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# Exploiting Landfill-Derived *Rhodotorula mucilaginosa* for Simultaneous Biofuel Synthesis and Leachate Remediation

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## ABSTRACT

Landfill leachate, a complex mixture resulting from decomposing waste, contains suspended and dissolved organic and inorganic compounds. This nutrient-rich environment facilitates the growth of diverse microbial communities that can utilize these compounds for sustenance. *Rhodotorula mucilaginosa* is a yeast with great potential in the field of biotechnology due to its ability to utilize diverse substrates and its strong resistance to environmental stress. This study was aimed at investigating the potential of *R. mucilaginosa,* a yeast strain isolated from landfill environments, for biofuel production and simultaneous pollutant reduction in leachate. Batch cultivations were conducted using leachate as the sole growth medium. Cultivation was conducted for 2, 4, 6, and 8 days to analyse the lipids from *R. mucilaginosa* biomass and the degradation of pollutants in the resulting leachate. Additionally, the fuel properties were determined to assess the quality of the biodiesel produced from *R. mucilaginosa* lipids. The obtained quality was compared with the American Society for Testing and Materials (ASTM D6751), the Indonesian National Standard (SNI 8968:2021), and the fatty acid methyl ester (FAME) derived from palm oil. Results demonstrated significant lipid accumulation by *R. mucilaginosa,* reaching 19% (w/w) after 144 hours (6 days) of cultivation. Gas chromatography-mass spectrometry (GC-MS) analysis revealed a FAME profile dominated by C16 and C18 fatty acids, suitable for biodiesel production. Concurrently, substantial reductions in leachate pollutant levels were observed, with decreases of 40.43% for chemical oxygen demand (COD), 86% for phosphate, 90% for ammonia, 53% for nitrate, and 64% for nitrite. These findings highlight the potential of *R. mucilaginosa,* isolated from landfill leachate, as a promising bioremediation agent for wastewater treatment and a sustainable source of lipids for renewable energy production.

**Keywords:** *Rhodotorula mucilaginosa*, lipid, leachate, biofuel, pollutant.

## INTRODUCTION

The increasing global demand for energy, driven by population growth, has resulted in a greater reliance on energy and the depletion of fossil fuel reserves. As a result, there is an urgent need for sustainable and efficient alternative energy sources to address both the growing energy demands and environmental concerns. Biofuels, particularly biodiesel, have emerged as a promising solution in the search for sustainable energy. Biodiesel offers non-toxicity, biodegradability, renewability, and environmental friendliness without contributing to net carbon dioxide emissions. (Singh et al.; Gohain et al., 2020). It can be directly substituted for diesel without requiring engine modifications. Research suggests that biodiesel can reduce carbon dioxide emissions by

78% compared to conventional diesel fuel (West et al., 2008). Currently, soybean and palm oil are the primary feedstocks for biodiesel production in America, Europe, and Asia, processed through transesterification. However, using these first-generation feedstocks, including edible vegetable oils, raises challenges such as higher food prices due to their dual role as food and fuel sources (Osorio-González et al., 2020). Second-generation feedstocks, such as waste cooking oil, animal fats, and inedible oils, offer a renewable alternative, but their long-term availability and associated costs are significant obstacles (Patel et al., 2020).

In the search for more sustainable feedstock sources, oleaginous microorganisms represent a third-generation option. These microorganisms, including bacteria, yeast, fungi, and algae, have lipid compositions similar to those derived from plants or animals (Mondal et al., 2017). They have become commercially viable sources for bio-oil production, with oleaginous yeasts demonstrating impressive lipid conversion capabilities, converting over 20% of their biomass into intracellular lipids in some strains (Kongruang et al., 2020). Ongoing research continues to explore the potential of these microorganisms and their substantial lipid yields (Singh et al., 2020).One of the most urgent challenges facing humanity today is waste management. The amount of waste produced rapidly increases due to rapid industrial expansion, population growth, and economic development. According to the World Bank, global annual waste generation has reached 2.01 billion metric tonnes, and it is projected to rise to 3.40 billion metric tonnes by 2050 (Kaza et al., 2018). Traditionally, a significant portion of this waste has been disposed of through incineration, chemical treatment, discharge into water bodies, or direct dumping into landfills. Shockingly, approximately 40% of the waste generated is left untreated, causing pollution and posing severe risks to the environment, air, water, soil, and human health (Tyagi and Kumar, 2021).

In developing and underdeveloped nations, such as Indonesia, solid waste management remains a significant challenge due to the need for environmentally friendly waste management techniques. In 2020, Indonesia generated 67.8 million tonnes of domestic waste, with food waste, plastic, wood, and paper being the most significant contributors. Food waste accounted for the most considerable portion at approximately 40%, followed by plastic at 17%, and wood and paper each at 16% (Farahdiba et al., 2023). Around 90% of solid waste is estimated to be disposed of in landfills.

Landfilling is often considered the simplest and most cost-effective method for solid waste disposal. However, this practice has significant environmental drawbacks. It contributes to releasing greenhouse gases, such as carbon dioxide ($CO_2$) and methane, which exacerbate global warming. Additionally, the decomposition of waste in landfills produces leachate, a liquid that can contaminate nearby soil and groundwater. Although various methods exist for extracting energy from landfill gas (Munawar and Fellner, 2013), the practice of collecting raw material from landfill leachate for energy stock remains uncommon.

While landfilling is a widely used method for managing solid waste worldwide, it poses a significant environmental challenge due to the generation of landfill leachate. Leachate is a complex liquid formed when water infiltrates and percolates through decomposed waste, causing the leaching of various pollutants (Baderna et al., 2011). It contains high concentrations of organic compounds like humic and fulvic acids and inorganic compounds such as heavy metals, ammonia, and salts. (Tsarpali et al., 2012). These contaminants make leachate a potential hazard, as its untreated discharge into water bodies can significantly degrade the quality of groundwater and surface water, posing risks to aquatic ecosystems and human health.

Leachate composition is complex and varies depending on the age and composition of the landfill. Therefore, effective treatment strategies must be implemented to mitigate environmental risks (Matejczyk et al., 2011; Silva et al., 2004). Organic compounds contribute to high levels of biochemical oxygen demand (BOD) and chemical oxygen demand (COD). At the same time, inorganic pollutants like heavy metals and ammonia can bioaccumulate in the food chain, leading to long-term ecological consequences. Therefore, it is crucial to employ suitable treatment methods to remove or reduce these pollutants before discharging leachate into water bodies. Failing to treat leachate adequately can result in severe water pollution, jeopardizing aquatic ecosystems, contaminating groundwater reserves, and posing risks to public health through potential exposure pathways. Therefore, developing and implementing effective leachate treatment strategies remain essential in responsible solid waste management practices. The unicellular yeast *R. mucilaginosa* belongs to the basidiomycete group of fungi. It is characterized by its distinctive pigmented colonies, ranging from pink to red. As a *basidiomycete, R. mucilaginosa* can thrive in both terrestrial and aquatic environments. This suggests that it has the potential for applications in heavy metal biotransformation processes and the bioremediation of contaminated liquids (Kurtzman et al., 2011). Due to their promising prospects in the field of biotechnology, these species have garnered increasing interest. Notably, oleaginous yeasts like *R. mucilaginosa* have the ability to accumulate significant amounts of triacylglycerols (TAGs) and various fatty acids. The specific composition of these compounds depends on the cultivation medium and the environmental stress conditions during growth (Jarboui et al., 2012; Rajpert et al., 2013). The similarity between the TAG composition produced by these yeasts and that of vegetable oils has encouraged research into their potential as sources for synthesizing fatty acid methyl ester (FAME) blends suitable for biodiesel production (Ruas et al., 2020). Several microorganisms, including *Rhodotorula gracilis, Cryptococcus curvatus, Yarrowia lipolytica, Rhodosporidium toruloides, Trichosporon capitatum, Cunninghamella echinulate, Candida curvata, and Mucor sp*., have been identified as efficient producers of TAGs and diverse fatty acids (Soccol et al., 2017). The remarkable ability of oleaginous yeasts like *R. mucilaginosa* to accumulate lipids has positioned them as promising candidates for sustainable biofuel production from renewable resources.

The primary objective of this research endeavour is to exploit the exceptional adaptability of *R. mucilaginosa* yeast, which demonstrates a dual capability of bioremediating contaminated surroundings and generating lipids for biofuel feedstock. In order to exploit these beneficial characteristics, the study employs a strain of *R. mucilaginosa* extracted from the Blang Bintang landfill in Aceh Besar, Indonesia. The primary aim is to utilize *R. mucilaginosa* obtained from the landfill to decrease the concentrations of pollutants in the leachate produced at the Blang Bintang landfill. This research also seeks to generate lipid-enriched *R. mucilaginosa* biomass, potentially a sustainable biofuel production feedstock. The study aims to contribute to advancing environmentally friendly strategies for waste management, pollution mitigation, and the transition to a more sustainable energy landscape by utilizing this versatile yeast's bioremediation and oleaginous capabilities.

## MATERIALS AND METHODS

### Yeast strain isolation

Leachate samples were collected from the stabilization pond treatment plant situated at the Provincial Domestic Waste Management Unit in Blang Bintang, Aceh Besar District, Indonesia. Aseptic techniques were used to obtain 100 μL samples of leachate, which were then inoculated into YEPGA (yeast extract, peptone, glucose, agar) media in sterile culture dishes. The samples were subsequently incubated at a controlled temperature of 28 °C for a period ranging from two to four days to facilitate microbial growth. Following the incubation period, the culture dishes were visually inspected for the presence of growing colonies. Representative colonies were selected and streaked onto fresh YEPGA agar plates using the quadrant streaking technique to isolate pure colonies. Phenotypic characterization of the isolated colonies was conducted by analysing their morphology, including shape, colour, margin, and elevation. Additionally, Gram staining was performed to characterize the microbial cells further. Yeast cell morphology was observed under a light microscope for detailed analysis (Jia et al., 2023). All observations and results were recorded systematically for subsequent analysis and interpretation.

### Batch cultivation of *R. mucilaginosa* for lipid production and pollutant degradation

The cultivation of *R. mucilaginosa* was conducted in Erlenmeyer flasks using leachate collected from the Blang Bintang landfill in Aceh Besar as the growth medium. Before inoculation, the leachate was diluted with an equal volume of distilled water, resulting in a 1:1 ratio of leachate to water. A 10% (v/v) inoculum of *R. mucilaginosa*, previously cultured in a yeast extract, peptone, and glucose (YEPG) medium, was introduced into 100 mL of sterilized diluted leachate contained within a 250 mL Erlenmeyer flask. An uninoculated flask containing only the diluted leachate served as a control. To investigate the influence of additional carbon sources, glucose was supplemented at 15% and 45% concentrations. The inoculated flasks were then transferred to a shaker incubator maintained at 28 °C with a continuous agitation speed of 140 revolutions per minute (rpm) to ensure proper aeration and homogeneous mixing. Throughout the experiment, all sampling procedures were carried out within a clean bench environment to prevent potential contamination (Irawati et al., 2017). The incubation process was conducted for varying durations of 2, 4, 6, and 8 days.

Upon completion of the designated incubation periods, the *R. mucilaginosa* biomass was harvested through centrifugation at 5000 rpm for 10 minutes, following the protocol described by Liang et al. (2013). The dry biomass content (g/L) was then determined gravimetrically using the method outlined by Uprety et al. (2017). While the supernatant obtained from the cultivation medium was used to analyse the concentrations of ammonia, nitrite, nitrate, phosphate, and the final chemical oxygen demand (COD), the lipid content of the dry *R. mucilaginosa* biomass was quantified using the Bligh and Dyer method (Gohain et al., 2020).

## Analytical procedure

The concentrations of ammonia, nitrite, nitrate, phosphate, and chemical oxygen demand (COD) in the supernatant were assessed using a Shimadzu UV-1800 ultraviolet-visible (UV-Vis) spectrophotometer. The concentration of ammonia was determined using the salicylate method (HACH Method 10031) with a measurement range of 0.4 to 50 mg/L and readings taken at a wavelength of 655 nm. The nitrate concentration was measured using the cadmium reduction method (HACH Method 8029), covering a range of 0.3 to 30 mg/L, with readings at 500 nm. The ferrous sulphate technique (HACH Method 8153) was employed to measure nitrite concentration, valid for concentrations between 2 to 250 mg/L, with measurements taken at 515 nm. For phosphate concentration, 5 mL of the supernatant was transferred to a 25 mL volumetric flask and diluted with mineral-free water up to the mark. The diluted sample was then analysed using the ultraviolet (UV) persulfate oxidation method (HACH Method 8007). The measurement range was 0 to 12.5 mg/L, and readings were conducted at a wavelength of 880 nm. The COD concentration was determined using the reactor digestion and colorimetry method (HACH Method 8000), covering a range of 20 to 1500 mg/L, with readings set at 620 nm. The supernatants were diluted accordingly if the concentrations fell outside the analytical range. The pollutant removal efficiency was calculated using Equation 1 in accordance with (Zhang et al., 2019).

$$Removal\ efficiency = 1 - \frac{C_t}{C_0} \times 100\% \quad (1)$$

where: $C_t$ and $C_0$ denote the concentration of the leachate quality indices (COD, phosphate, nitrate, nitrite, $NH_3$) in parts per million (ppm) at the time of $t$ and 0.

## Biodiesel production

The process of producing FAME from wet cells of *R.mucilaginosa* by direct microwave-assisted transesterification was conducted using an LG MC8289URC microwave oven. A mixture consisting of 5:1:5 v/w/v ratio of methanol, wet cells, and hexane was placed in a 100 mL screw bottle and immersed in a microwave bowl filled with water up to half its volume. This mixture was subjected to microwave irradiation at 900 W for 3 min, with 1 min cycles. Subsequently, 5 mL of methanol and 1% (w/w) NaOH catalyst were added to the mixture, which was then reacted again under microwave irradiation at 900 W for another 3 min and mixed using a vortex. After cooling the mixture to room temperature, 5 mL of warm water was added, and the top phase containing FAME and hexane was separated and evaporated (Siwina and Leesing, 2021). The fatty acid composition of *R.mucilaginosa* was analysed using a Shimadzu GCMS-QP2010 Ultra Gas Chromatography-Mass Spectrometry system equipped with an Rtx column (30 m × 0.25 mm, ID 0.25 μm).

## Determination of biodiesel fuel properties

After GC analysis, the fatty acid profile of *R. mucilagenos*a was utilized to determine the fuel properties of the corresponding biodiesel. The following parameters were calculated using previously established mathematical equations (Hoekman et al., 2012; Munch et al., 2015) Iodine value (*IV*), saponification value (*SV*), cetane number (*CN*), higher heating value (*HHV*), density (*D*), average degree of unsaturation of fatty acids (*AU*), kinematic viscosity (KV), cold filter plugging point (CFPP), and cloud point (*CP*). The *IV*, *SV*, *CN*, *HHV*, and *D* values can be calculated using Equations 2 to 6.

$$IV = \Sigma \frac{254 \times DB \times FC}{M} \quad (2)$$

$$SV = \Sigma \frac{560 \times FC}{M} \quad (3)$$

$$CN = 46.3 + \frac{5458}{SV} - \frac{0.255}{IV} \quad (4)$$

$$HHV = 49.43 - (0.041 \times SV) - (0.015 \times IV) \quad (5)$$

$$D = 0.8463 + \frac{4.9}{M} + (0.0118 \times DB) \quad (6)$$

where: *DB* is the number of double bonds in each FAME component, *FC* is the percentage of each fatty acid component by weight, and *M* is the molecular mass of FAME. *AU* and *KV* were calculated using Equations 7 and 8.

$$AU = \Sigma\, DB \times FC \quad (7)$$

$$KV = -(0.6316 - AU) + 5.2065 \quad (8)$$

Equations 9–11 determined the long-chain saturation factor (*LCSF*), *CFPP*, and *CP*.

$$LCSF = (0.1 \times C10{:}0) + (0.5 \times C18{:}0) \quad (9)$$

$$CFPP = (3.1417 \times LCSF) - 16.477 \quad (10)$$

$$CP = (0.526 \times C16{:}0) - 4.992 \quad (11)$$

The estimated fuel properties obtained were compared with the American Society for Testing and Materials (ASTM D6751), FAME derived from palm oil, and the Indonesian National Standard (SNI 8968: 2021) for biodiesel specifications.

## RESULTS AND DISCUSSIONS

### The isolated oleaginous yeast

In a previous study, we discovered a yeast strain in the leachate of the Blang Bintang landfill that has a remarkable ability to accumulate lipids. This yeast strain became the centrepiece of our current investigation. By conducting a thorough analysis using techniques such as BLAST and phylogenetic tree assessment, we determined that the yeast isolate belongs to the *R.mucilaginosa* strain. This discovery offers valuable information about the genetic lineage of the yeast species we are studying, laying the groundwork for future research to understand its lipid accumulation mechanisms and potential applications in biotechnology.

### Biomass and lipid production

The *R.mucilaginosa* yeast isolate was cultured in a landfill leachate medium. The medium was prepared by diluting the leachate with distilled water at a 1:1 ratio. A starter culture or inoculum was then introduced into the leachate, and growth was monitored at predetermined intervals of 2, 4, 6, and 8 days. To assess growth dynamics, optical density measurements, as well as the determination of cell biomass dry weight and lipid content, were conducted. The growth conditions achieved are depicted in Figure 1. Furthermore, the growth of *R.mucilaginosa* in leachate as a growth medium was investigated by supplementing glucose at concentrations of 15% and 45% (w/v) to evaluate its impact on biomass and lipid accumulation. In Figure 1, the growth curves depict the growth patterns of *R.mucilaginosa* under different nutritional conditions. The L curve represents the growth of *R.mucilaginosa* on landfill leachate media alone, while the L + 15 curve and L + 45 curve illustrate the growth on leachate media supplemented with 15% and 45% glucose, respectively. The results indicate that adding 15% glucose resulted in the highest biomass gain, reaching 0.89 g/L. This suggests that glucose supplementation enhances the growth of *R.mucilaginosa* compared to cultures without glucose, which yielded 0.86 g/L. However, a notable decrease in biomass was observed with the addition of 45% glucose, resulting in a biomass concentration of 0.54 g/L.

These findings highlight glucose as an optimal carbon source for promoting the growth of *R. mucilaginosa*, surpassing other carbon sources such as glycerol and molasses. The observed decrease in biomass at higher glucose concentrations (45%) may indicate a potential inhibitory effect or saturation of the microorganism's metabolic capacity under excessive glucose availability. Overall, understanding the impact of different carbon sources on the growth of *R.mucilaginosa* is crucial for optimizing its performance in various biotechnological applications, including wastewater treatment and bioremediation (Sineli et al., 2022).

In the life cycle of oleaginous yeasts like *R.mucilaginosa*, lipid accumulation progresses through three distinct phases: the balanced growth phase, the lipid accumulation phase, and the reserve lipid degradation phase. During the balanced growth phase, active growth occurs when all nutrients are plentiful. Subsequently, in the lipid accumulation phase, excess carbon sources prompt lipid storage as cell proliferation ceases, and resources are redirected toward lipid synthesis (Dourou et al., 2018).

The search for a substrate that is both cost-effective and sustainable for producing microbial oil is of utmost importance in the fields of biotechnology and industrial microbiology. Organic waste, including agricultural residues, food waste,

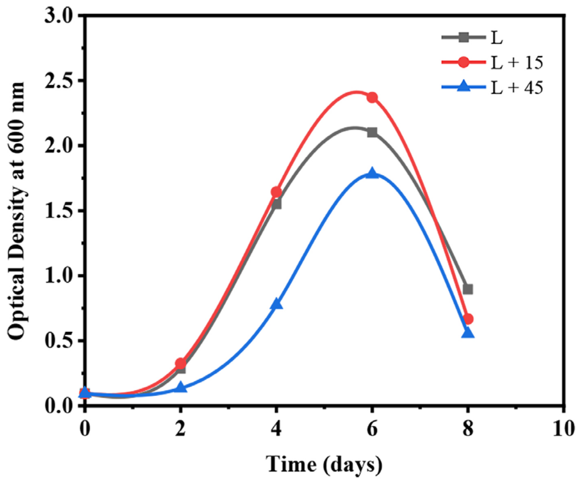


**Figure 1.** Changes in optical density of *R.mucilaginosa* yeast in the experimental media of leachate alone, leachate supplemented with 15 % and 45% glucose

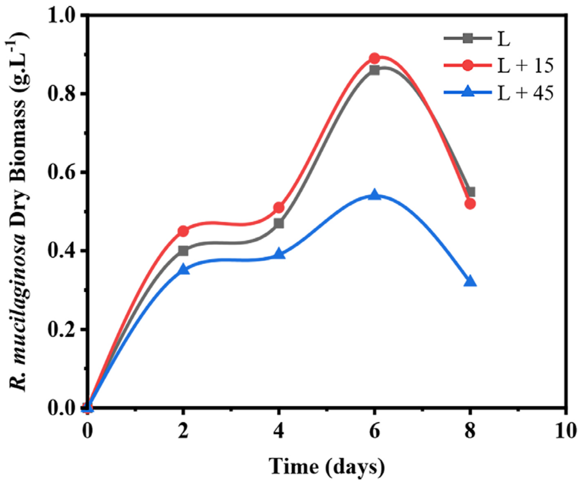


**Figure 2.** Dry biomass weight evolution during the growth of *R.mucilaginosa* in different experimental media of landfill leachate alone and leachate supplemented with 15 % and 45% glucose

and industrial effluents, is an appealing option due to its abundance and potential as a renewable resource for microbial cultivation. By using waste materials as substrates, we can enhance microbial lipid production, thereby contributing to the economic feasibility of large-scale production processes (Tomás-Pejó et al., 2021).

Leachate, a liquid seeping through landfill sites, represents a unique and underutilized resource in this context. Despite its complex composition and potential contaminants, leachate contains organic compounds that can be used as carbon sources for microbial growth. However, optimizing the use of leachate as a substrate requires careful consideration of factors such as nutrient availability, pH, and the presence of inhibitory substances.

The study found that using leachate as a substrate resulted in a biomass yield of 0.89 g/L when supplemented with 15% glucose, as demonstrated in Figure 2. While this yield is noteworthy, it is lower than the biomass obtained using alternative substrates like crude glycerol. For instance, *R.mucilaginosa* IIPL32 achieved a biomass yield of 19.7 g/L when cultivated on crude glycerol (Mishra et al., 2018). This difference highlights the significance of selecting and optimizing substrates to maximize microbial lipid production.

It is important to take a comprehensive approach to enhance biomass yields when using leachate as a substrate. This involves several key strategies. First, it is crucial to optimize the nutrient composition to align with the microbial strain's metabolic requirements. Second, adjusting the pH of the leachate is necessary to create an environment favourable for microbial growth and lipid accumulation. Additionally, detoxification methods are essential to remove inhibitory substances that could hinder microbial growth or lipid production. Furthermore, it is vital to meticulously adjust process parameters such as temperature, agitation, and aeration to support optimal microbial growth and facilitate efficient lipid synthesis. Moreover, genetic engineering techniques offer a promising avenue to enhance the capability of microbial strains to metabolize complex substrates present in leachate or increase their capacity for lipid accumulation. By integrating these multifaceted strategies, researchers aim to maximize the effectiveness and efficiency of microbial lipid production processes that use leachate as a sustainable substrate.

After drying, the biomass was analysed for lipid content, as shown in Figure 3. The figure shows that the highest fat content, 19%, was achieved after six days of cultivation, particularly with the addition of 15% glucose. This significant increase can be attributed to the peak growth observed on day 6. *R. mucilaginosa* likely released more extracellular enzymes during this time to break down complex nutrients into simpler forms, leading to an increase in fat content within the cells. This finding supports previous research conducted by Tsai et al., 2022, which also reported a similar pattern of biomass growth and fat production peaking at six days of incubation,

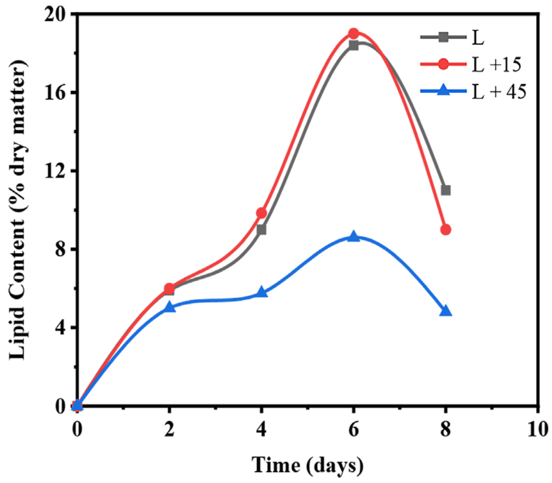


**Figure 3.** Lipid content of *R.mucilaginosa* from different experimental media of landfill leachate alone and leachate supplemented with 15% and 45% glucose

followed by a decline. It is worth noting that *Rhodotorula glutinis* exhibited the highest biomass and fat production after 120 hours of incubation, as observed by Sineli et al., 2022.

The decision to harvest *R.mucilaginosa* biomass on day eight was based on several key factors. Firstly, we observed a noticeable decrease in cell growth, indicating a higher cell death rate than cell proliferation. Secondly, signs of nutrient depletion became evident as the available nutrients were consumed. This was worsened by the increased turbidity of the media, which made it difficult for the cells to take up nutrients. As a result, the cells began to die, and further growth was halted. The optimal incubation period for *R.mucilaginosa* typically ranges from 5 days (Gohain et al., 2020) to 7 days (Ruas et al., 2020). This emphasizes the critical importance of harvesting biomass promptly to maximize both the yield of biomass and fat while minimizing nutrient depletion and cell death.

## Pollutant removal

Year after year, regulatory bodies worldwide are increasing their efforts to combat pollution, driven by a growing recognition of the severe environmental consequences associated with landfill leachate. This growing awareness highlights the urgent need for stricter regulations to reduce pollution levels. In this context, yeast emerges as a particularly promising solution. Its rapid growth surpasses that of molds, making it an attractive candidate for addressing pollution challenges. Additionally, yeast has demonstrated remarkable efficiency in metabolizing substrates, making it a valuable asset in waste remediation strategies. Its well-suited morphology further enhances its appeal, especially for large-scale cultivation projects, positioning yeast as a key player in the ongoing fight against environmental degradation (Arous et al., 2015). This assertion is supported by numerous studies utilizing certain oleaginous yeasts from the genus *Rhodotorula* for wastewater treatment and lipid production. These studies emphasize the diverse utility of *Rhodotorula* yeasts, demonstrating their ability to address environmental concerns while providing opportunities for sustainable lipid production. These findings highlight the potential of utilizing microbial resources for multiple purposes, reinforcing the effectiveness of yeast-based solutions in combating pollution and promoting resource efficiency (Arous et al., 2019).

Fundamental parameters, such as COD, BOD, the BOD/COD ratio, pH, suspended solids (SS), ammonia nitrogen ($NH_3$-N), total kjeldahl nitrogen TKN, and heavy metals, are crucial indicators of landfill leachate characteristics. These parameters form the basis for utilizing leachate as a substrate for cultivating *R. mucilaginosa* growth media, which helps reduce pollutant levels within the leachate. *R. mucilaginosa* has the ability to thrive and decompose compounds found in leachate, using them as essential nutrients for its growth. It specifically assimilates macro elements like carbon, nitrogen, and phosphorus, as well as important microelements such as Cu and Zn in the leachate. This inherent ability of *R. mucilaginosa* to metabolize diverse compounds highlights its potential as a bioremediation agent. It offers a sustainable approach to reducing landfill leachate pollutants while promoting microbial growth. This study evaluates the effectiveness of this process by measuring changes in COD, phosphate, $NH_3$, nitrate, and nitrite values.

Previous studies have demonstrated the potential of *R. mucilaginosa* to degrade pollutants in wastewater (Jarboui et al., 2012; Liang et al., 2021). Exploring the innovative research applications of *R. mucilaginosa* not only expands its utilization across diverse fields but also enhances its versatility for various objectives (Li et al., 2022). Certain microorganisms, called biological micropollutants, can significantly threaten human health when they contaminate water sources. However, these microorganisms also possess an extraordinary capability to transform toxic pollutants into harmless and stable substances. This phenomenon, known as pollutant degradation, aligns with the overarching goal of environmental remediation and fulfils the pressing requirement for contaminant breakdown. As highlighted by Gavrilescu et al. (2015), harnessing the inherent abilities of these microorganisms holds immense potential for mitigating the adverse impacts of pollution on the environment and human well-being.

The effectiveness of *R. mucilaginosa* in removing pollutants from leachate is demonstrated by the decrease in indicators such as COD, phosphate, ammonia, nitrate, and nitrite concentrations. These parameters are important in determining wastewater quality before it is released into the environment. In this study, the initial concentrations of these parameters were as follows: COD 1433 mg/L, phosphate 25.765 mg/L, ammonia 75.54 mg/L, nitrate 237.58 mg/L, and nitrite 123.1 mg/L. The successful reduction rates for COD, phosphate, ammonia, nitrate, and nitrite were found to be 40.43%, 86%, 90%, 53%, and 64% respectively. Figure 4 shows that *R.mucilaginosa* can effectively lower COD, phosphate, ammonia, nitrate, and nitrite levels in leachate. Additionally, the addition of glucose to the growth medium has minimal impact on the degradation of pollutants in leachate, except for nitrate degradation, which increases to 69.3% with a 15% glucose addition. However, this ability decreases after an incubation period exceeding six days, despite *R. mucilaginous* ability to use various carbon substrates such as glucose and xylose (Siwina and Leesing, 2021). *R.mucilaginosa* demonstrates its potential for reducing pollutant levels in olive mill wastewater, notably achieving a significant reduction in COD by 56.91%. This indicates the microorganism's effectiveness in degrading organic compounds present in the wastewater, thereby improving its quality (Jarboui et al., 2012). This underscores the potential of *R. mucilaginosa* as a versatile and efficient agent for pollutant remediation in diverse environmental contexts.

## Biodiesel production

The biodiesel production process using *R.mucilaginosa* biomass as a lipid source is a promising avenue in sustainable fuel production. *R. mucilaginosa* is a yeast species known for its ability to accumulate lipids rich in fatty acids, making it a valuable resource for biodiesel production. The GC-MS analysis of FAME provides insights into the composition of these lipids. As demonstrated in Table 1, the dominance of 9-Octadecadienoic acid (C18:1) in the FAME profile indicates a high content of unsaturated fatty acids desirable for biodiesel due to their low viscosity and good cold flow properties. Additionally, the presence of Hexadecanoic acid (C16:0), Octadecanoic acid (C18:0), and 9,12 Hexadecanoic acid (C18:2) further contributes to the overall fatty acid composition of the biodiesel.

Table 1 presents a detailed comparison of the saturated and unsaturated fatty acid profiles obtained from extracting *R.mucilaginosa* biomass in this study, along with findings from other researchers. Our study found 96.69% saturated and unsaturated fatty acids combined. This closely aligns with the 92.5% reported by Siwina & Leesing (2021) and surpasses the 81.57% reported by Dasgupta et al. (2017). The variation in fatty acid content can be attributed to the different characteristics of the substrates used in each study. Different substrates have unique carbon and nutrient profiles that significantly affect the fatty acid composition of *R. mucilaginosa* biomass.

The relatively low content of polyunsaturated fatty acids in the single-cell oil (SCO) derived from yeast in this study makes it particularly suitable as a feedstock for biodiesel production. It is well-documented that oils rich in polyunsaturated fatty acids are prone to oxidation, making them less ideal for biodiesel applications (Dey et al., 2011).

In comparison, fatty acids produced by other oleaginous species, such as *Trichosporon sp.* (Brar et al., 2017), *R. glutinis* ATCC204091 (Liu et al., 2015), *R. mucilaginosa* IIPL32 (Dasgupta et al., 2017), *R. mucilaginosa* Y-MG1 (Ayadi et al., 2019), and *R. taiwanensis* AM2353 (Miao et al., 2020), mainly consist of long-chain fatty acids with carbon chain lengths of C16 and C18. This pattern is consistent with the fatty acid profiles obtained in our study, further validating the effectiveness of *R. mucilaginosa* as a reliable source of fatty acids suitable for biodiesel production. Comparing the fatty acid composition of biodiesel from *R. mucilaginosa* KKUSY14 with other research findings helps understand the variability in lipid profiles and the potential for optimizing biodiesel production. The similarity of

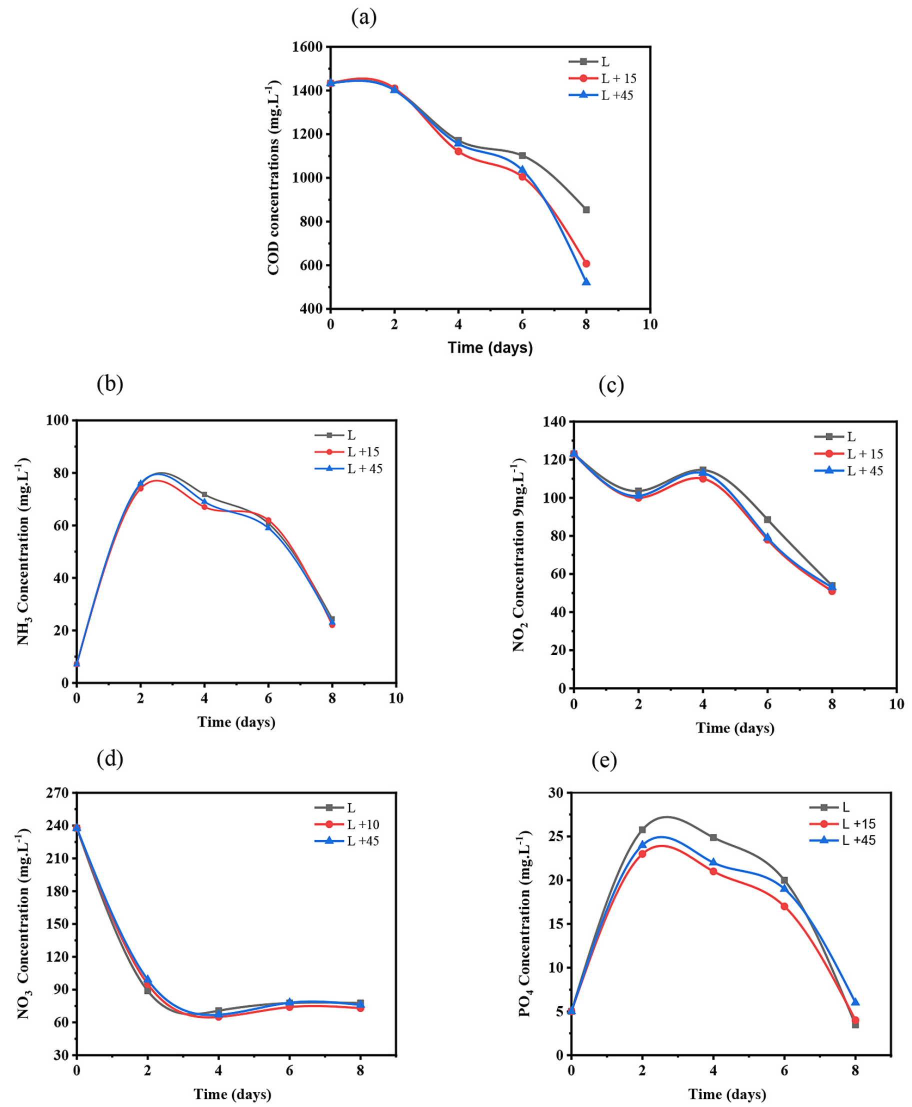


**Figure 4.** Changes in (a) COD, (b) ammonia, (c) nitrite, (d) nitrate and (e) phosphate concentrations

*R.mucilaginosa* fatty acids to vegetable oils underscores its potential as a sustainable alternative to traditional fossil fuels.

The composition of lipids used as biodiesel feedstock plays a crucial role in determining the quality and storage stability of the produced biodiesel. Ideally, lipids should be rich in saturated and monounsaturated fatty acids while containing lower levels of polyunsaturated fatty acids. Monounsaturated fatty acids, particularly C18:1, are beneficial for biodiesel quality as they contribute to good cold flow properties. On the other hand, a higher linolenic acid (C18:3) content can lead to rancidity and thickening of biodiesel during storage over extended periods (Knothe, 2009). In the case of biodiesel produced from *R. mucilaginosa* sourced from Blang Bintang landfill leachate, the absence of other polyunsaturated fatty acids and C18:3 in the fatty acid composition suggests that this biodiesel may exhibit improved storage stability over time. This finding indicates that *R.mucilaginosa* isolated from Blang Bintang landfill leachate in Aceh Besar holds promise as a viable raw material for biodiesel production, offering potential benefits in terms of quality and long-term storage suitability.

In order to ensure that the crude biodiesel produced from the transesterification process meets quality standards, it must undergo further processing to remove any excess water and impurities. In the United States, biodiesel must comply with the ASTM D6751 standard set by the American Society for Testing and Materials. This standard specifies the requirements for B100, which is 100% biodiesel. Similarly, other countries have their own standards. For example, in Indonesia,

**Table 1.** Comparison of fatty acid composition of *R.mucilaginosa*

| Substrate | Fatty acid profile of *R.mucilaginosa* | | | | | | References |
|---|---|---|---|---|---|---|---|
| | C16:0 | C16:1 | C18:0 | C18:1 | C18:2 | C18:3 | |
| Leachate | 15.26 | – | 4.06 | 77.37 | 3.31 | – | This study |
| Glucose | 30.3 | 9.9 | 7.6 | 44.7 | – | – | Siwina et al., 2021 |
| Sugarcane bagasse | 13.14 | 12.85 | – | 55.58 | 18.9 | – | Dasgupta et al., 2017 |
| Cornstalk and wheat straw | 16.62 | – | 1.63 | 69.45 | 5.78 | – | Enshaeieh et al., 2018 |
| Wheat bran | 11.94 | 0.72 | 11.06 | 66.12 | 7.1 | 0.88 | Ayadi et al., 2019 |

biodiesel must adhere to the Indonesian National Standard (SNI 8968:2021)

These standards are essential for ensuring the performance, safety, and environmental benefits of biodiesel. The ASTM D6751 standard covers various properties, including flash point, kinematic viscosity, sulphated ash, sulphur content, copper strip corrosion, cetane number, and free glycerine content, among others. Each of these parameters is crucial to ensure that biodiesel can be used effectively in diesel engines without causing any damage or excessive emissions.

In Indonesia, the SNI sets similar parameters that are tailored to local conditions and the specific types of feedstocks used in biodiesel production within the country. Meeting these standards requires strict quality control during and after the production process. This includes thorough purification steps such as water washing, dry washing with absorbent materials, and the use of ion exchange resins to remove contaminants. Additionally, proper storage and handling practices are necessary to prevent any post-production contamination and degradation of the biodiesel.

Overall, adherence to these national and international standards ensures that biodiesel is a viable and sustainable alternative to traditional diesel fuels. It promotes cleaner combustion and reduces dependency on fossil fuels.

The fatty acid profile of *R. mucilaginosa*-FAME was used to calculate various biodiesel fuel properties, including cetane number (CN), iodine value (IV), higher heating value (HHV), kinematic viscosity (KV), density (D), cloud point (CP), and cold filter plugging point (CFPP). Table 2 shows that the properties of *R. mucilaginosa*-FAME fuel closely resemble those of palm oil. Cetane number (CN) indicates the ignition delay of diesel fuel and biodiesel, with a higher CN corresponding to a faster ignition delay and lower emissions (Knothe, 2009). In this study, the resulting CN was 76.26 higher than that of FAME derived from palm oil having a value of 57.3, attributed to the high saturated fatty acid content of 77.7%. The iodine value (IV) determines the content of unsaturated fatty acids in FAME, with a higher IV indicating lower susceptibility to oxidation. The higher heating value (HHV) represents the amount of heat released by a unit of fuel after complete combustion. The study yielded an estimated HHV of 41.63 MJ/kg.

Kinematic viscosity (KV) plays a crucial role in the fuel injection process during combustion. Higher KV values are typically associated with lower unsaturated fatty acid content (Knothe, 2009). *R. mucilaginosa*-FAME exhibited a KV of 4.6 mm/s, which meets the ASTM D6751 standard limit. This study's

**Table 2.** Fuel properties of *R. mucilaginosa*-FAME (this study) and palm oil-FAME (Benjumea et al., 2008) compared to ASTM D6751 and SNI 8968:2021 standard

| Fuel properties | *R.mucilaginosa*-FAME | Palm oil-FAME | ASTM D6751 | SNI 8968:2021 |
|---|---|---|---|---|
| Cetane number | 76.29 | 57.3 | ≥40 | Min.70 |
| Iodine value (gi$_2$/100 g) | 61.23 | – | Not specified | Not specified |
| Higher heating value (MJ/kg) | 41.63 | 39.837 | Not specified | Not specified |
| Density (g/cm$^3$) | 0.886 | 0.864 | Not specified | 0.85–0.89 |
| Kinematic viscosity (mm/s) | 4.6 | 4.71 | 1.9–6 | 2.5–4.5 |
| Cloud point (°C) | 5 | 16 | – | Max.18 |
| Cold filter plugging point (°C) | 14.59 | 12 | Not specified | – |

estimated density obtained was 0.886 g/cm$^3$, aligning with the SNI standard. The cold flow properties of biodiesel, defined by cloud point (CP) and cold filter plugging point (CFPP), depend on the chain length and saturation of the acid content. Elevated cold flow properties can impede engine starting at low temperatures. *R. mucilaginosa*-FAME contains 19.6% SFA (C16:0 and C18:0), potentially contributing to its cold flow properties. According to the estimated CP of *R. mucilaginosa*-FAME at 5 °C and CFPP at 14.59 °C, the formation of wax crystals and clogging of engine filters may occur at low temperatures, limiting the use of biodiesel in cold climates. However, to address this issue, biodiesel can be blended with a suitable depressant or diesel fuel, as suggested by (Jeong et al., 2008).

## CONCLUSIONS

This study investigated the challenge of sustainable energy production by examining biofuel alternatives, specifically focusing on the utilization of *R. mucilaginosa* yeast isolated from the Blang Bintang landfill in Aceh Besar. The study has two main objectives: to reduce pollutant levels in landfill leachate and to generate biomass suitable for biofuel production. The findings demonstrate that *R. mucilaginosa* has the ability to accumulate fat, with levels reaching up to 19%, which meets the requirements for biodiesel production. The analysis of the FAME profile indicates that C16 and C18 fatty acids are dominant, further confirming its potential as a biodiesel feedstock. Furthermore, the study successfully decreased pollutant levels in the leachate. Notable reductions were observed in COD, phosphate, ammonia, nitrate, and nitrite, with percentages of 40.43%, 86%, 90%, 53%, and 64%, respectively. These results underscore the dual benefits of using *R. mucilaginosa*: it serves as both a renewable energy source and an effective method for remediating wastewater pollutants. In conclusion, *R. mucilaginosa* isolated from landfill leachate emerges as a promising solution for addressing energy and environmental challenges. Its ability to degrade pollutants in wastewater while simultaneously serving as a raw material for renewable energy production highlights its versatility and importance in sustainable resource management.

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