Riwayat

Original Article

Biodegradation of Diethyl Phthalate (DEP) Using Fungi Isolated from Leachate of Sarimukti Landfill, Bandung

Suci Keiva Mulyana[1]✉, Emenda Sembiring[2]
[1,2]Study Program of Environmental Engineering Ganesha Street Number 10, Bandung
Correspondence Author: sucikeivam@gmail.com✉

Abstract:

Phthalate compounds that are commonly found in everyday life are diethyl phthalate (DEP). This compound is dangerous because it is carcinogenic, xenoestrogenic and has endocrine disrupting effects. The accumulation of plastic in landfill causes this compound to be identified in leachate with an existing concentration of 1,277 mg/L. Biological treatment is one of the appropriate technologies in DEP removal. The treatment involves the degradation of DEP using fungi isolated from leacheate samples. Degradation tests were carried out at varying concentrations of 2 mg/l, 4 mg/l, 6 mg/l, 8 mg/l and 10 mg/l in a test time span of 15 days and analyzed every 3 days. Concentration testing was perfomed using High Performance Liquid Chromatography (HPLC). DEP removal efficiency reached 98-99% in each concentration variation on day 9 with a specific fungal growth rate of 0.0002-0.068/day.

Keywords: Fungal Isolate, Biodegradation, Phthalate, Diethyl Phthalate, Leachate.

Introduction

Industrial development in Indonesia increases every year with significant growth observed in the food, beverage, and household furniture industries, all of which heavily use plastic packaging. Plastic packaging is widely used because it can make human life easier. However, there are several disadvantages of plastic, including the use of non-environmentally friendly materials in the manufacturing process. One of the hazardous materials used in plastic production is phthalate (Sari, 2020). Phthalate is an organic compound extensively used in the production of plastics such as polyvinyl chloride (PVC). It is added to plastics to enhance their flexibility, durability, and workability during the manufacturing process of various products, including food and beverage packaging, household furniture, and even floor tiles (Okoli et al., 2014). The global

https://jurnal.usk.ac.id/riwayat

Received: June 9, 2024 – Revised: July 12, 2024- Accepted: September 29, 2024 - Published online: October 28, 2024

production of phthalates has steadily increased over the years due to their versatile applications in various industries (Shukur, 2024).

Phthalate are synthetic compound with a high octanol-water partition coefficient (log Kow) and low water solubility. Phthalate do not bind to the polymer matrix and are easily released from landfill into leachate (Wang et al., 2022). The migration and transformation phthalate in landfill are influenced by factor such as temperature, pressure, substrate concentration, oxidant concentration, reaction mixture composition, and the presence of chemical catalysts (Staples et al., 2000). Phthalates in waste can be leached into leachate, the low solubility increases the transportation of phthalates in the liquid phase, potentially contaminating soil and groundwater (Wei et al., 2019).

The main route of phthalate exposure is through water, as these chemicals enter the water system through sewage discharges, leaching from landfills, and various other sources of dispersal. Various field and laboratory studies reveal high exposure and apparent toxicity of phthalates, impacting human health and ecosystem functions. The main pathway of pthalates entering the human body is through the digestive system, although some can be assimilated through the respiratory system, and compound like DEHP can enter through the skin due to their more lipophilic (Luo et al. 2018). The types of phthalates are diethyl Phthalate (DEP), dimethyl Phthalate (DMP), dibutyl Phthalate (DBP), di(2-ethylhexyl)Phthalate (DEHP), butyl benzyl phthalate (BBP), diisodecyl phthalate (DIDP), diisononyl phthalate (DINP), di-n- hexyl-phthalate (DnHP) and di-n-octyl phthalate (DnOP). Common phthalate in everyday life are DEP, DMP and DEHP. The World Health Organization (WHO) identifies several human health risks of phthalates due to their carcinogenic, xenoestrogenic and endocrine distrupting, which interfere with the binding and action of natural hormones, thus disrupting physiological processes (González-Márquez et al., 2019a). The toxicity level of each phthalate compound varies depending on the chemical structure, concentration, duration and route of exposure.Acute toxicity data from Material Safety Data Sheet (MSDS) indicate that the LC50 of DEP for rats is 4.64 mg/l in 6h, while for other types like dibutyl phthalate (DBP) the LC50 is 15.68 mg/l in 4h and for the type of di(2-ethyl hexyl) phthalate (DEHP) the LC50 is 10.62 mg/l in 4h. In addition to its high toxicity, DEP is commonly found in everyday products and is categorized as a hazardous chemical in North America, being both carcinogenic and highly toxic. This contamination poses unknown long-term effects. Due to their toxic and ecologically hazardous nature, phthalates are designated as priority controlled organic pollutants by the Environmental Protection Agency (EPA). Thus, it is crucial to effectively and economically remove phthalate compounds from the environment to prevent negative impacts on ecosystems and human health.

Biodegradation using microorganisms is the most effective way for phthalate remediation. The biodegradation process utilizes microorganisms such as bacteria and fungi to break down phthalates into simpler and less harmful compounds, like phthalic acid, carbon dioxide, and water making biodegradation an effective method in reducing phthalates in the environment. Moreover, biodegradation is a natural process that does not require additional chemicals or high energy, making it more environmentally friendly than other treatment methods such as incineration or chemical treatment. Most studies on phthalate biodegradation have focused on bacterial microorganisms such as

research by Patil (2019) to biodegrade DEP with Bacillus sp. bacteria with a yield of 0.81 g/g and other studies using Bacillus sp. MY156 bacteria by Xie (2023) with a removal efficiency of 80% in 60h. However, there is limited research on the biodegradation of pththalate using fungi, even though fungi are one of the microorganisms that important role in biodegradation. Fungi have a efficient enzymatic system to secrete esterase that break ester bond in phthalate molecules. The resulting esterase effect will break the ester bonds that connect phthalate molecules and produce compounds that are more easily degraded by fungi. Previous research by González-Márquez et al. (2019) on the biodegradation of di(2-ethylhexyl) phthalate (DEHP) using Fusarium culmorum fungi found that DEHP was degraded into butanol, hexanol, catechol, and acetic acid, with the fungus able to reduce 92% of DEHP within 36 hours. Various fungi capable degrading plasticizers include Fusarium oxyxporum (Kim et al., 2003), Fusarium culmurom, Pleurotus ostreatus (Ahuactzin-Pérez et al., 2016), Neurospora crassa (Aguilar-Alvarado et al., 2015), Trichoderma harzianum, Polyporum brumalis and Saccharomyces cerevisiae. Many fungi also come from contaminated sources such as water, soil and leachate.

This research is a study of phthalate degradation by fungi isolated from landfill leachate. The selection of indigenous is based on their enhanced ability to degrade specific compunds, having adapted to their environmental conditions. Therefore, they have developed certain enzyme systems and metabolic mechanisms that are more effective in degrading certain compounds (Ayeni et al., 2022). The mechanism of phthalate degradation by fungi can be simplified as a fungi utilizing phthalate as the sole source of carbon and energy for their growth (González-Márquez et al., 2019). The results of this study are expected to provide information for managing phthalate by utilizing microorganisms in leachate water at Sarimukti Landfill Bandung.

Experimental Details

Isolation of Fungi From Leachate

Fungi in the leachate water were isolated on a nutrient-poor solid growth medium, Basal Salt Medium (BSM) to which a small concentration of DEP was added and then aerated using a 150 rpm shaker at room temperature. When turbidity began to appear in the BSM + DEP, a sample was taken from the flask and inoculated into Potato Dextrose Agar (PDA) in a petri dish, then incubated at room temperature until mold growth appeared. Fungi that grow on PDA media are subcultured to oblique PDA media and purified until a subculture is obtained which will be used as a stock subculture which will then be identified and used as a working culture (Trihartomo, 2024).

Fungal Identification

This identification by microscopic observation. Fungal culture from PDA media is taken and placed on a glass object that has been dripped with distilled water, then covered with a cover glass. Observation of the preparate using a microscope was carried out using an Olympus CX-22 light microscope with a magnification of 40x. Fungi were observed for morphology and then identified based on reference literature.

Biodegradation Process

Biodegradation tests were conducted on SBM liquid media with selected fungi that had the highest amount of biomass in media with high phthalate concentrations.

The test was conducted for 15 days and determined the kinetics of the removal rate, the amount of biomass and the final concentration of phthalate analyzed on days 0, 3, 5, 7, 9, 13 and 15 and variations in concentration of 2 mg/l, 4 mg/l, 6 mg/l, 8 mg/l and 10 mg/l (Wanggai, 2023). The determination of the concentration variation was also based on the concentration range of DEP in the leachate. The determination and calculation of the number of fungal cells was carried out by dry weight method and the final concentration was analyzed using High Performance Liquid Chromatography (HPLC). This HPLC analysis method was performed in reverse phase with a C18 column (Zorbax C18 column, Hewlett Packard, Santa Clara, CA) used to determine the level of degradation. Acetonitrile and water were used as solvents dissolving from 80 to 100% acetonitrile. Analysis was performed with a flow rate of 0.8 mL/min and detection at 280 nm. The degradation rate of phthalate was then calculated through peak area comparison (Mo et al., 2019a). After the biodegradation process, the removal efficiency was calculated using the following formula:

$$Efisiensi\ Penyisihan\ = \frac{Konsentrasi\ awal - konsentrasi\ akhir}{konsentrasi\ awal}\ x\ 100\%$$

Fungal Kinetics

The kinetics parameters determined are specific growth rate (μ), maximum specific growth rate (μmax), half saturation constant (Ks) and cell synthesis coefficient (Y).). The concept of determining the growth kinetics of microorganisms limited by the substrate is modeled by the Monod equation. The specific growth rate of fungi is given by:

$$\mu = \mu_{maks} \times \frac{S}{K_s + S}$$

where:

μ = Fungi growth rate (day)-1
μmaks = Maximum fungi growth rate
S = Substrate
Ks = Coefficient of Saturation

The specific growth rate (μ) was determined by fitting the biomass data over time using a logistic equation. Biomass yield (YX/S) was estimated as the coefficient of a linear regression of biomass concentration versus substrate concentration in grams biomass/g substrate consumed (Ahuactzin-Pérez et al., 2016b). Yield values were calculated based on the equation:

$$Y = \frac{X - X_0}{S - S_0}$$

where:

X = Biomass concentration at a certain time (mg/l)
Xo = Biomass concentration on day 0 (mg/l)
So = Day 0 substrate concentration (ppm)
S = Substrate concentration at a given time (ppm)

DEP Derived Compounds

Wang et al. 2018 described two pathways for DEP biodegradation: de-

esterification and trans-esterification. The de-esterification pathway degrades DEP into monoethyl phthalate (MEP), which is further converted into phthalic acid (PA) by esterase enzyme. The trans-esterification pathway degrades DEP into dimethyl phthalate (DMP), which also converts to PA with the help of esterase enzymes. The expected end product of this biodegradation process is that the DEP compound is hydrolyzed into the simplest compounds like CO2 and H2 O. In this study, the analysis of PA compounds which are derivatives of non-toxic DEP was analyzed using. Literature indicates that DEP biodegradation produces PA, which due to its simpler structure, can be further utilized by microorganism ultimately being hydrolyzed into non-hazardous CO2 and H2 O.

Results

Sub 1 Sample Characterization

Sample characterization is carried out to determine the properties of the sample. The characteristics reviewed in the characterization include temperature, temperature and TDS which are measured directly at the sampling location. In addition, measurements of Biological Oxygen Demand (BOD) parameters were also carried out. Chemical Oxygen Demand (COD), Total Suspended Solid (TSS), Total Dissolved Solid (TDS), Ammonia (NH3-N), Sulfate, Oil & Fat, KMNO4, Copper (Cu), Zinc (Zn), Iron (Fe), Lead (Pb) and Cadmium (Cd) conducted at the Environmental Engineering Water Laboratory, Bandung Institute of Technology. The sample characterization results are as follows:

Parameters	Unit	Value
pH	-	7,80
Temperature	°C	32,1
Turbidity	NTU	77
Biological Oxygen Demand (BOD)	mg/l	415
Chemical Oxygen Demand (COD)	mg/l	2814,68
Total Dissolved Solid (TDS)	mg/l	1292,8
Ammonia (NH_3 -N)	mg/l	1491
Sulfate	mg/l	306,13
Oils & Fats	mg/l	20,79
$KMNO_4$	mg/l	82,82
Copper (Cu)	mg/l	0,008
Zinc (Zn)	mg/l	0,427
Iron (Fe)	mg/l	7,452
Lead (Pb)	µg/l	6,194
Cadmium (Cd)	µg/l	1,175

The selection of test parameters on leachate water characteristics refers to Wanggai's (2023), sirenden (2022) and sari 2017 reseacrh/. Determination of characterization is based on parameters that affect the degradation of phthalate compounds by fungi (Afdal & Sari, 2017).

Parameters	Wanggai (2023)	Sirenden (2022)	Sari (2017)
pH	8,16	7.45	7,4
Temperature	31,6	-	30,2
Turbidity	63	-	-
Biological Oxygen Demand (BOD)	563,75	144	173,6
Chemical Oxygen Demand (COD)	3274,1	3756,6	345,8
Total Dissolved Solid (TDS)	6443	839	1880
Ammonia (NH_3 -N)	595,5	560	-
Sulfate	272,96	-	-
Oils & Fats	9,2	-	-
$KMNO_4$	63,2	-	-
Copper (Cu)	<0,5	-	1,0
Zinc (Zn)	0,000124	-	
Iron (Fe)	9,35	1,464	2,5
Lead (Pb)	0,7959	<0,001	1,3
Cadmium (Cd)	0,478	<0,003	-

Based on the data above, the value of the test parameters in the leachate characterization sample is indeed within the range of concentration values in other landfills. In general, the parameters reviewed in the sample characterization process are related to the processing needs, particularly biodegradation which requires factors affecting the isolation process of microorganism (fungi) and factors affecting the biodegradation process. At the leachate treatment plant (LTP) inlet, the pH is often neutral (6-9), with the leachate sample in this study having a pH of 7.80, consistent with previous studies by Wanggai (2023), Sirenden (2022), and Sari (2017).

One of the influential parameters in the biodegradation process is BOD and COD. BOD and COD concentrations indicate the oxygen requirement for microorganism to decompose organic matter. The ability to decompose contaminants (biodegradability) can be determined by the BOD/COD ratio. BOD/COD ratio used for aquaculture and biological processes is within the biodegradable range of >0.1 (Majeed Khan et al., 2011). Based on the test results, the BOD/COD ratio is 0.16 which indicates that the organic pollutants present in the leachate water sample are biodegradable and suitable for removal by biological treatment (Tamyiz, 2015). In research of (Sastrawidana et al., 2017) isolated fungi from milk wastewater in the concentration range of BOD 80-6000 mg/l and COD 150-12000 mg/l and Wanggai (2023) isolated bacteria at a concentration of BOD 563.75 mg/l and COD 3274.1 mg/l. Based on this literature, the BOD COD range in the sample is in accordance with the range for fungal isolation needs.

In addition, metal elements can also participate in many cellular metabolic pathways (Mo et al., 2019b). The effect on microbial growth and microbial activity depends mainly on the concentration of metal elements in the leachate. At low concentrations (100 µg/l) of Cu2+ , Zn2+ , Mn2+ , and Fe2+ , the degradation of DEP by microorganisms increases, whereas at high concentrations (500-1000µg/l), the degradation process is inhibited (Tao et al., 2020). Based on the analysis, these conditions are very suitable and conducive for DEP degradation.

Sub 2 DEP Existing Concentration

Diethyl Phthalate (DEP) is the primary compound to be removed in this study by biological treatment method using fungi. This existing concentration value will then become the range limit in the degradation experiment. DEP compounds were analyzed at several sampling location points. This sampling point variation was carried out to observe the relationship between DEP concentration and landfill age. The existing concentration of DEP can be seen in Table 2 below:

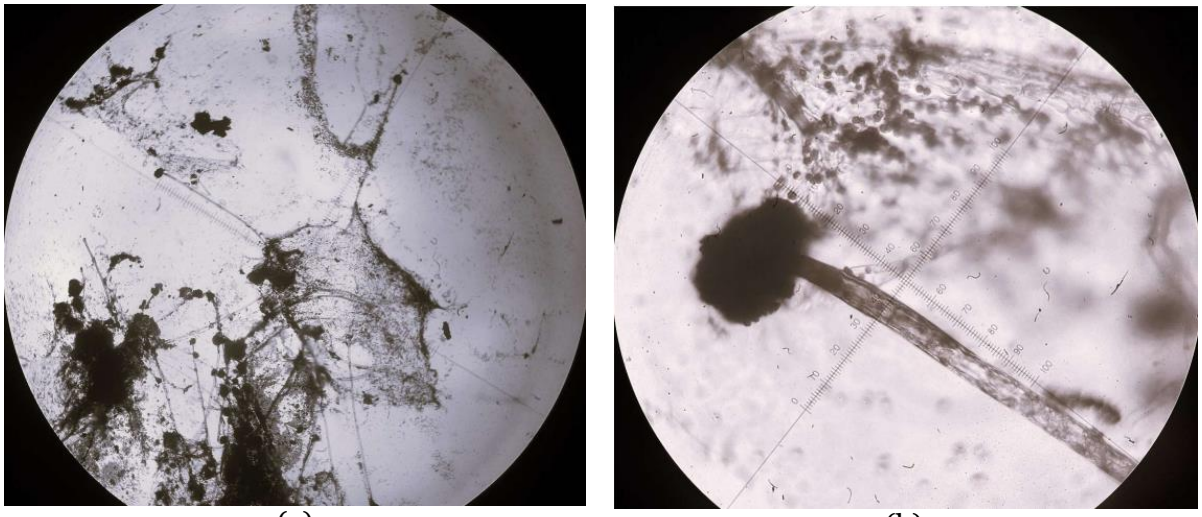
Table 3: Existing Concentration of DEP in Leachate

Parameters	Unit	Concentration				
		Sarimukti Landfill			Former Leuwigajah Landfill	
		Inlet	Zone 1	Zone 4	WWTP	Water Body
DEP	ppm	1,277	1,060	0,504	1,134	0,652

Based on the results of the research conducted, DEP concentrations were found in leachate samples are 0.504mg/l-1.277 mg/l. The presence of DEP in leachate comes from waste that contains DEP, especially plastic waste. In addition to the above research, research conducted by Fudala-Ksiazek et al. (2017) showed that DEP can be detected in leachate with varying concentrations including 0.35-59.75 µg/l and 0.26-6.98 µg/l in surface water and 6.0-7.7 µg/l in wastewater. The following are some studies that show DEP concentrations in leacheate in various parts of the world with both active and closed landfill categories including samples located in Poland (closed) of 3.71 g/l, Poland (active) 2.93 µg/l, Wuhan, China (closed) 2.01 µg/l, Wuhan, China (active) 0.66 µg/l and Japan (active) 1.60 µg/l, Japan (closed) 0.230 µg/l (Zhang et al., 2023).

Sub 3 Fungal Isolates

The process of isolation and identification of fungi obtained 2 types of fungi that will be used in the next stage of research. Based on the identification results, it was found that the fungi that were successfully isolated and had the potential to degrade phthalate from leachate samples were Rhizopus Arrhisuz and Penicillium Brefeldianum based on reference literature microbiology concepts and Applications by Micahel (1993). Rhizopus Arrhizus has a morphological form where zygospores are not found in this strain other than that, the colony is grayish brown. Rhizoids are brownish in color. Sporangiospores on stolons up to 1000 µm long, 18 µm wide with local swelling. Columella ellipsoidal with truncated base, height up to 130. This morphology can be seen below:

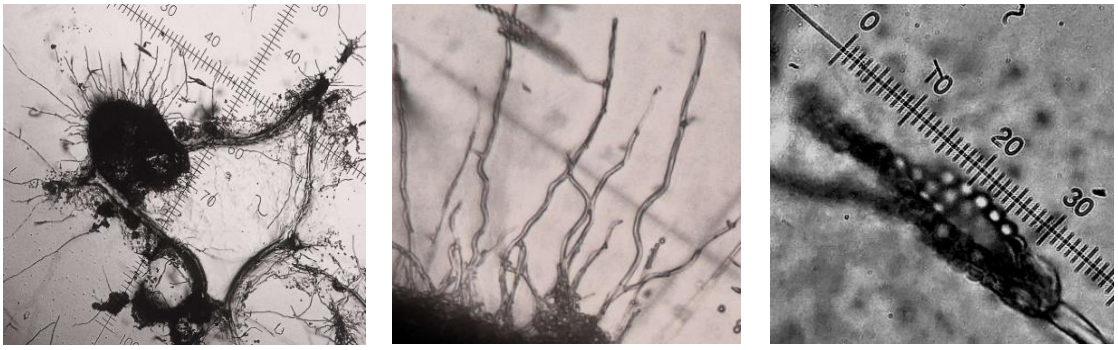


(a) (b)

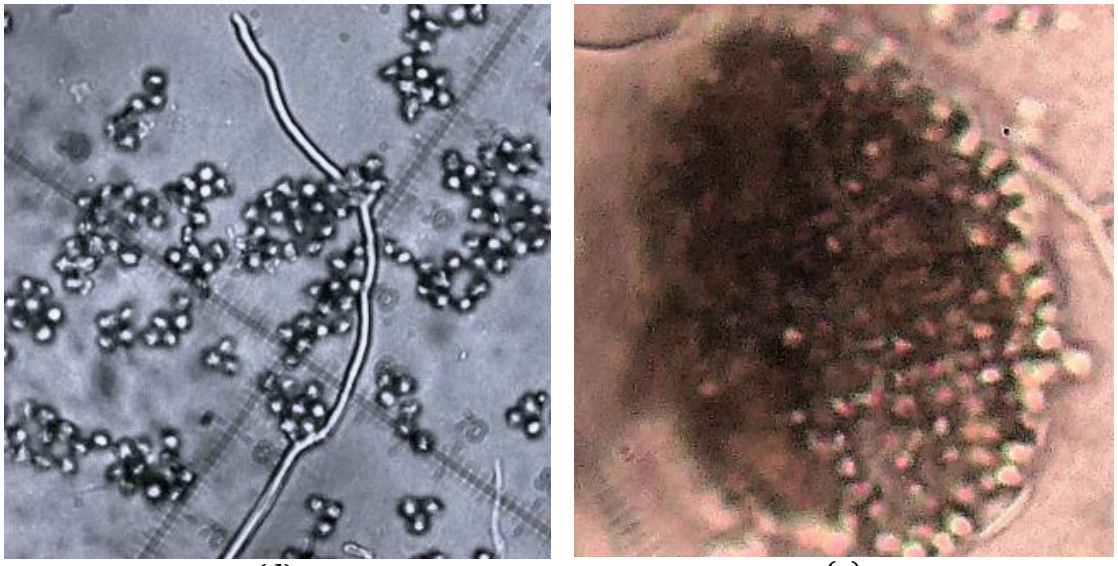
Figure 1. Sporangia, Sporongifor (a) 40x Enlargement (b) 400x Enlargement

Penicillium Brefeldianum with monovertitial conidiophores. Monoversialite conidiophores indicate that each conidiophore forms only one conidia at its tip, in contrast to other types of conidiophores which can form a larger number of conidia or in groups. In addition, sterigmata and conidial chains arise from unbranched conidiophores. In this species, mycelia and hyphae are septate and form a cleistothecium with spores measuring 3.5 μm. that morphology can be seen below:

Diethyl Phthalate (DEP) is the primary compound to be removed in this study by biological treatment method using fungi. This existing concentration value will then become the range limit in the degradation experiment. DEP compounds were analyzed at several sampling location points. This sampling point variation was carried out to observe the relationship between DEP concentration and landfill age. The existing concentration of DEP can be seen in Table 2 below:



(a) (b) (c)



(d) (e)

Figure 5. Morphological Characterization of Penicillium Brefeldianum (a) Hyphae and Conidiophores at 40x magnification, (b) Hyphae, Conidiophores, Metula, Sterigma and Conidium at 100x magnification, (c) Conidiophores, Metula, Sterigma and Conidium at 1000x magnification, (d) Hyphae and Spores at 1000x magnification, (e) Cleistothecium at 1000x magnification.

Determination of the use of fungi in the biodegradation process of 2 successfully identified fungi was carried out by testing the best fungi. This test was conducted by calculating VSS (mg/l) against time. VSS analysis was carried out by gravimetric method or dry weight weighing. The simple concept of this method is that the VSS value is determined from the difference in the weight of filter paper and sample with filter paper without sample. The following in Figure 6 is the growth curve of fungal isolate 1 and fungal isolate 2 obtained from the results of isolation and identification.The presentation of the research results and their discussion must be written in a single unit, after which the data on the research results are discussed in depth with citations in relevant journal articles.

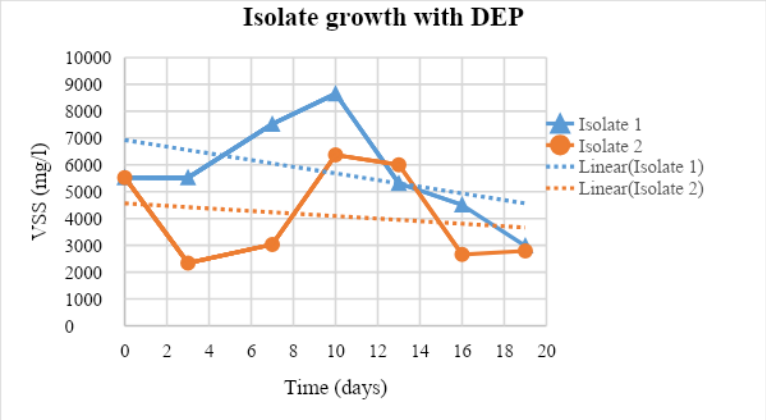


Figure 6. VSS of Fungi with DEP Substrate

From the experiments, it was found that isolate 1 was more able to adapt and use phthalate as a carbon source as seen from the amount of VSS from isolate 1 with the specific growth rate 0.0024/day. Based on the VSS and specific growth rate value the fungus used for the biodegradation process is isolate 1.

The process of isolating and identifying fungi from leachate samples resulted in two fungal species, Rhizopus Arrhizus and Penicillium Brefeldianum, which were selected for further biodegradation studies. These fungi were identified based on their morphological characteristics and previous literature, such as the microbiology concepts discussed by Michael in 1993. Rhizopus Arrhizus exhibited a distinctive morphology, with no zygospores present in this strain, and the colony appeared grayish-brown. Rhizoids, which are root-like structures, were brown in color. Sporangiospores formed on stolons that reached lengths of up to 1000 µm and were 18 µm wide, with localized swelling. The columella was ellipsoidal with a truncated base, measuring up to 130 µm in height. These morphological features are crucial in understanding how the fungus grows and reproduces, which directly affects its ability to degrade DEP in contaminated environments. The genus Rhizopus is known for its efficient enzymatic activity, particularly its ability to produce esterase, an enzyme critical for breaking down the ester bonds in phthalate molecules, making Rhizopus Arrhizus a promising candidate for DEP biodegradation.

Penicillium Brefeldianum, on the other hand, was identified by its monoverticillate conidiophores, meaning that each conidiophore produced only one conidium at its tip. This trait contrasts with other Penicillium species that may produce multiple conidia. Furthermore, Penicillium Brefeldianum had septate mycelia and hyphae, and it formed cleistothecia containing spores that measured 3.5 µm. The ability to form unbranched conidiophores suggests a high reproductive efficiency in challenging environments like landfills. Penicillium species are known for their resilience in harsh conditions, such as those found in landfill leachates, making Penicillium Brefeldianum another strong candidate for DEP degradation. However, further testing revealed that Rhizopus Arrhizus demonstrated superior adaptability in using DEP as a carbon source compared to Penicillium Brefeldianum.

The ability of both fungal species to degrade DEP was tested through Volatile Suspended Solids (VSS) analysis, which measured biomass production over time when the fungi were grown in media containing DEP. The VSS values were calculated using the gravimetric method, which involves weighing filter paper with and without the fungal sample to determine the difference in mass, thus estimating the amount of biomass produced. The experiments showed that Rhizopus Arrhizus (referred to as isolate 1) exhibited a higher VSS value, indicating greater biomass production and a higher specific growth rate of 0.0024/day. This suggests that Rhizopus Arrhizus was more effective at using DEP as a carbon source, likely due to its enhanced enzymatic capabilities, particularly the production of esterase. This ability to degrade DEP efficiently was in line with previous studies that reported similar findings for other Rhizopus species.

The graphical representation of the VSS data (Figure 6) further supported the observation that Rhizopus Arrhizus was more effective in utilizing DEP for growth. The growth curve for isolate 1 showed a consistent increase in biomass over the testing period, while isolate 2, Penicillium Brefeldianum, demonstrated an initial increase in biomass followed by a decline, suggesting that Penicillium Brefeldianum was less efficient in sustaining growth on DEP as a sole carbon source. Based on these findings, Rhizopus Arrhizus was selected for further biodegradation studies due to its

superior performance in degrading DEP and producing biomass. The results indicate that Rhizopus Arrhizus is better suited for DEP biodegradation in environments such as landfill leachates, where phthalates like DEP are prevalent contaminants.

Sub 4 Biodegradation of DEP Compounds by Fungi

Biodegradation experiments were conducted with initial DEP concentration variations of 2 mg/l; 4 mg/l; 6 mg/l; 8 mg/l and 10 mg/l. Concentration analysis was carried out using HPLC with an analysis time of 3 days. The final concentration of DEP during the test period are shown in the following graph:

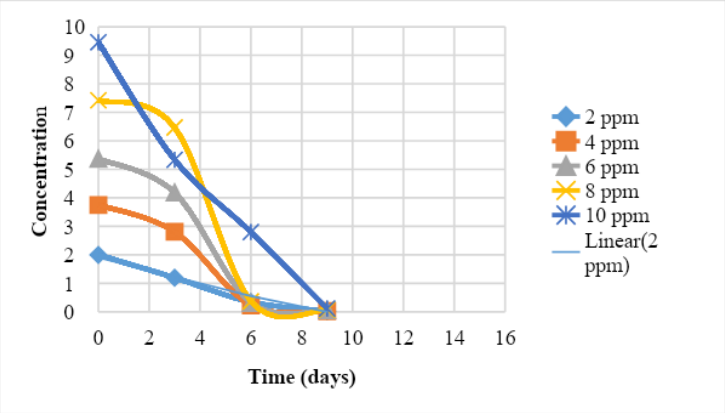


Figure 7. DEP Concentration Decrease Graph against Time

Based on the data obtained, the concentration of DEP during monitoring decreased. This means that the fungus used in the biodegradation process is able to utilize DEP as a carbon source in its growth. Ahuactzin-Perez et al (2016) found that the degradation of 1000 mg/l DEHP in 156 hours. Another study conducted by Marquez (2019) stated that the biodegradation of DEHP compounds using the fungus Fusarium Culmorum was able to remove 100 mg/l in 144 hours with a removal efficiency of 99.6%. In this study, the DEP concentration reached zero on the sixth day across all concentration variations.

Wang (2018) optimized DEP degradation by S. yanoikuyae SHJ under simulated shallow aquifer conditions, achieving complete degradation of 120 mg/L DEP in 36 hours (Y. Wang et al., 2018). Similarly, in this study, DEP concentrations were reduced to 0.23-2.80 mg/L over six days, reflecting an 82-95% degradation rate. Efficiency continued to increase, reaching 98-99% by day 9.

Biodegradation experiments were carried out with different initial DEP concentrations of 2 mg/l, 4 mg/l, 6 mg/l, 8 mg/l, and 10 mg/l to assess the fungi's ability to break down the compound. The DEP concentration was monitored every three days using High Performance Liquid Chromatography (HPLC), and the results demonstrated a consistent decrease in DEP levels over time. This indicates that the fungi utilized DEP as a carbon source to support their growth. In comparison with previous studies, Ahuactzin-Perez et al. (2016) observed the degradation of 1000 mg/l of DEHP within 156 hours, while Marquez (2019) reported that Fusarium Culmorum successfully degraded 100 mg/l of DEHP in 144 hours with a removal

efficiency of 99.6%. In the current study, the DEP concentration reached zero by the sixth day for all concentration variations, which suggests a faster degradation process compared to previous research on similar compounds. Additionally, Wang (2018) achieved complete degradation of 120 mg/l DEP in 36 hours using S. yanoikuyae SHJ in a simulated aquifer environment. This aligns with the present study's findings, where DEP concentrations were reduced to 0.23-2.80 mg/l within six days, reflecting a degradation rate of 82-95%. Furthermore, the removal efficiency continued to improve, reaching 98-99% by the ninth day across all concentration levels. This high efficiency demonstrates the fungi's strong capability to break down DEP and highlights the potential of using these fungi for bioremediation of phthalate-contaminated environments.

Sub 5 Fungal Kinetics

Observations of fungal growth on BSM media as shown in Figure 8 below

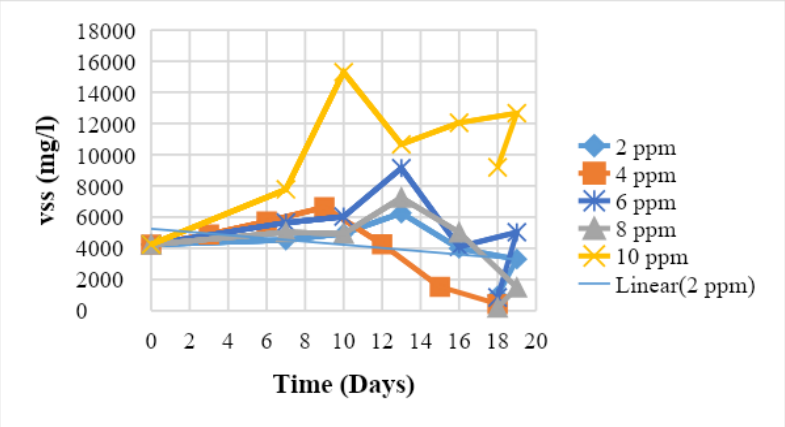


Figure 8. Fungal Growth at Various Concentrations

The figure above shows that the optimal growth occurred in media with the highest DEP concentration of 10 mg/l. This is supported by several previous studies such as Marquez (2019) degrading phthalate with initiation concentrations of 100-500 mg/l, Ahuactzin (2016) degrading phthalate at initiation concentrations of 500 and 1000 mg/l and Yue (2017) degrading phathalate at initiation concentrations of 500 mg/l with removal efficiencies above 95% respectively. The μ value was calculated by comparing the VSS of each concentration variation. The growth rate and Yield value of fungi at each concentration variation are presented in the table below:

Table 4. Specific Growth Rate of Fungi

Concentration Variation (ppm)	Specific Growth Rate (μ/day)	Yield (g VSS/g DEP)
2	0,0531	0.169
4	0,0658	0,543
6	0,0679	0.621
8	0,0812	0,108
10	0,1111	0,089

Ahuactzin-Perez (2016) reported the μ value of Fusarium Culmorum fungi was 0.056/day at a concentration of 500 mg/l and 0.08/day at a concentration of 1000 mg/l. In addition, Marquez's (2019) research showed the μ value in fungal growth with DEP as the only carbon source of 0.06/day. The data obtained in this study are in the range of 0.0531/day – 0.1111/day. Where the higher the concentration of DEP given, the greater the rate of fungi growth per day produced. This is in accordance with previous research conducted by Ahuactzin-Perez (2016) where the μ value at a concentration of 1000 mg/l is higher than the μ value at a concentration of 500 mg/l. The research conducted also obtained the same results where the growth rate at a concentration of 10 mg/l was greater than the growth rate with DEP at a concentration of 2 mg/l.

In addition to the above fungal growth kinetics parameters, fungal growth can be seen from the Y coefficient or commonly known as Growth Yield which can be seen in Table 4. The Y value indicates the amount of substrate converted into new cells. The Y value refers to how efficiently the fungus uses the available substrate in this case DEP to produce biomass. Growth yield is often measured by comparing the amount of biomass or product produced with the amount of substrate used. Metcalf & Eddy (2004) recommend Y values to be in the range of 0.3-0.8 g VSS/g COD for activated sludge. Previous research by Ahuactzin (2016) showed the value of Y in fungi to remove phthalate, which was 0.34 g VSS/g substrat. Meanwhile, the results of other studies by Marquez et al (2019) found a Y value of 0.05 g VSS/g substrat. In this study, the Y value of fungi on DEP substrate was in the range of 0.089-0.621 g VSS/g DEP.

The kinetic analysis of fungal growth in BSM media revealed that the optimal growth occurred in the media with the highest DEP concentration of 10 mg/l, as shown in Figure 8. This observation aligns with previous studies, such as Marquez (2019), who reported successful phthalate degradation at initiation concentrations ranging from 100 to 500 mg/l, and Ahuactzin (2016), who observed degradation at 500 and 1000 mg/l. Similarly, Yue (2017) demonstrated effective degradation of phthalate at 500 mg/l with removal efficiencies consistently above 95%. The specific growth rate (μ) of the fungi was calculated by comparing the Volatile Suspended Solids (VSS) data across different DEP concentration variations. The values ranged from 0.0531/day at 2 mg/l to 0.1111/day at 10 mg/l, as seen in Table 4.

These findings are in agreement with previous reports, such as Ahuactzin-Perez (2016), where the μ value of Fusarium Culmorum at a concentration of 500 mg/l was 0.056/day, increasing to 0.08/day at 1000 mg/l. Marquez's (2019) research further supports these results, indicating a μ value of 0.06/day when DEP was used as the only carbon source. The data from this study shows that the higher the DEP concentration, the greater the fungi's growth rate, which is consistent with the kinetic patterns observed in other fungal studies. This relationship indicates that DEP concentrations directly influence fungal growth, with higher substrate availability leading to increased metabolic activity and growth.

In addition to the specific growth rate, the growth yield (Y) was calculated to measure how efficiently the fungi converted the DEP substrate into new biomass. The

Y values, which represent the amount of substrate utilized to produce biomass, varied across DEP concentrations, ranging from 0.089 g VSS/g DEP at 10 mg/l to 0.621 g VSS/g DEP at 6 mg/l. These values indicate that the fungi were able to efficiently use DEP to grow, with the most effective yield occurring at intermediate concentrations. Previous research by Ahuactzin (2016) reported a Y value of 0.34 g VSS/g substrate for phthalate removal, while Marquez et al. (2019) found a lower yield of 0.05 g VSS/g substrate. The findings in this study are within the expected range for fungal growth, demonstrating that the fungi were capable of efficiently converting DEP into biomass across varying concentrations.

These results suggest that the fungi not only exhibited strong growth kinetics at higher DEP concentrations but also demonstrated efficient substrate utilization, making them suitable candidates for biodegradation processes in environments contaminated with phthalates like DEP. The growth yield and specific growth rates observed in this study provide important insights into how fungal biodegradation processes can be optimized by adjusting DEP concentrations, offering potential applications in environmental bioremediation strategies.

Sub 6 DEP Derived Compounds

The derivative compound tested to ensure the biodegradation process is PA with the concentration obtained after measurement using HPLC in each media of 0.0006 mg/l - 0.73 mg/l. In addition, another parameter that indicates the metabolic activity of microorganisms and their interaction with the substrate is the decrease in acidity (pH) in the media. The results showed that the concentration of 2 ppm with the lowest pH compared to the media at other concentrations on day 15 was 3.72. Research by Matsura et al. (1992) states that Phthalic Acid compounds are produced with a media pH range below 5 and optimum in the range of 2.2-3. In addition to the decrease in pH metabolic processes that indicate the occurrence of hydrolysis and the formation of final products H2O and CO2 can be observed visually characterized by the formation of water vapor in the test media used, particularly in media with a concentration of 2 mg/l.

The biodegradation of DEP resulted in the formation of phthalic acid (PA) as the primary derivative compound, which was measured using High Performance Liquid Chromatography (HPLC). The concentration of PA in the media ranged from 0.0006 mg/l to 0.73 mg/l, confirming that the degradation process successfully broke down DEP into less harmful compounds. Phthalic acid is a known byproduct of phthalate degradation, and its presence is an indicator of the enzymatic activity involved in breaking the ester bonds within the DEP molecule. The detection of PA across all concentration variations suggests that the fungi utilized DEP as a carbon source, leading to its hydrolysis into simpler, non-toxic substances.

Another critical factor observed during the biodegradation process was the decrease in pH, which reflects the metabolic activity of the fungi as they interact with DEP. The pH drop is significant because it indicates the production of acidic byproducts like phthalic acid during the breakdown of DEP. The lowest pH recorded was 3.72 in the media with a 2 mg/l DEP concentration on day 15, demonstrating that the fungi's metabolic processes led to increased acidity as DEP was degraded. According to research by Matsura et al. (1992), phthalic acid is typically produced in environments where the pH is below 5, with optimal production occurring at a pH

range of 2.2 to 3. This aligns with the findings of the present study, where the drop in pH supports the formation of phthalic acid as a key degradation product.

In addition to the decrease in pH, other metabolic indicators of fungal activity were visually observed in the test media. The presence of water vapor, particularly in media with a DEP concentration of 2 mg/l, suggested that hydrolysis was occurring, leading to the production of H2O and CO2 as final products. This visual evidence of water vapor formation further confirms the successful degradation of DEP into benign end products, consistent with the expected outcomes of microbial hydrolysis. These findings demonstrate that the fungi not only degraded DEP but also converted it into non-toxic substances, highlighting the potential of fungal bioremediation for treating DEP-contaminated environments. The combination of PA production, pH decrease, and the formation of H2O and CO2 illustrates the comprehensive breakdown of DEP and supports the effectiveness of the fungi in facilitating this process

Conclusion

Based on the results of research on the biodegradation of Diethyl Phthalate (DEP) compounds using fungi isolated from leachate of Sarimukti Landfill Bandung, it was found that the concentration of DEP in leachate water samples varied, with the highest at the inlet (1.277 mg/l) and the lowest value at zone 4 (0.504 mg/l). The fungus that was successfully isolated and used for the biodegradation process was Rhizopus Arrhizus. The DEP concentration reduction by the fungus reached a high removal efficiency, reaching 98-99% on day 9 at various DEP concentrations with a fungal specific growth rate of 0.0531-0.1111/day

References

Afdal, A., & Sari, R. N. (2017). Characteristics of leachate from Air Dingin Final Waste Disposal Site (TPA), Padang City, West Sumatra. In Proceedings of SNFA (National Seminar on Physics and its Applications) (Vol. 1, p. 8). https://doi.org/10.20961/prosidingsnfa.v1i0.4490

Aguilar-Alvarado, Y., Báez-Sánchez, M. del R., Martínez-Carrera, D. C., Ahuactzin-Pérez, M., Cuamatzi-Muñoz, M., & Sánchez, C. (2015). Mycelial growth and enzymatic activities of fungi isolated from recycled paper wastes grown on Di(2-ethylhexyl)phthalate. *Polish Journal of Environmental Studies*, 24(5), 1897-1902. https://doi.org/10.15244/pjoes/58808

Ahuactzin-Pérez, M., Tlecuitl-Beristain, S., García-Dávila, J., González-Pérez, M., Gutiérrez-Ruíz, M. C., & Sánchez, C. (2016a). Degradation of di(2-ethyl hexyl) phthalate by Fusarium culmorum: Kinetics, enzymatic activities and biodegradation pathway based on quantum chemical modelingpathway based on quantum chemical modeling. *Science of the Total Environment*, 566-567 (May), 1186-1193. https://doi.org/10.1016/j.scitotenv.2016.05.169

Ahuactzin-Pérez, M., Tlecuitl-Beristain, S., García-Dávila, J., González-Pérez, M., Gutiérrez-Ruíz, M. C., & Sánchez, C. (2016b). Degradation of di(2-ethyl hexyl) phthalate by Fusarium culmorum: Kinetics, enzymatic activities and biodegradation pathway based on quantum chemical modelingpathway based on quantum chemical modeling. *Science of the Total Environment*, 566-567, 1186-1193. https://doi.org/10.1016/j.scitotenv.2016.05.169

Ayeni, T. O., Arotupin, D. J., & Ayo, O. E. (2022). Biodegradation of polyethylene by

indigenous fungi from waste recycling site, South West, Nigeria. *Bulletin of the National Research Center,* 46(1). https://doi.org/10.1186/s42269-022-00871-4

Boonyaroj, V., Chiemchaisri, C., Chiemchaisri, W., Theepharaksapan, S., Yamamoto, K., 2012. Toxic organic micro-pollutants removal mechanisms in long-termoperated membrane bioreactor treating municipal solid waste leachate. Biores. *Technol.* 113, 174–180. http://dx.doi.org/10.1016/j.biortech.2011.12.127.

Clara, M., Windhofer, G., Hartl, W., Braun, K., Simon, M., Gans, O., Scheffknecht, C.,Chovanec, A., 2010. Occurrence of phthalates in surface runoff, untreated andtreated wastewater and fate during wastewater treatment. *Chemosphere* 78,1078–1084. http://dx.doi.org/10.1016/j.chemosphere.2009.12.052

Fauzan, R., Harsono, K., Meisandy, R. P., Barokah, M., & Muhaimin, M. I. (2024). Optimising Human Resource Management as an Effort to Improve Employee Performance through Digital Attendance. *Riwayat: Educational Journal of History and Humanities*, *7*(1), 16-25.

Fudala-Ksiazek, S., Pierpaoli, M., & Luczkiewicz, A. (2017). Fate and significance of phthalates and bisphenol A in liquid by-products generated during municipal solid waste mechanical-biological pre-treatment and disposal. *Waste Management*, 64, 28-38. https://doi.org/10.1016/j.wasman.2017.03.040

González-Márquez, A., Loera-Corral, O., Santacruz-Juárez, E., Tlécuitl-Beristain, S., García-Dávila, J., Viniegra-González, G., & Sánchez, C. (2019a). Biodegradation patterns of the endocrine disrupting pollutant di(2-ethyl hexyl) phthalate by Fusarium culmorum. *Ecotoxicology and Environmental Safety*, 170 (December 2018), 293-299. https://doi.org/10.1016/j.ecoenv.2018.11.140

González-Márquez, A., Loera-Corral, O., Santacruz-Juárez, E., Tlécuitl-Beristain, S., García-Dávila, J., Viniegra-González, G., & Sánchez, C. (2019b). Biodegradation patterns of the endocrine disrupting pollutant di(2-ethyl hexyl) phthalate by Fusarium culmorum. *Ecotoxicology and Environmental Safety,* 170 (November 2018), 293-299. https://doi.org/10.1016/j.ecoenv.2018.11.140

He, R., Tian, B.-H., Zhang, Q.-Q., Zhang, H.-T., 2015. Effect of Fenton oxidation onbiodegradability, biotoxicity and dissolved organic matter distribution o concentrated landfill leachate derived from a membrane process. *WasteManage*. 38, 232–239. http://dx.doi.org/10.1016/j.wasman.2015.01.006.

Imran, M. F. (2024). Criminological Examination of Physical and Psychological Violence Committed Against Children in the School Environment. *Riwayat: Educational Journal of History and Humanities*, *7*(1), 41-47.

Jonsson, S.J., Ejlertsson, J., Svensson, B.H., 2003. Transformation of phthalates inyoung landfill cells. *Waste Manage*. 23, 641–651. http://dx.doi.org/10.1016/S0956-053X(03)00099-0

Kalmykova, Y., Moona, N., Strömvall, A.-M., Björklund, K., 2014. Sorption anddegradation of petroleum hydrocarbons, polycyclic aromatic hydrocarbons,alkylphenols, bisphenol A and phthalates in landfill leachate using sand,activated carbon and peat filters. *Water Res.* 56, 246–257. http://dx.doi.org/10.1016/j.watres.2014.03.011.

Kim, Y. H., Lee, J., & Moon, S. H. (2003). Degradation of an endocrine disrupting chemical, DEHP [di-(2-ethylhexyl)- phthalate], by Fusarium oxysporum f. sp. pisi cutinase. *Applied Microbiology and Biotechnology,* 63(1), 75-80. https://doi.org/10.1007/s00253-003-1332-5

Majeed Khan, A., Shaheen, A., Ahmad, I., Malik, F., Hafiz, A., & Shahid, A. (2011). Correlation of COD and BOD of Domestic Wastewater with the Power Output of Bioreactor. *In J.Chem.Soc.Pak* (Vol. 33, Issue 2).

Mo, J., Zhang, Z., Wang, Z., Yu, Z., Li, S., Jiang, S. S., Liu, H., & Ao, J. (2019). Isolation and identification of a psychrotolerant dimethyl phthalate-degrading bacterium from selected frozen soil of high-latitude areas in China and optimization of its fermentation conditions using response surface methodology. *Biotechnology and Biotechnological Equipment*, 33(1), 1706-1720. https://doi.org/10.1080/13102818.2019.1696703

Net, S., Dumoulin, D., El-Osmani, R., Ouddane, B., 2014. Case study of PAHs, MePAHs, PCBs, phthalates and pesticides contamination in the Somme river water, France. *Int. J. Environ. Res.* 8, 1159–1170

Okoli, C. P., Adewuyi, G. O., Zhang, Q., Diagboya, P. N., & Guo, Q. (2014). Mechanism of dialkyl phthalates removal from aqueous solution using γ-cyclodextrin and starch based polyurethane polymer adsorbents. *Carbohydrate Polymers,* 114, 440-449. https://doi.org/10.1016/j.carbpol.2014.08.016

Patil, S. S., & Jena, H. M. (2019). Biodegradation of diethyl phthalate from synthetic wastewater in a batch operated internal loop airlift bioreactor. *International Biodeterioration and Biodegradation*, 143. https://doi.org/10.1016/j.ibiod.2019.104728

Romulo, C. S., & Dalimunthe, Z. (2024). Effect of related party transaction and tax haven utilization on tax avoidance moderated by Country-by-Country reporting. *Riwayat: Educational Journal of History and Humanities*, *7*(1), 26-40.

Royani, S., Fitriana, A. S., Bias, A., Enarga, P., & Bagaskara, Z. (2021). Assessment of Cod and Bod in Water in the Environment of the Kaliori Waste Final Processing Site (Tpa) in Banyumas Regency.

Sastrawidana, I. D. K., Maryam, S., & Sudiana, I. K. (n.d.). 2017. Red Pigments from Fungi Isolated from Soil of Dairy Waste Disposal Site.

Sibali, L.L., Okonkwo, J.O., McCrindle, R.I., 2013. Determination of selected phthalateesters compounds in water and sediments by capillary gas chromatography andflame ionization detector. J. Environ. Sci. Health Part A Toxicol. Hazard. Subst. Environ. Eng. 48, 1365–1377. http://dx.doi.org/10.1080/10934529.2013.781884

Tamyiz, M. (2015). Comparison of Bod/Cod Ratio in Upstream and Downstream Pond Areas on the Biodegradability of Organic Matter. *Journal of Research and Technology*, 1(1).

Tao, Y., Shi, H., Jiao, Y., Han, S., Akindolie, M. S., Yang, Y., Chen, Z., & Zhang, Y. (2020). Effects of humic acid on the biodegradation of di-n-butyl phthalate in mollisol. *Journal of Cleaner Production*, 249. https://doi.org/10.1016/j.jclepro.2019.119404

Wang, Y., Liu, H., Peng, Y., Tong, L., Feng, L., & Ma, K. (2018). New pathways for the biodegradation of diethyl phthalate by Sphingobium yanoikuyae SHJ. *Process Biochemistry*, 71, 152-158. https://doi.org/10.1016/j.procbio.2018.05.010

Wanggai, Jeane. 2023. Biodegradasi Senyawa Diethyl Phthalate dengan

Mikroorganisme Hasil Isolasi dari Air Lindi Tempat Pemrosesan Akhir (TPA) (Studi Kasus : TPA Sarimukti)

Westerhoff, P., Yoon, Y., Snyder, S., & Wert, E. (2005). Fate of endocrine-disruptor, pharmaceutical, and personal care product chemicals during simulated drinking water treatment processes. *Environmental Science and Technology*, 39(17), 6649-6663. https://doi.org/10.1021/es0484799

Zhang, Y., Gao, Y., Xi, B., Li, Y., Ge, X., Gong, Y., Chen, H., Chen, J., Tan, W., & Yuan, Y. (2023). Full life cycle and sustainability transitions of phthalates in landfills: A review. *Waste Management*, 170, 215-229. https://doi.org/10.1016/j.wasman.2023.09.013