EFFECT OF PROCESS PARAMETERS ON DECOLORIZATION OF FOOD GRADE COLOR USING YEAST

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ABSTRACT

Colour is the most attractive property of foods but at the same time it may act as serious pollutants when their origin is dyes and dye stuffs. To remove them is the biggest problem facing by the world today. In present study was carried out with the aim using yeast to remove food dye concentration from the industrial waste water. Potent yeast culture SPN20 selected on the basis of time and percentage of decolorization. It was found that a potent yeast isolate was able to decolorize Rasberry red food dye (94.05%) at 100 mg/l in static condition (in 100 ml MEB concentration in 30 hrs). The optimum condition for Rasberry red food dye decolorization and degradation was observed at static condition as dye concentration 100 mg/l 82.47 per cent (12.64 mg/l/h), pH 84.03 per cent (12.88 mg/l/h), temperature 87.63 per cent (13.43 mg/l/h), inoculum size 92.48 per cent (14.18 mg/l/h), Malt extract broth medium optimization 94.05% (14.42 mg/l/h). UV-Visible, HPTLC and FTIR analysis of untreated food dye revealed that the pollutant compounds which were present in untreated food dye were decreased on concentration or degrades to further metabolites during treatment. Phytotoxicity study demonstrated no toxicity of the biodegraded products with respect to plants viz. Phaseolus mungo.

KEY WORDS: Biodegradation, Decolorization, FTIR. HPTLC, Rasberry red food color

INTRODUCTION

Colour is the most attractive property of foods. To make food more attractive dyes have been widely used to highlight their original colors, or to provide different ones. This practice has been usually done to meet consumer expectations. However, an increasing concern, regarding the high concentrations of dyes in effluents of food industries, has emerged: once reaching surface and ground waters, such residues can cause serious and unpredictable damages to the aquatic life in general (Pavanelli *et al*., 2010). Natural colors are not hazardous to environment while colors produced from dyes and dyestuffs may act as serious pollutants. Azo dyes are less biodegradable because of their structures and though they represent, a potential important class of organic pollutant, little is known about their fate. Synthetic food dyes are most widely composed by aromatic rings and chromophore groups (e.g. azo, anthraquinone) and present high stability and xenobiotic characteristics, hence they are not easily degraded (Silva *et al*., 2008). The treatment of Azo dyes containing effluent was initially carried by using

physical and chemical treatment processes like adsorption, concentration, chemical transformation, but with time, potential hazards and disadvantages of these methods were noted as, formation of toxic sludge and formation of even more toxic metabolites. Alternatively, approach is shifting towards the use of conventional microbial decolorization and degradation methods to treat such effluents and wastewater containing dyes and toxic chemicals. The metabolites produced after biodegradation are mostly non toxic or comparatively less toxic in nature (Chaube *et al*., 2010).

MATERIALS AND METHODS

Food dye and chemicals

The food grade dye Rasberry red (a blend of NaCl and Carmoisine C.I. 14720, sunset yellow F. C. F. I. 15985) was purchased from General Stores Ahmedabad, Gujarat (India). The media components and chemicals were purchased from Hi media Labs, Bombay (India). All chemicals used were of highest purity analytical grade.

Collection of sample and media composition for screening of yeast

Soil samples were collected from Biogas center, Sadra (Gujarat), curd sample were collected from General store Sadra (Gujarat) and rotten fruits were collected from fruit market of Sadra (Gujarat) and stored at 4°C temperature in refrigerator. Decolorization was carried out in MALT EXTRACT BROTH medium of pH 6.5 ± 0.2 (37°C) contained g/l the following composition: Malt Extract (6.00), Yeast Extract (1.20), Maltose (1.80), Dextrose (6.00).

Isolation and screening of Food color decolorizing yeast

The microorganisms present in rotten fruits were enriched in a growth medium. The food colour decolorizing yeast cultures were carried out by enrichment culture technique using MEB with food colors (100 mg/l). The medium was autoclaved at 121°C for 15 minutes at 15 psi. Soil sample of 5 g and 5 ml Juice of rotten fruit were aseptically inoculated into 100 ml media in 250 ml Erlenmeyer flask, incubated under static condition at 37°C temperature, for 24 hrs. Yeast which showed decolorization in liquid medium was further transferred in fresh medium. A loopful sample from the decolorized liquid medium was streaked on the malt extract agar plates and incubated at 37°C for 24 hrs. The different colonies found on the plates after incubation were checked for ability of decolorization by yeast. All isolated yeast cultures were preserved on MEA slant. The slant preserve at 4°C temperature in refrigerator. This procedure was repeated at every 15 days.

Determination of optical density (OD) of culture supernant

Samples from experimental and control flasks were clarified. The OD of the supernant was determined with spectrophotometer (λmax540). Per cent dye decolorization was calculated as per the following formula:

$$\text{Per cent of decolorization} = \frac{(\text{Initial absorbance} - \text{Final absorbance})}{(\text{Final absorbance})} \times 100$$

Identification of potent yeast

Identification of potent yeast was done based on colony characteristic, gram staining and biochemical test.

Effect of physicochemical parameters on RRFD decolorization

Effect of various parameters such as dye concentration, inoculums size, temperature, pH and total media optimization on model dye RRFD decolorization was studied one at a time. The experiment was carried out in 250 ml Erlenmeyer flask containing 100 ml MEB medium and incubated at 37˚C temperature in static condition.

Effect of static and shaking condition on RRFD decolorization

Yeast was grown for 24 hrs in Erlenmeyer flask containing 100 ml MEB to study the effect of static and shaking condition on decolorization performance of yeast culture. After flasks was incubated at static as well as shaking condition at 37°C for 120 rpm on orbital shaker. The aliquot (3 ml) of the culture media was withdrawn at different time intervals, centrifuged at 5000 rpm for 15 min. Decolorization was monitored by measuring the absorbance of supernatant at the 540 nm.

Effect of food dye concentration on RRFD decolorization

In order to examine the effect of initial dye concentration on the decolorization in static condition, MEB medium was added with 100, 200, 300, 400 and 500 mg/l of the RRFD. The per cent decolorization was measured at different time interval. The optimum food dye concentration was used in all the experiments.

Effect of pH on RRFD decolorization

Different pH i.e. 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 were studied to check their effect on RRFD decolorization. The pH was adjusted using 1N HCL or 1N NaOH. After inoculation and incubation, assay for decolorization was carried out.

Effect of temperature on RRFD decolorization

Temperature is the key factor which regulates the rate of metabolic activity of organism. So, to determine the effect of temperature on RRFD decolorization, the flask was inoculated and incubated at different temperatures i.e. 34, 37, 40, 42 and 45°C.

Effect of inoculum size on RRFD decolorization

Density of cells in suspension is also one of the most important parameter for the decolorization of RRFD. The flasks were inoculated with different inoculum size i.e. 1, 2, 3, 4 and 5 per cent. After incubation assay for decolorization was carried out.

Effect of malt extract broth medium optimization on RRFD decolorization

The effect of malt extract broth medium on optimization of decolorization of RRFD at different concentration was studied with 1.7, 1.9, 2.1, 2.3, 2.5, 2.7, 2.9 and 3.1 g% concentrations were examined.

Comparison between unoptimized and optimized condition

The comparative study was carried out to find out effect of optimized decolorization and degradation on RRFD.

Analytical procedure

Decolorization was monitored by UV-Vis spectroscopic analysis; whereas biodegradation was monitored by High Pressure Thin Layer Chromatography (HPTLC) and Fourier Transform Infrared Spectroscopy (FTIR).

Toxicity study

Phytotoxicity tests were performed in order to assess the toxicity of the untreated and treated food dye. The phytotoxicity study was carried out (at room temperature) on *Phaseolus mungo* (35 Seeds) separately on 35 ml sample of control Rasberry red food dye and its degradation products (100 ppm) per day. Control set was carried out using distilled water at the same time. Germination (%) as well as the length of plumule (shoot) and radical (root) was recorded after 7 days. The germination percentage was calculated by using the following formula:

$$\text{Germination (\%)} = \frac{\text{No.of seeds germinated}}{\text{No.of seeds sowed} \times 100}$$

RESULTS AND DISCUSSION

Isolation and cultivation of food dye decolorizing yeast

Isolation from soil, curd and rotten fruits such as banana, orange, anar, apple, lemon, tomato and grapes were carried out by the enrichment culture technique using

RRFD as a sole source of energy. SPN20 selected for the further study on the basis of percentage of decolorization. The result shows the percentage of decolorization and decolorization rate of different yeast isolates (Table 1). Among the 20 yeast cultures, the culture designated as SPN20 was found to be most efficient since it could decolorize RRFD 94.05 per cent.

Identification of food dye decolorizing yeast

Twenty different yeast species were isolated from the different rotten fruits, soil and curd sample. Identification of potent yeast from rotten banana was done on the basis of cultural characteristic (Table 2), microscopic observation (Table 3) and biochemical test (Table 4**)**. The selected isolate SPN20 was most probably identified as *Candida norvegensis* on the basis of biochemical test.

Effect of physicochemical parameters on RRFD decolorization

Effect of static and shaking condition on RRFD decolorization

In this study, it was found that the shaking condition does not support rapid decolorization of the RRFD by yeast (SPN20) (Figure 1). Whereas under static condition,n decolorization occur very rapid (Figure 2). The results found in the study are very similar to Saratale *et al*. (2009b). They found that decolorization of Navy Blue HER was 100 per cent under static condition and 30 per cent under shaking condition. The growth of *T. beigelii* was also observed to be more under static condition (9.2 g/l) as compared to shaking condition (4.2 g/l). According to Kalyani *et al.* (2009), agitated culture of *Pseudomonas sp.* SUK1 showed almost no decolorization in 24 hrs, while the static culture decolorized more than 96 per cent of the initial dye concentration (300 mg/l) of Reactive Red 2 in 6 hrs.

Effect of food dye concentration on RRFD decolorization

Increase in dye concentration resulted in a significant change in percentage decolorization as well as the time required for decolorization. The percentage of decolorization (82.47 ± 0.05 %) and decolorization rate (12.64 mg/l/h) within 30 hrs (Figure 3). Similar results were observed in Bacterial isolate *Psychrobacter alimentarius* used for the degradation of reactive Black 5 (100 mg/l) dye in presence of high salt concentration (Khalid *et al.*, 2012). Dye concentration can influence the efficiency of microbial decolorization through a combination of factors including the toxicity imposed by dye at higher concentration (Sahasrabudhe and Pathode, 2011). The results indicated that increase in dye concentration might be affecting overall growth and enzyme systems involved in decolorization of RRFD, ultimately resulting into the decrease decolorization rate.

Effect of pH on RRFD decolorization

Generally, yeasts show better decolorization and biodegradation activities at acidic or neutral pH. The hydrogen ion concentration showed profound effect on the biological activities of the organism. In the present study, the maximum efficiency of percentage of decolorization (84.03 ± 0.02%) and decolorization rate (12.88 mg/l) was achieved at pH 6.0 within 30 hrs (Figure 4). Similar results have been reported by Du *et al.* (2011) for biodegradation of malachite green by *Pseudomonas sp.* strain DY1 under aerobic condition. Samthima *et al.* (2009) studied decolorization of synthetic dyes by white-rot fungus *Lentinus polychrous*. They found that the optimum pH for decolorization of Methyl Red was 5.0.

Effect of temperature on RRFD decolorization

Temperature is the key factor, which affect the cell and its metabolic reaction

during breakdown or utilization of complex carbon compound in form of food dye. So, as to investigate effect of temperature ranging from 34 to 45 °C and checked the percentage of decolorization and degradation. Maximum percentage of decolorization and degradation was observed at 40 °C (87.63 ± 0.04 %) (With decolorization rate 13.43 mg/l/h) (Figure 5). Wang and Yuen (2011) observed results are strongly supported to the results of present study. They suggested optimum temperature at 40 °C during their study on decolorization of the azo dye by bacterial isolate. At higher temperature, percentage of decolorization was rapidly decreased. Sahasrabudhe and Pathode (2011) also reported significant decolorization at 40 °C.

Effect of inoculum size on RRFD decolorization

Inoculum size play an important role because of sufficient cell biomass required to decolorize and degrade substrate in form of dye to degrade products. In order to find out the optimum inoculum needed for faster and higher percentage of decolorization by potent yeast SPN20, decolorizing ability was tested at different inoculum concentration from 1 to 5% (vv^{-1}). The decolorization rate increased with increase inoculum size, reached the maximum rate of decolorization 14.18 mg/l at 4 % (vv^{-1}) inoculum size with 92.48 ± 0.03 % of decolorization within 30 hrs (Figure 6). This type of results was shown by Tripathi and Srivastava, (2012). In inoculum size up to 5.5 per cent (vv^{-1}), increases the dye decolorization, but started decreasing upon further augmentation. The maximum decolorization was obtained at inoculum size 5.0 per cent (vv^{-1}). Orange G decolorization by *B. megaterium* ITBHU01 of 94.48 per cent was optimized. Ponraj *et al*. (2011) also showed decolorization high activity of *Bacillus sp*. (89.72%) in 4 per cent of inoculums.

Effect of malt extract broth medium optimization on RRFD decolorization

The effect of various concentration of the readymade Malt Extract Broth (MEB) medium was studied on the decolorization activity of the yeast (SPN20). It was found that as the concentration of Malt Extract Broth increases from 1.7 to 3.1 g%, decolorizations of the RRFD by the selected organism (SPN20) increases at specific level then it was decreases. Therefore, it indicated that 2.9 g% concentration of Malt Extract Broth is preferred by the yeast (SPN20) for maximum decolorization (Figure 7). Azo dye degradation by different microorganisms will generally respond differently towards different sources of carbon and nitrogen (Solís *et al.*, 2012). Carbon sources seemed to be effective to promote the decolorization probably due to the preference of the cells in assimilating the added carbon sources over using the dye compound as the carbon source (Chaube *et al.*, 2010).

Comparison between unoptimized and optimized condition

In the present study, yeast SPN20 gave the more decolorization in optimum condition as compared to un-optimized condition (Table 5). In the un-optimized condition, enzyme activity for decolorization is less probably due to some nutrients or condition which might be affect the dye concentration.

Analytical procedure

UV-Visible spectroscopy

The biodegradation of RRFD was monitored by UV-Vis analysis. Untreated RRFD (Figure 8 and 9) presented absorbance peaks at 540 nm. For treated RRFD after biodecolorization, the absorbance peaks in the visible region disappeared, indicating complete decolorization. In the U.V. spectra, the peak from 540 nm was disappeared.

HPTLC Analysis

Degradation activity of SPN20 was further supported by HPTLC. The effluent chromatograms were observed in UV light (254 nm) (Figure 10). The TLC plate was scanned at 254 nm for two different spots (Table 6). This suggests that there are new compounds formed due to degradation of pollutant compound. Similar results were observed by Sheth and Dave (2009) and Joshi *et al*. (2010).

FTIR analysis

The FTIR spectrum of control dye and metabolites by yeast was compared (Figure 11 and 12). The presence of newly formed peaks in test (20) at different cm^{-1} in comparison to control. The appearance of peaks in compared to control between 1650.24 to 1120.53 cm^{-1} may be due to enzymatic degradation of aromatic hydrocarbon by bacterial consortium. The presence of peaks from finger printing region in the range of 1650.24 to 1119.28 cm^{-1} in compared to control indicates the absence of azo (N=N) bonds, due to degradation of aromatic compounds and formation of new peaks represented charged aromatic. Two newly appeared peaks at 1650.24 and 1100.20 cm^{-1} in compared to control clearly indicates yeast isolate degraded aromatic compounds and produced primary aromatic amines with C-N stretch. From the observed analytical results, it indicated that appearance of new peaks and the absence of the majority of peaks from control sample representing the azo reductase activity catalyzed reductive cleavage of azo bonds. Several investigators (Saratale *et al*., 2009a; Balakrishnan *et al*., 2011; Tripathi and Srivastava, 2012) have reported similar patterns for FTIR spectra.

Toxicity study

Despite the fact that untreated dyeing effluents might cause serious environmental and health hazards, they are being disposed off in water bodies and this water can be used for the agriculture purpose in India. Use of untreated and treated dyeing effluents in agriculture has direct impact on fertility of soil. The relative sensitivity towards the food dye Rasberry red and its degradation products in relation to *Phaseolus mungo* were studied. The mean of plumule length and radical length of *Phaseolus mungo* were 13.9 and 4.3 cm, respectively, of 35 seeds in distilled water as a control with 100 per cent germination. The germination of *Phaseolus mungo* seeds inhibited 10 per cent when seeds treated with 100 ppm concentration of Rasberry red food dye, whereas the plumule length and radical length was found 4.9 and 1.4 cm, respectively with 100 per cent germination when treated with 100 ppm degradation products (Table 7). This study indicates the detoxification of Rasberry red food dye by *Candida norvegensis.* The results are similar to Kurade *et al.* (2013). They reported that phytotoxicity study assured the detoxification of Remazol red and Rubine GFL. Phytotoxicity study carried out with dye and dye metabolites using *Phaseolus mungo* indicated the detoxification of dye.

CONCLUSION

It was found that a potent yeast isolate SPN20 was able to decolorize Rasberry red food dye (94.05%) at 100 mg/l in static condition (in 100 ml MEB concentration in 30 hrs). The optimum condition for Rasberry red food dye decolorization and degradation was observed at static condition as dye concentration 100 mg/l 82.47 per cent (12.64 mg/l/h), pH 84.03 per cent (12.88 mg/l/h), temperature 87.63 per cent (13.43 mg/l/h), inoculum size 92.48 per cent (14.18 mg/l/h), Malt extract broth medium optimization 94.05% (14.42 mg/l/h). UV-Visible, HPTLC and FTIR analysis of untreated food dye revealed that the pollutant compounds which were present in untreated food dye were decreased on

concentration or degrades to further metabolites during treatment. Phytotoxicity study demonstrated no toxicity of the biodegraded products with respect to plants viz. Phaseolus mungo.

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REFERENCES

Balakrishnan, R. B.; Anand, K. P.; Saravanan, A. K. and Sirasanaganbla, U. B. (2011). Treatment of dye effluent by electrochemical and biological processes. *Open Journal of Safety Science and Technology*, **1**: 12-18.

Chaube, P.; Indurkar, H. and Moghe, S. (2010). Biodegradation and decolorization of dye by mix consortia of bacteria and study of toxicity on *Phaseolus mungo* and *Tritium aestivum*, *Asiatic J. Biotech. Resourc.*, **1:** 45-56.

Du, L. N.; Wang, S.; Li, G.; Wang, B.; Jia, X. M.; Zhao, Y. H. and Chen, Y. L. (2011). Biodegradation of malachite green by *Pseudomonas sp.* strain DY1 under aerobic condition: characteristics, degradation products, enzyme analysis and phytotoxicity. *Ecotoxicology*, **20**: 438–446.

Joshi, S. M.; Inamdar, S. A.; Telke, A. A.; Tamboli, D. P. and Govindwar, S. P. (2010). Exploring the potential of natural bacterial consortium to degrade mixture of dyes and textile effluent. *International Biodeterioration and Biodegradation*, **64**: 622-628.

Kalyani, D. C.; Telke, A. A.; Dhanve, R. S. and Jadhav, J. P. (2009). Eco-friendly biodegradation and detoxification of Reactive Red 2 textile dye by newly isolated *Pseudomonas sp*. SUK 1. *Journal of Hazardous Materials*, **163**: 735-742.

Khalid, A.; Kausar, F.; Arshad, M.; Mahmood, T. and Ahmed, I. (2012). Accelerated decolorization of reactive azo dyes under saline condition by bacteria isolated from Arabian Seawater Sediment. *Appl. Microbiol. Biotech.,* **96**: 1599-1606

Kurade, M. B.; Waghmode, T. R.; Tamboli, D. P. and Govindwar, S. P. (2013). Differential catalytic action of *Brevibacillus laterosporus* on two dissimilar azo dyes Remazol red and Rubine GFL, *J. Basic Microbiol.*, **53**: 136-146.

Pavanelli, S. P.; Bispo, G. L.; Nascentes, C. C. and Augusti, R. (2010). Degradation of food dyes by zero-valent metals exposed to ultrasonic and electro spray ionization mass spectrophotometry monitoring. *J. Brazilian Chem. Soc.*, **22**: 111-119

Ponraj, M.; Gokila, K. and Zambare, V. (2011). Bacterial decolorization of textile dye Orange 3R. *Int. J. Adv. Biotech. Res.*, **2**: 168-177.

Sahasrabudhe, M. M. and Pathode, G. R. (2011). Decolorization of C.I. Reactive Yellow 145 by *Enterococcus faecalis* strain YZ66: *Arch. Appl. Sci. Res.*, **3**(3): 403-414.

Samthima, R.; Khammuang, S.; and Svasti, J. (2009). Extracellular ligninolytic enzymes by *Lentinus polychrous u*nder solid state fermentation of potential agro industrial wastes and their effectiveness in decolorization of synthetic dyes. *Biotech. Bioprocess Engg.,***14**: 513-522.

Saratale, R. G.; Saratale, G. D.; Chang, J. S. and Govindwar, S. P. (2009b). Decolorization and biodegradation of textile dye Navy blue HER by *Trichosporon beigelii* NCIM-3326,

Journal of Hazardous Materials, **166**: 1421-1428

Saratale, R. G.; Saratale, G. D.; Kalyani, D. C.; Chang, J. S. and Govindwar, S. P. (2009a). Enhanced decolorization and biodegradation of textile azo dye Scarlet R by using developed microbial consortium-GR. *Bioresource Technology*, **100**(9): 2493-2500.

Sheth, N. and Dave, S. (2009). Optimization for enhance decolorization and degradation of Reactive Red BS C.I. 111 by *Pseudomonas aeruginosa* NGKTGS. *Biodegradation*, **20**(6): 827-836.

Silva, C. P.; Marmitt, S.; Haetinger, C. and Stulp, S. (2008). Amaranth food dye photochemical and photoelectron chemical degradation: Experiments and mathematical modeling, *Journal of WSEAS Translocations on Systems*, **7**: 793-803

Solís, M.; Solís, A.; Pérez, H. I.; Manjarrezb, N. and Floresa, M. (2012). Microbial decolourization of azo dyes: A review. *Process Biochemistry*, **47**: 1723-1748.

Tripathi, A. and Srivastava, S. (2012). Biodegradation of orange G by a novel isolated bacterial strain *Bacillus megaterium* ITBHU01 using response surface methodology, *African J. Biotech.*, **1**: 1768-1781

Wang, P. K. and Yuen, P. Y. (2011). Decolorization and biodegradation of methyl red by *Klebsiella pneumoniae* RS-13, *J. Basic Microbiol.*, **30**: 1736-1744.

Table 1: Different yeast capable of decolorizing RRFD dyes

Isolates	Time of Decolorization (Hrs)	Decolorization (%)	Average Rate of Decolorization (mg/L/h)
SPN 1	30	75.43	21.40
SPN 2	30	82.15	23.94
SPN 3	30	56.80	14.39
SPN 4	30	45.85	14.06
SPN 5	30	86.69	26.58
SPN 6	30	84.19	25.82
SPN 7	30	87.79	26.92
SPN 8	30	88.10	27.02
SPN 9	30	87.48	26.83
SPN 10	30	46.16	8.84
SPN 11	30	39.12	7.49
SPN 12	30	28.63	5.48
SPN 13	30	46.32	8.87
SPN 14	30	44.60	8.54
SPN 15	30	82.00	15.71
SPN 16	30	72.14	13.71
SPN 17	30	53.20	13.82
SPN 18	30	34.89	6.68
SPN 19	30	44.60	8.54
SPN20	30	94.05	14.42

Table 2: Cultural characteristics of SPN_{20}

Sr. No	Characteristics	SPN_{20}
1	Size	Big
2	Shape	Round
3	Margin	Entire
4	Elevation	Convex
5	Texture	Smooth
6	Opacity	Opaque
7	Pigment	White

Table 3: Microscopic observation of SPN_{20}

Sr. No	Characteristics	SPN_{20}
1	Gram's reaction	+Ve
2	Size	Big
3	Shape	Round
4	Occurrence	Single

Table 4: Result of biochemical test

Sr. No	Test	Result of SPN20
1	L-Lysine-ARYLAMIDASE	Negative
2	L-MALATE assimilation	Positive
3	Leucine-ARYLAMIDASE	Positive
4	ARGININE GP	Negative
5	ERYTHRITOL assimilation	Negative
6	GLYCEROL assimilation	Positive
7	Tyrosine ARYLAMIDASE	Positive
8	BETA-N-ACETYL-GLUCOSAMINIDASE	Negative
9	ARBUTINE assimilation	Negative
10	AMYGDALINE assimilation	Negative
11	D-GALACTOSE assimilation	Negative
12	GENTIOBIOSE assimilation	Negative
13	D-GLUCOSE assimilation	Positive
14	LACTOSE assimilation	Negative
15	METHYL-A-D-GLUCOPYRANOSIDE assimilation	Negative
16	D-CELLOBIOSE assimilation	Negative
17	GAMMA-GLUTAMYL-TRANSFERASE	Negative
18	D-MALTOSE assimilation	Negative
19	D-RAFFINOSE assimilation	Negative
20	PNP-N-acetyl-BD-galactosaminidase 1	Negative
21	D-MANNOSE assimilation	Positive
22	D-MELIBIOSE assimilation	Negative
23	D-MELEZITOSE assimilation	Negative
24	L-SORBOSE assimilation	Negative
25	L-RHAMNOSE assimilation	Negative
26	XYLITOL assimilation	Negative
27	D-SORBITOL assimilation	Negative
28	SACCHAROSE/SUCROSE assimilation	Negative
29	UREASE	Negative
30	ALPHA-GLUCOSIDASE	Negative
31	D-TURANOSE assimilation	Negative
32	D-TREHALOSE assimilation	Negative
33	NITRATE assimilation	Negative
34	L-ARABINOSE assimilation	Negative
35	D-GALACTURONATE assimilation	Negative
36	ESCULIN hydrolyse	Negative
37	L-GLUTAMATE assimilation	Positive
38	D-XYLOSE assimilation	Negative
39	DL-LACTATE assimilation	Positive
40	ACETATE assimilation	Positive
41	CITRATE (SODIUM) assimilation	Negative
42	GLUCURONATE ASSIMILATION	Negative
43	L-PROLINE assimilation	Positive
44	2-KETO-D-GLUCONATE assimilation	Negative
45	N-ACETYL-GLUCOSAMINE assimilation	Negative
46	D-GLUCONATE assimilation	Negative

Table 5: Comparison of unoptimized and optimized condition on RRFD decolorization

Sr. No	Parameter	Un-optimized	Optimized
1	% of decolorization	67.13 ± 0.04	94.05 ± 0.03
2	Average decolorization rate (mg/l/h)	10.29	14.49

Table 6: Scanning report of TLC revealed different peaks and R_f values of control and degraded products

Sample	Track	254 nm peck	254 nm R_f values
Control	1	1	0.20
		2	0.23
		3	0.35
		4	0.47
		5	0.60
		6	0.77
Test	2	1	0.54
		2	0.71
		3	0.77

Table 7*:* Phytotoxicity study of RRFD and degraded products on *Phaseolus mungo*

Parameters studied	Tap water	Untreated RRFD	Decolorized RRFD
Germination (%)	100	90	100
Plumule (cm)	13.9	4.9	13.8
Radical (cm)	4.3	1.4	4.1

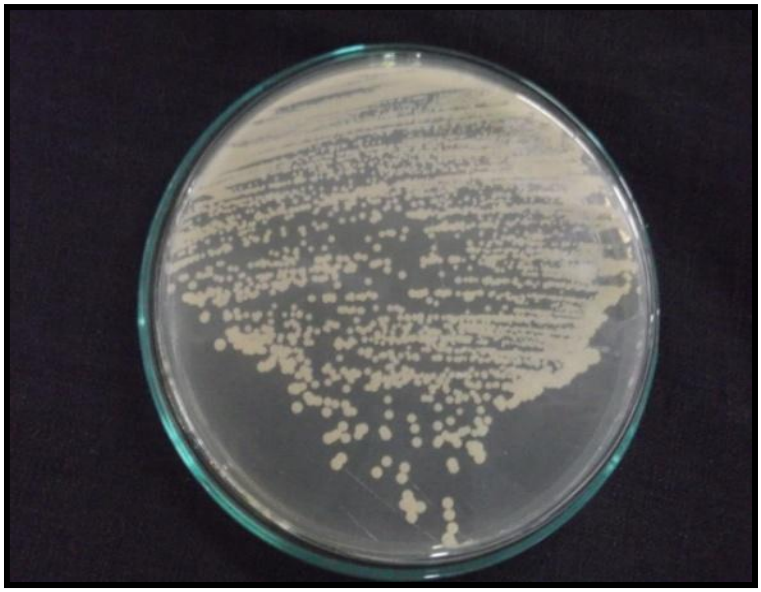


Plate 1 : Growth of SPN_{20}

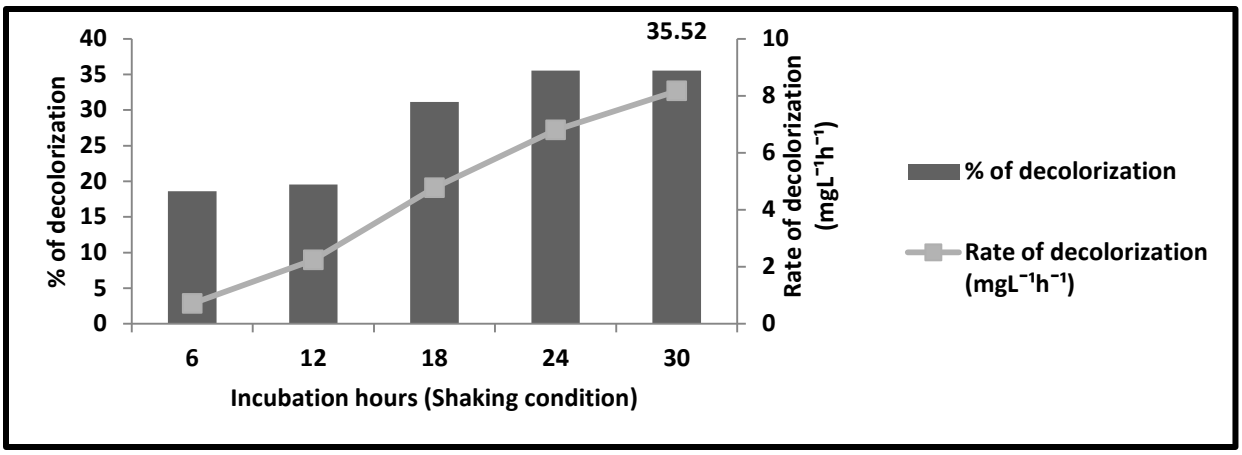


Fig.1: Effect of shaking condition on RRFD decolorization

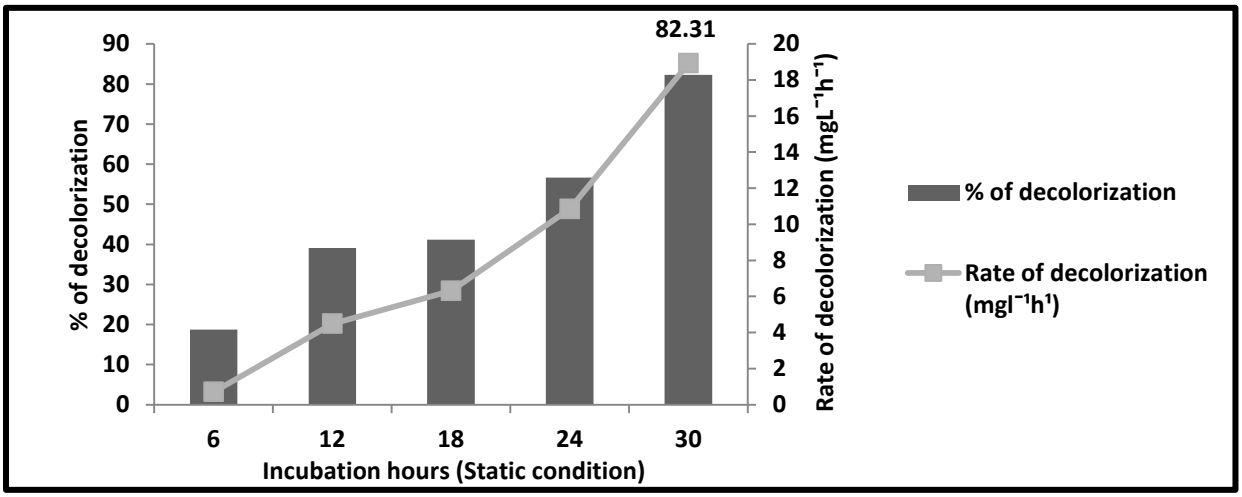


Fig. 2 : Effect of static condition on RRFD decolorization

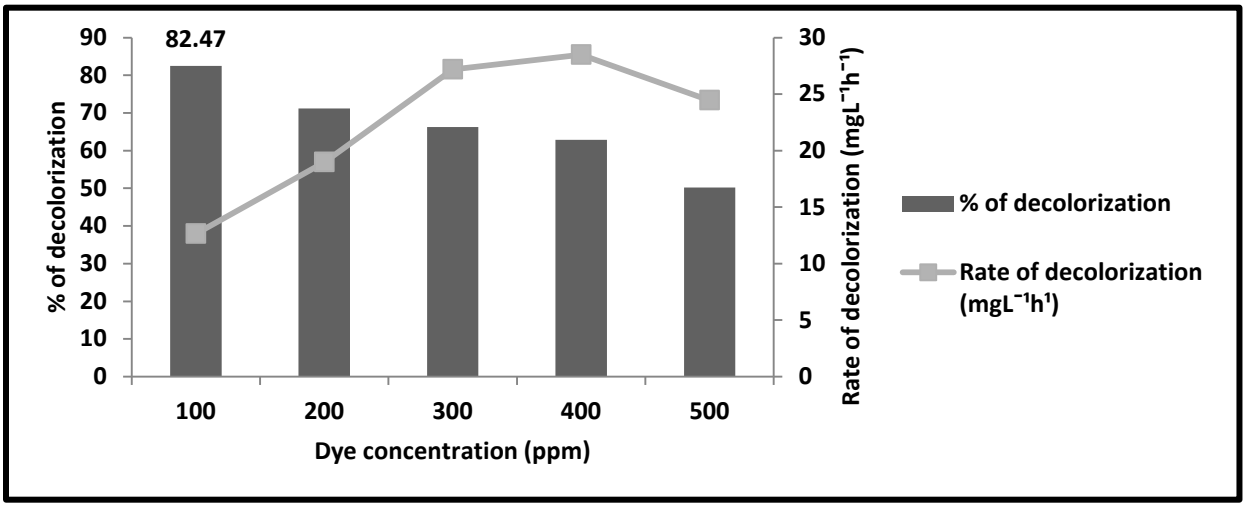


Fig. 3: Effect of dye concentration on RRFD decolorization

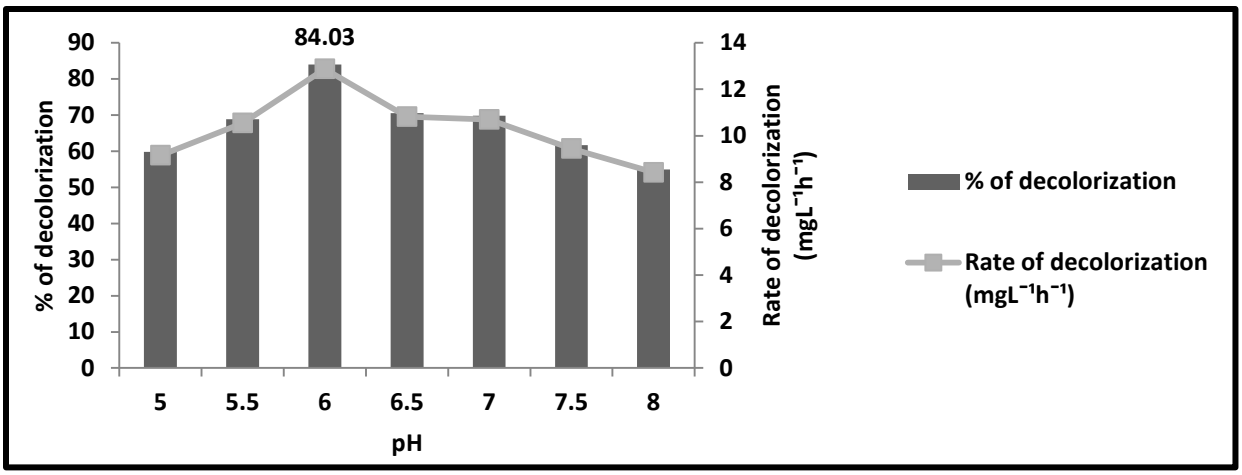


Fig.4: Effect of pH on RRFD decolorization

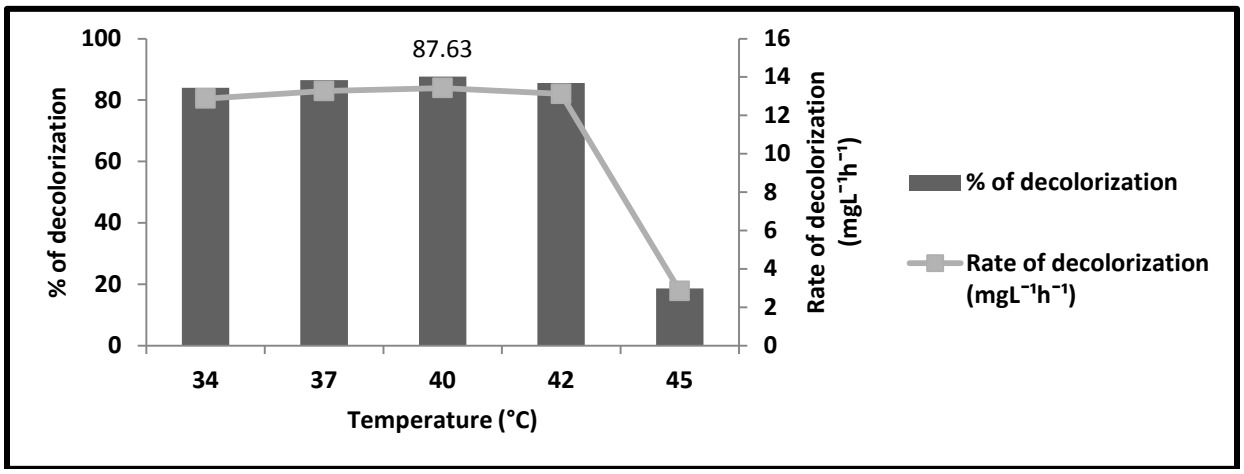


Fig. 5: Effect of temperature on RRFD decolorization

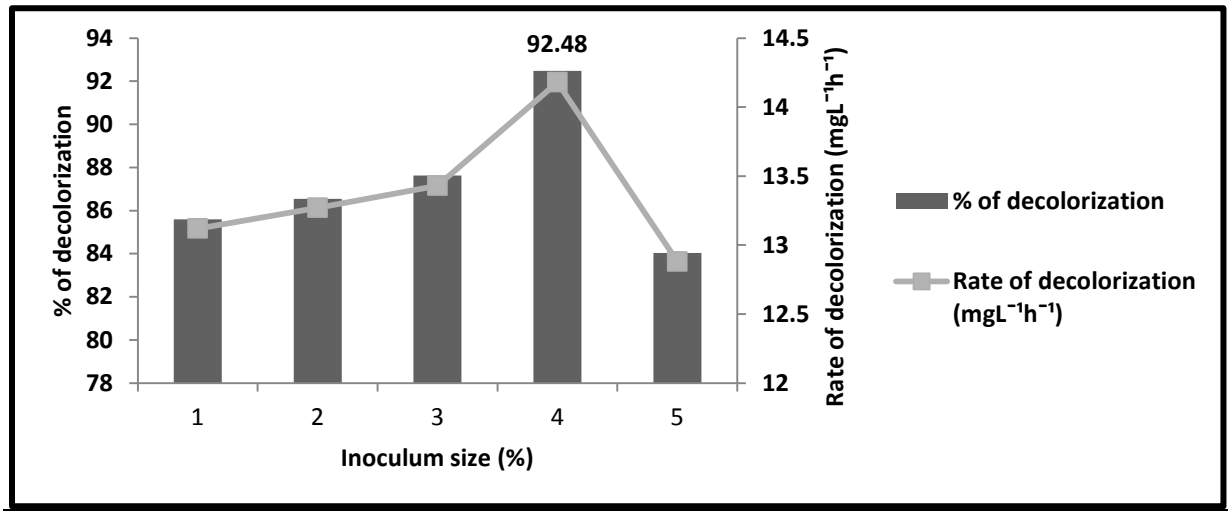


Fig. 6: Effect of inoculum size on RRFD decolorization

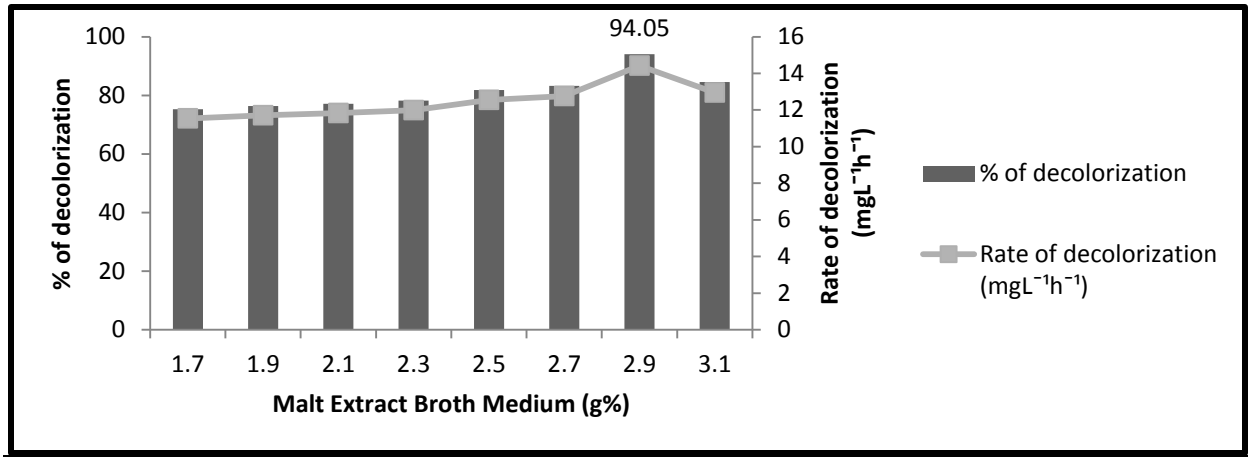


Fig. 7: Effect of Malt Extract Broth medium optimization on RRFD decolorization

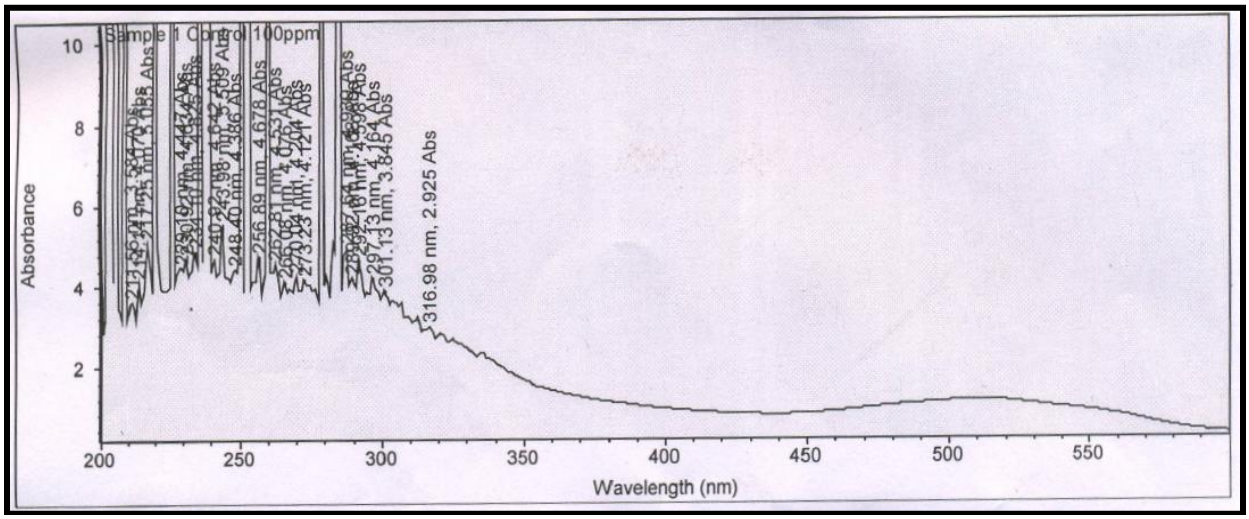
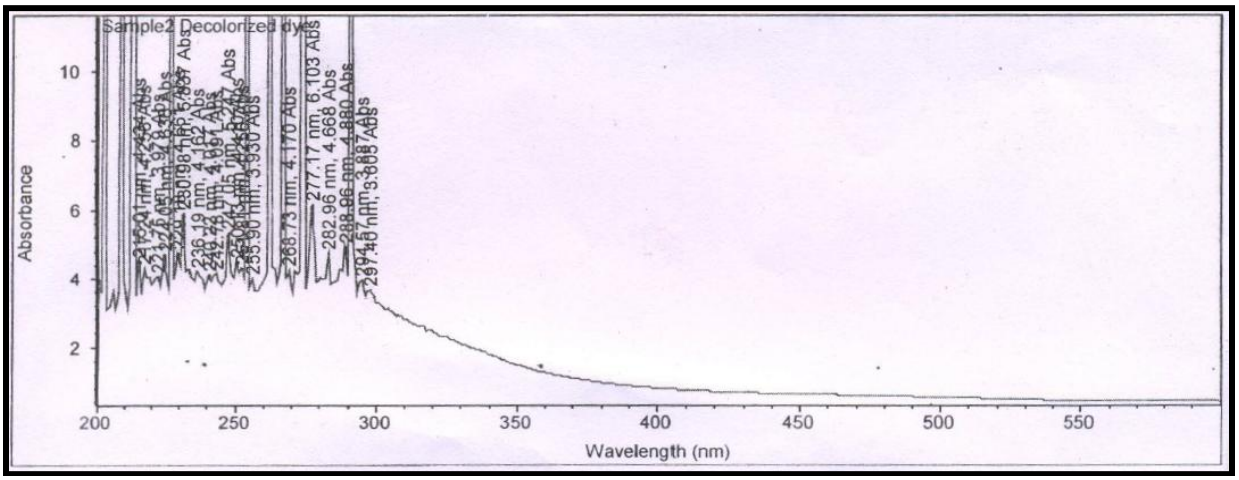


Fig. 8: UV-Visible image of control at 540 nm



Fig, 9: UV-Visible scanning image of decolorized test at 540 nm

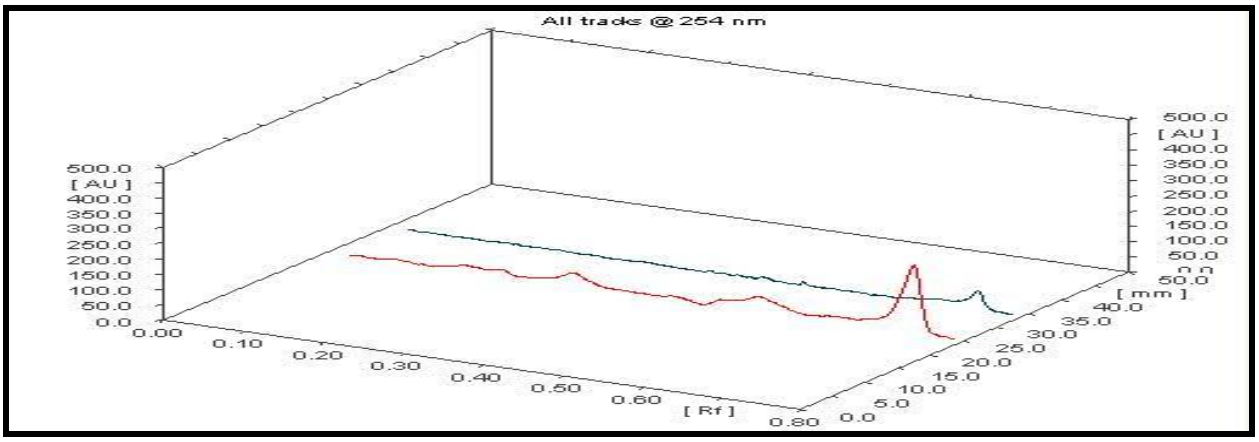


Fig.10: HPTLC analysis of control (Red color) & degraded products (Green color) at 254 nm

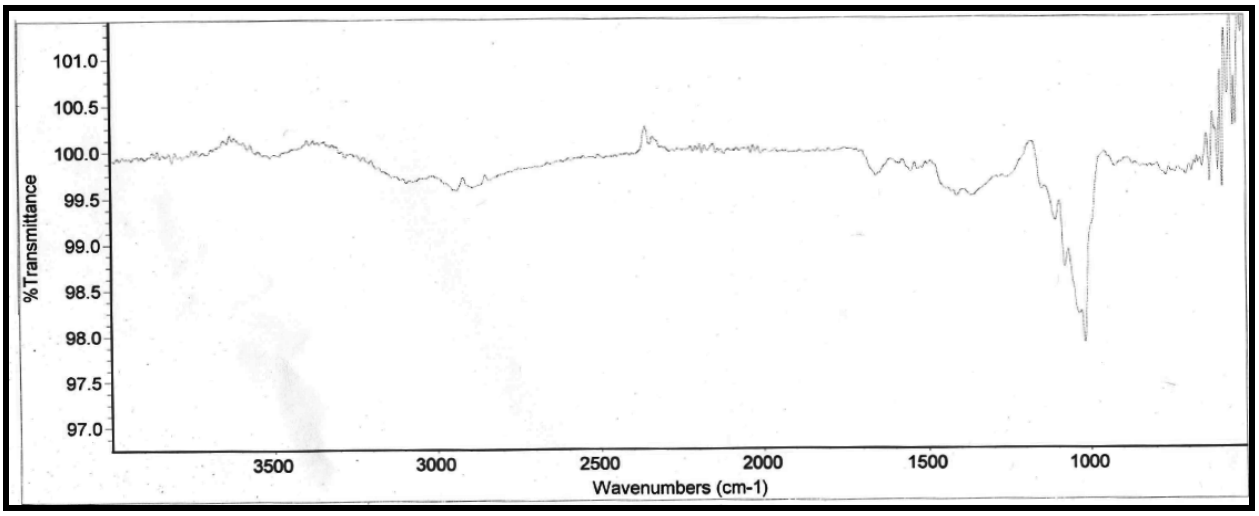


Fig. 11: FTIR spectrum of control (Rasberry red food dye)

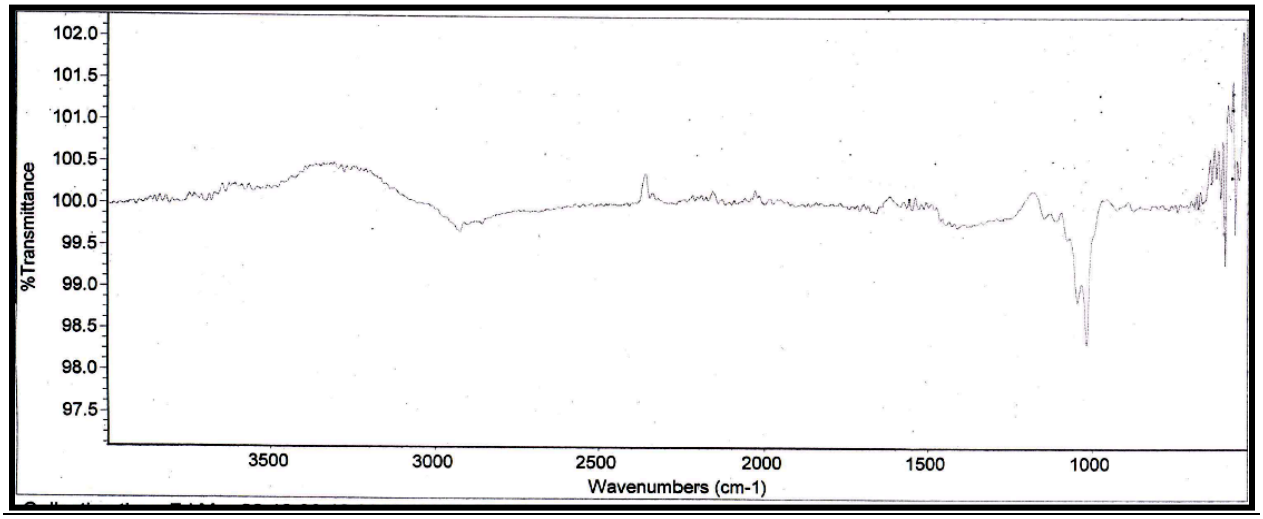


Fig. 12: FTIR spectrum of decolorized test broth

[MS received : November 07 , 2019] *[MS accepted : December 02, 2019]*