Vietnam Journal of Science and Technology ***60*** *(5) (2022) 813-824*

doi:10.15625/2525-2518/16417

PHYSIO-CHEMICAL PROPERTIES OF METHYL RED-DEGRADING STRAINS ISOLATED FROM TEXTILE WASTEWATER

Luong Thi Thu Huong[1], Dang Mai Anh[1], Nguyen Minh Thu[1], Nguyen Sy Nguyen[1], Ho Thi Loan[2], Ho Tu Cuong[1,*]

[1]*Institute of Environmental Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Viet Nam*

[2]*Institute of Ecological and Biological Resources, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Viet Nam*

[*]Email*: hotucuong@gmail.com*

Received: 19 August 2021; Accepted for publication: 20 October 2021

Abstract. Methyl red (MR)-degrading strains isolated from textile wastewater were identified and tested for physio-chemical properties. The bacterial colonies picked from the mineral medium supplemented with textile wastewater were transferred to the mineral medium containing MR for the test. Two strains, namely SYK and STXL2, demonstrating the ability of MR decolorization in the agar medium were selected and purified. Strains SYK and STXL2 incubated in the liquid mineral medium with MR were able to remove 100 % and 95.5 % of MR after 4 days of incubation, respectively. Gram staining of both strains confirmed that these two isolates were gram positive. The SYK strain has white, irregular and dry colonies while the STXL2 strain has orange, opaque, circular and glossy colonies. Both strains have short rod shape of cell under SEM images. The two strains were able to grow in the media such as Luria-Bertani medium, Meat-peptone broth, and Nutrient broth. The SYK strain was able to grow quickly in the medium with maltose while the STXL2 strain used glucose as the best carbon source. Biochemical tests showed that both strains had positive reaction in Voges-Proskauer test and negative reactions in lipase, cellulose, amylase, citrate, and gelatin hydrolysis tests. The SYK strain had positive reactions in protease, urease, and ammonia tests while the STXL2 strain had positive reaction in nitrate reduction test. The sequences of 16S rNA genes identified the SYK and STXL2 strains as *Streptomyces cellulosae* (100 % of similarity) and *Rhodococcus ruber* (99 % of similarity), and registered in the GenBank via access numbers MZ414193 and MZ414194, respectively.

Keywords: methyl red, textile wastewater, *Streptomyces cellulosae*, *Rhodococcus ruber*.

Classification numbers: 3.1.1, 3.5.3.

1. INTRODUCTION

Textile wastewater is considered the most polluting in the industry. Dyes could change water color into darker, hindering sunlight and oxygen absorption, affecting the growth of

aquatic species, especially photosynthetic species such as algae, which therefore also affects the ability of microorganisms to decompose organic pollutant. Not only affecting the self-cleaning ability of the aquatic environment, the organisms that the producers consume as well as the links in the food web of the ecosystem are also seriously affected. Tests carried out on aquatic plants and animals have previously shown that 37 % of the dyes are toxic, 2 % are very toxic and extremely toxic. Among the dyes, azo dyes, characterized by functional groups (–N=N–), account for the majority of the composition of dyes used in the textile industry [1]. These dyes are widely used because of their ease of production and low cost, as well as being able to mix colors to form different colors. However, many dyes in this group are serious environmental pollutants due to their carcinogenic or cytotoxic potential. MR, for instance, causes eye and skin sensitization, and the irritation of throat or gastrointestinal tract if inhaled or swallowed, or is even mutagenic in nature [1 - 2].

Several physical and chemical treatment methods for MR have been investigated recently. The chemical methods included the oxidation degradation of MR such as photocatalytic oxidation by ultraviolet or solar light in the presence of oxidant hydrogen peroxide and TiO_2 [3], [4] or Fenton oxidation [5], and electrochemical reduction of MR [6 - 7]. The other approach for MR removal from water was the application of adsorbents, for examples, activated carbon and multiwalled carbon nanotubes [8], iron oxide nanopartilces [9], and clay minerals [10]. The method of adsorption on the surface of porous solids such as activated carbon, organic metal frameworks based on iron nanoparticles, and clay minerals has a fast kinetics of removal, however, generates secondary pollutants such as sludges containing untreated materials. Oxidation methods consumed energy and needed complicated operation. Therefore, the greener and better treatment methods are still investigated and developed.

Microorganisms have also been studied and applied for the treatment of wastewater containing MR. It was reported that microorganisms were able to degrade MR into 2-aminobenzoic acid and N,N′dimethyl-p- phenyle-nediamine [2, 11 - 14]. The study showed that *Bacillus sp. strain UN2* was able to reduce up to 98 % of MR dye at a concentration of 100 ppm in 30 min under optimized pH and temperature conditions. This bacterium degraded MR into two prime metabolites: N,N′dimethyl-p- phenyle-nediamine and 2-aminobenzoic acid, possibly by the activities of three enzymes including azoreductase, laccase, and NADH-DCIP reductase [2]. Other microorganisms such as *Galactomyces geotrichum* MTCC 1360[14], *Rhodococcus* strain UCC 0016[13], *Acetobacter liquefaciens S-1* [12], and *Klebsiella pneumoniae RS-13*[11] showed the ability to decolorize up to 100 % within 1 h, and 24 h, 98 % within about 72 h, and 100 % within two days, respectively. Those studies suggested that the microbial application in the MR treatment was of great interest. Therefore, in this study, we are attempting to isolate the MR-reducing microorganism from the textile wastewater and characterize their physio-chemical properties in order to understand the optimal conditions for their growth.

2. MATERIALS AND METHODS

2.1. Materials

Mineral salt medium (MM) was used for bacterial isolation. It was prepared with 1 L of distilled water and the following components: 3.5 g of $Na_2HPO_4.2H_2O$; 1 g of KH_2PO_4; 0.5 g of $(NH_4)_2SO_4$; 0.1 g of $MgCl_2.6H_2O$; 50 mg of $Ca(NO_3)_2.4H_2O$; 1 mL of the solution of trace elements; and 15g of agar. The solution of trace elements was prepared with 1 L of distilled waterand included nitriloacetic acid - 1500 mg, $FeCl_2.4H_2O$ – 200 mg, $MgCl_2.6H_2O$ - 100 mg, $Na_2WO_4.2H_2O$ - 20 mg, $MnCl_2.4H_2O$ - 100 mg, $CoCl_2.6H_2O$ - 100 mg, $CaCl_2.2H_2O$ - 1000 mg,

$ZnCl_2$ - 50 mg, $CuCl_2.2H_2O$ - 2 mg, boric acid - 5 mg, $Na_2MoO_4.2H_2O$ - 10 mg, NaCl - 1000 mg, Na_2SeO_3 - 17 mg, and $NiCl_2.6H_2O$ - 24 mg. The pH of the medium solution was adjusted to 7.2 [15]. Textile wastewater was filtered with 0.02 µm Whatman Syringe filter as a substrate.

Luria-Bertani broth (LB), Nutrient broth (NB) and Meat extract peptone broth (MPB) were used for inoculated bacteria. The composition of LB included 5 g of Yeast extract (Hindawi, India), 10 g of NaCl, and 10 g of Tryptone (Merk, Germany) dissolved in 1 L of water. The composition of NB included 3 g of Yeast extract, 5 g of peptone, and 5 g of NaCl dissolved in 1 L of water. MPB included of 5 g of Peptone (Hindawi, India), 3 g of Meat extract (Hindawi. India), and 5 g of NaCl dissolved in 1 L of water [16 - 17].

Gause's medium was used for the biomass growth of isolated strains with the following components: 20 g of soluble starch, 1 g of KNO_3, 0.5 g of NaCl, 0.5 g of K_2HPO_4, 0.5 g of $MgSO_4$, 0.01 g of $FeSO_4$, and 1 L of H_2O [18].

For stock methyl red solution, 0.027 g of methyl red was dissolved in 10 mL of ethanol in a 100 mL volumetric flask and filled with distilled water.

All inorganic chemicals were purchased from either Fisher (UK) or Merck (Germany).

2.2. Methods

2.2.1. Analytical methods

Detection of MR: the MR solution was scanned in the wavelength range from 200 to 800 nm in a UV-Vis spectrophometer (Li-294, Lasany, India) for the typical peak of the wavelength. MR solutions with different concentrations were prepared for the standard curve at the typical peak of MR (430 nm). MR concentrations in the experimental solutions were calculated based on the standard curve.

To determine the efficiency of MR removal: the strains were incubated in the MM containing 0.04 mM of MR for 4 days. The samples were centrifuged at 12000 rpm (Eppendorf, Germany) to obtain the supernatant. Then MR concentrations in the supernatant were calculated based on the standard curve. The degradation efficiency of MR was calculated according to the following formula:

$$DE = (Ci-Cf)/Ci \times 100\ \%\ [13]$$

where DE is the degradation efficiency of MR, Ci is the initial concentration of the MR solution without inoculation, and Cf is the concentration of the MR supernatant from the solution after incubation.

For SEM images: cells were collected by centrifugation at 12000 rpm (Eppendorf, Germany) and washed with deionized water. The washed cells were suspended in deionized water and 10 µL of cells were dropped on a silica wafer and dried at room temperature overnight. SEM images were then obtained by scanning electron microscopy (SEM) (JSM-6510LV, JOEL, Japan) at 5 kV.

2.2.2. Experimental design

Isolation of microorganisms: Textile wastewater was collected from different stages of the wastewater treatment process at the treatment station in Bao Minh Industrial Park located in Nam Dinh province, Viet Nam, including untreated wastewater (labeled as CXL), pre-treated wastewater (labeled as STXL) and anaerobic-treated wastewater (labeled as SYK). Soil samples (labeled as RG) were also collected from Xuan Thuy national park, Nam Dinh province. One %

of the samples were enriched in the liquid MM supplemented with 20 % of the filtered textile wastewater for two days without shaking, and then were spread on MM agar supplemented with 20 % of the filtered textile wastewater as a substrate and incubated for 2-3 days at 35 and 45 ^{o}C. Ten different colonies of each sample grown on the agar media were selected for bacterial isolation and purification. The obtained isolates were tested for MR degrading ability by streaking the isolates on MM agar medium containing MR. The isolates with clear zone in the MR-MM agar medium were further tested in the liquid MM medium supplemented with 0.04 mM of MR for the confirmation of MR degrading efficiency.

Identification of isolates by 16S rDNA: Two isolates with the highest MR-reducing ability were selected and identified using 16S rDNA sequences. The isolates were cultured to produce pure biomass and their total DNA was extracted. In order to amplify ~1.5 kb gene from the isolates genomic DNA, 16S rDNA universal primer set [27F: 5'-AGA GTT RGA TCM TGG CTC AG-3' and 1492R: 5'-CGG YTA CCT TGT TAC GAC TT-3'] was used. Annealing stage was set at 55 °C. The PCR products were sequenced by Macrogen (Seoul, Korea). The partial sequence of 16S rDNA of each isolate was blasted in NCBI for the identification of the isolate. Then, the DNA sequences were aligned with highly identical sequences from NCBI database using ClustalW tool in BioEdit software v7.0.5.3 for sequence identity comparison[19]. The phylogenetic trees were constructed from aligned sequences using Mega X software[20]. Maximum likelyhood method and Tamura-Nei model were used to infer the evolutionary history and Bootstrap method with 1000 replications was applied for phylogenetic tree reconstruction.

Physio-chemical properties of selected isolates: The MR degrading isolates were prepared for morphological characteristics such as the observation of colony morphology on plate agar, gram staining, the observation of bacterial cell by SEM (JSM-6510LV, JOEL, Japan). Standard biochemical tests included fermentation (glucose) reactions, Voges Proskauer (VP) test, and tests for citrate, nitrate reductase, urease, ammonia, gelatin hydrolysis, amylase, protease, cellulase, and lipase activities were carried out as described by Li *et al*. [21].

Culture medium and carbon source utilization: two strains were inoculated in three culture media (Luria-Bertani broth, Nutrient broth, Meat extract peptone broth) and MM with 2 % of 6 types of carbonhydrates (glucose, saccharose, maltose, mannose, mannitol, and xylose). The growth of the isolates was evaluated by measuring the optical density of cells at a wavelength of 600 nm at 0, 8, 24, 72, and 96 h in a UV-Vis spectrophometer (Li-294, Lasany, India).

The optimal conditions for the growth of strains: two strains were inoculated in the either nutrient medium or meat extract peptone for investigating the optimal temperature (25 ºC - 35 ºC - 45 ºC - 55 ºC) and pH (2-4-6-7-8-10) conditions. The growth of the isolates was evaluated by measuring the optical density of cells at a wavelength of 600 nm at 0, 8, 24, and 72 h in a UV-Vis spectrophometer (Li-294, Lasany, India).

3. RESULTS AND DISCUSSION

3.1. Isolation and identification of methyl red reducing microorganisms

After being enriched in the liquid MM medium supplemented with filtered untreated wastewater, only five out of ten isolates were grown and formed a clear zone in the MM agar medium supplemented with MR (data not shown). These isolates were further selected for testing MR degradation. Figure 1 demonstrates the efficiency of MR removal in the liquid medium after 4 days of incubation with five different isolates. The SYK and STXL2 isolates were able to remove 100 % of MR from the liquid medium, while the remaining isolates could

only degrade around 30 % of MR (Figure 1). With the highest efficiency of MR degradation, these two isolates (SYK and STXL2) were selected for physio-chemical and molecular tests for their identification.

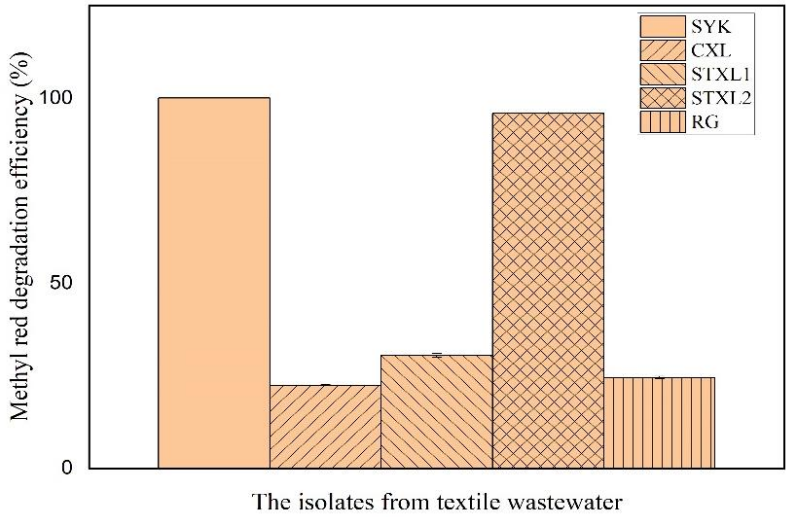

Figure 1. Efficiency of methyl red removal from liquid media of strains after 4 days of incubation.

On the agar medium, the SYK strain had white, irregular and dry colonies and the STXL2 strain appeared as orange, opaque, circular and glossy colonies. For gram staining, both strain cells were dyed with purple and the cell shape was not distinguished under light microscopy (Figure 2).

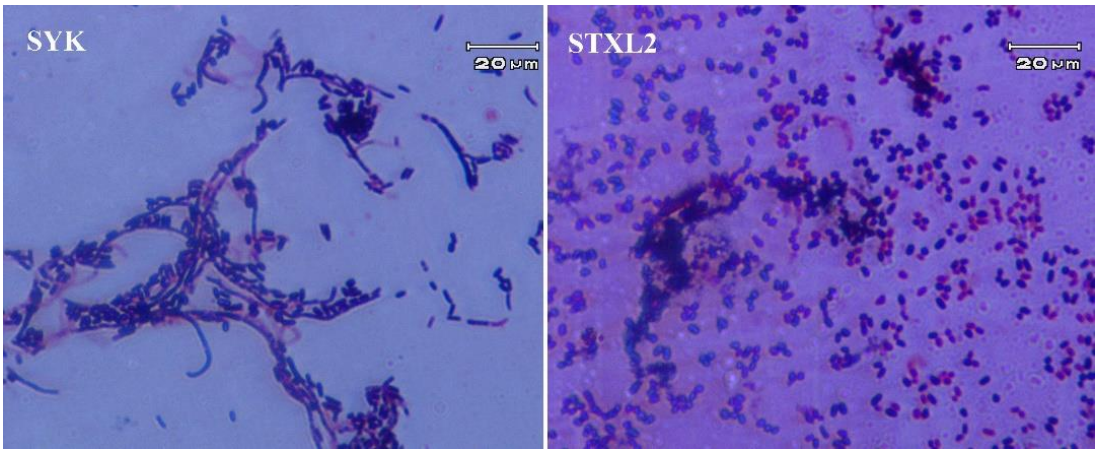

Figure 2. SYK and STXL2 strains gram stain.

Further analysis of cell shape under SEM (Figure 3) showed that two strains had short or short branched rod shape. The SEM image of STXL2 demonstrated a cell shape including short rod and branched cocci, meanwhile, SYK showed a single short rod with cili or cells linked together into a long chain of cells (Figure 3).

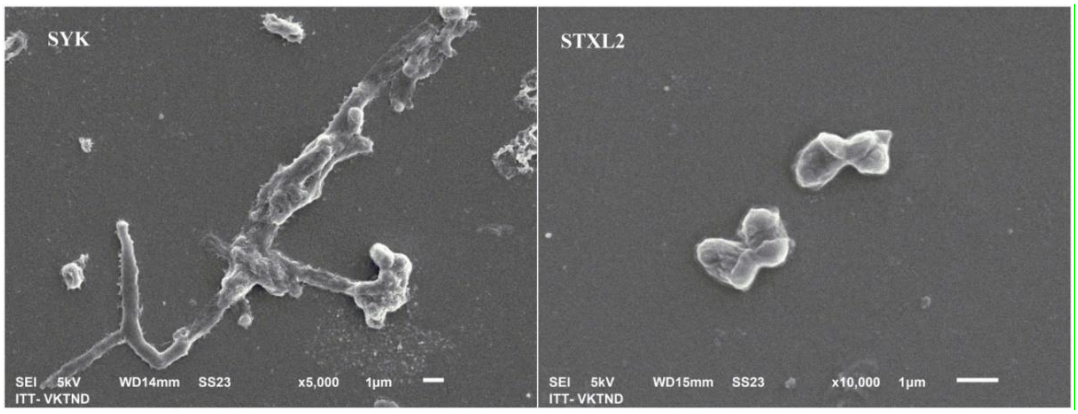

Figure 3. SEM images of SYK and STXL2 washed cells after 2 days of incubation in the Gause liquid medium.

The 16S rDNA gene sequence of the two strains shared the greatest similarity with members of the genus *Streptomyces* and *Rhodococcus*. The SYK strain had the 16S rDNA sequence that was 100 % identical to *Streptomyce cellulosae* (DSM 40802), and the 16S rDNA sequence of the STXL2 strain was 99 % similar to *Rhodococcus ruber* (DSM 43338). However, the phylogenetic analysis of *Rhodococcus* 16S rDNA gene sequences places strain STXL2 in a different clade from *R. ruber* and *R. aetherivorans* (Figure 4), implying that STXL2 is a new isolated strain.

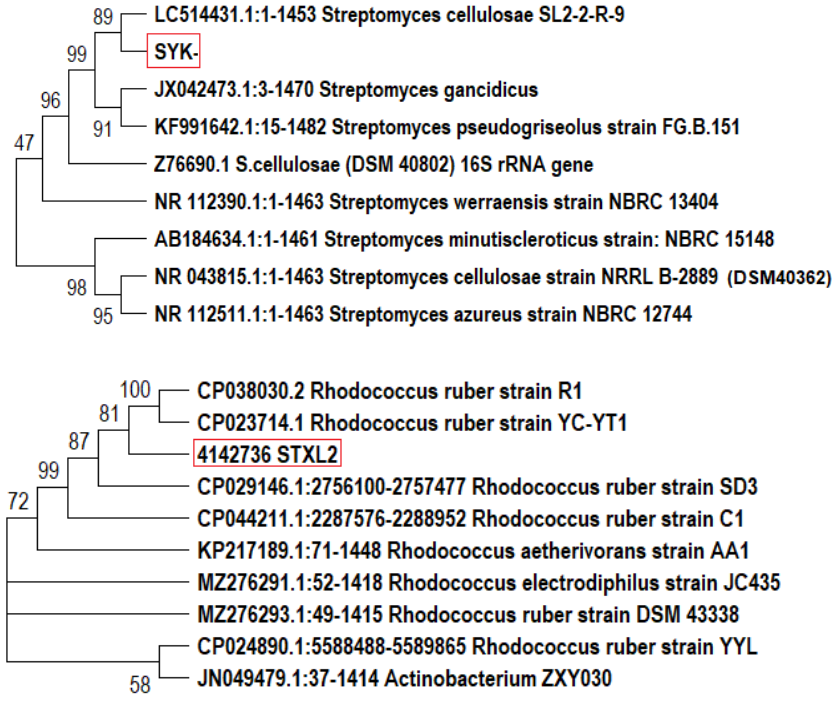

Figure 4. Phylogenetic tree of SYK (A) and STXL2 (B) strains. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches.

The biodegradation of methyl red by microbes was of interest not only to researchers but also to industry due to their cost effectiveness [14]. Various groups of microbes were able to degrade methyl red, including *Vibrio logei* and *Pseudomonas nitroreducers* [22], *Saccharomycescerevisiae* MTCC 463 [23], *Bacillus* sp strain UN2 [24], *G. geotrichum MTCC 1360, Rhodococcus strain UCC 0016, A. liquefaciens S-1, and K. pneumoniae RS-13*. In this study, we selected two out of the isolates from four different sources for further studies on their physio-chemical properties. Those strains based on the 16S rDNA molecular identification were similar to two reference strains *S. cellulosae* DSM 40362 and *R. ruber* DSM 43338. However, there was a difference in the cell shape between the two isolates and reference strains. The *S. cellulosae* DSM 40362 strain had a short rod shape with spore chain morphology [25], while the isolated *Strepomyces* existed more in a free cell form. The isolate similar to *Rhodococcus* was also different from the DSM 43338 reference strain in terms of morphology, one was short rod and the other was long rod [26]. The sequences of these two isolates were searched in GeneBank with more than 100 similar sequences (99 or 100 % of similarity), among them approximately ten sequences with a coverage value of 100 % were selected and used to build the phylogentic tree. The maximum likelyhood method and Tamura-Nei model were applied for the construction of the trees and clustering two isolates into different clades from the reference strains. It also revealed that the SYK strain was close to *S.cellulosae* SL2-2-R9 isolated from geothermal areas [25], while the STXL2 strain was in the same cluster with *R.ruber* strain R1 and strain YC-YT1 that were able to degrade lignin-derived aromatic compounds [27] and phthalate [28], respectively. However, it was found that the similarity of the 16S rRNA gene up to 99.9 % between strain 2AzMo and *R. wratislaviensis* IEGM 1112T (=NCIMB 13082T), 99.4 % between strain 2AzMo and *R. imtechensis* IEGM 940T (=RKJ300T), and 99.2 % to *R. koreensis* IEGM 962T (=DNP505T) was unable to identify the 2AzMo strain as the same strain as the others [29]. Therefore, the characteristics of MR degradation may place these two isolates as new strains.

3.2. Physio-chemical properties and growth conditions of selected isolates

Table 1. Biochemical properties of MR degrading strains isolated from textile wastewater and the data for *St. cellulosae* (*) and *R.ruber* (**) reference strains (ni: no information) were retrieved from the website: https://bacdive.dsmz.de/.

Tests	SYK	STXL2	DSM 40362*	DSM 43338**
Lipase	-	-	ni	-
Cellulase	-	-	-	-
Protease	+	-	ni	ni
Amylase	-	-	ni	ni
Gelatin hydrolysis	-	-	-	-
Citrate	-	-	-	+
Urease	+	-	-	-
Nitrate reduction	-	+	ni	ni
Ammonia	+	-	ni	ni
Fermentation	-	+	ni	ni
VP	+	+	-	-

Biochemical tests for the two strains were carried out to further identify the isolates. Table 1 demonstrates that strain SYK had positive tests with protease, urease, ammonia, and VP while the reference strain (S. cellulosae DSM 40362) was either negative in the tests or no information (for ammonia test). Similarly, the difference in biochemical tests between STXL2 and R. ruber DSM 43338 strains was found in the citrate and VP tests (Table 1).

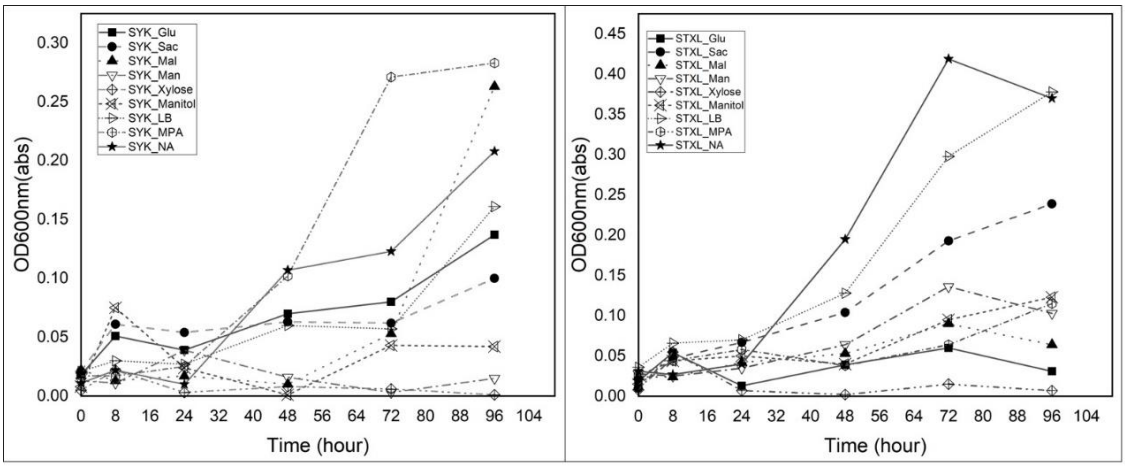

Figure 5. Culture medium and carbon source utilization of SYK and STXL2 strains, where Glu = glucose, S = saccharose, Mal = maltose, Man = mannose, LB = Luria-Bertani broth, MPA = Meat peptone broth, NA = Nutrient broth.

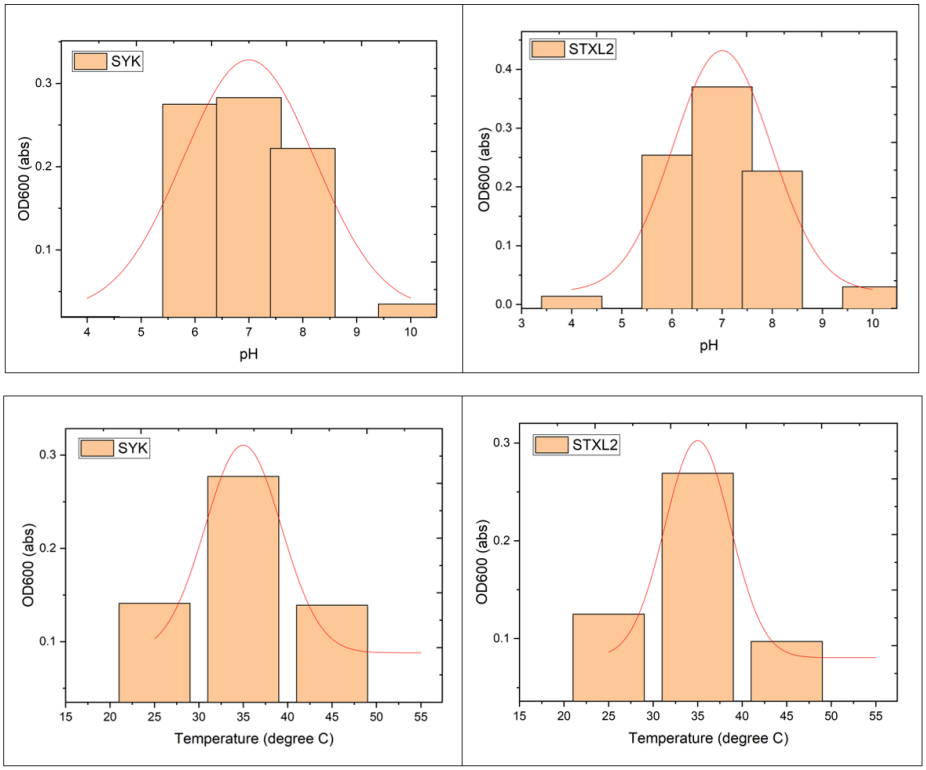

Figure 6. Effect of pH and temperature conditions on the growth of two strains SYK and STXL2.

The two isolates were investigated for their ability to grow in different media and carbon sources. Figure 4 confirms that both strains were able to grow in three media: LB, nutrient broth (NA), and meat peptone broth (MPB). However, the optimal medium for SYK and STXL2 was different. The SYK strain preferred MPA while STXL grew better in nutrient broth (Figure 5).

The optical density of both strains increased in the medium with saccharose and glucose, but decreased in the medium with xylose after 96 h of incubation (Figure 5). In addition, the cell biomass of the SYK strain also increased after 96 h of incubation with maltose as a carbon source although the cells seemed unable to develop in the medium supplemented with xylose, mannose, and mannitol. The STXL2 cell biomass increased weakly in the medium using mannose, mannitol, and maltose (Figure 5).

Two physiological properties (pH and temperature) were also investigated in order to find the optimal conditions for the cell growth. Figure 6 describes the effect of different pH values and temperature ranges on the growth of two strain cells. The Gaussian fitting curve showed that the optimum value of pH for SYK and STXL2 was 7.0, noticeably both strains still grew at a pH value of 10. As for the temperature effect, at 35 ^{o}C, the optical density of both cells reached maximum, implying that both strains were mesophilic.

The physio-chemical properties also further confirmed the difference between the two isolates (SYK and STXL2) and the two reference strains (DSM 40362 and DSM 43338) despite their molecular similarity. In addition, the S. cellulosae SL2-2-R-9 strain was thermophilic actinobacteria [25] and different from the mesophilic property of the SYK strain. Information on the biochemical properties of two closely related strains of Rhodococcus was not available, however, both strains possessed the ability of aromatic compound degradation. R. ruber YC-YT1 was reported to degrade phthalate ester, a plasticizer [28], and the analysis of R. ruber R1 genome demonstrated that this strain was able to degrade lignin-derived aromatics [27]. Interestingly, the other Rhodococcus strain that was able to degrade MR was mesophilic and neutral pH optimum for MR growth and degradation [13]. However, Maniyam et al. reported that this strain was able to use urea and ammonia, while STXL2 was not [13].

4. CONCLUSIONS

In this study, two isolates (SYK and STXL2) belonging to *Streptomyces* and *Rhodococcus* genus were able to completely degrade methyl red under aerobic conditions. These two strains were possibly classified as new trains closely related to *S. cellulosae* and *R. ruber*. The SYK and STXL2 strains were able to grow best in meat peptone broth and nutrient broth, respectively, implying biomass production for future application. Both strains were mesophilic with an optimal temperature of 35 ^{o}C and grew best in the pH range of 6.0 to 8.0, with an optimum at 7.0. The SYK strain was able to grow quickly in the medium with maltose while the STXL2 strain used glucose as the best carbon source. These properties are critical for future study on MR degradation.

Acknowledgements. Research funding was provided by the Basic Research Program of the Institute of Environmental Technology in 2021.

CRediT authorship contribution statement. Luong Thi Thu Huong: Methodology Investigation, Formal analysis, Funding acquisition. Dang Mai Anh: Formal analysis. Nguyen Minh Thu: Formal analysis. Nguyen Sy Nguyen: Formal analysis. Ho Thi Loan: Supervision. Ho Tu Cuong: Methodology, Supervision, Funding acquisition

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. M. R. H. Mas Haris and K. Sathasivam. - The removal of methyl red from aqueous solutions using banana pseudostem fibers, Am. J. Appl. Sci.. **6** (9) (2009), doi: 10.3844/ajassp.2009.1690.1700.
2. M. Zhao, P. F. Sun, L. N. Du, G. Wang, X. M. Jia, and Y. H. Zhao. - Biodegradation of methyl red by Bacillus sp. strain UN2: Decolorization capacity, metabolites characterization, and enzyme analysis, Environ. Sci. Pollut. Res., **21** (9) (2014), doi: 10.1007/s11356-014-2579-3.
3. H. Lachheb *et al.* - Photocatalytic degradation of various types of dyes (Alizarin S, Crocein Orange G, Methyl Red, Congo Red, Methylene Blue) in water by UV-irradiated titania, Appl. Catal. B Environ., **39** (1) (2002), doi: 10.1016/S0926-3373(02)00078-4.
4. X. H. Cheng and W. Guo. - The oxidation kinetics of reduction intermediate product of methyl red with hydrogen peroxide, Dye. Pigment., **72** (3) (2007), doi: 10.1016/j.dyepig.2005.09.022.
5. M. Azami, M. Bahram, and S. Nouri. - Central composite design for the optimization of removal of the azo dye, Methyl Red, from waste water using Fenton reaction, Curr. Chem. Lett., **2** (2) (2013), doi: 10.5267/j.ccl.2013.03.003.
6. M. Panizza and G. Cerisola. - Electrochemical degradation of methyl red using BDD and PbO2 anodes, Ind. Eng. Chem. Res., **47** (18) (2008), doi: 10.1021/ie8001292.
7. C. C. de O. Morais, A. J. C. da Silva, M. B. Ferreira, D. M. de Araújo, C. L. P. S. Zanta, and S. S. L. Castro. - Electrochemical Degradation of Methyl Red Using Ti/Ru0.3Ti0.7O2: Fragmentation of Azo Group, Electrocatalysis, **4** (4) (2013), doi: 10.1007/s12678-013-0166-x.
8. M. Ghaedi *et al.* - Activated carbon and multiwalled carbon nanotubes as efficient adsorbents for removal of arsenazo(III) and methyl red from waste water, Toxicol. Environ. Chem., **93** (3) (2011), doi: 10.1080/02772248.2010.540244.
9. S. Dadfarnia, A. M. Haji Shabani, S. E. Moradi, and S. Emami. - Methyl red removal from water by iron based metal-organic frameworks loaded onto iron oxide nanoparticle adsorbent, Appl. Surf. Sci., **330** (2015) (2015), doi: 10.1016/j.apsusc.2014.12.196.
10. D. F. Romdhane, Y. Satlaoui, R. Nasraoui, A. Charef, and R. Azouzi. - Adsorption, modeling, thermodynamic, and kinetic studies of methyl red removal from textile-polluted water using natural and purified organic matter rich clays as low-cost adsorbent, J. Chem., **2020** (2020), doi: 10.1155/2020/4376173.
11. P. K. Wong and P. Y. Yuen. - Decolorization and biodegradation of methyl red by Klebsiela pneumoniae RS-13, Water Res., **30** (7) (1996).
12. K. O. So, P. K. Wong, and K. -Y Chan. - Decolorization and biodegradation of methyl red by Acetobacter liquefaciens, Toxic. Assess., **5** (3) (1990), doi: 10.1002/tox.2540050303.
13. M. N. Maniyam, A. L. Ibrahim, and A. E. G. Cass. - Decolourization and biodegradation of azo dye methyl red by Rhodococcus strain UCC 0016, Environ. Technol. (United Kingdom), **41** (1) (2020), doi: 10.1080/09593330.2018.1491634.

14. S. U. Jadhav, S. D. Kalme, and S. P. Govindwar. - Biodegradation of Methyl red by Galactomyces geotrichum MTCC 1360, Int. Biodeterior. Biodegrad., **62** (2) (2008), doi: 10.1016/j.ibiod.2007.12.010.
15. H. B. Bui. - Isolation of cellulolytic bacteria, including actinomycetes, from coffee exocarps in coffee-producing areas in Vietnam, Int. J. Recycl. Org. Waste Agric., **3** (2014), doi: 10.1007/s40093-014-0048-0.
16. American Public Health Association, American Water Works Association, and Water Environment Federation. - Part 3000 Metals. 3111 Metals by Flame Atomic Absorption Spectrometry. 3111 B Direct Air - Acetylene Flame Method, Stand. Methods Exam. Water Waste Water (1992).
17. M. P. Macwilliams and M. Liao. - Luria Broth (LB) and Luria Agar (LA) Media and Their Uses Protocol, Am. Soc. Microbiol.,(2006).
18. J. Zhang and L. Zhang. - Improvement of an isolation medium for actinomycetes, Mod. Appl. Sci., **5** (2) (2011), doi: 10.5539/mas.v5n2p124.
19. T. A. Hall. - BioEdit: A user-friendly biological sequence alignment editor and analysis program for Window 95/98/NT, Inorganic Chemistry Frontiers, **4** (11) (2017), doi: 10.1039/c7qi00394c.
20. K. Tamura, G. Stecher, D. Peterson, A. Filipski, and S. Kumar. - MEGA6: Molecular evolutionary genetics analysis version 6.0, Mol. Biol. Evol., **30** (12) (2013), doi: 10.1093/molbev/mst197.
21. Q. Li, X. Chen, Y. Jiang, and C. Jiang. - Cultural, Physiological, and Biochemical Identification of Actinobacteria, Actinobacteria - Basics Biotechnol. Appl., (2016), doi: 10.5772/61462.
22. O. Adedayo, S. Javadpour, C. Taylor, W. A. Anderson, and M. Moo-Young. - Decolourization and detoxification of methyl red by aerobic bacteria from a wastewater treatment plant, World J. Microbiol. Biotechnol., **20 (**6) (2004), doi: 10.1023/B:WIBI.0000043150.37318.5f.
23. J. P. Jadhav, G. K. Parshetti, S. D. Kalme, and S. P. Govindwar. - Decolourization of azo dye methyl red by Saccharomyces cerevisiae MTCC 463, Chemosphere, **68** (2) (2007). doi: 10.1016/j.chemosphere.2006.12.087.
24. S. Zhao *et al.*. - Comparative proteomic analysis of Saccharomyces cerevisiae under different nitrogen sources, J. Proteomics, **101** (2014), doi: 10.1016/j.jprot.2014.01.031.
25. F. Ningsih *et al.*. - Isolation and 16S rRNA gene sequences analysis of thermophilic Actinobacteria isolated from soil in Cisolok geothermal area, West Java, Indonesia, IOP Conf. Ser. Earth Environ. Sci., **457** (1) (2020), doi: 10.1088/1755-1315/457/1/012015.
26. G. Alderson and M. Goodfellow. - The actinomycete-genus Rhodococcus: a home for the 'rhodochrous' complex, J. Gen. Microbiol., **100** (1977).
27. C. Farkas, R. A. Donoso, F. Melis-Acros, C. Garate-Castro, and D. Perez-pantoja. - Complete genome sequence of Rhodococcus ruber R1, a novel strain showing a broad catabolic potential toward lignin-derived aromatics, Microbiol. Resour. Announc., **5** (15) (2020). doi: 10.1128/MRA.00905-19.
28. T. Yang *et al.*. - Biodegradation of Di-(2-ethylhexyl) phthalate by rhodococcus ruber YC-YT1 in contaminated water and soil, Int. J. Environ. Res. Public Health, (15) (5) (2018), doi: 10.3390/ijerph15050964.

29. E. N. Kaparullina, Y. A. Trotsenko, and N. V. Doronina. - Characterization of Rhodococcus wratislaviensis, a New Gram-Positive Facultative Methylotroph, and Properties of Its C 1 Metabolism, Microbiol. Russian Fed. **88** (1) (2019). doi:10.1134/S0026261718060103.