Heliyon 5 (2019) e02302

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.heliyon.com

Production of pectin lyase from agricultural wastes by isolated marine *Penicillium expansum* RSW_SEP1 as dye wool fiber

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ARTICLE INFO

Keywords:
Agriculture
Microbiology

ABSTRACT

Enzyme technology is considered one of the most promising tools in the field of biotechnology as applied biocatalysts. The marine fungal strain *Penicillium expansum* RSW_SEP1 isolated from red sea water at Sharm El-Shiekh province and identified by 18S rRNA gene (accession number MH754656). The strain was able to produce pectin lyase from different agricultural wastes as the cheapest and most economical carbon source. The maximal enzyme activity of 88.83 U/mL was obtained from the pea peel (Pp) and sugar cane bagasse (Scb) at ratio 5:5 (w/w) and pH 6 by 8 mL of spore suspension as inoculum. Different concentrations of pectin lyase were examined on green dyed. The highest value of color strength (K/S) at 15% of wool fibers dyed with green algae by microwave after 5 min of the pretreated wool at pH 5.0. Different concentrations % of pectin lyase was examined on green dyed. The results indicated that, the highest value of color strength (K/S) at 15% of wool fibers dyed with green algae by microwave after 5 min of the pretreated wool at pH 5.0. This study focused on isolation and molecular identification of marine fungal strain as pectin lays producer from agriculture wastes and its application of dyeing wool fibers.

1. Introduction

Pectin Laos is one of the most promising industrial and biologically active enzymes belonging to pectin citrus family (Irshad et al., 2014). Pectin lyase (PL) is quite compelling as it acts straightforwardly on pectin without the requirement for past activity by different enzymes of the pectinolytic complex (Roberta et al., 2001). Microbial pectinase produce approximately 25% of the total worldwide enzyme sale Thangaratham and Manimegalai (2014). The pectinases that produced from fungi are utilized in the nutrition industry for the creation of olive oil, organic product squeezes, wine, in the illumination of juices, Pectin layer degradation in the textile industry which covers cellulose strands, in this way liberating the filaments for further handling (Roberta et al., 2001).

Pectin lyase has potential applications in improving of fiber quality cotton scouring, reducing the cationic request of pectic clarifications in paper dispensation, degumming of plant fibers, enhances the fermentation phase for tea and coffee process and handling of wastes from food dispensation industries (Gummadi and Sunil, 2007). Diverse research facility conditions are connected for the recuperation of fungal strains from marine substrates. The best assorted variety can be recuperated utilizing poor and rich medium, and diverse temperature, pH, and oxygen conditions (Rafaella et al., 2015).

A wide scope of agro-modern squanders, for example, oranges, rice straw, sugar cane bagasse, banana and coffee are a reasonable hotspot for their conceivable bioconversion into a scope of significant worth included result of interests like catalysts, synthetic compounds and numerous other fine materials. These enzymes not only provide as an economically viable alternative, but are environmentally friendly (Anwar et al., 2014; Giese et al., 2008; Ibrahim et al., 2013; Iqbal et al., 2011; Vikari et al., 2001). Eco friendly treatments such as polymers, enzyme and plasma are used instead of chemical treatments for surface modification of wool fibers to increase dye ability and fastness properties.

Surface adjustment techniques on fiber surface are important to continue to defeat shrinkage and hydrophilicity inconveniences of fleece strands (Onar and Sarıışık, 2004). Normal colors are natural well-disposed and non-poisonous, utilized for shading filaments to secure the earth and avert contamination brought about by enterprises (Hebeish et al., 2012; Ali et al., 2014). Green algae contains chlorophyll 1. Colorant shade extricated from green algae utilized for coloring pretreated fleece filaments by microwave warming. Ongoing improvements

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https://doi.org/10.1016/j.heliyon.2019.e02302
Received 26 March 2019; Received in revised form 29 April 2019; Accepted 9 August 2019

on plant based bioactive compounds have released up new chances in research. The greater part of the paper here focuses on the specialized subtleties of applying singular natural products. Natural dyes are utilized since point of reference dose for shading and for complexion and reproduction fibers (Ali et al., 2014).

Natural dyes unit ecological well disposed, short-lived and non-lethal. They're pulling in the consideration of people (El-Khatib et al., 2016). Numerous investigations on the machine of natural dyes are accounted for to ensure nature and avert contamination. As of late, the possibility of utilizing characteristic colors in material hue as UV- protection and antimicrobial has been examined. The advantages of using green algae as the wellspring of dyes and sustenance colorants matter are, in nourishment shading the vast majority of the dyes have the highest dietary benefit not at all like their made included substances. They are eco-accommodating in light of the fact that the procedure of creation of regular colors from fungi does not include the utilization of destructive or contaminating synthetic chemicals (Zhan and Zhao, 2009).

The aim of this research was a production of pectin lyase from the marine fungal strain *Penicillium expansum* MH754656 using different agricultural wastes as substrate and its application by using extracted natural pigments as chlorophyll from green algae for dyeing wool fibers.

2. Materials & methods

2.1. Microorganism

Marine fungal strain *Penicillium expansum* RSW_SEP1 isolated from red sea water at Sharm El-Shiekh province and identified by 18S ribosomal RNA gene.

2.2. Isolation medium

The medium composed of (g/l): Glucose 1.0, peptone 0.5, yeast extract 0.1 (800 ml sea water and 200 ml distilled water) and agar 15 (Jenkins et al., 1998).

2.3. Phylogenetic identification of fungal isolate

2.3.1. DNA isolation

DNA extraction was done by using the protocol of Gene Jet genomic DNA purification Kit (Thermo# K0791) as follows manufacture of the kit.

2.3.2. PCR amplification and sequencing

The PCR amplification of 18S rDNA region was carried out following the manufacture of Maxima Hot Start PCR Master Mix (Mix (Thermo) #K0221). The 18srDNA was amplified by polymerase chain reaction (PCR) using primers designed to amplify a 1500 bp fragment of the 18SrDNA region. The ITS1–5.8S–ITS2 genomic region was amplified from genomic DNA using the forward primer ITS1 (5-TCCGTAGGTGAACCTGCGG-3) and the reverse primer ITS4 (5-TCCTCCGCTTATTGATATGC-3) (White, 1990).

The PCR reaction was performed with 10μl of genomic DNA as the template, 1μL of 18S rRNA Forward primer, 1μl of 18S rRNA reverse primer 13 μl Water, nuclease-free and 25 μl Maxima® Hot Start PCR Master Mix (2X) in a 50μl reaction mixture as follows: activation of Taq polymerase at 95 °C for 10 min, 35 cycles of initial den. 95 °C for 10 min, dent. 95 °C for 30 Sec, annealing 55 °C for 1 min, extension 72 °C for 1min, final extension 72 °C for 15min. After completion, the PCR products were electrophoresed on 1% agarose gels, containing ethidium bromide (10 mg ml), to ensure that a fragment of the correct size had been amplified.

The amplification products were purified with K0701 GeneJET™ PCR Purification Kit (Thermo). Afterward, the samples become ready for sequencing in ABI Prism 3730XL DNA sequencer and analysis on GATC Company.

2.3.3. Phylogenetic analysis and tree construction

Phylogenetic data were obtained by aligning the nucleotides of different 18S rRNA retrieved from a BLAST algorithm (www.ncbi.nlm.nih.gov/BLAST), using the CLUSTAL W program version 1.8 with standard parameters. Phylogenetic and molecular evolutionary analyses were conducted using Mega 6 program (Tamura et al., 2013). All analyses were performed on a bootstrapped data set containing 500 replicates (generated by the program).

2.4. Fermentation conditions and substrates

Different agricultural wastes (potato peel, pea peel, apple peel, beet peel, orange peel carrot peel and sugar cane baggase) were used as carbon source and tested for pectin lyase production. All wastes were washed, dried at 70 °C in the oven and cut into small pieces before use.

The medium used for pectin lyase production was (g/L marine water): different agricultural wastes 20.0; $NaNO_3$ 2.0; K_2HPO_4 1.0; $MgSO_4$ 0.5; KCl 0.5; $FeSO_4$ 0.01 and autoclaved at 121 °C for 15 min. Erlenmeyer flasks (250 ml) containing 50 mL of fermentation medium was inoculated with two disks 6mm in diameter from the selected fungal isolate and incubated at 28 ± 2 °C for 7 days at 200 rpm.

2.5. Pectin lyase assay

The assay of pectin lyase was performed by the method described by Preiss and Ashwell (1963). A five hundred μL of enzyme was incubated for 1 h with 0.5 mL of 0.5% pectin in 50 mM Tris HCl buffer (pH 8) and 1 mL of 0.2 mM CaCl2. After 1 h, absorbance was measured at 548 nm against the blank. One unit of pectin lyase activity was defined as "the amount of enzyme present in 1 mL of original enzyme solution which released 1 μM of galcturonic acid in 1 min"

2.6. Optimization of enzyme production

2.6.1. A mixture of sugar cane bagasse (Scb) and pea peel (Pp)

A mixture of different proportions of **Scb** and Pp were studied as a next step, after selection of the most favorable substrates for pectin lyase production. Ten grams of Scb and Pp in different proportions (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9) in compared with 1 g of each waste alone.

2.6.2. Some of cultivation conditions for enzyme production

Various parameters were required for pectin lyase production. Different inoculum size using two disks of the selected fungi ranging from (2–10 mL) of fungal spores, studying the effect of different pH values (4.5–8.0), addition of equal weight of different amino acids (Hydrxyproline, L-serine, L-alanine, L-glutamine & proline).

2.6.3. Dyeing wool fibers pretreated with enzyme with colorant pigment extracted from green algae

2.6.3.1. Materials. Wool fibers 10/2, supplied by El Mahalla company-Egypt was used in this study.

2.6.3.2. Dyestuffs. Colorant pigment extracted from green algae used for dyeing pretreated wool fibers by microwave (Samsung M 245) heating with an output of 1,550 W operating at 2450 MHz. The wool fibers treated by enzyme at different concentrations (5–20%) for 5 min.

2.6.3.3. Dyeing wool fibers using microwave method. In a dye bath containing 2 g/L of the dye at liquor ratio 1:50, the wool fibers were dyed by microwave heating at pH 5, time for 5 min. The dyed samples were rinsed with warm water and then cold water, washed in a bath containing 5 g/L non-ionic detergent at 50 °C for 30 min, then rinsed and dried in air at room temperature.

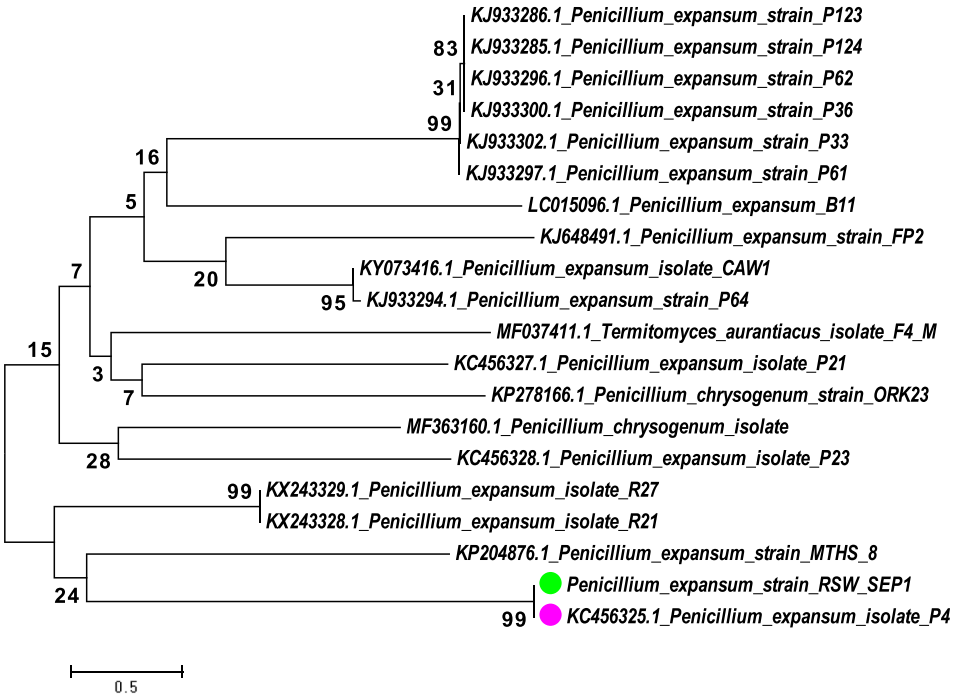

Fig. 1. Phylogentic tree showing the relationships of *Penicillium expansum* RSW_SEP1 with other *Penicillium* species.

2.6.4. Color measurements and analyses (Adeel et al., 2017)

2.6.4.1. Measurements of color strength (K/S value). An Ultra Scan PRO spectrophotometer was used to measure the reflectance of the samples and hence, the K/S was measured spectrophotometrically at wave lengths λmax 370 nm). The K/S of untreated and pretreated wool with the enzyme and dyed with Green Algae was evaluated.

2.6.4.2. CIELAB coordinates (L a* b*) measurements.* Measurements of un-dyed and dyed wool fibers were determined using an Ultra Scan PRO spectrophotometer (Hunter Lab) with a D65 illuminant and 108 standard observers.

3. Results and discussion

3.1. Fungal isolate identification

3.1.1. DNA isolation and PCR amplification

The DNA content of RSW_SEP1isolate was applied to PCR via common primers to amplify the ITS1 and ITS4 regions among the tiny and huge nuclear rDNA, counting the 18S rDNA. The previous primers amplified a DNA fragment of about 716 bp. The obtained data were in coincidence with Rasul et al. (2007) who mentioned that these primers are definite for fungi and amplified a DNA fragment of about 716 bp via various fungi.

The nucleotide sequence (716bp) of *Penicillium expansum* RSW_SEP1 strain was blasted with the available Gen bank resources using NCBI-BLAST search to compare the RSW_SEP1strain with those of member *Penicillium expansum* strains. The analysis indicated that the high sequence similarity species (99%) with *Penicillium expansum* strain.

3.1.2. Sequence alignment, phylogeny estimation and GC%

The phylogenetic tree (Fig. 1) showed that strains RSW_SEP1is most closely related to *Penicillium expansum*. Therefore, it was suggested a name *Penicillium expansum* RSW_SEP1. The nucleotide sequence of 18S rRNA genes for RSW_SEP1 strain has been deposited in Gen Bank under accession numbers: MH754656.

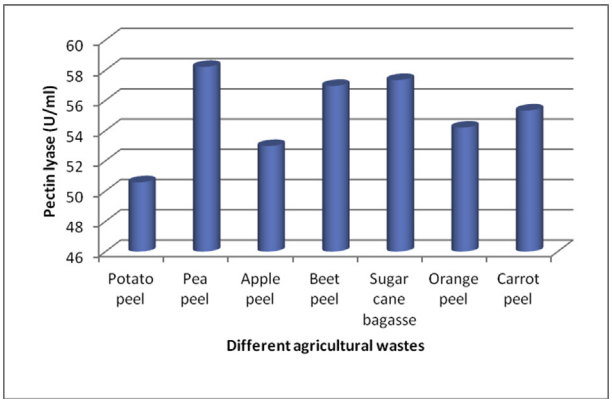

Fig. 2. Effect of different agricultural wastes on pectin lyase production from marine fungal strain *Penicillium expansum* MH75465.

Table 1
Effect of different ratios of pea peel and sugar cane bagasse on pectinlyase production from marine *Penicillium expansum* MH754656.

Different ratios	Pectin lyase activity (U/ml)
Sugar cane bagasse: Pea peel (Scb): Pp (w/w)	
9:1	54.72
8:2	58.40
7:3	60.46
6:4	66.33
5:5	69.76
4:6	60.42
3:7	58.40
2:8	54.03
1:9	50.14
1:0	58.22
0:1	57.35

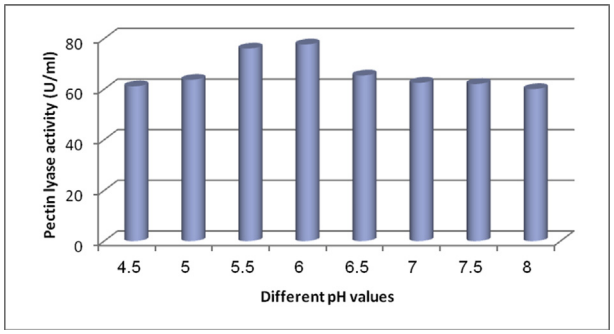

Fig. 3. Effect of different pH values on pectin lyase production from marine *Penicillium expansum* MH754656.

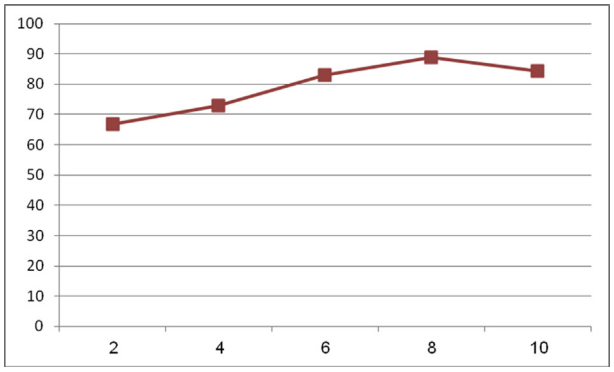

Fig. 4. Effect of different inoculum size on pectin lyase production from marine *Penicillium expansum* MH754656.

The percent of GC-content is an individual of several common features utilized to differentiate bacterial gene. The GC-content of the genomic DNA was 59.50 %mol% for RSW_SEP1 strain was taken from the phylogenetic analysis. These outcomes were in agreement with those by Nakase and Komagata (1971) who indicated that the GC contented of fungi varied from 34 to 63%. This variety is bigger than it is for each class. The compositional diversity also decreases from classes and subclasses to genera and species.

3.2. Optimization of enzyme production

3.2.1. Effect of different agricultural wastes on pectin lyase production from marine Penicillium expansum MH754656

Different agricultural wastes were used as economic and cheap carbon source for determination of pectin lyase production from marine fungal strain *Penicillium expansum* MH754656. The results in Fig. 2 showed that pea peel and sugar cane baggase were most promising for enzyme production produce (58.22 &57.38 U/ml) respectively followed by banger peel produce 56.96 U/ml. The other wastes showed moderate to low activity. These results were coincided with Safia et al. (2014) who showed that lemon peel was more promising for pectin lyase production from *Aspergillus oryzae*, also Sidra et al. (2013) obtained pectin lyase from *A. niger* using orange peel as carbon source. The same results were obtained by Sara et al. (2009) who illustrated that *Aspergillus flavipes* and *Aspergillus niveus* CH–Y-1043 strains produced pectin lyase using lemmon peel as sole carbon source and *Aspergillus brasiliensis* used orange peel for pectin lyase production (Janaina et al., 2017). In addition, *Pythium splendens* used cucumber, as carbon source for pectin lyase production (Wei-Chen et al., 1998).

3.2.2. Effect of different ratios between pea peel and sugar cane bagasse

Data recorded in Table 1 showed that equal weight of Pp and Scb (5:5 w/w) gave the optimum enzyme production of 69.76 U/mL in comparing with each waste alone followed by 6:4 (w/w) for Scb and Pp which produced of 66.33 U/ml. These results were coincided with Denis et al. (2002) who found that mixtures of mango peel or banana peels with sugar cane bagasse resulted an increase in pectin lyase production from *Penicillium viridicatum* RFC3 in comparison to fermentations without these residues. On the other hand, these results were in agreement with Natalia et al. (2004) who used three substrates include orange bagasse, sugar cane bagasse and wheat bran in fermentation medium inoculated with *Moniliella* SB9 for pectin lyase production.

3.2.3. Different pH values

Different pH values ranging from (4.5–8.0) were varying in fermentation medium for pectin lyase production activity as shown in Fig. (3). The increasing of pH values increased pectin lyase activity till reached pH 6.0 produce 77.70 U/ml then the enzyme activity gradually decreased. These results were not matching with the results obtained by Li et al. (2006) who found that pH 6.5 was optimized for the marine-derived fungus *Arthrinium c. f. saccharicola*. On the other hand, the optimum pH

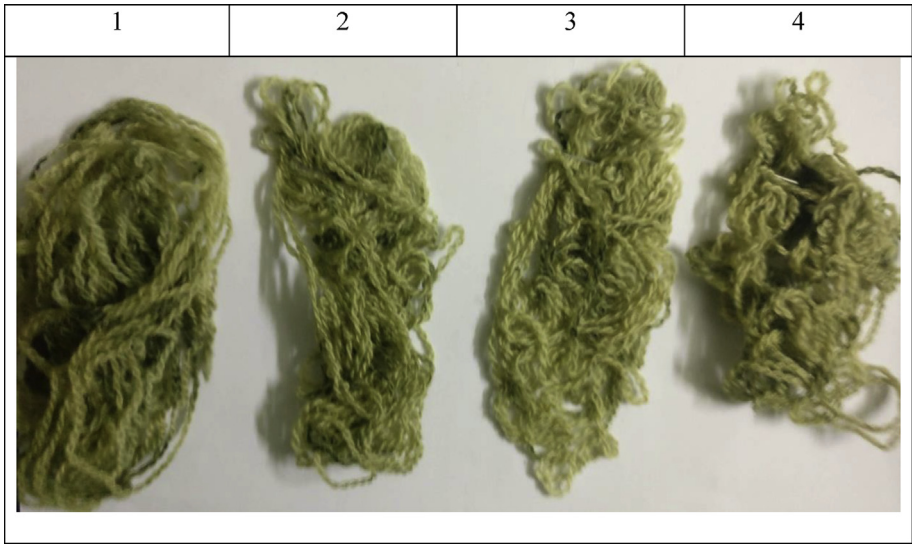

Fig. 5. Effect of concentration of enzyme on wool fibers dyed with green algae. Note: 1(5%), 2 (10%), 3(15%), 4(20%) conc.

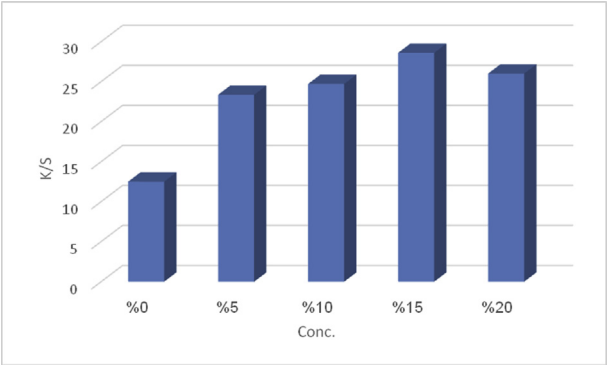

Fig. 6. Effect of enzyme concentration on wool fibers dyed with green algae by microwave method.

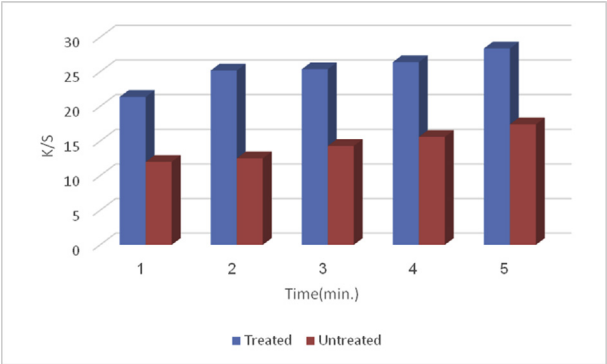

Fig. 7. Effect of time of dyeing on treated and untreated wool fibers dyed with green algae by microwave method.

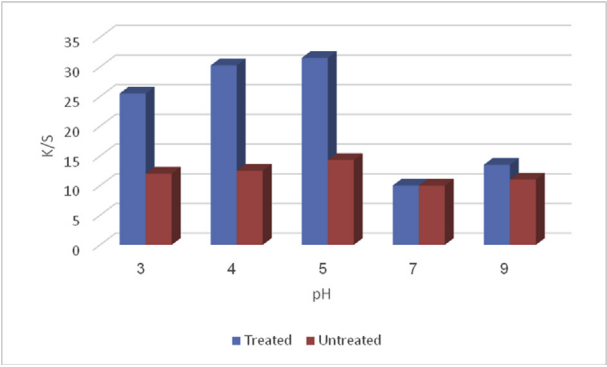

Fig. 8. Effect of pH of dyeing bath on treated and untreated wool fibers dyed with green algae by microwave method.

value of pectin lyase from Moniliella was 10.0 while from *Penicillium* sp. was 5.0 (Natalia et al., 2004). Genari et al. (1996) mentioned that the optimum pH of pectin lyase was obtained from *P. griseoroseum* in a medium with initial pH 6.8.

The variation in the fermentation medium pH levels makes microorganisms very susceptible to different pH values and also dependent on the nutritional composition and the physio-chemical of the supplementary growth substrates (Radha et al., 2005).

3.2.4. Effect of different inoculum size on pectin lyase production by Penicillium expansum MH754656

Data recorded in Fig.(4) showed that the increasing of inoculum size in the fermentation medium lead to an increase of pectin lyase production till reaching its maximum of 88.83 U/ml with inoculation of 2 disks (8 mm diameter) then the enzyme activity decreased. These results were in contrast with Sidra et al. (2013) who found that 4 ml inoculum of *Aspergillus niger* was optimized for pectin lyase production, also Safia et al. (2014) found that the optimum inoculum was one ml spore suspension of *Aspergillus oryzae*.

3.3. Effect of concentrations of enzyme on wool fibers dyed with green algae

The outcomes got demonstrated that, the pretreatment utilizing (5–20 %) convergence of enzyme gave the most noteworthy estimation of color strength (K/S) at 15% concentration of wool fibers colored with green algae by microwave as appeared in Fig.5 and 6. The Effect of microwave warming instrument is through ionic conduction, which is a sort of resistance warming. Contingent upon the quickening of the particles through the dye solution, it results in the crash of dye molecules with the particles of the fiber (Zhan and Zhao, 2009).

Microwave dyeing considers the dielectric and the warming properties of matter. The dielectric property alludes to the intrinsic electrical properties that influence the coloring by the dipolar pivot of the color and impacts the microwave field upon the dipoles. The fluid arrangement of dye has two segments which are polar, in the high recurrence microwave field. It impacts the vibrant vitality in the water particles and the dye molecules (Zhao and He, 2011).

The results in Fig. (7) showed that the effect of time dyeing wool fibers pretreated and dyed with green algae using microwave method. The results showed that the highest values of the color strength at 5 min. The outcome in Fig. (8) showed that the effect of pH of dyeing bath of wool fibers pretreated and dyed with Green algae using microwave method. The results showed that the highest values of the color strength at pH 5.0. Generally the treated samples gave higher results than the untreated wool fibers.

Declarations

Author contribution statement

Sherien M.M. Atalla: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Nadia G. EL Gamal: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Hassan M. Awad: Performed the experiments; Wrote the paper.

Nagia F. Ali: Performed the experiments; Performed the experiments.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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