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Potential application of *Rhodotorula mucilaginosa* derived from landfill leachate in sustainable biofuel production and wastewater treatment

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ABSTRACT

Landfill leachate, a byproduct of waste decomposition, poses significant environmental challenges owing to its complex organic composition, which facilitates microbial growth. Although classified as wastewater, landfill leachate contains organic compounds that promote microbial proliferation, rendering it a promising source for identifying yeasts with valuable metabolic capabilities. This study focused on isolating and analysing yeasts from leachate collected from the Blang Bintang landfill in Aceh Besar, Indonesia, specifically targeting their dual potential for biofuel production and wastewater treatment. *Rhodotorula mucilaginosa* was identified as the principal yeast strain using morphological and molecular techniques, including polymerase chain reaction and electrophoresis. When cultured in a nutrient-rich medium (yeast extract, peptone, glucose, and agar), *R. mucilaginosa* exhibited notable metabolic capabilities, a fat content of 21.6%, indicating its potential as a biofuel feedstock. Furthermore, trials employing synthetic wastewater demonstrated its capacity to significantly reduce pollutants, achieving reductions of 73.22% for phosphates, 43.17% for ammonia, 77.17% for nitrates, 18.75% for nitrites, and an impressive 96.36% for chemical oxygen demand. These findings underscore the dual functionality of *R. mucilaginosa* as a viable biofuel feedstock and an effective agent for wastewater treatment. This study suggests opportunities for developing integrated systems combining biofuel production and wastewater treatment, enhancing resource efficiency, and promoting environmental sustainability. This approach addresses energy needs while mitigating ecological pollution arising from leachate, presenting a comprehensive solution to two significant environmental challenges.

Keywords: biofuel, landfill leachate, lipids, *Rhodotorula mucilaginosa*, wastewater treatment.

INTRODUCTION

The dual challenges posed by climate change and the depletion of fossil fuels have catalysed extensive research into alternative energy sources. The escalating emissions of greenhouse gases have exacerbated global climate change, resulting in significant environmental degradation and adverse health effects in various species. In response to this pressing threat, an international consensus has emerged on the necessity of transitioning from finite carbon-based energy sources to renewable alternatives, including biofuels, solar, and wind power – critical options that offer the potential for sustainability and a reduced ecological footprint.

Biofuels are increasingly viewed as a promising renewable energy option capable of transforming the energy landscape [Panarello and

Gatto, 2023]. They offer several advantages, including carbon neutrality, compatibility with existing infrastructure, and the potential for domestic production, which reduces reliance on oil imports. Biofuels provide a pragmatic approach to decarbonisation in the transportation and industrial sectors without necessitating immediate, large-scale system changes. Nevertheless, first-generation biofuels, primarily sourced from crops like corn and sugarcane, have faced criticism regarding their carbon footprint due to associated land-use changes and intensive farming practices. Furthermore, the "food vs. fuel" debate raises ethical concerns about using arable land for fuel production, which could potentially undermine global food security [Fasanya et al., 2021]. This complex intersection of environmental, economic, and ethical issues has prompted a shift in research towards advanced biofuels made from non-food biomass, agricultural residues, and microbial systems that can produce biofuel precursors without competing with food needs.

Yeasts have emerged as particularly promising candidates for next generation biofuel production, offering several distinct advantages over conventional feedstocks. Their lipid composition closely resembles traditional plant oils, making them highly suitable for conversion into biodiesel that meets industry standards. Unlike crop-based feedstocks, yeasts exhibit rapid growth rates and can be cultivated year-round, independent of climatic conditions, ensuring consistent production and a stable supply chain. Furthermore, yeasts demonstrate remarkable substrate flexibility, effectively utilising a wide range of feedstocks, including agricultural and industrial waste streams. This adaptability aligns with circular economy principles by valorising waste materials while enhancing both the sustainability profile and economic viability of yeast-based biofuels. These combined advantages position yeasts as compelling alternatives that address the limitations of traditional crop-based biofuels through faster biomass accumulation, flexible cultivation requirements, and potential for genetic optimisation.

This international effort seeks to pave the way for a future that emphasises environmental stewardship and resilience. The ultimate objectives are to reduce the negative impacts of a warming planet and protect the shared environment for current and future generations. Although this crisis presents a significant challenge, a blend of optimism, innovation, and cooperation makes the transition to sustainability a more achievable goal. These efforts focus on implementing the systemic changes required for a sustainable future, prioritising climate actions that uphold human dignity. The most effective strategy for transformative environmental preservation involves fostering global partnerships to achieve sustainability.

Numerous studies have substantiated the viability of yeasts as effective biofuel feedstocks, demonstrating their diverse capabilities for lipid production. Research has revealed a wide range of lipid contents across various yeast species: *Rhodotorula glutinis* R4 produces approximately 7 g/L of lipids [Maza et al., 2020], *Cutaneotrichosporon oleaginosum* reaches 15.6 g/L [Liu et al., 2022], whereas *R. mucilaginosa* LP2 accumulates up to 41.3% of its dry weight as lipids [Liang et al., 2021]. Other notable examples include *Rhodotorula glutinis* at 35.15% [Fernandez-San Millan et al., 2020], *Rhodotorula toruloides* at 29.9% [Ruas et al., 2020], and *Candida tropicalis*, which boasts an impressive 79% lipid content [Dias et al., 2021]. In addition to their lipid-producing capacity, yeasts offer several advantages, including rapid growth rates, the ability to be cultivated on low-cost substrates, and the ease of cell separation from cultures [Khoo et al., 2023]. These findings highlight the significant potential of yeast species as efficient sources of biofuel, contributing to sustainable energy solutions [Wusnah et al., 2023]. Although various methods exist for treating municipal solid waste, including thermochemical and biological treatments [Liang et al., 2021; Liu et al., 2022], landfill disposal and incineration remain the most commonly used approaches [Zhou et al., 2017]. However, landfilling inevitably produces secondary pollutants, specifically leachate [Zhou et al., 2017; Dias et al., 2020], which is a complex, high-concentration organic wastewater containing various inorganic pollutants, including humic substances, heavy metals, inorganic salts, ammonia, and nitrogen [Zhou et al., 2017; Teng et al., 2021; Igwegbe et al., 2024]. Paradoxically, the composition of leachate, although environmentally concerning, presents an opportunity for microbial growth and potential biotechnological applications [Chem et al., 2021, Kinnunen and Hedrich, 2023], as it contains organic and inorganic materials that serve as nutrients for various microorganisms [Kinnunen and Hedrich, 2023] and promote microbial growth within the leachate environment [Wusnah et al., 2024].

Recognising this dual potential, researchers have begun exploring the utilisation of microbial communities from leachate for both waste treatment and biofuel production [Mishra et al., 2018; Ilmasari et al., 2023; Sartja et al., 2023; Teshnizi et al., 2025]. However, a significant knowledge gap remains regarding the specific capabilities of yeast strains isolated from landfill leachate in Indonesia, particularly their dual functionality in biofuel production and pollutant degradation. This study addresses this gap by focusing on the isolation, identification, and characterisation of *Rhodotorula mucilaginosa* from Blang Bintang landfill leachate in Aceh Besar, Indonesia. The goal was to identify yeast strains that could be used as raw materials for biofuel production while also demonstrating their ability to break down chemical compounds in simulated waste. This two-pronged approach aims to meet the growing demand for sustainable biofuel sources and address the pressing need for effective waste management solutions. By examining yeasts from landfill leachate, this study explores a unique connection between waste valorisation and renewable energy production. The discovery of yeast strains that can accumulate lipids and degrade pollutants could play a crucial role in creating more sustainable and integrated systems for waste management and biofuel production.

MATERIALS AND METHODS

Leachate sample collection and yeast strain isolation

Leachate samples taken from the Blang Bintang landfill in Aceh Besar, Indonesia, were used to isolate a novel oleaginous strain. The sampling procedure involved the vertical extraction of leachate using a sterile 1 L plastic container. Subsequently, 20 mL aliquots of filtered leachate were transferred into sterile sample bottles. To preserve the samples, 2–3 drops of Lugol's solution were added to each sample and gently mixed for 5–10 seconds. The labelled samples were then transported in a Styrofoam container filled with ice to maintain a low temperature. Before isolation, all materials and equipment were sterilised by autoclaving at 121 °C for 20 min [Gizaw et al., 2016]. Yeast was isolated using a medium consisting of yeast extract (1 g/L), peptone (1 g/L), glucose (10 g/L), and agar (13 g/L), referred to as yeast extract peptone glucose agar medium (YEPGA), which was prepared by dissolving the components in distilled water. The medium was sterilised by autoclaving at 121 °C for 20 min. Two distinct isolation techniques were employed: streak and paste plating. In the Streak Plating method, an inoculating loop is immersed in the leachate sample and subsequently streaked across the surface of the solidified agar medium. Conversely, in the pour-plating method, approximately 0.5 mL of the leachate sample was pipetted onto the centre of the agar medium surface and evenly distributed using a sterile spreader. Following inoculation, Petri dishes were sealed with Parafilm and incubated at 28 °C for 2–3 days. All plating procedures were performed under aseptic conditions under a laminar flow hood.

Morphological and molecular identification

Morphological observations of the isolates were conducted under a light microscope. Colonies grown on solid media were carefully examined to determine their shapes and colours. Each distinct colony was marked on the exterior of the Petri dish to facilitate subsequent subcultures. The subculture process involved transferring colonies to fresh solid media, after which the Petri dishes were sealed with Parafilm and incubated at 28 °C. This process was repeated until axenic cultures were obtained. Axenic cultures are characterised by uniform cell morphology and the absence of contaminating microorganisms. The isolated strain was designated *Rhodotorula mucilaginosa* Lindi-2-Doktor Ilmu Teknik (LD-2-DIT). The detailed morphological characteristics were examined using a light microscope (Olympus Model BX41TF). The observed features were compared with descriptions in the existing literature to aid identification based on morphological criteria.

Deoxyribonucleic Acid (DNA) extraction was conducted using the Zymo Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005). The extracted DNA was subsequently amplified through polymerase chain reaction (PCR) using 2x MyTaq HS red mix (Bioline, BIO-25048). Primers targeting the 18S recombinant ribonucleic acid (rRNA) gene were designed: 18S_E1772R (5'-CWDCBGCAGGTTCACCTAC-3') and E18F (5'-GATCCMGGTTGATYCTGCC-3'). The PCR reaction mixture, totalling 25 microliters (µL), comprised 12.5 µL of PCR master mix, 1 µL of each primer at 10 micromolar (µM), 1 µL

of yeast DNA template, and nuclease-free water to achieve the final volume. PCR amplification was executed under the following conditions: initial denaturation at 95 °C for 15 seconds, followed by 35 cycles of denaturation at 95 °C for 15 seconds, annealing at 56 °C for 30 seconds, and extension at 72 °C for 45 seconds. The resultant PCR products were sequenced and compared to known sequences using the BLAST tool provided in the GenBank public database.

R. mucilaginosa growth in yeast extract peptone glucose (YEPG) medium and synthetic wastewater

Pure isolates of *R. mucilaginosa* were cultured and propagated in a YEPG medium at 28 °C. To prepare starter cultures for liquid media cultivation, four colony strips of the isolate were inoculated into separate 100 mL aliquots of sterile liquid medium. The cultures were then incubated for 48 hours at 120 revolutions per minute (rpm) in a shaking incubator under 3000-lumen illumination. Subsequently, aliquots of the resulting starter cultures were inoculated into a fresh liquid medium for further cultivation. The cultures were monitored at 0, 2, 4, 6, and 8 days of incubation to track growth and metabolite production over time.

The cultivation of *R. mucilaginosa* in synthetic wastewater (SW) served two primary objectives: (1) to evaluate its efficacy in reducing pollutants in artificial wastewater and (2) to assess its biomass production capacity. Pollutant reduction was analysed by measuring changes in chemical oxygen demand (COD) as well as concentrations of phosphate (PO_4), ammonia (NH_3), nitrate, and nitrite. The composition of the artificial wastewater is presented in Table 1 [Jiang et al., 2019]. Furthermore, to investigate the effects on biomass yield and pollutant degradation efficiency, *R. mucilaginosa* was cultured in SW supplemented with 10% glucose (SW+10) [Prabhu et al., 2019]. This comparative approach aimed to elucidate the influence of glucose supplementation on biomass production and the organism's capacity to degrade pollutants in artificial effluents.

Analytical methods

Cell density was assessed spectrophotometrically at 600 nanometres (nm) (A600) using a BIOCHROM WPA Biowave Spectrophotometer. The culture medium was centrifuged at 4000 rpm for 10 min to ascertain the dry biomass weight. The resultant wet biomass was washed and dried at 60 °C for 12 h. All measurements were performed in triplicate. The lipid content in the dry biomass was determined using a modified Bligh and Dyer method [Saini et al., 2021]. A 0.5 g sample was dissolved in 100 mL of distilled water for 0.5 to 1 minute, followed by thermal treatment at 100 °C for 5 minutes to disrupt cell walls. Total intracellular lipid extraction was performed using a chloroform-methanol solvent mixture in a 1:1 volumetric ratio, ensuring efficient lipid solubilisation. The sample was centrifuged at 6500 rpm for 5 min to separate the mixture into an upper aqueous phase and a lower chloroform-rich phase, which was isolated using filter paper. The lower phase was re-extracted with 5 mL of chloroform to increase lipid recovery and centrifuged again. The final extract was filtered and quantitatively assessed by gravimetry, which involved evaporating the solvent to accurately measure the total lipid content.

Measurement of wastewater quality

The liquid growth medium solution of *R. mucilaginosa* was centrifuged at 4000 rpm for 10 min. After filtration, the supernatant was used to determine the COD, phosphate, ammonia, nitrate, and nitrite contents. The analytical methods used were based on the HACH method [Moretti et al., 2021]. The pollutant removal efficiency was calculated using Equation 1 [Jiang et al., 2019]:

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

where: C_t and C_0 denote the concentrations of artificial wastewater quality indices (COD, phosphate, ammonia, nitrate, and nitrite) in parts per million (ppm) at time t, and 0.

All wastewater quality parameter measurements were performed in triplicate (n = 3), and results are expressed as mean ± standard deviation. The detection limits and analytical precision for each method were validated according to standard quality control procedures.

Data analysis

All collected data are provided as mean values with their respective standard deviations and standard errors. Descriptive statistical studies

Table 1. Composition of the synthetic wastewater

Chemical compounds	Concentration as (mg/L)
Glucose	300
Ammonium chloride (NH_4Cl)	22.2
Potassium nitrate (KNO_3)	5.7
Sodium nitrite ($NaNO_2$)	1.1
Monopotassium phosphite (KH_2PO_3)	5.1
Boric acid (H_3BO_3)	2.86
Manganese (II) chloride tetrahydrate ($MnCl_2.4H_2O$)	1.81
Zinc sulphate heptahydrate ($ZnSO_4.7H_2O$)	0.222
Sodium molybdate dihydrate ($NaMoO_4.2H_2O$)	0.39
Copper sulphate pentahydrate ($CuSO_4.5H_2O$)	0.079
Cobalt nitrate hexahydrate ($Co(NO_3)_2.6H_2O$)	0.0494

were performed to summarise the central tendency and variability of essential data, including lipid content and pollutant removal. The computation of means, standard deviations, and standard errors was performed using spreadsheet software. Standard errors were calculated to indicate the precision of the mean estimations and are represented as error bars in all graphical displays. These statistical indicators collectively provide a comprehensive representation of data repeatability, reliability, and the uncertainty associated with the mean values.

RESULTS AND DISCUSSION

Morphological and molecular characterisation of strain LD-2-DIT

A Stereomicroscopic examination of the LD-2-DIT isolate revealed a distinctive colony morphology characterised by a central core surrounded by orange filaments. Figure 1 provides a comprehensive visual representation of the isolate's morphology, utilising three complementary imaging techniques: colony growth on solid YEPG medium, light microscopy, and scanning electron microscopy (SEM). Colonies grown on solid YEPG media (Figure 1a) exhibited a vibrant orange colour, a hallmark of *Rhodotorula* species. This distinctive colouration is due to the production of carotenoid pigments, which not only give the yeast its characteristic appearance but also play a role in protecting the cells from environmental stresses, such as UV radiation. The colonies consistently demonstrated a round shape with flat edges and glossy surfaces, typical of *R. mucilaginosa*. These observations were consistent with the findings of Ruas et al. [Ruas et al., 2020], who reported robust colony growth at 28 °C after 48 h of incubation.

Light microscopy at 400x magnification (Figure 1 b) revealed individual yeast cells that appeared ovoid to spherical in shape, consistent with the known morphology of *R. mucilaginosa*. The absence of hyphal structures confirmed the yeast's unicellular nature. The relatively uniform size of the cells suggests a healthy, actively growing culture. This morphological characteristic closely resembles that of *Rhodotorula mucilaginosa* strain S05, a novel strain identified by Gohain et al. [Gohain et al., 2020] capable of producing 22.21% lipids. The SEM image at 3000x magnification (Figure 1c) provides intricate details of the cell surface structure. The cells showed a smooth surface texture typical of *Rhodotorula* species. This high-resolution image allows for observing potential budding sites characteristic of asexual reproduction in yeasts. The three-dimensional perspective offered by SEM helps understand the spatial arrangement and interaction between individual cells. LD-2-DIT colonies exhibited optimal growth after a 2- to 3-day incubation period at 30 °C, a temperature conducive to the proliferation of lipid-producing yeasts. This temperature range was slightly higher than that reported in Ruas et al. [Ruas et al., 2020], which could be attributed to strain-specific adaptations or variations in culture conditions. It would be interesting to explore the effects of temperature on lipid production in future studies. Molecular analyses of the LD-2-DIT isolate were performed to complement and confirm the morphological observations. PCR amplification of the 18S rDNA of the isolate yielded two distinct fragments, as

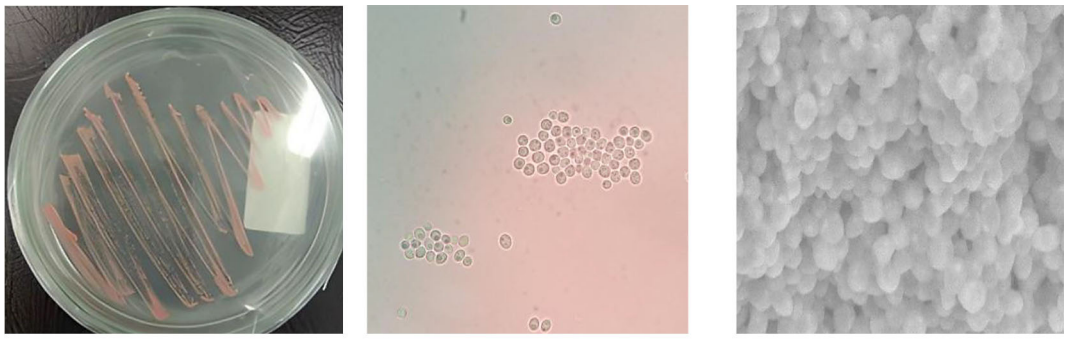

Figure 1. Characteristic cell morphology of *R. mucilaginosa.* (a) colony growth on solid YEPG media; (b) Cell morphology using a microscope with a magnification of 400 times; (c) overview of cell morphology using scanning electron microscopy (SEM) with a magnification of 3.000 times

shown in Figure 2. The observed fragments corresponded to the expected theoretical size, with the presence of single clear bands indicating optimal amplification conditions and high primer specificity for the target DNA. The clarity and specificity of these bands suggest several essential points. First, it showed high primer specificity, effectively targeting the desired regions of the 18S rDNA gene without non-specific binding. Second, the PCR conditions (temperature cycles, reagent concentrations, etc.) were well optimised. Lastly, the clear bands suggested that the extracted DNA was highly quality and free from significant contamination. The use of 18S rDNA for identification is particularly valuable because this gene is highly conserved among eukaryotes but contains variable regions allowing species-level discrimination. The successful amplification of these regions in our isolate provides a strong foundation for subsequent phylogenetic analysis. These primers, designed based on the *Rhodotorula* 18S rDNA gene sequence, effectively distinguish *Rhodotorula* from other yeast genera.

Phylogenetic analysis was performed using a 3000 bp sequence compiled from the two amplified fragments. As shown in Figure 3, the resulting phylogenetic tree offers crucial insights into the evolutionary relationships between our LD-2-DIT isolate and known yeast species. The tree revealed that the isolate LD-2-DIT shared the closest evolutionary relationship with *Rhodotorula mucilaginosa*, as supported by a robust bootstrap value of 99.77%. This high bootstrap value provides strong evidence of their close evolutionary relationship, significantly strengthening morphology-based identification. The next closest relative was identified as *Rhodotorula glutinis*, with a bootstrap support of 99.65%. While *R. glutinis* appeared as the next closest relative, the tree clearly distinguished LD-2-DIT as being more closely related to *R. mucilaginosa*. This distinction is essential because *R. glutinis* is another common *Rhodotorula* species with similar morphological characteristics.

The phylogenetic tree also provides a broader evolutionary context, showing the relationship of the isolate not only with other *Rhodotorula* species but also with more distantly related yeasts. This context helps us understand the unique characteristics and potential capabilities of *R. mucilaginosa* compared to other yeast species. *R. mucilaginosa* is classified as a unicellular basidiomycete yeast,

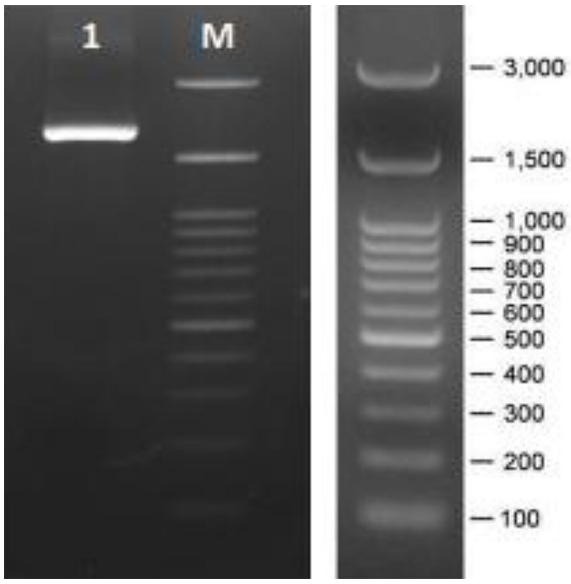

Figure 2. Amplification results of LD-2-DIT isolate

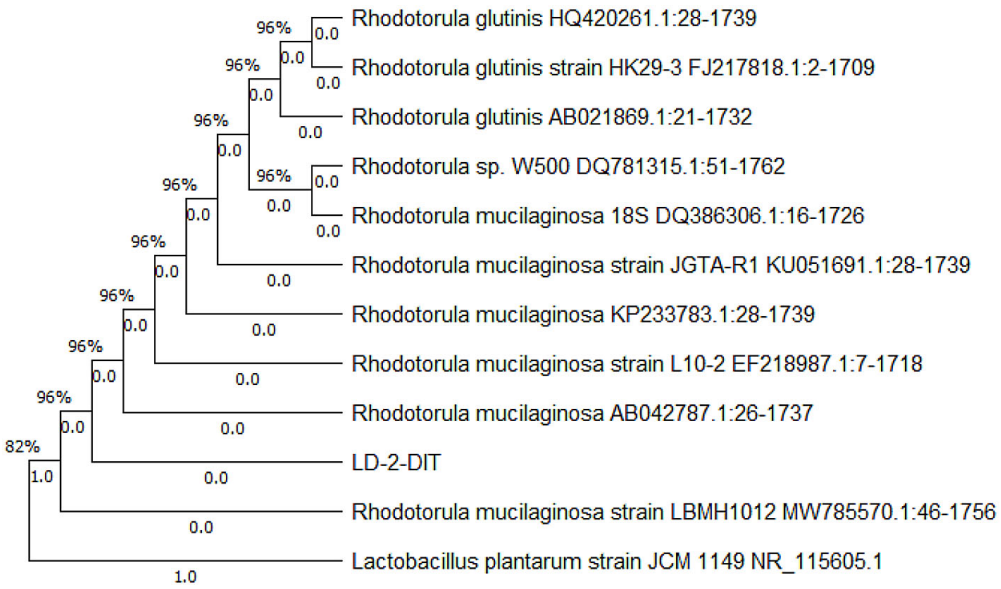

Figure 3. Molecular analysis of *R. mucilaginosa* phylogenetic tree using 18s rDNA sequences

characterised by its pink-to-red pigmented colonies. This species demonstrates remarkable adaptability and is capable of thriving in both terrestrial and aquatic environments [Ruas et al., 2020]. The high degree of similarity between the LD-2-DIT isolate and known *R. mucilaginosa* strains, both morphologically and genetically, strongly supports its identification as a member of the *R. mucilaginosa* genus. This comprehensive characterisation, combining morphological observations with molecular phylogenetic analysis, provides a solid foundation for further investigation into the biotechnological potential of the LD-2-DIT strain, particularly in areas such as lipid production and environmental applications.

Growth and lipid accumulation of *R. mucilaginosa*

This study investigated the growth and lipid accumulation of *R. mucilaginosa* in two distinct media: YEPG and synthetic wastewater. The composition of synthetic wastewater is presented in Table 1. Based on prior research conducted by Prabhu et al. [Prabhu et al., 2019], glucose was identified as the optimal carbon source for lipid accumulation in *R. mucilaginosa*. Consequently, synthetic wastewater was supplemented with 10% glucose to evaluate its effects on growth and lipid accumulation. Figure 4 illustrates the growth curves of *R. mucilaginosa* cultured in two distinct media conditions over 8 days, providing valuable insights into its growth characteristics and adaptability. In YEPG media (Figure 4a), the growth dynamics revealed a classical microbial growth pattern characterised by a notably brief lag phase, followed by a pronounced exponential phase during the initial 2–3 days. This rapid adaptation suggests the efficient utilisation of the nutrient-rich YEPG environment, ultimately achieving higher optical density measurements by day 6. This observation indicates superior biomass production capacity in the YEPG medium, which can be attributed to its readily available nutrients and growth factors.

The growth patterns in synthetic wastewater (Figure 4 b) exhibited distinct characteristics, reflecting the organisms' responses to a more challenging growth environment. The cultures demonstrated an extended lag phase and a more gradual exponential phase, which were particularly evident under conditions lacking glucose supplementation (black line). The introduction of 10% glucose into synthetic wastewater (blue line) did not enhance growth performance. Although a previous study by Prabhu et al. [Prabhu et al., 2019] indicated that glucose, as a carbon source, is instrumental for *R. mucilaginosa* growth and lipid accumulation, the current study illustrated that excess carbon can also inhibit microorganism growth. The observed variations in growth patterns between the YEPG and synthetic wastewater media can be attributed to several key factors. The complex nutrient profile of YEPG provides readily accessible nutrients and growth factors, whereas synthetic wastewater

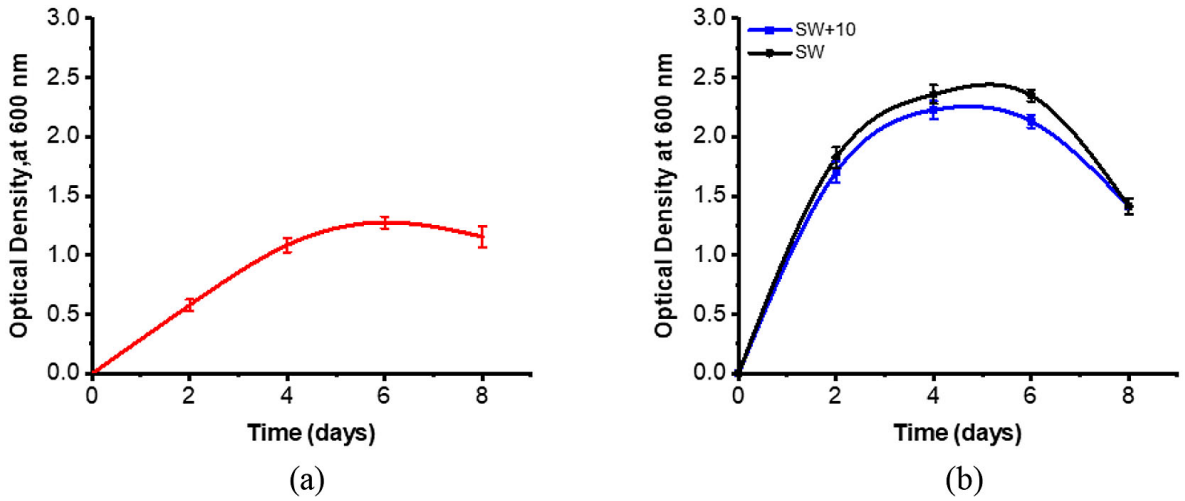

Figure 4. Growth curve of *R. mucilaginosa* in (a) YEPG media and (b) artificial wastewater

requires more extensive metabolic adjustments for nutrient utilisation. Additionally, synthetic wastewater may contain compounds that limit growth rates. Despite these challenges, *R. mucilaginosa* demonstrated sustained growth until day 8 in synthetic wastewater, albeit at lower optical densities. This persistence highlights the organism's robust adaptability to less-than-optimal growth conditions, suggesting its potential suitability for wastewater treatment. The ability to maintain growth in synthetic wastewater, particularly when supplemented with glucose, suggests that *R. mucilaginosa* could be effectively utilised in biotechnological applications involving wastewater remediation, potentially producing valuable biomass products. Figure 5 shows the time profile of lipid accumulation in the *R. mucilaginosa* biomass over the cultivation period. The data showed a progressive increase in lipid content, reaching a significant peak of 21.6% on day 6 of cultivation. This peak value is particularly noteworthy as it represents a considerable improvement over the 13.45% lipid content reported by [Tsai et al., 2022] under similar cultivation conditions. The lipid accumulation pattern exhibited three distinct phases: an initial gradual increase during the early cultivation period, a steeper accumulation phase between days 4 and 6, and a subsequent substantial decline. Notably, the lipid content decreased markedly after day 6, dropping from over 20% to about 8% in YEPG medium and from about 18% to 4–5% in synthetic wastewater (see Figure 5). This pronounced decrease may indicate cellular utilisation of stored lipids for maintenance of metabolism or stress response, a common phenomenon in oleaginous microorganisms during prolonged cultivation periods. The temporal alignment between the maximum lipid content (day 6) and the peak growth phase, as observed in Figure 4, is significant from a physiological perspective. This synchronisation suggests that lipid accumulation in *R. mucilaginosa* is growth-associated, with optimal lipid production occurring during the transition from the late exponential to the early stationary phase. This pattern is characteristic of oleaginous yeasts, in which nutrient limitation, particularly nitrogen depletion relative to carbon availability, often triggers an increase in lipid biosynthesis. The achievement of 21.6% lipid content demonstrated the robust lipid accumulation capacity of this *R. mucilaginosa* strain. This increased lipid production compared to previously reported values suggests the potential optimisation of cultivation conditions or strain-specific advantages.

Fig. 6 illustrates the biomass production of *R. mucilaginosa* across both media types. In alignment with the growth curves, biomass production reached its peak on day 6 in both media. Nonetheless, the synthetic wastewater medium (Figure 6b) yielded a slightly higher biomass (approximately 1.8–1.85 g/L) than YEPG (approximately 1.75 g/L), while the addition of 10% glucose to synthetic wastewater resulted in a reduced biomass (approximately 1.5 g/L). This discrepancy highlights the critical role of medium composition in optimising biomass production. The addition of 10% glucose to synthetic waste is likely to inhibit growth. This observation implies that

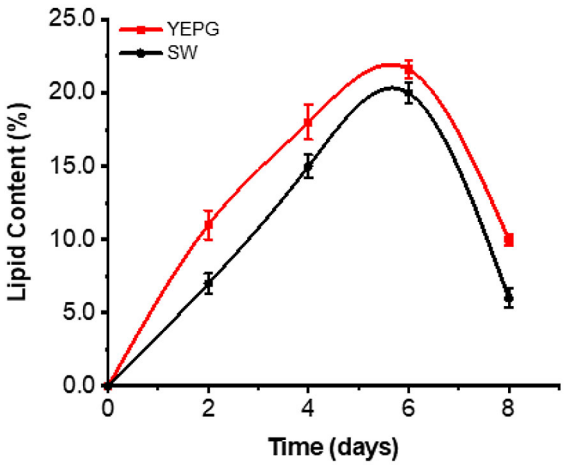

Figure 5. Lipid accumulation of *R. mucilaginosa* resulting from different growth media

while glucose is essential for lipid accumulation, excessive concentrations may exert inhibitory effects on overall growth.

The substantial lipid content (21.6%) and relatively short duration of biomass acquisition (6 days) highlight the potential of *R. mucilaginosa* as a promising source of biofuel feedstock. These findings are comparable to or exceed those reported for other *Rhodotorula* species. For example, Gohain et al.[Gohain et al., 2020] reported optimal harvesting times of 72–96 hours for *R. mucilaginosa* R2, whereas Sartaj et al. [Sartaj et al., 2022] identified a 6-day optimal cultivation period for *Rhodotorula glutinis* ISO A1. The results also highlight the advantages of *R. mucilaginosa* over other lipid-producing microorganisms, such as microalgae. While microalgae typically require 14–20 days for optimal biomass and lipid production [Karatay et al., 2016; Cobos et al., 2017], the *R. mucilaginosa* strain of the present study achieved high lipid content in just six days. This shorter cultivation time could significantly reduce production costs and increase process efficiency in industrial applications. However, it is essential to note that lipid content can vary considerably depending on the specific oleaginous yeast strain, growth conditions, and medium composition. The substantial decline in lipid content observed after day 6, particularly in cultures supplemented with 10% glucose, highlights the importance of optimising growth conditions to maximise and sustain lipid production. *R. mucilaginosa* remains a promising candidate for cost-effective and environmentally friendly biodiesel production due to its high peak lipid content, rapid growth, and ability to thrive in synthetic wastewater, making it a suitable candidate for this purpose. Future research should focus on optimising the growth conditions in synthetic wastewater to match or exceed the performance of the YEPG media. Additionally, investigating the fatty acid profile of accumulated lipids would provide valuable insights into the quality of potential biodiesel products. Furthermore, the ability of *R. mucilaginosa* to grow in synthetic wastewater suggests its possible applications in wastewater treatment coupled with biodiesel production. This dual-purpose approach can significantly enhance the economic and environmental sustainability of biodiesel production from microbial lipids.

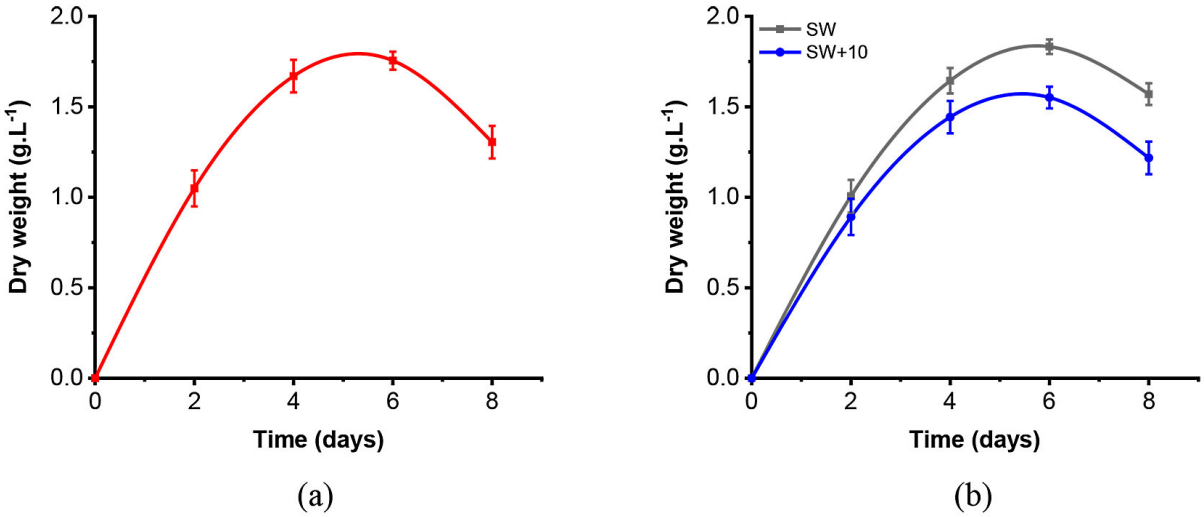

Figure 6. Biomass of *R. mucilaginosa* on (a) YEPG media and (b) artificial wastewater

Pollutant compounds removal

Rhodotorula mucilaginosa demonstrated a remarkable ability to remove pollutants from artificial wastewater, demonstrating its potential for wastewater treatment applications. The yeast's metabolic versatility allows it to decompose various compounds and utilise them as nutrients for growth. This process primarily involves the assimilation of macroelements, such as carbon, nitrogen, and phosphorus, as well as microelements, including copper and zinc. To assess the pollutant removal capacity of *R. mucilaginosa*, changes in key water quality parameters, including COD, phosphate, NH_3, nitrate, and nitrite, were monitored. The study was conducted under two conditions: artificial waste media without additional carbon sources and with 10% glucose supplementation. Without glucose supplementation, *R. mucilaginosa* exhibited relatively impressive pollutant removal efficiencies. A reduction of 65.63% in COD, 37.74% in ammonia, and 41.10% in nitrate was achieved. The lack of phosphate reduction is likely due to the limited carbon present in the growth medium, which may hinder the cells' ability to take up phosphate efficiently due to energy constraints. The significant increase in nitrite likely resulted from the partial reduction of nitrate to nitrite without further reduction, possibly because the same limited carbon availability affects the electron transport chain. The addition of 10% glucose significantly enhanced the overall removal efficiency. Figure 7 illustrates that COD removal increased markedly to 96.36%, indicating enhanced cellular metabolic activity. Phosphate removal increased to 73.22%, suggesting that improved nutrient uptake was facilitated by sufficient carbon availability. After 6 days of incubation, ammonia removal experienced a modest increase of 43.17%, whereas nitrate removal significantly increased to 77.17%. Moreover, nitrite accumulation increased only slightly (18.75%). The observed enhancement associated with glucose supplementation is likely attributable to the increased energy availability, as glucose serves as a crucial energy source for cellular processes. Furthermore, the incorporation of glucose results in an elevated carbon-to-nitrogen (C: N) ratio, which fosters a more favourable balance between carbon and nitrogen, thereby promoting optimal metabolic activity. These results, illustrated in Figure 7, revealed several interesting patterns in the nutrient removal capabilities of *R. mucilaginosa*. Yeast demonstrated high efficiency in reducing COD and ammonia levels, particularly in the presence of additional glucose. The addition of 10% glucose to the media led to a significant increase in the removal efficiencies of most parameters. This suggests a substantial potential for the application of *R. mucilaginosa* in treating various carbon-rich wastewaters. This observation indicates that while *R. mucilaginosa* can utilise multiple carbon sources, including glucose and xylose [Siwina and Leesing, 2021], there may be an optimal carbon concentration for nutrient removal. Excess carbon may enhance yeast metabolic activity, potentially increasing nutrient uptake mechanisms. The temporal dynamics of nutrient removal should also be taken into account.

The yeast removal efficiency peaked around day 6 of incubation, after which the performance declined. This trend was observed under both glucose-supplemented and unsupplemented conditions, indicating a consistent metabolic pattern regardless of the availability of additional carbon sources. Notably, despite the relatively lower nutrient removal efficiency in the non-glucose-supplemented media, the relative biomass yields were not significantly different, as shown in Figure 6. This suggests that *R. mucilaginosa* maintained its growth and biomass production, even when its nutrient removal capacity was reduced. The performance of *R. mucilaginosa* in this study compares favourably with previous research, such as that reported by Boutafda et al. [Boutafda et al., 2019] demonstrated that *R. mucilaginosa* reduced COD levels in olive mill wastewater by 56.91%. Our study revealed a significantly higher COD reduction (96.36%) in glucose-supplemented media, highlighting the potential of our isolated strain for wastewater treatment. These findings underscore the importance of optimising the growth conditions and media composition to maximise *R. mucilaginosa's* pollutant removal efficiency. Future research should focus on understanding the metabolic pathways involved in nutrient assimilation and how various environmental factors, including carbon source availability and concentration, influence these pathways. The dual capability of *R. mucilaginosa* in both wastewater treatment and biomass production presents exciting opportunities for integrated biotechnological applications, potentially leading to the development of systems where wastewater treatment and biofuel production occur simultaneously, thereby enhancing resource efficiency and environmental sustainability.

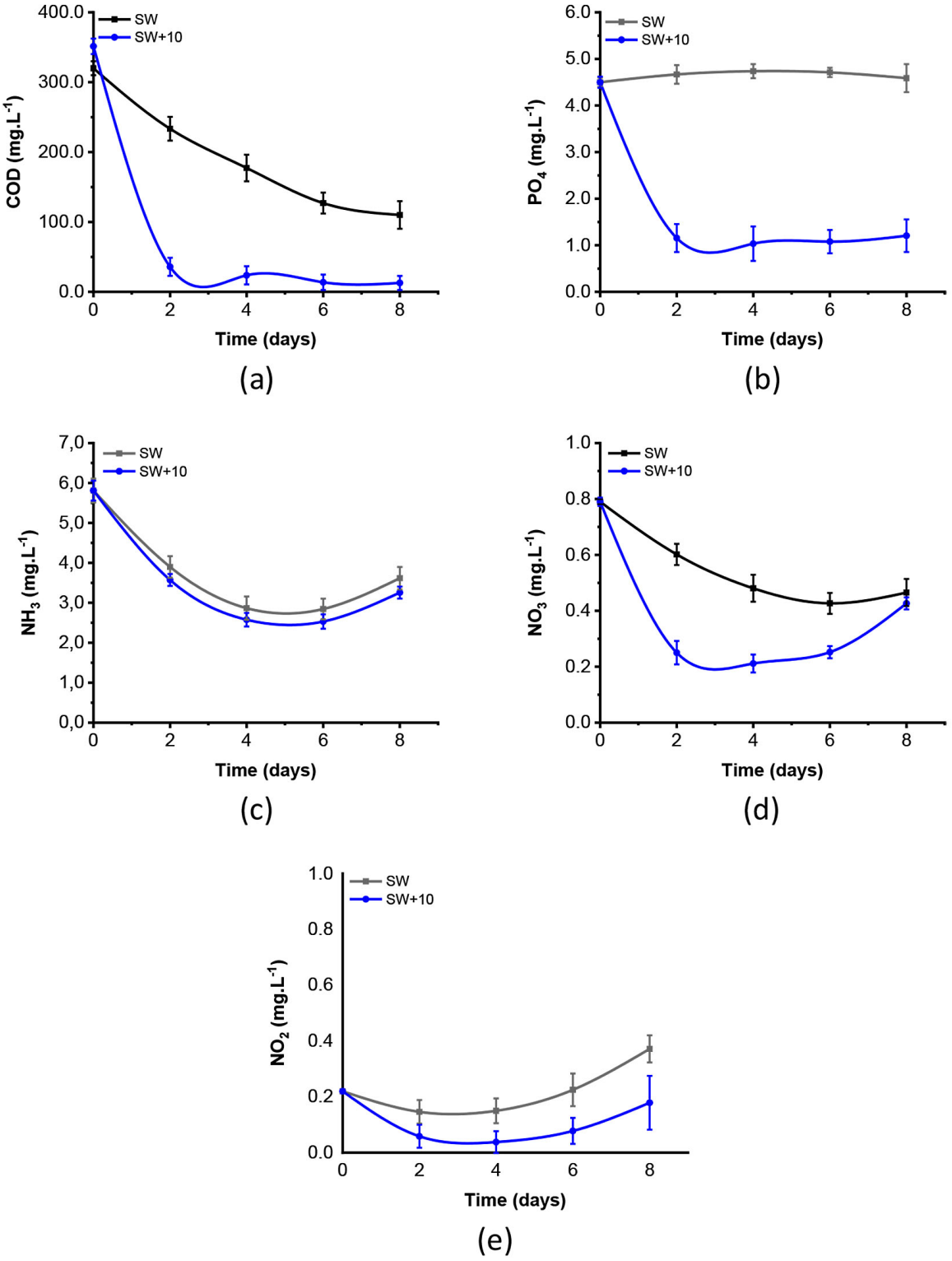

Figure 7. Temporal changes in (a) COD, (b) phosphate, (c) ammonia, (d) nitrate, and (e) nitrite

CONCLUSIONS

Landfill leachate, a nutrient-rich liquid resulting from waste decomposition, is a potential reservoir for diverse microorganisms with biotechnological applications. This study successfully isolated and characterised *Rhodotorula mucilaginosa* LD-2-DIT from the leachate of the Blang Bintang landfill in Aceh Besar, Indonesia. This novel yeast strain demonstrated remarkable dual functionality, exhibiting significant potential for both biofuel production

and wastewater treatment. Analysis of *R. mucilaginosa* LD-2-DIT biomass revealed a promising composition for biofuel feedstock, with a substantial fat content of 21.6%. These characteristics suggest that this strain could be an excellent candidate for biodiesel and bioethanol production, addressing the increasing global demand for renewable energy sources. Concurrently, the strain exhibited exceptional capabilities for reducing pollutant levels in artificial waste media, achieving removal efficiencies of up to 96.36% for COD and 43.17% for ammonia. These results indicate the considerable potential for application in wastewater treatment processes, particularly for carbon-rich effluents. The dual functionality of *R. mucilaginosa* LD-2-DIT makes it a valuable asset for sustainable biotechnology applications. Its ability to metabolise pollutants suggests that it might utilise actual wastewater as a nutrient source, thereby simultaneously treating wastewater and producing valuable biomass. This synergistic approach opens up possibilities for developing integrated systems wherein wastewater treatment and biofuel production occur concurrently, enhancing resource efficiency and promoting environmental sustainability. Future research should prioritise the optimisation of growth conditions to maximise the pollutant removal efficiency and lipid accumulation. Additionally, investigating this strain's performance in real wastewater samples from various industries would provide valuable insights into its practical applications. Genetic engineering approaches could potentially enhance the strain's lipid production or pollutant removal capabilities, further increasing its biotechnological value. Pilot-scale studies are crucial for evaluating the feasibility of large-scale applications and addressing any challenges that arise from scaling up the process. In conclusion, *R. mucilaginosa* LD-2-DIT is a promising biological agent for addressing two significant global challenges: wastewater management and renewable energy production. Its unique capabilities underscore the potential of microbial biotechnology to develop sustainable solutions for environmental and energy concerns. As research continues to explore and harness the potential of microorganisms from diverse environments, strains such as *R. mucilaginosa* LD-2-DIT will pave the way for innovative, eco-friendly technologies that can contribute to a more sustainable future.

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