Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2013.822333

52 (2014) 6632–6638
October

Taylor & Francis
Taylor & Francis Group

Feasibility of different carbon sources for growing microbial biomass in aerobic batch reactor and their application for dye removal from contaminated wastewater

Kapil Kumar[a,*], M.G. Dastidar[a], T.R. Sreekrishnan[b]

[a]*Centre for Energy Studies, Indian Institute of Technology Delhi, Hauz Khas, New Delhi 110016, India*
Tel. +91 11 8475805221; Fax: +91 11 26581121; email: Kapil.iitd05@gmail.com
[b]*Department of Biochemical Engineering & Biotechnology, Indian Institute of Technology Delhi, Hauz Khas, New Delhi 110016, India*

Received 18 March 2013; Accepted 9 June 2013

ABSTRACT

In search for a cheaper energy source, this study was undertaken to investigate the potential of mixed culture for the decolorization of Methylene blue (MB) and Remazol black B (RBB) using glucose, molasses, and cheese whey as an energy source for microbial growth in aerobic batch reactor. The experiments were performed with synthetic solutions of both the dyes ranging from 25 to 300 mg l^{-1}. The results show that the decolorization and microbial growth was affected by the increase in concentration of dyes leading to the decreased decolorization of dyes. The presence of glucose was found to be more efficient as compared to molasses and cheese whey. After 36 h, mixed culture was able to decolorize MB up to 83, 59, and 54% and RBB up to 80, 58, and 52% in the presence of glucose, molasses, and cheese whey, respectively, at 300 mg l^{-1} initial dye concentration. The maximum specific uptake was 57, 51, and 52 mg g^{-1} in presence of glucose, molasses, and cheese whey, respectively, at 300 mg l^{-1} initial dye concentration of RBB. The maximum specific uptake was 58, 51, and 53 mg g^{-1} in the presence of glucose, molasses, and cheese whey, respectively, at 300 mg l^{-1} initial dye concentration of MB. The results of this study shall be useful to develop a suitable decolorization process for the treatment of dye-contaminated wastewater or wastewater contaminated with a variety of dyes.

Keywords: Dyes; Mixed culture; Decolorization; Biomass; Molasses; Cheese whey

1. Introduction

Textile and dyeing industries have been listed under seventeen highly polluted industries by Central Pollution Control Board in India [1]. The dye-contaminated wastewater discharged into open waters presents an esthetic problem. As dyes are designed to be chemically and photolytically stable, they are highly persistent in natural environments. The release of dyes may, therefore, presents an ecotoxic hazard and introduces the potential danger of bioaccumulation that may eventually affect man by transport through the food chain. Textile finishing wastewater, especially, dye house effluents contain different classes of organic dyes and chemicals, and thus, they are

*Corresponding author.

colored and have extreme pH, chemical oxygen demand (COD), biological oxygen demand (BOD), different salts, surfactants, heavy metals, mineral oils, etc. cause the reduce in light penetration affecting photosynthetic activity in aquatic life and may also be toxic to some aquatic life [2–8]. The removal of the polluting dyes and other contaminants from effluents is an important problem, particularly for small-scale textile industries where working conditions and economic status do not allow them to treat their wastewater before disposal, and they have no choice other than discharging the effluents into the main stream of water resources. Most physicochemical methods for dye removal have drawbacks, because they are expensive, have limited versatility, are greatly interferred by other wastewater constituents, and/or generate waste products that must be handled [9–14]. Alternatively, biological treatment may present a relatively inexpensive way to remove dyes from wastewater. Microbial process for decolorization and degradation is an environment-friendly and cost-competitive alternative process over chemical decomposition processes [15].

In most of the studies reported on decolorization by various microorganisms using pure and mixed culture, glucose has been used preferably as an energy source. There is also a need to search for cheaper energy source for the development of an economically viable bioremediation process for the wastewater contaminated with dyes. For the growth of microorganisms, molasses, and cheese whey which are generated as waste in food industries can also be used as an energy source as an alternative of glucose. The disposal of cheese whey into the environment is a matter of serious concern due to the presence of high organic matter content such as lactose, protein, vitamins, BOD in the range 40–60 $g\,l^{-1}$ and COD in the range 50–80 $g\,l^{-1}$ [16–19]. The growth of certain microorganisms is favored in the cheese whey due to its composition and hence biological treatment of whey has been reported to be a favorable process [20,21]. Further, molasses is a by-product of sugarcane industry, which has low price as compared to other sources of sugar, and due to the presence of several other compounds and vitamins, it can be used as growth medium for microorganisms to develop an economic viable treatment process for dye contaminated wastewater [22]. Scanty information is available in the literature on the treatment of dye-contaminated wastewater using molasses and cheese whey as an energy source. The treatment technique using these sources can be considered as economically more viable with simultaneous benefit of pollution minimization. The batch decolorization was performed in the present study using synthetic solutions of RBB and MB dyes at different initial dye concentrations in the presence of glucose, molasses, and cheese whey as energy source for microorganisms.

2. Materials and methods

2.1. Synthetic solutions of dyes

In the present study, Remazol Black B (RBB molecular formula: $C_{26}H_{21}N_5Na_4O_{19}S_6$, molecular weight: 991.82 and pKa 6.9) and Methylene blue (MB Molecular formula: $C_{16}H_{18}N_3SCl$, Molecular weight:319.85 and pKa 3.8) were used. Stock solutions of RBB and MB dyes of 1,000 $mg\,l^{-1}$ concentration were prepared by dissolving the appropriate quantities of powdered dyes in tap water, and the solutions of the desired concentrations for various experiments were obtained by successive dilution. The dyes were procured from textile engineering department of Indian Institute of Technology Delhi (IIT Delhi) and were of analytical grade with 95% purity. The chemical structure of the dyes is shown in Fig. 1(a) and (b) [23,24].

2.2. Composition of cheese whey and molasses

The aerobic mixed culture used in this study was grown in glucose, cheese whey, and molasses. The characteristics of glucose, cheese whey, and molasses are shown in Tables 1–3, respectively.

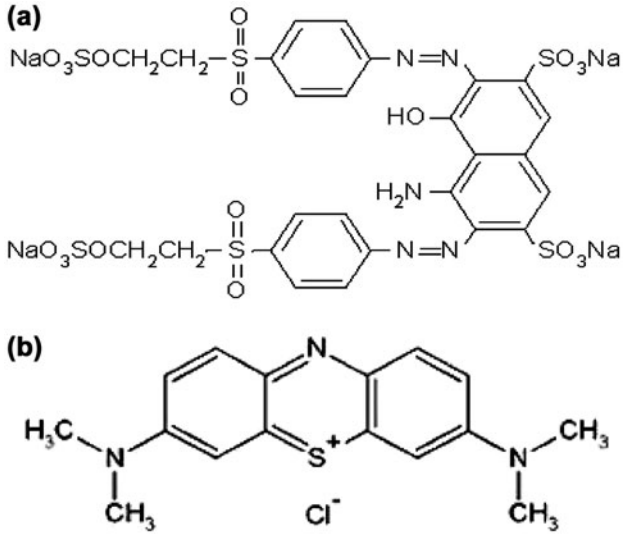

Fig. 1. (a) Structure of Remazol Black B. (b) Structure of MB.

Table 1
Glucose medium composition

Component	Concentration ($g\,l^{-1}$)
Glucose	10
Yeast extract	0.340
NH_4Cl	0.840
KH_2PO_4	0.134
K_2HPO_4	0.234
$MgCl_2.6H_2O$	0.084

Table 2
Cheese whey composition

Component	Concentration
BOD ($g\,l^{-1}$)	38
COD ($g\,l^{-1}$)	64
Total phosphorous content ($mg\,l^{-1}$)	136
Total nitrogen content ($mg\,l^{-1}$)	867
Total sugar content ($g\,l^{-1}$)	48

Table 3
Molasses composition

Component	Value
BOD ($g\,l^{-1}$)	30
COD ($kg\,l^{-1}$)	100
Total phosphorous content ($g\,l^{-1}$)	0.10
Total nitrogen content ($g\,l^{-1}$)	1.5
Total sugar content ($g\,l^{-1}$)	477

2.3. Aerobic mixed culture acclimatization and inoculum preparation

A primary requirement of this work was to develop an acclimatized culture that can decolorize higher concentrations of RBB and MB. Acclimatization was done in shake flasks with RBB dye, and the same inoculum was used for all the experiments performed with MB dye. The synthetic dye solutions and the activated sludge samples were stored at 4°C. A 10% (v/v) inoculum was used in all the experiments conducted in 500-ml Erlenmeyer flasks stoppered with cotton plugs, which were used as the completely mixed reactors. The working volume of the liquid was 200 ml. To check whether there was any decrease in dye concentration due to volatilization, control flasks (without inoculum) were operated under the same conditions. For enrichment of the culture, the heterogenous population was first grown aerobically in a medium containing 1% (w/v) glucose as the carbon and energy source and $25\,mg\,l^{-1}$ RBB dye. During acclimatization period, the amount of glucose was regularly checked and maintained at 1%. The culture was gradually exposed to increasing concentrations of RBB dye in order to acclimatize the microbial culture to the higher concentrations of dye. Successive transfers of the culture into fresh glucose medium containing higher concentrations of RBB, up to $300\,mg\,l^{-1}$, were done at 37°C. This acclimatized microbial culture was used in all the experiments conducted in batch mode for decolorization of the dyes (RBB and MB).

2.4. Aerobic batch decolorization using synthetic dye solutions

The decolorization experiments were performed in batch mode in 500-ml Erlenmeyer flasks. A working volume of 200 ml was employed throughout the study. The glucose media and dye (concentration according to the requirement, i.e. 10, 20, or $50\,mg\,l^{-1}$) were added to the flasks. The flasks were incubated with 10% (v/v) acclimatized inoculum. After adding glucose media, inoculum and required concentration of dye, the flasks were kept in an orbital shaker at 180 rpm and 30°C. The initial pH of the solution was adjusted using 1 N hydrochloric acid or sodium hydroxide as per the requirement. In addition, control flasks containing only dye and media and without inoculum were also kept under the same conditions to see the abiotic decolorization, if any. All the experiments were performed in duplicate.

2.5. Analytical methods

At different time intervals, the samples were withdrawn from the flasks and centrifuged at 5,000 rpm for 10 min. to precipitate suspended biomass. The concentration of dye in the supernatant was determined by reading absorbance at 595 nm. This absorbance was compared with standard curve plotted using different concentrations of the dye. The measurement of absorbance and centrifugation were done by using systronic UV-VIS spectrophotometer 117 and Hitech model centrifuge, respectively.

3. Results and discussions

The percentage decolorization with time at pH 7.0 and 30°C using different carbon sources at $25\,mg\,l^{-1}$ initial concentration of RBB and MB is shown in Fig. 2. It is clear from the figure that maximum decolorization was achieved with glucose as the carbon

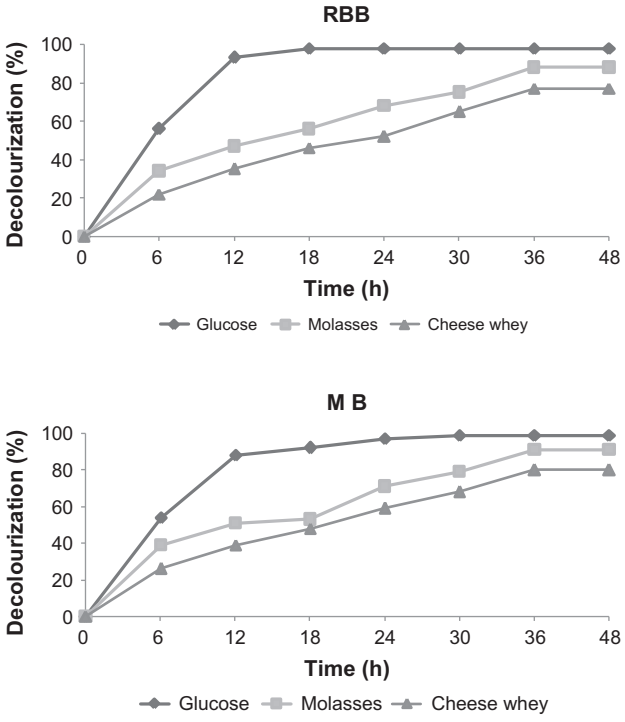

Fig. 2. Effect of carbon source on decolourization of RBB and MB with time at 25 mg l^{-1} initial dye concentration using TS.

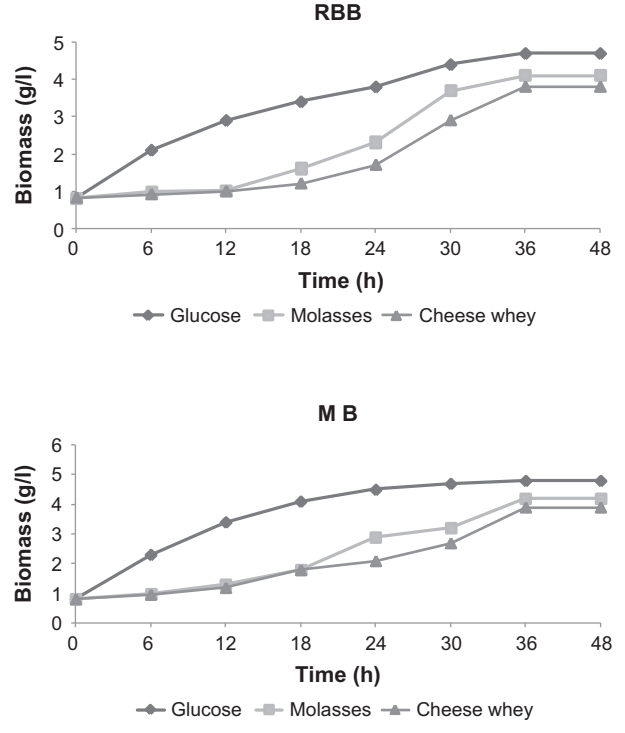

Fig. 3. Change in biomass concentration with time in presence of different carbon sources at 25 mg l^{-1} initial dye concentration (RBB and MB) using TSL.

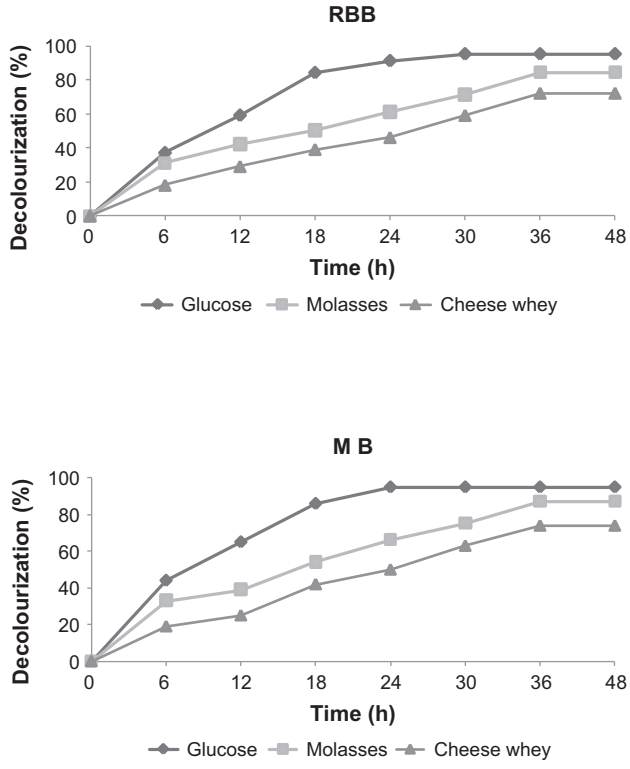

Fig. 4. Effect of carbon source on decolourization of RBB and MB with time at 100 mg l^{-1} initial dye concentration using TSL.

source (supplement) followed by molasses and cheese whey for both the dyes (RBB and MB). Moreover, rate of decolorization was faster with glucose in comparison with molasses and cheese whey. The maximum decolorization after 48 h was found to be 99% for MB and 98% for RBB in the presence of glucose, while it was 91% and 88% for MB and RBB, respectively, in the presence of molasses. On the other hand, the minimum decolorization was achieved for both the dyes under similar experimental conditions in the presence of cheese whey and it was 80% and 77% for MB and RBB, respectively. The lower decolorization in the presence of molasses and cheese whey might be due to the lower growth of microorganisms.

Fig. 3 shows the change in biomass concentration with time in the presence of different carbon sources and at 25 mg l^{-1} initial concentration of RBB and MB. The figure clearly indicates that biomass concentration increased with time till 36 h and then became constant for all the carbon sources for both the dyes. However, the lag phase was longer in case of cheese whey and molasses. Maximum biomass concentration (around 4.7 g l^{-1}) was found in the presence of glucose as compared to molasses (4.1 g l^{-1}) and cheese whey (3.5 g l^{-1}).

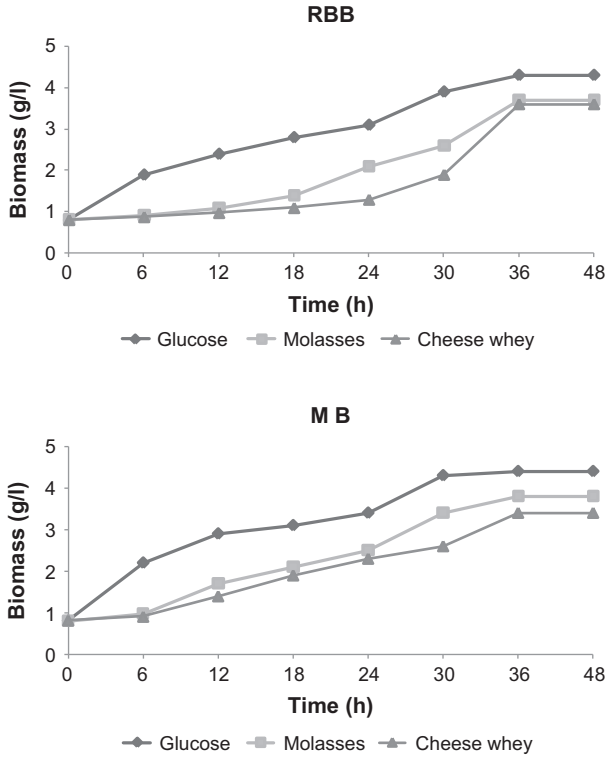

Fig. 5. Change in biomass concentration with time in presence of different carbon sources at 100 mg l^{-1} initial dye concentration (RBB and MB) using TSL.

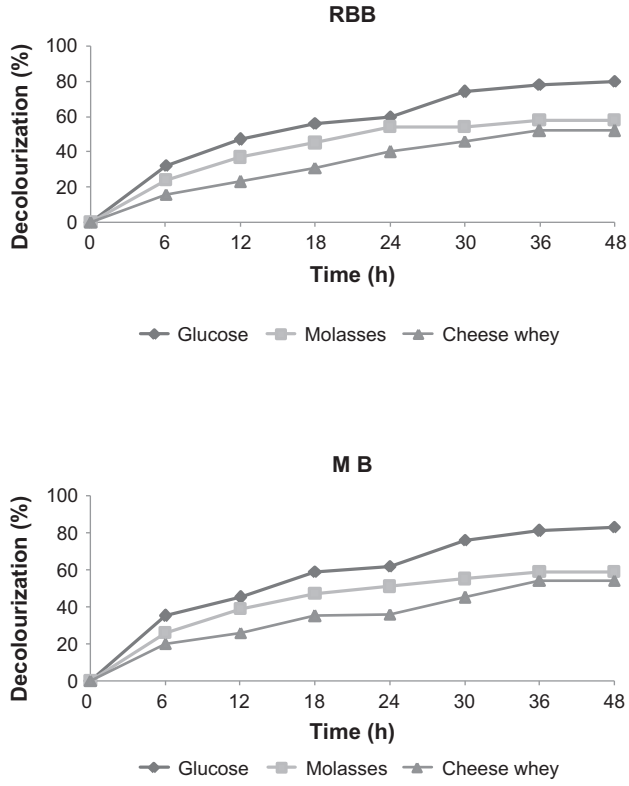

Fig. 6. Effect of carbon source on decolourization of RBB and MB with time at 300 mg l^{-1} initial dye concentration using TSL.

Fig. 4 shows the percentage decolorization of RBB and MB with time at 100 mg l^{-1} initial dye concentration in the presence of different carbon sources. The Figure clearly indicates that percentage decolorization decreased for both the dyes with increase in dye concentration although percentage decolorization increased till 36 h for both the dyes using all the carbon sources. After 36 h, the maximum decolorization was found to be 95% for RBB and MB in presence of glucose. Molasses could decolorize 84 and 87% of RBB and MB, respectively, whereas cheese whey resulted in lower decolorization of 72 and 74% for RBB and MB, respectively, after 36 h under similar experimental conditions. The lower biomass concentration (2.7 mg l^{-1}) was also found in the presence of cheese whey, resulting in lower decolourization (Fig. 5). Fig. 5 shows the change in biomass concentration with time in presence of different carbon sources at 100 mg l^{-1}initial concentration of RBB and MB. It is clear from the figure that maximum growth of biomass (4.3 g l^{-1}) took place in presence of glucose followed by molasses (3.7 g l^{-1}) and cheese whey (2.7 g l^{-1}). The biomass concentration increased with time till 36 h then remained constant for all the carbon sources studied. Lower biomass concentration was found at higher dye concentration in comparison with higher biomass concentration at lower dye concentration (25 mg l^{-1}) in the presence of all carbon sources which resulted in lower percentage decolourization at higher dye concentration for both the dyes (RBB and MB).

Fig. 6 shows the percentage decolorization of RBB and MB with time in the presence of different carbon sources at 300 mg l^{-1} initial dye concentration. Figure clearly indicates that percentage decolorization increased with time till 36 h and then remained constant for all carbon sources. Rate of decolorization was faster with glucose in comparison with molasses and cheese whey. Maximum decolorization was found with glucose followed by molasses and cheese whey. The decolorization after 36 h was found to be 80 and 83% for RBB and MB respectively in the presence of glucose, while as it was 58 and 59% for RBB and MB respectively in the presence of molasses. The decolorization was only 52 and 54% for RBB and MB, respectively, in the presence of cheese whey after 36 h. The reason for lower decolorization at higher dye concentration is due to the lower biomass growth rate caused by the inhibitory effect of the dye. Similar kinds of

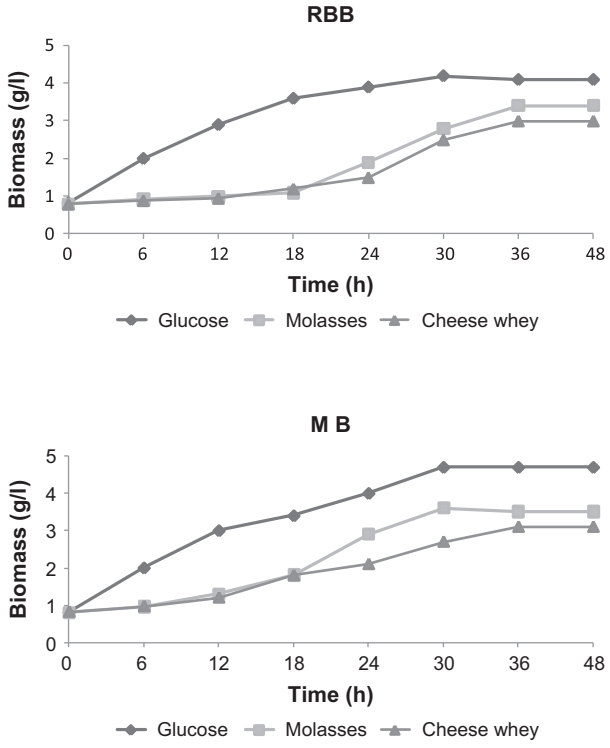

Fig. 7. Change in biomass concentration in presence of different carbon sources at 300 mg l^{-1} initial dye concentration (RBB and MB) using TSL.

Table 4
Maximum specific dye uptake (mg g^{-1}) in presence of different carbon sources at different initial concentrations of RBB and MB

Carbon source	Maximum specific dye uptake (mg/g)					
	RBB (mg l^{-1})			MB (mg l^{-1})		
	25	100	300	25	100	300
Glucose	5.21	22	57	5.1	21.59	57.85
Molasses	5.36	22.7	51	5.4	22.89	50.57
Cheese whey	5.06	20	52	5.1	21.76	52.25

results have also been reported in the literature [25–27].

Fig. 7 shows the change in biomass concentration at 300 mg l^{-1} initial dye concentration in the presence of different carbon sources. It is clear from the figure that biomass increased with time for all carbon sources and maximum biomass (4.1 g l^{-1}) was found in the presence of glucose followed by molasses (3.4 g l^{-1}) and cheese whey (3 g l^{-1}). Decolorization was higher in the presence of glucose followed by molasses and cheese whey.

It is clear from Table 4 that maximum specific uptake increased with increase in dye concentration for both the dyes. Maximum specific uptake for RBB increased from 5.21 mg g^{-1} to 52 mg g^{-1} with increase in dye concentration increased from 25 mg l^{-1} to 300 mg l^{-1}. The maximum specific uptake of MB increased from 5.1 mg g^{-1} to 52.25 mg g^{-1} with increase in dye concentration from 25 mg l^{-1} to 300 mg l^{-1}.

4. Conclusion

The results of the study showed that the mixed culture was able to grow and decolorize both the dyes (RBB and MB) not only with glucose but also with molasses and cheese whey as supplementary carbon sources. After 36 h, mixed culture was able to decolorize MB up to 83, 59, and 54% and RBB up to 80, 58 and 52% in the presence of glucose, molasses, and cheese whey, respectively, at 300 mg/l initial dye concentration. The maximum specific uptake was found to be 57, 51, and 52 mg/g in the presence of glucose, molasses, and cheese whey, respectively, at 300 mg/l initial dye concentration of RBB. The maximum specific uptake was found to be 58, 51, and 53 mg/g in the presence of glucose, molasses, and cheese whey, respectively, at 300 mg/l initial dye concentration of MB. The results of the above study will help to develop a suitable treatment strategy for the treatment of wastewater contaminated with dyes using cheaper carbon sources like cheese whey and molasses instead of glucose for the growth of microorganisms. This will decrease the cost for treatment of dye-contaminated wastewater as well as will be helpful to solve the problem of disposal and treatment of these wastes.

Acknowledgment

One of the authors Mr. Kapil Kumar is highly grateful to All India Council for Technical Education New Delhi, India, for providing financial support for the study.

References

[1] Central Pollution Control Board (C.P.C.B), Biological Treatment of Textile mill effluent. A Case Study, IMPACTS/5, 2000–2001.
[2] V. Golob, A. Vinder, M. Simonic, Efficiency of the coagulation/flocculation method for the treatment of dye bath effluents, Dyes Pigm. 67 (2005) 93–97.
[3] I.M. Banat, P. Nigam, D. Singh, R. Marchant, Microbial decolorization of textile-dye-containing effluents: A review, Bio. Technol. 58 (1996) 217–227.
[4] Y. Fu, T. Viraraghavan, Fungal decolorization of wastewaters: A review, Bio. Technol. 79 (2001) 251–262.

[5] I.M. Robinson, G. McMullan, R. Marchant, P. Nigam, Remediation of dyes in textile effluent: A critical review on current treatment technologies, Bio. Technol. 77 (2001) 247–255.
[6] S.D. Lambert, N.J.D. Graham, C.J. Sollar, G.D. Fowle, Evaluation of inorganic adsorbents for the removal of problematic textile dyes and pesticides, Water Sci. Technol. 36 (1997) 173–180.
[7] G. Mishra, M.A. Tripathy, A critical review of the treatment for decolorization of textile effluent, Colourage 40 (1993) 35–38.
[8] Y.M. Sloka, A.M. Le Marechal, Methods of decoloration of textile wastewaters, Dyes Pigm. 37 (1997) 335–356.
[9] S.H. Lin, F.C. Peng, Continuous treatment of textile wastewater by combined coagulation, electrochemical oxidation and activated sludge, Water Res. 30 (1996) 587–592.
[10] B. Manu, S. Chaudhari, Anaerobic decolorization of simulated textile wastewater containing azo dyes, Bio. Technol. 82 (2001) 225–231.
[11] E. Razo-Flores, M. Luijten, B. Donlon, G. Lettinga, J. Field, Biodegredation of selected azo dyes under methanogenic conditions, Water Sci. Technol. 36 (1997) 65–72.
[12] J.K. Glenn, M.H. Gold, Decolorization of several polymeric dyes by the lignin degrading basidiomycete Phanerochaete chrysosporium, Appl. Environ. Microbiol. 45 (1983) 1741–1747.
[13] P.A. Ramalh, H. Scholze, M.H. Cardoso, M.T. Ramalho, A.M. Oliveira-Campos, Improved conditions for the aerobic reductive decolourisation of azo dyes by Candida zeylanoides, Enzyme Microbiol. Technol. 31 (2002) 848–854.
[14] C. Meehan, I.M. Banat, G. McMullan, P. Nigam, F. Smyth, R. Marchant, Decolorization of Remazol Black-using a thermotolerant yeast Kluyveromyces marxianus IMB3, Environ. Int. 26 (2000) 75–79.
[15] P. Verma, D. Madamwar, Decolourization of synthetic dyes by a newly isolated strain of Serratia marcescens, World J. Microbiol. Biotechnol. 19 (2003) 615–618.
[16] M.I. Berruga, A. Jaspe, C. SanJose, Selection of yeast strains for lactose hydrolysis in dairy effluents, Int. Biodeterior. Biodegrad. 40 (1997) 119–123.
[17] S. Devi, P. Banumathi, M. Jothi, Studies on development and evaluation of whey based fruit beverage, Bev. food world 31 (2004) 44–45.
[18] A.E. Ghaly, R.K. Singh, Pollution potential reduction of cheese whey through yeast fermentation, Appl. Biochem. Biotechnol. 22 (1989) 181–203.
[19] G.L. Reddy, B.V.R. Rao, K.S.R. Reddy, D. Venkayya, Development of a whey beverage, Ind. J. Dairy Sci. 40 (1987) 445–450.
[20] S. Hwang, H.L. Hansen, Modeling and optimization in anaerobic bioconversion of complex substrates to acidic and butyric acids, Biotechnol. Bioeng. 54 (1997) 451–460.
[21] R. Rech, C.F. Cassini, A. Secchi, M. Ayub, Utilization of proteinhydrolyzed cheese whey for production of b-galactosidase by *Kluyveromyces marxianus*, J. Ind. Microbiol. Biotechnol. 23 (1999) 91–96.
[22] R.S. Makkar, S.C. Cameotra, Utilization of molasses for biosurfactant production by two Bacillus strains at thermophilic conditions, J. Am. Oil Chem. Soc. 74 (1997) 887–889.
[23] D.A. Oxspring, G. Mcmullan, W.F. Symth, R. Marchant, Decolourisation and metabolism of the reactive textile dye, Remazol Black B, by an immobilized microbial consortium, Biotech. Lett. 18 (1996) 527–530.
[24] L. Bandounas, M. Pinkse, J.H. de Winde, H.J. Ruijssenaars, Identification of a quinone dehydrogenase from a *Bacillus* sp. involved in the decolourization of the lignin-model dye, Azure B, New Biotechnol. 30 (2013) 196–204.
[25] A. Zumriye, G. Donmez, Combined effects of molasses sucrose and reactive dye on the growth and dye bioaccumulation properties of Candida tropicalis, Process Biochem. 40 (2005) 2443–2454.
[26] G. Donmez, Bioaccumulation of the reactive textile dyes by *Candida tropicalis* growing in molasses medium, Enzyme Microb. Technol. 30 (2002) 363–366.
[27] D. Cetin, G. Donmez, Decolorization of reactive dyes by mixed cultures isolated from textile effluent under anaerobic conditions, Enzyme Microb. Technol. 38 (2006) 926–930.