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Optimization of coagulant dosage and decolourisation of BMDS spent wash using external membrane bio reactor (E-MBR)

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ABSTRACT

During the recent days, investigations in pollution control of industrial effluents become one of the pinnacle problems of the society. Molasses used in distilleries for production of alcohol by fermentation process, generating highly organic and colored wastewater which is released as distilled spent waste. Treatment with microorganism is widely used to reduce 65-70% COD and 80-85% BOD level from the wastewater by converting the organic content present in it. This work assess the optimization ratio of coagulant and flocculant by RSM-CCD (10% PAC, 10% Maxfloc 508 and 0.2% Maxfloc C22). The R^2 value of coagulation was calculated as 0.99194 and Adjusted R^2 value of 0.984695 with predicated R^2 value as 0.938909. The microorganism isolated from UASB sludge was used as a microbial degradation of the spent wash waste after coagulation and further on subjected for EMBR and filtration. The suitability of E-MBR for distillery wastewater treatment for the maximum decolorization upto 90% using membrane filtration that can be adopted to treat this bio-methanated distillery spent wash (BMDS) water and to make digested spent wash fit for reclamation. A laboratory scale External Membrane Bio reactor (E-MBR) is used to treat the distillery waste water collected after digestion. The performance of this study is evaluated based on the removal of colour, pH changes observed at various stages of this treatment process and to optimize the coagulant dosage.

Keywords: RSM-CCD, Bacterial identification, COD, BOD, BMDS, E-MBR, reclamation, anaerobic digestion.

INTRODUCTION

The process of manufacture of alcohol from molasses is mainly used for the production of industrial alcohol. The molasses are diluted with water and nutrients are added in fermenter then allowed to ferment under controlled conditions of temperature and pH. The liquid is then distilled for recovery of alcohol. In this process the reducing sugars are broken down to ethyl alcohol and carbon dioxide. The bottom residue from the column is the spent wash [1]. Molasses spent wash is a dark brown colored effluent, conventionally treated by facultative and anaerobic digestion for generation of methane and then taken for further treatment prior to disposal. The bottom residue contains very high levels of COD, BOD also contains high solids as well as high potassium, phosphorus, nitrogen content, low pH and dark in colour [2, 3].

Table 1: Characteristics of distillery waste water

Parameter	Raw Molasses Spent wash	Spent wash (after anaerobic digestion)
Colour	Dark brown	Dark brown
pH	3.8 – 4.4	7.2 – 7.5
Chemical oxygen demand, mg/L	90,000 – 110,000	40,000 – 50,000
Biological oxygen demand, mg/L	40,000 – 50,000	10,000 – 15,000

Membrane technologies are receiving special recognition as alternatives to conventional water treatment and as a means of polishing treated waste water effluent for reuse applications [4]. MBR has proven to be effective for treatment of many industrial wastewaters. MBR system can be classified into two major categories. The first category is normally referred to as the external membrane configuration. This involves the use of polymeric membranes located external to the bioreactor. The second category is internal or submerged membrane system, where the membranes are directly submerged in the bioreactor [5].

The external membrane configuration is preferred, due to higher flux, low area requirement, longer life, capable of operating at high solid concentration and low capital cost. In view of these factors, we have chosen to carry out this study by selecting an external membrane configuration system. The main objective of this work is to enhance the treatability of spent wash in MBR after microbial degradation. Colour removal and COD reduction are of prime importance in this study on treatment of distillery wastewater [6, 7].

MATERIALS AND METHODS

Sample Collection and Preservation

The spent wash was collected from a distillery unit (M/s. Padmadevi Sugars Ltd) in Kanchipuram district of Tamil Nadu. Samples were in the form of liquid untreated effluent and sludge were collected in sterile containers and preserved at 4°C for further use.

Chemicals & Membrane

All the chemicals were of highest purity available and were of analytical grade, MERCK, Mumbai. Coagulants used for the study were purchased from M/s. Thermax Limited, Pune. Hollow fiber membranes have a smaller range of pore sizes (0.01 to 0.1 micron) used which are capable of removing viruses as well as some colour, odor, and organics removal [8].

Coagulation & flocculation experiment:

The jar tests were carried out on diluted spent wash using the commonly available coagulants of such as alum, poly aluminium chloride (PAC), lime and ferric chloride [9,10]. To enhance the colour removal we have used Maxfloc 508 and Maxfloc C22 in conjunction with PAC. Maxfloc products are used for reducing the dose of the PAC. Maxfloc 508 is a medium molecular weight highly cationic polyelectrolyte that effectively coagulates, precipitates coloring matter, suspended solids which attribute to COD and BOD. Maxfloc 508 solution of 5 to 10% concentration is used in this study. Maxfloc C22 is a high molecular weight, low cationic charge polymer in powder form. For this study, response surface methodology was performed in various concentration of this product is used to improve the efficiency of solid - liquid separation process.

Twenty Lab runs have been carried out using the following chemicals at various concentrations, dosage, combinations to arrive at the optimum chemical dosage and also to reduce the colour significantly in the spent wash [11, 12].

Table 2 Coded level for independent factors used in Experimental Design

Study Type			Response Surface		
Initial Design			Central Composite		
Design Model	Quadratic				
Factor	Name	Low coded	Low Actual	High Coded	High Actual
A	10% PAC	-1	1	1	3
B	10% MF-508	-1	2	1	6
C	0.2% MF-C22	-1	30	1	50

The quadratic equation

$Y= \beta_o+ \beta_1A + \beta_2B + \beta_3C + \beta_4A^2 + \beta_5B^2 + \beta_6C^2 + \beta_7AB+ \beta_8BC + \beta_9AC$

Where Y is the measured response, A, B and C are the coded independent input variables, β_o is the intercept term, β_1, β_2, and β_3 are the coefficients showing the linear effects, β_4, β_5 and β_6, are the quadratic coefficients showing the squared effects and β_7, β_8 and β_9 are the cross product coefficients showing the interaction effects [13].

Enrichment & Isolation of Spent Wash decolorizing bacteria.

UASB sludge was used for enrichment of decolorizing bacterial cultures by enrichment culture techniques. Microorganisms having the potential for decolourising spent wash were isolated by means of enrichment techniques. MSM supplemented with glucose (0.5 % w/v) and yeast extract (0.2 % w/v) ammonium phosphate (0.01% w/v), NaCl (0.02% w/v), $MgSO_47H_2O$ (0.02% w/v), KCl (0.01% w/v) Sludge (1000 ppm) was used for the study [14, 15]. The culture flasks were incubated on orbital shaker at 37°C. After incubation a loop-full of medium was streaked onto sterile nutrient agar plates and incubated at 37°C for 24 to 48 h, and 1 ml of the enriched culture was transferred to fresh medium. Well grown bacterial colonies were picked and further purified by streaking. The pure cultures of individual bacterial strains were maintained by streaking on nutrient agar slant and stored at 4°C. Identification of the bacterial isolates was carried out by the routine bacteriological methods i.e., by the colony morphology, preliminary tests like Gram staining, capsule staining, endospore staining, motility, catalase and oxidase, plating on selective medias and performing biochemical tests [16, 17]. These purified organisms were subjected for 16s rDNA identification [18].

DNA was isolated from organism. A large fragment of the 16S rRNA gene was amplified by PCR using the universal primers. The PCR product after purification is sequenced using a Terminator Cycle Sequencing Ready Reaction Kit and a model 3100 automatic sequencer. The closest known relatives of the new isolates were determined by performing a sequence database search. The sequences of closely related strains were retrieved from GENBANK and the Ribosomal Database Project (RDP) libraries [19]. Multiple gene alignment was also done for the nucleotide sequence to identify the similarity among the organism.

Coagulation Experiment

The experimental setup consist of HDPE containers for chemical mixing tank and feed tank followed by membrane bioreactor, membrane module, sand filter, activated carbon filter, micron cartridge filter. The chemical mixing tank is a cylindrical container placed vertically for the dosage of coagulant/flocculant. The optimum concentration of coagulant was prepared. The supernatant solution obtained after chemical addition is also collected and fed into the feed tank for further studies [20, 21].

UV-VIS Spectrophotometer

Intensity of colour was measured by checking the OD values of the test solutions. Except filtered water samples, supernatant and colored samples were centrifuged prior to determination of absorbance for eliminating the hindrance due to suspended particles in the sample. The supernatant was diluted and the absorbance was measured using UV-Visible spectrophotometer at the maximum wavelength of 600nm [22]. The removal of colour was evaluated in terms of reduction of absorbance from the original waste water in comparison with the supernatant and the outlet samples collected from subsequent units.

Decolorization of effluent by consortium of bacteria

All experiments were performed in triplicate. The percentage of discoloration was calculated according to the formula below:

$$Percentage\ Decolourisation = \frac{A_o - A_x}{A_o} \times 100$$

where, A_o, initial absorbance; A_x, absorbance at each time.

External Membrane Bio Reactor (E-MBR)

The outlet from this filter feed tank is connected to a feed pump of suitable capacity (20-30 liters per hour) and the outlet from this pump is fed to cylindrical vessel (bio-reactor) and the inoculums was added. The outlet was connected to a hollow fiber membrane made up of polysulphone followed with a sand filter [23]. The final clear outlet from this membrane module which is often termed as membrane permeates was collected. The outlet from the sand filter is connected to a 5 micron cartridge filter to remove the suspended particles that are present in the sand

filter outlet. The outlet from this cartridge filter is fed to an activated carbon filter containing granular activated carbon made out of coconut shell carbon having the particle size of 4 x 16 mesh sizes approximately. The outlet from the carbon filter is connected to 1 micron cartridge filter for removing the fine suspended particulates that are passing out **(Figure. 1).**

Fig 1 Experimental setup of EMBR

RESULTS AND DISCUSSION

Poly aluminium chloride (PAC) was found to be effective in terms of decolorization with less sludge formation. Out of various trials conducted, results of trial is found to be effective and satisfactory in terms of flocs formation, faster settling of flocs, maximum separation of supernatant solution, noticeable colour removal and less sludge volume. The required coagulant / flocculent chemicals such as 10% poly aluminium chloride (PAC), 10% Maxfloc 508 and 0.2% Maxfloc C22 solutions **(Table 3)** are added to this diluted spent wash at appropriate dose levels and mixed thoroughly in mixing tank and allow to settle.

Table 3: Optimization of Coagulant Dosage

Std	Factor 1	Factor 2	Factor 3	% Discoloration	
	A:10% PAC	B:10% MF-508	C:0.2% MF-C22	Actual	Predicted
1	1	2	30	30	30.2
2	3	2	30	30	30.6
3	1	6	30	30	30.2
4	3	6	30	40	40.6
5	1	2	50	40	38.2
6	3	2	50	40	38.6
7	1	6	50	30	28.2
8	3	6	50	40	38.6
9	0.318207	4	40	20	21.3
10	3.681793	4	40	30	30.4
11	2	0.636414	40	30	30.9
12	2	7.363586	40	30	30.9
13	2	4	23.18207	50	48.4
14	2	4	56.81793	50	53.3
15	2	4	40	60	60.0
16	2	4	40	60	60.0
17	2	4	40	60	60.0
18	2	4	40	60	60.0
19	2	4	40	60	60.0
20	2	4	40	60	60.0

Table 4: ANOVA table for the factorial design

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	3546.203	9	394.0226	136.8276	< 0.0001	significant
A-10% PAC	99.25857	1	99.25857	34.46837	0.0002	
B-10% MF-508	0	1	0	0	1.0000	
C-0.2% MF-C22	29.28932	1	29.28932	10.17096	0.0097	
AB	50	1	50	17.36292	0.0019	
AC	0	1	0	0	1.0000	
BC	50	1	50	17.36292	0.0019	
A^2	2094.066	1	2094.066	727.182	< 0.0001	
B^2	1524.913	1	1524.913	529.5388	< 0.0001	
C^2	149.0061	1	149.0061	51.7436	< 0.0001	
Residual	28.79701	10	2.879701			
Lack of Fit	28.79701	5	5.759401			Non-significant
Pure Error	0.0078	5	0.0054			
Cor Total	3575	19				
Std. Dev.	1.696968		R-Squared		0.991945	
Mean	42.5		Adj R-Squared		0.984695	
C.V. %	3.992866		Pred R-Squared		0.938909	
PRESS	218.3986		Adeq Precision		32.19238	

The R^2 value of coagulation was calculated as 0.99194 and Adjusted R^2 value of 0.984695 with predicated value as 0.938909.

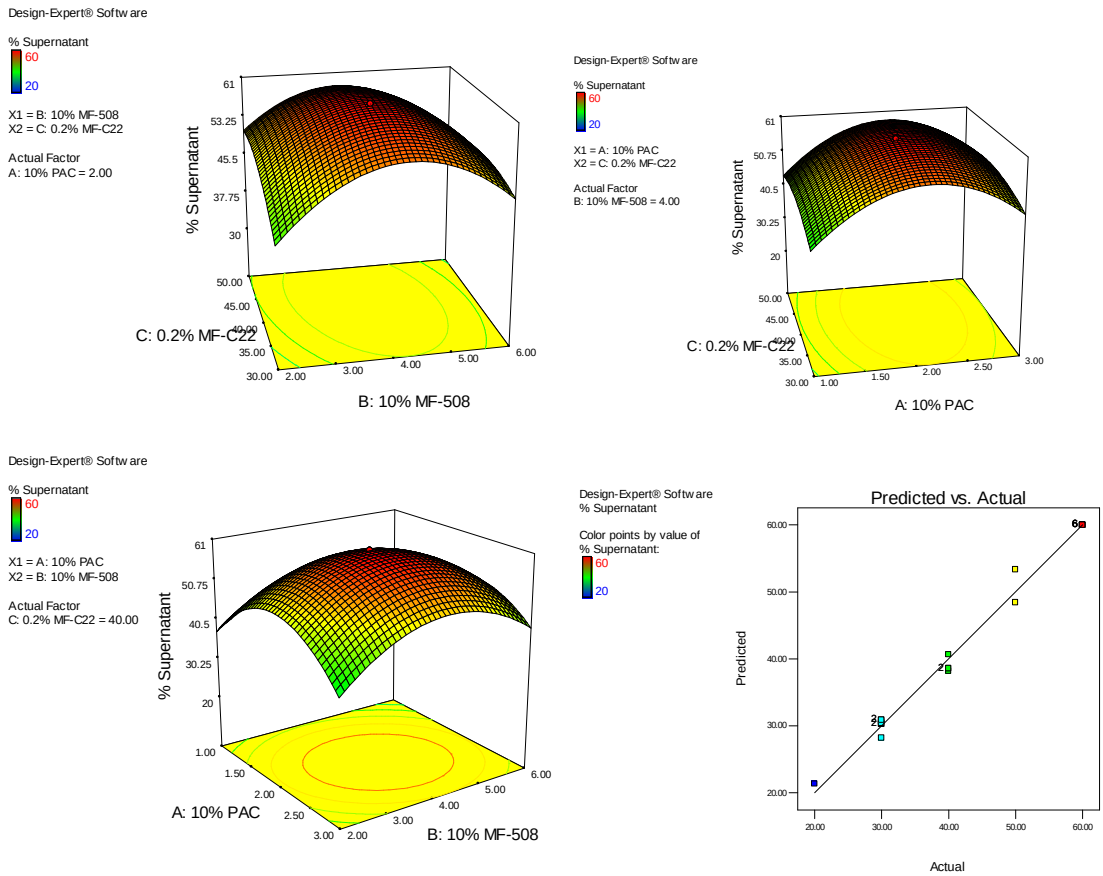

Figure 2: Response Surface plots showing the mutual effect of factors on the decolourisation

Identification of Organism

The organism were isolated from the Sludge was subjected with following biochemical test. The results are given in Table 5.

Table 5: Morphological, physiological and biochemical features of isolates from UASB sludge

S.NO	Test	*Bacillus licheniformis*	*Bacillus funiculus*	*Ps. aeruginosa*
1	Gram Staining	Gram positive rod	Gram positive rod	Gram negative rod
2	Cell morphology	Bacilli in chain	Bacilli in chain	Short rod in single
3	Motility	Positive	Positive	Positive
4	Endospore staining	Positive	Positive	Negative
5	Starch Hydrolysis Test	Positive	Positive	Negative
6	Catalase	Positive	Positive	Positive
7	Oxidase	Positive	Positive	Positive
8	Indole	Negative	Negative	Negative
9	MR (Methyl Red)	Positive	Positive	Negative
10	VP (Voges Proskauer)	Negative	Negative	Negative
11	Citrate Utilization Test	Negative	Negative	Positive
12	Oxidase	Positive	Positive	Positive
13	H2S production	Negative	Negative	Negative
14	Gas production	Negative	Negative	Negative
15	Glucose	Positive	Positive	Negative
16	Lactose	Negative	Negative	Negative
17	Sucrose	Positive	Positive	Negative
18	Maltose	Positive	Negative	Negative
19	Mannitol	Positive	Negative	Negative

16s rRNA sequencing

The isolated organism were isolated as pure culture and 16s rRNA sequencing was performed. The organisms were identified as *Bacillus licheniformis*,*Bacillus funiculus* and *Ps. aeruginosa.* The FASTA format were given below

***Bacillus licheniformis* strain 16S ribosomal RNA gene, partial sequence**

GGACCGATTTTCGGGAATTATTGGGCGTAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCC
CGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGT
GTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGAC
GCTGAGGCGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGT
GCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCAAACGCATTAAGCACTCCGCCTGGGGAGTAC
GGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCG
AAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTCCCCTTCGG
GGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC
GAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCG
GAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGGC
AGAACAAAGGGCAGCGAAGCCGCGAGGCTAAGCCAATCCCACAAATCTGTTCTCAGTTCGGATCGCAGTC
TGCAACTCGACTGCGTGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCC
GGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTTG
GAGCCAGCCGCCGAAAAGGGGGGGAA

***Bacillus funiculus* strain 16S ribosomal RNA gene, partial sequence**

AACATGGTTTTCCGGATATTGGGCGTAAAGCGCGCGCAGGCGGTTCCTTAAGTCTGATGTGAAAGCCCAC
GGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAAAGCGGAATTCCACGTG

TAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTTCTGGTCTGTAACTGACG
CTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTG
CTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACG
GCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGA
AGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACTCTAGAGATAGAGCGTTTCCCCTTCG
GGGGGACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGGTTAAGTCCCG
CAACGAGCGCAACCCTGATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAA
CCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATG
GACGGTACAAAGAGTCGCGAGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTA
GGCTGCAACTCGCCTACATGAAGCCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTT
CCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGCAACACCCGAAGTCGGTGAGGTAACCGT
AAGGAGCCAGCCGCCTTAAAGGGTGGGGTTG

***Pseudomonas aeruginosa* strain 16S ribosomal RNA gene, partial sequence**
ATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGATGAAGGGAGCTTGCTCCT
GGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACG
GGCGCTAATACCGCATACGTCCTGAGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCC
TAGGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGAT
GATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAA
TGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTT
GGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCAACAGAATAAGCACCGGCTAACTTC
GTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAG
GTGGTTCAGCAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTACTGAGCTAG
AGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTG
GCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATA
CCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTA
ACGCGATAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGC
ACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGA
ACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTG
TCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAAC

CLUSTAL W Multiple Sequence Alignments

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Sequence type explicitly set to DNA
Sequence format is Pearson
Sequence 1: gi|330414750|gb|JF682389.1|      936 bp
Sequence 2: gi|345505202|gb|JF683611.1|      941 bp
Sequence 3: gi|727929342|gb|KM491169.1|     1088 bp
Start of Pairwise alignments
Aligning...

Sequences (1:2) Aligned. Score: 93.91
Sequences (1:3) Aligned. Score: 50.64
Sequences (2:3) Aligned. Score: 49.95

There are 2 groups
Start of Multiple Alignment

Aligning...
Group 1: Sequences:   2      Score:16920
Group 2: Sequences:   3      Score:10222
Alignment Score 11359
```

CLUSTAL W is a reliable, practical and efficient tool for multiple sequences alignment, though caution should be taken to judge the significance and validity of the results. The organism was subjected for clustalW and multiple sequence alignment was done to investigate the similarity between the sequence shows a higher similarity among them. There was a massive reduction in the color was absorbed after microbial treatment.

After settling, water and sludge gets separated and the supernatant solution is drawn for further treatment. The supernatant solution was fed to bio-reactor tank and seeded with nutrients and microorganisms. Permeate was collected at a flow rate of 12 l/h continuously and pass through series of filters such as sand filter, 5 micron filter, activated carbon filter and 1 micron cartridge filter. The outlet samples from each filtration unit is collected separately and taken for testing.

Figure 3: Colour intensity at each unit outlet

Figure 3 shows the colour intensity of the outlet stream from each unit operation. The maximum decolourisation was obtained by EMBR around 89% for 1:1 dilution of spent wash.

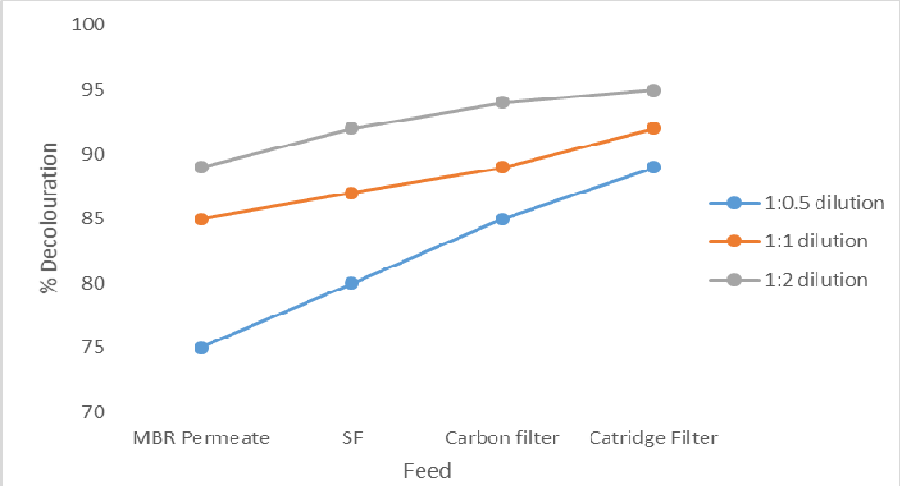

Figure 4: Percentage decolourisation at each unit outlet

CONCLUSION

The coagulant / flocculent chemicals such as 2 g/l of 10% poly aluminium chloride (PAC), 4 g/l of 10% Maxfloc 508 and 40 mg/l of 0.2% Maxfloc C22 solutions was optimized to yield the maximum supernatant solution. The optimization was done by using RSM with R^2 value of 0.99194. Significant removal of colour reduction is noticed at each stage of the process in a gradual manner. The membrane permeate water is found to be clear. This result displays an efficiency of 90% for the reduction of colour from the waste water sample taken initially for this study.

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