Results & Discussions

Chapter-4

CHAPTER 4

4.1 Isolation, screening, and evaluation of the decolorizing capacity of dye degrading bacteria

4.1.1 Isolation and Screening of Dye Decolorizing Bacterial Strains

Distinct colonies of bacteria having a zone of decolorization on the dye-agar Petri plates at 37° C after 48 h of incubation were selected for isolation. Sixty different colonies based on their morphology were further subcultured. And among them, 13 pure bacterial cultures were selected for purification. All isolates were named according to their morphology and shown in **Table 4.1**. Dye decolorization was studied for two model dyes using these 13 isolates and results were shown in **Table 4.1.** All the bacterial strains have the potential for decolorization of dye-containing nutrient medium, however, their decolorization efficiency varied substantially. Out of these 13 bacterial strains, two of them showing the highest decolorization for model dyes (**Figure 4.1**) and were selected for further studies.

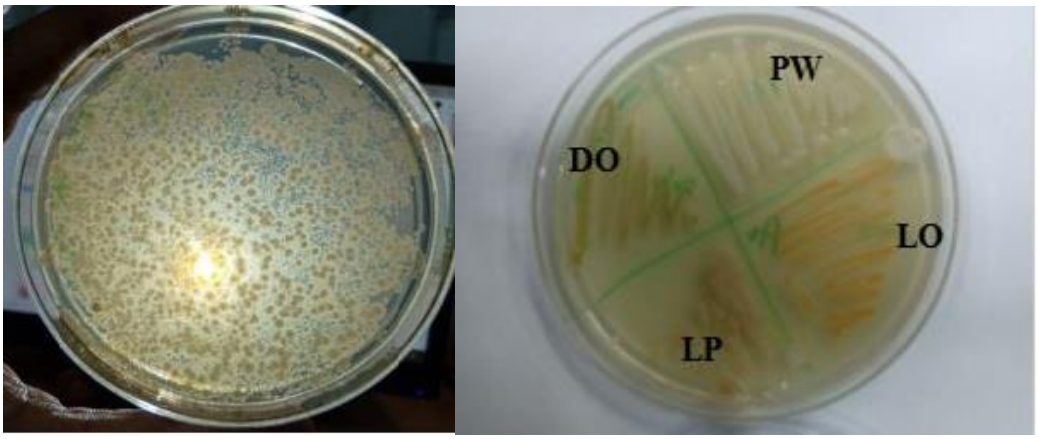

Figure 4.1 isolated strains

Table 4.1: Decolorization efficiency of all the isolated strains for RO 16 and RR 120 dyes for 100 ppm for 24 h.

S.no	Isolated strains	% decolorization	% decolorization
1	LO (light orange)	56	80
2	DO (dark orange)	81	50
3	PW (pure white)	30	20
4	LP (light pink)	23	27
5	DP (dark pink)	25	18
6	PW (peach white)	16	21
7	LY (light yellow)	29	35
8	WO (white orange)	41	26
9	WY (white-yellow)	32	37
10	WP (white pink)	30	15
11	WW1(white)	29	35
12	WW2 (white)	14	27
13	WW3 (white)	17	20

4.1.2 The Identification of Isolated Microorganisms

The results obtained from the 16S rRNA gene sequence for DO strain confirmed the presence of *Acinetobacter pitii*. *Acinetobacter species* are gram-negative, non-fermenting bacteria. They are coccobacilli and are found in soil, freshwater, and natural habitat (Jain et al., 2016). The morphological characteristics of the *Acinetobacter pitii* were observed by FE-SEM analysis **(Figure 4.2**). **Figure 4.2(a)** shows coccobacillus structure **Figure 4.2(b)** is the image of cellulose filter to clear the difference with and without the presence of bacteria.

Till now, these species were not reported for application in microbial fuel cells to degrade dye along with the production of electricity. A lot of research is going on this organism in the area of biodegradation of toxic waste (Liu et al., 2017; Potivichayanon et al., 2006).

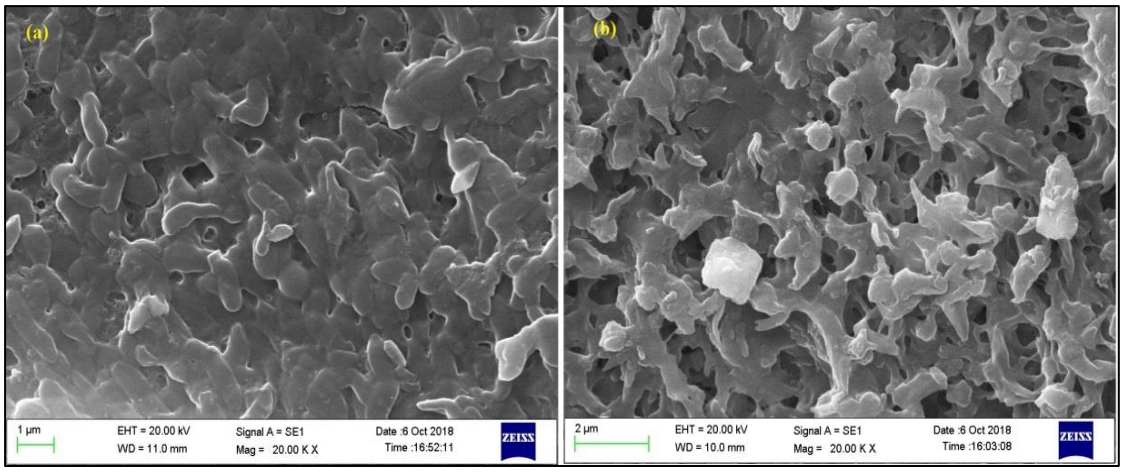

Figure 4. 2(a) SEM images of *Acinetobacter pitii* at 20 K X on the surface of cellulose filter 0.2 microns, 4.2(b)SEM images of cellulose filter 0.2 microns with growth media without bacteria at 20KX

These isolates are capable of degrading RO 16 dye up to a concentration of 500 ppm efficiently. Approximately 100 % color removal was observed for 100 ppm of dye for this isolate within 24h in the batch study. High removal of 80% and 70% were also observed at 250 and 500 ppm concentrations of dye when the batch time was increased from 24h to 72h. The phylogenic tree of ***Acinetobacter pitii*** is shown in **Figure 4.3**

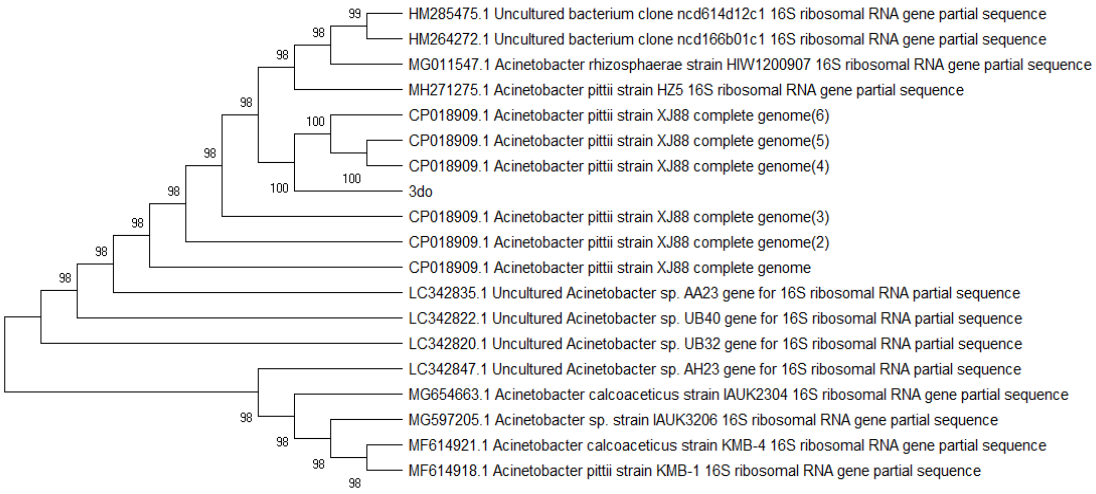

Figure 4.3 Phylogenic tree of the bacterial isolate (*Acinetobacter pitii*)

Another isolated LO strain was found to be the most efficient isolate for the degradation of RR120 and identified as *Staphylococcus equorum* using the 16S rRNA gene sequence which is a gram-positive facultative anaerobe. They are spherical (cocci) in shape and remain in the grape-like cluster and confirmed by FE-SEM (**Figure 4.4**). These species are a member of the coagulase-negative staphylococcus group and are abundantly found on the skin and mucous membranes of various animal and human clinical materials. They are mainly responsible for the aroma of fermented food like cheese and sausages (Irlinger et al., 2012; Nováková et al., 2006). The phylogenic tree is shown in **figure 4.5**

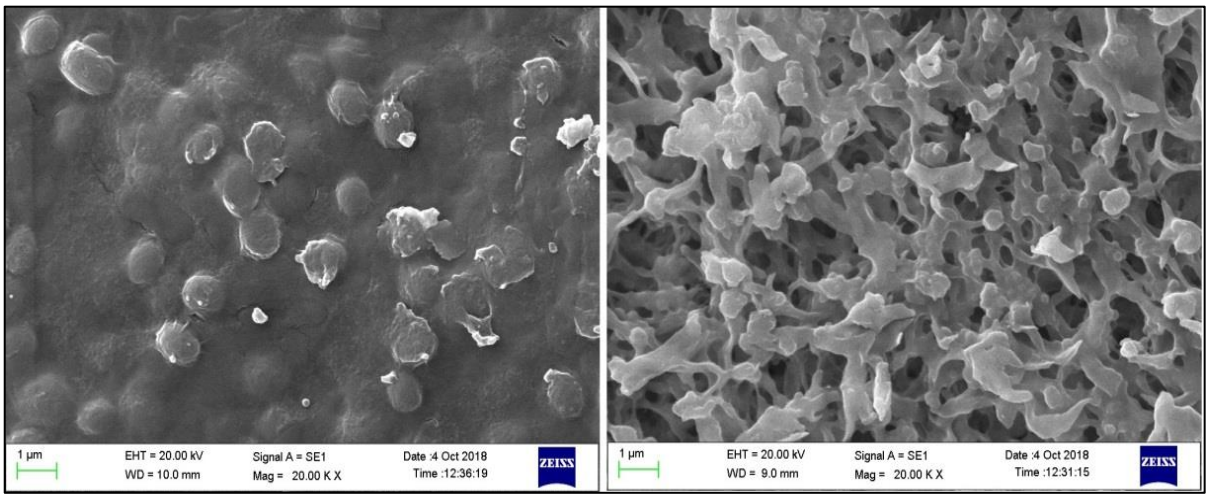

Figure 4.4 (a) SEM images of *Staphylococcus equorum*ta at 20 K X on the surface of cellulose filter 0.2 microns, (b) SEM images of cellulose filter 0.2 microns with growth media without bacteria at 20KX

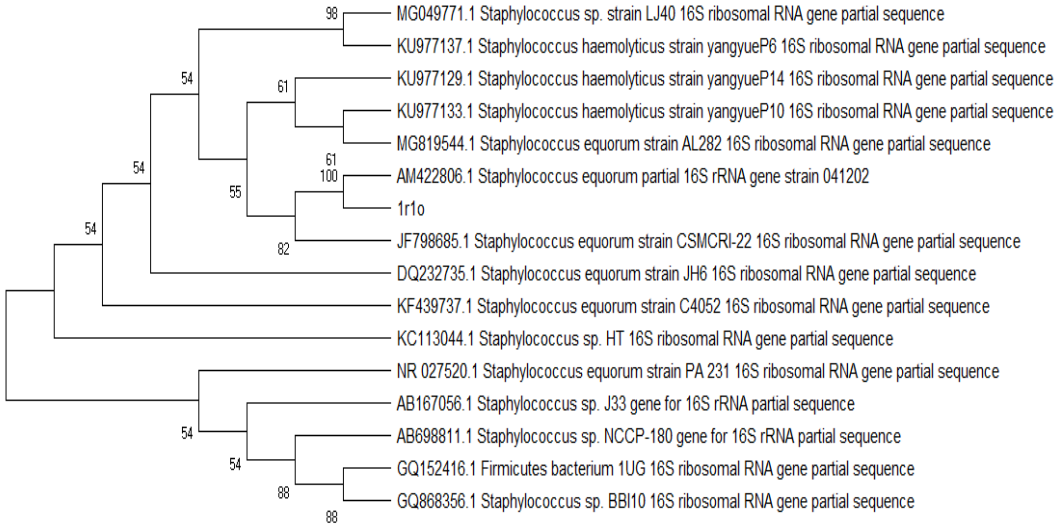

Figure 4.5: Phylogenic tree of the bacterial isolate (*Staphylococcus equorum* RAP1)

Details about the results of the maximum identification score of the isolates are given in Appendix 1.

4.1.3 Dye Decolorization Studies

To carry out an efficient MFC experiment, it is necessary to know the best suitable environment for dye degradation using isolated strains. For this, a batch study is performed under different environmental conditions. Batch decolorization studies have been carried out using two isolated strains for two model dyes. At the same time, MFC is also operated with the same strain and dye. A control (without inoculation) was also run parallel under the same condition to check the abiotic decolorization of the dye. All the experiments were carried out in static conditions at 25 ±5°C.

- **Time**

To evaluate the effect of batch time on degradation, 100 ppm dye solution of RO 16 and RR 120 were taken along with nutrient media in the conical flask (batch) and MFC. A control (without inoculums) run was also operated in parallel mode. All systems were left

to achieve saturation (maximum) level of degradation. And results are shown in **Figure 4.6.**

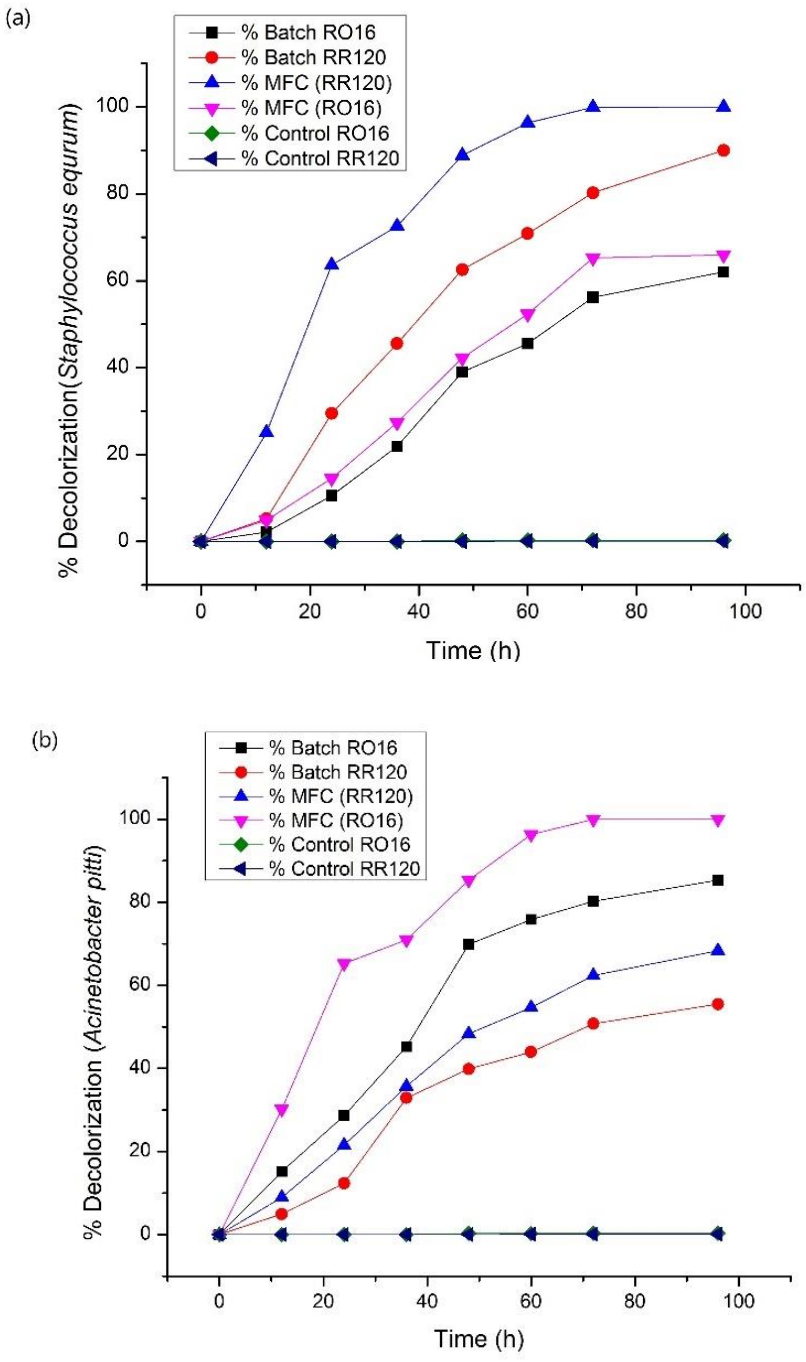

Figure 4.6 Study on isolated strains (a) *Staphylococcus eqrum* and (b) *Acinetobacter pitti* for degradation of RO16 dye and RR120 Dye in batch and MFC for 100 ppm initial concentration of dyes

It was cleared from **figure 4.6** that *Staphylococcus equorum* has higher decolorization for RR120 dye and *Acinetobacter pitti* for RO16. During the experiment, dye degradation was also observed in MFC and better degradation of dyes was found in MFCs as compared to batch studies. The % decolorization increased with and tending towards constant value. In the batch system % dye decolorization was found to be 80.23 % using *Staphylococcus equrum* for RR120 and 81 % using *Acinetobacter pitti* for RO16 after 72 h of operation. In the MFC 100 %, decolorization of both dyes was achieved in 72 hours which indicates the better performance of MFC as compared to the batch system for dye degradation. As discussed earlier that MFCs utilize oxygen as an electron acceptor. Hence, dye degradation in MFC could be expected higher than batch biodegradation (Ilamathi and Jayapriya, 2017; Yaqoob et al., 2020).

- **pH**

To optimize the pH, Concentration and time were fixed to 100 ppm and 72 h, and the rest condition was kept the same. Experiments were carried out at pH 5, 7, and 9. The pH values were selected keeping in mind that most of the enzymes responsible for dye reduction are active near-neutral pH (Nisar et al., 2017). It was observed (**Figure 4.7**) that the highest degradation for both the strain for model dyes was found to be maximum for 7 pH. This result is supported by many pieces of literature (Liu et al., 2019; Singh et al., 2015).

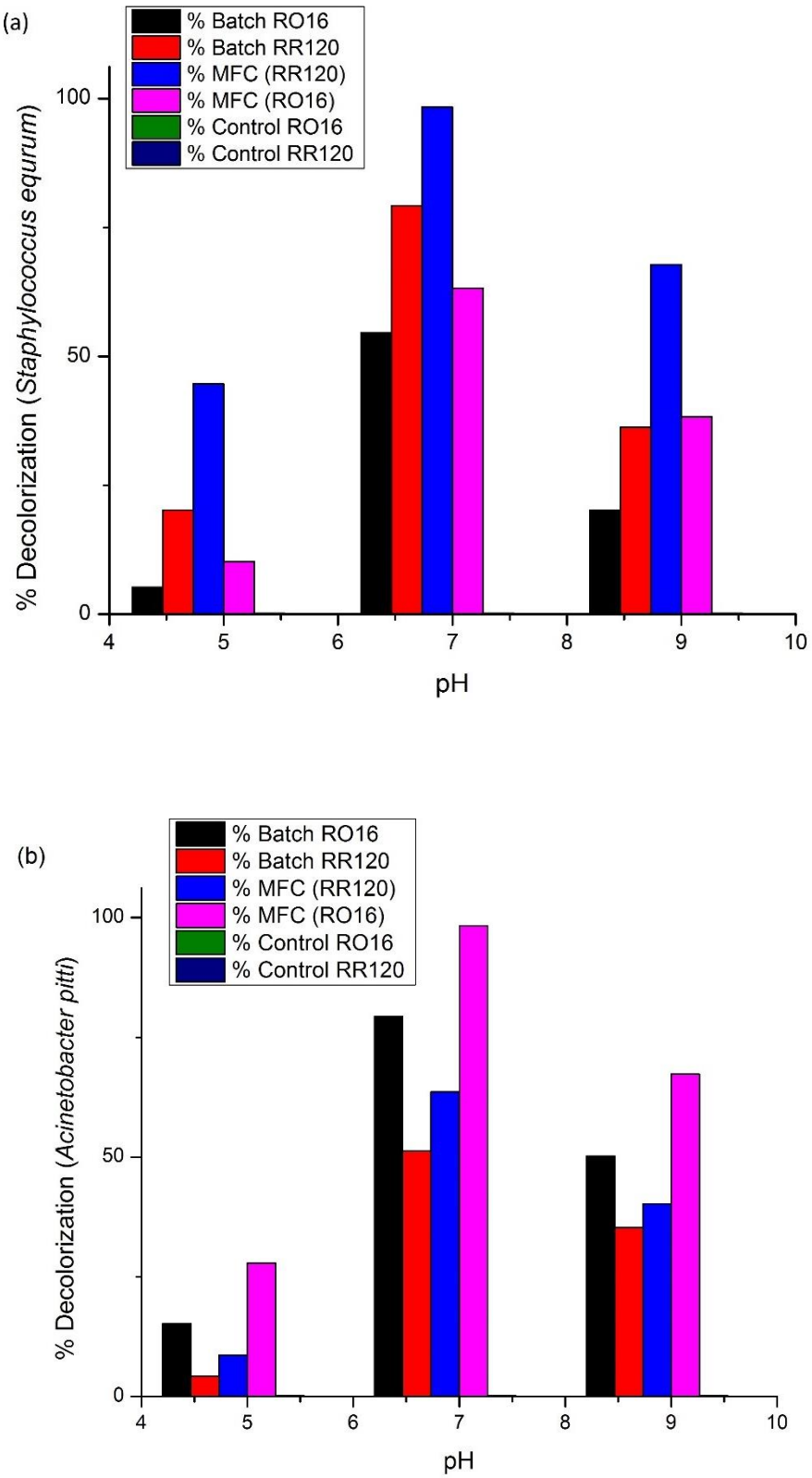

Figure 4.7 Study on isolated strains (a) *Staphylococcus eqrum* and (b) *Acinetobacter pitti* for degradation of RO16 dye and RR120 Dye in batch and MFC for 100 ppm initial concentration of dyes at 72 h

- **Concentration**

The bacterial species can withstand only up to a certain level of concentration (tolerance limit) of dye and beyond that level, the degradation rate decreases substantially. The tolerance limit is a very important factor for the real-time application of MFCs. Most of the color waste effluents contain dye in the range of 10 ppm to 250 ppm (O'Neill et al., 1999). % Decolorization (**Figure 4.8**) of RO 16 using *Acinetobacter pitti* was observed by varying its concentration in the range of 100-600 ppm and found that there was a constant decrease in % decolorization from 100 to 500 ppm but after 500 ppm a sharp decrease in % decolorization was observed. The result suggests that *Acinetobacter pitti* has a tolerance limit of approximately 500 ppm for RO 16 dye. In the case of RR120 same phenomena were observed after 300 ppm so, here we can conclude that *Staphyloccous equrum* has a tolerance limit of up 300 ppm for RR120 dye.

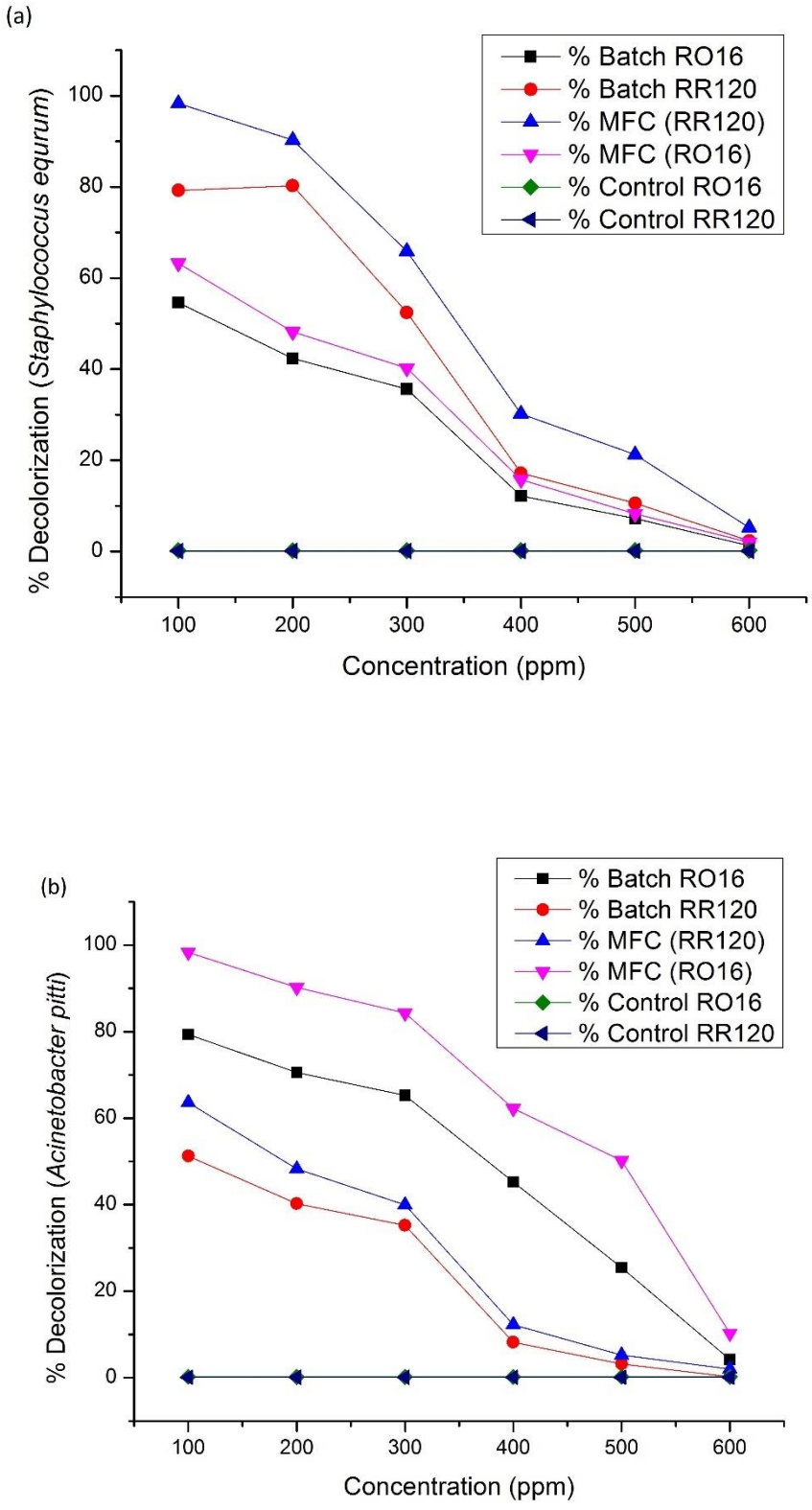

Figure 4.8 Study on isolated strains (a)*Staphylococcus eqrum* and (b)*Acinetobacter pitti* for degradation of RO16 dye and RR120 Dye in batch and MFC for 7 pH at 72 h.

4.1.4 Concluding remarks

Strains isolated from the primary clarifier of wastewater treatment plants have an excellent tolerance limit for the degradation of selected model dyes. Two most dye decolorizing potent strains LO and DO were found to be suitable for decolorizing model dyes and through 16s rDNA molecular characterization techniques identified as *Staphylococcus equrum* and *Acinetobacter pitti*. They can degrade a very high concentration of RO16 and RR120 dye. From the batch study, it was concluded that *Staphylococcus equrum* can degrade RR120 in a concentration range of 100 ppm to 300 ppm at slightly neutral pH and *Acinetobacter pitti* can degrade a very high concentration of RO16 100 to 500 ppm efficiently at neutral pH. Considering the results obtained through batch study, our further studies were carried out by taking into consideration the range of concentration of dyes, time, and pH for both model dyes with these isolated bacterial strains.