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[Cláudia Mouro](#) , [Ana P. Gomes](#) , Rita V. Costa , [Farzaneh Moghtader](#) , [Isabel C. Gouveia](#) \*

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Article

# Sustainable Bioactive Dyeing of Textiles: A Novel Strategy Using Bacterial Pigments, Natural Antibacterial Ingredients, and Deep Eutectic Solvents

Cláudia Mouro, Ana P. Gomes, Rita V. Costa, Farzaneh Moghtader and Isabel C. Gouveia \*

FibEnTech Research Unit, Faculty of Engineering, University of Beira Interior, 6200-001 Covilhã, Portugal

\* Correspondence: igouveia@ubi.pt (I.C.G.); Tel.: +351-27-531-9825

**Abstract:** Textile industry is one of the major industries responsible for environmental pollution globally. This is mainly due to the use of synthetic dyes, toxic ingredients and solvents for dyeing/treatments/protocols of textiles. Replacement of those non ecofriendly ingredients would be a very challenging target to develop novel sustainable textile dyeing strategies. Using biobased and bioactive pigments, antibacterial ingredients and especially natural solvents are recently pointed out as substances to be included in the related formulations. In this study the dyeing ability of the crude gel prodigiosin produced by non-pathogenic bacteria *Serratia plymuthica* as a natural pigment was investigated on various multifiber fabrics at different conditions (temperature and pH) and by using salts (NaOH and Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>) and alternative mordants (a conventional: FeSO<sub>4</sub> and a new bio-mordant: L-Cysteine (L-Cys)) in the recipes with different compositions and especially replacing of organic solvent with a new type of gel-based Choline chloride (ChCl) : Lactic acid (LA) (1 : 2) deep eutectic solvents (DES) as a dyeing medium. For determination of the dyeing process performances, the color strength (*K/S* value) of dyed fabric and the dyeing equalization (*dE*) parameters were evaluated. The fastness properties of the dyed fabrics which displayed a higher *K/S* value and good *dE* were evaluated. The nylon fabrics dyed with 3.0% *owf* L-Cys at pH = 8.3 improved the color fastness to washing, while the gel-based ChCl : LA (1 : 2) DES dye bath provided a better color fastness to light. Moreover, the nylon fabrics under these conditions exhibited remarkable antimicrobial activity against *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*). As conclusion, the use of the crude gel prodigiosin pigment revealed an advantageous ability to dye the textile materials, responding to consumers' demand for more ecological and sustainable products. Furthermore, the natural reducing agent L-Cys, never tested before as a bio-mordant, and the use of gel-based DES as a dyeing medium demonstrated enhanced colorimetric and antibacterial properties on dyed nylon with crude gel prodigiosin pigment.

**Keywords:** Bacterial pigments; Crude gel prodigiosin pigment; Natural-dyeing; Bio-mordant; L-Cysteine; Gel-based deep eutectic solvent

## 1. Introduction

Nowadays, the textile industry is a major contributor to global pollution, due to the use of synthetic and toxic dyes and also several hazardous chemicals/ingredients during the dyeing and finishing processes that cause serious adverse environmental impacts [1,2]. Particularly, the textile dyeing processes discharge contaminants into water, soil, and the atmosphere and require excessive consumption of water and energy, that have a quite dangerous and negative impact on environmental and human health [1–4]. In this context, and in line with increasingly stringent government policies and regulations, it is crucial to develop and provide more environmentally friendly and economically sustainable strategies [2,5]. In addition, allied with this, consumers are concerned about environmental issues in the textile industry and are willing to pay more for materials that are less harmful and toxic to the environment [5].

Thus, the re-introduction of natural dyes, as an alternative option to the petrochemical-based synthetic dyes, that have been dominating the modern textile industry since their arise, due to their low manufacturing costs, processability, and desirable coloring characteristics, has been explored to achieve economically and environmentally sustainable dyeing processes [1,2,4]. In fact, the recent interest in natural dyes over dangerous synthetic dyes is due to their better biodegradability, non-toxicity, environmental safety, and lower incidence of allergic reactions [1,4–6]. Nevertheless, natural dyes, particularly those derived from plants, display a few inherent drawbacks, such as low affinity for the textiles, poor color fastness properties, limited range of color, low yields, problems in the reproduction of shades, long extraction processes, and seasonal variations [1,4]. Accordingly, to overcome such limitations and improve the textile dyeing processes with green natural dyes, different mordants, such as metallic salts and natural tannin-containing plants have been applied to enhance the dyeability and fastness features of the textile materials [1,4]. However, most of these mordants usually alter the hue and darkness or brightness of the dyed textile fibers [1]. Therefore, in this study, the amino acid L-Cysteine (L-Cys), widely used as a reducing agent, was tested as a new natural bio-mordant, which has not been applied before to the best of our knowledge. Besides, gel-based deep eutectic solvents (DES), formed by a hydrogen bond donor and a hydrogen bond acceptor, which have been aimed to be used as novel environmentally friendly and water-free dyeing mediums, gained our attention due to their ability to improve the color fastness properties without changing the dyeing properties [7,8].

In turn, the high demand for natural dyes has led to the analysis of new sources for their extraction [2,4,6]. Among them, microbial pigments have been highlighted by several authors due to their diversity, greater productivity, rapid growth in the culture medium, and efficient control of the fermentation process [4]. Furthermore, microbial pigments can be produced using low-cost fermentation media, namely agro-industrial wastes [9–11].

Microbial pigments can be produced by bacteria, fungi, yeast, and algae with various chemical structures and a large array of colors [4]. Moreover, the pigments produced from natural sources present interesting bioactive properties in addition to their color features, such as antioxidant, antimicrobial, anticancer activity, insect-repellent, and ultraviolet protection [1,2,4,5,12,13].

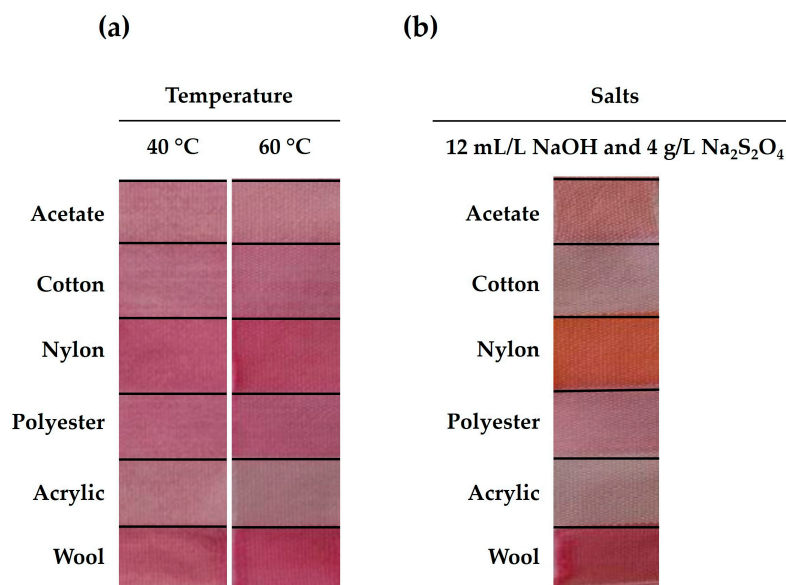
Over the years, a wide range of bacteria has been reported for their ability to synthesize multifunctional secondary metabolites in response to external stimuli with a high growth rate [2,12,13]. Concerning that, this study aimed to dye a multifiber fabric with a crude gel prodigiosin pigment, a bacterial metabolite commonly produced by numerous species, including *Serratia* sp., and which is recognized for its attractive red color and a broad spectrum of bioactive properties, such as antimicrobial, antifungal, anticancer, antimalarial, and immunosuppressive activities [2,4,13]. Furthermore, the prodigiosin pigment has been widely extracted and purified by using conventional organic solvents, like acidified ethanol and methanol [14–22], chloroform [18,22], acetonitrile [18,22], dimethyl sulfoxide (DMSO) [15,18,22], ethyl acetate [19], petroleum ether [19], diethyl ether [19], and acetone [15,19] which are harmful to the human health and environment. Accordingly, to the authors' knowledge, the present study is the first report to use a crude gel prodigiosin pigment obtained directly from medium-agar plates, without further adopting solvent extraction and purification methods. Moreover, the effect of the dyeing conditions, such as the temperature, the salts (NaOH and Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>) and mordants (FeSO<sub>4</sub> and L-Cys) addition, as well as the use of gel-based Choline chloride (ChCl) : Lactic acid (LA) (1 : 2) DES as a dyeing medium were investigated, and the crude gel prodigiosin pigment collected directly from the culture medium revealed to have several economic and environmental benefits and a high potential to replace the synthetic dyes used in the textile industry. Mainly, the new bio-mordant, L-Cys shown to have a good green dyeing performance, in preference to nylon, and proved to be suitable to strengthen the inherent antibacterial properties of the prodigiosin pigment. In addition, the use of water-free gel-based ChCl : LA (1 : 2) DES dyeing medium effectively improved the light fastness properties of dyed nylon fabrics and reinforced their remarkable antibacterial activity. Therefore, the sustainable dyeing process for dyeing nylon with the crude gel prodigiosin pigment using DES can significantly reduce the water consumption and effluent emission, which is crucial to develop novel green dyeing solutions.

## 2. Results and Discussion

### 2.1. Evaluation of the color strength and equalization of the dyed fabrics

#### 2.1.1. The effect of temperature

The multifiber fabrics dyed with the crude gel prodigiosin pigment at 40 °C and 60 °C revealed various tones for the different textile fibers, Figure 1a. Such occur essentially due to the different interactions established between the pigment and the fiber, as well as the color of the fiber itself. In addition, the performance of the bacterial pigments, namely the color depth and dyeing rate, depends significantly on the dyebath temperature [2].



**Figure 1.** Influence of temperature (a) and of salts addition (NaOH and Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>) (b) on multifiber dyeing with the crude gel prodigiosin pigment.

According to the literature, high temperatures, above 80 °C, are more suitable for dyeing textile fibers with prodigiosin [23–26]. However, more environmentally friendly and sustainable dyeing methods have emerged as a more efficient and economical alternative to conventional processes. Concerning that, lower temperatures, like 40 °C and 60 °C, were applied, and their effect on the color strength (*K/S*) of the dyed multifiber fabrics was evaluated and summarized in Table 1a. The temperatures of 40 °C and 60 °C showed similar results for the multifiber fabrics. Nevertheless, the crude gel prodigiosin pigment presented greater affinity for the wool and nylon fibers ( $K/S_{Wool_{40\text{ °C}}} = 1.56$ ;  $K/S_{Wool_{60\text{ °C}}} = 1.38$ ;  $K/S_{Nylon_{40\text{ °C}}} = 1.17$ ;  $K/S_{Nylon_{60\text{ °C}}} = 1.20$ ). Besides, more uniform colors were observed at 60 °C, resulting in an improvement in dyeing equalization (*dE*) values ( $dE_{Wool_{60\text{ °C}}} = 0.05$ ;  $dE_{Nylon_{60\text{ °C}}} = 0.04$ ). Therefore, 60 °C was chosen to achieve a better crude gel prodigiosin dyeing effectiveness.

**Table 1.** Color Strength (*K/S*) measurements and the dyeing equalization (*dE*) values on multifiber dyeing with the crude gel prodigiosin pigment at 40 °C and 60 °C (a) and after salts addition (b).

	Temperature (°C)		Salts
	40	60	
(a) Acetate	<i>K/S</i>	0.49	12 mL/L NaOH and 4 g/L Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub>
	<i>dE</i>	0.10	
Cotton	<i>K/S</i>	0.78	Acetate
			<i>K/S</i>
			<i>dE</i>
			Cotton
			<i>K/S</i>
			0.59
			0.19
			0.25

	<i>dE</i>	0.10	0.08		<i>dE</i>	0.16
Nylon	<i>K/S</i>	1.17	1.20	Nylon	<i>K/S</i>	1.25
	<i>dE</i>	0.16	0.04		<i>dE</i>	0.14
Polyester	<i>K/S</i>	0.68	0.72	Polyester	<i>K/S</i>	0.39
	<i>dE</i>	0.09	0.05		<i>dE</i>	0.07
Acrylic	<i>K/S</i>	0.51	0.30	Acrylic	<i>K/S</i>	0.14
	<i>dE</i>	0.06	0.03		<i>dE</i>	0.10
Wool	<i>K/S</i>	1.56	1.38	Wool	<i>K/S</i>	1.60
	<i>dE</i>	0.12	0.05		<i>dE</i>	0.05

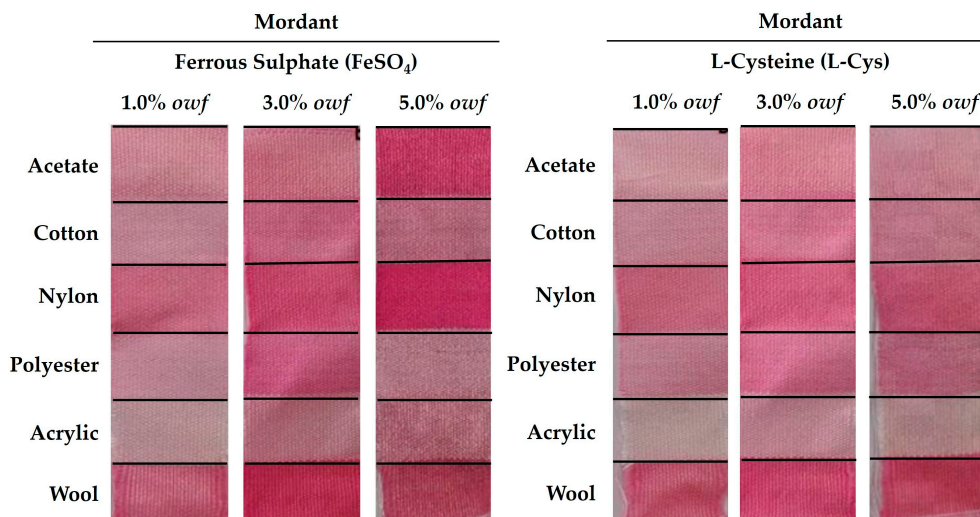
## 2.1.2. The effect of the dyeing aids addition

### 2.1.2.1. The addition of salts

In order to improve the crude gel prodigiosin pigment solubility, sodium hydroxide (NaOH) and sodium hydrosulphite ( $\text{Na}_2\text{S}_2\text{O}_4$ ) were used as alkali and a reducing agent, respectively. Regarding that, the addition of these aides changed the dye bath's pH of 7.0 to 12.5, resulting in different interactions between the crude gel prodigiosin pigment and the several fibers, Figure 1b. The nylon fabric presented the most significant difference, exhibiting a fluorescent orange tone. Such occurs essentially because prodigiosin pigment is sensitive to pH changes, which results in color change for a blue-purple tone at acidic pH's, pink-red at neutral pH's, and an orange-yellow tone at alkaline pH's [24,26,27]. Nevertheless, the crude gel prodigiosin pigment showed greater affinity for nylon ( $K/S_{\text{Nylon}} = 1.25$ ) and wool ( $K/S_{\text{Wool}} = 1.60$ ) fibers, Table 1b. However, the *dE* of the different fabrics (Table 1b) was higher than the obtained for the dyed samples without auxiliaries at 60 °C (Table 1a), resulting in less homogeneous dyeing samples. Hence, the addition of these salts did not prove to be beneficial for the dyeing process with the crude gel prodigiosin pigment.

### 2.1.2.2. The addition of mordants

Mordants are often used as additives in dyebaths to improve the binding of natural pigments to fibers. Concerning that, mordants form complexes with natural dyes capable of attaching to the fiber surfaces, improving the exhaustion of dyebaths and the washing fastness of the dyed fabrics. Nevertheless, the various forms and types of mordants can influence the shades and hues of the fabrics, resulting in brighter or darker colors, or even differences in the natural color of the pigment, which can be a beneficial or an unwanted effect [2,4]. Moreover, the mordant concentration is also a crucial factor that can cause an increase in the color intensity during the textile dyeing [4]. Therefore, the effect of the addition of mordants, like  $\text{FeSO}_4$  and L-Cys, on the *K/S* values and *dE* of the dyed fabrics was investigated at three different concentrations (1.0%, 3.0%, and 5.0% *owf*), Figure 2 and Table 2.



**Figure 2.** Influence of mordants addition (Ferrous Sulphate ( $\text{FeSO}_4$ ) and L-Cysteine (L-Cys)) at 1.0, 3.0, and 5.0% owf on multifiber dyeing with crude gel prodigiosin pigment.

**Table 2.** Color Strength (K/S) measurements and the dyeing equalization (dE) values on multifiber dyeing with the crude gel prodigiosin pigment after mordants (Ferrous Sulphate ( $\text{FeSO}_4$ ) and L-Cysteine (L-Cys)) addition at 1.0, 3.0, and 5.0% owf.

		Mordants					
		Ferrous Sulphate ( $\text{FeSO}_4$ )			L-Cysteine (L-Cys)		
		1.0%	3.0%	5.0%	1.0%	3.0%	5.0%
Acetate	K/S	0.75	1.65	6.43	0.47	0.63	0.70
	dE	0.15	0.08	0.17	0.13	0.08	0.10
Cotton	K/S	0.55	0.90	1.23	0.59	0.86	0.94
	dE	0.14	0.11	0.43	0.04	0.04	0.09
Nylon	K/S	1.39	2.02	6.81	1.12	1.72	1.61
	dE	0.09	0.09	0.16	0.06	0.02	0.07
Polyester	K/S	0.48	0.60	0.77	0.68	0.76	0.91
	dE	0.10	0.15	0.30	0.07	0.04	0.19
Acrylic	K/S	0.27	0.66	0.92	0.19	0.21	0.35
	dE	0.09	0.06	0.55	0.05	0.02	0.12
Wool	K/S	1.47	2.44	2.06	1.69	2.08	2.48
	dE	0.10	0.11	0.96	0.06	0.04	0.11

To achieve bright and strong colors with natural dyes, metallic mordants such as the conventional  $\text{FeSO}_4$ , that is known as a blue-green vitriol water-soluble dulling mordant, have been commonly used [4]. However, the solubility of the  $\text{FeSO}_4$  mordant in the aqueous crude gel prodigiosin pigment bath was low, resulting in a heterogeneous coloration at a rate directly proportional to the increase in mordant concentration, Figure 2. Similar results were previously reported by Burkinshaw et al. and Mongkholrattanasit et al., who observed a shade of dark greyish-brown in the fabric of relatively low K/S when  $\text{FeSO}_4$  mordant was added [28,29]. Such occur essentially due to the ability of the pigment molecules to form a metallic complex with positively charged ions ( $\text{Fe}^{2+}$ ), as previously described by Uddin [30]. Hence, when the  $\text{FeSO}_4$  mordant was added to the crude gel prodigiosin dye bath solution different shades of color were observed in the

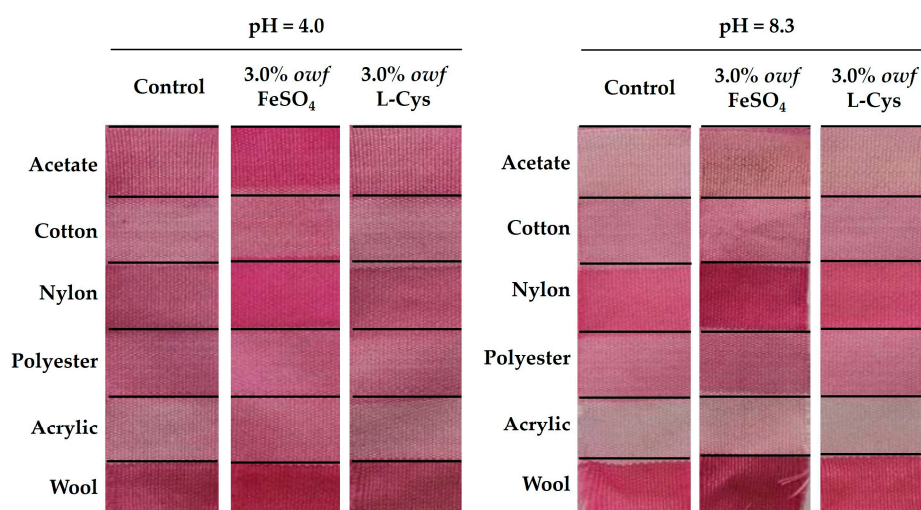
distinct fibers and a blackening of the color shades was revealed when the mordant concentration increases of 1.0% *owf* to 5.0% *owf*, Figure 2. Similar observations have also been reported by other researchers in the literature [4]. Moreover, the lower concentration of  $\text{FeSO}_4$  (1.0% *owf*) resulted in a smoother and similar dyeing among the fabrics, while a higher concentration of  $\text{FeSO}_4$  (5.0% *owf*) presented a deep but uneven dyeing, Figure 2 and Table 2.

In addition, the crude gel prodigiosin pigment with 1.0% and 3.0% *owf* of  $\text{FeSO}_4$  exhibited the highest affinity for nylon and wool fibers (Table 2), resulting in the highest *K/S* values ( $K/S_{\text{Nylon}_1.0\% \text{FeSO}_4} = 1.39$ ;  $K/S_{\text{Nylon}_3.0\% \text{FeSO}_4} = 2.02$ ;  $K/S_{\text{Wool}_1.0\% \text{FeSO}_4} = 1.47$ ;  $K/S_{\text{Wool}_3.0\% \text{FeSO}_4} = 2.44$ ), as well as, lower *dE* ( $dE_{\text{Nylon}_1.0\% \text{FeSO}_4} = 0.09$ ;  $dE_{\text{Nylon}_3.0\% \text{FeSO}_4} = 0.09$ ;  $dE_{\text{Wool}_1.0\% \text{FeSO}_4} = 0.10$ ;  $dE_{\text{Wool}_3.0\% \text{FeSO}_4} = 0.11$ ).

On the other hand, bio-mordants, like L-Cys have been regarded as a sustainable and ecologically alternative to metallic mordanting. The dyeing of the fabrics with the crude gel prodigiosin pigment using L-Cys as bio-mordant resulted in different tones of pink. These different tones were similar for the three different concentrations of prodigiosin, Figure 2. Nevertheless, nylon and wool displayed a better color strength ( $K/S_{\text{Nylon}_1.0\% \text{L-Cys}} = 1.12$ ;  $K/S_{\text{Nylon}_3.0\% \text{L-Cys}} = 1.72$ ;  $K/S_{\text{Nylon}_5.0\% \text{L-Cys}} = 1.61$ ;  $K/S_{\text{Wool}_1.0\% \text{L-Cys}} = 1.69$ ;  $K/S_{\text{Wool}_3.0\% \text{L-Cys}} = 2.08$ ;  $K/S_{\text{Wool}_5.0\% \text{L-Cys}} = 2.48$ ), and an improved dyeing equalization (*dE*) in the presence of 3.0% *owf* of L-Cys ( $dE_{\text{Nylon}_3.0\% \text{L-Cys}} = 0.02$  and  $dE_{\text{Wool}_3.0\% \text{L-Cys}} = 0.04$ ).

Therefore, as an efficient dyeing depends on the color intensity of dyed fibers and a good equalization, 3.0% *owf* of L-Cys should be chosen to achieve a better dyeing process with the crude gel prodigiosin pigment.

Nevertheless, bacterial pigments, such as prodigiosin are sensitive to pH alterations, resulting in color changes. Namely, pink prodigiosin pigment at acidic pH changes to blue-purple and at alkaline pH's to orange-yellow, respectively [24,26]. Thus, in order to analyse the effect of dyebath pH on the color characteristics, two different pH values (pH = 4.0 and pH = 8.3) were studied in the various types of fiber under different conditions (without and with simultaneous mordanting of 3.0% *owf* of  $\text{FeSO}_4$  and L-Cys), Figure 3 and Table 3.



**Figure 3.** Multifiber dyeing with and without mordants (3.0% *owf* Ferrous Sulphate ( $\text{FeSO}_4$ ) and 3.0% *owf* L-Cysteine (L-Cys)) addition at pH = 4.0 and pH = 8.3.

**Table 3.** Color Strength (*K/S*) measurements and the dyeing equalization (*dE*) values on multifiber dyeing with the crude gel prodigiosin pigment at pH = 4.0 and pH = 8.3 with and without adding mordants (3.0% *owf* Ferrous Sulphate ( $\text{FeSO}_4$ ) and 3.0% *owf* L-Cysteine (L-Cys)).

pH					
4.0			8.3		
Control	3.0% <i>owf</i> $\text{FeSO}_4$	3.0% <i>owf</i> L-Cys	Control	3.0% <i>owf</i> $\text{FeSO}_4$	3.0% <i>owf</i> L-Cys

Acetate	<i>K/S</i>	1.71	3.81	1.53	0.51	1.01	0.58
	<i>dE</i>	1.21	0.19	1.16	0.11	0.12	0.11
Cotton	<i>K/S</i>	0.86	1.46	0.83	0.90	1.21	0.97
	<i>dE</i>	0.83	0.62	0.80	0.17	0.23	0.13
Nylon	<i>K/S</i>	1.73	3.30	1.59	1.87	3.67	2.30
	<i>dE</i>	1.01	0.13	1.00	0.09	0.11	0.05
Polyester	<i>K/S</i>	1.10	1.11	0.97	0.95	1.28	0.97
	<i>dE</i>	0.41	0.22	0.33	0.12	0.26	0.11
Acrylic	<i>K/S</i>	0.57	1.04	0.61	0.24	0.42	0.26
	<i>dE</i>	0.17	0.23	0.15	0.17	0.20	0.11
Wool	<i>K/S</i>	2.33	3.22	2.04	2.38	3.11	2.49
	<i>dE</i>	0.70	0.35	0.84	0.25	0.33	0.27

At pH = 4.0, the crude gel prodigiosin pigment dyeing of the fabrics with the metallic mordant ( $\text{FeSO}_4$ ) was favour in comparison with the dyeing process with bio-mordant (L-Cys) and without the addition of mordant, revealing a greater pink color strength (*K/S*), less fiber oxidation, and more efficient dyeing, Figure 3. Moreover, the dyeing with  $\text{FeSO}_4$  demonstrated a superior stability of the pink color of crude gel prodigiosin pigment in the different fibers, particularly in nylon and wool ( $K/S_{\text{Nylon}_3.0\% \text{FeSO}_4, \text{pH}=4.0} = 3.30$ ;  $K/S_{\text{Wool}_3.0\% \text{FeSO}_4, \text{pH}=4.0} = 3.22$ ;  $dE_{\text{Nylon}_3.0\% \text{FeSO}_4, \text{pH}=4.0} = 0.13$ ;  $dE_{\text{Wool}_3.0\% \text{FeSO}_4, \text{pH}=4.0} = 0.35$ ). Therefore, the acidic pH provided more efficient dyeing in the presence of the metallic mordant, increasing its solubility and improving its equalization (*dE*), Table 3. Similar data were reported by Uddin, who revealed that mordants' metal ions act as electron acceptors for electron donors and produce coordination bonds with the dye molecules, making them insoluble in water [30]. In this way, metallic mordants, such as  $\text{FeSO}_4$ , can improve the dye uptake and retention, resulting in a higher depth of shade and greater color fastness properties. Furthermore, Alihosseini et al. showed that prodigiosin pigments produced from a strain of *Vibrio* sp. isolated from marine sediments were able to dye wool, nylon, acrylics, and silk fibers [23]. However, Alihosseini et al. found enhanced dyeing characteristics. The reason for this was because different prodigiosin-producing strains were used and the prodigiosin pigments exhibited different levels of purification [23]. Thus, the bacterial pigment color can be influenced by their nature and consequently, various shades of the same pigment can be obtained on similar fabrics [2].

On the other hand, the infeasibility of dyeing with the crude gel prodigiosin pigment at acidic pH for the fibers dyed simultaneously with L-Cys and without the addition of mordant was confirmed with the high values of *dE* and consequently in a lower equalization of dyeing.

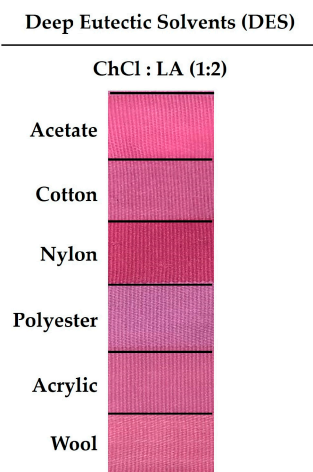
In turn, at pH = 8.3, the dyeing process with the crude gel prodigiosin pigment was favoured in the three different conditions (without the addition of mordants, with the addition of L-Cys, and  $\text{FeSO}_4$ ), although this pH aid the dyeing with the bio-mordant, because pH = 8.3 is related to the p*K*<sub>a</sub> of L-Cys. The fibers dyed with the crude gel prodigiosin pigment in the presence of L-Cys and without the addition of any auxiliary revealed similar and efficient dyeing, Figure 3 and Table 3. Moreover, the dyeing was homogeneous when L-Cys was added, revealing good equalization values ( $dE_{\text{Acetate}_3.0\% \text{L-Cys}, \text{pH}=8.3} = 0.11$ ;  $dE_{\text{Cotton}_3.0\% \text{L-Cys}, \text{pH}=8.3} = 0.13$ ;  $dE_{\text{Nylon}_3.0\% \text{L-Cys}, \text{pH}=8.3} = 0.05$ ;  $dE_{\text{Polyester}_3.0\% \text{L-Cys}, \text{pH}=8.3} = 0.11$ ;  $dE_{\text{Acrylic}_3.0\% \text{L-Cys}, \text{pH}=8.3} = 0.11$ ;  $dE_{\text{Wool}_3.0\% \text{L-Cys}, \text{pH}=8.3} = 0.27$ ).

However, when the  $\text{FeSO}_4$  was used, the oxidation of the fibers increased, mainly in the wool and nylon due to the higher adsorption to the surface of fibers. Nevertheless, although a stronger color strength of the nylon and wool fibers was obtained ( $K/S_{\text{Nylon}_3.0\% \text{FeSO}_4, \text{pH}=8.3} = 3.67$ ;  $K/S_{\text{Wool}_3.0\% \text{FeSO}_4, \text{pH}=8.3} = 3.11$ ), the *dE* was higher ( $dE_{\text{Nylon}_3.0\% \text{FeSO}_4, \text{pH}=8.3} = 0.11$ ;  $dE_{\text{Wool}_3.0\% \text{FeSO}_4, \text{pH}=8.3} = 0.33$ ) Figure 3 and Table 3.

Hence, the choice of the ideal conditions for the dyeing process with the crude gel prodigiosin pigment depends on the color intensity and the uniformity of the dyes. So, it was decided to proceed with 3.0% owf L-Cys at pH = 8.3 for dyeing the nylon and wool fabrics.

### 2.1.3. The effect of the use of gel-based deep eutectic solvents (DES) as a prodigiosin dyeing medium

Recently, gel-based DES have received an increasing attention as a promising green alternative to conventional solvents in order to extract specific alkaloid compounds from different sources, namely from plants, like proline : oxalic acid (1 : 1), ChCl : LA (1 : 2), ChCl : Fructose (5 : 1) [32–34]. In addition, gel-based DES prepared from a hydrogen bond acceptor and a hydrogen bond donor, such as ChCl: urea : glycerol and ChCl : ethylene glycol, can act as water-free solvents able to dissolve dyes without changing their dyeing properties [7]. Hence, in this study, the gel-based ChCl : LA (1 : 2) DES mixture was selected as a dyeing medium for the first time to dissolve the crude gel prodigiosin pigment, a bacterial alkaloid. According to Figure 4 and Table 4, the multifiber fabric dyed with the crude gel prodigiosin pigment dissolved by gel-based ChCl : LA (1 : 2) DES exhibited different shades. Additionally, the strongest red-pink color was obtained in nylon ((K/S<sub>Nylon\_gel-based ChCl : LA (1:2) DES</sub> = 3.33). This value was higher than those found on nylon dyed with the crude gel prodigiosin pigment containing 3.0% owf L-Cys at pH = 8.3 (K/S<sub>Nylon\_3.0% L-Cys\_pH=8.3</sub> = 2.30). Moreover, the ChCl : LA (1 : 2) DES-based gel used to dissolve the crude gel prodigiosin pigment increased the final equalization (dE) in the dyeing process of the multifiber fabric, reaching lower values for the different fibers (dE<sub>Acetate\_gel-based ChCl : LA (1:2) DES</sub> = 0.02; dE<sub>Cotton\_gel-based ChCl : LA (1:2) DES</sub> = 0.02; dE<sub>Nylon\_gel-based ChCl : LA (1:2) DES</sub> = 0.01; dE<sub>Polyester\_gel-based ChCl : LA (1:2) DES</sub> = 0.01; dE<sub>Acrylic\_gel-based ChCl : LA (1:2) DES</sub> = 0.01; dE<sub>Wool\_gel-based ChCl : LA (1:2) DES</sub> = 0.01), in comparison with the values obtained for the fabrics dyeing with the crude gel prodigiosin pigment containing 3.0% owf L-Cys at pH = 8.3 (dE<sub>Acetate\_3.0% L-Cys\_pH=8.3</sub> = 0.11; dE<sub>Cotton\_3.0% L-Cys\_pH=8.3</sub> = 0.13; dE<sub>Nylon\_3.0% L-Cys\_pH=8.3</sub> = 0.05; dE<sub>Polyester\_3.0% L-Cys\_pH=8.3</sub> = 0.11; dE<sub>Acrylic\_3.0% L-Cys\_pH=8.3</sub> = 0.11; dE<sub>Wool\_3.0% L-Cys\_pH=8.3</sub> = 0.27).



**Figure 4.** Influence of the use of gel-based deep eutectic solvents (DES) as a dyeing medium on the multifiber fabric dyeing with crude gel prodigiosin pigment.

**Table 4.** Color Strength (K/S) measurements and the dyeing equalization (dE) values on multifiber fabric using the gel-based deep eutectic solvents (DES) as a prodigiosin dyeing medium.

<b>Gel-based Deep Eutectic Solvents (DES)</b>		
<b>ChCl : LA (1 : 2)</b>		
<b>Acetate</b>	<i>K/S</i>	2.76
	<i>dE</i>	0.02

<b>Cotton</b>	<i>K/S</i>	1.60
	<i>dE</i>	0.02
<b>Nylon</b>	<i>K/S</i>	3.33
	<i>dE</i>	0.01
<b>Polyester</b>	<i>K/S</i>	1.22
	<i>dE</i>	0.01
<b>Acrylic</b>	<i>K/S</i>	1.55
	<i>dE</i>	0.01
<b>Wool</b>	<i>K/S</i>	1.60
	<i>dE</i>	0.01

Therefore, these results demonstrate the potential of the gel-based DES to act as a water-free dyeing medium able to decrease the enormous amounts of water required for the dyeing process. In addition, these new dyeing medium can simultaneously be used as a mordant, providing better absorption and fastness properties [4,7]. For example, Zheng et al. used a ChCl/Ethylene glycol DES to dissolve disperse dyes without changing their chemical structure and original dyeing properties. Moreover, they revealed that after dissolve the dyes into DES mixture, the particle sizes of the disperse dyes were significantly decreased, improving their permeability and consequently the *K/S* values on polyester fabrics as well as the wet rubbing fastness [7]. In fact, their findings showed that green dyeing processes arise as a highly promising approach to reducing water pollution and dyeing costs, as well as meeting the demand for environmental protection.

## 2.2. Evaluation of fastness properties of dyed fabrics

### 2.2.1. Assessment of the washing fastness properties

In this section, the washing fastness of the fabrics that obtained the deepest coloration and better uniformity was evaluated, i.e. the nylon and wool dyed with the crude gel prodigiosin pigment with 3.0% owf L-Cys at pH = 8.3 and the nylon dyed using the gel-based ChCl : LA (1 : 2) DES as dyeing medium. These fabrics were evaluated before and after washing according to ISO 105-C06:2010 and the color difference ( $\Delta E^*$ ) and color fastness ( $\Delta Color$ ) were determined, Table 5.

**Table 5.** The washing fastness properties of the nylon and wool with 3.0% owf L-Cysteine (L-Cys) at pH = 8.3 and the nylon dyed using the gel-based ChCl : LA (1 : 2) DES.

	<b>Fastness Index</b>				
	$\Delta L^*$	$\Delta a^*$	$\Delta b^*$	$\Delta E^*$	$\Delta Color$
<b>Prodigionsin_3.0% owf L-Cys_Nylon_pH = 8.3</b>	1.66	-0.77	2.64	3.21	3
<b>Prodigiosin_3.0% owf L-Cys_Wool_pH = 8.3</b>	3.31	-6.56	2.86	7.88	2
<b>Prodigiosin_gel-based ChCl : LA (1 : 2) DES_Nylon</b>	11.12	-1.15	1.09	10.77	1-2

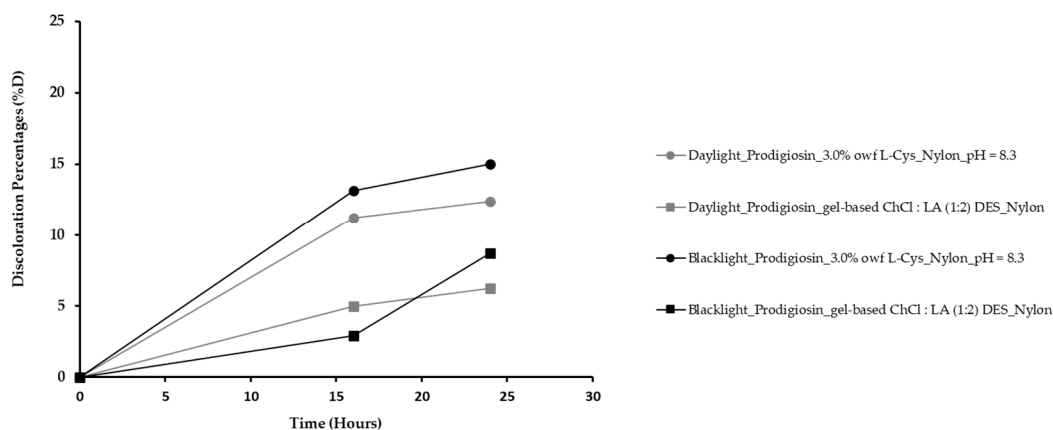
The obtained results showed that the selected fabrics displayed an  $\Delta E^*$  relatively high, i.e. higher than 1, which reveals that a significant color difference was observed after the washing, particularly when the nylon was dyed with the crude gel prodigiosin pigment using the gel-based ChCl : LA (1 : 2) DES as a dyeing medium ( $\Delta E^*_{Nylon\_gel-based\ ChCl:LA\ (1:2)\ DES} = 11.12$ ). In addition, the  $\Delta L^*$  parameter, which measures the lightness difference, was also considered an important factor since the lightness (L)

provides a suitable roadmap for the depth of shade, particularly for the colors that easily change the shade and consequently exhibit high values of  $\Delta a^*$  and  $\Delta b^*$  after washing [24]. Concerning that, it is possible to identify a satisfactory washing fastness for the nylon and wool dyed with the crude gel prodigiosin pigment with 3.0% owf L-Cys bio-mordant at pH = 8.3 ( $\Delta L^*_{\text{Nylon}_3.0\% \text{ L-Cys}_{\text{pH}=8.3}} = 1.66$  and  $\Delta L^*_{\text{Wool}_3.0\% \text{ L-Cys}_{\text{pH}=8.3}} = 3.31$ ). However, the nylon dyed with the gel-based ChCl : LA (1 : 2) DES dyebath presented poor washing fastness ( $\Delta L^*_{\text{Nylon}_{\text{gel-based ChCl : LA (1:2) DES}} = 11.12$ ), which was confirmed by visual assessment of the fabrics when the washed samples were set side by side with the unwashed ones in the Color-Chex lightbox and was evaluated the  $\Delta\text{Color}$  according with CIELab values of the gray-scale using the CIE illuminant D65. In fact, a better fastness index was observed for the nylon dyed with 3.0% owf L-Cys at pH = 8.3 ( $\Delta\text{Color} = 3$ ) in comparison to the wool, which revealed a lower fastness index ( $\Delta\text{Color} = 2$ ), Table 5. Furthermore, the nylon dyed using the gel-based ChCl : LA (1 : 2) DES showed the lowest one ( $\Delta\text{Color} = 1-2$ ), resulting in a greater release of pigment during the washing.

Therefore, the nylon dyed with 3.0% owf L-Cys at pH = 8.3 demonstrated retain a higher amount of crude gel prodigiosin pigment on its surface, which is not so lost after washing, making the prodigiosin dyeing process more efficient. Such data is in accordance with the results published by Liu et al., which reported that the acrylics and polyamide (nylon) dyed with a high purity prodigiosin of the mutant strain of *Serratia marcescens* jx1-1 showed good washing fastness [35].

### 2.2.2. Assessment of the light fastness properties

Commonly, the colored fabrics after light exposure fade upon the presence of oxygen and humidity. In addition, bacterial or natural pigments exhibit poor light stability toward the light in comparison with the most synthetic dyes [4]. Therefore, in this study, the nylon samples dyed with the crude gel prodigiosin pigment containing 3.0% owf of L-Cys at pH = 8.3 and using the gel-based ChCl : LA (1 : 2) DES were directly exposed to blacklight and daylight during 0, 16, and 24 h to accelerate their fading, as shown in Figure 5 through of the discoloration percentages (%D).



**Figure 5.** Evaluation of the light fastness properties of the nylon dyed with the crude gel prodigiosin pigment, with 3.0% owf of L-Cys at pH = 8.3 and using the gel-based ChCl : LA (1 : 2) DES. Discoloration percentages (%D) of each sample after daylight and blacklight exposure.

After 24 h, the color fading was similar for both samples incorporated with L-Cys bio-mordant due to the L-Cys antioxidant-reducing power. However, the nylon dyed with prodigiosin at pH = 8.3, exposed to artificial daylight, and containing L-Cys, suffered a slightly lower color change in comparison with the blacklight sample. Thus, for the nylon containing L-Cys as bio-mordant, it is proposed that L-Cys oxidizes and forms cystine, which is more susceptible to UV light and less to the other lights, resulting in lower discoloration percentages [36]. Moreover, Koch et al. revealed that L-Cys is more susceptible to dark light [37]. This result is consistent with the slight increase in the percentage of discoloration (%D) for nylon dyed with the crude gel prodigiosin pigment containing 3.0% owf of L-Cys at pH = 8.3 exposed to blacklight, Figure 5. Therefore, the addition of the L-Cys bio-mordant can improve the fastness to light of nylon fibers dyed with prodigiosin, a photosensitive

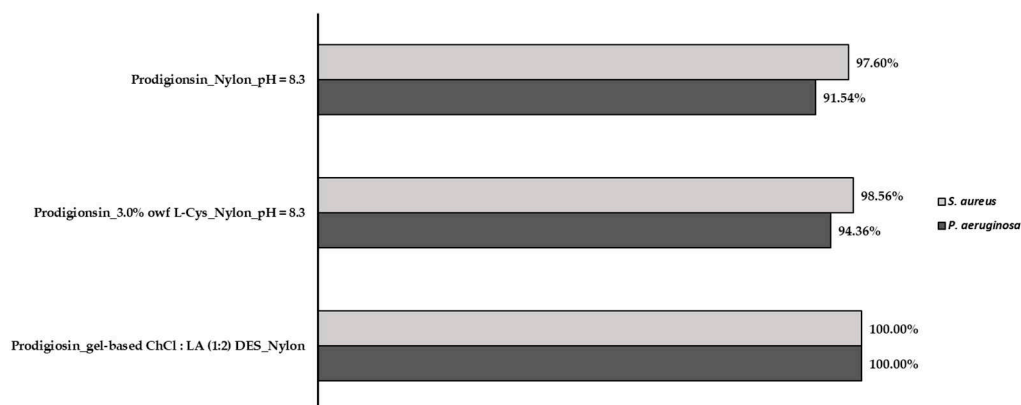
pigment, and makes this work innovative to obtain better natural dyeing. Furthermore, the samples dyed with crude gel prodigiosin pigment using the gel-based ChCl : LA (1 : 2) DES exhibited a very good light fastness after exposed to blacklight and daylight, reaching %D of 8.70% and 6.22%, after 24 h, respectively. Furthermore, the obtained results showed that the nylon dyed with the crude prodigiosin pigment using the gel-based ChCl : LA (1 : 2) DES present light fastness properties similar to that found in the nylon dyed with 3.0% *owf* of L-Cys at pH = 8.3, being more susceptible to blacklight, Figure 5. However, a improvement in the color light fastness was observed due to the incorporation of the gel-based ChCl : LA (1 : 2) DES as dyebath, thus confirming their capability to improve the colorfastness to light.

### 2.3. Evaluation of the antibacterial activity of dyed fabrics

The antibacterial activity of the nylon dyed with the crude gel prodigiosin pigment with and without the addition of 3.0% *owf* of L-Cys at pH = 8.3, as well as using the gel-based ChCl : LA (1 : 2) DES was determined against two bacteria, *S. aureus* (gram-positive) and *P. aeruginosa* (gram-negative), after 24 h.

According to the results presented in Figure 6, a positive inhibitory effect against both strains was shown. Both nylon samples dyed with the crude gel prodigiosin pigment with and without L-Cys exhibited greater efficacy against gram-positive bacteria (*S. aureus*), demonstrating an inhibitory effect of 98.56% and 97.60%, respectively. On the other hand, a lower inhibitory effect was observed for the gram-negative bacteria (*P. aeruginosa*) (94.36% for nylon dyed with the prodigiosin pigment containing 3.0% *owf* L-Cys and 91.54% for nylon dyed with the crude gel prodigiosin pigment without the addition of auxiliaries). These results are in accordance with the data previously reported by Ren et al. and Matwally et al., who reported that the prodigiosin exhibits the ability to induce the production of autolysins in gram-positive bacteria, like *S. aureus*, causing cell lysis and consequently cell death [4,38]. Besides, prodigiosin pigment can penetrate the cell membrane and inhibit specific target enzymes, like topoisomerase IV and DNA gyrase, avoiding cell growth [4]. However, this effect is not observed in gram-negative bacteria, like *P. aeruginosa*. For this type of bacteria, prodigiosin can hinder RNA and protein synthesis, cell division, membrane integrity, and cellular respiration [4].

Moreover, the nylon dyed with the crude gel prodigiosin pigment with the addition of L-Cys displayed an enhanced antibacterial effect in comparison with the nylon without the addition of auxiliaries, like the L-Cys bio-mordant. This result can be explained by taking into account the high reactivity between the thiol (-SH) groups of L-Cys and the sulfhydryl groups (-SH) present in the cell membrane, which lead to a drastic decrease in enzymatic activity and bacterial metabolism and cause leakage of cellular content and cell death [39].



**Figure 6.** Antibacterial activity evaluation: Percentage of bacterial reduction of the nylon dyed with the crude gel prodigiosin pigment with and without addition of 3.0% *owf* of L-Cysteine (L-Cys) at pH = 8.3 and using the gel-based ChCl : LA (1 : 2) DES against *S. aureus* and *P. aeruginosa*.

In turn, the obtained results showed that the nylon dyed with the crude gel prodigiosin pigment using the gel-based ChCl : LA (1 : 2) DES presented an superior inhibitory effect in bacterial growth,

resulting in an antibacterial efficiency of 100% for *S. aureus* and *P. aeruginosa*, when compared to the control group that does not receive the prodigiosin dyeing and the use of auxiliaries. The percentage of inhibition of the *S. aureus* and *P. aeruginosa* growth is in accordance with the data noticed by Mouro et al., where an inhibitory effect of 100% was observed against *S. aureus* and *K. pneumoniae* when nanofibers of polyvinyl alcohol (PVA) were blended with the L-Cys : LA DES containing dissolved wool keratin [40]. In addition, in the literature, it has been described that ChCl-based DES improved the antibacterial activity against several gram-positive and gram-negative bacteria [41]. Moreover, LA has been demonstrated to be lethal to microorganisms due to its ability to diffuse to the inside of the cell through the cell membrane and ionize. Also, the acidic pH can result in DNA damage and affect the interactions with the membrane enzymes and proteins, causing changes in cell membrane structure and permeability, and leading to the leakage of the cellular constituents and cell death [42].

### 3. Conclusions

Conventionally, textiles are dyed with synthetic dyes which are very effective but unfortunately cause severe environmental pollution. Bacterial pigments are important alternatives, however they traditionally are extracted using toxic organic solvents hazardous for the environment. In this study, the crude gel of prodigiosin pigment produced from non-pathogenic bacteria *Serratia plymuthica* was successfully used to dye the multifiber fabric in order to develop an eco-friendly and efficient dyeing process, as an alternative to the those hazardous substances and protocols for sustainable future. The influence of different parameters were studied, including the effect of temperature and pH that was changed by using different salts (NaOH and Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>) with different concentrations, and two mordants (FeSO<sub>4</sub> and L-Cys) with different compositions, and a gel-based ChCl : LA (1 : 2) DES as a promising water-free dyeing medium.

The results attained showed that the functionalization process at 60°C for 60 min, using 3.0% *owf* L-Cys at pH = 8.3 was suitable to obtain colored materials, particularly nylon and wool fabrics, with high *K/S* values and improved *dE*, as well as nylon fibers dyed using the gel-based ChCl : LA (1 : 2) DES as a dyeing medium. In addition, the nylon dyed with 3.0% *owf* L-Cys at pH = 8.3 displayed a superior fastness to washing, while the use of gel-based ChCl : LA (1 : 2) DES demonstrated exceptional light fastness. Moreover, the nylon dyed with the crude gel prodigiosin pigment, with the addition of the bio-mordant L-Cys and using the water-free gel-based DES, exhibited remarkable antibacterial activity against *S. aureus* and *P. aeruginosa*.

Therefore, it was concluded that the crude gel prodigiosin pigment, applied without the use of solvent extraction processes for pigment recovery, was potentially useful to dye nylon fabrics. In addition, the use of L-Cys - for the first time of in the related literature - as bio-mordant, demonstrated to be a sustainable and ecological alternative to metallic mordants according to the color features, fastness properties, and antibacterial capability. Likewise, the use of water-free dyeing media via green solvent solutions, like gel-based ChCl : LA (1 : 2) DES can help to reduce water pollution and wastewater - means that reduced environmental pollution.

It was proposed that, a screening of suitable gel-based DES, namely composed by amino acids, like L-Cys, should be evaluated in a near future as water-free and green dyeing mediums in order to develop environmentally friendly dyeing processes. In addition to the large number of potential combinations which may form potential gel-based DES dyeing media, ultrasound assisted dyeing methods should also be investigated as an opportunity to save electrical energy and thermal energy by varying process parameters of dyeing process in textile industry.

### 4. Materials and Methods

#### 4.1. Materials

SDC Multifiber DW was manufactured by James Heal. Sodium chloride (NaCl), ethanol, dipotassium hydrogen phosphate (K<sub>2</sub>HPO<sub>4</sub>), DL-Lactic acid 90% (LA), and Choline chloride 99% (ChCl) were acquired from Fisher Scientific (Fisher Scientific, Leicestershire, UK). Ferrous sulphate (FeSO<sub>4</sub>), L-Cysteine (L-Cys), sodium hydroxide (NaOH), sodium dithionite (Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub>), peptone,

nutrient agar (NA), nutrient broth (NB), brain heart infusion agar (BHI), hydrochloric acid (HCl), glycerol, and tween-80 were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA). Agar-agar was acquired from Labkem (Labkem, Barcelona, Spain). ECE reference detergent was provided from Bayer.

#### 4.2. Production and recovery of prodigiosin pigment

Prodigiosin pigment was produced by *Serratia plymuthica*, gently provided by Peter Askew (Industrial Microbiological Services Ltd, Hampshire, UK), using Peptone Glycerol Phosphate (PGP) medium (5 g/L peptone, 10 mL/L glycerol, 2 g/L  $K_2HPO_4$ , and 15 g/L agar-agar) at 20 °C in the absence of light [13]. The pigment was directly collected from the medium-agar plates and used as a crude gel prodigiosin pigment.

#### 4.3. Preparation of the crude gel prodigiosin pigment solution and dyeing process

The crude gel prodigiosin pigment bath solution was prepared in an acidified ethanol/distilled water (1:99) system with a concentration of 30% (*w/v*). In turn, the dyeing processes were performed using a 4 cm x 10 cm multifiber fabric composed of acetate, cotton, nylon, polyester, acrylic, and wool on the Datacolor AHIBA IR equipment (Datacolor company, USA) by the exhaustion method. The dyeing duration (60 min), bath ratio (1:50), rate of temperature rise (2 °C/min) and stirring rate (20 rpm) were kept constant throughout the experiments. After dyeing processes, the multifiber fabrics were rinsed with distilled water twice and then dried at room temperature.

##### 4.3.1. Optimization of dyeing conditions

###### Step 1: The effect of temperature

In order to evaluate the influence of temperature in the dyeing process, multifiber fabrics were dyed with the crude gel prodigiosin pigment at temperature of 40 and 60 °C.

###### Step 2: The effect of the dyeing aids addition

- The addition of salts: Salts often used in dyeing textile fibers, like sodium hydroxide (NaOH) (12 mL/L) and sodium dithionite ( $Na_2S_2O_4$ ) (4 g/L), were added to the aqueous crude gel prodigiosin pigment bath in order to evaluate the effectiveness of these salts in the dyeing process.

- The addition of mordants: The mordant process was performed by the simultaneous mordanting methodology using two different mordants: Ferrous Sulphate ( $FeSO_4$ ), a metallic mordant, and L-Cysteine (L-Cys), an amino acid bio-mordant, at 1.0%, 3.0%, and 5.0% over the weight of the fiber (*owf*) under the same dyeing conditions. Moreover, the effect of the crude gel prodigiosin pigment bath pH on dyeing multifiber fabrics was evaluated at pH = 4.0 and pH = 8.3 in order to achieve an acidic pH during dyeing with the  $FeSO_4$  metallic mordant and the alkaline pKa of bio-mordant L-Cys, respectively.

###### Step 3: The effect of the use of gel-based deep eutectic solvents (DES) as a prodigiosin dyeing medium

Firstly, the choline chloride (ChCl) (hydrogen bond donor—HBD) was combined with lactic acid (LA) (hydrogen bond acceptors—HBA) in a 1 : 2 molar ratio in order to produce the gel-based DES mixture. The DES components were added to a sealed lab flash and heated at 80 °C under magnetic stirring until a perfectly clear and transparent gel-liquid was formed. Then, 30% (*w/v*) of crude gel prodigiosin pigment dissolved in the pre-prepared gel-based ChCl : LA (1 : 2) DES was used as a green dyeing medium. The multifiber fabric was dyed under the same dyeing conditions, namely at a liquor ratio of 1:50, with a dyeing temperature and time of 60 °C and 60 min, respectively.

#### 4.4. Characterization of the dyed fabrics

##### 4.4.1. Color strength measurements of dyed fabrics

The color yield of the crude gel prodigiosin pigment dyed multifiber fabrics was assessed by the color strength ( $K/S$ ) from the reflectance values using a Datacolor 110 spectrophotometer (Datacolor company, USA) at 535 nm. The  $K/S$  values were determined using the Kubelka–Munk Equation (1):

$$K/S = \frac{(1 - R)^2}{2R} \quad (1)$$

Where  $R$  represents the observed reflectance of the dyed fabrics,  $K$  the absorption coefficient, and  $S$  the scattering coefficient. The  $K/S$  values were reported as the average of at least three measurements at different positions ( $n=3$ ). The different measurements at various points of the samples were also used to verify the dyeing equalization ( $dE$ ).

#### 4.4.2. Evaluation of fastness properties of dyed fabrics

Upon finishing the dyeing processes, fastness to washing and light was studied. For color fastness to washing, the dyed fabrics with superior performance during the crude gel prodigiosin pigment dyeing, namely the wool and nylon fabrics dyed with 3.0% *owf* L-Cys at pH = 8.3 and the nylon dyed using the gel-based ChCl : LA (1 : 2) DES as a dyeing medium, were washing with a commercial ECE detergent (4 g/L) according to ISO 105-C06:2010 for 30 min at 40 °C using a sample dyeing machine Linitest Device. After washing, the samples were rinsed in distilled water and dried at room temperature.

The color difference ( $\Delta E$ ) of the samples, before and after washing, was determined from the colorimetric properties using the CIELab values ( $L^*$ ,  $a^*$ , and  $b^*$ ) from the Equation (2):

$$\Delta E = (\Delta a^{*2} + \Delta b^{*2} + \Delta L^{*2})^{1/2} \quad (2)$$

Where  $a^*$  represents redness and greenness,  $b^*$  indicates yellowness and blueness, and  $L^*$  shows lightness from black (0) to white (100).

The evaluation of the  $\Delta E$  was carried out in the Datacolor spectraflash SF300 spectrophotometer and the fastness index ( $\Delta Color$ ) in the Color-Chex lightbox from CIELab values of the gray-scale using the CIE illuminant D65.

In turn, for light fastness, the nylon fabric dyed with the crude gel prodigiosin pigment with the addition of the 3.0% *owf* of L-Cys bio-mordant at pH = 8.3 and using the gel-based ChCl : LA (1 : 2) DES as a dyeing medium, was tested following a method adapted ISO 105-B02:2014. Briefly, the nylon samples (7.5 cm × 7.5 cm) were exposed to artificial daylight and blacklight, in a Color-Chex lightbox for 0, 16, and 24 h. After that, the fading degree was assessed by measuring the sample  $K/S$  using a Datacolor 110 spectrophotometer (Datacolor company, USA) and the discoloration percentages (% $D$ ) determined using the following Equation (3):

$$\%D = \frac{K/S_i - K/S_f}{K/S_i} \times 100 \quad (3)$$

Where  $K/S_i$  is the sample color strength before exposure to artificial lights and  $K/S_f$  is the sample color strength after exposure to artificial daylight or blacklight.

#### 4.4.3. Evaluation of the antibacterial activity of dyed fabrics

The antibacterial efficiency of the nylon dyed with the crude gel prodigiosin pigment, with and without the addition of 3.0% *owf* L-Cys bio-mordant at pH = 8.3, and using the gel-based ChCl : LA (1 : 2) DES as a dyeing medium was evaluated against *Staphylococcus aureus* ATTC 6538 (*S. aureus*) and *Pseudomonas aeruginosa* PA25 (*P. aeruginosa*) according to Japanese Industrial Standard JIS L 1902:2002, a standard method widely used to test the antibacterial activity of textiles. Briefly, a bacterial suspension ( $1 \pm 0.3 \times 10^5$  CFU/mL) was prepared and inoculated on nylon square samples. Then, the samples were subjected to vigorous vortex mixing for 30 s in a neutralizing solution and serial dilutions were prepared with 0.85 (*w/v*) NaCl, plated in agar plates, and incubated for 18–24 h at 37 °C. The antimicrobial activity was quantitatively expressed in percentage of bacterial growth inhibition (% *Inhibition*) using the Equation (4):

$$\% \text{ Inhibition} = \frac{C - A}{C} \times 100 \quad (4)$$

Where C is the average value of CFU of undyed nylon sample, and A is the average value of CFU of nylon dyed with the crude gel prodigiosin pigment with and without the addition of 3.0% *owf* L-Cys at pH = 8.3 and using the gel-based ChCl : LA (1 : 2) DES dyeing medium.

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**Data Availability Statement:** Data that support the findings of this study are included in the article.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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