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# FUNGAL DECOLORIZATION OF ANAEROBICALLY BIODIGESTED DISTILLERY EFFLUENT (ABDE) FOLLOWING COAGULANT PRETREATMENT

**Sushil Kumar Shukla[1], *Ashutosh Tripathi[2] and P.K. Mishra[3]**
[1]Assistant Prof., Center for Environmental Sciences, Central University of Jharkhand, Ranchi, India
[2]Assistant Prof., Amity Institute of Environmental Sciences, Amity University, Noida-125, Gautam Buddh Nagar, U.P.(201303), India
[3]Professor, Department of Chemical Engineering, IIT (BHU), Varanasi (221005), India
E-mails: [1]shuklask2000@gmail.com, [1]sushil.shukla@cuj.ac.in, [3]pkmishra.che@iitbhu.ac.in
[2]tripathiashutos@gmail.com, [2]atripathi1@amity.edu (**Corresponding Author*)

**Abstract:** The Spent wash produced from distillery effluents (DSW) is bioassimilated with the help of anaerobic biodigestor. The anaerobically biodigested distillery Effluent (ABDE) thus produced is associated with high COD, BOD and color. COD and BOD reduction from ABDE poses a tough challenge to the environmentalists. The problem gets magnified in countries like India due to limitation of treatment cost. ABDE needs to be diluted extensively for microbial growth to occur which further limits the option of biological treatment. In the present work, ABDE was maximally decolorized by 97.2% with the help of combined coagulant as well as biological treatment with the help of a potent fungal species, *Aspergillus niger* ATCC No. 26550 and NCIM No. 684, moreover with limited dilution. Decolorization parameters were optimized for screening the significant factors. Coagulants such as potash alum, ferric chloride and aluminum chloride have been studied in the present work. The optimum values for the important factors to achieve maximum decolorization of coagulant pretreated ABDE by 63.4% were 10 g/L Sucrose, 2 g/L $NH_4NO_3$, 10% inoculum volume, pH 5, temperature $30^0$C at 85% ABDE: 15% Water of ABDE dilution. The coagulation treatment result depicts that coagulation by alum alone turn into 78.5 % COD reduction as well as 92.4 % color reduction. The total decolorization obtained after complete treatment (coagulation+fungal) was 97.2%, which indicates fungal decolorization after pretreatment with alum is a feasible alternative for the treatment of ABDE. Furthermore, FTIR spectrum of the treated ABDE also confirms degradation of the melanoidin.
**Keywords:** Anaerobically Biodigested Distillery Effluent (ABDE), Potash Alum, *Asperillus niger,* Decolorization.

## INTRODUCTION

Effluent originating from distilleries known as spent wash has very high levels of BOD, COD, color as well as high potassium, phosphorus and sulfate content [1]. A typical cane molasses based distillery generates 12-15 L of spent wash per liter of ethanol produced (2). In Indian scenario situation is quite challenging as there are around 212 distilleries which

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generates around 30 billion litre of spent wash annually. Spent wash is a strongly acidic, dark brown colored, hydrophilic viscous liquid waste with strong objectionable odour (3). Spentwash disposal into the environment is hazardous. Disposal of distillery spentwash on land reduces soil alkalinity and inhibit seed germination [4]. Color of the spent wash reduces sunlight penetration in water bodies causing reduced photosynthetic activity and dissolved oxygen concentration which affects aquatic life [5]. Color of spentwash is largely due to melanoidin [6].Melanoidin is one of the biopolymers, which are difficult to decompose by microorganisms. Several basidiomycetes and ascomycetes type fungi have been used in the decolorization of melanoidins in connection with color and COD reduction of wastewaters from distilleries [7]. *Coriolus sp.* No.20, in class basidiomycetes, was the first strain reported for its ability to remove melanoidins from molasses spentwash [8]. Miranda et al. [9] studied color elimination from anaerobically and aerobically treated beet molasses spentwash using *Aspergillus niger* and the nutrients used for this process were sucrose, $NH_4NO_3$, $KH_2PO_4$ and $MgSO_4 \cdot 7H_2O$ with an initial pH of 5. Decolorization of molasses wastewater from an alcoholic fermentation factory by *Trametes versicolor* was studied with a low sucrose concentration and adding $KH_2PO_4$ as the only nutrient [10]. Shayegan et al. used *Aspergillus species* isolated from the soil for decolorization of anaerobically digested and aerobically treated distillery wastewater. With diluted wastewater at optimum values of nutrients, 75% decolorization was achieved which reduced to 40% on using undiluted wastewater [11].

Many physicochemical treatment methods are available for the degradation and decolorization of spentwash [12]. The advantage of these physicochemical methods in combination with fungal treatment is that, it can effectively decolorize undiluted wastewater; otherwise dilution at field scale will increase the amount of wastewater considerably. Alum is very effective coagulant for decolorization of DSW [13, 14]. In the present study, ABDE was pretreated with different coagulants and fungal decolorization was performed. As medium components and other parameters play a very important role in enhancing the decolorization, therefore, their optimization study is very important. Process optimization by the traditional 'one-factor-at-a time' technique was used in the present work.

## Materials & Methods

### Wastewater:

Anaerobically biodigested Distillery Effluent (ABDE) was obtained from a cane molasses-based Lord's distillery, Nandganj, Ghazipur (India). ABDE was characterized and analyzed for pH, total solids (TS), chemical oxygen demand (COD), biological oxygen demand

(BOD), based on the Standard Methods for Examination of Water and Wastewater [15] (Table 1).

| S. No. | Parameter | ABDE |
|---|---|---|
| 1. | Color | Dark Brown |
| 2. | pH | 7.65 |
| 3. | Alkalinity(mg/L) | 2300 |
| 4. | Total solids(mg/L) | 51100 |
| 5. | Total dissolved solids (mg/L) | 44600 |
| 6. | Total Suspended solids(mg/L) | 6500 |
| 7. | BOD(mg/L) | 6200 |
| 8. | COD(mg/L) | 42500 |

**Table 1:** Composition of anaerobically biodigested distillery effluent obtained from Lords distillery, Nandganj, Ghazipur, U.P.

**Coagulant pretreatment**

ABDE was pretreated with coagulants such as potash alum, ferric chloride and aluminium chloride (commercial) at different coagulant doses**.** pH of ABDE was adjusted with 35% HCl. Coagulation was done in jar test apparatus (VELP Scientifica, Model JLT6, France). Coagulants were added to the effluent and flash-mixed for 2min at 100 rpm and, thereafter, slowly mixed at 30 rpm for 30 min. The supernatant obtained after 1 h settling was used for fungal treatment.

**Microorganism and inoculums**

The decolorization of ABDE was carried out using *Aspergillus niger* ATCC No. 26550 and NCIM No. 684 .The strain was obtained from NCL, Pune, It was maintained on PDA incubated at 30°C for 4-5 days and stored under refrigeration at 4-6°C.

**Flask cultures**

Screening and optimization studies were performed in 250 mL shake flasks with 100 mL of ABDE after coagulation. The different concentrations of nutrients such as carbon, nitrogen, MgSO4 sources were added. After sterilization in autoclave, flasks were inoculated with fungal pellets (10%). Flasks were, then, put inside the shaker at 180 rpm at $30^{0}$C. Samples were withdrawn every 24 h for observing maximum percentage decolorization.

**Decolorization assay**

At regular intervals 5mL sample were taken from shake flasks and centrifuged at 10,000 rpm for 10 minute. The supernatant after centrifugation was diluted 10 times and used for color reduction measurement. The absorbance was measured at 475nm using Systronics double beam spectrophotometer (2202). The decolorization yield was expressed as the percentage decrease in absorbance at 475nm related to the initial absorbance at the same wavelength. All experiments were performed in triplicates and samples were withdrawn at regular interval of time (24 hr) for decolorization measurements.

$$\% \text{ Decolorization} = [(\text{initial OD} - \text{final OD}) / \text{initial OD}] \times 100$$

A Nicolet 5700 spectrophotometer was used to record FTIR spectra of ABDE pre and post treatment. The spectra were recorded in the wave number range of 4000–500 $cm^{-1}$.

**Results and Discussion:**

The fungal decolorization of ABDE was performed with 100 times dilution and 63.4% decolorization was obtained. Dilution by such extent is not favorable so filtrate obtained after coagulation with alum was used for aerobic decolorization of ABDE. The characteristics of ABDE before pretreatment with alum are given in Table 1. 92.45% color removal and 78.5% COD reduction was observed after pretreatment with alum. Color removal and COD reduction was due to precipitation of hydrocolloids and the dissolved matter.

**Optimization of Dose and pH of Coagulants:**

Optimum dose for potash alum was found to be 8 g/100 ml as can be seen from Figure 1(a). The observed COD and color reduction were 78.5% and 92.45 %, respectively. The observed COD and color reduction were 66.8% and 74.6%, respectively for aluminium chloride at optimum dose of 3.5 g/100 ml as shown in Figure1(b) The optimum dose for ferric chloride was found to be 3.0 g/100 ml, and the COD and color reduction were 59.0% and 67.0 % respectively as shown in Figure 1(c). It is clear that the dose of potash alum used was nearly thrice as compared to other coagulants used but the cost of potash alum is 20 times less than aluminium chloride and ferric chloride, so it will be most economical option for large scale application. In addition to this, optimum pH for coagulation using potash alum is closer to the pH of effluent stream in industrial condition. Keeping in view the above results, potash alum was used as coagulant for further experiments.

It was observed that optimum pH for coagulation by potash alum was 8, aluminium chloride was 6 and for ferric chloride was 4. It is noteworthy that optimum pH in case of potash alum

was closer to actual condition of ABDE (pH=7.65), thus avoiding the need of addition of chemicals for pH adjustment.

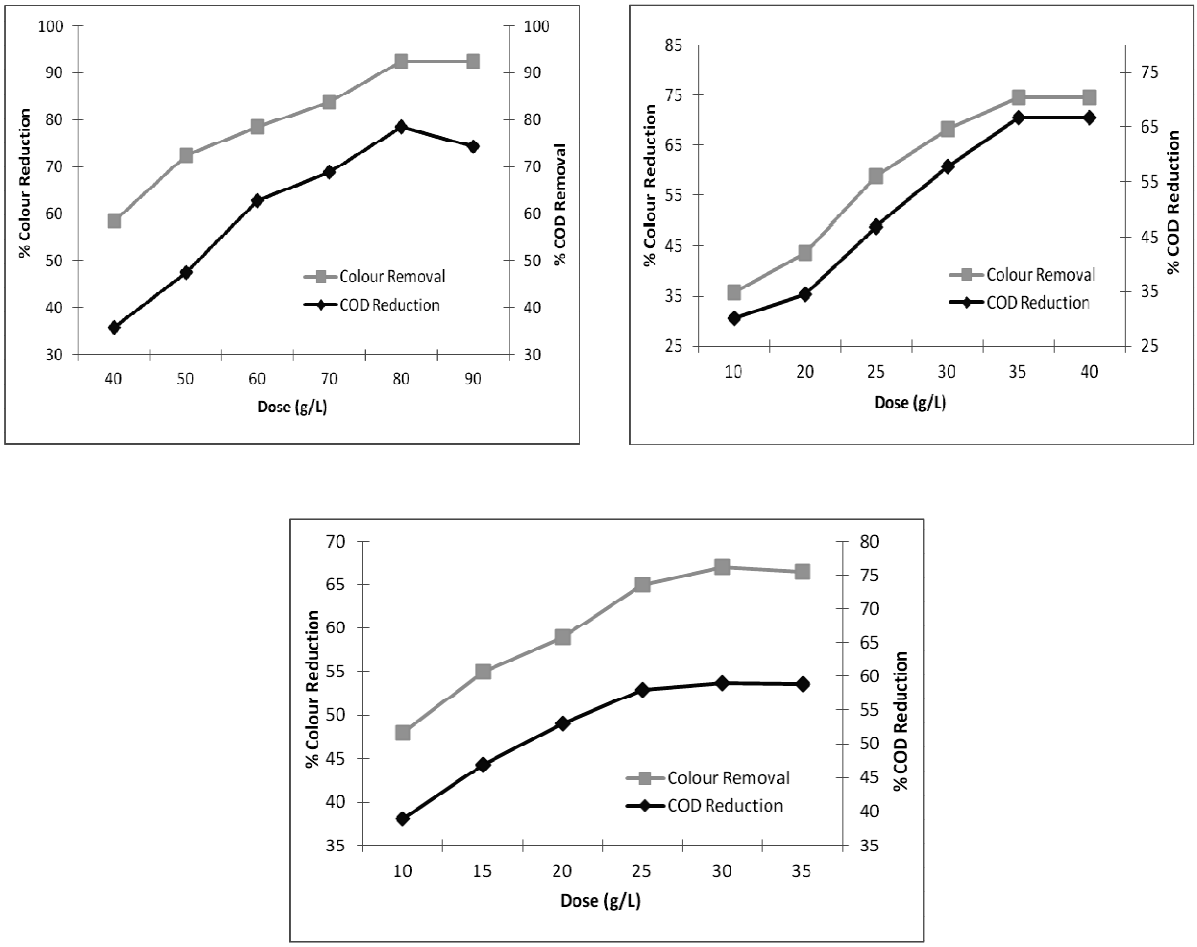


**Fig.1.** Effect of dose of (a) Alum, (b) Aluminium chloride and (c) Ferric chloride on percentage decolorization and COD reduction

**Aerobic Degradation of Coagulated ABDE:**

The filtrate of coagulated ABDE was used for biological study. Various parameters influencing decolorization using *Aspergillus niger* were optimized. Throughout the experiments, small (2-6 mm diameter), compact and uniform pellets of the fungus were used. Best color removal was observed after 3-4 days in each case. The experiments were repeated thrice to validate the results

**Concentration (v/v) Optimization of Coagulated ABDE**

Results of decolorization studies performed for different dilutions of the coagulated ABDE are shown in Figure 2(a). It is seen that maximum color removal of 63.5 % was observed at 15 % dilution. The color removal decreased at higher dilution, which may be due to less available equivalent glucose in molasses. At lower dilution, due to higher concentration of

melanoidin and its antioxidant properties, suppressed activity of microbes resulted in less color removal. Figure 2(a) also shows that color reduction obtained is dependent on microbial concentration obtained at same dilution, which is controlled through the metabolic pathway. Figure 2(b) supports the mechanism as decreased growth of biomass is observed due to less available sugar sources.

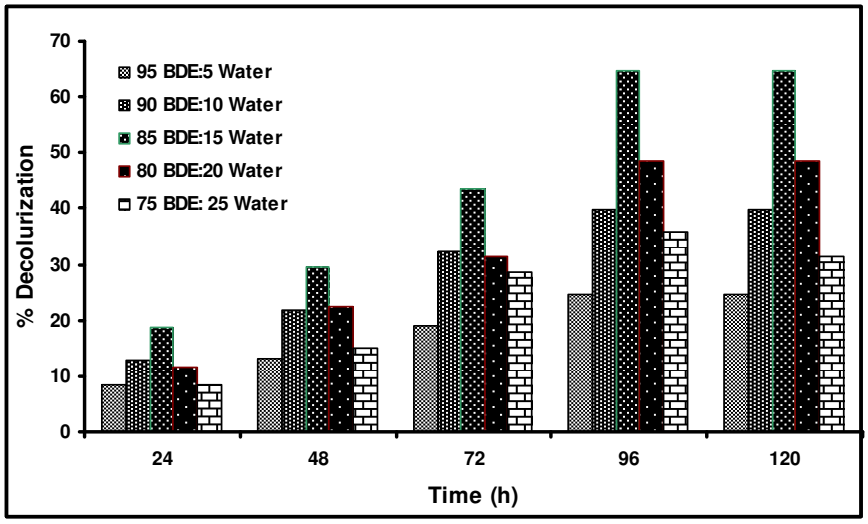


**Figure 2(a):** Percentage decolorization of ABDE by *Aspergillus niger* and Biomass production

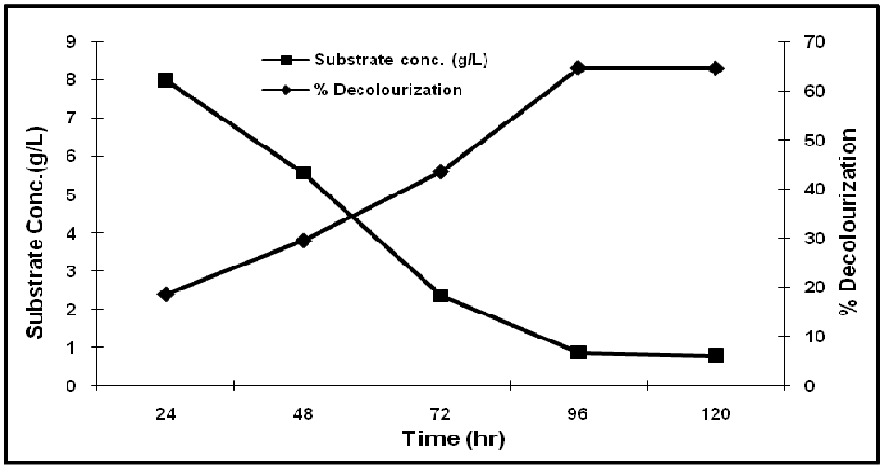


**Figure 2(b):** Comparison of Biomass production and Sucrose uptake rate by *Aspergillus niger*

**Optimization of Carbon and Nitrogen Sources:**

Batch fermentation runs were conducted in triplicate in shake flasks using various carbon sources like glucose, sucrose, fructose and galactose at 10 g/L [16]). Figure 3(a) presents the effect of carbon sources on decolorization of coagulated ABDE. It clearly shows that sucrose and glucose are effective carbon source as they render more decolorization as compared to

two other carbon sources used. Sucrose is the best carbon source as compaired to glucose as shown in Figure. After the initial acclimatization period, degradation started as observed after 24 hrs, there after it increased rapidly up to 96 hrs, and approached to an asymptotic value. Slight increase in decolorization (nearly 1%) between 96 to 120 hrs is likely to be due to the growth of the cultures on the retractile carbon component of the digested ABDE. This indicates that decolorization may occur as a result of secondary metabolic reaction. It may be due to sugar oxidase as suggested by different scientist [17, 18]).

Various nitrogen sources like urea, ammonium nitrate ammonium phosphate, ammonium sulphate at the level of 2 g/L have been used to evaluate their effectiveness in microbial decolorization of ABDE [16]. Ammonium Nitrate show maximum decolorization at 2 g/l as compared to other nitrogen sources as shown in Fig. 3(b).

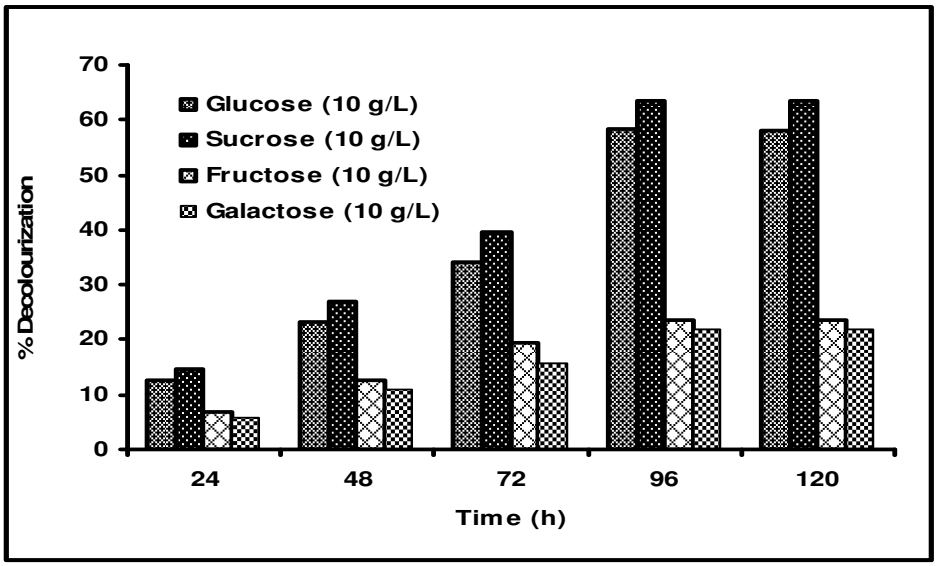


**Figure 3(a).** Effect of carbon sources on decolorization of coagulated ABDE

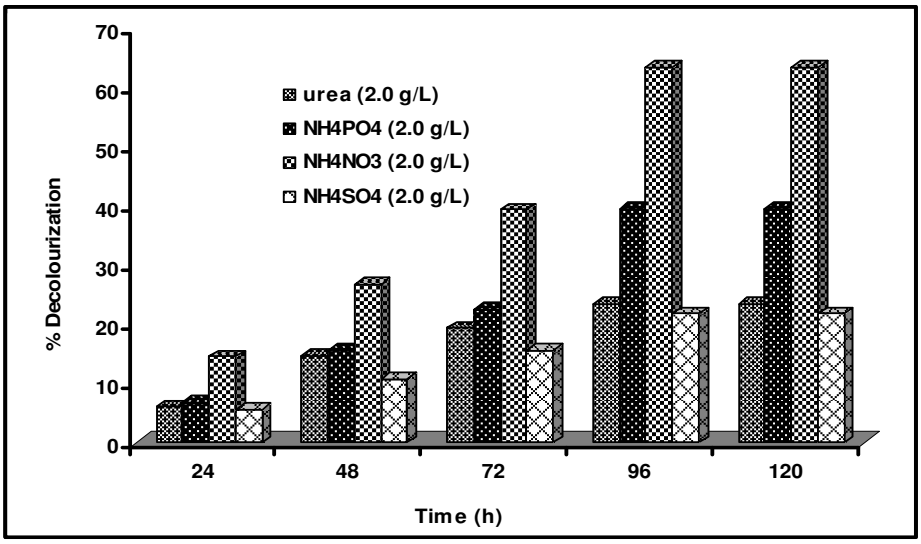


**Figure 3(b).** Effect of nitrogen sources on decolorization of coagulated ABDE

**Optimization of the Process Temperature:**

The degradation of melanoidin is a temperature dependent process. Optimum temperature for maximum activity of *Aspergillus niger* was determined by measuring color removal at different temperatures as shown in Figure 4. Increasing trend in activity has been observed till 30°C there after decreasing trend was observed due to denaturing of enzyme at higher temperatures. Optimum temperature thus obtained was 30°C.

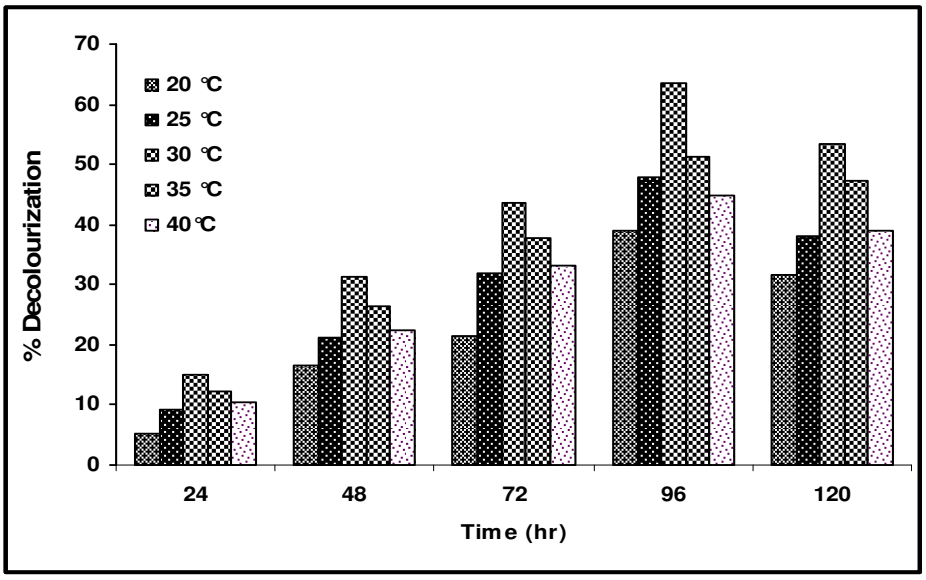


**Figure 4.** Effect of temperature on decolorization of coagulated ABDE

**Optimization of the Process pH**

Figure 5 shows the effect of pH on color removal efficiency of coagulated ABDE. It can be seen from these figures that maximum color removal has been obtained at pH 5. Reduction in color removal beyond this pH can be attributed to the sensitivity of fungus to pH condition.

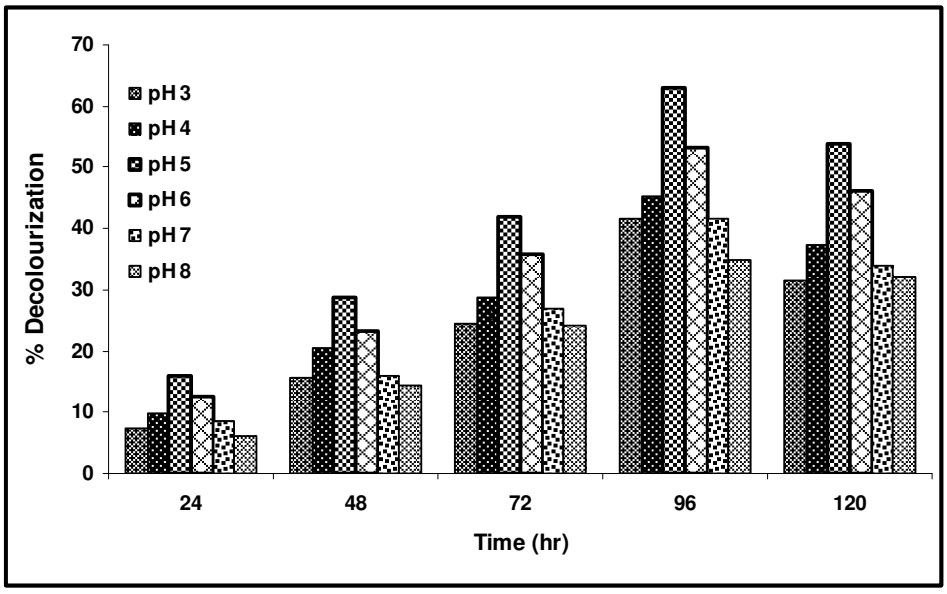


**Figure 5.** Effect of pH on decolorization of coagulated ABDE

**Optimization of Inoculum Level on the Decolorization of ABDE:**

Batch studies were also performed to optimize inoculum volume. Inoculum levels of 2.5 to 15 (v/v) were used to measure color removal and sucrose consumption for coagulated ABDE. Figure 6 presents the results obtained by these experiments. It can be clearly seen that maximum decolorization of 63.4% has been achieved at 10% inoculum level. Sucrose consumption rate were also maximum at this inoculum level which remained almost constant there after, signifying no further microbial activity beyond this inoculum volume**.**

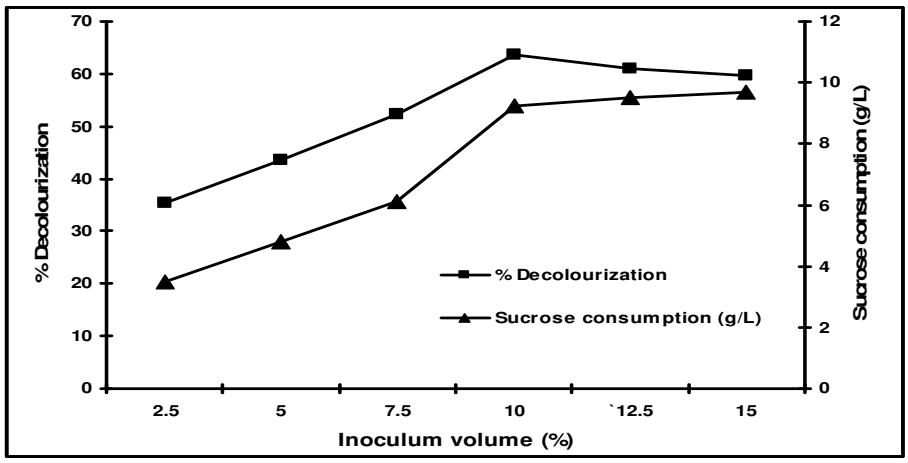


**Figure 6.** Effect of inoculum level on decolorization of coagulated ABDE

**Characterization of Effluent by FTIR Spectra:**

FITR spectra of synthetic solution of melanoidin, untreated and aerobic treated ABDE are shown in Figure 8 (a), 8 (b) and 8 (c) respectively. The spectra of synthetic melanoidin and

untreated ABDE are characteristically similar, in contrast the spectrum of treated ABDE is quiet different. The characteristic peak at wave no. 2354 $cm^{-1}$, which is maximum pronounced in the case of synthetic melanoidin and also prominent in the case of untreated ABDE, is absent in the case of treated ABDE which indicates its almost complete removal during the treatment process. The color reduction was also supported by UV-Visible spectra of the treated and untreated ABDE. These observations collectively prove the efficacy of coagulation followed by aerobic treatment.

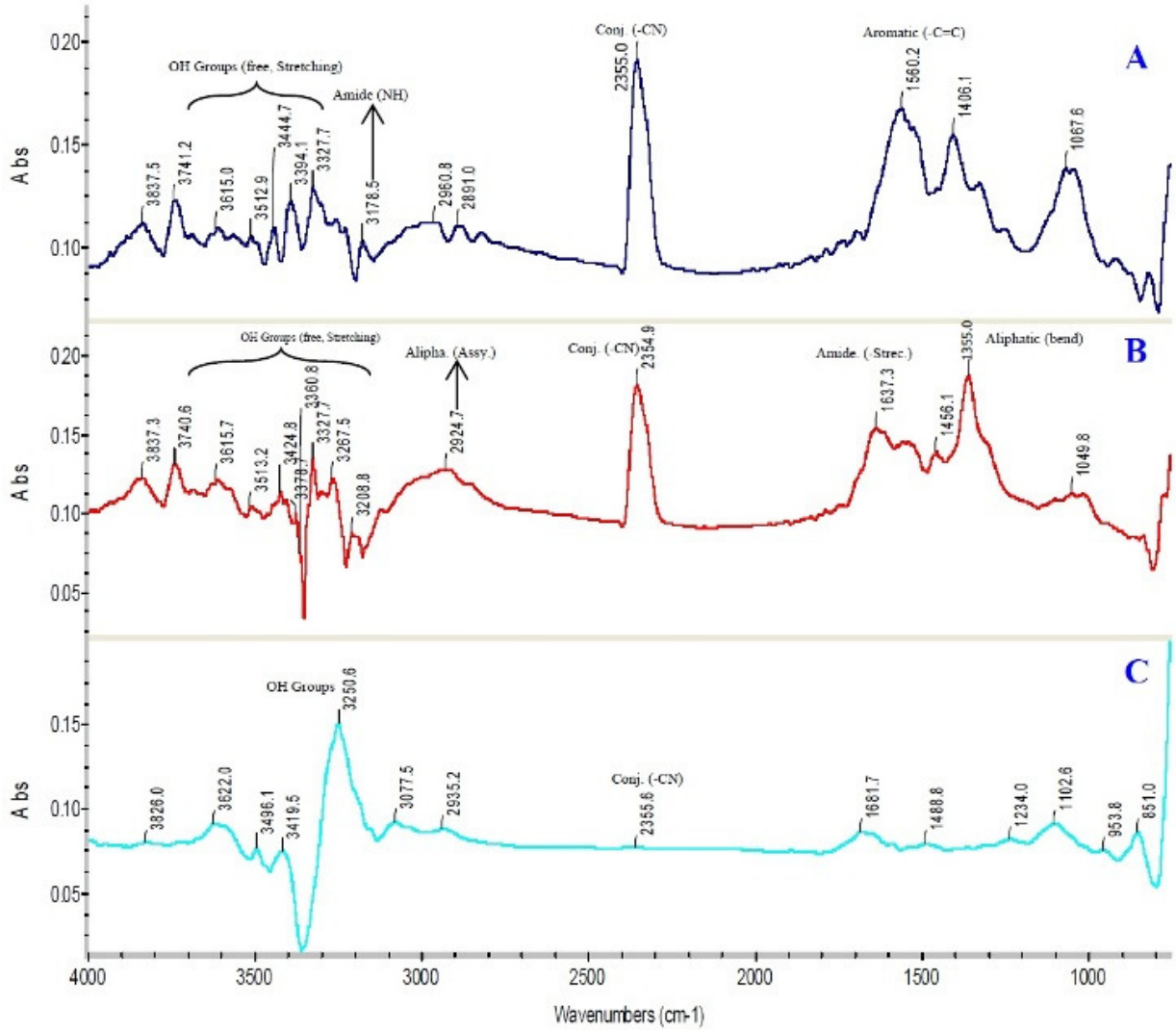


**Figure 8.** FTIR Spectra of (a) Melanoidin, (b) Untreated ABDE and (c) Treated ABDE of distillery effluent

## Conclusion

Extensive dilution required for treatment of ABDE using aerobic biodegradation, makes it unfit or commercially unviable option. Coagulation as pretreatment before aerobic biodegradation seems to be an attractive option as it reduces required dilution for aerobic

treatment substantially in addition to reduction in color by 92.45% and COD by 78.5 %. The remaining color and organic load present in the coagulated ABDE have been reduced by aerobic degradation employing *Aspergillus niger*. The total decolorization obtained after complete treatment (coagulation+fungal) was 97.2%, which indicates fungal decolorization after pretreatment with alum is a viable option for the treatment of anaerobically biodigested distillery Effluent (ABDE).

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