

PAPER • OPEN ACCESS

Preliminary Study of Local Bacteria Isolates in Decolorizing Liquid Waste from Boiling Process of Batik Industry

To cite this article: I Noviar *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **305** 012017

View the [article online](#) for updates and enhancements.

Preliminary Study of Local Bacteria Isolates in Decolorizing Liquid Waste from Boiling Process of Batik Industry

I Noviar, E Munir*, K Nurtjahja

Department of Biology, Faculty of Mathematics and Natural Sciences,
Universitas Sumatera Utara, Medan, Indonesia

*E-mail: erman@usu.ac.id

Abstract. Liquid waste from boiling process of batik industry contains toxic compounds including wax, dye, soda ash, metal, cloth etc. If it is disposed into the environment without any treatment it will pollute the environment and threaten lives. Several methods to treat liquid waste of batik industry have been implemented, including by physical, chemical, and biological processes. The study to obtain potential bacteria in decolorization of liquid waste from boiling process of batik industry has been initiated. Bacteria were isolated from ditch soil around local batik industry in Medan. Fifteen bacterial isolates were obtained. Screening to obtain potential isolates to decolorize waste was done by culturing the isolates in solid minimal salt media containing increases concentration of liquid waste starting from 25%, 50% to 75%, no any other carbon source added to the media. Six isolates showed the growth in media containing 75% waste. All six isolates were then tested for the growth in minimal salt medium broth containing 75% of waste. Three isolates SP2, SP3, and SP4 exhibited good growth at this waste level media. These isolates were considered as potential bacteria and are subjected to be used for further decolorization analyses. Molecular identification of isolates based on 16S rRNA is also in process.

1. Introduction

Batik industry plays significant roles in national economic development, recruiting workers, and fulfilling domestic needs for clothing materials in Indonesia. During the process of manufacturing it uses many chemicals and dyes which still exist at high amount in its liquid waste. Unfortunately, chemical dyes are less favorable for the growth of microorganisms. It possess high toxicity towards mammals and aquatic organisms [1]. The azo- groups of dyes including naphthol is very toxic and are considered carcinogenic compound [2]. The boiling process of batik industry consumes large amounts of water, and the wastewater is disposed to the environment without any treatment, particularly at home batik industry. Therefore the liquid waste of textile industry poses environmental problem [3].

Around 40 - 65 L of wastewater are produced per kilograms of cloth during boiling process of batik industry [4]. The wastewater contains a large amount of dyes residues with various concentrations depending on the type of dye molecules [5]. Wastewater of batik industrial also contains recalcitrant chemicals which are difficult to degrade in nature [6]. However, some evidences have been reported that certain microorganisms including bacteria have indicated their ability to degrade and decolorize dyes containing in wastewater of batik industry. *Pseudomonas* isolated from liquid waste is able to degrade the residual batik or malam by reducing the color intensity or decolorization [7]. *Bacillus* and *Aspergillus* were able to decolorize textile dye waste by percentages of 31 and 41%, respectively [8]. *Micrococcus* was reported to decolorize azo dyes by percentage of 95% [9]. *Bacillus*, *Escherichia coli*



and *Pseudomonas fluorescens* are able to decolorize and degrade the reactive dyes [10]. *Aeromonas hydrophila* was reported as the best azo-dye removal in anaerobic conditions [11].

Extracellular enzymes produced by bacteria in decolorization study are reported to degrade toxic compounds completely both in aerobic and anaerobic environment [12]. On the other hand, environmental conditions such as pH, dye concentration, carbon source and nitrogen are needed to be optimized to get good result of decolorization process [13]. Then based on our current understanding, the potential decolorizing bacteria may be isolated in local sites of wastewater environment or sites around industrial processes. In this preliminary study, we reported a number of potential soil bacterial strains capable of growing on liquid waste from boiling process of batik industry.

2. Materials and Methods

2.1. Isolation and characterization of bacterial isolates

Bacteria were isolated from soil around drainage line of wastewater of local batik industrial located in Medan Tembung Sub-district, Medan, North Sumatera. Environmental condition of sampling sites were recorded (pH 6–6.8 and temperature 26–28 °C). Composite of soil samples were made from two random sites and stored in cool temperature prior laboratory use. Ten grams of soil were added into 90 mL NaCl 0.9% in 250-mL flask followed with mild agitation for 15 min. Serial dilution was made through up to 10^8 in reaction tubes. One mL of sample from selected dilution was spreaded on top of Nutrient Agar (NA) and plates were incubated at ambient temperature (28 ± 2 °C) for 24 hr. Distinctive colonies based on their morphological characters were picked and grown in new NA plate for pure culture.

2.2. Screening for the growth ability soil bacteria in wastewater containing media

The step was also targeted to evaluate growth response or the ability of isolates to grow in waste containing media. The screening of potential decolorizing bacterial strains was determined by two stages, first using solid and then in liquid medium supplemented with wastewater of boiling process of batik industry. Pure isolates obtained from isolation step were cultured in Minimal Salt Medium Agar (MSMA) supplemented with various concentration of wastewaters with an increase concentration from 25, 50, to 75%. Zero point one percent of glucose was included to initiate the growth. Cultures were incubated in ambient temperature for 7 days. Daily observation was conducted to assess the growth of bacteria colonies expressed as ‘growing’/ (+) and ‘no growth’/ (–).

2.3. Screening potential for decolorizing ability

Potential strains based on quantitative test were obtained through the number of colonies observed during day 6 and 12 of incubations on Minimal Salt Medium Broth (MSMB). One loopful of bacteria was initially grown in 5 mL tube containing Nutrient Broth (NB) for 24 hr until reaching optical density of 0.5 McFarland standard. One mL of overnight culture was inoculated into 100-mL flask containing 25 mL of MSMB supplemented with 75% of wastewater without addition of glucose. The flask was incubated in ambient condition for 12 days under 120 rpm agitation. Serial dilution was made through 10^1 – 10^8 in reaction tubes containing 9-mL of physiological saline solutions. One mL of aliquot was spreaded on top of Plate Count Agar (PCA) and incubated for 24 hr. Colonies were counted and expressed as colony forming units per mL aliquot or CFU/mL.

3. Results and Discussion

3.1. Morphological characteristics of bacterial isolates

The study obtained 15 soil bacterial isolates from drainage line of wastewater of local batik industry. Each isolate was characterized based on its colony performances (Table 1). Most isolates exhibited irregular colony with different form of edge from entire to filamentous. Most colony was yellowish white with flat elevation. Based on the colony performance, it has been assumed that the isolates were

difference each other. Compared to other studies, our result may reflect a higher number of isolates recovered from wastewater samples. Nine bacterial isolates were reported to harbor the soil around contaminated site of dye-containing wastewater [14]. Meanwhile, ten bacterial isolates were reported to harbor the soil of landfill site in North Sumatera [15]. Forty eight bacterial isolates were reported to sludge samples were collected from Ankleshwar Industrial Estate, Ankleshwar, Gujarat, India around which many textile processing units are situated [16].

Table 1. Morphological characters of isolated soil bacteria strains

Isolate Code	Morphological characters			
	Form	Edge	Elevation	Color
SP 1	Irregular	Undulate	Flat	Milky white
SP2	Irregular	Lobate	Flat	Yellowish white
SP3	Irregular	Undulate	Flat	Yellowish white
SP4	Irregular	Undulate	Flat	Yellowish white
SP5	Circular	Entire	Flat	Yellowish white
SP6	Circular	Curled	Flat	Yellowish white
SP7	Irregular	Lobate	Flat	Yellowish white
SP8	Circular	Curled	Raised	Yellowish white
SP9	Irregular	Undulate	Flat	Milky white
SP10	Irregular	Undulate	Raised	Milky white
SP11	Circular	Entire	Convex	Yellowish white
SP12	Circular	Undulate	Raised	Yellowish white
SP13	Irregular	Undulate	Flat	Milky white
SP14	Circular	Curled	Raised	Milky white
SP15	Filamentous	Filamentous	Flat	Milky white

3.2. The ability of bacterial isolates to grow in wastewater containing media

The growth of tested bacterial isolates was observed qualitatively as shown by growing colonies on MSMA plus glucose (0.1%) (Table 2). At a concentration of 25 and 50 % waste, all isolates exhibited the same growth responses. Six isolates were unable to grow at those waste concentrations and nine isolates grew at both concentrations. At much higher waste concentration (75%), eight of nine isolates were able to grow after 7 day of incubation. When glucose was not included to the cultures, only six isolates (SP1, SP2, SP3, SP4, SP6 and SP11) showed the growth. This result indicates, the isolates may have the ability to utilize the waste materials for their growth. Furthermore it shows that the number of isolates able to grow reduces when concentration of waste increases, in other word, the growth of isolates was affected by the presence of nutrient in medium and the concentration of waste which might contain toxic compounds. Environmental condition including organic content affects the growth of biodegradative bacteria [17]. A high organic content may lower the oxygen concentration leading to decline of microorganismal growth [18]. Hence, the overall capacity of biodegradative bacteria in degrading of certain waste may be attributed to their adaptive properties towards stress condition or contaminated habitat [19].

3.3. Screening potential for decolorization ability of isolates

The screening was done in MSMB containing higher concentration of wastewater (75%), no other carbon sources supplemented to the culture, result is shown in Table 3. From six isolates (SP1, SP2, SP3, SP4, SP6 and SP11) showing the growth in plate containing 75% wastewater as described above, three isolates SP2, SP3 and SP4 exhibited higher growth based on the number of cell, the other three isolates were also detected their growth but at a lower rate. Then the growth was relatively constant from the 6 to the 12 days of cultivation. These results indicate the isolates utilized organic compounds including dyes existed in waste for the growth. Then, these isolates, SP2, SP3 and SP4, were considered as potential isolates and used for further analyses. This result may also indicate the bacteria

could decolorize dyes existed in waste media. Similar results was also reported, where bacteria strains isolated from dye-contaminated using MSMB [14]. In addition, hydrolase-producing bacteria isolated from organic waste were able to produce amylase, cellulase and protease indicating their ability to degrade complex nutrients [16].

Table 2. Qualitative screening result of isolated soil bacteria

Isolate Code	MSMA I	MSMA II	MSMA III	MSMA IV
SP1	+	+	+	+
SP2	+	+	+	+
SP3	+	+	+	+
SP4	+	+	+	+
SP5	-	-	-	-
SP6	+	+	+	+
SP7	+	+	+	-
SP8	+	+	+	-
SP9	-	-	-	-
SP10	+	+	-	-
SP11	+	+	+	+
SP12	-	-	-	-
SP13	-	-	-	-
SP14	-	-	-	-
SP15	-	-	-	-

Notes: MSMA I (25% wastewater + 0.3 gr glucose), II (50% wastewater + 0.3 gr glucose), III (75% wastewater + 0.3 gr glucose), IV (75% (-) glucose)

Tabel 3. Growth of selected isolates in MSMB containing 75% wastewater

Code of Isolate	CFU/ml	
	Day-6	Day-12
SP1	ND	1.23×10^5
SP2	2.92×10^5	2.66×10^5
SP3	4.50×10^4	1.6×10^4
SP4	2.88×10^5	2.01×10^5
SP6	2.10×10^4	1.70×10^4
SP11	2.00×10^3	1.40×10^4

3.4. Biochemical characterization of potential strains

Three isolates with better growth performances (SP2, SP3 and SP4) were further analysed for their biochemical characters, result is shown in the Table 4.

Table 4. Gram staining and biochemical characters of potential isolates

Isolate Code	Gram staining	Cell morphology	Biochemical analyses						
			Amylase	Gelatinase	Citrate Utilization	H ₂ S		Motility	Catalase
						Slant	Butt		
SP2	Negative	Rod	+	-	-	Yellow	Yellow	+	-
SP3	Negative	Rod	-	-	+	Yellow	Yellow	-	+
SP4	Negative	Rod	-	-	-	Red	Yellow	+	-

Result as in Table 4 indicates that all selected isolates were basil of Gram negative but the characters biochemical features were quite different among isolates. It indicates that Sp2, SP3 and SP4 may be different each other. Molecular identification based on 16S rRNA has been under our investigation, result will be reported somewhere. Some gram negative bacteria capable of decolorization have been reported by many groups including *Pseudomonas* [7] and *Escherichia coli* [10].

4. Conclusions

Fifteen bacterial isolates were obtained from soil of from drainage line of wastewater of local batik industry. The isolates were able to grow and used carbon sources in wastewater of boiling process of batik manufacture. Six isolates exhibited the growth in media containing 75% waste, and three isolates (SP2, SP3, and SP4) were considered potential for decolorization based on the growth (CFU/ml) in wastewater containing media.

Acknowledgement

This work was partly supported by University of Sumatera Utara under TALENTA Grant 2017 and part of magister thesis of first author.

References

- [1] Kusumawati N. 2008. Studi degradasi zat pewarna naftol menggunakan Ferrat (FeO₄)²⁻. *Inotek*, 12: 1-9
- [2] Sunarya IK. 2012. Zat warna alam alternatif warna batik yang menarik. *Inotek*. 16: 103-121.
- [3] Andleeb S, Atiq N, Ali MI, Ur-Rehman F, Hameed A, Ahmad S. 2010 Biodegradation Of anthraquinone dye by *Aspergillus niger* Sa1 in self designed fluidized bed bioreactor. *J. Environ. Health*. 7: 371-376.
- [4] Manu B, Chaudhari S. 2003 Decolorization of indigo and azo dyes in semicontinuous reactors with long hydraulic retention time. *Process Biochem* 38:1213-1221.
- [5] Cervantes FJ, Santos ABD. 2011. Reduction of azo dyes by anaerobic bacteria: Microbiological and Biochemical Aspects. *Rev Environ Sci Biotechnol* 10:125-137
- [6] Suprihatin H. 2014. Kandungan organik limbah cair industri batik jenis Sidoarjo dan alternatif pengolahannya. Pusat Penelitian Lingkungan Hidup Universitas Riau.
- [7] Citrapancayudha DR, Soetarto ES. 2016. Biodegradation of wax residue on semi-solid waste of batik industry by bacteria. [Thesis] Fakultas Biologi UGM Yogyakarta.
- [8] Maruthupandy M, Avila AJ, Muthusamy A. 2012. Decolorization of textile dye effluent using *Bacillus* sp. and *Aspergillus* sp. *Indo-Global Res. J. of Pharm. Sci.* 2: 217-221.
- [9] Olukanni OD, Osuntoki AA, Gbenle GO. 2006. Textile effluent biodegradation potentials of textile effluent. *African J. Biotechnol.* 5: 1980-1984.
- [10] Sriram N, Reetha D, Saranraj P. 2013. Biological degradation of reactive dyes by using bacteria isolated from dye effluent contaminated soil. *Middle East J. Sci. Res.* 17: 1695-1700.
- [11] Chen CK, Wu YJ, Liou D.J, Hwang JC. 2003. Decolorization of the textile dyes by newly isolated bacterial strains. *J. Biotechnol.* 101: 57-68.
- [12] Velan M, Revathi M, Saravanan M, Chiya AB. 2012. Removal of copper, nickel, and zinc ions from electroplating rinse water. *Clean Soil, Air, Water* 40: 66-79.
- [13] Ardihina A. 2007. Dekolorisasi batik tulis menggunakan jamur indogenous hasil isolasi pada limbah yang berbeda. Bogor; ITB: 2-3
- [14] Pokharia A, Ahluwalia SS, 2013. Isolation and screening of dye decolorizing bacterial isolates from contaminated sites. *Textiles Light Indust. Sci. Technol.* 2: 54-61.
- [15] Munir E, Sipayung FC, Priyani N, Suryanto D. 2018. Potential of bacteria isolated from landfill Soil in degrading low density polyethylene plastic. *IOP Conference Series: Earth and Environ. Sci.* 126: 012144.

- [16] Shah MP, Patel KA, Nair SS, Darji AM. 2014. Decolorization of remazol black-B by three bacterial isolates. *Intern. J. Environ. Bioremed. Biodegrad.* 2: 44-49
- [17] Suriawiria U. 1995. Pengantar Mikrobiologi Umum. Penerbit Angkasa. Bandung.
- [18] Kristanto P. 2004. Ekologi Industri. Andi, Yogyakarta.
- [19] Bala JD, Lalung J, Ismail N. 2014. Biodegradation of palm oil mill effluent by bacterial. *Intern. J. Sci. Res. Publ.* 4: 1-10.