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“NATURAL & SYNTHETIC DYES: A STUDY ON STANDERIZATION OF METHODS OF APPLICATION OF ECO-FRIENDLY O MEET THE REQUIREMENTS FOR EXPORT MARKET”

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ABSTRACT

Regular colors are involves those colorants that are gotten from creature or vegetable issue without compound handling. They are chiefly a severe colors albeit some tank, dissolvable, shade and corrosive sorts are known. Common colors utilized in the colouration of materials, nourishments, medications, and beautifiers. Little amounts of colors are additionally utilized in a colouration of paper, cowhide, shoe finish, wood, stick, candles, and so forth. In the prior days, colors were gotten uniquely from a characteristic sources. Be that as it may, common colors experience the ill effects of certain characteristic inconveniences of a normalized application and the normalization of the color itself as colors gathered from comparative plants or regular sources are affected and exposed to the notions of atmosphere, soil, development strategies and so forth. Thus for regular colors to be genuinely popularized and to assume a serious position concerning the engineered colors, the normalization strategies play an exceptionally critical and indispensable job. In this paper we will demonstrate some customary and frequently utilized techniques application, the distinguishing proof strategies and furthermore normalization cycle of the common colors. Regular colors contains those colorants that are gotten from creature or vegetable issue without substance handling. They are chiefly a stringent colors albeit some tank, dissolvable, shade, and corrosive sorts are known. Regular colors can utilized for coloring practically a wide range of common strands. Late examination shows that they can likewise be utilized to color some engineered filaments. Aside from their application in materials, common colors are likewise utilized in the Coloration of food, prescriptions, craftsmanship articles, and in calfskin preparing, and a significant number of the color yielding plants are utilized as meds in different customary Medicinal treatments.

Characteristic colors are gotten from common assets; these are extensively named plant, creature, mineral, and microbial colors. Normal colors can be utilized for coloring practically a wide range of common filaments. Ongoing examination shows that they are additionally used to color some manufactured strands. Separated of their application in materials, normal colors are likewise utilized in the shading of food, medications, workmanship articles, and in cowhide preparing, and a large number of the color yielding plants are utilized as drugs in different conventional restorative treatments. This book section endeavors to audit the order of normal colors and different maintainability issues associated with their creation and candidate.

Keyword: Colorants, Restorative Treatments, Colors

I. INTRODUCTION

Material preparing industry is a one of the major natural polluters as the gushing from these ventures contains weighty heap of synthetic compounds including colors utilized during material handling. There are two fundamental approaches

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to restrict of the ecological effect of material handling. One is to build adequately huge and exceptionally successful affluent treatment plants, and other route is to utilize colors and synthetic compounds that are climate inviting. The rich biodiversity of our nation has given us a lot of crude materials. However maintainable linkage must be created between development, assortment and their utilization. Common colors can deliver exceptional tasteful characteristics, which, joined with the moral essentialness of an item that is ecologically amicable, gives increased the value of material creation as craftwork and as an industry. Numerous colors are accessible from tree squander or can be handily developed in market gardens. In zones where engineered colors, mordents and different added substances are imported and accordingly moderately costly, normal colors can offer an alluring other option. As of late there has been a recovery of the developing enthusiasm on the use of characteristic colors on normal strands because of overall ecological awareness. They revealed that as of late various business dyers and little material fare houses have begun taking a gander at the conceivable outcomes of utilizing common colors for standard premise coloring and printing of material to beat ecological contamination related with manufactured colors. For effective utilization of characteristic colors, the fitting and normalized coloring methods should be embraced without scarifying required nature of colored materials. Subsequently, to acquire fresher shades with satisfactory shading quickness conduct and reproducible shading yield, proper logical strategies or methodology should be gotten from logical investigations on coloring techniques, coloring measure factors, coloring energy and similarity of particular common colors. A need has additionally felt to reinvestigate and remake of the customary cycles of normal coloring to control every treatment and pre-coloring measure.

II. RESEARCH METHODOLOGY

As colors have complex compound structures, their substance names are hard to comprehend and recall and basic names are in the neighborhood dialects and are territory explicit, a shading file has been produced for recognizing the colors. It fills in as reference for both the synthetic and specialized properties of colors. Prior distributed by the Society of Dyers and Colourists (SDC), United Kingdom, it is currently mutually distributed by SDC and the American Association of Textile Chemists and Colourists (AATCC), United States. In the shading file, Within the application class, the colors are organized by the tone. In this manner a color has a CI constitution number allocated to its synthetic constitution and furthermore a name in the shading file which indicates the kind of color. For instance, the CI number of normal indigo color is 75780 with the name CI Natural Blue 1 in which CI signifies Color Index, Natural demonstrates kind of color, Blue shows the tone, and 1 is the distinguishing number. Normal colors have been assembled as a class in the shading list. In Volume 3 of the shading record, 32 characteristic reds, 6 normal oranges, 3 regular blues, 5 common greens, 29 common yellows, 12 regular earthy colors, 6 normal blacks, and 1 normal white have been recorded. Normal colors have an unpredictable chemical constitution. In contrast to manufactured colors, they are generally not a solitary element but rather a blend of firmly related synthetic mixes. Based on significant substance constituents present, they are separated into:

- Indigoid colors
- Anthraquinone colors
- Naphthoquinone, Benzoquinone dyes
- Flavonoid colors
- Carotenoid colors
- Tannin-based colors.

III. RESULT AND DISCUSSION

3.1 Adsorption of colors onto sunflower seed shells

The adsorption of colors onto SS was led as demonstrated in a past paper 7. The pace of adsorption followed a pseudo-second-order motor model and the intra-particle dissemination was discovered to be the rate-controlling stage. Furthermore, the balance information fitted well both the Freundlich and multilayer adsorption isotherm conditions.

3.2 Dye decolouration and laccase creation

So as to tackle the removal issue of the colored SS created from the adsorption cycle, the decolouration of the adsorbed colors by the white-rot organism *T. pubescens* was examined. Societies with non-dyed SS were additionally performed

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for correlation. Fig. 3.1 shows ESEM .SS has a heterogeneous surface and micro-pores (about 1.5 μm) as observed from its ESEM photo. After color adsorption, SS introduced a smoother surface and littler micro-pores (about 1 μm) on account of the pressing of the color what made surface inconsistencies less sharp. After parasitic treatment colored SS were completely colonized by the organism and micro-pores were completely secured. Besides, the measure of SS debased by parasitic proteins following 12 days of development was assessed by contrasting the underlying and finishing dry load of the SS. The mean measure of debased SS (RDS) was resolved to be 0.024 corrupted grams per unique gram of help.

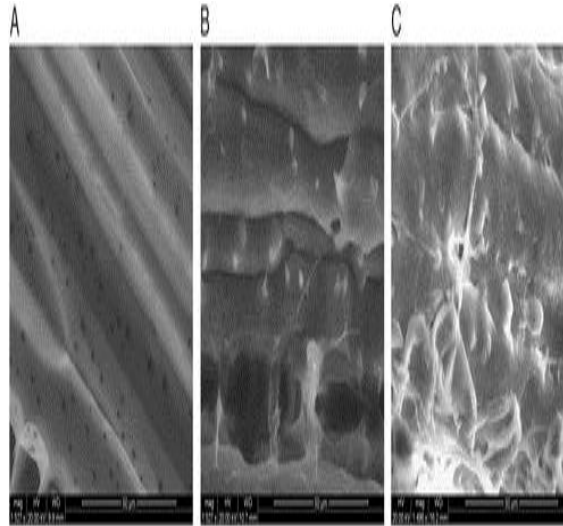


Figure 3.1 ESEM photographs of the original SS

Absorption spectra of desorption medium from *T. pubescens* are shown in Fig. 3.2. The absorbance peaks in the absorption spectrum obtained at day 0 showed the presence of dye in the desorption medium.

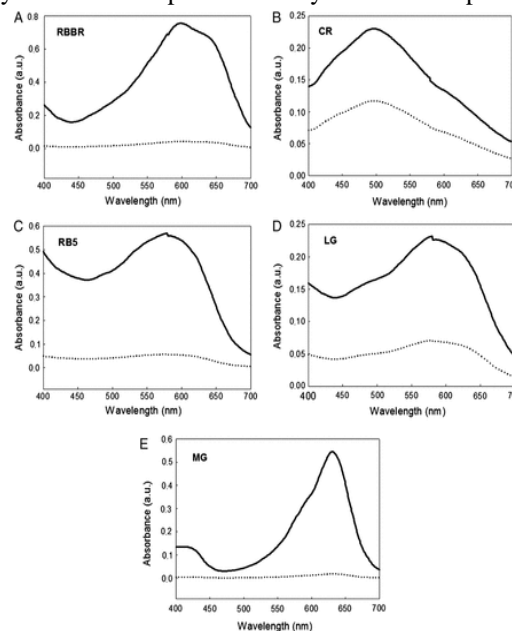


Figure 3.2 Absorption spectra of desorption medium

As shown in Fig. 3.2, laccase production began between the 2nd and the 3rd cultivation day (around 120 U/L) and, then, it sharply increased up to a maximum activity of nearly 7000 U/L at the end of the cultivation. Although the laccase activities obtained with the non-dyed SS were higher than those attained with the dyed ones (Fig. 2.4), the latter led to activities high enough to consider this material suitable as a support-substrate for laccase production under semi-solid-state conditions. The effect of using shells dyed with a single

dye (RBBR or RB5) or shells dyed with a mixture of dyes (RBBR, RB5, CR, LG and MG) on laccase production was minimal. In addition, the laccase activities obtained were about 147-fold higher than that obtained in submerged cultures using stainless steel sponges as supports. Also, they were considerably higher than those attained under semi-solid-state conditions using other agro-wastes as support-substrates such as banana skin and wheat bran .

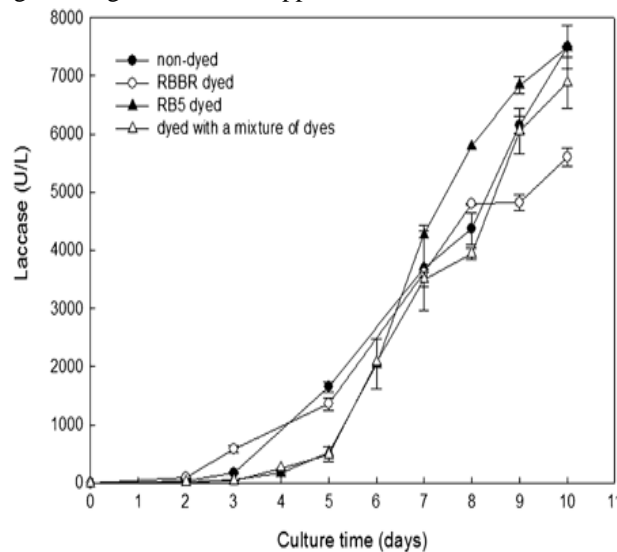


Figure 3.3 Laccase chart

Laccase production by *T. pubescens* grown under semi-solid-state conditions: (—●—) non-dyed SS; (—○—) RBBR-dyed SS; (—▲—) RB5-dyed SS; (—△—) SS dyed with a mixture of dyes. Laccase activities obtained with non-dyed SS are higher than those attained with the dyed ones. It should be pointed out that laccase activities are ca. 147-fold higher than those obtained in submerged cultivation. In view of the above results further experiments in order to enhance laccase production using dyed SS as support-substrates were performed. For this SS dyed with RB5 were selected due to this dye is widely used in the textile industry.

3.3 Effect of inducer addition on laccase production

It is known that the addition of inducers into the culture medium considerably influences the production of laccase. Thus, we studied the addition of five potential laccase-inducing substances into the culture medium such as Cu^{+2} , 2,5-xylidine, soy oil, coconut oil and tannic acid. The inducer concentration and the time point of the inducer addition (at the time of inoculation, trophophase and idiophase) were also investigated. As shown in Fig. 3.4 the highest laccase activity (25773 U/L) was obtained by adding 0.5 mM Cu^{+2} to the culture medium on the 3rd day of cultivation. This activity is more than 3-fold higher than that obtained in cultures with no inducer addition. There was not a linear correlation between biomass and laccase production, so the influence of inducer compounds on laccase production was not due to increased biomass production (Fig. 2.4).

Other authors have also reported the positive effect of Cu^{+2} as an inducer of laccase activity in fungi belonging to the *Trametes* genus. Experiments with purified laccase showed that Cu^{+2} not only induces laccase by the expression of laccase genes, but it also positively affects the activity and stability of the enzyme.

Effect of different inducers on laccase and biomass production by *T. pubescens* grown under semi-solid-state conditions using RB5-dyed SS as support-substrates. The highest laccase activity is obtained for the medium supplemented with 0.5 mM Cu^{+2} which is at least 3-fold higher than those obtained in cultures with no inducer addition. These results show that there is no linear correlation between biomass and laccase production. Duplicate experiments were run for comparison and samples were analysed twice. The values in the figures correspond to mean values with a standard deviation lower than 15%.

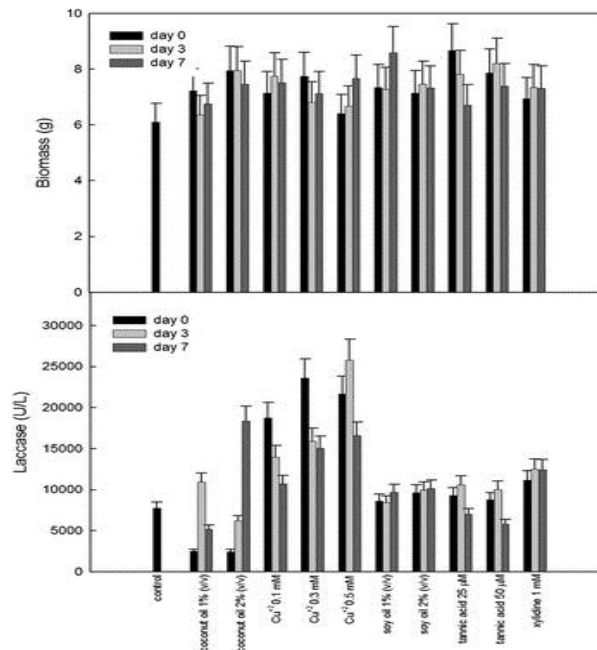


Figure 3.4 Laccase Biomass Chart

3.4 Joint effect of inducer addition on laccase production

In view of the above results the joint effect of supplementing the medium with Cu^{+2} plus xylidine and Cu^{+2} plus tannic acid was investigated. Thus, the experiments were performed with 0.5 mM Cu^{+2} plus 1 mM xylidine and with 0.5 mM Cu^{+2} plus 50 μM tannic acid added on the 3rd day of cultivation. As can be observed in Fig. 3.5 the joint addition of Cu^{+2} (0.5 mM) and tannic acid (50 μM) increased the laccase activity by 4-fold in relation to the reference cultures (Fig. 3.4). However, this increase was not acute (17%) in relation to the addition of only 0.5 mM Cu^{+2} (Fig. 3.4). The fact that Cu^{+2} acted synergistically with tannic acid to increase laccase activity is very interesting, since tannic acid is a non-toxic compound whereas xylidine is a toxic one.

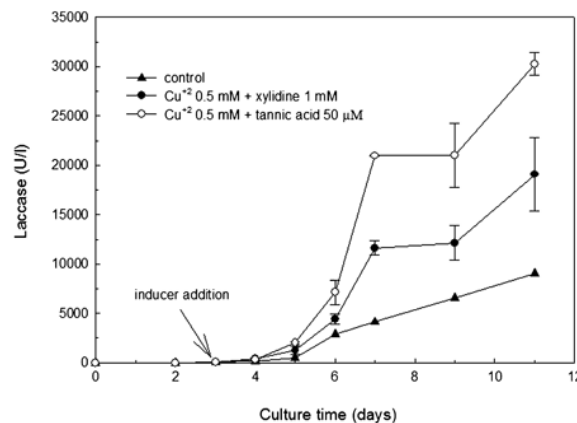


Figure 3.5 Laccase Activity Chart

Joint effect of Cu^{+2} plus tannic acid and Cu^{+2} plus xylidine added at the 3rd cultivation day on laccase production by *T. pubescens* grown under semi-solid-state conditions using RB5-dyed SS as support-substrates: control (—▲—); 0.5 mM Cu^{+2} +1 mM xylidine (—●—); 0.5 mM Cu^{+2} +50 μM tannic acid (—○—) Fig. 3.6 shows the protease production in cultures with and without inducers (Cu^{+2} and tannic acid). Protease production was lower in the induced cultures, which was likely the reason of the higher laccase activities produced in such cultures. This is in agreement with a paper by Palmieri et al. in which was stated that the positive effect of copper on laccase stability may be due to inhibition by Cu^{+2} of an extracellular protease.

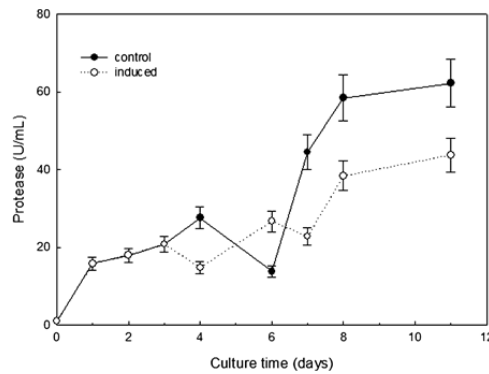


Figure 3.6 Protease Chart

Protease production by *T. ubescens* grown under semi-solid-state conditions using RB5-dyed SS as support-substrates: control cultures (—●—); copper-induced cultures (---○---

3.5 Dye decolouration and laccase production at reactor scale

The above process was scaled-up to laboratory reactors. The static tray bioreactor, also known as koji bioreactor, was the reactor configuration utilised since it is the generally used bioreactor for solid-state cultures. Tray bioreactors are very simple in design, with no forced aeration or mixing for the solid support-substrate. The design of tray bioreactors has remained almost unchanged over the last decades. Tray bioreactors are extensively used for the production of fermented oriental foods and enzymes. The results obtained are shown in Fig. 2.7. They were quite similar to those attained at flask scale which shows the potential of this process for its implementation at industrial scale.

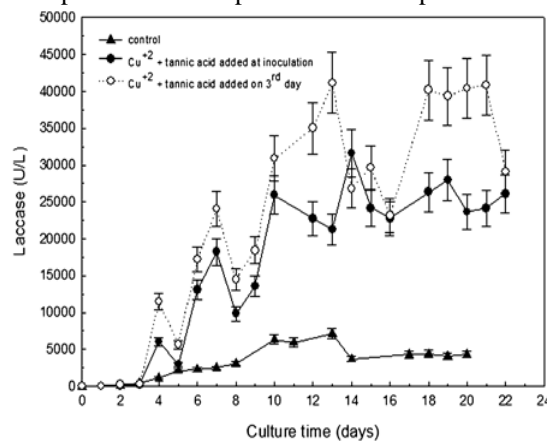
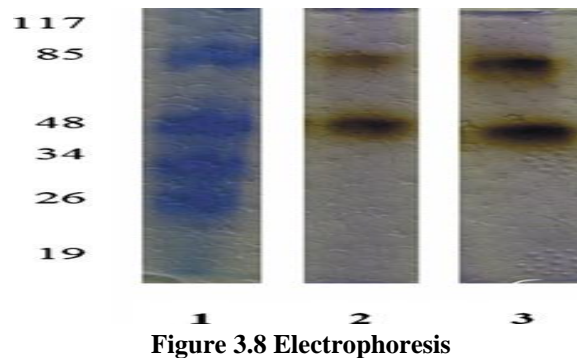


Figure 3.7 Laccase Parameters

Laccase production by *T. pubescens* grown on RB5-dyed SS in a tray bioreactor under semi-solid-state conditions: control (—▲—); 0.5 mM Cu²⁺+50 μM tannic acid added at the inoculation (—●—); 0.5 mM Cu²⁺+50 μM tannic acid added on the 3rd cultivation day (---○---

3.6 Electrophoresis of the extracellular crude obtained

Samples showing the highest laccase activities from the cultures without inducers and supplemented with 0.5 mM Cu²⁺ and 50 μM tannic acid on the 3rd cultivation day were collected, centrifuged and ultra-filtrated in 20 mL-Vivaspin tubes (Sartorius AG, Göttingen, Germany) with a membrane cut-off of 10 kDa. The resulting crude showed a specific laccase activity of 40 U/mg. From the activity staining it was clear that laccase enzymes were the main ligninolytic enzymes produced in both cultures with molecular weights of around 83 kDa and 50 kDa (Fig.3.8).



Guaiacol stained SDS-PAGE gel. Lane 1: molecular weight standards (kDa); lane 2: extracellular crude from cultures without inducers; lane 3: extracellular crude from cultures with inducers (Cu^{+2} and tannic acid added on the 3rd cultivation day)

3.7 UV-visible absorption spectrum of the extracellular crude obtained

The UV-visible absorption spectrum of the crude obtained showed two peaks at 280 and 620 nm and a shoulder at 330 nm (Fig. 3.9). The peak at 620 nm is typical for the type I Cu (II), which is responsible for the deep blue colour of the enzyme [24](#) and the shoulder at 330 nm indicated the presence of the type III binuclear Cu (II) pair [25](#). The spectral characteristics of laccase from *T. pubescens* were similar to that observed for other fungal laccases [26](#), [27](#).

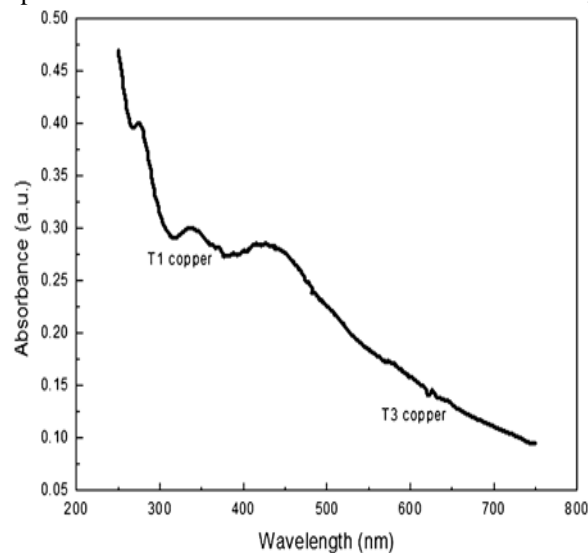


Figure 3.9 Absorbance

UV-Visible absorption spectrum of crude laccase (235 mg/mL in succinic buffer pH 4.5) from induced cultures of *T. pubescens*.

IV. CONCLUSION

Brief data about regular colors, their sources, application methodology, and different focal points and detriments of utilizing them was examined in this part. Albeit numerous weaknesses, for example, helpless quickness properties and utilization of prohibited metal salts and so forth can be effectively overwhelmed by examination and mindfulness, others, for example, nonreproducibility of shades and improving the accessibility would require higher exploration and mechanical speculations. Directly an around 1 % portion of materials is just being colored with common colors generally in the cabin area by conventional craftsmans, aficionados, and little entrepreneurs. Selling of regular color bearing materials and their cleansed extricates is, nonetheless, being done at a little industry level. Numerous makers in the United States, India, China, and different nations are occupied with this movement and their items are accessible on the Internet. The repetitive application cycle and nonreproducibility of shades and lacking accessibility are a portion of

the elements liable for their nonadoption in standard material preparing. Nonetheless, at the current degree of color asset accessibility, their appropriation by the material business isn't alluring additionally as that would bring about an ecological fiasco by method of loss of biodiversity and consumption of woodland spread notwithstanding the colossal natural preferred position offered by them as far as the lower contamination of the profluent whenever utilized appropriately. This favorable position can be used by the customary craftsmans in safeguarding their environmental factors from the evil impacts of contamination brought about by engineered colors as they don't approach costly emanating treatment plants required for manufactured colors. The advantages of examination directed on the advancement of improved application procedures for better speed and ecological consistence should contact these individuals so they can gain their occupations and the customer additionally get the advantage of really naturally agreeable materials. The accessibility of regular colors should be expanded in a supportable way by using the side-effects and squanders from agribusiness and agroprocessing enterprises and reasonable assortment of backwoods produce. This might be enhanced by developing significant color bearing plants on badlands and peripheral grounds along these lines giving an elective money yield to cultivators. Foundation of legitimate portrayal and confirmation conventions for common colors would improve customer trust in normal colored materials and would profit the two makers and clients. On the off chance that normal color accessibility can be expanded by the above-depicted measures and the expense of filtered colors can be carried down with an appropriate affirmation. Because of its ease and high adsorption limit (around 4 mg/g), SS was discovered to be a promising material for color expulsion from wastewater. Therefore, the color adsorbed onto SS was decolourised by the white-rot organism *T. pubescens*, which, likewise, created high titres of laccase chemicals. Further, the framework was effectively scaled-up to research center bioreactors. Notwithstanding, a few viewpoints identified with improving the bioreactor plan, optimising more process parameters and greater automation are needed for the industrial exploitation of this process.

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