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Microbial Degradation Of Scarlet RR Collected From Textile Dye Effluent

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ABSTRACT

Worldwide, several thousand tons of synthetic dyes are produced annually. The chemical or physico-chemical treatment methods are inefficient, expensive, have limited applicability, and cannot be applied to a large-scale effluent treatment process. The alternative approach is bioremediation, which is cheaper, sustainable, and eco-friendly technique. In present research two plausible bacterial isolates were selected to decolorize Scarlet RR dye. At static conditions, successful decolorization was achieved and decolorization percentage varied from 81% to 97, while at pH 7 and temperature 37 °C maximum decolorization is observed. Biodegradation of dye was confirmed by FTIR and GC-MS analysis. The present work can resolve leading problems touching contamination of the water bodies because of textile effluent discharge.

Keywords: Azo dye, Decolorization, Biodegradation, FTIR, GC-MS

1. INTRODUCTION

The textile industry alone constitutes 66% of the dye consumption. Approximately, 10% of the dye stuff reaches water bodies during production and dyeing process [1,2]. These dyes are complex organic compounds which are harmful when released in environment through any disposal method. These dyes have been found to be carcinogenic by many of the researchers [3,4]. The microorganisms are being employed for bioremediation of wastes of many industries, be it heavy metals, antibiotic degradation etc., this microbial degradation has been used for dye degradation as well. A dye is a colored substance which absorbs a particular wavelength and gives color corresponding to that wavelength [5]. There are two types of dyes- natural dyes (obtained from leaves, wood, and bark) and synthetic dyes [6]. About 50% of dyes were lost in effluent after dyeing process [7-8]. The chemical or physico-chemical treatment methods are inefficient, expensive, have limited applicability, and cannot be applied to a large scale effluent treatment process [9,10,11]. The alternative is bioremediation as it is cheaper, uses less chemicals and also is eco-friendly [12,13]. Much of the research work has been done for the bioremediation of certain dyes and many fungal and bacterial species, including gram-positive and gram-negative have been reported to be capable of degrading textile dyes [14,15]. Research was carried out by Khehra et al. [16] to isolate micro-organism capable of degrading textile dye Brown 3 REL. Azo dyes are aromatic compounds (azo linkages (R1-N=N-R2), which are the mostly used dyes in industries. Azo dyes are aromatic compounds with one or more azo linkages (R1-N=N-R2). Monoazo dyes have single N=N group, whereas diazo have two N=N bonds. Azo dyes are made up of aromatic amines. Azo group, N=N is generally attached to benzene and

naphthalene rings. This reactive azo group is responsible for imparting color to the dye, which varies in intensity [17,18]. According to Brown et al. [19], azo dyes constitute more than 50% of the dyes used in industries. Azo dyes contaminate the ground water and rivers. These dyes are resistant to degradation as they are resistant to light and temperature and so remain stable in the environment for a long time [20]. The toxicity of these azo dyes is a serious environmental concern as these are found to be carcinogenic and mutagenic, in particular substituted benzene [21,22]. Substituted benzene and naphthalene rings of azo dye have been found to be carcinogenic [23]. Further, azo dyes themselves are not carcinogenic but their metabolites are carcinogenic [24]. Scarlet RR is azo dye, which has been reported to be carcinogenic by many research workers [25,26].
In the present study, previously isolated PGPR of the pseudomonad species (*Pseudomonas pseudoalcaligenes* PS5) which were earlier reported to degrade not only acephate but additionally also has the ability to persist against heavy metal contaminated industrial soils were used to evaluate the bioremediation potential of *Pseudomonas pseudoalcaligenes* PS5 against scarlet RR for efficient decolourization and degradation.

This work provides the promising purpose of *Pseudomonas pseudoalcaligenes* PS5 in the field remediation of dyes from environment contaminated with it and its possible role of assistance in biodegradation of other xenobiotics compounds also.

2. MATERIALS AND METHODOLOGY

2.1 Dye stuff and chemicals

Scarlet RR was was generously provided from Jai Ambey Textiles, Amritsar, Punjab (India) manufactured by Colortex dye stuff company, Surat, India. From Loba Chemie (Mumbai, India) and Himedia (Mumbai, India) , other chemicals, media and solvent were purchased.

2.2 Organism and culture condition

In our previous work, we carried out characterization of selected strain through 16S rRNA sequencing. Two universal primers 1492R (ACCTTGTTACGACTT) and 27F (AGAGTTTGATCMTGGC TCAG) were used to obtained partial sequence and results shows the isolated strain belong to Pseudomonas pseudoalcaligenes PS5 (Genbank accession number KJ588061.1). This culture was kept in a rotating shaker at 200 rpm for 24 hours at 37 ˚C. Then, 1ml of this suspension culture was taken and added to a fresh medium containing Scarlet RR dye which is to be decolorized. Then at the intervals of 24, 48, 72, 96, upto 408 hours, culture was withdrawn and subjected for centrifugation at 10,000 rpm for 15-20 minutes. The supernatant volume of 4 ml was used to measure the decolorization process [27,28].

2.3 Decolorization experiment

Suspension of isolate were prepared by inoculating an individual bacterial colony (obtained on agar plates) in nutrient broth, under shaker at 200 rpm for 24 h. 1 ml of this suspension culture was added to a fresh medium containing dye which is to be decolorized. Then at the intervals of 24, 48, 72, 96, upto 408h, culture was withdrawn and centrifuged at 10,000 rpm for 15-20 minutes. The λ_{max} (maximum wavelength) of the dye *Scarlet RR* is 470 nm. Decolorization percentage was calculated by the formula suggested by Saratale et al. [29].

Decolorization (%) = (Initial absorbance – Observed absorbance) × 100

Initial absorbance

Comparative analysis of dyes before and after incubation in presence of bacteria was carried out by following the protocol of Asad et al. [30] and Kurade et al. [31].

2.4 Effect of pH on Decolorization

Decolorization of Scarlet RR which is thought to be recalcitrant, was observed using UV-Vis Spectrophotometer at different pH values [32].

2.5 Fourier transformed infrared spectroscopy (FTIR) Analysis

Decolorization was quantitatively analyzed and monitored by FTIR. FTIR analysis was carried out for the treated samples, to check removal or transformation of harmful functional groups. For this, control and degraded dye samples were centrifuged at 5000 rpm for 30 min and extraction was done by adding an equal amount of ethyl acetate to the supernatant and dried over Na_2SO_4 and kept in a 60°C oven. Spectrum was compared in the mid-IR region from 500 to 4000 cm^{-1}.

2.6 Gas Chromatography (GC) Analysis

The metabolite identification was carried out by gas-chromatography mass spectrometry (GC-MS) by adding HPLC grade methanol to the dried samples [33]. The identification of metabolites were analysed using gas chromatography (GC-MS) of Thermo Fisher Scientific, model (polaris-Q) equipped with flame ionization detector and the column used (DB-5, 30m × 0.25mm × 0.25µm) by following the method of Saratale et al. [29]. Temperature was kept initially at 70 °C for two minutes, and then raised upto 300 °C with an accrual of 10 °C/ min and the temperature of injection port was maintained at 240 °C. The flow rate of helium as a carrier gas was 1.0 ml/ min. Mass/charge (M/Z) ratio of range 10-610 was used for MS analysis [34].

3. RESULT

3.1 Decolorization of Scarlet RR dye

Decolorization studies were carried out by noting down the absorbance value for the samples after inoculation with the bacterium. Media used was nutrient broth at pH = 7 and temperature used was 30 °C at static condition. Scarlet RR was decolorized to light pink color, color variation was seen after 48 hr of incubation.

3.2 Effect of pH on decolorization of Scarlet RR dye

The effect pH on the decolorization of Scarlet RR dye was studied over decolorization percentage. Decolorization and degradation was achieved at a wide range of pH from 3 to 10. All experiments were done in triplicates along with the uninoculated control. Decolorization in both the isolates follows polynomial trends when plotted against pH (Fig. 1). Decolorization increased above pH 5 and decreased below pH 5 in most of the cases. It was

found that pH (3-6) was the most unacceptable pH for decolorization of dyes. Maximum decolorization was observed at pH 7 and temperature 37 °C by W2 isolate.

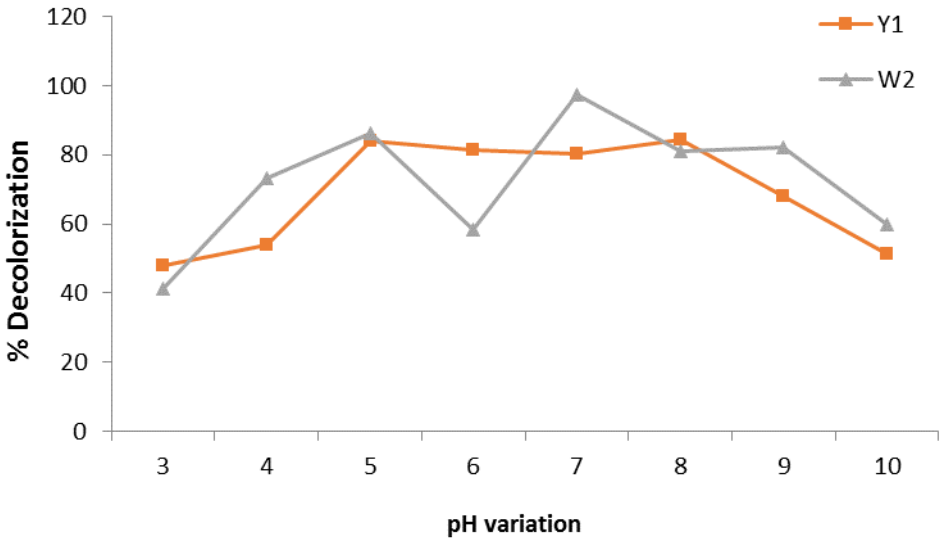

Figure 1. Effect Of Ph On Decolorization By Bacterial Isolates (Y1 And W2**)**

3.3 Spectrophotometric analysis

Decolorized medium was taken and centrifuged at 5000 rpm for 20 minutes and supernatant was used to measure OD. We obtained maximum decolorization of Scarlet RR dye by W2 isolate at pH 7 and temperature 37 °C (Fig. 2). Marked decrease in the absorbance of degraded samples was observed at λ_{max} (470 nm) of the dye. This can be an outcome of cleavage of azo bond. Change in absorbance readings for the dye indicates a conspicuous change in the structure of the dye.

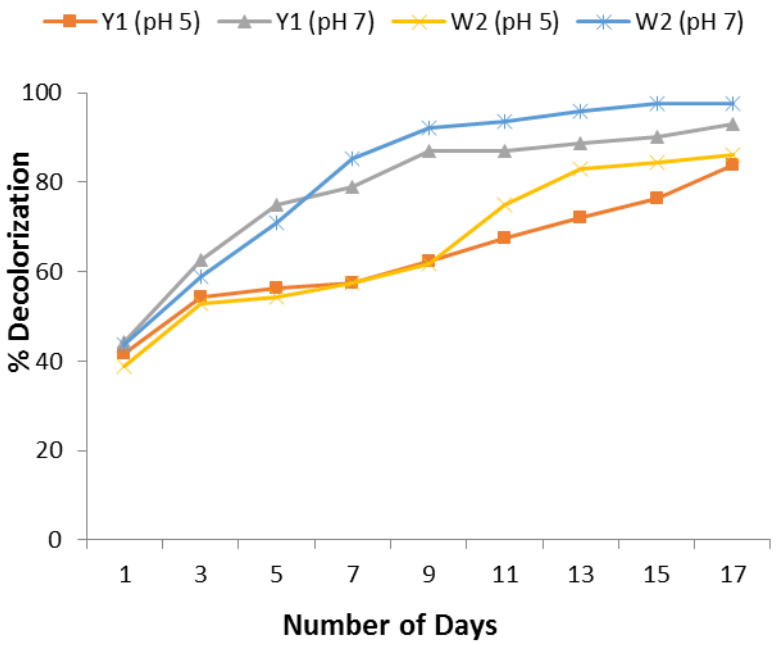

Figure 2. Decolorization of Scarlett RR by bacterial isolates (Y1 and W2) at pH (5 and 7) at 37 °C temperature

3.4 FTIR analysis

FTIR spectrum of both the control and degraded samples were compared. FTIR results confirmed the biodegradation of Scarlet RR by the selected strain. O-H Stretching, C-N stretching, N-H stretching, C-C stretching and –C ≡ C–H: C–H bend appeared at 3450.77, 1932.74, 1645.33, 1614.47, 1437.02, 1215.19, 1138.04, 630.74, 495.72 cm^{-1}. Greater number of peaks was found to shift in the metabolized samples. Intensity of peaks was greatly reduced in the degraded metabolites (Fig. 3 a and b).

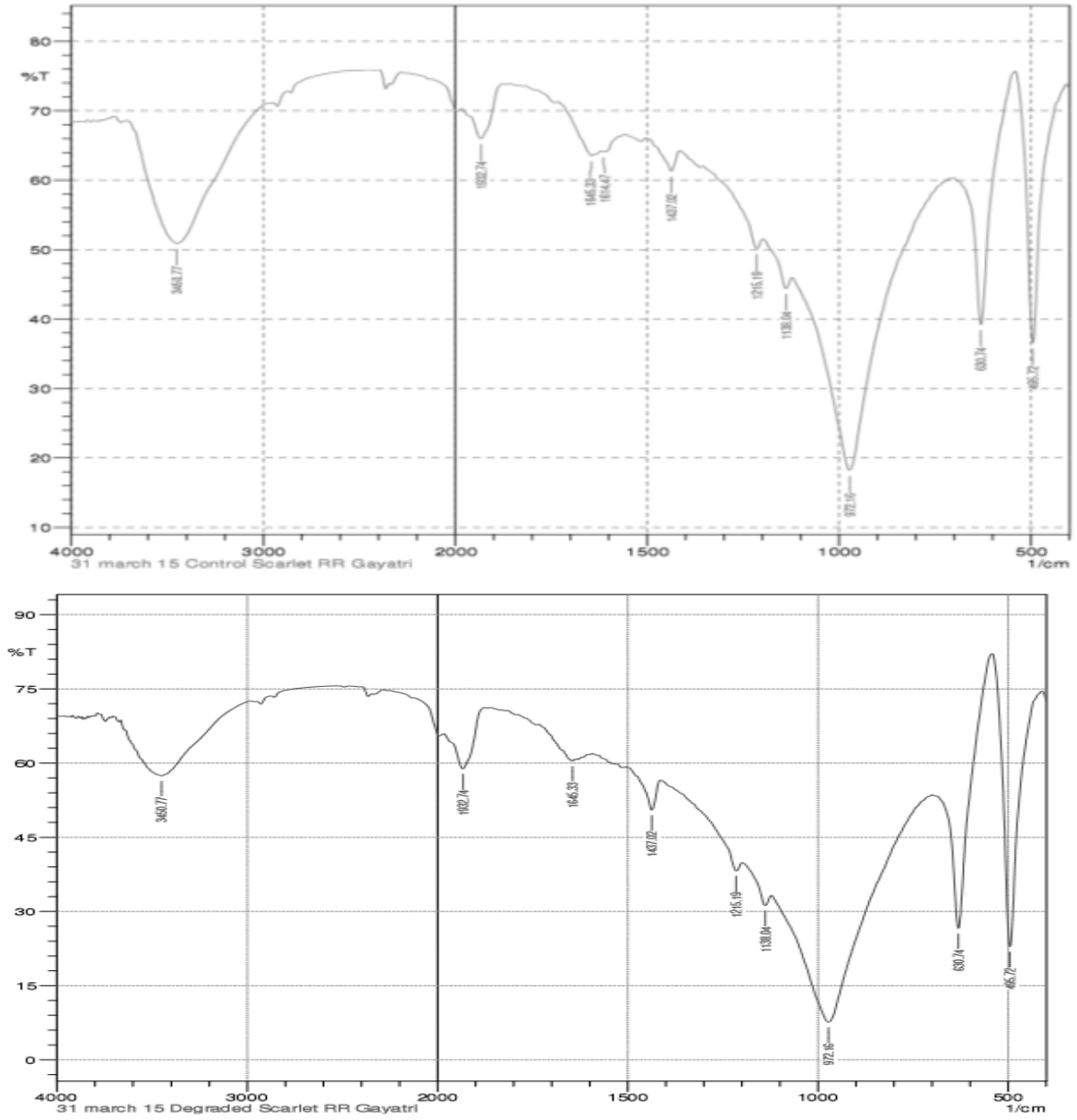

Figure 3. FTIR spectrum of control Scarlet RR dye (a) control and (b) and its metabolites obtained after decolorization using Y1 bacterial isolate

3.5 GC-MS analysis

Further confirmation of biodegradation was done by GC-MS analysis. Mass spectra of control and degraded samples of Scarlet RR are shown in fig 4 and 5. Degraded metabolites were recognized by GC-MS analysis. Different mass spectra were obtained when control and degraded samples were analysed in GC-MS. M/Z ratio obtained were significantly different in control and degraded samples. Peaks at 503, 399, 415 were completely eradicated in case of degraded scarlet RR dye. This again shows complete eradication of toxic azo bond. This result is in compliance with the FTIR results obtained for Scarlet RR dye.

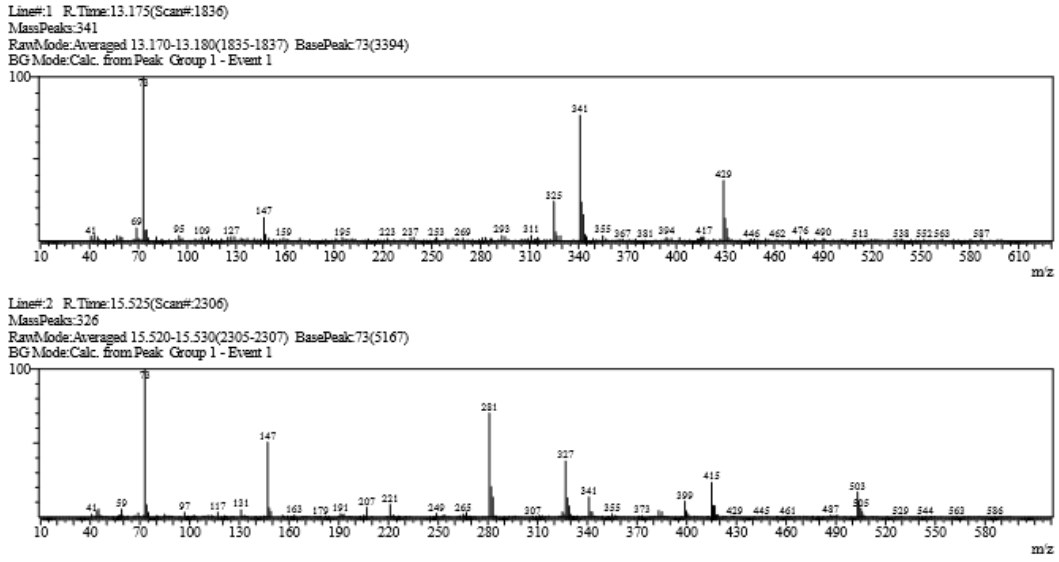

Figure 4. Mass spectrum analysis of Scarlet RR dye (a) control and (b) its metabolites obtained after decolorization using Y1 bacterial isolate

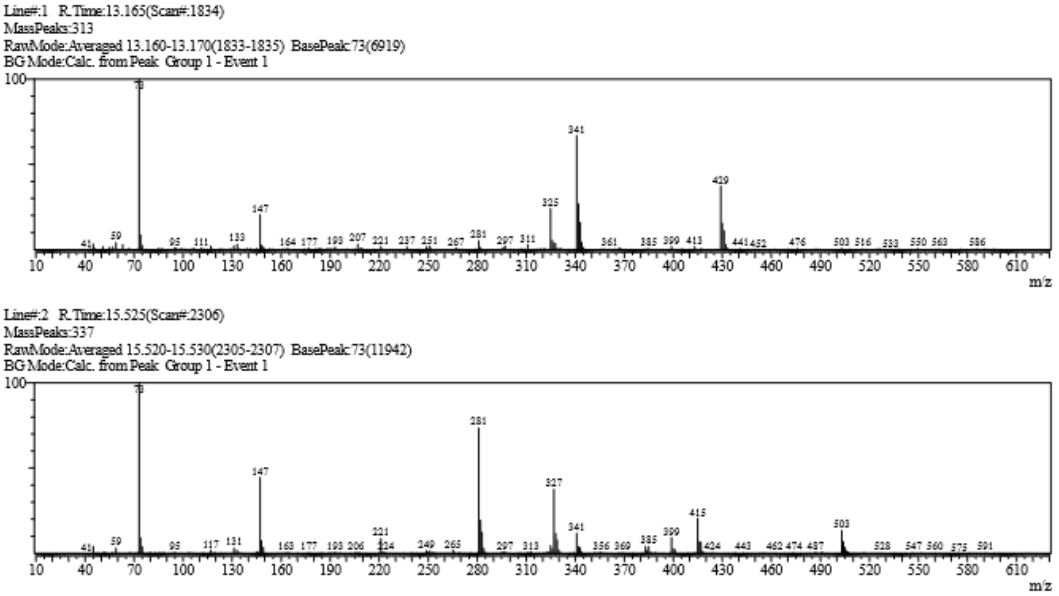

Figure 5. Mass spectra analysis of Scarlet RR dye (a) control and (b) its metabolites obtained after decolorization using W2 bacterial isolate

4. DISCUSSION

It has been proclaimed repeatedly that bacteria dwell in textile effluents to utilize its ingredients for their survival. Similarly, the textile dye effluent examined in this work was found to accommodate an assorted turf of microbes. These bacteria are native inhabitants of the textile effluent as the latter serves as their supply of essential nutrients. This finding is in compliance with certain previous studies [35,36]. Industrial dye effluent and sludge generated by effluent treatment plant is loaded with dye degrading and decolorizing microbial populace. Two plausible isolates were selected on the basis of ability to decolorize the dye and were inoculated in the presence of Scarlett RR dyes. At static conditions, successful decolorization was achieved and decolorization percentage varied from 81% to 97%. Maximum

decolorization was observed at pH 7 and temperature 37 °C. But, one thing was observed that prolonged incubation time is required for complete degradation. Spectrophotometric analysis done after 5 days did not yielded the complete degradation, so with the prolonged incubation, newly formed metabolites were also degraded. Similar results of decolorization studies have been reported earlier by Ito et al. [37]. FTIR spectrum of both the control and degraded samples were compared. FTIR results confirmed the biodegradation of Scarlet RR by strain. O-H Stretching, C-N stretching, N-H stretching, C-C stretching and –C ≡ C–H: C–H bend appeared at 3450.77, 1932.74, 1645.33, 1614.47, 1437.02, 1215.19, 1138.04, 630.74, 495.72 cm^{-1}. Greater number of peaks was found to shift in the metabolized samples. Intensity of peaks was greatly reduced in the degraded metabolites. Similar results have been produced by Patel et al. [38]. They reported metabolization of Acid Maroon dye by bacterial consortium EDPA. FTIR results indicated the wreckage of azo bond and main chromophore was destroyed. Further confirmation of biodegradation was done by GC-MS analysis. We used GC-MS of agilent technologies for the recognition of degraded metabolites. Different mass spectra were obtained when control and degraded samples were analysed in GC-MS. M/Z ratio obtained were significantly different in control and degraded samples. Peaks at 503, 399, 415 were completely eradicated in case of degraded scarlet RR dye. Lade et al. [39], also found certain metabolites by comparing the m/z ratios. Similar results have been reported by many of the research workers [40,41]. We can recommend further research to develop a customized alternative treatment for textile dye degradation. This can resolve leading problems touching contamination of the water bodies because of textile effluent discharge.

Bacteria are helpful in many other ways and it is found bacteria isolated from heavy metal soils has potential of plant growth promoting traits [42]. Various zinc solubilizing bacteria have been found to promote growth and nutrition of rice plants [43].

Various studies have been done on scarlet RR azo dye and other textile dyes along with some plant derived dye and useful informations have been gathered in this aspects [44-47].

Conflict of interest

Authors declare that no conflicts of interest exist.

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