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Bioremediation of Azo dye methyl red using Enterobacter cloacae (GIDC p_1)

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Abstract

Surat is the city where maximum textile industries have been found and it represents flourishing textile industries in State of Gujarat. It occupies a very important place in Indian economy in terms of its share and value added and export earnings. Wastewater discharged from these industries contains a variety of polluting substances and the most common and undesirable is dye. Presence of even < 1 ppm dye is harmful to aquatic and terrestrial life so their removal is the alarming point. Usually the removal of dye can be carried out using physical, chemical, and biological methods. But biological methods are quite impactful. Many studies have reported that the microorganisms are powerful biocatalyst for removal of dye containing wastewater. Present study is based on the biodegradation of Azo dye i.e. Methyl red by the isolate Enterobacter cloacae which was isolated from the textile effluent of Sachin GIDC Surat. These strains was identified by molecular and biochemical methods. Degradation was studied with respect to various factors such as pH, temperature, static and shaking conditions, and different carbohydrate and nitrogen source. Results from this research study showed that dye was significantly degraded around 90 to 95% at the optimal factors i.e. pH 7, Temperature 30℃ in static condition and with the carbohydrate source glucose and nitrogen source as urea. These data indicates a unique characteristic of the strain useful for the degradation.

Keywords: Textile Effluent, Degradation, Methyl Red, Azo dye

Introduction

Environment pollution has been recognised as one of the major problem in the world. As of population increases the need of water. Environmental biotechnology is expanding in tine treatment of textile effluent released by the textile industries using biological methodsof removal system.

Bacteria, Fungi, Actinomycetes are most commonly used for the treatment of textile effluent and decolourisation method. Because of the omnipresent property of different and numerous bacterial Spps make them important for the treatment of this industrial waste water. They can decolorize textile dyes and only few are able to mineralize those compounds into carbon dioxide and water. Therefore scrutinizing the bacterial community from the dye enriched environment is the most important and tedious job for discovering the novel biocatalyst and to isolate the decolourising potential species a screening method is required.

Industrial waste products and effluents are considered as undesirable by product for economic development and technology. When these waste products are not handled properly and not disposed properly lead to harmful effects to both human and aquatic life as well as adverse effect on the environment. Exposure to these harmful textile effluent leads to health effects such as Headache, Nausea, Lung and skin irritation leads to serious ailments like congenital malformations.

Pollution of water is at present one of the major questionable activity. Colour is one of the most obvious indicator of water pollution and they discharge highly coloured synthetic dye effluent can be damaging to the receiving water bodies.

Azo dyes are largest commercials dyes available and most widely used in textiles, foods, cosmetics, plastic laboratory, leather, paper, printing, colour photography(M.A,Syed et al 2009). Because of this widespread application in textile and dyestuff industries. And because of its application the removal of azo dyes mostly physical and chemical methods. But the biggest disadvantages are that they are cost prohibitive and often lead to extra solid wastes. So, the biological methods are considered to be the best methods for removal of azo dye degradation.

Due to maximum use of these dyes in these industries, so they are the main source of textile effluent. There is around 4,50,000 tons dyes been produced annually and worldwide and > 11% is lost in processing and application.

The present study shows that the strain isolated from the textile effluent has the highest degradation capacity and it can be quite useful for the further research purposes. From the literature review and study it has been found that the strain isolated from the effluent was not been studied previously.

Materials and methods

Collection of Textile effluent

Textile effluents were collected from effluents site as a source of bacteria from Sachin G.I.D.C., Surat, and Gujarat, India. It was collected in sterile plastic container.

Reagents

Azo dye is used in the study. Dye was commercially graded and supplied by the dealer of "HI MEDIA" India. Methyl Red azo dye is used in the study.

Isolation of Dye Decolourising and Degrading Bacteria

A 10% v/v enrichment bHM broth was transferred into 250 ml flask containing 100ml BHM broth with respective dye and incubated for 24 hours at R.T. under static condition. Strains capable of utilizing fresh dyes as a nutrient source were consider and repeatedly inoculated using 0.1% inoculum in a fresh BHM broth containing azo dye and were monitored visually during and after 24 to 48 hours. Strains showing high decolourising potential and give reproducible results with chosen and selected for further studies.

Partial identification of isolates

The loopful of rowth obtained after incubation was streaked on BHM dye agar plates were incubated at R.T. for 24 hours and morphology by the colonies obtained after incubation was studied. Partial identification of isolates was carried out by 16srRNA sequence. Growth of the pure culture obtained i.e. *Enterobacter cloacae* was maintained on BHM medium at ambient temperature.

Dye decolourising assays

The flask containing BHM medium with dye was inoculated using 0.1% v/v inoculum of isolate bacterial suspension. The decolourisation was determined by

measuring the difference between initial and final optical density at specific nm.

The % of decolourisation was calculated as:-

% of decolourisation = Initial observation– Observed observation/Initial observation x 100.

Effect of various physiochemical parameters on dye decolourisation degradation

1. **Dye decolourisation under static and shaking condition**

   Decolourisation and degradation of dye was studied under 2 different condition i.e. Static and Shaking to find out the mode of degradation. Influence of shaking was studied on a rotatory shaker 120 rpm at room temperature for stationary/ static condition experiment was performed without agitation and simple incubator was used for carrying out an experiment.

2. **Effect Of pH**

   To study the effect of pH on dye decolourisation and degradation process modified BHM basal medium was used along with dye at different pH i.e.5, 7 and 9. pH of the medium was adjusted with 1N HCl and 1N NaOH. The flask were inoculated with the *Enterobacter cloacae* and incubated at R.T. under static condition and observed for mode of degradation and decolourisation.

3. **Effect Of different Temperature**

   To study the effect of different temperature on decolourisation and degradation process, modified basal BHM medium was use along with the Methyl red dye and different temperature i.e. at 25°C, 30° C and 35° C at desired pH under static condition to observe the pattern of decolourisation.

4. **Effect of Carbon Source**

   Modified basal BHM medium was inoculated with the inoculum of the *Enterobacter cloacae* was used for the various co-substrates i.e. Glucose, Lactose and Sucrose as a carbon source. In addition control flasks were also kept only dyes and media and without inoculum to observe the decolourisation and degradation if any.

5. **Effect of Nitrogen Sources**

   To study the effect of different Nitrogen source on degradation and decolourisation process. Modified basal BHM medium was inoculated with various co substrates urea, yeast extract and peptone to observe the effect of decolourisation and degradation process.

   At different time intervals the samples were withdrawn from the flask and centrifuged at 5000 rpm for 10 to 15 minutes to precipitate suspended biomass. And the concentration of the dye in the supernatant was determined by reading wavelength. Absorbance was compared with the control medium.

Results and discussion

1. **Isolation screening and identification of dye degrading bacteria**

   The sample was collected from textile dye industry site at Surat Sachin GIDC Gujarat India. Textile industries have shown increase in yarn production and output of fabric materials. Its clearly observed that the textile industry play an important role in economical position. (M. Ponraj et al 2011).

   Effluent was collected from the disposal site so that the screening and isolation of dye degrading bacteria would increase. And these industries are major sources of soil and water pollution. Large amount of dye containing water is discharge by these textile units and these leads to water pollution problem.

Isolation was carried out on Modified Bushnell Hass medium containing methyl red dye at 100 mg/l. 0.5ml of enriched sample was spread on BH Agar medium with respect to respective dye and plates were incubated at 30˚C for 24 hours. Isolated organisms were preferred for screening. 1˙ Screening was done on basis of zone of decolourisation. And these screening was tested for decolourisation on the sterile modified Bushnell Hass medium.

5ml of sample was removed from each enriched medium and observed for the measurement of decolourisation on Shimadzu Spectrophotometer. Decolourisation was observed at the interval of every 6 hours at 520 nm. GIDC P1 was one of the isolate been isolated from the textile effluent and studied further.

The bacteria culture was identified by microscopic, biochemical characteristics and as well as by molecular biology testing and identified result was Enterobacter cloacae.

Table 1: Modified Bushnell Hass medium.

Components	Weight g/l
$MgSO_4$	0.2
$CaCl_2$	0.02
KH_2PO_4	1.0
K_2HPO_4	1.0
NH_4NO_3	1.0
$FeCl_3$	0.05
Dextrose	0.50
Yeast Extract	0.50

2. Partial Identification Of the isolate

Strain GIDC P1 with strong degradation and decolourisation ability with Azo dye Methyl red was isolated. Significant decolourisation and degradation was observed after 24 hour incubation and maximum value was observed within 30 hours of incubation.

So, for the identification of the novel organism for its great characteristics of degradation process based on the molecular biological techniques the given organism was identified as *Enterobacter cloacae.*

Table 2: Biochemical results of GIDC P1.

Test	Results
Gram reaction	Gram negative rods shape
Arrangement	Single
Motility	Motile
Glucose	A
Lactose	A/G
Sucrose	A
Maltose	A
MR	-Ve
VP	+Ve
Citrate	+Ve
Catalase	+Ve

A = Acid, A/G = Acid And Gas, +Ve = Positive , -Ve = Negative

Effect of Various Physiochemical Parameters

1. Effect of static and shaking condition on dye degradation and decolourisation:

Degradation and decolourisation of azo dye methyl red was found to be more in the static condition then in the shaking condition. According to the literature review (*Hwang et al 2009)* they found that the *citrobacter spps* was found to degrade more effectively in static condition. Oxygen was favourable to the growth of bacteria but decrease the yield of degradation and decolourisation process. But the study of previous literature shows that the strain GIDC P1 (*Enterobacter cloacae)* was not been studied much.so it found to be the novel organism. And the appropriate condition for its characteristics was Static condition which showed the maximum effect on the degradation and decolourisation process.

The sequence is as under

Length: 1200 basepairs:

NNNNNNTANNNNNNCNGNAANATGCAAGTCGNNCGGTAGCACAGAGAGCTTGCTCTCGGGTGACGAG
TGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAG
GGGGATAACTACTGGAAACGGTAGCTAATACCGCATAATGTCGCAAGACCAAAGAGGGGGACCTTCG
GGCCTCTTGCCATCAGATGTGCCCAGATGGGATTAGCTAGTAGGG
GGGGTAACGGCTCACCTAGGCGACAATCCCTAGCTGGTCTGAGAGGATGACCAGCCCCACTGGAACTG
AGACACGGTCCAGACTCCTACGGGAGGCAGCGGGGGGGAATAT
TGCACAATGGGCGCAAGCCTGATGCACCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTA
CTTTCAGCGGGGAGGAAGGTGTTGTGGTTAATAACCCCAGCAATT
GACGTTCCCCGCAAAAAAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAA
GCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTG
TCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCAAAACTGGCAGGCTGGAGTCTT
GTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTANA
GATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCG
TGGGGAGCAAACAGGATTATATACNCCTGGTAGTCCACGCCGTA
AACGATGTCGATTTGGAGGTTGTGCCCTTGAGGCGTNCTTCCGGAGCTAACGCGTNAATCGACCGCCT
GGGGGAGTACGGCCGCNAGGTTAAAANTCANATGAANTGACGG
GGGGCCCGCACNACGCGGTGNANNATGTGNTTTTATTTNGNNGCNNNANNAGANCNTTACCTGGTCNT
GACNNCNCANNANTNNCANANATGNATTGGTGCCTTCTGNAC
TATGAAACAGGTGCTGCATGGNTGTCNTCNGCTCGTNTGTGAAATGNTGGATNNTNCCNCNANNNNCG
CACCNTNTTCNTTNTGCNNNNGTAGGCNGGNNNNNNNNNAN
TNNNGNNNACTGANANGNGGGANNNNNCANNNNNNTNNNNNNNNNGGGNNNNNNNTNNNAANGNN
NTANNNANNNNNACNCGCNNANNNA

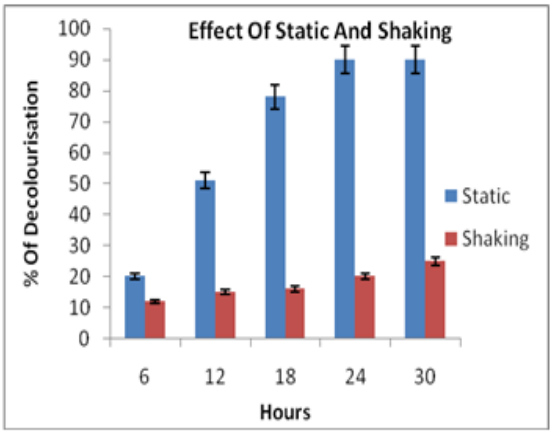
Sequence analysis of 16sr RNA sequence of GIDCP1 strain had highest similarity with the species *Enterobacter cloacae* (99%).

2. Effect Of pH:

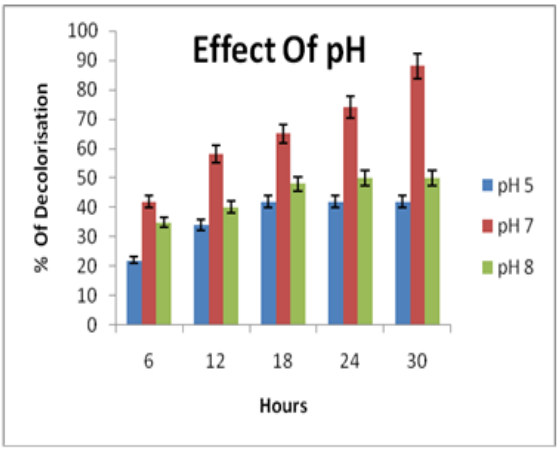
The best decolourisation was achieved at pH 7.0 within 24 to 30 hours of incubation. This could be because of the fact that the optimum pH for the growth of *Enterobacter spps* is neutral. The rate of colour removal was quite less at acidic condition, pH tolerance of dye degradation and decolourisation is quite important because azo dye bind to cotton fibres by addition or substitution under alkaline condition (*Hwang et al 2009).*

There are some species which shows the negative effect on dye decolourisation at pH range 7 to 9. But the spps shows the positive impact on degradation and decolourisation between 5to 7 using dye Remazol Black B (*Shahid Mahmood et al 2011).* While literature review also showed that the *P. putida* have an excellent degradation capacity at pH 7.0. Showing 37.5% degradation within 10 hours (*Bhatt et al 2012).*

Similarly *B. cereus* too showed decolourisation of two different azo dyes at neutral pH. So from the research it can be said that the isolates strain GIDC P1 i.e. *E. cloacae* favourable pH is 7 and it varies according to different species and organisms.



Graph 1: Effect of Static and Shaking Condition.



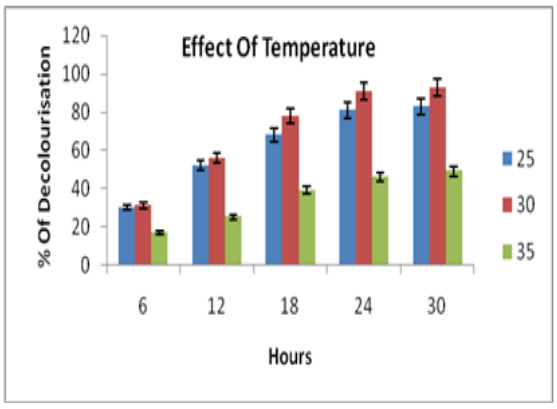
Graph 2: Effect of pH.

3. Effect of Temperature:

E. cloacae showed strong decolourisation and degradation at 30 C. 93% degradation was carried out at 30 C in 30 hours. While at the temperature 25 C don't so much degradation. While at 35 C the degradation and decolourisation was found to be 49% only.

Literature studies showed that Citrobacter spps showed good degradation at the temperature range between 27 to 30 C (Hwang et al 2009) while B.subtilis shows degradation at thetemperature range of 45 C (G.milliki et al 2012) because of its ability to survive at high temperature and become spores.

While for the azo dye Remazol Black B the strains isolated from the effluent so the better degradation capacity at 25 C (Shahid Mahmood et al 2011).



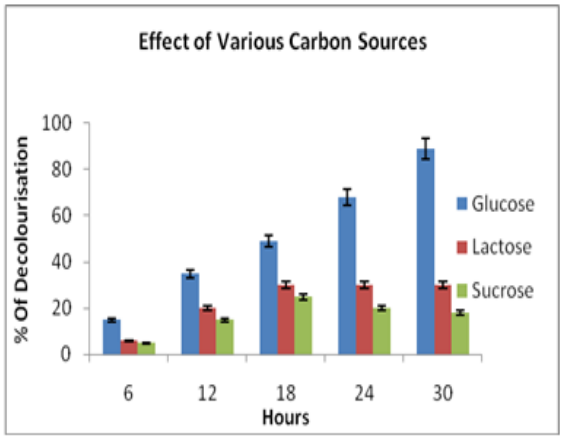
Graph 3: Effect of Temperature.

4. Effect of Carbon sources:

Three different carbon sources are Glucose, Lactose, and Sucrose were utilized for the observation of 3 different carbon sources on the dye decolourisation and degradation. All the above 3 carbon sources were inoculated in 1% w/v. After incubation it was observed that the glucose gave the best degradation. After 24 hours it showed around 68%degradationwhile at the end of 30 hours it showed the 89% of degradation.

While the other 2 sugars Lactose and Sucrose did not so much degradation. From the different literatures referred glucose was found to be the optimum carbon sources for the growth and it also showed a positive effect on the degradation and decolourisation process.

Starch was found to be another carbon source which was been used by the Bacillus spps. For the growth and showed positive effect on growth and degradation process. (G Milliki et al 2012).



Graph 4: Effect of carbon sources.

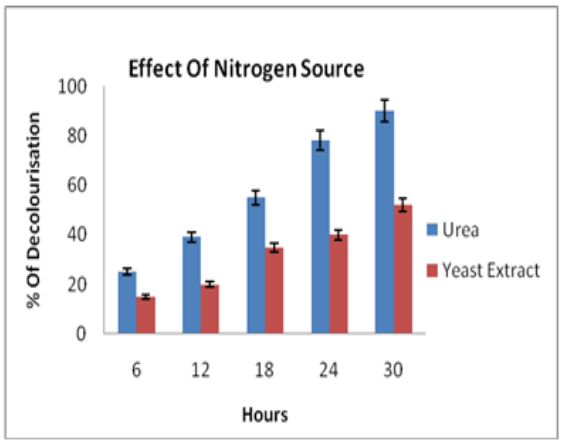
5. Effect of Nitrogen sources:

Two different nitrogen sources were been for the observation or effect on the degradation and decolourisation process. i.e. Urea and yeast extract. Using urea as a nitrogen source showed the degradation 90% in 30 hours. While using yeast extract as a nitrogen source showed only 52% degradation at the end of 30 hours.

Different nitrogen sources rather than yeast extract and urea are beef extract, peptone, tryptone. According to the different Organisms the nitrogen also varies and also its effect that we can see in our results above.

Many bacteria such as Bacillus, Klebseilla, Salmonella gave the best result using beef extract but the results and effect varies according to the species (M. Ponraj et al 2011).

So for the E.cloacae the given strain urea found to beoptimum as a nitrogen source for degradation point of view.



Graph 5: Effect of Nitrogen source.

Conclusion

Textile dye Methyl Red one of the Azo dye is degraded under static conditions with a coordinated effect of bacteria which was isolated from the textile dye effluent. The strain GIDCP1 was isolated from the textile effluent and was identified using various biochemical and molecular identification technique as *Enterobacter cloacae.*

Various factors were studied to see the effect of various physiochemical effect on dye degradation process and it was found that it gave better degradation with the factors of Static condition, pH-7.0,Temperature 30˚C, Carbon source as Glucose and Nitrogen Source as Urea. It gave 95 to 98% degradation and decolourisation in 30 hours of the incubation period.

Competing interests

The authors declare that they have no competing interests.

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