BIODEGRADATION OF BLACK AND REMAZOL RED TEXTILE DYES BY BACTERIAL STRAINS ISOLATED FROM RIVER CONTAMINATED TEXTILE DYES EFFLUENT

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Abstract

Research on biodegradation of black and red Remazol dyes in textile wastewater containing black and red Remazol dyes in concentrations of 500 ppm, 1000 ppm and 1500 ppm was carried out for 14 days in singly and consortium. Strains isolated from effluent containing Remazol black and red dyes. Selected isolates were identified using the API test and known as Bacillus subtilis, Bacillus licheniformis, Bacillus coagulans, and Bacillus cereus. Biodegradation in a consortium can reduce respectively; decolorization reached were 64.18%, 60.11% and 54.27% in Remazol black, and reached 65.46%, 62.92% and 55.90% in Remazol red. COD 77.63-72.92%, BOD 77.31-75.46%, TSS 67,68-52.72%, ammonia 62.85-53.69%. Biodegradation of textile wastewater containing red Remazol decreased respectively, COD 78.6-72.8%, BOD of 78.07-76.33%, TSS 70.14-58.72%, and ammonia 68.51-55.47%. Biodegradation of red Remazol textile dyes results in a greater reduction in concentration than in black Remazol. Also, biodegradation in a consortium results in a greater decrease in dye concentration than singly.

Key words*: biodegradation, consortium, decolorization, textile dyes, wastewater.*

INTRODUCTION

The textile factory produces dominant liquid waste containing dyes caused by the dyeing process to increase the commercial value of the fabric. Textile waste water, in general, has a very high concentration of organic matter such as protein, lipids, and cellulose so that COD (Chemical Oxygen Demand) and BOD (Biochemical Oxygen Demand) become high. Decreased river water quality occurs because the waste that enters the body of water contains various organic materials, heavy metals, fats, and minerals.

Textile waste treatment needs to be done to minimize pollution by wastewater by providing effective and efficient wastewater treatment technologies that are environmentally friendly. The processing of liquid waste is carried out biologically, which is expected to reduce the cost of treatment, and the results of waste biodegradation become safer (Srinivasan et al., 2014). Indonesia is a country that has the potential for creative industries, namely the batik industry, which is also found in all regions and it is part of economic activities that can improve the welfare of the community. In Indonesia, pollution of water bodies such as rivers is caused by textile effluent and the textile home industry were discharged into water bodies without being treated.

The batik industry effluent has large volume, concentrated colour, pungent odour and temperature, acidity (pH), and high in some parameter such as Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Suspended Solid (TSS).

Dyeing effluents have become a vital source of water pollution. Due to the xenobiotic properties and toxicity to all life forms including humans, removal of undesirable colour and associated toxicity is crucial (Yaseen and Scholz, 2018).

Various pollutants contained in textile wastes mainly are recalcitrant organics, colours, toxicants, and surfactants, chlorinated compounds, pH, and salts. Especially azo dyes (N = N group), which is a large group of synthetic dyes with a variety of colour and structure. Azo dyes are estimated to be around 60-70% of all dyes used in food and textile manufactures, and nearly 2-50% of these dyes are lost and enter waste discharges (Uday et al., 2016). Remazol black has a diazo structure and Remazol red has a mono azo structure were reactive azo dyes (Benkhaya Rabet and El Harfi, 2020). The characteristic chemical structure (such as $-C=C-$, $-N=N-$ and $-C\equiv N-$) of azo dyes makes them recalcitrant to biological break down (Singh and Singh, 2015).

The batik industry uses a lot of Remazol dyes because it is faster in the process and more economical than dyes naphthol or indigo sol in batik colouring, Remazol dyes are used, because Remazol dyes are faster in processing and are more economical than naphthol or indigo sol dyes. The advantage of Remazol dye is that the dye is strongly bonded to the fabric, gives good colour, and does not fade easily it is used mainly for dyeing cellulose, cotton, rayon and wool fibres (Dewi et al., 2018).

The toxicity of azo reactive dyes according to EU criteria for hazardous substances is relatively low. However, a very small amount (10-50 mg/l^{-1}) of dye is visible in water, decreasing the transparency of the water, inhibit the solubility of gases, thus interfer the activity of photosynthesis of microalgae (Chequer et al., 2013). Also, azo dyes have a negative impact due to biotransformation products such as aromatic amines show carcinogenic and mutagenic effects, and toxic (Brüschweiler and Merlo, 2017). Bioremediation refers to cost-effective and environment-friendly method for converting the toxic, recalcitrant pollutants into environmentally benign products through the action of various biological treatments or their metabolite. Bacteria, fungi, and yeast are known to decolorize dyes; these microorganisms develop a system of enzyme decolorization and mineralization of azo dyes (Deshmuk et al., 2016).

Biological decolorization has been employed under either aerobic or anaerobic environment. This usually involves tolerating bacteria or other microbes that can be genetically engineered to provide degrading potential strains and they are working singly or in consortia. Some strains, such as *Bacillus cereus, Pseudomonas putida, Pseudomonas fluorescence, Pseudomonas desmolyticum and Bacillus* sp. have been used in the biodegradation of azo dyes. These microbial consortia were recommended for environmental remediation to degrade variety of pollutants (Telke et al., 2014).

Microorganisms developed enzyme systems for the decolorization and mineralization of azo dyes under certain conditions and involving azo reductases and laccases. Laccases have been shown to decolorize a wide range of industrial colours Dyes that have complex aromatic molecular structures are more stable and difficult to biodegrade (Kannan et al., 2013). Species *Bacillus coagulans, Bacillus pumilus, Nitrosomonas* sp*., Pseudomonas* sp*., Bacillus licheniformis, Bacillus strearothermophyllus, Bacillus brevis, Enterobacter aerogenes* and *Cellulomonas* also can degrade textile waste. Some bacterial species that are used to degrade textile waste containing dyes include the consortium of *Pseudomonas aeruginosa* and *Bacillus subtilis.*

Bacteria can produce extracellular enzymes such as proteases, lipases, amylases, and cellulases. The enzyme can break down complex organic compounds into simpler compounds (Telke et al., 2014). Due to the chemical complexity of dyes, it is necessary to develop more efficient microbial processes for decolorization. Some of reports suggest that the average decolorization rate of the bacterial consortium was significantly higher than that observed for individual bacterial cultures (Sghaier et al., 2019).

Therefore, the purpose of this study was to evaluate the ability of strain was isolated from mixed wastewater effluent with Remazol black and Remazol red.

The isolate were *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus coagulans* in consortia and singly to decolorize and to degrade of dye-effluent generated by the local textile industry that contaminated with Remazol black and Remazol red.

MATERIALS AND METHODS

The study was carried out experimentally in a laboratory to evaluate the ability of bacterial isolates from textile effluent to decolorize and to degrade of Remazol black red and. Research and data analysis using a completely randomized design (CRD) factorial AxB pattern with three replications.

The factor I is the type of bacteria (A) consisting of: *Bacillus subtilis, Bacillus licheniformis, B. coagulans*, and a consortium of three species of bacteria.

Factor II namely (B0): 100% textile waste, (B1): Textile waste + 500 ppm black remazol, (B2): Textile waste + 1000 ppm black remazol, (B3): Textile waste + 1500 ppm black remazol, (B4): Textile waste + 500 ppm red remazol, (B5): Textile waste + 1000 ppm red remazol, (B6): Textile waste + 1500 ppm red remazol. Remazol black and remazol Red dyes, obtained from the home industry batik company Trusmi, Cirebon. Decolorization analysis was measured with a spectrophotometer (SHIMADZU-1700, Japan) at 597 nm. The decolorization was expressed as percent (%) decolorization and estimated as (Ai - At)/Ai x 100, where Ai is the initial of absorbance of the dye solution and At is absorbance at cultivation time.

Data analysis was performed using the Variance Analysis (ANOVA) test to determine the effect of treatment on the measured parameters and it is followed by Duncan's multiple range test with a level of 5%.

The parameters measured in this study are the population of bacteria (Total Plate Count), BOD (Biochemical Oxygen Demand), COD (Chemical Oxygen Demand), Do (Dissolved Oxygen), TSS (Total Suspended Solid), degrees of acidity, temperature, and ammonia. Decolorization efficiency analysis using visible spectrophotometric method.

RESULTS AND DISCUSSIONS

Microbial selection tolerant of black and red Remazol dyes isolated from river water mixed with textile waste

Strains selected that are tolerant to black and red Remazol dyes at concentrations of 500 ppm, 1000 ppm and 1500 ppm (Tabel 1).

Tabel 1. Tolerance of isolates to Remazol black and red dyes at different of concentrations.

		Black Remazol concentration (mg/l)			Red Remazol concentration (mg/l)		
No	Strain	500	1000	1500	500	1000	1500
1	*B. subtilis*	+	+	+	+	+	+
2	*B. licheniformis*	+	+	+	+	+	+
3	*B. coagulans*	+	+	-	+	+	-

The Tabel 1 showed that *Bacillus subtilis, Bacillus licheniformis* and *Bacillus coagulans* can grow on dyes, which means that the three bacterial isolates can degrade black Remazol dyes and red and can use carbon and nitrogen as their energy sources. *B subtilis, P. putida, B. coagulans* and *B. licheniformis* are found in textile effluent.

These three strains are known to produce enzymes azo reductase and also produce several extracellular proteins such as amylase, aminopeptidase, metal protease, lactamase, endo-N-acetyl glucose amidase, and lipase. Bacillus licheniformis also contributes to the nutritional cycle and has antifungal activity. Bacillus licheniformis secretes extracellular amylase, β-glucanase, and protease enzymes, while *Bacillus subtilis* produces protease enzymes *B. coagulans, B. subtilis, B. licheniformis, B. amyloliquifaciens, B. cereus, B. megaterium, B. caldolyticus, B. polymyxa, B. pumilus, B. circulans, B. firmus, B. brevis, B. macerans, B. stearothermophilus* produce various enzymes such as a-Amylase, b-Amylase, Arabinase, Cellulase, Chitinase, Chitosanase, Dextranase, Galactanase.

Bacillus coagulans are known to be able to degrade lipids and tolerate acids (Elshaghabee et al., 2017). The ability of the strain to tolerate, decolorize azo dyes at high concentration gives it an advantage for treatment of textile industry.

Bio-decolorization of Remazol black and red dyes by three isolates selected singly and consortium

Biodegradation can be interpreted as a process decomposition of substance by activities microbes, which result in the transformation of the structure of a compound such that changes in molecular integrity or breakdown of materials that occur when microorganisms use an organic substance as a source of carbon and energy.

One of the main physical features of textile waste water is colour and must be removed before being discharged into nature.

Azo reactive dyes are one of the synthetic dyes that are very commonly used in the textile dyeing industry.

A significant proportion of these dyes were entering into the surrounding environment in the form of wastewater. Black and red Remazol dyes are one of the substances that colour and contained in the textile waste water.

In this study, the biodegradation of Remazol black and Remazol red dyes by three isolates carried out singly and consortium and showed that all strains were able to use Remazol black and Remazol red textile dyes contained in wastewater at concentrations of 500 ppm, 1000 ppm and 1500 ppm (Tabel 2).

Tabel 2. Bio-decolorization of Remazol black and Remazol red dyes (% reduction) by *Bacillus subtilis, Bacillus licheniformis, Bacillus coagulans* singly and consortium

Treatment	Remazol black (%) reduction	Remazol red (%) reduction
Control 500 ppm	0	0
Control 1000 ppm	0	0
Control 1500 ppm	0	0
B. subtilis 500 ppm	60.26	62.04
B. subtilis 1000 ppm	57.37	58.23
B. subtilis 1500 ppm	52.67	53.53
B. licheniformis 500 ppm	55.03	57.21
B. licheniformis 1000 ppm	52.55	54.31
B. licheniformis 1500 ppm	48.45	50.86
B. coagulans 500 ppm	58.70	60.75
B. coagulans 1000 ppm	54.37	56.24
B. coagulans 1500 ppm	51.13	51.96
Consortium 500 ppm	64.18	65.46
Consortium 1000 ppm	60.11	62.92
Consortium 1500 ppm	54.27	55.90

The treatment of Remazol black and Remazol red dyes by the consortium of bacteria *Bacillus subtilis, Bacillus licheniformis,* and *Bacillus coagulans* at concentrations of 500 mg/l, 1000 mg/l and 1500 mg/l, percentage of decolorization reached were 64.18%, 60.11% and 54.27% in Remazol black, and reached 65.46%, 62.92% and 55.90% in Remazol red. These results indicate that the decolorization of dyes by the consortium is higher than individual isolates.

The consortium can decolorize at a higher rate compared to the individual bacterial. This high decolorization is due to the synergistic effect. Individual strains can attack dye molecules at various positions or can use decomposition products produced by other strains, and this is synchronization in AZO dye decolorization (Shah et al., 2014; Karim et al., 2018). Biodegradation by the consortium is considered more effective in decomposing organic matter such as cellulose, lipids, and protein. Because the consortium is a combination of several strains that carry out biodegradation activities synergistically, isolates carry out metabolic activities together and complement each other (Thakur et al*.*, 2012). Decolorization by pure as well as mixed cultures have required complex organic carbon sources, such as, yeast extract, peptone, or a combination of complex organic source and carbohydrate (Telke et al., 2014). Some bacteria can utilize these dyes as a source of nutrients in the form of a single carbon, while other bacteria only break down the azo with the azo reductase enzyme. Azo bonds (N = N) present in these compounds are resistant to break, with the potential for the persistence and accumulation in the environment. Bacteria are precious biodegradation agents for the decolorization of textile dyes that pollute the environment. Bacterial genera, such as *Pseudomonas, Bacillus, Rhodococcus*, are reported to be bacteria that rapid decolorize of azo dyes. The azo dye decolorization by bacteria has been associated with the production of oxidoreductive enzymes, such as lignin peroxidase, laccase, azo-reductase and other non-specific reductases. Some review suggested that azo dye decolorization was associated with reductive cleavage of the azo bond by reductase (Telke et al., 2014.)

Consortium of *B. coagulans, B. pumilus, B. subtilis and Nitrosomonas* sp. very effective in decomposing Remazol blue dyes in industrial effluent (Amniati, 2013). The result also shows that the higher the concentration of blue Remazol dye, the smaller the degradation efficiency. This consortium can decipher industrial waste with a maximum concentration of Remazol blue dye of 2500 mg/l produced decolorization 84.9%, decreased on BOD levels by 57.9%, decreased on COD by 37.7%; and a decrease in TSS by 52%. Mixed bacterial consisted of five bacterial strains as *B. vallismortis, B. pumilus, B. cereus, B. subtilis* and *B. megaterium* exhibited excellent potential (43-71%) in aerobic decolorization of azo dyes (Lade et al., 2015).

Bacterial strains are able to decipher azo dyes by aerobics and use these dyes as a source of carbon and nitrogen (Coughlin et al., 2002; Sudha, 2014). The content of dyes in the waste water of 1 mg/L has caused water to appear coloured, while the dye content in textile waste generally ranges between 20-200 mg/l. Decolorization ability is different for each type of azo dyes. Azo-reductase is reported to be the critical enzyme expressed in bacterial azo dyes degrading and catalyses the reductive cleavage of the azo bond resulted in aromatic amines are metabolized under aerobic conditions and subsequently simpler compounds, then leading to the removal of colour (Telke et al., 2014). The basic step in the decolorization and degradation of azo dyes is breakdown of azo bonds, leading to removal of colour. Azo dyes are known to undergo reductive cleavage whereas the resultant aromatic amines are metabolized under aerobic conditions (Kumari et al., 2016). Microorganisms degrade a wide range of mono-azo, di-azo, tri-azo, and poly-azo textile dyes used in dyeing and printing industries (Sudha et al., 2014). Azo-reductase is reported to be the key enzyme expressed in bacterial azo dyes degrading and catalyses the reductive cleavage of the azo bond. Azo-reductase activity had been identified in a number of bacterial species recently, such as *Staphylococcus aureus, Shewanella putrefaciens, Shewanella* and *Pseudomonas* spp. (Shah et al., 2014).

Ammonia levels in textile waste biodegradation

High ammonia levels are an indication of organic material pollution from domestic, industrial, and agricultural wastes. The elimination of nitrogen is achieved with the means of a two-step process: nitrification followed by heterotrophic denitrification. The first one converts ammonia into nitrates (NO_3^-) under anoxic condition while the second one converts the resulting NO_3^- (electron acceptor) into nitrogen gas (N_2) with the presence of organic carbon (electron donor) (Benneouala et al., 2017).

Unionized ammonia is toxic because nitrification microorganisms are needed to oxidize ammonia to nitrite and nitrate. Ammonia in water can be in the form of NH_3 molecules (non-dissociation/unionization) and the form of ammonia ions (dissociation) in the form of NH_4^+. Both forms of ammonia are very dependent on pH conditions and water temperature. Cell walls cannot be penetrated by ammonia ions (NH_4^+), but ammonia NH_3 will easily diffuse through the network if the concentration is high and potentially toxic to fish. The results of biodegradation of dyes in textile effluent showed that the consortium of *Bacillus subtilis, Bacillus licheniformis and Bacillus coagulans* on Remazol black at a concentration of 500, 1000 and 1500 mg/l for 14 days can reduce ammonia levels were 62.85%, 56.44%, 53.69% respectively. While on Remazol red can reduce ammonia levels was 68.51%, 54.76%, 70.72% respectively. This result shows that biodegradation by consortium can reduce ammonia higher than singly, also can be seen that the decrease in ammonia levels in Remazol red is more considerable than Remazol black (Table 3).

Tabel 3. Ammonia levels on biodegradation of black and red Remazol dyes (%) by three strains in a consortium and individually

Treatment	Remazol black ammonia (%) reduction	Remazol red ammonia (%) reduction
Control 500 ppm	1.36	2.05
Control 1000 ppm	0.15	1.51
Control 1500 ppm	0.14	73.43
B. subtilis 500 ppm	55.89	62;18
B. subtilis 1000 ppm	52.08	36.3
B. subtilis 1500 ppm	51.53	65.12
B. licheniformis 500 ppm	52.13	46.25
B. licheniformis 1000 ppm	50.48	55.86
B. licheniformis 1500 ppm	50.28	71.27
B. coagulans 500 ppm	61.55	62.25
B. coagulans 1000 ppm	55.09	59.13
B. coagulans 1500 ppm	51.52	47;20
Consortium 500 ppm	62.86	68.51
Consortium 1000 ppm	56.44	54.76
Consortium 1500 ppm	53.69	70.72

Decreasing ammonia levels will reduce ammonia toxicity and improve wastewater quality. Referring to Government Regulation of the Republic of Indonesia No. 82 of 2001 concerning Management of Water Quality and Water Pollution Control, the quality standard of COD class III parameters is 0.5 mg/l, it can be concluded that biodegradation products of textiles effluent that contain black and red Remazol have not met the quality standard but have been able to reduce ammonia levels. Lowering the toxicity of ammonium in textile effluent, nitrification by nitrifying microorganisms is an attempt to eliminate ammonium biologically. In nitrification, ammonia will be oxidized to nitrite and then to nitrate, and nitrifying bacteria used the organic compounds in textile effluent as a source of nutrition.

Biological Oxygen Demand (BOD) levels in textile effluent biodegradation

Biochemical Oxygen Demand (BOD) is the amount of dissolved oxygen needed by aerobic microorganisms to oxidize organic matter to carbon dioxide (CO_2) and water (H_2O). The higher the BOD concentration of water, shows the concentration of organic matter in water is high, which is an indicator that the waters have been polluted.

Tabel 4. BOD levels (mg/l) on biodegradation of Remazol black and red dyes (%) by three strains in a consortium and individually

Treatment (ppm)	Remazol black BOD % reduction	Remazol red BOD % reduction
Control 500 ppm	7.73	1.57
Control 1000 ppm	4.94	0.02
Control 1500 ppm	1.05	0.02
B. subtilis 500 ppm	73.58	74.10
B. subtilis 1000 ppm	71.85	72.10
B. subtilis 1500 ppm	71.81	71.92
B. licheniformis 500 ppm	72.90	72.63
B. licheniformis 1000 ppm	71.62	71.64
B. licheniformis 1500 ppm	69.31	70.55
B. coagulans 500 ppm	75.06	75.32
B. coagulans 1000 ppm	7278	73.90
B. coagulans 1500 ppm	72.53	72.03
Consortium 500 ppm	77.31	78.06
Consortium 1000 ppm	76.59	76.70
Consortium 1500 ppm	75.45	76.33

Table 4 shows that the biodegradation of Remazol black and red Remazol in textile wastewater by a consortium and individual *Bacillus subtilis, Bacillus licheniformis,* and *Bacillus coagulants* with a concentration of 500 ppm, 100 ppm, and 1500 ppm obtained BOD levels in black Remazol, respectively 77.31%, 76.59%, 75.45% while BOD levels in red Remazol BOD levels were 78.06%, 76.7% and 76.33%, respectively. The removal of organic matter and nutrients from the wastewater is an important aspect of biological treatment.

A decrease in BOD levels around 70 percent shows the consortium, and individuals can degrade dyes up to 1000 mg/l. A significant reduction in the BOD value indicates that organic matter in waste acts as a substrate for the metabolism of aerobic microorganisms. The decrease in BOD levels in the consortium is more significant than the reduction of BOD levels individually. Decreased levels of BOD might be due to their synergistic effect on pollutant removal. bacterial consortium proved as efficient organic biodegradation than that of the monocultures.

The developed bacterial consortium was more efficient in biodegradation pure dye solutions as well as mixture of all dyes, which indicates that microbial consortium is more powerful agent to treat dying wastewater than single bacterial inoculums, and all the isolates used showed compatibility with each other. Various microorganisms including, yeasts *Proteus* sp*., Enterococcus* sp*., Streptococcus* sp*., Bacillus subtillis* and *Streptococcus* sp., can degrade azo compounds, and after the bacterial degradation, they were also found less toxic than original dye (Singh and Singh, 2015).

The developed bacterial consortium was much more efficient in biodegradation and decolorizing single dyes as well as mixture of dyes than monocultures indicating the potential of mixed microbial consortium as potent bioremediation agent for cost effective removal of diverse dyes from dying effluent. The dye effluents are high in colour, pH, suspended solids (SS), chemical oxygen demand (COD), biochemical oxygen demand (BOD) and metals, temperature and salts. Therefore, during the treatment processes, it is important to monitor and compare these parameters with the standard concentrations before discharging the corresponding effluent to the receiving water body (Karim et al., 2018).

Chemical Oxygen Demand (COD) levels in biodegradation of textile effluent

The effluents from textile industries are varied in composition, which may not be biodegradable. The level of COD is a critically important factor in evaluating the extent of organic pollution in textile wastewater. Chemical Oxygen Demand (COD) is the amount of oxygen needed to oxidize organic compounds chemically. COD is a test conducted to determine the content of biodegradable (easily decomposed) and non-biodegradable (non-biodegradable) organic compounds (Myszograj et al., 2017). In environmental chemistry, the chemical oxygen demand (COD) test is commonly used to indirectly measure the number of organic compounds in water, making COD a useful measure of water quality. It is expressed in milligrams per litter (mg/l), which affects the mass of oxygen consumed per litter of solution (Kosseva et al., 2013). COD is always indicated as pollution loads resulting from each processing operation of various raw materials. Therefore, COD removal is needed with more effective microorganisms for treatment. High levels of COD depend on the type of fibre, dyes, additives, and various textile operations that are routinely carried out.

Tabel 5. COD levels (mg/l) on biodegradation of three strains in a consortium and individually

Treatment (ppm)	Remazol black COD % reduction	Remazol red COD % reduction
Control 500 ppm	0.65	0.24
Control 1000 ppm	0.19	0.04
Control 1500 ppm	0.00	0.03
B. subtilis 500 ppm	69.53	70.3
B. subtilis 1000 ppm	67.66	69.08
B. subtilis 1500 ppm	66.68	67.19
B. licheniformis 500 ppm	65.96	67.92
B. licheniformis 1000 ppm	62.72	63.06
B. licheniformis 1500 ppm	61.78	61.88
B. coagulans 500 ppm	68.70	69.52
B. coagulans 1000 ppm	67.98	68.95
B. coagulans 1500 ppm	65.91	66.49
Consortium 500 ppm	73.91	73.77
Consortium 1000 ppm	71.74	71.29
Consortium 1500 ppm	68.91	71.00

Biodegradation of Remazol black and Remazol red dyes by a bacterial consortium of *Bacillus subtilis, Bacillus licheniformis* and *Bacillus coagulans* at concentrations of 500 ppm, 1000 and 1500 ppm COD levels obtained, respectively 73.91%, 71.74%, 68.91%, 73.77%, 71.29%, 70.00% (Table 5). The decrease in COD value indicates the degradation of dyes and organic matter in the waste by microorganisms. Biodegradation reaches up to 70% because microorganisms decompose finely dispersed materials, colloids, and solutes through metabolism.

Although pure culture is reported to be effective in treating textile wastewater, a mixed culture or consortium would probably be more effective in degrading toxic compounds in the textile wastewater. A mixed culture can adapt better to changing conditions during growth. For example, anaerobic, facultative species will grow after aerobic species, and facultative species will survive with or without oxygen.

Bacillus sp., *Pseudomonas* sp., *and Pseudomonas luteola* are widely used in industrial waste management and can reduce levels of dissolved COD by 57.5%, which is carried out for 12 days (Dwipayana, 2010).

Bacillus sp. can integrate the components of cellulose, tolerant to acids, and can break down urea into ammonia, carbon dioxide, and water to reduce the levels of BOD and COD of industrial liquid waste. The genus *Bacillus* has interesting physiological properties because each type has different abilities, including (1) able to degrade organic compounds such as protein, starch, cellulose, hydrocarbons, and dyes, (2) able to produce antibiotics, (3) plays a role in nitrification and denitrification, (4) nitrogen-fixing, (5) oxidizing selenium, (6) oxidizing and reducing manganese (Mn). Some genera of Bacillus differ in their growth properties, some of which are mesophilic, namely *Bacillus subtilis*, facultative thermophilic, for example *Bacillus coagulans*, thermophilic for example *Bacillus thermophilus*. It also has different enzymatic abilities in producing enzymes, including in producing amylase, protease, and lipase enzymes such as *Bacillus licheniformis, Bacillus cereus, Bacillus subtilis, Bacillus coagulans, Bacillus pumilus, Bacillus samithi, Bacillus brevis.* These bacteria can decompose dyes, such as Remazol, indigo sol, and naphtol. Microbial consortia were recommended for environmental remediation to degrade a variety of pollutants. The different conditions of textile wastewater after certain times may affect the growth of the consortia.

Industrial wastewater has a very high content of organic matter, one of which is cellulose content. The process of decomposition of the waste requires the help of cellulase enzymes that can break the glycosidic bonds. *Bacillus subtilis* and *Bacillus coagulans* are known to be the Bacillus class, which are capable of producing high amounts of cellulase enzymes. The reduced BOD and COD levels indicate the reduction of the toxic substances in the effluent. The mechanism of decolorization may be through two steps, either through adsorption and other enzymatically.

Different enzyme systems are known to exist in individual bacteria that are responsible for degradation. A consortium of the bacterium gives a synergistic effect both enzymatically as well as the availability of surface areas, which may be liable for the enhanced rate of decolorization.

Total Suspended Solid **(TSS) levels in biodegradation of textile effluent**

Total Suspended Solids (TSS) is the portion of fine particulate matter that remains in suspension in water.

TSS are particles that are larger than 2 microns found in the water column. Anything smaller than this is called a dissolved solid.

The majority of suspended solids are made up of inorganic materials, although bacteria and algae can contribute to total solid levels.

These solids include anything floating through the water such as gravel, silt, sand or clay. TSS is the term used to refer to the solid particles suspended in water. It is defined as the total amount of solid material, suspended in water, that is retained by a filter.

The decrease in TSS percentage (%) produced by biodegradation of Remazol black and red Remazol dyes by *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus coagulans* in a consortium at various concentrations of each 67.5%, 66.83%, 52.71%, 70.13%, 60.93% and 58.72%.

Tabel 6. The TSS levels (mg/l) on biodegradation of Remazol black and Remazol red dyes (%) by three Isolates in a consortium and individually

Treatment (ppm)	Remazol black TSS % Reduction	Remazol red TSS % reduction
Control 500 ppm	0.73	0.185
Control 1000 ppm	0.13	0.13
Control 1500 ppm	0	0.03
B. subtilis 500 ppm	57.27	57,57
B. subtilis 1000 ppm	55.49	56.11
B. subtilis 1500 ppm	55.33	55.59
B. licheniformis 500 ppm	55.88	57.05
B. licheniformis 1000 ppm	53.48	55.16
B. licheniformis 1500 ppm	52.94	53.82
B. coagulans 500 ppm	56.75	57.45
B. coagulans 1000 ppm	55.95	55.26
B. coagulans 1500 ppm	50.53	54.79
Consortium 500 ppm	67.5	70.13
Consortium 1000 ppm	66.83	60.93
Consortium 1500 ppm	52.71	58.72

The results show that the biodegradation with mixed bacteria showed more significant than individual.

A decrease in TSS levels indicates that the synergistic effect of bacterial combination brings about enhanced performance for effecttive biodegradation. The decrease in TSS level an occur because the organic materials contained in the waste have been broken down by waste degrading bacteria and produce compounds that can be used for bacterial growth.

As in this study, showed that bacteria from different genera can work together in an environment and survive through the interaction of the metabolite because a mixed culture has more competence and has a higher tolerance to toxic metabolites.

Some strains, such as *Bacillus cereus, Pseudomonas putida, Pseudomonas fluorescence, Pseudomonas desmolyticum* and *Bacillus* sp. have been used in the biodegradation of azo dyes.

These microbial consortia were recommended for environmental remediation to degrade variety of pollutants (Kumari et al., 2016). *Bacillus subtilis* and *Bacillus coagulans* have high cellulolytic ability, therefore dissolved solids of cellulose can be decomposed. Also, *B.*

subtilis and *B. coagulans* belong to a group of *Bacillus* genus bacteria that can decompose crude fibres and lignin that are difficult to decompose through the process of delignifycation and hydrolysis of cellulose.

CONCLUSIONS

B. subtilis, B. licheniformis and *B. coagulans* isolated from the river were contaminated from textile effluent, can grow, and tolerate Remazol black and red Remazol dyes up to 1500 mg/l. A consortium of these species can decolorize black Remazol and red Remazol up to 1000 mg/l, with a reduction percentage ranging from 50-60%. Bio-decolorosation by the consortium is more effective than monoculture. Also, the consortium can reduce levels of BOD, TSS, BOD and ammonia > 70% at concentrations of Remazol black and red Remazol up to 1000 mg/l.

ACKNOWLEDGEMENTS

This study was supported by the Academic Leadership Grant, an internal from the University of Padjadjaran with the title: "Isolation, Identification, Characterization of The Potential and Application of Microorganism to Processing Industrial and Domestic Waste" supervised by professor dr. Jetty Nurhayati.

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