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Full Length Research Paper

Marine bacterial prodigiosin as dye for rubber latex, polymethyl methacrylate sheets and paper

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Prodigiosin is known for its immunomodulatory, antibacterial, antimycotic, antimalarial, algicidal and anticancer activities. Here, we reported the evaluation of prodigiosin pigment as a dyeing agent in rubber latex, paper and polymethyl methacrylate (PMMA) so that it can be considered as an alternative to synthetic pigments. Maximum color shade was obtained in rubber sheet prepared with 0.5 parts per hundred gram of rubber (phr) pigment and PMMA sheet incorporated with 0.08 µg pigment. Results indicate scope for utilization of prodigiosin as dye for PMMA and rubber and also prodigiosin dyed paper as a pH indicator. Further, being a natural and water insoluble pigment, it is ecofriendly.

Key words: Prodigiosin, *Serratia* sp., dye, rubber, polymethyl methacrylate.

INTRODUCTION

Natural pigments and synthetic dyes are extensively used in various industries including food, textile, paper, rubber, in agricultural practice, and water science and technology. However, the effluents released from the dyeing units of these industries contain synthetic dyes that are toxic and cause extensive environmental pollution besides polluting the ground water resources of drinking water and agriculture practices. Consequently, synthetic dyes have a significant negative impact on the environment (Tibor, 2007; Balakrishnan et al., 2008). To alleviate the problems caused by the synthetic dyes and chemicals, alternative eco-friendly technologies and use of natural pigments that are biodegradable are preferred. In this context, natural pigments have drawn the attention of industries as safe alternative. As per the available data, Europe imports US $ 53 million worth of natural dyes and the major importing countries include Germany (32%), France (17%), Italy (14%), USA (12%) and U.K. (10%). The largest dye suppliers include Mexico, Peru, China and India, each exporting dyes worth US $ 15 million to Europe.

Natural biocolorants obtained from plants, animals and microorganisms are possible alternatives to synthetic dyes and pigments currently employed in various industries (Mapari et al., 2005). Among the natural sources, microorganisms offer great scope and hope as compared to other resources. The genetic diversity in microbes, ease of their cultivation, extraction and sophistication in technologies has made their choice more feasible (Juailova et al., 1997). In fact, microorganisms produce a large variety of stable pigments such as carotenoids, flavonoids, quinones and rubramines, and fermentation production results in higher yields of pigments and lower residues as compared to that obtained from plants and animals (Durán et al., 2002). Thus, biosynthesis of dyes and pigments via fermentation processes has attracted

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more attention in recent years (Durán et al., 2002; Hobson and Wales, 1998). It may be also noted that prodigiosin is known to have several biological activities such as immunomodulatory, antibacterial, antimycotic, antimalarial (Lazaro et al., 2002; Pandey et al., 2003), anticancer (Montaner and Perez-Tomas, 2001) and algicidal activities (Kim et al., 2007).

Currently, synthetic dyes are used to impart color for rubber products. Polymethyl methacrylate (PMMA) is a transparent thermoplastic, often used as a light or shatter-resistant alternative to glass. These sheets are also given different color shades. Similarly, papers with different colour shades are manufactured. In all these three products, use of prodigiosin as a natural dye has not been reported so far to the best of our knowledge. In this context, we report here the prospects of using prodigiosin, produced by *Serratia* sp. BTWJ8 isolated from marine sediment, as dye for imparting different shades of color to rubber latex, polymethyl methacrylate sheets and paper.

MATERIALS AND METHODS

Purification and characterization of the pigment

The pigment produced by *Serratia* sp. BTWJ8 was purified and characterized as described previously (Jissa et al., 2011). The purified pigment was used for the following application studies:

Dyeing of rubber latex

The colouring dye was prepared by ball milling using the compounding ingredients as mentioned below: Natural rubber latex (60% dry rubber content) 100 (phr), sulphur (50%) 1.5 phr, ZnO (50%) 0.9 phr, accelerator (50%) 0.7 phr, and antioxidant (50%) 0.5 phr. The compounding ingredients were then subjected to sonication for 30 min to make homogenous pigment dispersion. Using this pigment dispersion, four different concentrations viz: 0.0, 0.16, 0.3 and 0.5 phr namely 1, 2, 3 and 4 respectively were prepared to obtain varied color shades. The mixes were then casted onto Petri plates to make required rubber sheets, and kept for 24 h at room temperature (RT) (28 ± 2°C). The rubber sheets with 1 mm thickness were incubated at 70°C for 2 h in hot air oven for proper vulcanization of the rubber. Mix 1 was used as the control.

Dyeing of polymethyl methacrylate sheet

Bacterial pigment in methanol (40 µg/L) was used as the stock solution for imparting colour onto the polymethyl methacrylate sheets prepared using 10% solution of PMMA in chloroform. Towards imparting varied colour shades, four different concentrations of bacterial pigment dispersion viz: 250 µl (0.01 µg; w/v), 500 µl (0.02 µg; w/v), 1 ml (0.04 µg; w/v) and 2 ml (0.08 µg; w/v) were added to PMMA solution from the stock solution separately, mixed well, poured into a watch glass, and kept for 3 h at RT (28 ± 2°C). The watch glasses were covered with a glass plate in order to prevent air contact.

Dyeing of paper

Eight types of paper with different qualities like 'art paper', 'JK paper', 'sunlight', '6.9 SPB', '7.8 SPB', '11 Kg JK', '21.3 Kg JK' and '18.6 Kg SPB' commercially available in the market were selected and used in the present study. All the paper materials were cut into equal size of 2 cm^2. Bacterial pigment in methanol (40 µg/L) was used as the stock solution. An aliquot of 200 µl (0.008 µg; w/v) of the stock solution was applied on to the different paper materials on a warm surface and allowed to dry at RT for 15 min to impart colour. Paper material without pigment was kept as the control. After dyeing, acidic (pH 2.0), neutral (pH 7.0) and alkaline (pH 10.0) solutions were spotted over all the paper materials to evaluate the dyed paper as probable pH indicators.

RESULTS AND DISCUSSION

Synthetic dyes made from nonrenewable sources such as fossil fuels are used extensively in the textile, rubber, paper and plastic industries and the industrial effluents loaded with these dyes has created alarming situation with respect to environmental health. Wastewater from printing and dyeing units is often rich in color, containing residues of reactive dyes and chemicals. The toxic effects of dyestuffs and other organic compounds, as well as acidic and alkaline contaminants in these dye effluents reach a stage where they are not treated effectively before their disposal into environment. Considering the ill effects of synthetic dyes on human beings and ecosystem, Germany banned the use of numerous specific azo-dyes for their manufacturing and applications and most of the countries brought effective laws and regulations related to the customer health and safety and protection of eco-system (Nimkar and Bhajekar, 2006; Premi, 1996). The present trend of work culture, safety and eco-requirements will continue to dominate the trade and the processor will need to understand the changes that need to be effected to satisfy these requirements (Burdhan, 2002). As a consequence, there is a renewed interest in the use of natural pigments as dyes, which is normally biodegradable in the environment.

Red pigment isolated from *Serratia* sp. BTWJ8 purified and identified as prodigiosin (Jissa et al., 2011) was used for the application studies. Attempt made to evaluate the probable use of this pigment as a coloring agent showed very promising results with rubber, PMMA and paper. Since there was no suitable methodology available in literature, to the best of our knowledge we employed reliable methodologies standardized in our laboratory for dyeing experiments.

The impact of rubber and its products is on the rise in our day-to-day life and the use of rubber is widespread, ranging from household to industrial products, entering the production stream at the intermediate stage or as final products. Studies conducted with rubber products show that prodigiosin is an effective dye for inclusion in rubber products (Figure 1). The maximum color was

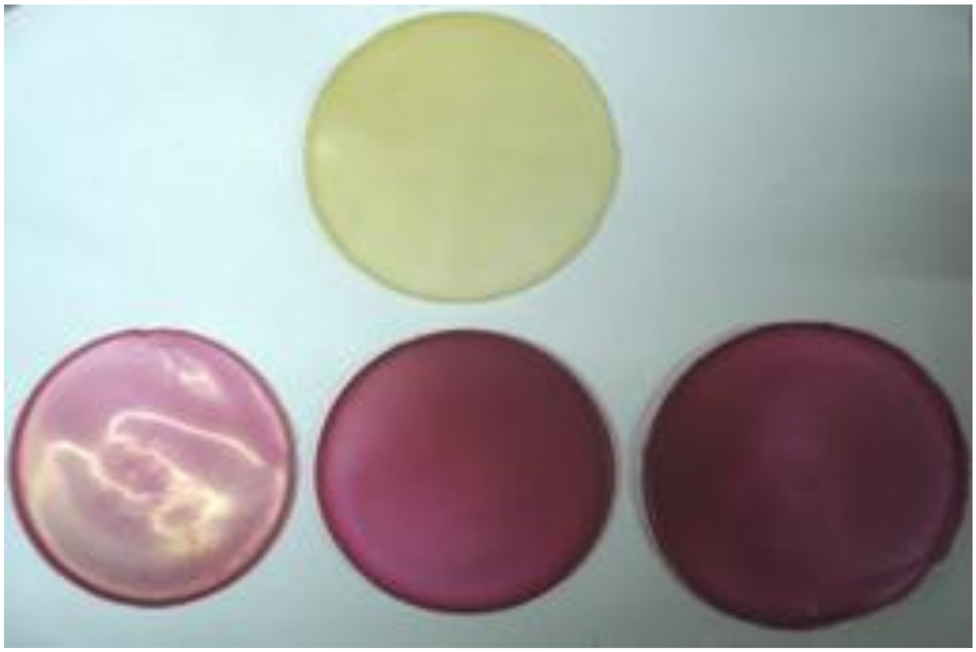

Figure 1. Rubber sheets dyed with bacterial pigment. Compounding ingredients were prepared by ball milling as follows: Natural rubber latex (60% dry rubber content), 100 parts per hundred gram of rubber (phr), sulphur (50%) 1.5 phr, ZnO (50%) 0.9 phr, accelerator (50%) 0.7 phr, antioxidant (50%) 0.5 phr. From this, 0.0, 0.16, 0.3 and 0.5 phr pigment dispersion, prepared by means of sonication for 30 min, was used to prepare Mix 1, 2, 3 and 4, respectively. Mix 1 was used as the control.

obtained in rubber sheet prepared with Mix 4 that contained 0.5 phr pigment, followed by 0.3 phr and the minimum color shade was obtained with Mix 2 incorporated with 0.16 phr pigment. The results indicate that different color shades can be produced by varying the concentration of pigment in rubber latex. Studies conducted with rubber products show that prodigiosin is an effective dye for inclusion in rubber products.

PMMA or poly methyl 2-methylpropenoate, a synthetic polymer of methyl methacrylate, commonly called acrylic glass or simply acrylic is widely used in the lenses of exterior lights of automobiles and also plastic optical fiber used for short communication. The results obtained for the studies conducted with PMMA (Figure 2) indicated probable scope for exploiting this pigment as a natural dye in synthetic plastic materials. The topmost color intensity was noticed with PMMA sheet incorporated with 0.08 µg pigment followed by those sheets with 0.04 and 0.02 µg pigments. The least color shade was observed with PMMA sheet integrated with 0.01 µg pigment. Hence, it is proposed that different color shades can be produced by varying the concentration of red prodigiosin in PMMA solution in chloroform.

Paper continues to remain as a popular medium for printing, writing and also as a packaging material. So, colorants for paper industry have a bright but challenging prospect. In the present study, we evaluated eight types of paper with different qualities commercially available in the market. All the paper materials were cut into equal sizes of 2 cm^2. Bacterial pigment in methanol (40 µg/L) was used as the stock solution; 200 µl (0.008 µg; w/v) of the stock solution was applied on the different paper materials on a warm surface and allowed to dry at RT for 15 min. Paper material without pigment was kept as control. After dyeing, acidic (pH 2.0), neutral (pH 7.0) and alkaline (pH 10.0) solutions were spotted over all the paper materials. It was observed that the color of all the prodigiosin dyed paper materials recorded a change from white to red, pink and yellowish orange, respectively with acidic (pH 2.0), neutral (pH 7.0) and alkaline (pH 10.0) solutions strongly suggesting that prodigiosin dyed paper can be used as a pH indicator. The results obtained for

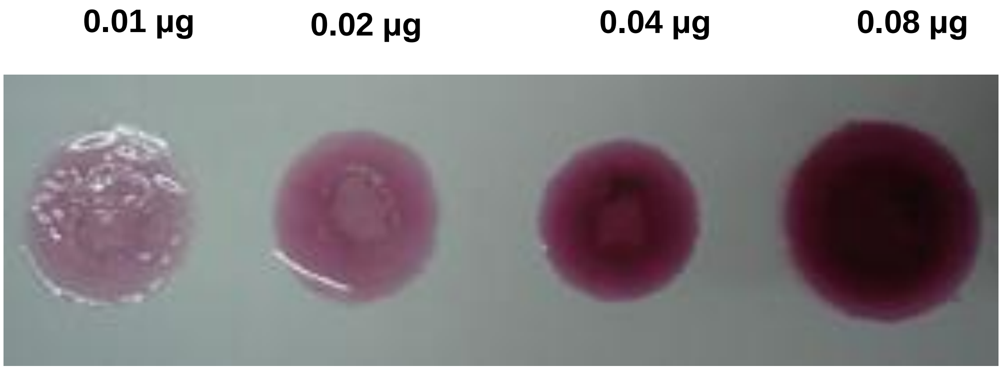

Figure 2. Polymethyl methacrylate dyed with bacterial pigment. Bacterial pigment in methanol (40 µg/L) was used as the stock solution. Aliquots of 10% solution of PMMA was prepared in chloroform 250 µl (0.01 µg), 500 µl (0.02 µg), 1 ml (0.04 µg) and 2 ml (0.08 µg) of bacterial pigment were added to 10% PMMA solution in chloroform separately, mixed well and poured into a watch glass and kept for 3 h at RT (28 ± 2°C). The watch glasses were covered with a glass plate in order to prevent air contact.

the studies conducted with paper indicated that the pigment can be used as a dye for preparation of colored paper.

Based on the results, it is concluded that prodigiosin produced by marine *Serratia* sp. BTWJ8 has the potential for use as a natural dyeing agent for the preparation of colored rubber latex products, PMMA with different color shades and pH indicator paper. Being a natural pigment, it is definitely harmless and would be ecofriendly.

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