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ORIGINAL PAPER

Effect of different operational conditions on the decolorization of molasses spent wash using once developed soil inoculum

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Abstract A stirred vessel coupled with membrane unit containing cellulose acetate (0.45 μm) membrane was used to study the decolorization of anaerobically digested molasses spent wash (MSW). The soil collected from the MSW disposal site was used as inoculum to study the decolorization without addition of any additives. The same inoculum was used over a period of 163 days at room temperature to study the decolorization of 12.5–50% (v/v) MSW using different operational conditions. The reactor was entered in to the inhibition mode after the feeding of 50% MSW, which was restored 100% without changing any operational condition. The maximum decolorization obtained for 12.5% (v/v) MSW was 77.22 ± 0.13%. The decolorization achieved for 25, 37.5, and 50% (v/v) MSW was 70.41 ± 0.12, 56.47 ± 0.17, and 48.78 ± 0.09%, respectively. Increase in the utilization of protein and reducing sugar was observed up to 25% MSW whereas, higher concentration showed decrease in the utilization. Results indicate 63% removal of chemical oxygen demand for 12.5% (v/v) MSW. Membrane flux which was significantly reduced after the feeding of 50% MSW was regenerated without changing the washing procedure, however, 35% decrease in sample flux was observed over the continuous use of membrane for the period of 198 days.

Keywords Molasses spent wash · Soil as inoculum · Stirred vessel · Decolorization · Membrane flux

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Introduction

Most of the distilleries in India use cane molasses as the principal raw material for the alcohol production. Generally, around 10–15 l of molasses spent wash (MSW) are generated for the production of one liter of ethanol (Kumar et al. 1997). MSW has dark color and is recalcitrance in nature due to the presence of melanoidins brown polymers which are formed by the Maillard amino-carbonyl reaction (Wedzicha and Kaputo 1992). It is difficult to treat by normal biological processes such as activated sludge or anaerobic lagooning (Singh and Nigam 1995). It has high pollution potential because of its extremely high biochemical and chemical oxygen demand (Chaudhari et al. 2008). So far, different studies have been carried out to solve the effluent treatment problem of the distillery industries but with limited success. Lots of efforts are being made to utilize microbes for the decolorization and mineralization of MSW. A thermophilic strain of *Aspergillus fumigatus* cultivated on a glycerol-peptone medium decolorizes molasses

melanoidin to an extent of 75% at 45°C within 3 days with shaking (Ohmomo et al. 1987). Almost 85% color from MSW was removed after 10 days by using the white-rot fungus *Phanerochaete chrysosporium* grown in a medium containing 6.25% MSW (Fahy et al. 1997). Similarly, 71.5% decolorization was achieved using white-rot fungi at 6.25% MSW concentration, however, it was decreased significantly at higher (12.5 and 25% v/v) concentrations (Kumar et al. 1998). In general, the need of additional carbon and diluted MSW are two major shortcomings in microbial decolorization (Kumar et al. 1997), which remains still unsolved. Thus, there is a need to develop an efficient method for MSW treatment for its safe disposal. In the present study we have used polymeric membrane to verify its utility in such operation. Because, lots of studies have been carried out recently using membranes for the effective removal of organic, inorganic contaminants and biological material from wastewaters (Chae and Shin 2007). However, membrane fouling remains a major problem in widespread application of membrane which reduces productivity and increases maintenance and operating costs (Lee et al. 2007). Therefore, we have given more thrust in understanding the flux pattern of the membrane to judge its utility for long term use in such operations. The aim of the present study was to find out more practical and economical way for the decolorization of MSW so, the concerned industry may be encouraged to adapt it voluntarily. We have studied MSW decolorization using different operational conditions and a simple reactor system. This paper presents a novel experimental approach by using soil as inoculum instead of any isolated microorganism and optimization of decolorization without addition of any additional carbon or nitrogen source in the system.

Materials and methods

Experimental set up

As shown in Fig. 1, the experimental setup was used for the study which includes reactor comprising a modified glass vessel of 1 l volume with broad conical neck and outlets at bottom and on top. It was calibrated with the known volumes for the level indications and was kept on magnetic stirrer driven at constant speed of 200 rpm. To avoid the falling of any object in the

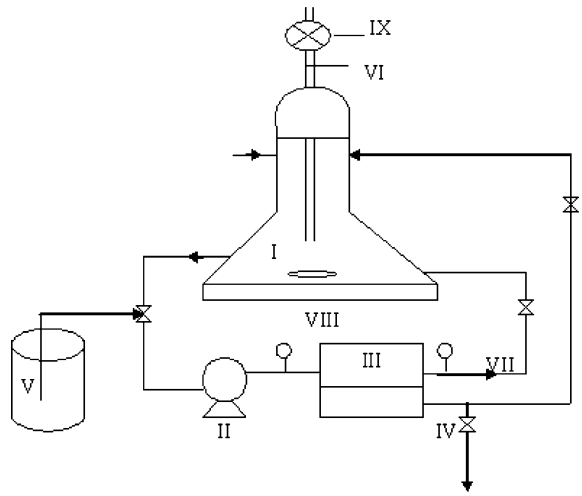

Fig. 1 Schematic diagram of reactor. *I* Reactor containing feed, *II* peristaltic pump, *III* membrane unit, *IV* permeate, *V* washing/cleaning solution, *VI* vent, *VII* rententate, *VIII* magnetic stirrer, *IX* prefilter

reactor, the top opening of the reactor was closed with cotton plug and vent connected to air micro filter (procured from Millipore corp.). Peristaltic Pump (Watson–Marlon, Model 67213) was calibrated for various flow rates. The tubing used was silicon soft rubber tubing and the pump was run at constant speed. The membrane unit used was flat sheet module and the membrane was cellulose acetate (pore size 0.45 μm; active surface area 72 cm^2; procured from Millipore corp.). All the experiments were carried out under nonsterile conditions, without supply of artificial air and at room temperature (25 ± 3°C).

Reactor feed preparation

Anaerobically digested MSW was collected from the bio-methanation plant at Vasantdada Shetkari S·S.K. Ltd, Sangli, India and used as such without any pretreatment. Reactor feed was prepared by mixing 12.5% (v/v) of MSW which was prepared using tap water and 2.5% (w/v) soil. The soil sample which was collected from the MSW disposal site and showed higher MSW decoloriztion activity in our earlier study (Adikane et al. 2006) was used as it is to prepare reactor feed. The MSW and soil mixture was kept on rotary shaker at 200 rpm for 24 h and then the entire mixture was filtered through Whatman filter paper

(pore size 0.78 μm)/cotton to remove coarse material and treated as reactor feed.

Sampling and membrane washing

Prior to sampling, the reactor feed was allowed to recirculate through the membrane for 1 h at 0.5 bar outlet pressure by connecting the retentate and permeate port to the reactor assembly. The permeate 5–10 ml was collected as per experiment requirement and flux was measured and termed as "sample flux". After measuring the absorbance for color, permeate was stored in a deep freezer (below 0°C) to carry out other measurements. After disconnecting the retentate and permeate port, 200 ml NaCl (0.85%), sodium doducil sulphate (0.1%) was recirculate (15 min) subsequently each for two times thorough the membrane unit. Then the distilled water (200 ml) was recirculate (10 min) three to four times thorough the membrane and "final flux" was measured. Later on, 0.1% formaldehyde recirculate (10 min) thorough the membrane and the retentate and permeate port was closed after putting off the pump. The membrane was allowed to remain in formaldehyde till the next sampling. Prior to the next sampling, formaldehyde was removed and membrane was thoroughly washed by recirculating distilled water and flux was measured and termed as "initial flux". The first "initial flux" was measured when membrane was fixed to the module.

Analytical methods

The analysis of collected samples was carried out to monitor the MSW decoloriztion using different methods. The estimation of color was carried out as reported earlier (Kumar et al. 1997) by measuring optical density (OD) of collected sample at 475 nm using a UV/Vis Spectrometer (Unican, UK). The decolorization activity was expressed as % decolorization [% decolorization = 100 – (OD obtained at respective day × 100/OD obtained at '0' day)]. The methods reported earlier were used for the estimation of nutritional elements such as protein, reducing sugars (Adikane et al. 2006) and total sugar (Dubois et al. 1956) to measure their utilization and expressed as % utilization [% utilization = 100 − (concentration obtained at respective day × 100/concentration obtained at '0' day)]. The estimation of COD was carried out using conventional titration method (Trivedy and Goel 1986).

Results and discussion

The purpose of this study was to develop an economical and simple method for the effective MSW decolorization. Therefore, the optimization of MSW decolorization was studied under nonsterile conditions, without adjustment of pH, without supply of artificial air, at room temperature, using soil as sole inoculum and without addition of any carbon or nitrogen source in the system. The membrane was used only in sampling to avoid the loss of biomass thus the reactor could be used with or without membrane unit. The utilization of protein, reducing sugar and total sugar was studied because generally microbes utilize these ingredients for the growth. Therefore, this was used as an indirect evidence for the understanding of biomass growth pattern in the present study.

Fed batch study

In this study, sampling was done every day and feed (12.5% MSW) was added on the 7 day. Because, 7 day incubation period for maximum decolorization of anaerobically digested MSW was reported earlier (Kumar et al. 1997; Adikane et al. 2006). Almost 60% utilization of reducing sugar and protein along with 56.6% decolorization was obtained on the 7 day (Fig. 2a). On the 14 day, 60% utilization of reducing sugar and protein along with 58.5% decolorization was obtained. However, increasing trends in the utilization of reducing sugar, protein and MSW decolorization was observed on the 21, 28, and 35 day. In comparison with the 7 day, almost 10% increase in decolorization (66.8%) and around 7% increase in reducing sugar and protein utilization was obtained on the 35 day. The higher utilization of reducing sugar and protein suggest increase in biomass growth. Although there was increase in decolorization but it is evident from the results that the 7 day batch time is not sufficient to achieve significant decolorization. The flux pattern of the membrane shows initial steep decrease in flux (Fig. 2b). Around 30, 20, and 17% decrease in initial flux, final flux and sample flux was observed, respectively. However, after initial steady decrease up to

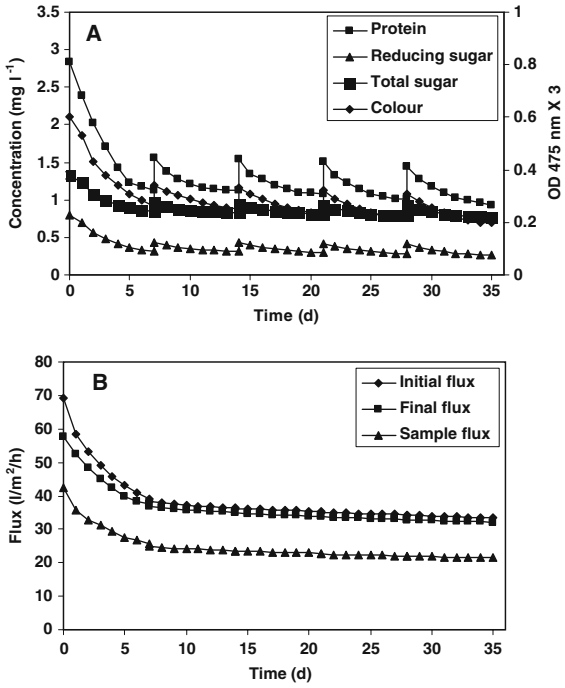

Fig. 2 MSW decolorization using fed batch mode of operation. **a** Decolorization, **b** flux profile; initial reactor feed volume 330 ml; sample volume 10 ml 24 h^{-1}; feed volume that was added on the 7 day subsequently 80 ml 12.5% (v/v) MSW; total duration of study 35 days

around 7 day it remains stable till 35 days. The flux obtained after 7 day may be referred as stabilized flux. The initial steep decrease in flux may be due to the irreversible blockage of membrane pores which is common phenomena observe in polymeric membranes (Mulder 1996). In general, the process flux (sample flux) remains below 5% of initial flux in microfiltration and ultrafiltration operations (Mulder 1996). However, sample flux was around 30% in the present case. The results clearly indicate that the membrane can be used for longer time.

Batch study

The average of the decolorization obtained on the 7, 14, 21, 28, and 35 day in the above experiment (Fed batch study) was around 60 ± 4%. This was significantly less (20%) than the decolorization which was obtained (81%) for 12.5% MSW after 18 days incubation in our earlier test tube study (Adikane et al. 2006). Therefore, this experiment was planed to achieve higher decolorization. The reactor feed was prepared by filtration through cotton to save the filtration time and to reduce overall operational cost. The reactor was run in a batch mode of operation to assess the time required for higher decolorization. The higher decolorization (73%) was obtained on the 15 day (Fig. 3a). The average decolorization obtained for the 15–28 day was 73.46 ± 0.34%. This was significantly higher than the decolorization obtained in the above study (Fed batch study) where batch time was 7 day. This indicates that the batch time around 15 day may be sufficient for the maximum decolorization of 12.5% MSW. The average utilization of protein, reducing sugar and total sugar obtained for the 15–28 day was 78.47 ± 0.44, 77.36 ± 1.5, and 51.98 ± 3.94%, respectively. Whereas the average utilization of protein, reducing sugar and total sugar obtained for the 1–15 day was 67.17 ± 13.0, 63.35 ± 6.8, and 38.84 ± 6.8%, respectively. In comparison to the 15–28 day, around 11, 14, and 13% less utilization of protein, reducing sugar and total sugar, respectively, was obtained for the 1–15 day. This suggests that there is a close relation between utilization of the nutritional elements and decolorization. The significant decolorization (73.46 ± 0.34%)

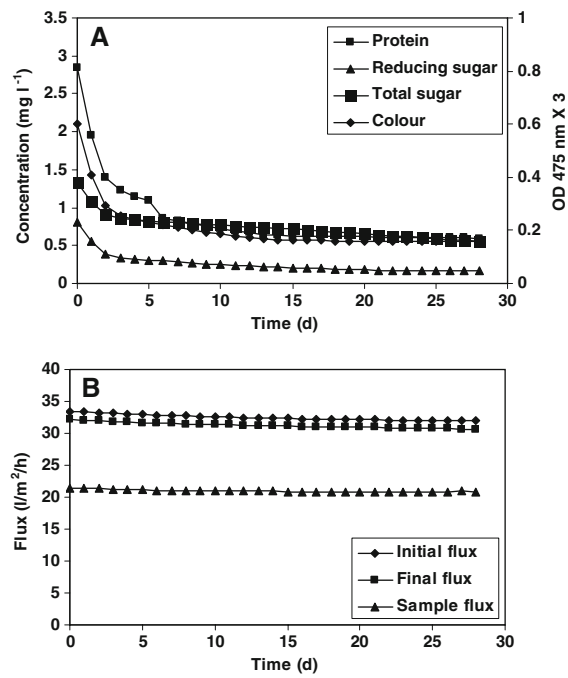

Fig. 3 MSW decolorization using batch mode of operation. **a** Decolorization, **b** flux profile; initial reactor feed volume 400 ml; sample volume 5 ml 24 h^{-1}; total duration of study 28 days

obtained for the 15–28 day support this observation. The steady membrane flux pattern was observed through out the experiment (Fig. 3b) which was similar to the stabilized flux obtained in earlier experiment (Fed batch study). Sofar, membrane was used continuously for 63 days (3.1 & 3.2). This indicates better flux regeneration capacity of the membrane.

Effect of MSW concentration

This study was planed to check the reactor and membrane behavior at higher MSW concentration. The same reactor feed (250 ml) left in the above study ("Batch study") was used to evaluate the performance of the biomass. So far, most of the microbial decolorization studies have been carried out at low (6–25%) MSW concentration (Ohmomo et al. 1987; Kumar et al. 1998; Fahy et al. 1997; Adikane et al. 2006). However, it is desirable to have a methodology for the decolorization of higher MSW concentration as to make it commercially viable. The reactor was run in a fed batch mode and the sampling and addition of feed was done after every 24 h. Experiment was started by the addition of 12.5% MSW and it was continued till the constant four consecutive readings obtained. The reactor was reached nearly to steady state on the 18 day (Fig. 4a). The average decolorization for 12.5% MSW calculated for the 4 days (18–21 days) was 76.95 ± 0.08%. Therefore, it was decided to stop the addition of feed on the 21 day and the next addition of feed containing 25% MSW was started on the same day. Similarly, the addition of feed containing 37.5 and 50% MSW was started on 42 and 63 day, respectively. The average decolorization calculated for the 4 days for 25% (39–42 days), 37.5% (60–63 days), and 50% (81–84 days) MSW was 70.41 ± 0.12, 56.47 ± 0.17, and 48.78 ± 0.09%, respectively. In comparison with the batch study, the decolorization obtained for 12.5% MSW was around 3.5% higher. However, the decolorization obtained for 25% MSW (70.41 ± 0.12%) may be considered as significant. Because, the decolorization was achieved in the absence of any additional carbon or nitrogen source which was not reported so far. It appears that the present reactor can be effectively used for the biodegradation of 25% MSW. However, decrease in the utilization of nutritional elements was also observed at higher MSW concentration. In comparison with 12.5% MSW, the 11.72, 5.3, and 33.63% decrease in the utilization of protein, reducing sugar and total sugar, respectively was observed at 50% MSW. However, in comparison with the 25% MSW there was 4.35, 6.13, and 9.10% decrease at 37.5% MSW and 13.35, 9.0, and 27.97% decrease at 50% MSW in the utilization of protein, reducing sugar and total sugar, respectively. This indicates the inhibition of biomass growth at 37.5 and 50% MSW concentration which results in lower decolorization. The visible observation of the reactor during the study also supports this hypothesis. Interestingly in comparison with 12.5% MSW, there was 1.7, 3.8% increase in the utilization of protein, reducing sugar, respectively whereas 5.5% decrease in the utilization of total sugar at 25% MSW. This may be the reason behind the significant decolorization (70.41 ± 0.12%) obtained at 25% MSW concentration. Surprisingly, nearly constant readings were

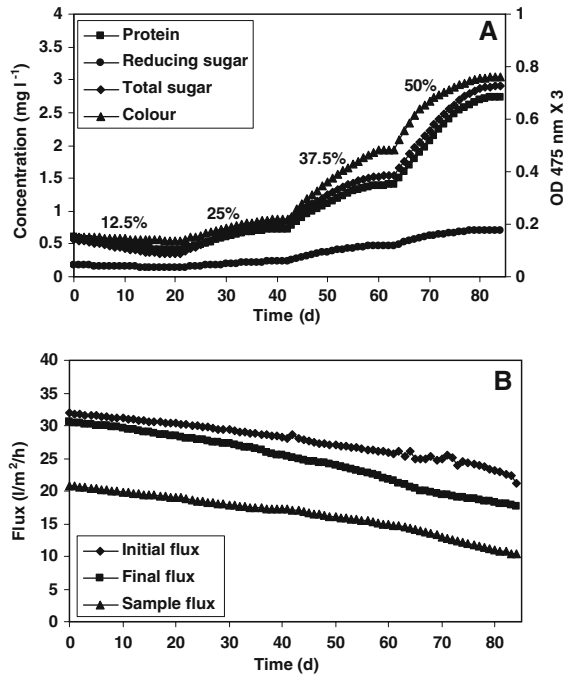

Fig. 4 Effect of different concentration of MSW on decolorization. This study is a continuation of earlier study (Fig. 3); **a** decolorization, **b** flux profile; reactor feed volume 250 ml; sample volume 10 ml 24 h^{-1}; feed volume that was added after every 24 h up to 21 day 10 ml 12.5% (v/v) MSW; feed volume that was added after every 24 h on 21 up to 42 day 10 ml 25% (v/v) MSW; feed volume that was added after every 24 h on 42 up to 63 day 10 ml 37.5% (v/v) MSW; feed volume that was added after every 24 h on 63 up to 84 day 10 ml 50% (v/v) MSW; total duration of study 84 days

obtained at all the MSW concentration after the 18 day (Fig. 4a). This suggests that the proper management of feeding rate/batch time may help to achieve significant decolorization of higher MSW concentration using present reactor system. There was significant effect of different MSW concentration on the flux profile (Fig. 4b). It was observed that as the concentration increases flux decreases. In comparison to the flux obtained on 28 day of batch study, the decrease in initial flux for 12.5, 25, 37.5, and 50% MSW concentration was 4.5 ± 0.27, 10.99 ± 0.77, 19.15 ± 0.9, and 30.22 ± 2.42%, respectively. The decrease in final flux for 12.5, 25, 37.5, and 50% MSW concentration was 6.49 ± 0.46, 16.57 ± 0.64, 29.67 ± 1.07, and 40.99 ± 0.55%, respectively. And the decrease in sample flux for 12.5, 25, 37.5, and 50% MSW concentration was 7.88 ± 0.52, 17.01 ± 0.25, 28.77 ± 0.69, and 48.94 ± 1.13%, respectively. Around 50% decrease in average sample flux was observed at 50% MSW concentration. The average sample flux was calculated for the 4 days as average decolorization was calculated above. This indicates significant fouling of the membrane at 50% MSW concentration.

The reactor regeneration study

In the above experiment (Effect of MSW concentration), there was significant increase in color, protein, reducing sugar and total sugar on the 84 day (Fig. 4a). It appears that the reactor enters in inhibition mode. Therefore, this experiment was planed to bring back the reactor in active mode without disturbing the existing experimental conditions. To achieve this, it was decided to add water till the reactor reaches to the state of 0 day of the above experiment (Effect of MSW concentration). The daily feeding and sampling was done instead of changing all the volumes to maintain the similarity in mode of operation. This may help to compare the performance of the reactor with the above studies. On the 30 day, 87.5, 86.91, 88.63, and 89.26% decrease in color, protein, reducing sugar and total sugar was obtained, respectively (Fig. 5a). The average decolorization obtained for the first 15 days was 42.49 ± 22.87 and 81.32 ± 4.3% for the next 15 days. This indicates the completion of reactor regeneration process. Similarly, the regeneration of membrane flux was also observed (Fig. 5b). In comparison with the 0 day of the above experiment (Effect of MSW concentration), 100% regeneration of sample

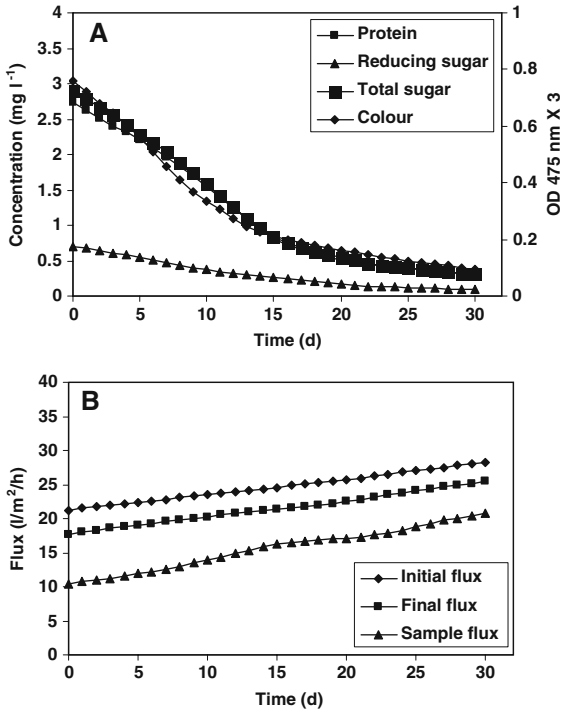

Fig. 5 Regeneration of reactor. This study is a continuation of earlier study (Fig. 4); **a** decolorization, **b** flux profile; reactor feed volume 250 ml; sample volume 10 ml 24 h^{-1}; feed volume that was added after every 24 h up to 30 day 10 ml distilled water; total duration of study 30 days

flux was obtained. Whereas 11.5 and 16.7% decrease in initial and final flux was also observed, respectively. However, the 100% regeneration of sample flux suggests good working life of the membrane.

The reactor reusability study

It appears that the above experiments (The reactor regeneration study) help to bring back the reactor to active mode. However, this experiment was planed to verify whether the decolorization capabilities of the reactor restored completely or not. The reactor was run in a fed batch mode for the 21 days. The sampling and addition of feed (12.5% MSW) was done after every 24 h. The reactor reached nearly to the steady state on 11 day (Fig. 6a). The average decolorization calculated for 11–21 days was 77.22 ± 0.13%. This was little higher to the decolorization (76.95 ± 0.08%) obtained in the earlier study for 12.5% MSW (Effect of MSW concentration). The average utilization of protein, reducing sugar, total sugar calculated for

11–21 days was 85.21 ± 0.78, 82.36 ± 0.46, 72.41 ± 1.7%, respectively. The results clearly indicate that the decolorization capability of the reactor was restored completely and reactor can be used effectively without changing the inoculum. So far, the inoculum which was generated in the above study (Batch study) was used over a period of 163 days at room temperature. This indicates that the once developed biomass can be used for longer time and there is no need to add inoculum again and again to achieve maximum decolorization. However, the flux pattern of the membrane showed decreasing trend (Fig. 6b). In comparison with the respective flux obtained on the last day of the above experiment (The reactor regeneration study), there was almost 12, 10, and 21% decrease in initial, final and sample flux, respectively. However, in comparison to the stabilized flux values obtained in the fed batch study, the overall decrease in flux was 35, 38, and 34% for initial, final and sample flux, respectively. This indicates that the overall fouling of the membrane was restricted to 35% which means almost 65% active flux of the membrane remains intact after 198 days of use. This may be attributed to the operation of membrane strictly at constant pressure (0.5 bar) through out the study.

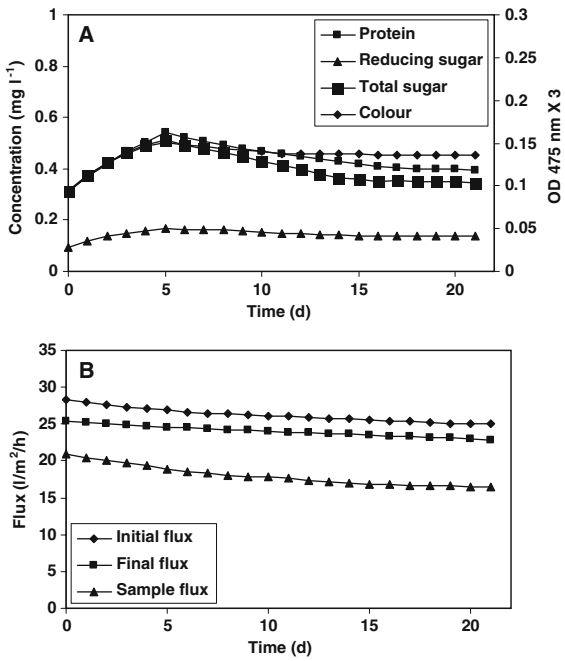

Fig. 6 Verification of reactor reusability. This study is a continuation of earlier study (Fig. 5); **a** decolorization, **b** flux profile; reactor feed volume 250 ml; sample volume 10 ml 24 h^{-1}; feed volume that was added after every 24 h up to 21 day 10 ml 12.5% (v/v) MSW; total duration of study 21 days

The mass balance and biomass characterization

After continuous use of reactor for 163 days using once developed soil inoculum, it is essential to know the mass balance of the components which generally used to sustain the biomass growth and nature of the biomass present in the reactor. To verify this, the reactor was harvested on the last day of the above study (The reactor reusability study) by centrifuging the mixture (treated MSW and biomass) present in the reactor vessel at 10,000 g and analysis of different components were carried out from the supernatant. Table 1 shows the results, 63% removal of COD was observed which suggests that the present system is also suitable for the effective biodegradation of MSW. This appears to be significant COD removal as it was achieved in absence of any nutritional supplement. However, 51% removal of COD from MSW was reported (Mohana et al. 2007) using bacterial consortium within 72 h in the presence of nutritional supplement (0.5% glucose etc.). To understand the nature of biomass present in the reactor, a loopful of suspension from the reactor vessel prior to harvesting was streaked on nutrient agar plates and different microbial cultures were isolated using standard microbial techniques (next publication will cover all the details). The MSW decolorization was carried out using each isolated culture and the cultures which showed higher decolorization (>30%) were selected

Table 1 Mass balance of different components utilized by biomass

Sample	Protein (g l^{-1})	Reducing sugar (g l^{-1})	Total sugar (g l^{-1})	COD (mg l^{-1})
Reactor feed	2.8	0.8	1.3	4,515
Reactor harvest	0.39	0.14	0.35	1,670
Utilized by biomass	2.4	0.66	0.95	2,845

for identification studies. Two bacterial and two fungal strains were identified as *Bacillus subtilis*, *Bacillus sphaericus and Aspergillus nidulans*, *Aspergillus terreus,* respectively which showed higher decolorization, further studies are in progress.

Conclusion

It is evident from the results that the significant decolorization of MSW can be achieved by using simple stirred vessel and once developed biomass using soil as sole inoculum in absence of any additives. The nutritional elements utilization pattern observed in the present study strongly suggest that the biomass growth in the reactor can be controlled by using proper feeding rate and MSW concentration. Interestingly, there was no foul smell in the collected samples as well as in and around the reactor which was present during the dilution of MSW. This suggests that the present system is capable of effective biodegradation of pollutants present in the MSW.

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