Abstract

Introduction

Azo dyes are released into wastewater streams without any pretreatment and pollute water and soil environments. To prevent contamination of our vulnerable resources, removal of these dye pollutants is of great importance. For this purpose, wastewater samples were collected from dye-contaminated sites of Ankleshwar, Gujarat, India. The aim of this study is to discuss a selection of bacterial strains efficient in decolourisation of Remazol Black-B.

Materials and methods

About 50 bacterial isolates were isolated through enrichment and then tested for their potential to remove Remazol Black-B azo dye in liquid medium. Three bacterial isolates capable of degrading Remazol Black-B azo dye efficiently were screened through experimentation on modified mineral salt medium.

Results

Isolate ETL-A was able to completely remove the Remazol Black-B dye from the liquid medium in 18 hours. Furthermore, the isolate showed the best performance at the dye concentration of 100 mg L\(^{-1}\) medium (pH 7) and at temperature 35 °C. Similarly, yeast extract proved to be the best carbon source for decolourisation purposes.

Conclusion

The results imply that the isolate ETL-A could be used for the removal of the reactive dyes from textile effluents.

Introduction

Due to rapid industrialisation, a lot of chemicals including dyes are manufactured and used in day to day life\(^1\). Synthetic dyes are extensively used in textile, dyeing, paper printing, colour photography, food, cosmetics and other industries. Approximately 10 000 different dyes and pigments are used industrially and over 0.7 million tons of synthetic dyes are produced annually, worldwide\(^2\). Explosion of population coupled with industrial revolution results in pollution of water, air and soil. The discharge of pollutants from various industries poses a threat to the biodiversity of the earth. The textile finishing generates a large amount of dyes containing wastewater from dyeing and subsequent steps that forms one of the largest contributions to water pollution\(^3\). The traditional textile finishing industry consumes about 100 litres of water to process about 1 kg of textile material. The new closed-loop technologies such as the reuse of microbially or enzymatically treated dyeing effluents could help in reducing this enormous amount of water consumption\(^4\). It was already reported that 10%-15% of dyes are lost in the effluent during the dyeing process\(^5\). Azo dyes have been used increasingly in industries because of their ease and cost effectiveness in synthesis compared to natural dyes. However, most azo dyes are toxic, carcinogenic and mutagenic\(^6\). Azo bonds present in azo dyes are resistant to breakdown, with the potential for the persistence and accumulation of high levels of dye in the environment\(^7\). These dyes cannot be easily degraded, while some are toxic to higher animals\(^8\). Azo dyes are very stable in acidic and alkaline conditions and are resistant to temperature and light. However, they can be degraded by bacteria under aerobic and anaerobic conditions\(^9\). Azo dyes are environment pollutants\(^10\) and they contribute to the mutagenic activity of ground and surface water that are polluted by textile effluents\(^11,12\). The complex aromatic substituted structures make conjugated systems and are responsible for intense colour and high water solubility\(^13\). Their discharge into surface water also leads to aesthetic problems, obstructing light penetration and oxygen transfer in to water bodies\(^14,15\). Several physicochemical techniques have been proposed for treatment of coloured textile effluents. These include adsorption on different materials, oxidation and precipitation by Fenton’s reagent, bleaching with chloride or ozone, photo degradation or membrane filtration\(^16\). The economic and safe removal of the polluting dyes is still an important issue. Because all these physicochemical methods are very expensive and result in the production of large amount of sludge, they create the secondary level of land pollution. In this situation bioremediation is becoming important, because it is cost-effective, environmentally friendly and produces less sludge\(^17\). Therefore, in such situations, biological treatment may be a real hope. These methods have the advantages of being environment friendly. Microorganisms have developed enzyme systems for the decolourisation and mineralisation of azo dyes under certain environmental conditions\(^18-20\). So, this study was designed to isolate efficient azo dye decolourising bacterial strains from the textile effluents. Since the...
bacterial isolates were originated from the dye-contaminated textile wastewater of local industry, they can easily adapt to the prevailing local environment. Therefore, such bacteria can be used to develop an effective biological treatment system for the wastewaters contaminated with azo dyes. This study discusses some bacterial strains efficient in decolourisation of Remazol Black-B.

**Materials and methods**

The protocol of this study has been approved by the relevant ethical committee related to our institution in which it was performed.

**Sampling**

Water and sludge samples were collected from Ankleshwar Industrial Estate, Ankleshwar, Gujarat, India around which many textile processing units are situated. Samples were taken from drains at different locations and sampling sites were selected on the basis of the allocation of outlet from textile units. Electrical conductivity and pH were determined to assess the presence of total suspended solids and acidity or alkalinity of the collected samples (Table 1).

**Isolation of azo dye decolourising bacteria**

Bacterial strains were isolated from wastewater and sludge samples of dye industry units. Isolates from each inoculum source were first enriched using MSM medium amended with an azo dye Remazol Black-B as the sole source of C and N. Dye was added at a concentration of 150 mg L⁻¹. The cultures containing 200 mL of MSM broth with dye in 500 mL Erlenmeyer flasks were inoculated with 10 mL volume of wastewater or sludge suspensions. The flasks were incubated at 32°C for seven days under static conditions. After incubation, cell suspensions from each flask were plated onto MSM agar medium and incubated at 32°C for 24 hours. Microbial colonies that appeared on the agar medium were washed gently with sterile water and resuspended into the flasks containing fresh MSM broth spiked with the Remazol Black-B dye. About 50 actively growing colonies were selected for purification.

**Identification**

The most effective dye-decolourising bacterial strain ETL-A was identified by carbon source utilisation patterns using Biolog GN2 microplate (Biolog, USA) and the analysis of 16S rDNA sequences. For the 16S rDNA sequence analysis, bacterial genomic DNA was extracted and purified using a Wizard Genomic DNA Prep. Kit (Promega Corp., Madison, USA). Two primers annealing to the 5' and 3' end of the 16S rRNA gene were 5'-AGTTTGATCCTTGCGTCAG-3' (positions 9–27 (Escherichia coli 16S rDNA numbering)) and 5'-AGAAGGAGGTGATCCAGCC-3' (positions 1542–1525 (E. coli 16S rDNA numbering)), respectively. Polymerase chain reaction was performed as follows: predenaturation at 95°C for 5 minutes, 30 cycles at 95°C for 40 seconds, 55°C for 40 seconds and 72°C for 2 minutes. The polymerase chain reaction product was subcloned into pGEM-T easy vector (Promega, Madison, USA) and its nucleotide sequence was determined by Bangalore Genei Ltd. (Bangalore, India). The partial rDNA sequences were analysed using a BLAST search algorithm to estimate the degree of similarity to other rDNA sequences obtained from the NCBI/GenBank. Phylogenetic trees were constructed by the ClustalX program. Physiological characteristics were determined according to the procedures outlined in Bergey’s Manual of Determinative Bacteriology.

**Purification of bacterial isolates**

Selected isolates were purified by streaking on MSM medium containing agar at the concentration of 20 g L⁻¹. Streaking was done thrice in a zig-zag manner. The purified cultures were preserved in a refrigerator for subsequent study.

**Screening efficient azo dye decolourising bacterial isolates**

Screening was done to find out the efficient bacterial strains capable of decolourising the Remazol Black-B azo dye using modified MSM. For this purpose, 50 isolates that had the ability to decolourise Remazol Black-B from all samples were selected. After that the decolourisation ability of each isolate was tested in the liquid medium. Media inoculated with the respective inocula were inoculated at 35°C for 24 hours. After 24 hours, the respective cells were harvested by medium centrifugation at 10 000 rpm (REMI R-23, India) for 10 minutes. Then decolourisation was determined with the help of a spectrophotometer (SHIMADZU, Japan) at 597 nm. Uninoculated blanks were run to determine abiotic decolourisation. The three most effective bacterial isolates (ETL-A, ETL-B and ETL-C) from the final screening were further examined for their decolourisation.

---

**Table 1** Total soluble salts (TSS) and pH of the dye-contaminated textile effluent and sludge

| Sampling site               | TSS | pH | Notes |   |
|-----------------------------|-----|----|-------|---|
| Near K Patel dye unit       | 58  | 8.2| Effluent | |
| Near Dynamic dye unit       | 78  | 8.5| Effluent | |
| Near Harpal dye unit        | 152 | 10.3| Sludge | |
| Near Chemcrux unit          | 118 | 9.4| Effluent | |
| Near Suyog dye unit         | 98  | 8.7| Sludge | |

Licensee OA Publishing London 2013. Creative Commons Attribution License (CC-BY)

For citation purposes: Shah MP, Patel KA, Nair SS, Darji AM. Selection of bacterial strains efficient in decolourisation of Remazol Black-B. OA Biotechnology 2013 Mar 01;2(2):14.
potentials in test tubes at different time periods. Ten millilitres of the sterilized MSM broth containing Remazol Black-B at the concentration of 100 mg L\(^{-1}\) was added to autoclaved test tubes supplemented with 0.5% yeast extract as a co-substrate. The medium was inoculated with the respective bacterial strains by adding inocula of uniform cell density (OD: 0.6) at 597 nm. The test tubes were tightly sealed and incubated at 35°C under static conditions. Uninoculated test tubes with MSM containing azo dye plus yeast extract were incubated under similar conditions to check for abiotic decolourisation of dye. Decolourisation was measured after 6, 12, 18 and 24 hours at 597 nm by spectrophotometer as described\(^{20}\).

Optimisation of environmental factors for efficient decolourisation

Factors like substrate concentration, temperature and pH were optimised during the experimentation for different carbon sources (glucose, yeast extract, mannitol and maltose) at the concentration of 4 g L\(^{-1}\) were also tested as co-substrates in the decolourisation process. Optimisation studies included various concentrations of dye (50, 75, 100, 125, 150, 200 and 250 mg L\(^{-1}\)), pH values (5, 6, 7, 8, 9) and temperatures (25°C, 30°C, 35°C, 40°C, 45°C). All the bacterial isolates ETL-A, ETL-B and ETL-C were tested to optimise their decolourisation efficiency. While culture conditions were the same as used in the decolourisation experiment, that is, MSM was used along with the 100 mg L\(^{-1}\) of Remazol Black-B azo dye. Uninoculated blanks were run to check the abiotic decolourisation during the experimentation.

Statistical analysis

Data were entered in a Microsoft\textsuperscript{®} Excel 2007 spreadsheet.

Results

Efficiency of the bacterial isolates to decolourise Remazol Black-B was examined by measuring colour intensity in a liquid medium. Based upon the relative decolourisation efficiency of different isolates, three of the most efficient isolates (ETL-A, ETL-B and ETL-C) with more than 80% decolourising efficiency were selected for further experiments (data not shown).

Biodecolourisation of Remazol Black-B by selected bacterial isolates

Biodecolourisation of Remazol Black-B by the selective bacterial isolates (ETL-A, ETL-B and ETL-C) was confirmed by conducting another experiment in liquid medium at different time periods (Figure 1). It was found that different bacterial isolates had variable potential to remove Remazol Black-B in the growing cultures. The most efficient bacterial isolate to decolourise the Remazol Black-B was ETL-A with 98% colour removal efficiency in 18-hour incubation period while remaining isolates displayed maximum decolourisation in 24 hours. Isolate ETL-B was the second most efficient bacterial isolate and it decolourised the Remazol Black-B up to 94% in 24 hours. Similarly, ETL-C isolates had decolourisation potential of 80%.

Identification

Sequence analysis of 16S rDNA showed that the strain ETL-1982 had highest similarity with the species Bacillus spp. (96%) which has been proved to have decolourising ability against azo dyes. Based on the phenotypic characteristics and phylogenetic analysis, strain ETL-A was identified as Bacillus (Figure 2).

**Figure 1:** Biodecolourisation of Remazol Black-B.

**Figure 2:** Phylogram (neighbor-joining method) showing genetic relationship between strain ETL-A & other microorganisms based on the 16S rRNA gene sequence analysis.
Factors affecting biodecolourisation of Remazol Black-B in liquid medium

The potential of selected isolates (ETL-A, ETL-B and ETL-C) was further investigated for the optimisation of various incubation/environmental conditions for decolourising the azo dye in liquid medium. It was evident (Figure 3) that Remazol Black-B azo dye decolourisation sharply increased up to 100 mg L\(^{-1}\) of substrate concentration and maximum decolourisation was observed at 100 mg L\(^{-1}\) of substrate concentration. Then, there was a gradual decrease in the azo dye decolourisation. Isolate ETL-A was the most efficient azo dye decolourising strain with more or less complete removal of the colour, that is, 100% decolourisation at 100 mg L\(^{-1}\) and minimum decolourisation was recorded at 50 mg L\(^{-1}\) while after 100 mg L\(^{-1}\) substrate concentration, again ETL-A showed a decreasing trend. Isolate ETL-B was the second at the rank with 90% decolourisation at 100 mg L\(^{-1}\). But, ETL-C showed a different trend from the other isolates, it indicated enhanced decolourisation up to 200 mg L\(^{-1}\) (82%).

Types of carbon sources

Effects of different carbon sources such as maltose, mannitol, glucose and yeast extract were evaluated on Remazol Black-B decolourisation by bacterial isolates (Figure 4). It was found that maximum decolourisation occurred with 4% yeast extract in all selected strains (85%–95%) that was followed by glucose in which decolourisation occurred in the range of 20%–25%. However, least decolourisation was observed in the case of mannitol (10%–15%). Similarly, maltose application also showed decolourisation in the lower range (up to 18%).

Effect of pH

For studying effect of pH value, different levels of pH ranging from 5 to 9 were used and incubation of all selected isolates was done at these levels (Figure 5). Initially with the increase in pH value from 5 to 7, decolourisation increased and maximum decolourisation occurred at pH 7.

Similarly, further increase in pH from 7 to 9 had a negative effect on decolourisation capacity of various isolates. The maximum decolourisation was observed with the isolate ETL-A (98%) at pH 7, while minimum decolourisation occurred at pH 9. Similar trends in the remaining isolates ETL-B and ETL-C were observed at pH 7. Overall, it was noted that all the bacterial isolates showed optimum decolourisation from pH 5 to 7.

Effect of incubation temperature

Five levels (25°C, 30°C, 35°C, 40°C and 45°C) of temperature were used for assessing optimal biodecolourisation of Remazol Black-B by selected bacterial isolates. It is evident (Figure 6) that when the temperature was raised from 25°C to 35°C there was an inconsistent trend in decolourisation from the different isolates. The ETL-A and ETL-B isolates showed gradual increase in decolourisation, while one isolate ETL-C displayed maximum decolourisation at 25°C. The remaining two bacterial isolates (ETL-A and ETL-B) with a gradual rise from 25°C to 35°C showed maximum decolourisation at 35°C. As the temperature increased further from 35°C to 45°C, there was a sharp decline in decolourisation capacity in all the isolates. It was also observed that with the rise in temperature, abiotic decolourisation also increased. Maximum decolourisation was observed with the isolate ETL-A (98%) at 35°C and it is followed by ETL-B (94%) at the same temperature. Least decolourisation was observed at 45°C in all the selected isolates.

Discussion

Industrial effluent is not stable and it varies often in a wide range depending upon the process practiced. South-Asian countries are experiencing severe environmental problems due to rapid industrialisation. This phenomenon is very common where the polluting industries like textile
Environmental problem and a public health concern. A major portion of this wastewater contains azo dyes which are increasingly used in industries because of their ease and cost-effectiveness in synthesis compared to natural dyes. Relative effectiveness of the isolated bacteria for the decolourisation of Remazol Black-B clearly implies that these can be effectively used for the removal of Remazol Black-B from contaminated industrial wastewater. Azoreductase is reported to be the key enzyme expressed in azo dye-degrading bacteria and catalyses the reductive cleavage of the azo bond. Azoreductase activity had been identified in several species of bacteria recently, such as Staphylococcus aureus, Shewanella putrefaciens, Shewanella strain J18 143 and Pseudomonas spp. It was indicated that increase in substrate concentration from its optimum level had a negative effect on decolourisation capacity of isolates. Investigations with different dye concentrations in other experiments also reported higher net colour removal efficiencies at lower dye concentrations. Decrease in decolourisation ability at high substrate concentration might be due to the toxicity of the dye (and co-contaminants)29. Azo dyes generally contain one or more sulphonic acid groups on aromatic rings, which might act as detergents to inhibit the growth of microorganisms30. Another reason of the toxicity at higher concentration may be due to the presence of heavy metals (metal-complex dyes) and/or the presence of non-hydrolysed reactive groups, which may retard the bacterial growth (reactive dyes)32. Similarly, reduction in decolourisation at low concentration of the substrate might be due to the decrease in enzyme ability to recognise the substrate efficiently. Whereas in the case of different carbon sources tested on yeast extract proved to be the best amongst tested carbon sources. Our results were in agreement with the research conducted by other authors.

Figure 5: Effect of different sources of carbon on decolourisation of Remazol Black-B.

Figure 6: Effect of incubation temperature.
in which the bacterial strains grew well and completely decolourised K-2BP when either yeast extract or peptone was present in the medium; however, glucose, glycerol, sucrose, lactose and starch resulted in lower rates of growth and decolourisation of these dyes. Other studies also reported the maximum decolourisation of azo dyes in the presence of yeast extract by bacteria.\textsuperscript{13,14} In the case of pH as a variable, decolourisation was on a higher side at pH 7. Whereas higher pH values (alkaline conditions) decreased the decolourisation efficiency of all the tested isolates. So, from this study, it could be concluded that neutral pH supported bacterial activity to decolourise Remazol Black-B in liquid medium.\textsuperscript{15,16} Temperature is another very important parameter for anaerobic treatment of wastewaters. Selected isolates were mesophilic bacteria because they all showed better decolourisation in the temperature range of 25°C–35°C. Similar results were also reported by Guo et al.\textsuperscript{17} The mesophilic range is traditionally used since it is generally thought that maintaining high temperature would be uneconomical, while degradation within the psychrophilic range is too slow. Overall, one of the selected isolates (ETL-A) of bacteria was able to completely remove the colour of the dye in 18 hours. However, these isolates should be tested at a large-scale treatment system to examine their potential for bioremediation of dye-polluted wastewaters.

\textbf{Conclusion}

This study reveals that the selected three cultures can be used successfully for decolourising Reactive Black-B dye. The cultures exhibited maximum decolourisation ability at a pH of 7 for all the three isolates, and 35°C for ETL-A, ETL-B and 25°C for ETL-C. Moreover, 4 g/L yeast extract was found to be optimum for decolourisation. In conclusion, bacterial species can be studied further for bioremediation of dye-polluted waters including rate of degradation of azo dyes other than the selected Black dye through an application of bioaugmentation.

\textbf{References}

1. Moorthi PS, Selvam SP, Sasikalaveni A, Murugesan K, Kalaiachelian PT. Decolourization of textile dyes and their effluents using white rot fungi. Afr J Biotech. 2007; 6(4):424–9.
2. Rafi F, Fraeankalin W, Cerniglia CE. Aerobic Decolorization of Reactive Azo Dyes in Presence of Various Cosubstrates. Appl Environ Microbiol.1990;56:2146–64.
3. Bhati HH, Akram N, Asgher M. Optimization of culture conditions for enhanced decolourization of Glicarun Red FN-2BL by Schizochyllum commune IBL-6. Appl Biochem Biotechnol. 2008;149:255–64.
4. Abdulla E, Tzanov T, Costa S, Robra K, Cavaco A, Gubitz G. Optimization of cultural conditions for decolorisation of textile azo dyes by Bacillus Subtilis SPR42 under submerged fermentation. Appl Environ Microbiol.2000;66(80):3357–63.
5. Zollinger H. Colour chemistry synthesis properties and application of organic dyes and pigments. New York: VCH; 1991. pp. 92–102.
6. Pinheiro HM, Touraud E, Tomas O. Aromatic amines from azo dye reduction: status review with emphasis on direct UV spectrophotometric detection in textile industry wastewater. Dyes Pigments. 2004;61(2):121–39.
7. Talarpothi AM, Donnelly T, Anderson G. Color removal from a simulated dye wastewater using a two phase anaerobic packed bed reactor. Water Res. 2001;35:425–32.
8. Fu Y, Viraraghavan T. Fungal degradation of dye wastewaters: a review. Biore- sour Technol. 2001;79:251–62.
9. Wong P, Yuen P. Decolorization and biodegradation of Methyl red by Klebsiella pneumoniae RS-13. Water Res. 1996;30(7):1736–44.
10. Stolz A. Basic and applied aspects in the microbial degradation of azo dyes. Appl Microbiol Biotechnol. 2001;56:69–80.
11. Rajaguru P, Vidya L, Baskaraseethupathi B, Kumar PA, Palanivel M, Kalaiselvi K. Genotoxicity evaluation of polluted ground water in human peripheral blood lymphocytes using the comet assay. Mut Res. 2002;517:29–37.
12. Umbuzeiro GA, Freeman H, Warren SH, Oliveira DP, Terao Y, Watanabe T, et al. The contribution of azo dyes to the mutagenic activity of the Cristais River. Chemosphere. 2005;60:55–64.
13. Khehra MS, Saini HS, Sharma DK, Chadha BS, Chimni SS. Biodegradation of azo dye C.I. Acid Red 88 by an anoxic-aerobic sequential bioreactor. Dyes Pigments. 2006;70:1–7.
14. Slokarc YM, Le Marechal AM. Methods of decoloration of textile wastewater. Dyes Pigments. 1998;37:335–56.
15. Bae JS, Freeman HS. Aquatic toxicity evaluation of new direct dyes to the Daphnia magna. Dyes Pigments. 2007;73:91–5.
16. Robinson T, McMullan G, Marchant R, Nigam P. Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative (review). Biore Tech. 2001;77(3):247–55.
17. Chen KC, Wu YJ, Liou DJ, Hwang SCJ. Decolourization of the textile azo dyes by newly isolated bacterial strains. J Bio- tech. 2003;101:57–68.
18. Hao JJ, FQ Song, Huang E, Yang CL, Zhang ZJ, Zheng Y, et.al. Production of lactic acid by a newly isolated deuteromycete fungus Pestalotiopsis sp. and its decolourization of azo dye. J Ind Microbiol Bio- tech. 2001;24:333–40.
19. Pandey AP, Singh, Lyengar L. Bacterial decolourization and degradation of azo dyes. Int Biodeterioration Biodegrad. 2007;59:73–84.
20. Khalid AM, Arshad, Crowley DE. Accelerated decolourization of structurally different azo dyes by newly isolated bacterial strains. Appl Microbiol Biotechnol. 2008;78:361–9.
21. Thomson JD, Gibson TJ, Plewniak F, Jeannouf F, Higgins DG. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 1997;25(24):4876–82.
22. Staley JR, Boone AR, Brenner DJ, Vos PD, Garrity GM, Goodfell M, et al. Bergey’s Manual of Determinative Bacteriology. 2nd ed. Springer; 2001.
23. Jiumkins R. Pretreatment of textile waste water. Proceedings 37th Industrial waste Conference Purdue Uni. Lafayette, Ind.; 1982. pp. 37–139.
24. Vaidya AA, Dayte KV. Environmental pollution during chemical processing of synthetic fibres. Colourage. 1982;14:3–10.

Licensee OA Publishing London 2013. Creative Commons Attribution License (CC-BY)

**FOR CITATION PURPOSES:** Shah MP, Patel KA, Nair SS, Darji AM. Selection of bacterial strains efficient in decolourisation of Remazol Black-B. OA Biotechnology 2013 Mar 01;2(2):14.
25. Ajayi SO, Osibanjo O. The state of environment in Nig. Pollution studies of textile industries in Nig. Monogra. Am Public Health Assoc. standard 1980;1:76–86.
26. Nachiyar CV, Rajkumar GS. Purification and characterization of an oxygen insensitive azoreductase from Pseudomonas aeruginosa. Enzyme Microb Technol. 2005;36:503–9.
27. Li T, Guthrie JT. Colour removal from aqueous solution of metal complex azo dyes using bacterial cells of Shewanella strain J18. 143. Bioreosour Technol. 2010;101:4291–5.
28. Lin J, Zhang X, Li Z, Lei L. Biodegradation of Reactive blue 13 in a two-stage anaerobic/aerobic fluidized beds system with a Pseudomonas sp. isolate. Bioresour Technol. 2010;101:34–40.
29. Cruz A, Buitron G. Biodegradation of Disperse Blue 79 using sequenced anaerobic/aerobic biofilters. Water Sci Technol. 2010;44:159–66.
30. Kapdan IK, Oztekin R. Decolourization of textile dyestuff Reactive Orange 16 in fed-batch reactor under anaerobic condition. Enzyme Microb Technol. 2003;33:231–5.
31. Sponza DT, Isik M. Reactor performances and fate of aromatic amines through decolourization of Direct Black 38 dye under anaerobic/aerobic sequential. Process Biochem. 2005;40:35–44.
32. Chen KC, Wu JY, Liou DJ, Hwang SCJ. Decolourization of the textile dyes by newly isolated bacterial strains. J Biotechnol. 2003;10:57–68.
33. Guo J, Zhou J, Wang D, Tian C, Wang P, Uddin MS. A novel moderately halophilic bacterium for decolorizing azo dye under high salt condition. Biodegradation. 2008;19:15–19.
34. Hu TL. Degradation of azo dye RP2B by Pseudomonas luteola. Water Sci Technol. 1998;38:299–306.
35. Mali PL, Mahajan MM, Patil DP, Kulkarni MV. Biodecolorization of members of triphenylmethane and azo groups of dyes. J Sci Ind Res. 1999;59:221–4.
36. Chang JS, Kuo TS, Qu Y, Gao J, Wang P, Zhang H. Azo dye decolorization with a mutant Escherichia Coli strain. Biotechnol Lett. 2000;22:807–12.
37. Varel VH, Hashimoto AG, Chen YR. Effect of temperature and retention time on methane production from beef cattle waste. Appl Environ Microbiol. 1980;40:217–22.