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# INDUSTRIAL APPLICATIONS OF RED PIGMENT PRODIGIOSIN EXTRACTED FROM *Serratia marcescens*

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## ABSTRACT

**In present study, the industrial applications of red pigment prodigiosin, extracted from *Serratia marcescens*, were explored. Twelve textile fabric samples (santoon, georgette, bubble, cotton 20/20, cotton polyester, lycra, butter crape, hosiery, satin, linen, cotton and polyester) were used to check the fabrics staining potential of prodigiosin under acidic, basic and neutral conditions. Out of the selected fabrics, the pigment gave attractive red shade to bubble, hosiery, cotton 20-20 and lycra in all the sets. Colour intensity decreased in santoon, cotton polyester, cotton and polyester. Very faint colouration was noticed in satin, linen, butter crape and georgette. Application of prodigiosin as food colorant in bakery products revealed successful use of pigment in decoration of cakes and other bakery products. Different concentrations (1:1 and 1:2) of pigment were used in paper making process. The products were exposed to sunlight and fluorescent light for 4 h in which sunlight caused higher fading effect. Prodigiosin was also used in soap making at 1, 2 and 3 mg $L^{-1}$ concentration and it gave attractive colour shades to the final products. For candle making, different concentrations (1:5, 2:5 and 3:5) of pigment used were and the study showed that the prodigiosin can be an attractive choice for making colourful candles of different shapes. The potential of prodigiosin as ink for highlighter pen was tested by preparing different compositions in presence of citric acid and glycerol. Results suggested that the concentration >0.25% can be readily used in highlighter pen.**

**Keywords:** Bakery industry, paper industry, prodigiosin, *Serratia marcescens*, textile industry

## INTRODUCTION

Various biopigments have potential industrial applications till most of the industries now a day's preferred to use synthetic pigments instead of these naturally derived ones. These synthetic dyes have many limitations, like their production process involves the use of hazardous chemicals, leading not only to the safety concerns of workers, but also may generate hazardous wastes and cause various types of environmental pollutions. Most of the synthetic dyes are neither eco-friendly nor economical. There are continuous efforts to explore the biopigments which can successfully satisfy the requirements of industries. Bacterial pigments cover the wide spectrum of colours and show better biodegradability and higher compatibility with the environment, so offer promising alternative for pigment use in industries.

The textile industry uses approximately 1.3 million ton dyes, pigments and dye precursors, valued at around US $ 23 billion. Until the second half of the 19$^{th}$ century, all dyes used in textiles were naturally derived. However, with the synthesis of mauveine by Perkin in 1856, the synthetic dye industry grew enormously and almost wiped out the use of natural dyes in industries (Venil *et al*.,

2013). Biosynthesis of colorants like prodigiosin (a natural dye) for use in textile industry has recently received renewed interests (Mapari *et al*., 2005). Many researchers have reported the practical use of prodigiosin, derived from *S. marcescens,* for colouring different fabrics like pure cotton, pure silk, pure rayon, jacquard rayon, acrylic, cotton, silk satin, polyester, etc. (Yusof, 2008; Ahmad, *et al*., 2012).

The development of appealing colourful foods is important in successful product marketing. The biopigments are used as additives, colour intensifiers, antioxidants, etc. These biopigments are considered safe when used as natural food colorants and may benefit human health as well as preserve the biodiversity. Biopigments use can help in minimizing the use of synthetic colorants which release harmful chemicals into the environment (Neeraj *et al*., 2011). The successful marketing of pigments derived from microbes, both as a food colour and a nutritional supplement, reflects the presence and significance of niche markets in which consumers willingly pay a premium for natural ingredients. The number of approved colorants for the food industry is also limited. Commercial production of bright red pigment prodigiosin through microbial fermentation also has applications in candle industries for colouring candle (Ahmad *et al*., 2012), in bakery for decoration of cakes and pastries, in paper industries (Martinko and Madigan, 2006), in highlighter and ball ink pen and for production soaps and oil colours (Venil *et al*., 2013).

The success of any pigment produced by fermentation depends upon its acceptance by consumers, regulatory approval and the size of capital investment required in launching the product in market (Dufosse *et al*., 2009). So from ecological perspective, the substitution of chemical dyes with natural products in textile dyeing may not only be a feasible strategy to reduce the health risk and ecosystem pollution, but also an opportunity for new markets and new businesses (Venil *et al*., 2021). The present investigation was aimed to study the potential applications of red pigment prodigiosin in textile, bakery, paper and pulp industries, soap and candle making, ink and highlighter pen and in the production of oil colours so as to explore a sustainable eco-friendly way to colour the products of daily use with bacterial pigments prodigiosin.

## MATERIALS AND METHODS

In present study, the red pigment prodigiosin extracted from bacterium *Serratia marcescens* was used (Phatake and Dharmadhikari, 2016; Bhagwat and Padalia, 2020). The pigment was produced in large quantity in well-optimized peanut broth (Phatake and Dharmadhikari, 2015) and characterized by TLC, HPTC and FTIR. The experimentation part was performed in the laboratory facility of Vidya Pratishthan School of Biotechnology (VSBT), Baramati, Pune, Maharashtra (India) during the year 2016-17.

### *Application of prodigiosin in textile fabrics*

Twelve different fabric samples *viz*., santoon, georgette, bubble, cotton 20/20, cotton polyester, lycra, butter crape, hosiery, satin, linen, cotton and polyester were purchased from a local market in Baramati, Maharashtra (India). The fabrics samples were uniformly cut into the pieces of 4 x 3 cm size. The crude pigment solution of prodigiosin was used as dyeing agent. All dyeing experiments were carried out using fabric samples that were previously wetted in hot water. The dyeing solution to fabric (w/w) ratio (liquor ratio) was kept at 50:1. For dyeing process, the crude pigment solution of methanol: water (1:1, v/v) with 1% solid mass of sample fabrics was used in a dyeing bath. All the test cloth samples were dyed under neutral, acidic and basic pH conditions. For dying under neutral condition, the pH of crude pigment extract was adjusted to 7. The test cloths were dyed under acidic conditions in a methanol: water solution of extract that was first adjusted to pH 3.5 by using 0.1 M $H_2SO_4$ solution. For dying under basic condition, the pH of methanol: water extract of pigment was adjusted to 8.2 by using 0.1M sodium hydroxide solution. Finally, the sample cloths were dyed in this solution by using a standard protocol (Alihossein *et al*., 2008). For dying, the clothes were soaked in

pigment solution at 80ºC for 60 min in water bath. The dyed fabrics were rinsed in warm water and detergent solution, and rinsed in cold water and dried at room temperature (Yusof, 2008). All the experiments were carried out in triplicates and average values were used to calculate standard errors.

***Use of prodigiosin in bakery***

The natural pigments were used in bakery products like biscuits, cookies, cakes pastries, cakes mixes and for decorating the cakes. For use of biopigments in bakery, the extracted dry pigment powder was mixed with cream in variable concentration, which was further used for decoration of cakes and other bakery products. Cream without pigment served as a control. Finally, this cream mixture with pigment was filled in the cone and applied on the cake for top decoration and for name writing.

***Application of prodigiosin in paper***

To evaluate the potential use of prodigiosin in paper making, various concentrations of prodigiosin pigment (1:1 and 2:1) and one teaspoon of corn starch were homogeneously blended in one half of thick pulp, whereas in the other half no pigment was added and it served as control. The pulp was then evenly spread onto a net to drain the excess water, followed by 24 h drying (Martinko and Mardigan, 2006). To check the effect of radiation on paper colour, prodigiosin dyed paper was exposed to sunlight and fluorescent light for 4 h. While the paper unexposed to light served as control (Scheyer and Chiweshe, 2001).

***Use of prodigiosin in soap making***

Traditionally, soaps are made from triglycerides (oils and fat) and artificial colorants which are costly and hazardous to the environment. Natural colorants like bio-pigments can readily be used for soap making. For this, 30 g sodium hydroxide was added to 100 mL water and the solution mixed well. This mixture was then added in 50-60 mL hot coconut oil and heated at 100°C. In above mixture 30 g sodium chloride and different concentration of extracted pigment (1, 2 and 3 mg 100 $mL^{-1}$) was added immediately. The same procedure was used for the preparation of control soap (without pigment). The mixtures were poured into the mold and cooled for 30 min. After cooling soap were gently removed from the mold and tested for colour intensity (Venil, *et al*., 2013).

***Prodigiosin in candles making***

For application of bacterial pigment in candle making, commercial candles wax (50 g) were placed in two separate beakers and heated until completely melted. One beaker was used as control and in other one the extracted pigment was added in different concentrations (1:5, 2:5 and 3:5). The mixtures were homogenized and poured into the mold. The wicks were immediately placed into the centre of mold and the candles were left to cool at room temperature for 1 h. After cooling candles were carefully removed from the molds (Ahmad *et al*., 2012).

***Use of prodigiosin in highlighter pen***

The potential of prodigiosin as ink for highlighter pen was evaluated by preparing different compositions in presence of citric acid and glycerol. For this, dry powder of pigment in different concentration (0.125, 0.25, 0.50, 1.0 and 2.0%) was used. The prepared ink was then used to highlight printed words. The effect of different concentrations of pigment and solvents i.e. ethyl acetate and glycerol used in highlighter ink was also studied (Ahmad *et al*., 2012).

***Application of prodigiosin in the production of oil colour***

Linseed oil (flaxseed oil) gives a shining effect and makes oil colour application easy. Moreover, to produce colour with appropriate consistency and concentration, a fixed amount of oil has to be used in colour production. In this study, the isolated pigment from bacteria in the form of powder was mixed with linseed oil in 3:1 ratio and its application as oil colour was studied on paper. To protect the biological nature of the obtained colour, adding other filler compounds in it were avoided (Pakzad *et al*., 2016).

## RESULTS AND DISCUSSION

The isolated *Serratia marcescens* strain produced large quantity of prodigiosin (1501.12 units $cell^{-1}$) by previously optimized parameters in peanut broth (Phatake and Dharmadhikari, 2015). The thin layer chromatographic (Rf value, 0.62) confirmed that produced pigment was prodigiosin. The extracted pigment was analysed by using two most frequently used modern analytical techniques HPTLC and FTIR. In HPTLC study the pigment from cell supernatant and pellet was scanned at multiple wavelengths (450, 500, 550, 600 and 650 nm). In both the cases peak height increased up to 550 nm (lambda max of standard pigment). The further increase in wavelength caused spontaneous decrease in absorbance. The FTIR spectrum obtained from an alcoholic extract of red pigment prodigiosin showed a broad range of envelope around 3900.45-3307.12 $cm^{-1}$ centered at 3556.06 $cm^{-1}$ attributed to –N–H stretch in the skeleton of pigment matching with the standard one.

***Application of prodigiosin in textile industry***

Chemically synthesized colorants used in textile industries are harmful to the animal health including human, and environment. So there is a need to develop natural colorants which can replace these synthetic dyes because they are safe to use in textile industries. In present study, prodigiosin was used to colour 12 fabrics samples collected from the local market. The colouring potential of prodigiosin was assessed at acidic, neutral and basic conditions. The study showed intense coloration in all the fabrics at neutral pH, followed by acidic condition; while the colour intensity notably decreased under basic conditions (Fig. 1-3). On the basis of a pattern of coloration, fabrics were divided into three groups as dark, medium and faint colour. Out of the various fabrics selected, pigment gave attractive red shade to bubble, hosiery, cotton 20-20 and lycra in all the three sets. The colour intensity decreased in santoon, cotton polyester, cotton and polyester as compared to the first group. Very faint colour was observed in 3rd group which contained satin, linen, butter crape and georgette.

In dying process, the chemical nature of textile materials is important as it affects the mechanism by which the dyes are absorbed. Since cotton is made from cellulosic materials, it requires dye molecule which contains groups capable of forming hydrogen bond with the hydroxyl group on cellulose molecule (prodigiosin molecule is unable to form hydrogen bond). The dyeing of cotton depicted considerable difficulty as the number of dyes having an attraction for cotton was few.

Many fibres from group one are made up of polyamide. Since the pigments consisted of amine as a functional group, therefore in acrylic dyeing process the pigments get attached by the formation of hydrogen bond to the amide group in the fibre, resulting in good coloration of these fabrics. The effect of washing on colour intensity was also determined by exposing the treated fabrics to water and detergent. There was no significant colour loss or variation in colour intensity after washing and shade drying in all fabrics.

Krishnamurthy *et al.* (2002) have used prodigiosin from marine *Serratia* sp. for application as dye in textile industry. The results showed that pigment could be used for imparting red colour to various grades of textile materials as natural dye. After washing the colour was observed to be stable. Ahmad *et al*. (2012) used prodigiosin to colour five types of fabric namely acrylic, polyester microfiber, polyester, silk and cotton by direct boiling the fabrics with the bacterial cells and observed attractive and stable shades of coloration pattern after dyeing. The colour fastness test was conducted by using the standard soap solution to monitor the colour change by comparing the colour intensity of fabrics before and after washing.

Alihosseini *et al*. (2008) used bright red pigment to stain fibers including wool, nylon, acrylics, and silk, and reported that wool, silk, nylon 66, acrylic and modacrylic fabrics were coloured to a pretty deep shade; whereas cotton, viscose, and polypropylene could only be stained by the same dyeing solution. Shalinimol and Annadurai (2012) used wool, silk, nylon, cotton and polyester fabrics to dye by prodigiosin obtained from *S. marcescens* MTCC 10774. The colourfastness of dyed fabrics was verified by dipping the fabrics in commercial detergent solutions for 48 h and by rubbing. Various


**Fig. 1: Different textile fabrics (control samples) used for testing**


**Fig. 2: Dyed fabrics samples before washing (acidic pH)**


**Fig. 3: Dyed fabrics samples after washing (acidic pH)**

pink shades of fabrics were obtained. The shade of dyed fabric was found mainly dependent on the amount of pigment used.

***Use of prodigiosin in bakery products***

In present study, prodigiosin was successfully used in bakery for giving faint and dark red shade to the creams used in cake decoration (Fig. 4). The study showed that the pigment can be a good alternative for synthetic dyes in a bakery for future. Thus bacterial colorants in addition to being eco-friendly can serve the dual need for visually appealing colours and probiotic health benefits in food products (Nagpal *et al*., 2011).

The development of foods with attractive appearance is one of the important goals of food industry (Aberoumand, 2011). Any food with good nutritive value is not accepted by customers if it does not have appealing colour. Some biologically synthesized biopigments such as carotene from fungus *Blakeslea trispora* in Europe or pigments from *Monascus* in Asia are used in food industry. These pigments ensure good appearance with additional nutritive and medicinal values such as antibiotic, antioxidants, etc. The customers are crazy toward natural products (pigment) because of non-lethal effect in comparison to the synthetic chemicals. The prodigiosin has successfully been applied to yogurt, milk and carbonated drinks (Namazkar and Ahmad, 2013).


Control Test I Test II Test III

**Fig. 4: Application of prodigiosin pigment in bakery products**

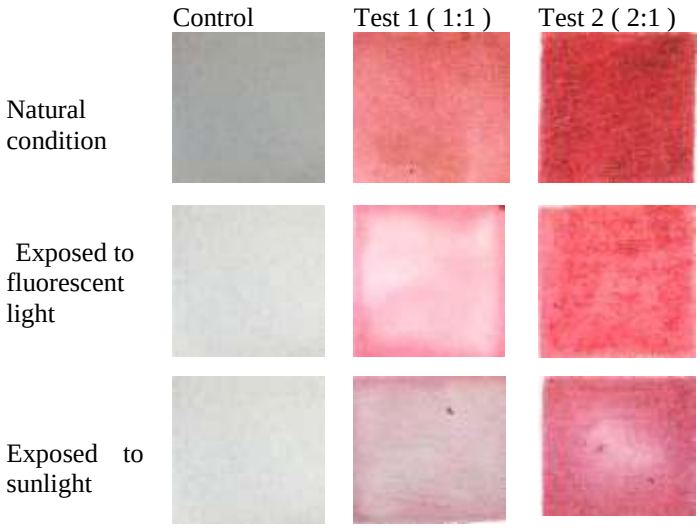


**Fig. 5: Use of prodigiosin in paper making**

***Use of prodigiosin in paper making***

In present study prodigiosin (red pigment) in variable concentrations was used for making filter paper. The pigment gave attractive colour shades to the paper at 1:1 and 2:1 concentration. After production, the paper was exposed to sunlight and fluorescent light for 4 h, while paper unexposed to light acted as a control. The initial intense red colouration of paper was substantially reduced to light red upon exposure to both sunlight and fluorescent light, but the sunlight exerted higher fading effect (Fig. 5). This effect can be attributed to the wider range of light wave-lengths of sunlight as compared to the fluorescent light (Scheyer and Chiweshe, 2001). Ahmad *et al*. (2012) evaluated the potential of prodigiosin in colouring paper and they reported similar observations with negative effect of sunlight on the shades. The produced paper needs to be further assessed for its antimicrobial activity which will be additional benefit and the area of interest for researchers.

***Prodigiosin in soap making***

Soaps are available in market with variable colours and shades. In present study different concentration of prodigiosin (1, 2 and 3 mg $L^{-1}$) were assessed for making soap in laboratory. Prodigiosin reportedly has antioxidant activity so has promising potential for use in cosmetics and skin-related products. The produced soap showed faint to bright red colour pattern depending upon the concentration used (Fig. 6). Ahmed *et al*. (2012) reported that the soap solution prepared by mixing 5 g standard soap with 2 g anhydrous $Na_2CO_3$ in 1 L distilled water and heated at $60^0$C for 15 min for homogenization produced distinct attractive colour in test soap as compared to the control.

Control soap (without pigment) | Test soap I (with 1 mL $L^{-1}$ pigment) | Test soap II (with 2 mL $L^{-1}$ pigment) | Test soap III (with 3 mL $L^{-1}$ pigment)

**Fig. 6: Use of pigment prodigiosin in soap making**

***Application of prodigiosin in candles making***

The use of pigments in candle making was studied by taking 3 concentrations of prodigiosin (1:5, 2:5 and 3:5). For each concentration four different shapes of wax candle were prepared (Fig. 7). After drying colour intensity of each group was measured. The study showed that as the concentration of pigment increased, the colour intensity increased in all the 3 groups with quite attractive colour in candle at lower concentration thus revealing the effectiveness of pigment in the production process. Ahmad *et al*. (2012) evaluated the potential of prodigiosin in colouring candles and pencil case pouch. The candles in all shapes produced by them showed attractive shades when compared with control.

Test 1 (1:5) | Test 2 (2:5) | Test 3 (3:5)

**Fig. 7: Application of pigment prodigiosin in candle making**

***Use of prodigiosin in highlighter pen***

The prepared ink was used to highlight printed words (Fig. 8). The effect of solvent used i.e. ethyl acetate and glycerol on produced ink was also investigated. Based on the smearing effect, glycerol performed better as solvent as compared to the ethyl acetate. The solvent can include any liquid capable of transferring the colorant and acid buffer to the substrate, a good solvent is a liquid which can evaporate in short time and have a low degree of smear. Out five different concentrations of pigment used, 0.50 to 2.0% concentration was found effective to be used in highlighter pen (Fig. 8).

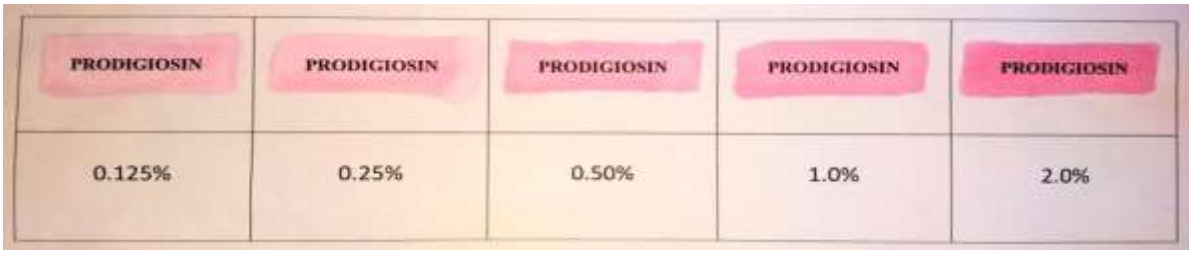


**Fig. 8: Application of pigment in a highlighter pen**

**Table 1: Composition of highlighter ink**

| Components (% by wt) | Ink 1 | Ink 2 | Ink 3 | Ink 4 | Ink 5 |
|---|---|---|---|---|---|
| Dry pigment | 0.00125 | 0.0025 | 0.005 | 0.010 | 0.02 |
| Citric acid | 0.0008 | 0.001672 | 0.00338 | 0.0067 | 0.0136 |
| Glycerol | 0.9979 | 0.9958 | 0.9916 | 0.9833 | 0.9664 |
| Total | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Pigment (%) | 0.125 | 0.25 | 0.50 | 1.00 | 2.00 |

Table 1 shows the five different types of the ink prepared by using different concentration of pigment. According to which final quantity of other components are also adjusted in final preparation.

***Application of prodigiosin in production of oil colour***

Different materials like oils, resins, solvents and other additives are used as intermediate to cause the binding of pigment particles, their consolidation on background and facilitating the application of colour. Linseed oil is stable and dries more quickly than other vegetable oils (Pakbaz, 2006). Our results showed that the extracted pigment dissolved well in the linseed oil and the bio-oil colour had desirable transparency, consistency and fluidity. The colour intensity increased with increase in concentration from 1:1 to 3:1 (Fig. 9). Pakzad *et al*. (2016) extracted biopigment carotenoid from *S. aureus* by methanolic extraction and used it for the preparation of oil colour by using linseed oil (flaxseed oil). They found that the combination of extracted pigment with linseed oil created a colour similar to cadmium yellow on paper so suggested its use as an oil colour for painting.

[A] Test 1 (1:1) [B] Test 2 (2:1) [C] Test 3 (3:1)

**Fig. 9: Mixing of isolated pigment prodigiosin with linseed oil in 1:1, 2:1 and 3:1 ratio for preparation of oil colours**

**Conclusion:** The application of extracted prodigiosin in colouration of textile fabrics was explored for staining at acidic, basic and neutral pH. Pigment gave attractive red shade to bubble, hosiery, cotton 20-20 and lycra. Color intensity decreased in santoon, cotton polyester, cotton and polyester while very faint coloration was observed in satin, linen, butter crape and georgette and basic and neutral pH, so acidic pH is recommended for getting the attractive colour shades in selected fabrics. The study presented the insights into the application of pigment in bakery for colouring different food products like cakes, pastries, etc. In paper making, the pigment gave notable colour shades to the paper at 1:1 and 2:1 concentration. The prodigiosin proved effective in soap preparations, candle making, highlighter pens and oil paint production.

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