituents is a complex process due to different interactions such as co-metabolism, inhibition, cross induction, and non-interaction affecting the removal of target contaminants [22]. Therefore, a better understanding of the contamination of MTBE and mixtures is required to successfully establish bioremediation schemes.

The objective of this research was to evaluate the ability of pure strains and mixed consortium to degrade MTBE, followed by establishment of kinetic models to understand the synergism of a mixed consortium. Furthermore, the interactions between MTBE and other gasoline components in biodegradation were studied. The effect of different concentrations of metals in gasoline-contaminated environments on MTBE biodegradation were investigated. In addition, the influences of pH and temperature were tested, and the tolerance of a mixed consortium to various conditions was compared. Finally, the development of MTBE bioremediation was prospected.

2. Materials and Methods

2.1. Chemical Materials

Methyl *tert*-butyl ether (purity, 99%), benzene (purity, 99%), toluene (purity, 99.5%), dodecane (purity, 99.5%), and other chemical reagents were purchased from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China). The metals tested included iron, zinc, copper, lead, cadmium, and manganese. They were added to the mineral salt medium in the form of soluble salts: $ZnCl_2$, $CuCl_2$, Pb $(NO_3)_2$, $CdCl_2$, and $MnCl_2$. The biochemical reagent used was purchased from Sangon Biotech (Shanghai, China).

2.2. Microorganisms and Media

Three MTBE-degrading strains (*Stenotrophomonas* L1, *Pseudoxanthomonas* M3, and *Sphingobacterium* M10) used in this study were isolated and activated from the culture preserved in a −80 °C refrigerator in the laboratory [23]. The mineral salt medium (MSM)

used for microorganism growth and MTBE degradation in this study was contained as follows (g/L): KH_2PO_4 0.7, K_2HPO_4 0.85, $(NH_4)_2SO_4$ 1.23, $CaCl_2 \cdot H_2O$ 0.03, $FeSO_4 \cdot 7H_2O$ 0.001, and 5 mL trace element solution containing the following trace elements (mg/L): H_3BO_3 60, $CoCl_2 \cdot 6H_2O$ 40, $ZnSO_4 \cdot 7H_2O$ 20, $MnCl_2 \cdot 4H_2O$ 6, $NaMoO_4 \cdot 2H_2O$ 6, $NiCl_2 \cdot 6H_2O$ 4, and $CuCl_2 \cdot 2H_2O$ 2. The membrane-filter sterilized solution of each of the metal salts was prepared by dissolving the metal with a calculated concentration and added to sterile MSM medium. Different concentrations of MTBE were added into MSM as a carbon and energy source. The Luria-Bertani (LB) formulation included the following components (g/L): Tryptone 10, yeast extract 5, and NaCl 10. The pH value of mediums was adjusted to 7.0 by adding either the NaOH or HCl solution. All apparatus and medium were autoclaved at 121 °C for 20 min in advance.

2.3. Microbial Cultivation

The continuous degradation experiments were performed as reported previously [24]. The strains were inoculated into LB broth overnight at 25 °C. After washing, 1 mL of culture was then inoculated into 20 mL of MSM liquid medium in 100 mL serum bottles sealed with Teflon Mininet valves. Three pure isolates were cultured separately and then mixed to avoid any competitiveness that may occur during growth. One millilitre of mixed culture was prepared by mixing three pure cultures in the ratio of 1:1:1. Initial cell suspension was standardized to an optical density at 595 nm (OD_{595}) of 0.13. The bottles were incubated at 25 °C, shaken at 160 rpm for 72 h. The subculturing was performed by increasing the MTBE concentration from 5 to 50 mg/L. If needed, BTEX or dodecane was also added. The variation of strain growth and substrate concentration with each tube was monitored periodically. The cultures were harvested by centrifugation at 6000 rpm for 15 min in a Sorvall™ ST 40 Centrifuge (ThermoFisher Scientific, Cambridge, MA, USA).

2.4. Analytical Methods

The cell growth was measured spectrophotometrically by monitoring the optical density of the culture at 595 nm (OD_{595}) in an iMark Microplate Reader (BioRad, Hercules, CA, USA). The pH value was detected by digital pH meter (Jingke Instrument Co., Ltd., Shanghai, China).

The MTBE concentration was determined by headspace Gas Chromatography-Mass Spectrometer (GC-MS) analysis, as described by [24]. Briefly, after centrifugation at 6000 rpm for 15 min in a Sorvall ST 40 Centrifuge (Thermo-Fisher Scientific, Waltham MA, USA), the supernatant was filtrated through 0.22 μm membrane filters. One millilitre of filtered supernatant was added into a 2 mL headspace bottle and then injected by 50 μL microinjector. The concentration of MTBE was measured by gas chromatograph equipped with a HP-5MS capillary column (30 m length, 0.25 mm ID, 0.25 μm film) and a TraceISQ (ThermoFinnigan, Austin, TX, USA) mass spectrometric detector. The injection port was set in splitless mode, and the temperatures of the injector and ion source were 200 °C and 230 °C, respectively. The oven temperature program was held at 40 °C and incrementally increased to 70 °C at 10 °C/min. The flow rate of carrier gas (Helium 5.0) was 1.0 mL/min, and the mass spectrometry was operated in electron impact mode at 70 eV in selected ion monitoring (SIM) mode at73 m/z for MTBE. The formula 1 was used to calculate MTBE degradation efficiency:

$$Q = \frac{S_0 - S}{S_0} \times 100\% \quad (1)$$

where Q represents the degradation efficiency (%); S represents the residual MTBE concentration (mg/L); and S_0 represents the initial concentration (mg/L).

The computer-based statistical analyses were performed with SPSSAU software, and the analysis of variance (ANOVA) was used to estimate the differences between treatments at a significance level of 0.01 or 0.05.

2.5. Kinetic Analysis

The MTBE biodegradation was quantitatively described by four kinetic models. The first-order model can be determined by the following equation [25]:

$$S = S_0\, e^{-kt} \tag{2}$$

where S represents the MTBE concentration (mg/L); S_0 represents the initial concentration (mg/L); k is the degradation rate constant (mg/(L·h)); and t is the time (h).

In 1999, the Quiroga-Sales-Romero model was proposed based on the matrix consumption process [26]. The model can be used to simulate the kinetic behaviour of MTBE degradation, expressed as:

$$S = \frac{h(S_0 - q) - q(S_0 - h)e^{(\mu_{max} t)}}{(S_0 - q) - (S_0 - h)e^{(\mu_{max} t)}} \tag{3}$$

where S represents the MTBE concentration (mg/L); S_0 represents the initial concentration (mg/L); q represents the residual minimum concentration that cannot be degraded by microorganisms (mg/L); h represents the maximum substrate concentration that can be degraded by microorganisms (mg/L); t is the time (h); and μ_{max} is the maximum growth rate of microorganisms (h^{-1}).

The other two common models for the biodegradation of a single substrate, the Logistic model and Boltzmann model [25], are shown as:

$$y = A_2 + \frac{A_1 - A_2}{1 + \left(\frac{x}{x_0}\right)^p} \tag{4}$$

where A_1 represents the initial concentration (mg/L); A_2 represents the yield concentration (mg/L); x is the time (h); p is the power coefficient; and x_0 is the half-degradation time (h).

$$y = A_2 + \frac{A_1 - A_2}{1 + e^{(x - x_0)/dx}} \tag{5}$$

where A_1 represents the initial concentration (mg/L); A_2 represents the yield concentration (mg/L); x is the time (h); x_0 is the half-degradation time (h); and dx is the time constant.

The kinetic parameters in Equations (2)–(5) were determined numerically by fitting the experimental biodegradation data to the solution of the corresponding equation. By determining which of three models provide the most accurate fit to experimental data, the characteristic of MTBE degradation at different concentrations can be further determined. All of the calculations were performed by fitting the data with Origin v2018 software (OriginLab, Northampton, MA, USA).

3. Result and Discussion

3.1. The Degradation of MTBE by Three Pure Strains

Three MTBE-degrading strains were domesticated by gradually increasing the initial concentration of MTBE from 5 to 50 mg/L in the culture with MTBE as the sole carbon source. The biomass of three strains at different MTBE concentrations is shown in Figure 1a. It was found that a higher biomass was obtained in the culture with MTBE concentrations of 10–30 mg/L. The growth of strains was stimulated at MTBE concentrations of 5–20 mg/L, while was suppressed at more than 40 mg/L, suggesting that MTBE at concentrations higher than 40 mg/L was toxic to strains and inhibited the growth. In contrast, the M3 strain had a higher biomass at lower concentrations (5–10 mg/L), and the M10 strain preferred to grow in a medium supplemented with high concentrations (30–50 mg/L) of MTBE. Consequently, the concentration of MTBE had a significant effect on the biomass of strains ($p < 0.05$).

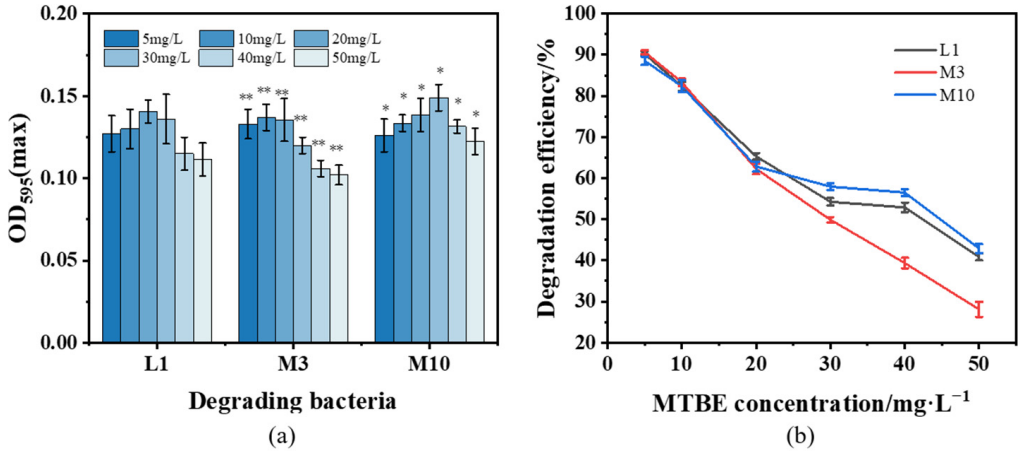

Figure 1. The growth and removal of MTBE with different concentration by three pure strains. The (**a**) biomass (OD_{595}) and (**b**) degradation efficiency of MTBE was determined after incubation for 72 h at 25 °C under shaking condition (160 rpm). The error bars represented standard deviation of three independent data. * represents $p < 0.05$; ** represents $p < 0.01$.

The degradation curves of three strains at different concentrations of MTBE are presented in Figure 1b. It can be shown that all three strains could degrade MTBE through direct metabolism when MTBE was added as the sole carbon source. At concentrations of 5–10 mg/L, the removal efficiency of MTBE was above 80% for all three strains, with the M3 strain being higher. However, the increase in MTBE concentration caused a significant decrease in MTBE removal, especially for the M3 strain. The degradation efficiency was reduced to 40–50% as the MTBE concentration further increased up to 40–50 mg/L. At concentrations of 30–50 mg/L, the M10 strain displayed a higher removal of MTBE, which could be attributed to the better growth of the M10 strain. ANOVA confirmed that there was a significant difference in MTBE degradation efficiency between the experimental groups with different MTBE concentrations ($p < 0.01$). Although all three strains could degrade MTBE, exceeding the threshold concentration for the strains' metabolism caused a negative impact on both strain growth and MTBE removal. Therefore, it is important to explore methods to improve the degrading ability of MTBE by strains.

3.2. The Mixture of Three Pure Cultures Enhanced the Removal of MTBE

After seven generations of continuous subcultivation and domestication, three strains were highly adaptable to MTBE, and showed different growth and degrading characteristic to different concentrations of MTBE. Hence, three strains were mixed and co-cultured to evaluate the degrading ability of the defined mixed consortium. Figure 2a shows the degradation efficiency of MTBE by three strains and their mixtures. Among them, the M3 strain adapted to the low concentration, yielding 1.646 mg/L of MTBE after 72 h of cultivation at an initial concentration of 10 mg/L. At low concentrations (5–10 mg/L), the *p*-value was greater than 0.05, indicating that the difference in MTBE removal efficiency between mixed and pure cultures was not significant. As the concentration of MTBE increased to 30 mg/L, the degradation efficiency by the mixed consortium was about 20% higher than that of any individual. However, the MTBE removal was negatively affected at a high dosage of MTBE at 50 mg/L, decreasing the removal efficiency to 30–40%. In comparison, the mixture of three pure cultures enhanced MTBE removal at 30 mg/L. At high concentrations (30–50 mg/L), the *p*-value was less than 0.01, suggesting that there was a significant difference in MTBE removal between the mixed and pure cultures.

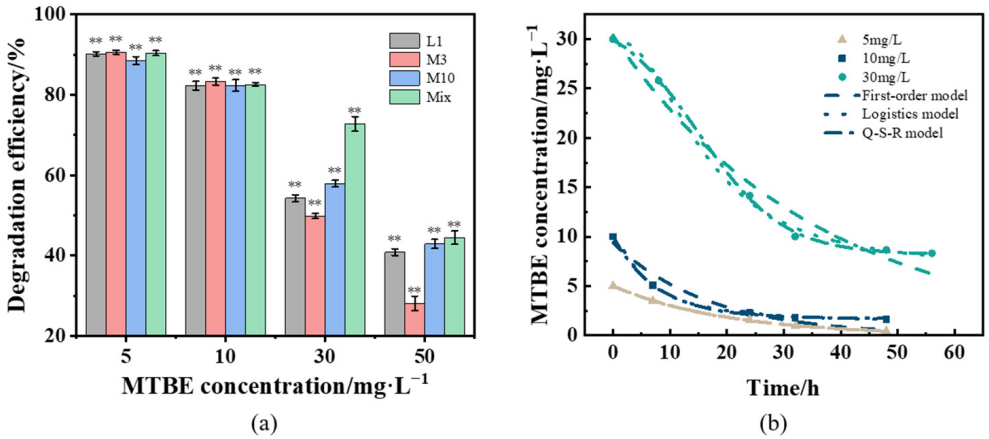

Figure 2. The comparison of the degrading capacity of pure strains and their mixture under different concentrations. (**a**) The efficiency of MTBE degradation by pure strains and their mixture. (**b**) Time course for MTBE degradation by mixed consortium and simulation results for three models. Symbols present the experimental data and curves show their statistic tendencies. ** represents $p < 0.01$.

Similarly, a study compared the removal of 500 mg/L pyrene and 1% w/v tetracosane by 15 oil-degrading microorganisms and their mixture and proposed that the mixed culture SMC had the best performance in hydrocarbons' removal, which promoted the degradation of pyrene and tetracosane by about 13% and 15%, respectively, compared with the best pure strain [27]. In a separate study, six strains with a high degrading ability of petroleum hydrocarbon were selected for the mixed consortium cultivation. Since metabolites produced by one strain could be further decomposed by another, a considerable promotion in degradation efficiency (1.6 to 1.85-fold) was attained by mixed consortium composed of all isolates [28]. While toxic metabolites disturbed biodegradation either by being adsorbed to the cell surface or by inducing mutations in catalytic enzymes, the mixed consortium could prevent the accumulation of toxic intermediates and increase the number of catabolic pathways available for pesticide biodegradation, thus showing an improvement in both microbial survival and removal efficiency [29]. In addition, Kurade et al. [30] detected that the activities of enzymes in consortium were highly induced, and the expression and accumulation of bioconversion enzymes may also be responsible for the higher efficiency of consortium. Studies have shown that while pure strains can metabolize compounds, the consortium can expand the extent of degradation owing to the varied enzymatic systems in different organisms [31]. To sum up, the cooperative activities in the mixed consortium enhanced the degradation efficiency of MTBE, especially at higher concentrations (30–50 mg/L).

In addition, the effect of the MTBE concentration on biodegradation can be encoded in terms of kinetic parameters obtained by fitting the data (Figure 2b). There was no apparent lack of fit, indicating that both the parameters and models could be adequately applied to describe the trends of MTBE degradation. For MTBE removal by mixed consortium, the data were fitted reasonably well by the Logistic model ($R^2 = 0.9997$) at a MTBE concentration of 10 mg/L, and the Quiroga-Sales-Romero model ($R^2 = 0.9993$) simulated the data more accurately as the concentration increased to 30 mg/L. One of the characteristics of MTBE removal at 5–10 mg/L by mixed consortium was the lack of lag phase; that is, there was no delay in MTBE degradation at the beginning of the run. It is speculated that a synergism was developed between the mixture, resulting in a faster adaption to MTBE. As the MTBE concentration increased from 5 to 30 mg/L, the rate constant (k) of the mixed consortium increased firstly from 0.05 to 0.06 mg/(L·h) and then reduced to 0.03 mg/(L·h) (Table 1). It implies that the supplement of a carbon source could promote MTBE degradation at 5 mg/L. However, the stimulation was reversed into suppression at higher MTBE concentrations (30 mg/L). Compared with three pure strains, summarized in Table 2, the degradation rate of the mixed consortium was significantly raised by 0.04 mg/(L·h) at 10 mg/L. It indicates

that although the efficiency of MTBE biodegradation by mixed consortium did not increase, the rate increased (at 10 mg/L). Traditionally, the time (x_0) required to reach half the degraded concentration is also applied as an indicator to estimate the degradation rate, and Equations (4) and (5) were utilized to determine x_0. Notably, the x_0 varied dramatically with MTBE concentrations, and the x_0 was less than 20 h at all conditions, indicating that mixed consortium exhibited a strong degrading ability of MTBE. Furthermore, compared with three pure strains, the x_0 of mixed consortium was shortened by about 20 h at 10 mg/L. Some bacteria in consortium can degrade metabolites formed by another to reduce the accumulation and toxicity of metabolites, thus increasing the degradation rate [32]. This is consistent with the result characterized with rate constant. Additionally, the maximum growth rate of strains (μ_{max}) was calculated by Equation (3), and the μ_{max} of three pure strains and mixed consortium was 0.04, 0.06, 0.04, and 0.13 h^{-1}, respectively (at 10 mg/L). This corroborates the conclusion mentioned above that low concentrations (5–10 mg/L) were more suitable for the growth of the M3 strain. Although mixing three pure cultures did not increase the biomass, it accelerated the growth of bacteria. The results can be supported by the view that more enzymes are induced to catalyse the biodegradation through the synergism between organisms in mixed consortium [33]. As a result, the mixture of three pure cultures enhanced MTBE biodegradation, not only promoting the growth of bacteria, but also improving the degradation rate of MTBE, especially at low concentration (10 mg/L).

Table 1. The kinetic parameters corresponding to the degradation curve by a mixed consortium at various concentrations.

Concentrations	5 mg/L	10 mg/L	30 mg/L
First-order model			
S_0 (mg/L)	5.00 ± 0.05	9.39 ± 0.92	30.37 ± 1.60
k (mg/L·h)	0.050 ± 0.001	0.059 ± 0.013	0.028 ± 0.003
R^2	0.9992	0.9391	0.9675
Quiroga-Sales-Romero model			
S_0 (mg/L)	4.99 ± 0.10	9.98 ± 0.21	29.94 ± 0.40
q (mg/L)	−0.06 ± 0.22	1.72 ± 0.56	8.20 ± 0.32
μ_{max} (h^{-1})	0.05 ± 0.01	0.13 ± 0.07	0.14 ± 0.01
R^2	0.9993	0.9991	0.9993
Logistic model			
A_1 (mg/L)	5.00 ± 0.12	10.00 ± 0.13	29.93 ± 0.77
x_0 (h)	19.08 ± 5.92	5.92 ± 0.34	16.22 ± 1.53
R^2	0.9991	0.9997	0.9973

Table 2. The kinetic parameters corresponding to the degradation curve by pure strains at 10 mg/L.

Type of Bacteria	L1	M3	M10
First-order model			
S_0 (mg/L)	10.24 ± 0.39	10.78 ± 0.48	10.41 ± 0.50
k (mg/L·h)	0.020 ± 0.002	0.021 ± 0.002	0.019 ± 0.002
R^2	0.9778	0.9716	0.9634
Quiroga-Sales-Romero model			
S_0 (mg/L)	9.69 ± 0.39	10.03 ± 0.24	9.75 ± 0.47
q (mg/L)	0.71 ± 1.10	1.15 ± 0.40	0.49 ± 1.68
μ_{max} (h^{-1})	0.04 ± 0.02	0.06 ± 0.01	0.04 ± 0.02
R^2	0.9897	0.9963	0.9838
Boltzmann model			
A_1(mg/L)	12.43 ± 3.38	11.24 ± 0.88	11.80 ± 3.15
x_0 (h)	26.38 ± 11.77	31.73 ± 3.19	33.82 ± 10.70
R^2	0.9897	0.9963	0.9838

3.3. The Mixture of Three Pure Cultures Effectively Removed MTBE in the Co-Occurrence of Other Gasoline Components

Currently, most researches have focused on the degradation of target pollutants, but the effect of coexistent pollutants are neglected. MTBE is always detected in the environment together with other gasoline components, such as alkanes, BTEX, and heavy metals [34]. Therefore, it is important to explore the interaction between MTBE and other organics or metals to stimulate the in situ bioremediation of MTBE.

The individual and simultaneous removal of MTBE with other gasoline organic components is shown in Figure 3a, and the highest degradation efficiency was achieved by adding MTBE alone. In binary mixtures with BTEX, the degradation efficiency of MTBE was 5–10% lower, with a greater negative impact on the M10 strain. Furthermore, dodecane was unfavourable to MTBE removal with a 35–50% reduction of degradation efficiency, and M3 strain was more susceptible to dodecane. The addition of both alkanes and BTEX led to a reduction in MTBE biodegradation, and the effect of each substrate on MTBE removal decreased in the order of dodecane > BTEX. The significant differences in degradation efficiency of the experimental groups containing different carbon sources were verified by ANOVA ($p < 0.01$). One of the possible reasons was that three strains used in this study could utilize dodecane and BTEX as the carbon source for growth (Figure S1). Compounds with a higher affinity for bacteria were found to compete with MTBE for the action of enzymes to reduce the MTBE removal [35]. Moreover, it can be speculated that strains preferentially metabolized aliphatic hydrocarbons rather than ethers, which is consistent with previous studies.

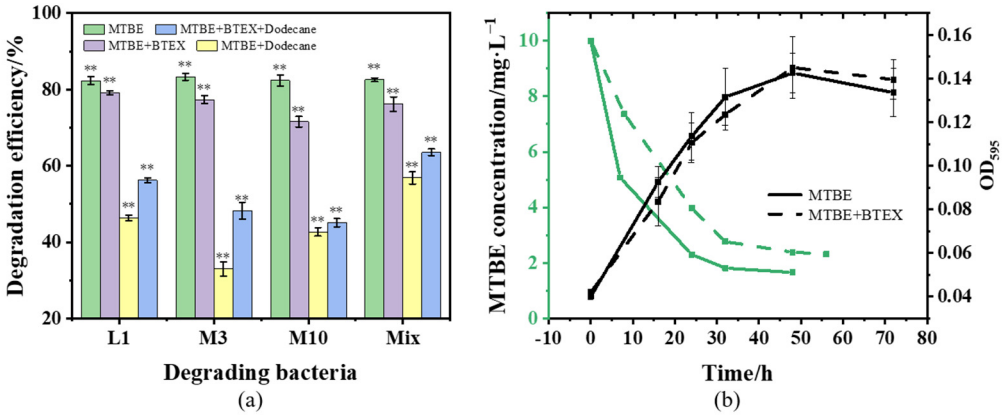

Figure 3. Effect of gasoline organic components on MTBE removal. (**a**) Biodegradation of MTBE in the presence of various gasoline organic components. (**b**) Time course for MTBE degradation and strain growth with or without the addition of BTEX. ** represents $p < 0.01$.

Since other studies have mainly focused on the effect of BTEX on MTBE removal, a system containing multiple substrates was proposed in this study. In ternary mixtures, the degradation efficiency of MTBE was lower than adding BTEX alone, but slightly higher than mixing with dodecane. This may be due to the synergistic effect provided by the mixing of alkanes and aromatics. It was reported that both hydroxylase genes related to the alkanes and dioxygenase genes related to the biodegradation of aromatic hydrocarbons were detected simultaneously in the mixture system [36]. What is more, the results illustrated that the degradation efficiency of MTBE was improved by about 10% with the mixed consortium compared with pure strains in the ternary system. In particular, the mixed consortium promoted MTBE removal in either binary or ternary mixtures containing dodecane. Some researchers support the cultivation of a mixed consortium for the bioremediation in the co-occurrence of other pollutants. Patel et al. [37] proposed that the enrichment of the mixed bacterial cultures DAK11 could effectively degrade PAHs at 500 mg/L, even in the presence of various co-contaminants like petroleum, mono-aromatic hydrocarbons, and heavy metals. Chakraborty [38] illustrated that para-nitrophenol was

degraded co-metabolically by a mixed culture composed of four bacteria, and the mixed culture tolerated and eliminated the negative effects of other co-contaminants.

In addition, the effect of BTEX on MTBE degradation rate was further discussed. The trend of residual MTBE concentrations and corresponding biomass as a function of time is present in Figure 3b. It can be seen that MTBE was metabolized in the binary system containing BTEX, but the degradation of substrate was not directly proportional to the increase in biomass. The lag between the increase in biomass and the decrease in concentration may be attributed to the production of intermediates. Moreover, the growth trend and biomass of bacteria were similar before and after BTEX addition. It is supposed that BTEX had no obvious impact on the growth of bacteria. However, it came clear that BTEX delayed the removal of MTBE, and the degradation rate of MTBE in binary mixture was slower than that of MTBE alone. The mechanism of BTEX delaying the degradation of MTBE may be due to the competitive binding between substrates to the active site [39].

In general, toxic intermediates can be metabolized through the synergy between enzymes. However, the coexistence of benzene and toluene with MTBE leads to the accumulation of metabolites with a high toxicity to bacteria, thus inhibiting the removal of MTBE [39]. Similarly, NKNU02 was identified as a MTBE-degrading strain, which could degrade MTBE at a concentration up to 100 mg/L. However, the removal of MTBE by NKNU02 was partially inhibited, decreasing from 60% with MTBE alone to 15% with a mixture of toluene, benzene, o-xylene, and m-xylene [15]. Although MTBE could be degraded by EH831 isolated from oil-contaminated soil without any lag period, the removal of MTBE by EH831 was reduced with the addition of benzene, ethylbenzene, or xylene. In particular, MTBE was not able to be biodegraded in quaternary or quinary mixtures containing ethylbenzene [14]. Deeb et al. [35] showed that PM1 could grow on MTBE as carbon source, however, the removal of MTBE by PM1 was completely restrained at toluene concentrations above 60 mg/L or in the presence of 20 mg/L ethylbenzene and xylene. Additionally, the degradation rate of MTBE was significantly slowed down when PM1 was incubated in a mixture of MTBE/benzene or MTBE/toluene. Further, they suggested that although the biodegradation of BTEX and MTBE mainly proceeds through two independent and inducible pathways, the removal of MTBE in groundwater may be delayed until MTBE has migrated outside the BTEX plume. Among BTEX compounds, ethylbenzene and xylene exhibit a greater negative effect on MTBE biodegradation than benzene and toluene [35,39]. Furthermore, the effect of BTEX on MTBE biodegradation depends on the microbial species and the concentration of contaminants. For example, the diversity of microbial communities' results in a different selectivity and priority for MTBE and BTEX. Moreover, if the concentration of MTBE is high enough or the proportion of BTEX is low, the system will not be disturbed. In a word, the effect of gasoline organic compositions on MTBE degradation deserves extensive attention if the environment is seriously contaminated with gasoline. In addition, the mixture of three pure cultures exhibited a higher adaptability and enhanced MTBE removal in the co-occurrence of other gasoline organic components. Hence, a synergetic system containing a mixed consortium is recommended for MTBE bioremediation, either as a single substrate or as a mixture containing gasoline components.

3.4. The Mixture of Three Pure Cultures Effectively Removed MTBE in the Presence of Heavy Metals

The co-contamination of organic and inorganic (such as MTBE and heavy metals) has been detected all over the world. Heavy metals are toxic to microorganisms, thus disturbing the biodegradation system. Exploring the interaction between bacteria and metals contained in gasoline on MTBE removal may develop a practical method for in-site remediation of MTBE contaminated sites.

The capabilities of strains to degrade MTBE in the presence of metal ions (Fe^{2+}, Mn^{2+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, and Cd^{2+}) detected in gasoline-impacted soils and water was investigated. The growth response of strains to six metal ions with different concentrations in a MSM

medium containing MTBE as carbon source is presented in Figure 4a. As can be seen, the maximum biomass varied in the range of 0.1045–0.183 and 0.0755–0.143 for metal concentrations of 1 and 20 mg/L, respectively. At a metal concentration of 1 mg/L, the growth of strains exhibited similarity and there was no substantial change in the biomass of three tested metal ions (Pb^{2+}, Cu^{2+} and Cd^{2+}). In comparison, Zn^{2+} caused a sharp decrease in biomass, indicating that strains grew well in the presence of metal ions other than Zn^{2+} at concentration below 1 mg/L. Furthermore, the biomass decreased with increasing metal concentration, while the biomass treated with Fe^{2+} hardly changed. The growth of strains was inhibited by replacing essential ions or blocking the important functional group with the introduction of metal ions except for Mn^{2+} and Fe^{2+} at concentration up to 20 mg/L [40]. Under two test concentrations, the strains treated with Fe^{2+} and Mn^{2+} grew better, while Zn^{2+} had a stronger inhibitory effect on the growth of strains.

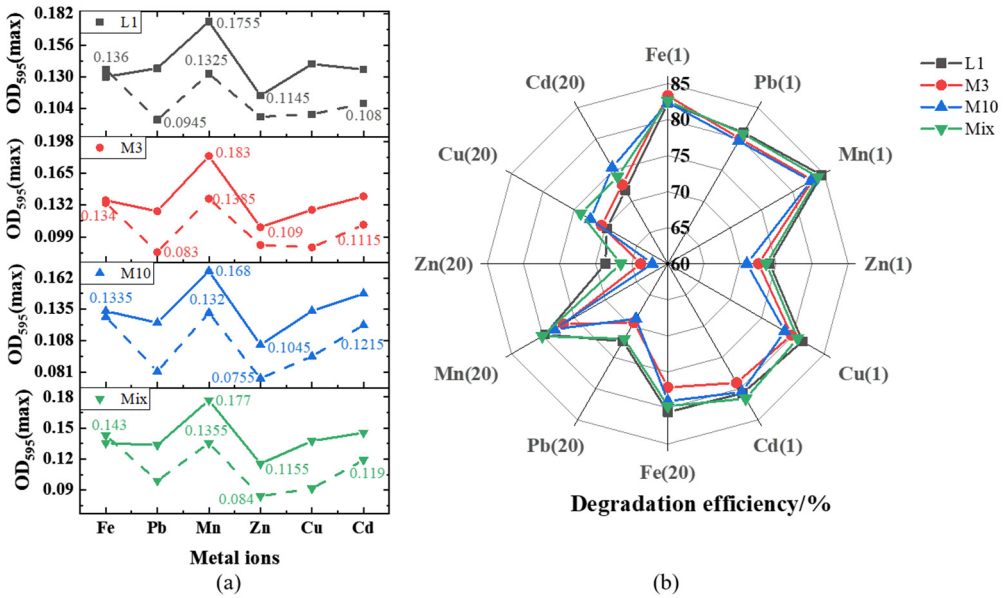

Figure 4. Effect of metal ions on degrading bacteria. The (**a**) maximum cell density of strains and (**b**) the yield of MTBE degradation with various metal ions, the concentrations of metals were controlled at 1 mg/L (dotted line) and 20 mg/L (solid line), respectively.

Several interactions were observed during MTBE biodegradation, including enhancement and inhibition (Figure 4b). The introduction of Mn^{2+} at a concentration of 1 mg/L promoted the growth of strains and consequently offered a stimulating effect on MTBE biodegradation, while suppressing the removal at a high metal concentration (20 mg/L). Similarly, the removal of MTBE was enhanced at a low concentration (1 mg/L) of Fe^{2+}, and the addition of Fe^{2+} had no obvious inhibitory effect until a concentration of 20 mg/L was reached. However, other metal ions did not cause any stimulation. For Pb^{2+}, Cu^{2+} and Cd^{2+}, the removal of MTBE changed little at a metal concentration of 1 mg/L, but was inhibited as the metal concentration increased to 20 mg/L. It was found that Zn^{2+} was detrimental to MTBE biodegradation, and its introduction restrained MTBE removal even at a concentration of 1 mg/L. Additionally, the inhibition was getting severe at a concentration of 20 mg/L; that is, the degradation efficiency was negatively correlated with the concentration of Zn^{2+}. As shown in Figure 4b, the system was upset at 20 mg/L for all metal ions tested, and the MTBE degradation efficiency decreased constantly in the following order: $Zn^{2+} > Pb^{2+} > Cu^{2+} > Cd^{2+} > Mn^{2+} > Fe^{2+}$. Among then, Zn^{2+} had the strongest inhibitory effect and was the only metal ion that showed inhibition at 1 mg/L. The four metal ions (Zn^{2+}, Pb^{2+}, Cu^{2+}, Cd^{2+}) induced inhibition and showed a dose-dependent response, and the other Fe^{2+} and Mn^{2+} exhibited weaker negative effects.

The results in this study are consistent with reports published previously. Hassen et al. [41] selected several metal-resistant bacteria from naturally polluted environments and determined the minimum inhibitory concentrations of Cu^{2+}, Cd^{2+}, and Zn^{2+} for *Pseudomonas aeruginosa* (strain S7) were 1.6, 1.5 and 1.5 mg/L, respectively. Further, *Pseudomonas aeruginosa* (strain S6) isolated from raw wastewater in Tunis city exhibited a relatively high resistance to various metals. Bioassays were performed to assess the responses of *Micrococcus* and *Pseudomonas* to the metals added singly or in combination to a crude oil/mineral salts medium. It was found that Zn^{2+} and Pb^{2+} induced significant decreases in the density of the two bacteria, while Cu^{2+} and Mn^{2+} did not [42]. Lin et al. [40] illustrated that the addition of 1 mg/L Mn^{2+} offered a slight stimulating effect on MTBE degradation, while Zn^{2+} reduced MTBE removal at the same concentration. The degradation rate of MTBE decreased at Zn^{2+} concentrations of 10 mg/L and 50 mg/L, and the inhibition was stronger at high concentration (50 mg/L).

Unlike organics, metals are not biodegradable, so they inhibit microbial growth when accumulated to toxic levels. While some metal ions are necessary for bacteria metabolism as they provide essential elements for synthesis and action of enzymes and proteins, they may be replaced by metal ions existing in the environment, resulting in the loss of functionality [43]. The promotion of MTBE biodegradation is mainly due to the better cell growth and enzyme synthesis [44]. However, the toxicity of metal ions dominants at high concentrations and their binding to groups on enzyme molecules associated to MTBE biodegradation or cellular metabolism leads to the inactivation and denaturation of enzymes [44]. Moreover, the sequestration of metal ions prevents cell metabolism, such as abiotic oxidation followed by precipitation. The reduction in MTBE removal is the result of adsorption of metal ions into microbial cells and precipitation of metal ions on the cell surfaces [44]. More seriously, some metal ions change the transcription mechanism in cells, thus fundamentally suppressing the cell division [45]. Therefore, the original bacteria are partially inactivated in the presence of metals.

In addition, the effect of metals on MTBE biodegradation was similar in terms of either the three pure strains or the mixed consortium. The degrading ability of bacteria was unaffected until the threshold concentration of metals was reached. The threshold required for inhibition depends on the concentration and type of metals as well as the bacteria. Within the appropriate concentration, the enzymatic system in propagated cells is not suppressed, and the metabolic reaction proceed normally. Above the threshold concentration, the activity of cells is restrained and no new cells can be synthesized, resulting in MTBE being degraded only after additional bacteria reproduction [40]. What is more, compared with pure strains, the degradation efficiency of MTBE by mixed consortium was higher with the addition of 20 mg/L Mn^{2+}, Pb^{2+} and Cu^{2+}. That is, mixed consortium had stronger resistance to metal ions, especially for the high concentration (20 mg/L) of Mn^{2+}, Pb^{2+}, and Cu^{2+}. The results revealed that mixing three pure cultures effectively removed MTBE in the presence of heavy metals, and attention should be paid to MTBE bioremediation in gasoline-contaminated environment with the presence of various organics and heavy metals.

3.5. The Mixture of Three Pure Cultures Improved the Adaptability to Variable Temperature and pH

There are various environment factors that can affect the MTBE biodegradation, and temperature and ambient pH are chosen for discussion here. Figure 5a shows the maximum biomass of strains and the removal of MTBE under different temperatures. It is speculated that low temperature was not conductive to the growth of strains, resulting in a reduction in biomass at 15 °C. Moreover, the results showed that the tested temperature ranges from 15 °C to 35 °C was adequate for all strains to degrade MTBE. The removal of MTBE was decreased at 15 °C due to the inhibition of enzymes activity involved in bacterial metabolism at low temperatures. With the increase of temperature, the enzyme activity was improved, so that the degradation efficiency increased gradually, reaching a maximum

at 25 °C. However, as the temperature further raised to 35 °C, the removal efficiency reduced due to the inactivation of enzymes at high temperature [46]. Furthermore, the mixed consortium promoted MTBE removal at either low or high temperature, and the degradation efficiency was increased by about 10% compared with pure strains at 35 °C, suggesting that mixing three pure cultures enhanced the tolerance to temperature.

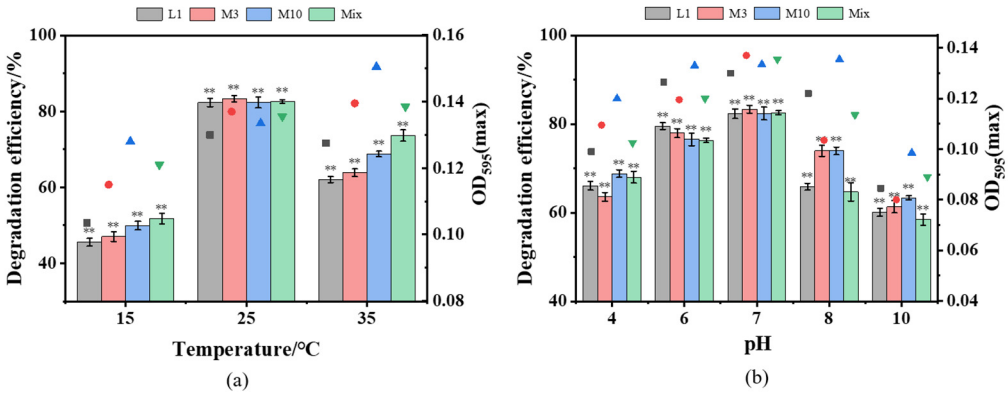

Figure 5. Tolerance of strains to temperature and pH. The growth of strains (scatter chart) and the yield of degradation (bar chart) carried out on various (**a**) temperature and (**b**) pH. ** represents $p < 0.01$.

Another common factor is the ambient pH, and the effect of pH on the growth of strains and MTBE removal is shown in Figure 5b. It is illustrated that all strains were sensitive to the ambient pH ($p < 0.05$), and the growth of strains was significantly inhibited under either a strong acid or strong base. Furthermore, the removal of MTBE changed within a pH range of 4–10, and the degradation efficiency was higher under neutral conditions (pH = 7). The biodegradation of MTBE was decreased due to the inactivation of strains in strongly acidic or basic conditions, and the resistance of strains to acid conditions was much higher than that of basic conditions. It was confirmed that both temperature and ambient pH affected MTBE biodegradation ($p < 0.01$). Moreover, compared with pure strains, a mixed consortium improved the adaptability to unfavorable conditions, especially at low or high temperatures. Additionally, further researches to promote MTBE biodegradation by regulating other environment factors are needed.

4. Conclusions

This study was conducted to evaluate the degradation potential of co-cultures of three strains in a system contaminated with MTBE and other gasoline components. The conclusions are as follows:

(1) A mixed consortium was successfully constructed by mixing three pure strains, which increased the degradation efficiency by about 20% at a concentration of 30 mg/L. Furthermore, the synergistic system containing the mixed consortium degraded MTBE with a rate three times faster than any of its individual components at 10 mg/L, and showed a shorter, if any, lag periods during MTBE degradation. This confirms the feasibility of cultivating a mixed consortium, which can improve the efficiency and rate of biodegradation.

(2) The addition of alkanes, BTEX or metals affected MTBE removal. Therefore, the effect of co-contamination of gasoline components is supposed to be considered in MTBE biodegradation.

(3) Compared with pure strains, a mixed consortium improved MTBE removal in the ternary system and exhibited stronger resistance to metals. Furthermore, a mixed consortium improved the degradation efficiency at either low or high temperatures, showing a wider adaptability to environmental conditions. This supports the mixing

of different pure strains for MTBE bioremediation in case of coexistence of other contaminants in practical applications.

(4) In addition to mixed bacterial cultures, other synergistic systems containing fungi-bacteria also deserve further investigation to develop an efficient biological system for MTBE biodegradation and mineralization.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app13042144/s1, Figure S1: Time course for strains growth with the addition of (a) dodecane, (b) benzene and (c) toluene.

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References

1. Van, D.; Pijls, C.; Sinke, A.; Langenhoff, A.; Smidt, H.; Gerritse, J. Anaerobic degradation of a mixture of MtBE, EtBE, TBA, and benzene under different redox conditions. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 3387–3397.
2. Badra, J.; Alowaid, F.; Alhussaini, A.; Alnakhli, A.; AlRamadan, A.S. Understanding of the octane response of gasoline/MTBE blends. *Fuel* **2022**, *318*, 123647. [CrossRef]
3. Levchuk, I.; Bhatnagar, A.; Sillanpää, M. Overview of technologies for removal of methyl tert-butyl ether (MTBE) from water. *Sci. Total Environ.* **2014**, *476–477*, 415–433. [CrossRef] [PubMed]
4. Russo, A.V.; Lobo, D.N.D.; Jacobo, S.E. Removal of MTBE in Columns Filled with Modified Natural Zeolites. *Procedia Mater. Sci.* **2015**, *8*, 375–382. [CrossRef]
5. Zadaka-Amir, D.; Nasser, A.; Nir, S.; Mishael, Y.G. Removal of methyl tertiary-butyl ether (MTBE) from water by polymer–zeolite composites. *Microporous Mesoporous Mater.* **2012**, *151*, 216–222. [CrossRef]
6. Zhang, L.E.; Qin, J.; Zhang, Z.; Li, Q.; Huang, J.; Peng, X.; Qing, L.; Liang, G.; Liang, L.; Huang, Y.; et al. Concentrations and potential health risks of methyl tertiary-butyl ether (MTBE) in air and drinking water from Nanning, South China. *Sci. Total Environ.* **2016**, *541*, 1348–1354. [CrossRef]
7. Hassen, J.A.; Gross, C.P. MTBE: Groundwater remediation technologies. *Remediation* **2010**, *10*, 129–139. [CrossRef]
8. Attarian, P.; Mokhtarani, N. Comparison of co-metabolic and direct metabolic biodegradation of MTBE: Monitoring main intermediates and SBR stable operation. *Environ. Technol. Innov.* **2021**, *22*, 101475. [CrossRef]
9. Xu, X.; Zhou, H.; Chen, X.; Wang, B.; Jin, Z.; Ji, F. Biodegradation potential of polycyclic aromatic hydrocarbons by immobilized *Klebsiella* sp. in soil washing effluent. *Chemosphere* **2019**, *223*, 140–147.
10. Kane, S.R.; Chakicherla, A.Y.; Chain, P.S.; Schmidt, R.; Shin, M.W.; Legler, T.C.; Scow, K.M.; Larimer, F.W.; Lucas, S.M.; Richardson, P.M. Whole-genome analysis of the methyl tert-butyl ether-degrading beta-proteobacterium *Methylibium petroleiphilum* PM1. *J. Bacteriol.* **2007**, *189*, 1931–1945. [CrossRef]
11. Muller, R.H.; Rohwerder, T.; Harms, H. Degradation of fuel oxygenates and their main intermediates by Aquincola tertiaricarbonis L108. *Microbiology* **2008**, *154*, 1414–1421. [CrossRef] [PubMed]
12. Streger, S.H.; Vainberg, S.; Dong, H.; Hatzinger, P.B. Enhancing transport of hydrogenophaga flava ENV735 for bioaugmentation of aquifers contaminated with methyl tert-butyl ether. *Appl. Environ. Microbiol.* **2002**, *68*, 5571–5579. [CrossRef] [PubMed]
13. Franois, A.; Mathis, H.; Godefroy, D.; Piveteau, P.; Monot, F. Biodegradation of methyl tert-butyl ether and other fuel oxygenates by a new strain, Mycobacterium austroafricanum IFP 2012. *Appl. Environ. Microbiol.* **2002**, *68*, 2754–2762. [CrossRef] [PubMed]
14. Lee, E.-H.; Cho, K.-S. Effect of substrate interaction on the degradation of methyl tert-butyl ether, benzene, toluene, ethylbenzene, and xylene by *Rhodococcus* sp. *J. Hazard. Mater.* **2009**, *167*, 669–674. [CrossRef] [PubMed]
15. Chen, S.C.; Chen, C.S.; Zhan, K.-V.; Yang, K.-H.; Chien, C.-C.; Shieh, B.-S.; Chen, W.-M. Biodegradation of methyl tert-butyl ether (MTBE) by *Enterobacter* sp. NKNU02. *J. Hazard. Mater.* **2011**, *186*, 1744–1750. [CrossRef] [PubMed]

16. Eixarch, H.; Constantí, M. Biodegradation of MTBE by Achromobacter xylosoxidans MCM1/1 induces synthesis of proteins that may be related to cell survival. *Process Biochem.* **2010**, *45*, 794–798. [CrossRef]
17. Ghorbannezhad, H.; Moghimi, H.; Dastgheib, S.M.M. Evaluation of heavy petroleum degradation using bacterial-fungal mixed cultures. *Ecotoxicol. Environ. Saf.* **2018**, *164*, 434–439. [CrossRef]
18. Patel, A.B.; Singh, S.; Patel, A.; Jain, K.; Amin, S.; Madamwar, D. Synergistic biodegradation of phenanthrene and fluoranthene by mixed bacterial cultures. *Bioresour. Technol.* **2019**, *284*, 115–120. [CrossRef]
19. Kamyabi, A.; Nouri, H.; Moghimi, H. Synergistic Effect of *Sarocladium* sp. and *Cryptococcus* sp. Co-Culture on Crude Oil Biodegradation and Biosurfactant Production. *Appl. Biochem. Biotechnol.* **2017**, *182*, 324–334. [CrossRef]
20. Zhang, X.; Kong, D.; Liu, X.; Xie, H.; Lou, X.; Zeng, C. Combined microbial degradation of crude oil under alkaline conditions by Acinetobacter baumannii and *Talaromyces* sp. *Chemosphere* **2021**, *273*, 129666. [CrossRef]
21. Iturbe, R.; Flores, R.M.; Torres, L.G. Soil and Water Contamination Levels in an Out-of-Service Oil Distribution and Storage Station in Michoacan, Mexico. *Water Air Soil Pollut.* **2003**, *146*, 261–281. [CrossRef]
22. Patel, V.; Jain, S.; Madamwar, D. Naphthalene degradation by bacterial consortium (DV-AL) developed from Alang-Sosiya ship breaking yard, Gujarat, India. *Bioresour. Technol.* **2012**, *107*, 122–130. [CrossRef] [PubMed]
23. Li, S.S.; Zhang, D.; Wei, Y. Enhanced Biodegradation of Methyl tert-butyl-ether by a Microbial Consortium. *Curr. Microbiol.* **2013**, *68*, 317–323. [CrossRef] [PubMed]
24. Li, S.; Wang, D.; Du, D.; Qian, K.; Yan, W. Characterization of co-metabolic biodegradation of methyl tert-butyl ether by a *Acinetobacter* sp. strain. *RSC Adv.* **2019**, *9*, 38962–38972. [CrossRef] [PubMed]
25. Simkins, S.; Alexander, M. Models for mineralization kinetics with the variables of substrate concentration and population density. *Appl. Environ. Microbiol.* **1984**, *47*, 1299–1306. [CrossRef]
26. Ouiroga, J.M.; Perales, J.A.; Romero, L.I.; Sales, D. Biodegradation kinetics of surfactants in seawater. *Chemosphere* **1999**, *39*, 1957–1969. [CrossRef] [PubMed]
27. Ghorbannezhad, H.; Moghimi, H.; Dastgheib, S.M.M. Evaluation of pyrene and tetracosane degradation by mixed-cultures of fungi and bacteria. *J. Hazard. Mater.* **2021**, *416*, 126202. [CrossRef]
28. Kshirsagar, S.D.; Mattam, A.J.; Jose, S.; Ramachandrarao, B.; Velankar, H.R. Heavy hydrocarbons as selective substrates for isolation of asphaltene degraders: A substrate-based bacterial isolation strategy for petroleum hydrocarbon biodegradation. *Environ. Technol. Innov.* **2020**, *19*, 100832. [CrossRef]
29. Briceño, G.; Schalchli, H.; Mutis, A.; Benimeli, C.S.; Palma, G.; Tortella, G.R.; Diez, M.C. Use of pure and mixed culture of diazinon-degrading Streptomyces to remove other organophosphorus pesticides. *Int. Biodeterior. Biodegrad.* **2016**, *114*, 193–201. [CrossRef]
30. Kurade, M.B.; Waghmode, T.R.; Patil, S.M.; Jeon, B.-H.; Govindwar, S.P. Monitoring the gradual biodegradation of dyes in a simulated textile effluent and development of a novel triple layered fixed bed reactor using a bacterium-yeast consortium. *Chem. Eng. J.* **2017**, *307*, 1026–1036. [CrossRef]
31. Kaustuvmani, P.; Rupshikha, P.; Kalita, M.C.; Suresh, D. Development of an Efficient Bacterial Consortium for the Potential Remediation of Hydrocarbons from Contaminated Sites. *Front. Microbiol.* **2016**, *7*, 1092.
32. Fulekar, H.M. Microbial degradation of petrochemical waste-polycyclic aromatic hydrocarbons. *Bioresour. Bioprocess.* **2017**, *4*, 28. [CrossRef] [PubMed]
33. Attarian, P.; Mokhtarani, N. Feasibility study of aerobic cometabolism biodegradation of MTBE by a microbial consortium: Biomass growth and decay rate. *J. Water Process Eng.* **2021**, *44*, 102338. [CrossRef]
34. Zhang, L.L.; Zhu, R.Y.; Chen, J.M.; Cai, W.M. Biodegradation of methyl tert-butyl ether as a sole carbon source by aerobic granules cultivated in a sequencing batch reactor. *Bioprocess Biosyst. Eng.* **2008**, *31*, 527–534. [CrossRef]
35. Deeb, R.A.; Hu, H.Y.; Hanson, J.R.; Scow, K.M.; Alvarez-Cohen, L. Substrate Interactions in BTEX and MTBE Mixtures by an MTBE-Degrading Isolate. *Environ. Sci. Technol.* **2001**, *35*, 312–317. [CrossRef]
36. Izquierdo, A.R.; Vila, J.; Petit, C.; Peyret, P.; Koch, A.; Grifoll, M. Microbial populations and functions associated with the degradation of aliphatic and aromatic hydrocarbon oil fractions. *J. Biotechnol.* **2015**, *208*, S51. [CrossRef]
37. Patel, A.B.; Mahala, K.; Jain, K.; Madamwar, D. Development of mixed bacterial cultures DAK11 capable for degrading mixture of polycyclic aromatic hydrocarbons (PAHs). *Bioresour. Technol.* **2018**, *253*, 288–296. [CrossRef]
38. Chakraborty, B. Kinetic study of degradation of p-nitro phenol by a mixed bacterial culture and its constituent pure strains. *Mater. Today Proc.* **2016**, *3*, 3505–3524. [CrossRef]
39. Lin, C.-W.; Cheng, Y.-W.; Tsai, S.-L. Multi-substrate biodegradation kinetics of MTBE and BTEX mixtures by *Pseudomonas aeruginosa*. *Process Biochem.* **2007**, *42*, 1211–1217. [CrossRef]
40. Lin, C.-W.; Chen, S.-Y.; Cheng, Y.-W. Effect of metals on biodegradation kinetics for methyl tert-butyl ether. *Biochem. Eng. J.* **2006**, *32*, 25–32. [CrossRef]
41. Hassen, A.; Saidi, N.; Cherif, M.; Boudabous, A. Resistance of environmental bacteria to heavy metals. *Bioresour. Technol.* **1998**, *64*, 7–15. [CrossRef]
42. Benka-Coker, M.O.; Ekundayo, J.A. Effects of heavy metals on growth of species of *Micrococcus* and *Pseudomonas* in a crude oil/mineral salts medium. *Bioresour. Technol.* **1998**, *66*, 241–245. [CrossRef]
43. Jansen, E.; Michels, M.; Til, M.; Doelman, P. Effects of heavy metals in soil on microbial diversity and activity as shown by the sensitivity-resistance index, an ecologically relevant parameter. *Biol. Fertil. Soils* **1994**, *17*, 177–184. [CrossRef]

44. Lin, C.-W.; Cheng, Y.-W.; Tsai, S.-L. Influences of metals on kinetics of methyl tert-butyl ether biodegradation by *Ochrobactrum cytisi*. *Chemosphere* **2007**, *69*, 1485–1491. [CrossRef] [PubMed]
45. Gopinath, K.P.; Kathiravan, M.N.; Srinivasan, R.; Sankaranarayanan, S. Evaluation and elimination of inhibitory effects of salts and heavy metal ions on biodegradation of Congo red by *Pseudomonas* sp. mutant. *Bioresour. Technol.* **2011**, *102*, 3687–3693. [CrossRef]
46. Mouafo Tamnou, E.B.; Tamsa Arfao, A.; Nougang, M.E.; Metsopkeng, C.S.; Noah Ewoti, O.V.; Moungang, L.M.; Nana, P.A.; Atem Takang-Etta, L.-R.; Perrière, F.; Sime-Ngando, T.; et al. Biodegradation of polyethylene by the bacterium *Pseudomonas aeruginosa* in acidic aquatic microcosm and effect of the environmental temperature. *Environ. Chall.* **2021**, *3*, 100056. [CrossRef]