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Bio-Decolorization and Degradation of Reactive Blue 222 by a Novel Isolate *Kucoria marina* CU2005

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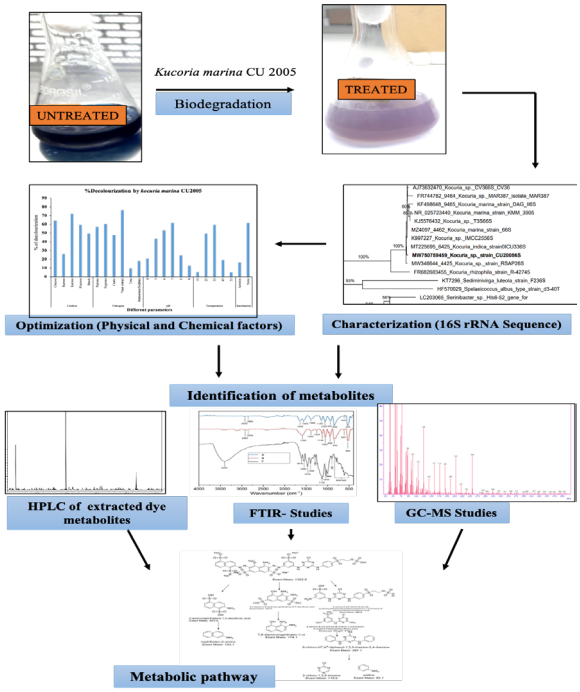
Graphical abstract:



Abstract:

In this study, a novel bacterial strain, *Kucoria marina* CU2005, was isolated and identified using 16S rRNA gene sequencing from an industrial wastewater sludge sample capable of degrading Reactive Blue 222 (RB222) dye. Batch mode bio stimulation studies were performed with minimal salt media to optimize key physiological parameters for effective decolorization of RB222. When cultured at 35 °C and pH 7 under static conditions, this bacterium decolorized 82 percent of the dye after 24 hours. Decolorization was monitored using UV-vis spectrophotometry. Isolate's ability to decolorize the complex dye was attributed to its degradation potential rather than a passive surface adsorption. FTIR, HPLC, GC-MS studies were used to confirm microbial dye metabolism. The results indicated breakdown of dye upon decolorization as some peaks were shifted and generation of aromatic amine for monosubstituted benzene ring as intermediates of dye degradation in decolorized solutions. This study has shown the potential of *Kucoria marina* CU2005 to decolorize RB222 dye at a better pace and efficiency than previously reported bacterial strains. Thus, we propose that our isolated strain can be utilized as a potential dye decolorizer in environmental biotechnology as effluent treatment for decolorization of RB 222.

Keywords: Reactive blue 222, Biodegradation, *Kucuria marina*, Optimisation, Dye metabolites

Introduction

In today's world, with rapid industrialization there is extensive application of synthetic chemicals used in various industries. These recalcitrant compounds are persistent in nature affecting human health and environment.

Water being the essential for the survival and existence of any form of life on earth. Textile industries are the oldest industry contributing majorly to Indian economy. Currently these industries are at high risk as the dyes used in textile industries are toxic and carcinogenic to living forms. Textile industries are consuming the highest amount of water and it's been estimated that on an average 100 liters of water is required to produce 1 Kg of fiber thus identified as major water polluting industries (1).

Synthetic dyes are used expansively over natural dye across the globe owing to its major benefits such as the low cost in its production, dying, good fastness with vibrant shades. Nearly 20% of dyes used in industries are lost as significant component in industrial effluent. These dyes being complex aromatic compounds tend to resist degradation and accumulates in water and soil. This interferes with water properties such as pH, Chemical Oxygen Demand and Biological Oxygen Demand (2).

This dye effluent also considered to be a large source of aromatic amines which are the intermediate products of dye degradation and mutagenic in nature. These industrial effluents can also affect the quality of water and soil used in irrigation which in turn can reduce the crop yield and quality(3). These dyes are associated with health risk to humans as they can affect vital organs such as liver, kidney, gall bladder digestive tract and skin. Reactive dyes can cause DNA damage leading to various types of cancers and confirms genetic toxicity of dye (4). In vitro studies with Reactive blue19 on 3D dermal structure was tested using comet assay which confirmed its genotoxicity against humans (5). Reactive dyes are also proved to be cytotoxic proven with human keratinocyte HaCaT cell line. Studies on textile industry workers emphasis the occupational disease such as skin dermatitis and bladder cancer due to prolonged exposure to these reactive dyes (6).

To sustain the valuable resources of the environment for future. There are different methods of treatment of industrial effluent for its effective reuse. The most recommended is bioremediation mediated by microbes over physical or chemical methods. Bacteria are the best mediators of remediation of recalcitrant and toxic dyes. The complete process of industrial effluent treatment becomes effective and economical when bacterial strain are employed as they can easily adopt to varying environmental conditions and quickly catalysis the biotransformation of textile dye to non-toxic end products (7). Current research aims at the isolation of the potential bacterial strain that can decolorize and degrade RB222 from industrial effluent waste by enrichment method. The isolate was characterized by 16S rRNA sequencing. Physical and chemical factors were examined for *Kucoria marina* CU2005 for its best efficiency in decolorization and degradation of RB222. FTIR, HPLC, and GC-MS tools were used to confirm the degradation of dye and to propose the dye intermediates.

Materials and methods

Chemicals and culture medium

MSM broth (M1253) comprising of Sodium chloride (2.5g l^{-1}), Disodium phosphate (33.5g L^{-1}), Potassium phosphate (15g L^{-1}) and Ammonium chloride (5g L^{-1}) used in dye degradation studies was procured from Hi-Media Laboratories (Mumbai, India). Reactive Blue 222 dye was obtained from Praasa overseas (Gujarat, India). All other chemicals and Methanol and Ethyl acetate used in this research were of analytical grade from Merck (India).

Isolation of bacteria by enrichment method

Industrial wastewater sludge was collected from industrial area Peenya, Bangalore, India. 5% (w/v) of sludge was inoculated into MSM broth containing 100mgL^{-1} and incubated at 37ºC on an orbital shaker incubator at 120 rpm for 24 h. From this

10%(v/v) was sub-cultured into MSM broth containing 200 $mgL^{-1}$and incubated under same condition as mentioned previously to enrich dye degrading bacteria for next 24 h. Further from this fresh culture 10% (V/V) was inoculated into MSM media with higher concentration of dye (500 mgL^{-1}) and maintained at same conditions as mentioned earlier. After 24 h of incubation period dye decoloring bacteria was screened on solid MSM media with 100 $mgL^{-1}$of reactive blue 222 dye. Plates were incubated under static conditions in bacterial incubator at 37ºC for 48 h to identify the dye decolorizing bacterial colonies.

Identification of rb 222 dye decolorizing bacterial strain

Genomic DNA was obtained from the pure culture of bacterial isolate (B5) and subjected to integrity check on 1.0% agarose gel. 16S rRNA genes were specifically amplified using BDT v3.1 cycle sequencing kit and the sequence was deciphered on ABI 3730xl Genetic analyzer by Sanger's di-deoxy chain termination method. Forward and reverse sequencing reactions was used to search the GenBank database using BLAST tool. Related strain's 16s rRNA sequences were obtained from the GenBank database and sequence aligned using MEGA X. Using the aligned data, a phylogenetic tree was constructed using MEGA X with 500 bootstrap replicates (8,9).

Effect of physiochemical factors on dye decolorization

100 ml of MSM broth was prepared in 250 ml conical flask with 100 mgL^{-1} RB222 dye. Media was inoculated with 4% (v/v) inoculum to study the influence of different physical and chemical factors in dye decolorization. pH (4–9), temperature (15–55 °C), co-substrate in media such as nitrogen source (peptone, tryptone, casein, yeast extract, urea, and ammonium sulphate), carbon source (glucose, sucrose, lactose, fructose, and starch), dye concentrations (100-1000 mgL^{-1}) were investigated. These cultures were maintained under static incubation at 37ºC for 24 h. To examine the effect of aeration in dye decolorization broth was maintained under shaker incubator at 120 rpm for 24 h. All experiments were carried out in triplicate, and the uninoculated MSM media were kept under the same conditions as the control. Decolorization percentage was calculated using the formula:

$$\text{Decolorization rate (\%)} = (C-T)/C \times 100$$

Control=Absorbance of uninoculated MSM media with dye.

Test=Absorbance of decolorized MSM media

Extraction and analysis of metabolites

A chromatographic method was used to separate and identify intermediate dye metabolites and their transformation products. In brief, MSM broth with 200 mg L^{-1} was inoculated with 4% (V/V) bacterial isolate and the dye intermediate metabolites were extracted with ethyl acetate solvent after the incubation period. Broth was centrifuged at 10,000rpm for 15 min and the supernatant was collected and mixed with equal volume of ethyl acetate and mixed for 20 min using magnetic stirrer. Organic layer with dye metabolites was evaporated and crystals were obtained using rotatory evaporator. The fine crystals were subjected to FTIR, HPLC and GC_MS studies (10). FTIR analysis (Shimadzu-IrSpirit with ATR accessory) was performed using KBr disk method in the mid-IR range (400-4000cm^{-1}) and the percentage of transmittance was recorded (11). High-performance liquid chromatography (Shimadzu-LCMS 8040) was performed with 50% methanol and 50% water as mobile phase and analytes were separated in C 18 column. To examine the dye degradation UV detector was set at λmax of RB-222 (615 nm), and dye degraded metabolites (280 nm), respectively.

Gas chromatography–mass spectrometry (GC–MS) analysis was performed in the Shimadzu GC-MS QP2010SE model with a 30 m × 0.25 mm x 0.25um Shimadzu 5Sil-MS

column. A sample volume of 1 µL was injected and the injector temperature was maintained at 280 °C. Helium was the carrier gas, flown at 1 mL min^{-1}. The temperature of the column was set at 60 °C held for 4 minutes, and successively increased to 200 °C at a ramp rate of 10 °C min−1, holding 3 minutes, followed by 200 °C-280 °C at a ramp rate of 10 °C min−1, holding the temperature for 9 minutes. Mass spectra were obtained at 70 eV. GC–MS spectra were analyzed using the software Shimadzu post run analysis (12). The peaks in the spectra were identified with the help of NIST2017.LIB library.

Statistical analysis

All data from dye decolorization assays were tested for statistical significance by comparing the means of different test conditions using One-way ANOVA. The data were considered significant if $p < 0.05$.

Results and discussion

Primary screening and decolorization studies

Reactive Blue 222 RB222) dye has a molecular weight of 1323.1 g/mol and is a complex diazo dye. RB222 has a maximum absorbance at 615 nm. Textile industrial sludge sample was inoculated into MSM media with RB222 as sole carbon source. Subculturing with increasing dye concentrations in MSM media at regular intervals was used to obtain a potential isolate. Bacterial colony (B5) on MSM agar media with RB222 at 100 mg L^{-1} exhibited a prominent dye decolorization in reference to control without inoculum. An assay with MSM broth and RB222 was also performed to quantify its decolorization efficiency (100 mgL-1). Under static conditions, *Kucoria marina* CU 2005 (B5) showed 61% dye decolorization after 24 hours. The dye decolorization ability of the isolate can be improved by further optimizing the conditions. The concentration of dye in industrial effluent typically ranges from 10 to 25 mg L^{-1}, but in these studies, the initial concentration was 100 mg L^{-1}. Bacteria are the best mediator for bioremediation as they quickly adapt to the environmental conditions and decolorize the dye in short period of time (13).

The isolate B5 is of significance in bioremediation of the most widely used textile dye RB222. As per the literature review, the least biological degradation of reactive 222 has been reported while the passive process of dye removal using biosorption was carried out using Dead biomass of *Rhizopus arrhizus* NCIM997. RB222 are recalcitrant and resist its degradation and hence a two-step bioremediation method was standardized for degradation which employed both chemical and biological methods. Primarily dye was treated with a strong oxidizing reagent such as Fenton's reagent followed by treatment with mixed fungal culture of *P. ostreatus IBL-02 (PO)* and *P. chrysosporium IBL-03 (PC*). In another study basidomycetes *P. ostreatus* could decolourise RB222 by 76.39% when immobilized on lignocellulose support of *Luffa cylindrica.* The Abiotic control experiment resulted in 71.30% dye decolorization. This clearly indicates decolorization was by passive process mainly attributed to absorptive capacity of support medium that is *Luffa cylindrica* (14, 15). All these studies emphasis the need to identify a pure potential bacterial culture capable of decolorizing and degrading the RB222 which is toxic and highly resistance to degradation.

Identification of RB 222 degrading bacteria

The 16S rRNA analysis of isolate was performed by Barcode Biosciences, Bangalore, India. The consensus from the forward and reverse sequencing reactions was used to search the GenBank database using BLAST. The BLAST search showed that the hit with the highest identity of 99.78% was *Kocuria sp*. Strain ZJFT1123, accession number MH298683.1, with 98% coverage. The 16s rRNA sequences of related strains were obtained from the GenBank database and sequence alignment was performed using MEGA X. The aligned dataset consisted of 58 taxa and 1511 nucleotides. Using the aligned data, a Neighbor-Joining tree

was constructed in MEGA X with 500 bootstrap replicates. The model of sequence evolution used was maximum composite likelihood and to rate variation gamma distribution with gamma value of 1 was used (16, 17).

From BLAST search and Neighbour-Joining phylogenetic tree, the newly isolated strain was found to be *Kocuria sp*. From the phylogenetic tree, the newly isolated strain falls into the genus *Kocuria* with 100 % bootstrap support (Fig. 1). Therefore, using 16s rRNA sequencing the bacterium was found to be a strain of *Kocuria sp* which was deposited in NCBI database with accession number MW750759459.

Figure. 1 Phylogenetic tree of the isolate B5 Optimization for dye decolorization

Effect of aeration

Isolate B5 showed 61% of RB222 decolorization under static conditions within 24 h while its decolorization efficiency was greatly reduced to 16% under shaking conditions (Fig. 2).

Kocuria rosea (MTCC 1532) and *Kocuria rhizophila* were reported enhanced decolorize of methyl orange, persian blue and reactive orange 13 under static condition compared to shaking conditions. This kind of response is due to an increase in dissolved oxygen in media with shaking that can inhibit dye reduction catalyzed by azo reductase and hence aeration of culture was avoided in rest of the experiments. Many researchers had also reported similar results emphasizing that dye degradation happens at its best under anaerobic conditions presence of excess oxygen prevents the liberation of electrons for breaking the azo bond in the dye (18, 19).

Effect of temperature

Temperature can significantly influence microbial activity such as its growth, metabolism and bioremediation of xenobiotic compounds and hence considered to be key physiological factor. The isolate B5 decolorization was monitored over a temperature range of 15-55º C. There was gradual increase in decolorization with increase in temperature from 15 to 35º C which was recorded from 5 to 59% at dye concentration of 100 mg L^{-1}. Further raise in incubation temperature from 45 to 55º C had resulted in decline of dye decolorization from 19 to 5% (Fig 2). The results obtained was due to decline in cell viability and the enzymes get denatured. At low temperature transporter channels on microbial cell membrane will shut down and also results in freezing of microbial cell content (20).

Biological decolorization of dyes catalysed by microbes require optimal temperature as they reach to maximum biomass. Majorly of the microbial enzymes associated with dye degradation performs its best at its optimal temperature with excellent metabolic turnover. A pattern was observed in microbial decolorization, with raise in incubation temperature there was increase in the percentage of decolorization up to certain temperature (optimal range) beyond which dye decolorization drastically reduce. Results obtained in this study were in agreement with

previous studies as dye decolorization was very efficient by bacterium Similarly, bacterium *Kocuria kristinae* RC3 could also potentially degrade amido black 10B at 37ºC with incubation period of 24 h (21).

Effect of pH

Different batch static experiment was conducted to examine effect of pH ranging from 4 to 9 on dye decolorization. Optimal dye decolorization was recorded at pH 6-7. The efficiency drastically reduced when the pH of medium was acidic and basic. This is due to difference in transport of dye across the cell membrane with pH variation. RB222 decolorization by B5 was observed to be 61% at pH7 within 24 h (Fig. 2). This confirms bacterial growth, and its enzyme activities are optimal at neutral. *Bacillus albus* MW407057 also showed similar kind of results in methylene blue dye degradation which was reported to be high (98%) at pH 7 and reduced below and above this neutral pH (22). This can be attributed to biological activity of bacterial enzymes to be best at this pH while deviation from this had hampered its efficiency in dye degradation. These reactive azo dyes also get protonated at acidic pH resulting in structural variation that hampers its uptake and decolorization by bacterial isolated which is considered to be the important rate limiting parameter in decolorization (23). *Kocuria indica* DP-K7 degraded methyl red a pH7 effectively *Kocuria rosea* MTCC 1532 was able to optimally degrade methyl orange at pH6.8 (9, 24).

Effect of co-substrate

Static batch fermentation with varying carbon and nitrogen and was set to evaluate the decolorization of RB222 by B5. Significant difference in decolorization percentage was observed with change in carbon and nitrogen sources. Among the tested carbon sources (Glucose, Sucrose, Lactose, Fructose, Starch) lactose was found to be the ideal carbon source with highest percentage of RB222 dye decolorization recorded as 72% within 24 h (Fig. 2). Similarly, among the nitrogen sources (peptone, tryptone, yeast extract, casein, urea, ammonium sulphate) examined. Yeast extract complemented the microbial degradation which was recorded as 76% within 24 h while urea has inhibited microbial decolorization. Comparatively yeast extract was found to be better co substrate in the metabolism of the dye. Yeast extract is nutritionally complex not only acts as nitrogen source but also the carbon source (25). Generally azo dyes contain less of carbon and hence utilising them as sole nutritional component becomes challenging for microorganisms. Metabolism of lactose and yeast extract generates NADH as by product which in turn serves as electron donor in azo dye degradation.

This kind of results were previously reported with *Kocuria rosea* (MTCC 1532) towards decolorization of methyl orange in presence of yeast extract as co substrate. Studies emphasised the co-substrate metabolism by aerobic process will result in depletion of oxygen encouraging anaerobic degradation of azo dyes (24, 26).

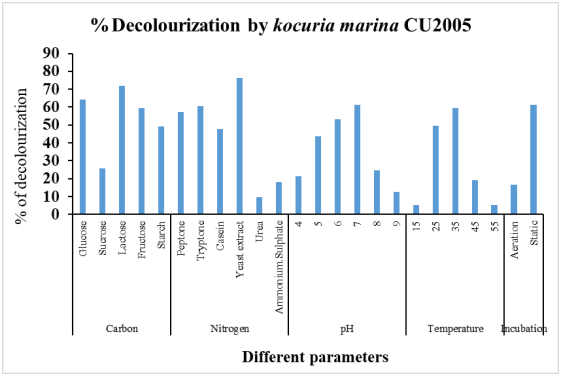


Figure 2 Decolorization of Reactive Blue 222 by Kucoria marina CU2005 at different physical and chemical parameters

Effect of dye concentration and complexity

Initial dye concentration of 100-1000 ppm was considered for the study. Microbial dye decolorization efficiency gradually reduced

with increase in dye concentration. B5 reported maximum percentage of decolorization in MSM with 100 mg L^{-1} and least efficiency was recorded at 1000 mg L^{-1}. Results obtained were 62%, 52%, 39%. 22%, 10% at the dye concentration of 100 ml L^{-1}, 250 mg L^{-1}, 500 mg L^{-1}, 750 mg L^{-1} and 1000 mg L^{-1} respectively (Fig. 3). Microbial decolorization was found to be inversely proportional to dye concentration. Studies have emphasized that initial concentration of dye can affect the rate of decolorization that can vary with respect to microbial species. Research in this aspect had revealed that increased dye concentration can inhibit dye decolorization which is attributed to the toxic effect of dye on the growth and metabolism of microorganisms. Further the enzymatic dye degradation mediated by azoreductase, laccase and peroxidases activities drastically reduce as active sites of these enzymes are saturated resulting in decrease in dye degradation (27).

Another important parameter associated with dye that can greatly influence dye decolorization is the chemical nature of the dye. Complex dyes with higher molecular weight are resistant to bioremediation and the rate of degradation is low compared to simple and low molecular weight dyes (28) . RB222, being a diazo dye with electron withdrawing group SO3H as aromatic ring substituent can significantly inhibit enzymatic oxidation of dye (29). RB222 dyes with reactive group sulfonic acid (SO3H) attached to aromatic ring resist microbial

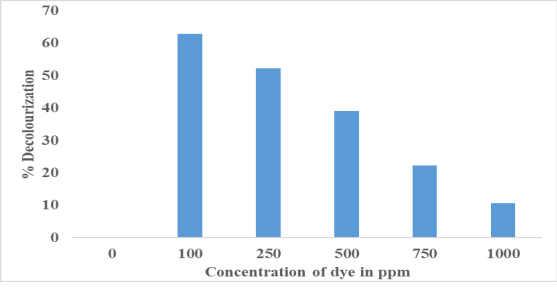


Figure 3. Decolorization of Reactive Blue 222 by Kucoria marina CU2005 at different dye concentrations

Reactive blue 222 decolorization at optimal conditions and growth kinetics

From the above studies optimized temperature, pH, aeration, initial dye concentration, carbon and nitrogen sources had enabled effective dye decolorization. Batch fermentation with 100 mg L^{-1} RB222 in MSM media supplemented with 1% lactose and yeast extract at pH 7 maintained at 35ºC recorded the microbial decolorization as 82% after 24 h. This was the optimal decolorization of RB222 by isolate. Growth of the isolate B5 and dye decolorization was recorded. Cell density was measured at 600 nm and the RB222 decolorization was read at its absorbance maxima 615nm at regular time interval (Fig.4) Microbial growth and RB222 decolorization were the function of time which increased with time. The exponential growth of B5 was observed as the MSM media with lactose and yeast extract as supplements that had supported the microbial growth and dye metabolism (23). The microbial cell pellet obtained after centrifugation didn't retain any dye color indicating the process was not biosorption and it was biodegradation. Further in this study FTIR, HPLC and GC-MS were used to confirm dye degradation.

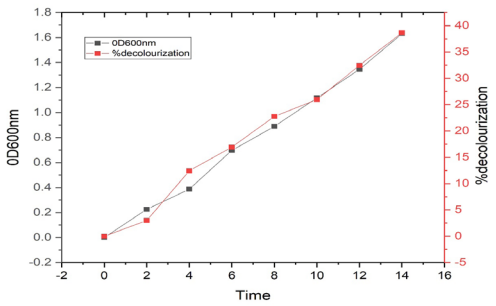


Figure 4. Growth of kinetics of dye degradation by Kucoria marina CU2005 at pH7 and 35ºC

Dye degradation studies

FTIR spectrum of RB222 dye degraded metabolite obtained after microbial degradation was compared with original dye.

The metabolites obtained after 48 h(A) and 72 h(B) were subjected to FTIR analysis to monitor the progress in biological degradation of RB222. There was a significant variation indicating dye degradation (Fig. 5). Original dye spectrum (C) showed a significant 1610 cm^{-1} peaks in the fingerprint region giving us clear indication of the functional groups. 3400 cm^{-1} for O-H stretching of phenol, 1550-1610 cm^{-1} indicating azo (N=N) group, 1200-1000 cm^{-1} shows alkane C-H wagging and range from 900-500 shows alkene C-H bending. Dye metabolite recorded at two different time intervals (48 &72 h) showed appearance of new peaks 2920 and 2850 cm^{-1} for C-H stretching of alkanes, 1580, 1560 cm^{-1} representing aromatic ring, 1390 cm^{-1} indicating the generation of aromatic amine and 620 cm^{-1} for monosubstituted benzene ring as intermediates of dye degradation. Absence of peak 3400 cm^{-1} and 1610 cm^{-1} indicates phenolic compounds and azo bonds degradation. This change in molecular structure of the starting material during the progress of biodegradation of dye was confirmed. Biodegradation of reactive green by *Marasmius* sp. BBKAV79 was also studied using FTIR (30).

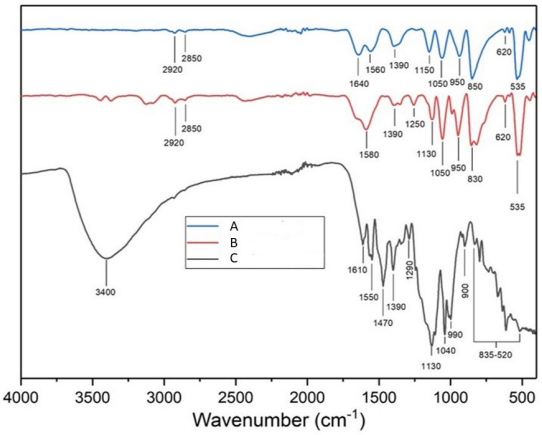


Figure 5. FTIR spectrum of dye metabolites

A-72 h after microbial degradation, B- 48 h after microbial degradation and C-Parental dye(RB222)

HPLC analysis for RB222 was studied at its absorbance maxima 615nm which showed a sharp peak corresponding to undegraded dye (Fig. 6a) and no peaks at 280nm (Fig. 6b). Further the extract of dye metabolite after degradation when examined at 615nm did not show any peak indicating complete degradation of dye to intermediate metabolites that were not chromogenic, to identify these non-chromogenic intermediates produced metabolites were examined under UV detector at 280nm the chromatogram showed multiple peaks with different retention time was 0.6, 5 and 11 mins (Fig.6c). While the parental RB222 when examined at 280 nm chromatogram showed no peaks (Fig6b). This emphasises that new peaks were produced were due to microbial degradation by *Kucoria marina* CU2005.

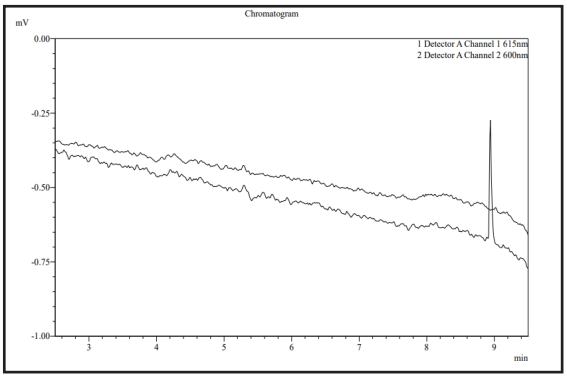


Figure 6a. HPLC chromatogram of RB222 at 615nm.

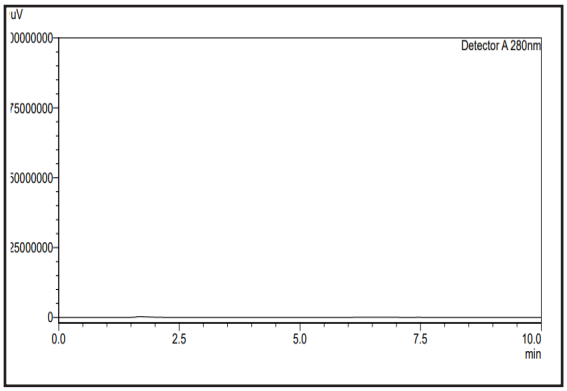


Figure 6b. HPLC chromatogram of RB222(Parental dye) at 280nm

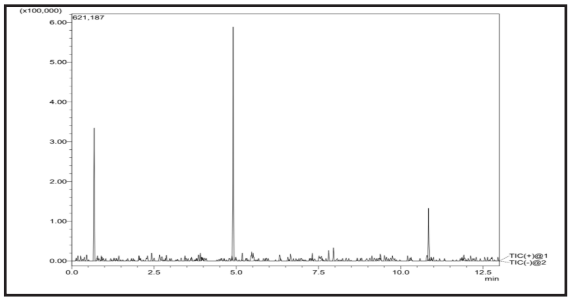


Figure 6c. HPLC chromatogram of RB222 dye intermediate metabolites (Nonchromogenic) at 280nm

GC-MS studies were performed to identify the metabolic intermediates of RB222 obtained by microbial degradation. GC-MS spectrum showed several peaks indicating partial mineralization of RB222 (Fig 7). Initially the azo bond is broken down producing aromatic amines which is further metabolized by the isolate under static conditions. Based on the GC-MS chromatogram data the intermediates produced were identified and the degradation pathway was proposed (Fig. 7). The azo bond of RB222 is degraded by *Kucoria marina* CU2005 to three compounds of lower molecular weight. Fragmentation of RB222 was predicted based on the m/z values of intermediate compounds (31, 32). The intermediate metabolites identified were CI; 2-chloro-1, 3, 5-trazine, naphthalene-2-amine (M.W-303), CII; 3,4-diamino-5-hydroxynaphthalene-2,7 disulfonic acid (M.W-334), CIII; 3-amino-5-4-chloro(6-(4(-(sulfooxy) ethyl)sulfonyl)phenyl)amino)-1.3.5 triazin-2-yl) amino) benzenesulfon -ic acid (M.W-580). These intermediates further undergo chemical transformation by to C-N bond cleavage and desulfonation producing compounds such as CIV; Napthalen-2-amine (M.W-143), CV; 7,8-diaminonapthalen-1-ol(M.W-174), and CVI; 3-amino 5-((4-amino-6-ch;oro-1,3,5-triazin-2-yl)amino)benzenesulfonic acid (M.W-316), CVI is metabolized to 6-chloro N^2,N^4-diphenyl-1.3.5-triazine-2,4 –diamine (M.W-297). Further desulfonation of CI and CII yields naphthalene-2-amine (M.W- 143) and 7, 8 –diaminonapthalen-1-ol (M.W-174). Deamination and ring opening of CIV yields 2-chloro-1, 2, 3-triazine (M.W-115) and aniline (M.W-93). Parental dye was mineralized to give new products by microbial degradation and the products obtained were of lower molecular weight and low toxicity compared to parental dye of high molecular weight.

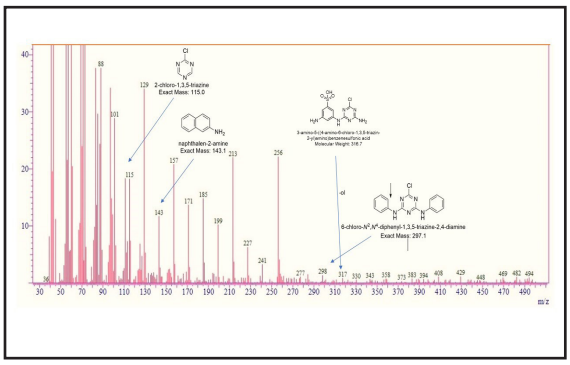


Figure 7. Gas chromatography and Mass spectroscopy of RB222 dye intermediates by Kucoria marina CU2005

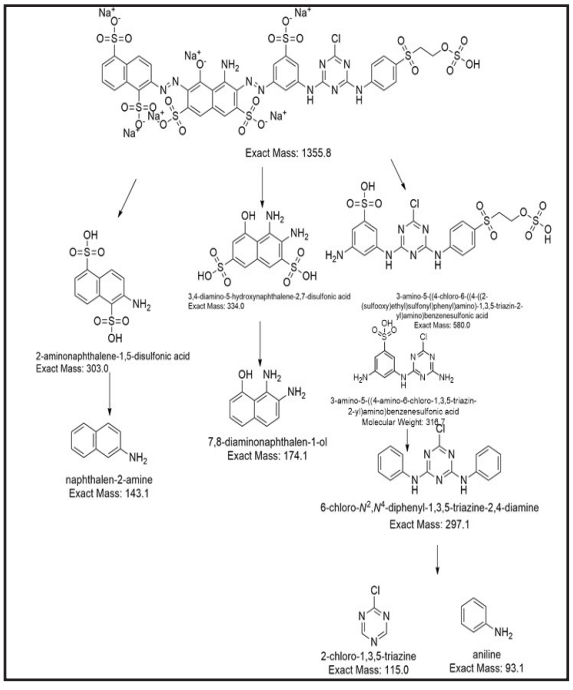


Figure 7. Proposed RB222 dye Biodegradation pathway by Kucoria marina CU2005

Conclusion

Novel bacteria isolate *Kucoria marina* CU2005 isolated from industrial waste exhibited a potential to decolorize and degrade RB222. Optimizing the crucial physical and chemical factor such as pH, temperature and co substrate had enhanced microbial degradation recorded as 82%. One of the significant features of the isolate was its ability to with stand higher concentration of dye up to 1000 mg L^{-1} . These emphases the application of the isolate in industrial effluent treatment. The co metabolism, biodegradation and cell growth studies confirm the active participation of the isolate in dye degradation with generation of dye intermediates identified by HPLC and GC-MS.

Conflict of Interest: Authors declare no conflict of interest

Authors contribution:

All the authors of the manuscript titled "Bio-decolorization and degradation of reactive Blue 222 by a novel isolate *Kucoria marina* CU2005" have contributed in one way or the other towards the work. First and third authors were instrumental in conceptualization of idea and analysis, second author assisted in writing and review of the manuscript, third and fourth authors were involved in supervision, validation, and project administration**.**

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