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Research Article

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Utilization of fruit wastes as substrates for the decolourization of reactive blue textile dye by *Bacillus licheniformis* MTCC 429

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ABSTRACT

In the present study, the potential of Bacillus licheniformis MTCC 429 to decolourize reactive blue dye used in textile industry was evaluated. The influence of different incubation time, pH, temperature, inoculum concentration and carbon sources was studied to find the optimum conditions required for maximum decolourization of reactive blue dye using B. licheniformis MTCC 429. Incubation time of 24 hrs, with pH 7, temperature 35°C and 5% inoculum were observed to be the optimum decolourizing conditions. Glucose showed the maximum dye decolourization when compared to other carbon sources tested. The fruit wastes such as sapota and apple peel waste extracts were tested as substrate for the growth and dye decolourization ability of the bacteria. The sapota peel waste extracts served as better substrate which exhibited 91% decolourization ability when compared to apple waste. The results of the present study evidenced the potential of B. licheniformis MTCC 429 to be used in the biological treatment of textile effluents containing recalcitrant textile dyes.

Key words: *Bacillus licheniformis MTCC 429, reactive blue, decolourization, fruit waste*

INTRODUCTION

According to a recent survey, nearly 10,000 dyes are used in the textile industry and most of them are recalcitrant in nature[1]. These dyes after processing are discharged as waste effluents that have been estimated as 2,80,000 tonnes every year worldwide. The discharge of such dyes into water bodies have many adverse effects over aquatic life by blocking light penetration and also by altering pH, BOD and COD of the water[2,3]. Due to lack of adequate treatment procedures, these dyes can persist in the environment for a very long time[4].

By considering the adverse effects of such dyes including their toxicity, colour, mutagenicity, etc., many investigations have been done by various researchers for evaluating dye decolourization and degradation ability using different microorganisms[5]. Generally, reactive dyes are extensively used in textile industries due to their bright colour, low energy consumption and their covalent bonding ability with fabrics[6]. These kinds of dyes are mostly synthetic and consist of two key components: chromophore, that imparts the colour and auxochrome which renders the solubility and charge[7]. Their aromatic molecular structure makes them more stable and is difficult to degrade. The dye, reactive blue is found in thousands of textiles, foodstuffs, and pharmaceutical wastewater. In addition, reactive blue was also used as aquatic algaecide by various professionals[8].

In the present investigation, the ability of *Bacillus licheniformis* MTCC 429 to decolourize reactive blue dye was screened and the various operational parameters affecting the decolourization of reactive blue

dye were also optimized. In addition, the effect of sapota and apple peel waste extracts on the growth and dye decolourization efficiency of *B. licheniformis* MTCC 429 were also investigated.

MATERIAL AND METHODS

Chemicals and dye:

All the chemicals and reagents used in the present study were of analytical grade and purchased from Sigma Aldrich (USA) and Merck (India).

Microorganism:

For the present study, *Bacillus licheniformis* MTCC 429 was procured from MTCC, IMTECH, Chandigarh. The culture was maintained in nutrient agar slants at 4 °C till further use.

Screening for dye decolourization:

Bacillus licheniformis MTCC 429 was tested for its growth and decolourization ability on nutrient agar medium amended with 0.01% of reactive blue dye. The dye amended NA plates were streaked with the bacterial culture and incubated for 24 hrs at 37°C. The plates were then observed for the clearance zone around the bacterial colony for their decolourization ability.

Dye decolourization study:

Quantitative dye decolourization study was carried out in 250 ml conical flasks containing 100 ml of nutrient broth amended with reactive blue dye at a concentration of 0.1 g/L. The flask was inoculated with 1 ml of overnight bacterial inoculum and then incubated in rotary shaker incubator for 3 days at 37°C. During incubation, about 5 ml aliquots of samples were withdrawn at every 6 hours interval for determining the dye decolourization ability[9]. The obtained culture medium was centrifuged at 8000 rpm for 15 minutes and decolourization percentage was assessed spectrophotometrically by measuring absorbance of the supernatant at the absorption maxima (λm) of reactive blue using the formula:

$$\text{Decolourization percentage} = \frac{\text{Initial OD} - \text{Final OD}}{\text{Initial OD}} \times 100$$

Optimization of dye decolourization:

The effect of various operational parameters on dye decolourization was assessed by growing bacterial culture in dye amended growth medium. Initially, the pH of the medium was adjusted with different pH ranging from 6.0 to 9.0 (6.0, 6.5, 7.0, 7.5, 8, 8.5, 9.0) and the decolourization percentage was calculated. For the investigation of optimum temperature for dye decolourization, the bacterial culture was inoculated in the growth media and incubated at different temperature ranging from 25°C to 55°C. The inoculum concentration was varied to determine the optimum inoculum size for the better dye decolourization. The effect of different carbon sources on dye decolourization was also studied using different carbon source including starch, glucose, sucrose, mannose and lactose (0.1%). For all the parameters studied, the decolourization percentage was calculated using the formula described earlier.

Preparation of sapota peel and apple peel extracts:

The fruit wastes such as sapota peels and apple peels were collected from local market and juice shops. The collected fruit peels were washed using tap water followed by distilled water. The fruit peels were then shade dried at room temperature till the moisture content is completely removed. After complete drying, the fruit peels were ground into fine powder using mixer grinder. The fruit peel extract was prepared by mixing one gram of the each fruit peel powder with 100 ml of distilled water separately and left for two days for extraction. After two days, the apple and sapota peel extracts were filtered, sterilized and used for dye decolourization studies[10].

Fruit peel extracts in dye decolourization:

The effect of sapota peel extracts and apple peel extracts on dye decolourization was determined by amending different concentrations of the fruit peel extracts in Luria bertoni (LB) broth. For this purpose, 10 to 100 % fruit peel extracts (sapota and apple) in LB broth were prepared separately and amended with reactive blue dye at 0.01% concentration. The medium was inoculated with 5% inoculum and incubated for 24 hrs under shaken condition. After 24 hrs of incubation, the absorbance was recorded and the dye decolourization percentage was calculated using the formula described earlier.

RESULTS AND DISCUSSION

The improper disposal of textile effluent into environment has polluted not only the surface water but also the subsoil water to be contaminated. The level of the available effluent treatment procedures has not been satisfactory at most of the industrial units. Most of the units illegally let the untreated effluent into nearby river sources which caused serious impact on agriculture as well as aquaculture. These effluents also result in serious health problems including skin allergies and lung infections. It is mandatory to treat such dye waste by biological methods are found to be cost effective and simple to use for this purpose. Various microbes were reported to utilize a variety of chemical compounds including dyes as their sole carbon source.

The bacterial isolate, *B. licheniformis* MTCC 429 showed a better decolourization of reactive blue dye in plate assay (Fig. 1). The bacteria were tested for their dye decolourizing ability on liquid medium and showed complete decolourization of reactive blue.

In this course of study on decolourization of reactive blue, *B. licheniformis* MTCC 429 showed maximum decolourization (73%) during 24th hr of incubation (Fig. 2). The rate of decolourization increased till 24 hrs and no further decolourization occurred after that. The capability of *B. licheniformis* MTCC 429 to decolourize textile dyes is directly correlated to their ability to produce ligninolytic enzymes. Zille and co-workers evidenced the relationship between dye decolourization and production of ligninolytic enzymes[11]. The dye concentration also plays a significant role in the growth and dye degradation ability of the bacteria[12]. The results elucidate that *B. licheniformis* MTCC 429 was capable of decolourizing reactive blue textile dye.

In microorganisms, the environmental pH directly influences their growth and metabolism by responding to even slight pH alterations by biochemical adaptation mechanisms. From the available reports, it was found that alteration in pH significantly affect the decolourization efficiency of bacteria. *Bacillus licheniformis* MTCC 429 showed a maximum dye decolourization (74%) in the medium adjusted with pH 7 (Fig. 3). Various researchers have reported that the optimum pH required by different microorganisms for the decolourization of textile dyes[13, 14]. The dye decolourization efficiency of most of the bacteria is favored in pH range of 3–8[15]. Most of the azo dye degrading bacterial species reported are able to decolourize the dye at neutral pH[16].

Temperature plays an important role in growth of the microorganisms; hence, it is taken into consideration for the optimization. The influence of temperature on reactive blue decolourization was studied at the temperature range from 25ºC to 50ºC. The rate of decolourization increased till the optimum temperature of 35°C (78%) attained and beyond which a decrease in dye decolourization was observed (Fig. 4). For *Bacillus* sp., the optimum temperatures for dye decolourization have been reported in the range between 25 and 37°C[17]. It has also been reported that the temperature below 25°C and above 50°C inhibits the bacterial growth and dye degrading enzyme synthesis[18].

Varying volumes of bacterial inocula were used to inoculate the decolourization medium and the flasks were incubated for 24 hrs at their optimum pH (7.0) and temperature (35°C). The variation in dye decolourization with respect to varying inoculum size under optimum conditions was displayed in Fig. 5. The better decolourization (80%) of the reactive blue dye after 24 hrs of incubation time period was observed in the flask which received 5% inoculum of *B. licheniformis* MTCC 429.The rate of dye decolourization gradually increased until 5% inoculum was added to the decolourization medium. The further increase in inoculum concentration decreased the dye decolourization efficiency of *B. licheniformis* MTCC 429 which may be due to the depletion of nutrients. For optimal dye decolourization using microorganisms, requires an optimum amount of the microbial cells[17]. Among the carbon sources tested, glucose was found to be the better carbon source for the decolourization of reactive blue by *B. licheniformis* MTCC 429 (Fig. 6).

The adsorption and removal of synthetic dyes from water bodies involve various complex and cost intensive methods. In the present research, it was investigated the decolourization efficiencies of eco-friendly fruit peel extracts as substrates for the growth and decolourization of reactive blue dye by *B. licheniformis* MTCC 429. The sapota and apple peel extracts were prepared and tested as substrate at

different concentrations in LB broth for bacterial growth and dye decolourization efficiency. The sapota peel extract at the concentration of 60% in LB broth showed better dye decolourization (91%) when compared to other concentrations. Whereas, apple peal extract at the concentration of 70% in LB broth showed optimum dye decolourization of 85% (Fig. 7). Both the fruit peel extracts showed promising dye decolourization properties and on comparison, sapota peel extract was proven to be a better substrate when compared to apple peel extract for the reactive blue dye decolourization by *B. licheniformis* MTCC 429. This might be due to the high sugar content in sapota peel extract which facilitated the better utilization for the growth as well as dye decolourization process. Several other studies also supported the present findings as Kulandaivel and co-workers[s] who have used agro-industrial waste as substrate for *Bacillus* sp. for the decolourization of crystal violet, Congo red, methylene blue and safranin[19]. Similarly, Marina and co-workers (2012) used sugar beet waste as substrate for the growth and decolourization of textile violet dye by *Aspergillus Ochraceus*[20].

Fig. 1: Bacillus *licheniformis* MTCC 429 grown on NA medium amended with reactive blue dye

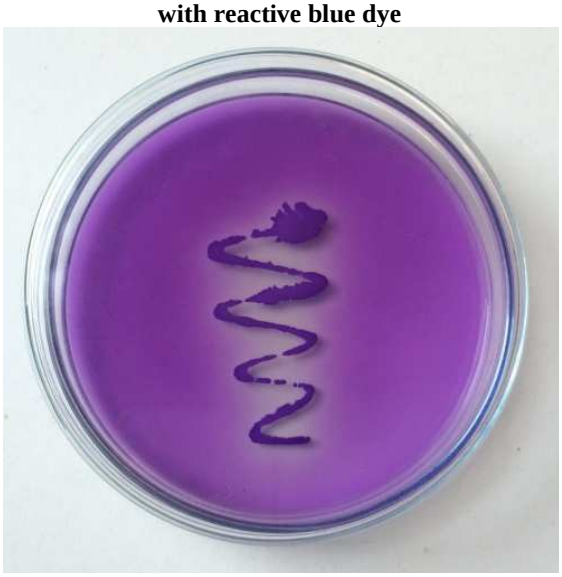


Fig. 2: Effect of incubation time on dye decolourization efficacy of *B. licheniformis* MTCC 429

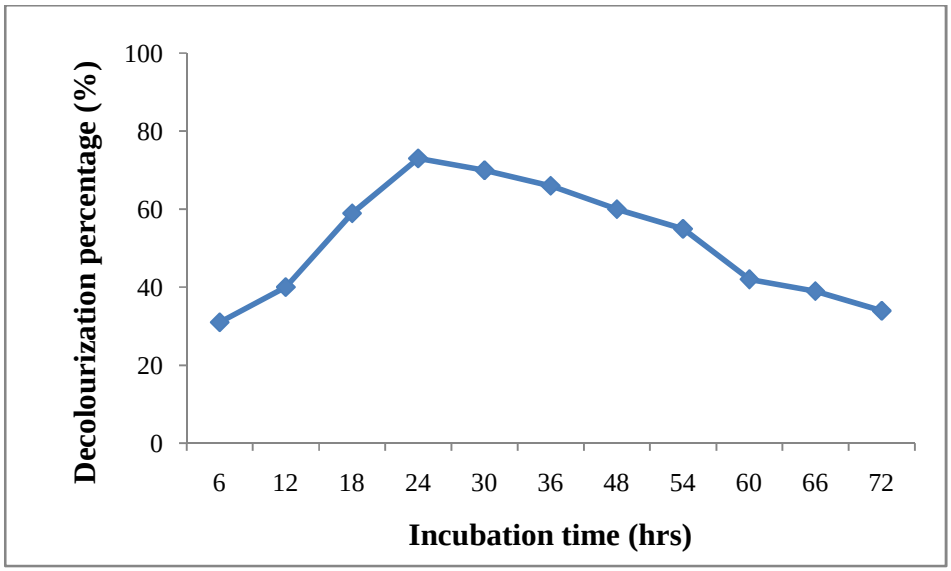


Fig. 3: Effect of pH on dye decolourization efficacy of *B. licheniformis* MTCC 429

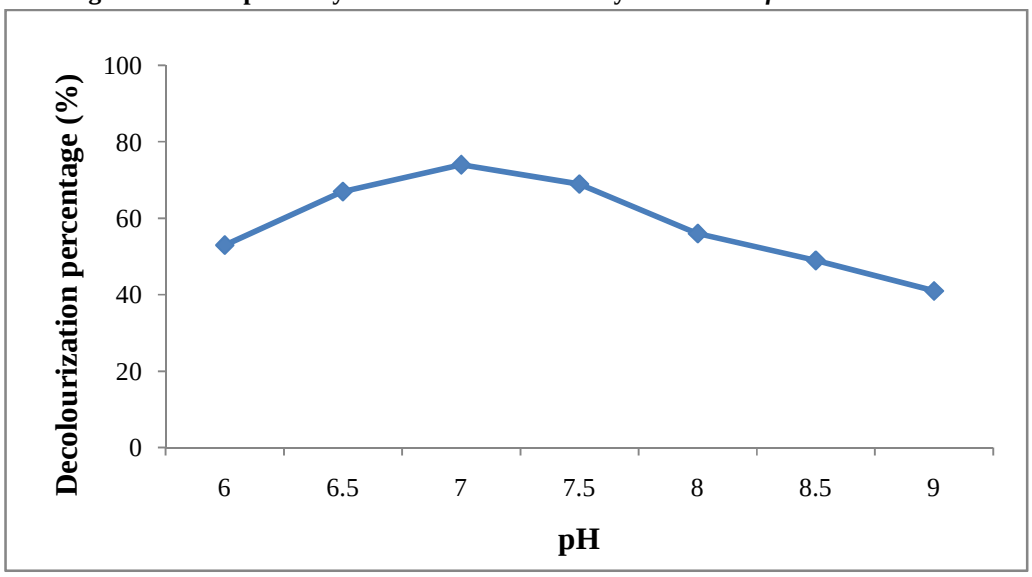


Fig. 4: Effect of temperature on dye decolourization efficacy of *B. licheniformis* MTCC 429

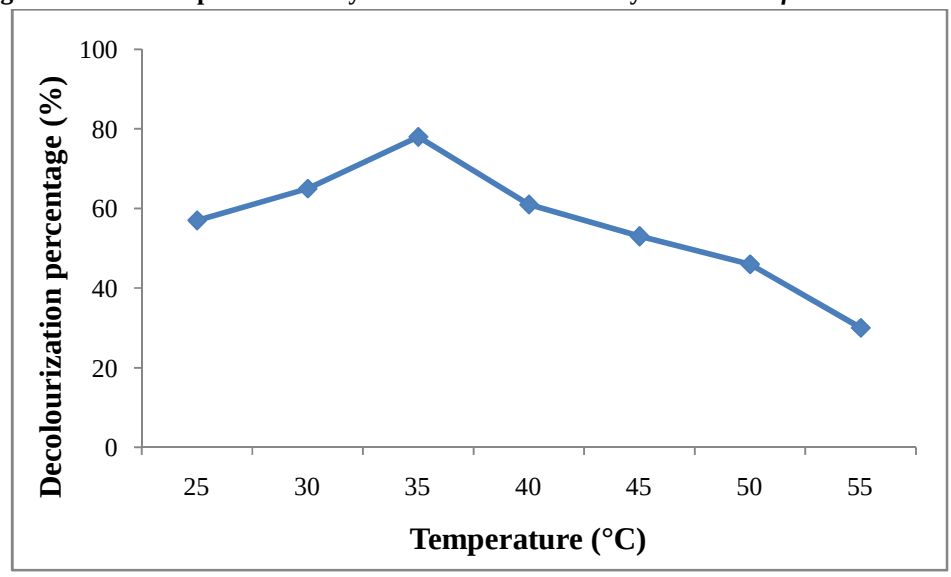


Fig. 5: Effect of inoculum percentage on dye decolourization efficacy of *B. licheniformis* MTCC 429

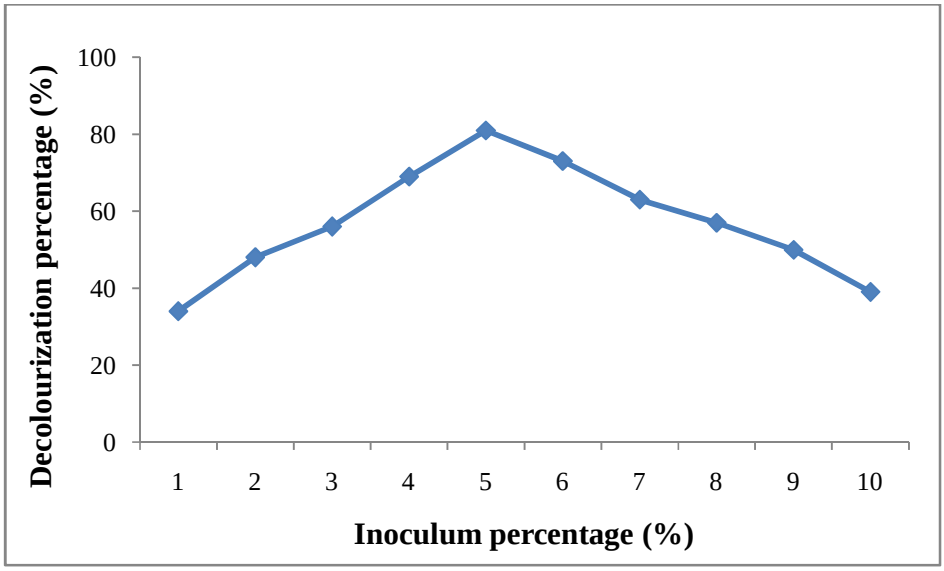


Fig. 6: Effect of different carbon sources on dye decolourization efficacy of *B. licheniformis* MTCC 429

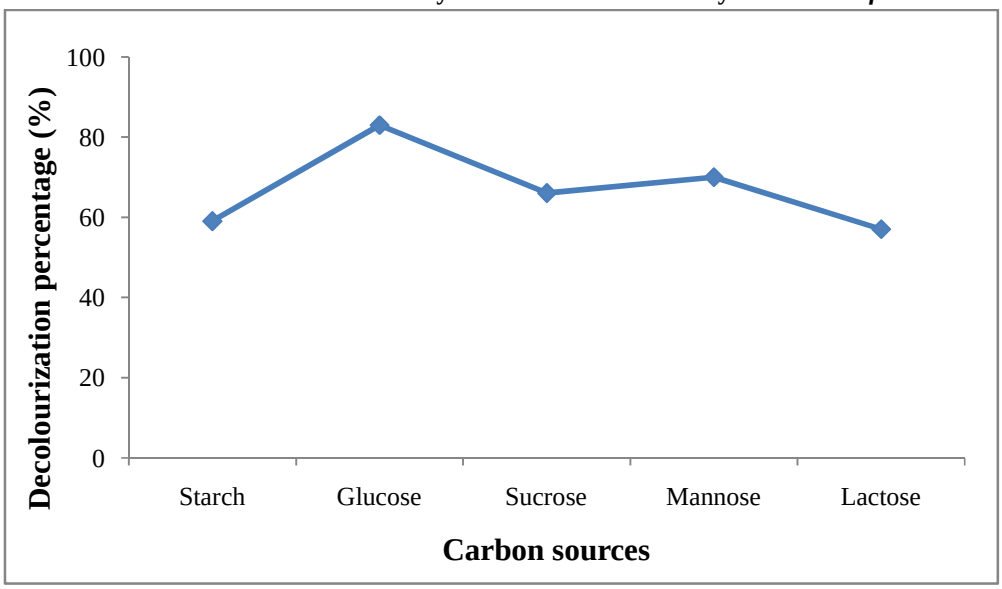
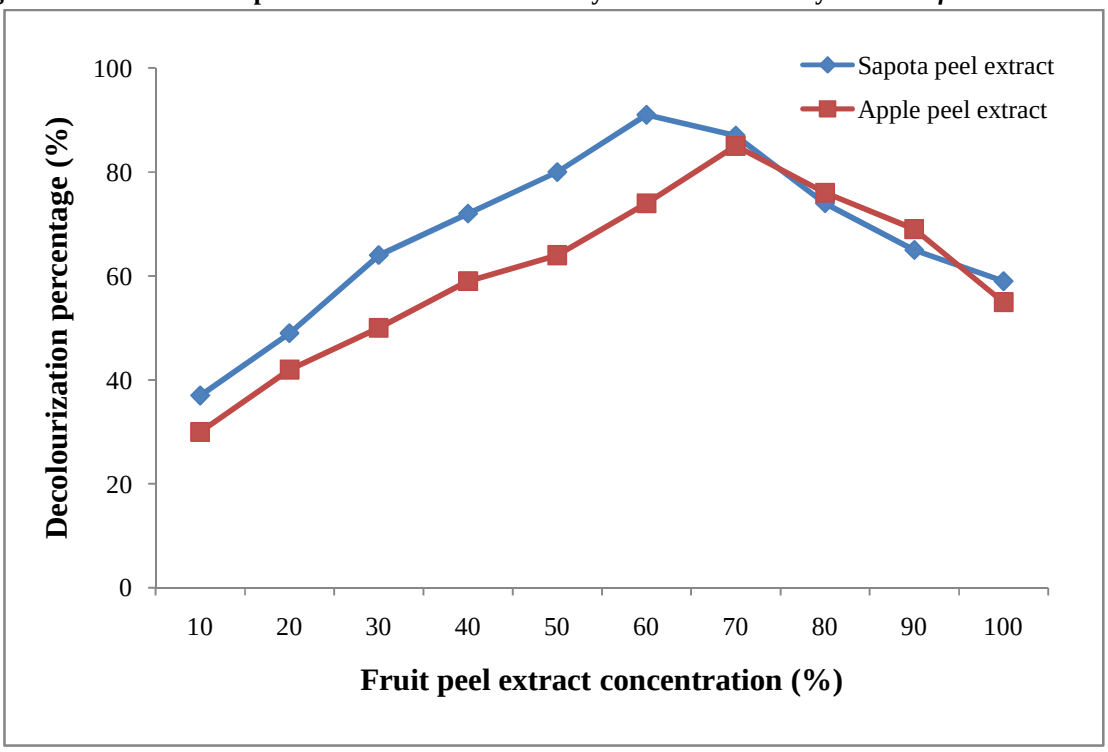


Fig. 7: Utilization of fruit peel extract for reactive blue dye decolourization by *B. licheniformis* MTCC 429



CONCLUSION

The textile dye, reactive blue is completely degradable under aerobic conditions with the aid of *Bacillus licheniformis* MTCC 429. The process parameters for the decolourization of reactive blue using *B. licheniformis* MTCC 429 was optimized and better decolourization of the dye was achieved in 24 hrs at pH 7 and 35°C using 5% inoculum which was correlated with the spectrophotometric studies. The efficiency of sapota and apple peel extracts as substrates for the growth and dye decolourization properties of *B. licheniformis* MTCC 429 was also evaluated. The *B. licheniformis* MTCC 429 with its dye decolourizing efficiency has an excellent scope for use in the treatment of industrial effluents that contain untreated residual textile dyes.

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