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Enhancement in biodegradability of distillery wastewater using enzymatic pretreatment

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Abstract

A combined treatment technique consisting of enzymatic hydrolysis, followed by aerobic biological oxidation was investigated for the treatment of alcohol distillery spent wash. The enzyme cellulase was used for the pretreatment step with an intention of transforming the complex and large pollutant molecules into simpler biologically assimilable smaller molecules. Batch experiments were performed in order to analyze the influence of various parameters like pretreatment time, enzyme concentration and pH during the pretreatment step on the subsequent aerobic oxidation kinetics. The rate of aerobic oxidation was enhanced by 2.3 fold for the pretreated sample as compared to the untreated sample when the pH during the pretreatment step was maintained at a value of 4.8. Similarly, a two fold increase in the aerobic oxidation rate was found when the effluent was pretreated with the enzyme, without any pH control (i.e. effluent pH of 3,8). The study indicated that the enzymatic pretreatment of the effluent could be one of the successful pretreatments which can lead to enhancement of the rate of the subsequent aerobic oxidation.

Keywords: Distillery spent wash; Enzyme; Cellulase; Biodegradability and pre-treatment

1. Introduction

In the Indian context, all the ethanol produced in the country is by way of the fermentation of cane-sugar molasses and its subsequent distillation. Molasses (a by-product of the sugar industry) contains roughly 50% fermentable sugars (sucrose, glucose and fructose) and is one of the important raw materials used in the production of ethanol owing to its cheapness and ready availability. During the course of alcohol production around 12–13 l of effluent (termed as spentwash) are generated per liter of alcohol. The spentwash is highly colored and is characterized by a high concentration of dissolved solids (of which 50% may be present as reducing sugars), high ash content, high temperature and low pH. The pollution caused by this effluent is the single most important detrimental aspect, which has affected the growth of this industry (Inamdar, 1991).

The characteristics of the distillery wastewater vary considerably according to the fermentation feed stock, the location and the fermentation/distillation processes adopted. The spentwash contains dissolved impurities from the cane juice, the nutrients added during the molasses fermentation, by-products of the fermentation and the decomposition products. The suspended impurities like dust, cellulosic fibers, etc. are usually removed before the concentration (evaporation) of the juice. However, water soluble hemicelluloses, proteins, gums, organic non-sugars and minerals from the cane juice are present in the spentwash in their original or converted forms exerting a chemical oxygen demand (Thampi, 2000) during subsequent treatment.

For a batch fermentation process, distillery spent wash with a typical BOD of 35,000–50,000 mg/L and a COD of 80,000–100,000 mg/L is obtained. On the other hand for a continuous fermentation process, the values of the BOD and COD vary in the range of 60,000–100,000 and 160,000–200,000 mg/L respectively (Thampi, 2000).

Primarily as a result of the difficulties of treating the stillage (spent wash) and due to a high concentration of the dissolved organic and inorganic matter, a host of utilization and treatment schemes (like recycle, direct land application,

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Notations

S/S_o	Residual substrate concentration, in terms of COD
X/X_o	Residual biomass concentration, in terms of MLSS
X_o	Biomass concentration, in terms of MLSS
MLSS	Mixed liquor suspended solids, mg/L
BOD	Biochemical oxygen demand, mg/L
COD	Chemical oxygen demand, mg/L
TDS	Total dissolved solids, mg/L
ASN6	Culture isolated from aeration tank of the effluent treatment plant of Asian Paints, Mumbai
ETP	Effluent treatment plant
FPU	Filter paper unit
IU	International Unit
PTFE	Polytetrafluoroethylene
DO	Dissolved oxygen, mg/L
AO	Batch subjected to Aerobic oxidation
E	Enzyme pretreated sample

evaporation and combustion, aerobic and anaerobic biological methods, thermal and thermochemical methods) have been proposed. Recent developments indicate that the use of hybrid methods involving combinations of these partial treatment schemes are more successful in the complete treatment of this effluent (Sheehan and Greenfield, 1980; Thampi, 2000).

In the present work, the possibility of using an enzyme to enhance the biodegradability of the distillery-spent wash has been investigated. This integrated technique is being considered in light of a new possibility offered by enzymes used in the field of wastewater treatment.

The enzymatic treatment falls between the physicochemical and biological treatment processes if one looks at the advantages offered by it (Karam and Nicell, 1997). In other words, enzymatic treatment has technological advantages and yet is in its infancy, requiring economical considerations (in terms of the process development) in order to apply it on the plant scale. The potential advantages of enzymatic treatment (when used as a standalone technique) as compared to the conventional treatment include: applicability to biorefractory compounds; operation either at high or low contaminant concentrations; operation over a wide range of pH, temperature and salinity; absence of shock loading effects; absence of delays associated with the acclimatization of biomass; reduction in the sludge volume (no biomass is generated) and the ease and simplicity of controlling the process (Karam and Nicell, 1997). In recognition of these potential advantages, recent research has focused on the development of enzymatic processes for the treatment of wastewaters, solid wastes, hazardous wastes and soils. Karam and Nicell (1997) have summarized the enzymes utilized in the field of wastewater treatment according to the categories of specific waste types and their origins in an excellent review. In an another review, Aitken (1993) has presented waste treatment situations that may be appropriate for the use of enzyme technology along with the criteria for selection of the enzymes that may show a near-term applicability.

A large number of enzymes (e.g. peroxidases, oxidoreductases, cellulolytic enzymes cyanidase, proteases, amylases, etc.) from a variety of different sources have been reported to play an important role in an array of waste treatment applications (Klibanov et al., 1980; Klibanov and Morris, 1981; Aitken and Irvine, 1989; Duff et al., 1994; Ferrer et al., 1991; Dec and Bollag, 1994).

The literature also cites examples of the use of integrated enzyme/biological techniques, leading to an improved biological oxidation process. Jung and coworkers (2002) treated dairy wastewater containing different oil and grease contents in batch activated sludge systems with and without an enzymatic pre-hydrolysis stage (with 0.2% w/v of fermented babassu cake containing *Penicillum restrictum* lipases). It was found that when the oil and grease concentration in the control bioreactor was increased (400, 600 and 800 mg l^{-1}), the COD removal efficiency fell (86, 75 and 0% respectively). However in the reactor fed with pre-hydrolyzed wastewater, COD removal efficiency was maintained (93, 92 and 82% respectively at the similar COD loading). At an oil and grease concentration of 800 mg l^{-1}, the control bioreactor presented a final volatile suspended solids concentration ten times greater than that obtained in the bioreactor fed with pre-hydrolyzed wastewater, indicating the effectiveness of using the enzymatic pool produced by the fungus *P. restrictum* in the biological treatment of dairy wastewaters with high oil and grease content (Jung et al., 2002).

In another integrated process (enzymatic/biological), wastewaters containing high levels of oil and grease (900 mg l^{-1}) were treated in an upflow anaerobic sludge blanket reactor (UASB) utilizing lipases from a babassu cake fermented with *P. restrictum*. The technique resulted in almost 90% COD removal, 90% VSS reduction and 75% turbidity reduction, in relation to the raw wastewater (Cammarota et al., 2001).

In the present study, keeping the complexity of the distillery spent wash in mind, the efforts were directed towards enzyme-catalyzed in situ transformation of pollutants present in the distillery-spent wash into biologically amenable products, thus possessing higher metabolic value (bioacceptability) for the microbes in the subsequent biological oxidation step.

2. Experimental

2.1. Materials

2.1.1. Distillery wastewater

The distillery wastewater was procured from Terna Shetkari Sahakari Sakhar Karkhana, Osmanabad, Maharashtra, India. The waste was filtered to remove the suspended solids, and this was taken for further study. Table 1 shows the characteristics of the raw distillery spent wash obtained after filtration.

2.1.2. Culture for aerobic oxidation

The aerobic oxidation was carried out using a single strain (named ASN6). The organism was isolated from the activated sludge of an effluent treatment plant (Asian Paints, Mumbai). The strain was identified as a potential degrader of this effluent after it underwent differential screening and enrichment techniques utilizing the effluent as the sole carbon source in the medium. The staining procedures and the biochemical tests revealed that the strain was a facultative Gram positive bacterium belonging to the genus *Bacillus*. This pure culture was used rather than the mixed culture (as is the usual practice) to specifically maintain a constant (similar) microbial environment in the biodegradation studies.

2.1.3. Cellulase enzyme

The enzyme used in the study was cellulase (a kind gift from M/s Advanced Biochemicals, Mumbai, India). The enzyme used consisted of a blend of enzymes viz., endoglucanase (EC 3.2.1.4), cellobiohydrolase (EC 3.2.1.91) and β-glucosidase (EC 3.2.1.21) and had an average activity (assayed) of 100 FPU/g of the powder.

2.1.4. Bioreactor

All the biodegradability studies were performed in a bioreactor made of glass having a capacity of one liter. The lid of the reactor had four different ports. The impeller was introduced in the bioreactor through the central port with the help of a Teflon stuffing box. The remaining three ports were used for air sparging, for sampling and as an air outlet (passed through an air filter), which was also used for the addition of inoculum and for pH measurements during the biological aerobic treatment. Air was sparged in the bioreactor using a fish tank aerator and a pre-calibrated rotameter. The sterility of the medium was maintained by filtering the air through 0.22 μm membrane filters (PTFE Pall filters, Gelman Laboratories, USA). The end of the sparger tube (single-point-type) was made narrow in order to ensure the generation of small air bubbles, enhancing the gas-liquid mass transfer rates. The impeller was attached to a motor (REMI Equipments, India). The speed of the impeller was kept constant (at 700 rpm) with the help of the speed controller.

Table 1
Characteristics of the raw distillery spent wash

Parameter	Values
Color	Brown
pH	3.8–4.0
COD (mg/L)	100,000–110,000
TDS (mg/L)	98,000–110,000
TSS (mg/L)	3000–5,000
TOC (mg/L)	55,000–56,000
Ammoniacal N (mg/L)	12,000–14,000
Reducing Sugars (mg/L)	9000–10,000
Chloride* (mg/L)	4000–6,000
Sulphate* (mg/L)	4500–6,000
BOD_5* (mg/L)	30,000–45,000

*Data obtained from the factory report.

2.2. Experimental protocol

2.2.1. Optimization of cellulase activity

Before commencing the enzymatic pretreatment studies, the optimization of the pH and the temperature for the maximum enzyme activity was carried out for the enzyme which was used in the study. The enzyme was assayed at different pH, ranging from 3.0–6.0, using citrate buffer (0.05 M). The enzyme was also assayed at different temperatures, namely 30, 40, 50 and 60 °C. These studies indicated that a pH of 4.8 and a temperature of 50 °C were optimum for maximum enzyme activity under the assay conditions. These optimized parameters were used in further studies irrespective of the difference in the substrates during the assay (filter paper) and the actual treatment (spent wash).

2.2.2. Preparation of distillery waste for enzyme treatment

The raw effluent sample was centrifuged (REMI Equipments, India) at 5000 rpm for 20 min to remove the suspended solids. The raw untreated effluent was diluted with water to bring down the COD to an approximate value of 9000–10,000 ppm. In real practice, it is impractical to use such high dilutions, but these concentrations ($\sim$10,000 ppm) are typical of residual COD concentrations remaining after major biological treatments (say anaerobic) and hence chosen in this study.

2.2.3. Enzymatic pretreatment step

The pretreatment step was carried out in a constant temperature water bath maintained at 50 °C. The contents were continuously stirred during the treatment with the help of a magnetic stirrer. The effluent sample was contacted with the enzyme under different operating conditions of treatment time, temperature and the enzyme concentration. The pH values were adjusted with the help of NaOH solution (2 N). 1 mL sample aliquots were withdrawn at regular time intervals. The samples were analyzed for the enzyme activity, COD and sugars. At the end of the pretreatment step, the pH of the effluent was measured. This enzyme pre-treated effluent sample was then subjected to

the aerobic oxidation to study the effect of the enzyme pretreatment on the rates of aerobic biological oxidation.

2.2.4. Preparation of the inoculum for the biodegradability studies (Culture acclimatization)

For the biodegradability studies, the seed inoculum was prepared. The isolate ASN6 was stored on nutrient agar (NA) slants (7 mL) at 4 °C. The culture from two such slants was inoculated to the sterile diluted waste, under aseptic conditions and it was incubated on an orbital shaker (180 rpm) at ambient temperature (30–34 °C) for 12 h (a period determined after the enrichment studies). The inoculum volume was kept constant at 50 mL for every batch experiment.

2.2.5. Aerobic oxidation of the pretreated samples (Biodegradability studies)

Biodegradability is a parameter for evaluating the possibility of using the aerobic oxidation step. This step evaluated the biodegradability of the enzymatically pretreated samples. The initial volume of the effluent in the reactor was kept constant at 650 mL for every batch. The enzyme-pretreated sample (E) and also the untreated effluent were first sterilized. It is to be noted that this step of sterilization (heat treatment) may bring about some physico-chemical changes in the effluent, yet the relative merits or demerits of the enzyme pretreatment can still be judged accurately, without having to bother about the alteration due to external contamination. Inoculum was aseptically transferred to the sterilized effluent and the degradation was carried out under aerobic conditions.

The contents were maintained well mixed with the help of a 4 bladed downpumping pitched blade turbine, rotating at 700 rpm, and the air was sparged at the bottom of the impeller by using a single point sparger. This way, a DO level above 2 ppm was maintained during each run. The initial pH of the effluent was maintained at 7.5. Four-milliliter aliquots were withdrawn at regular time intervals and every sample was analyzed for COD, MLSS and sugars. During the run, periodic pH measurements were also carried out.

The biodegradation effectiveness of the enzyme pretreated samples was compared with the results obtained with the untreated sample. The untreated sample was directly taken for the aerobic oxidation (AO) step by prior centrifugation, dilution of the original waste to the same concentration and sterilization. It was observed that autoclaving of the effluent sample led to a decrease in its pH value by one unit. Hence, for all the biodegradability studies the initial pH of the sample was maintained at a value of 8.5 so that the pH of the sterilized sample dropped to 7.5 (which was then the starting pH for the subsequent aerobic oxidation step).

The effects of different pretreatment conditions like pH during the pretreatment step, the effect of the enzyme concentration and the pretreatment time on the initial rates of subsequent biological oxidation steps have been studied.

2.3. Analysis

COD determination was done according to the Standard Methods of Analysis of Wastewater and Water, (APHA, 1998). For the MLSS measurements, 4 mL of sample was centrifuged at 5000 rpm and the cell weight was found out by dry cell weight method. The pH was monitored during the aerobic oxidation step with the help of a digital pH meter (Equiptronics, India) under aseptic conditions.

Cellulase assay was carried out using the Filter Paper Assay technique (FPU Assay) according to the procedure mentioned by Ghose (1987). Because the FPU assay is non-linear, (the use of the International Unit (IU) *per se* is incorrect as this unit is based on the initial velocities of the reaction) it has been recommended that the results be expressed simply as units per milliliter. One unit is defined as 1 μmol min^{-1} of substrate converted to 0.18 mg min^{-1} of the product (reducing sugars as glucose) during the hydrolysis reaction under the reaction conditions (pH 4.8, 0.05 M citrate buffer, 50 °C). FPU (units ml^{-1}) is calculated (Ghose, 1987) as:

$$\mathrm{FPU} = \frac{0.37}{\text{enzyme concentration to release 2.0 mg glucose}}.$$

3. Results and discussion

3.1. Effect of pretreatment conditions on the effluent

The distillery spent wash was contacted with the enzyme during the pretreatment step. It was found that the enzyme pretreatment step did not change the COD value of the effluent. The initial COD values matched with those obtained at the end of the pretreatment step. It was also observed that there was a marginal increase in the pH of the effluent. An increase of 0.5–0.8 pH units at the end of the pretreatment was obtained. This effect was found to be independent of the different pretreatment conditions studied, viz., the effect of enzyme concentration, the effect of pretreatment time and the effect of the pH during the treatment. This observation was as expected since the enzyme pretreatment was expected to break down the cellulosic material present in the distillery spent wash but not the mineralization of the pollutants.

Most of the cellulosic materials in the spent wash consist of three major organic components: cellulose, hemicellulose and lignin. A variation in the composition depending on the source of the material has been reported during the enzymatic hydrolysis of these materials (Magee and Kosaric, 1985).

3.2. Effect of pH during pretreatment step on the subsequent aerobic oxidation

It was important to study the COD removal efficiency dependence on the conditions of the pretreatment step. The effluent was contacted with the enzyme at two different pH values, one represented the pH for optimum enzyme activity (pH: 4.8) and the other represented the pH of the raw untreated effluent (pH: 3.98). 50 FPU of the enzyme was loaded for the pretreatment step and the pretreatment was carried out for 24 h. The pretreated samples were then subjected to aerobic oxidation. Fig. 1 shows the % COD reduction obtained in the case of the enzymatically pretreated effluent at two different pH values as compared to the untreated sample. Each of these data points are an average of three such experiments. The average values and error bars are also indicated in the figure. Fig. 1 indicates that the enzymatic pretreatment step has enhanced the biodegradability of the effluent. It was found that the maintenance of optimum pH of the enzyme (pH: 4.8) favored the maximum subsequent removal of COD by aerobic oxidation. At the end of 24 h of aerobic oxidation, the % COD reduction obtained was 28.8, and 23.0% for enzymatically-pretreated samples at the pH of 4.8 and pH of 3.98 respectively as compared to 18.3% for the untreated effluent (control). At the end of 72 h of aerobic oxidation, the pretreatment condition of pH 4.8 yielded a maximum COD reduction of 54.3% as compared to 44.6 and 47.3% for the batches pretreated at the pH of 3.98 and the untreated batch respectively.

Fig. 2 shows the first order degradation plot for the enzymatically-pretreated samples and that of the control. This figure clearly quantifies the observations reported in the Fig. 1. Due to the complexity of the effluent, the optimum pH for treatment may be different and not always correspond to the optimum pH of the enzyme activity. From Fig. 2 it can also be observed that among the two pretreatment conditions studied, the enzyme pretreatment condition at the pH of 4.8 yielded 2.3 times higher initial rate of aerobic oxidation as compared to the untreated effluent sample. In the case of the batch, maintained at pH 3.98, only a 1.7 fold increase in the initial rate of aerobic oxidation has been observed. Hence, the subsequent pretreatment experiments were carried out at the pH value of 4.8.

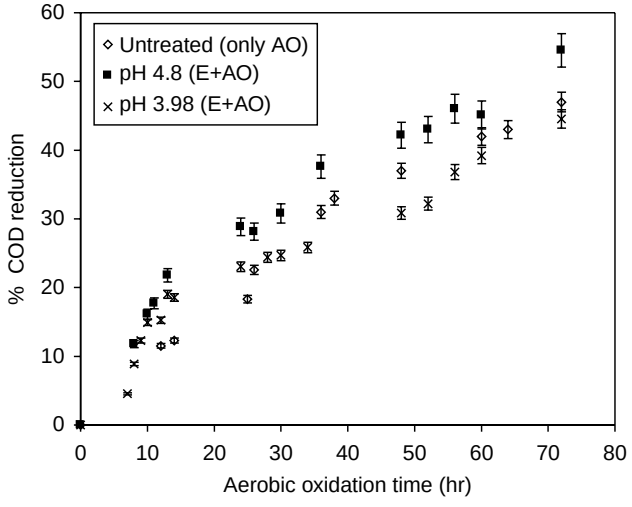

Fig. 1. % COD reduction for the enzymatically pretreated samples as compared to the control at different pH (Enzyme dose: 50 FPU and Pretreatment time: 24 h).

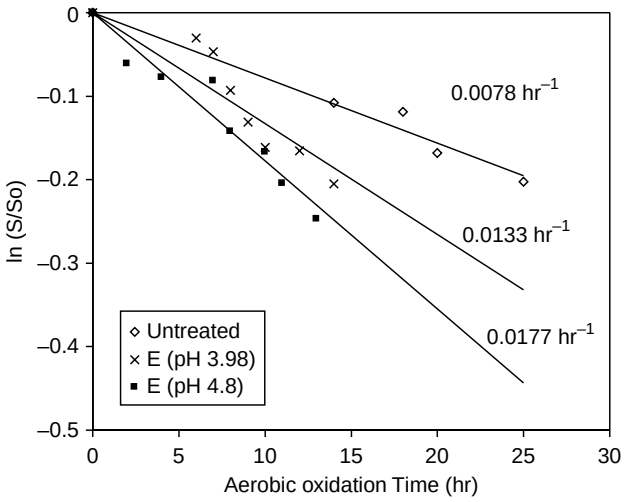

Fig. 2. First order degradation plot for the enzymatically pretreated sample at different pH (Enzyme dose: 50 FPU and Pretreatment time: 24 h).

3.3. Effect of enzyme concentration on the subsequent aerobic oxidation

The effect of the enzyme concentration on the subsequent biological oxidation process was also studied. In this regard the enzyme concentration was varied between 25 FPU to 100 FPU in order to maximize the initial enzymatic reactions. The pH during the run was kept at 4.8 (as found earlier) and the pretreatment was carried out for 24 h. The pretreated samples were then subjected to aerobic oxidation. Fig. 3 demonstrates the first order degradation plot for varying enzyme dosage. The enzymatic hydrolysis of cellulose is a complex process requiring the participation of several enzymes. The present study involves three types of enzymes i.e. cellobiohydrolase, endoglucanase and β-glucosidase. These are all hydrolytic enzymes and they act either in a sequential manner or simultaneously (Enari, 1983). In the present study, it was found that the enzyme dosage beyond 50 FPU did not result in an increase in the initial rate of aerobic oxidation. The enzyme dose of 50 FPU under the pretreatment condition yielded the maximum initial rate of subsequent aerobic degradation.

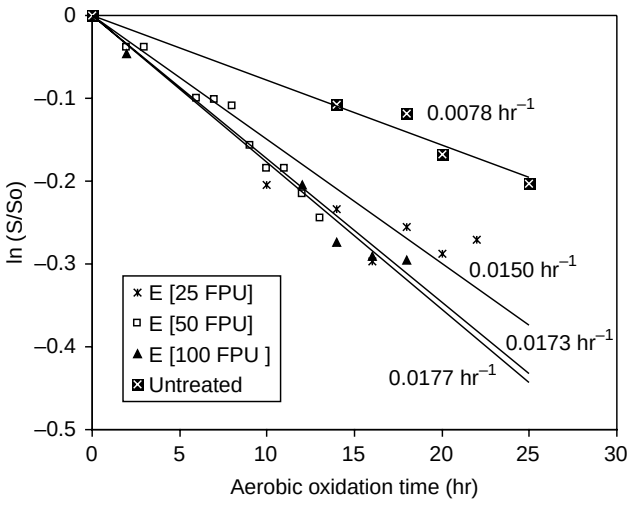

Fig. 3. First order degradation plot depicting the effect of enzyme dose on biological oxidation (pH: 4.8 and pretreatment time: 24 h).

3.4. Effect of the pretreatment time on the subsequent aerobic oxidation

In order to maximize the production of biologically degradable products during the enzymatic treatment, the distillery spent wash was subjected to different enzyme-contacting periods. The enzyme dose of 50 FPU was kept as an optimum and the operating pH was maintained at 4.8. The enzymatic pretreatment was carried out for two different periods, 12 and 24 h. The first order degradation plot demonstrating the effect on the subsequent aerobic initial oxidation rate is shown in Fig. 4. From the figure it is clear that the initial degradation rates were substantially higher, almost 2.3 times higher than for the untreated effluent sample but almost the same for the effluent treated for 12 and 24 h. No significant difference in 12 and 24 h of treatment suggests the possibility of an excess amount of enzyme during the treatment. Thus, we found that 12 h pretreatment with 50 FPU of cellulase yielded nearly the same oxidation rates as the 24 h pretreatment with 25 FPU of cellulase. This indicates that these reactions are first order with respect to the enzyme concentration and hence the treatment time and the enzyme concentrations are interchangeable. The results indicate that by manipulating the enzyme concentration, the treatment time can be reduced further to say 3 or 6 h and can still give enhanced biological oxidation rates. The above observation also allows us to represent these enzymatic reactions (i.e. breakdown of complex pollutant molecules to simpler ones) in the form of the first order plots as shown earlier.

4. General discussion

During the aerobic oxidation runs of the different batches studied, samples were withdrawn and were analyzed for MLSS levels along with the COD. In all the batch tests performed, the initial biomass concentration (X_0) was maintained in the range of 60–100 ppm through the inoculation. During the transient reaction of oxidation, the biomass responded differently by evolving into different growth patterns before the runs were stopped (at 72 h). The evolution of biomass during the aerobic oxidation of the samples pretreated with enzymes at different pH is shown in the Fig. 5. The rate of the substrate consumption appears to be significantly affected by the pH of the enzymatic pretreatment. This also suggests that the enzymatic reaction products are likely to be different.

In their review, Kovarova-Kovar and Egli (1998) have cited many such results, where the kinetic behavior of

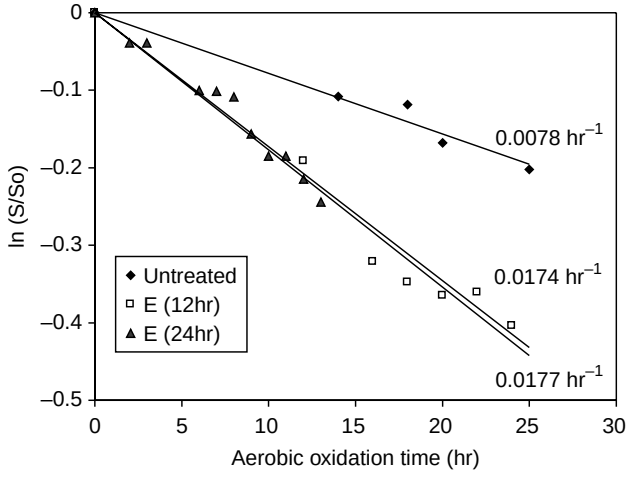

Fig. 4. Effect of enzyme pretreatment time on biological oxidation (pH: 4.8, Enzyme dose: 50 FPU).

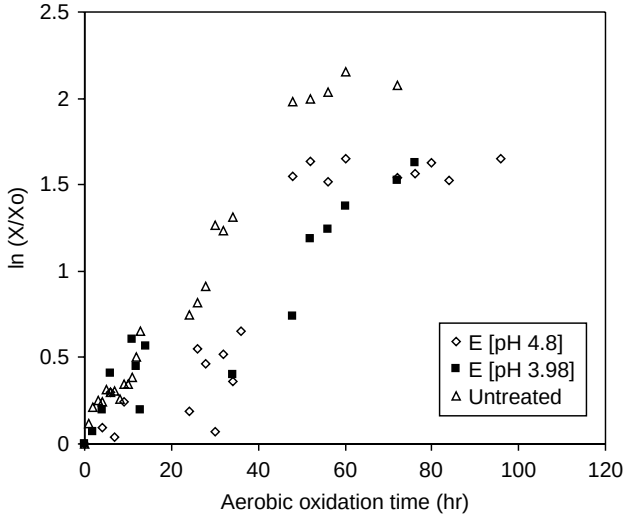

Fig. 5. Evolution of biomass for batches studied at different pH.

a microbial cell could not be described by a single set of kinetic rate constants. It has also been stated that the growth rate during the cultivation using two or three sugars (different substrates) is not controlled in the same way as that with individual sugars but is controlled by either the total sugar concentration or the concentration of a gross parameter like dissolved organic carbon, available as a substrate. Frequently, an increase in the maximum specific growth rate has been observed, when a culture is exposed to a mixture of carbon sources in comparison to growth with either of these substrates as single carbon sources, indicating simultaneous operation of two or more catabolic pathways (Kovarova-Kovar and Egli, 1998).

Bacterial degradation of large molecular weight compounds is initially very different from the degradation of small molecular weight compounds. Many small compounds can be transported (diffused) intact across the outer cell membrane, whereas larger molecules must be hydrolyzed by one or more enzymes to sub-units small enough to be transported into the cell. Molecules larger than 1000 amu (atomic mass unit) can be considered as macromolecules (Haldane and Logan, 18731).

Polysaccharide degradation thus can require a large amount of hydrolytic enzymes to fully degrade the polymer (Haldane and Logan, 1994). Based on the past studies, one can conclude that the treatability of the effluent wastewater depends strongly on the molecular weight distribution of the contaminants (Levine et al., 1985; Haldane and Logan, 1994; Ubukata, 1997; Eliosov and Argaman, 1995; Confer and Logan, 1997; Grady et al., 1984).

During the biological treatment, the molecular weight distributions in the wastewater change as a result of new cell synthesis, flocculation, adsorption and biochemical transformation/oxidation. The enzymatic breakdown of macromolecules is also feasible and has been reported in the literature. The size ranges of organic material in the wastewater play a key role in the biological treatment processes. Bacteria can take up molecules with a molecular mass of less than 10^3 amu such as amino acids, volatile fatty acids, and glucose. Polymers such as starch and proteins cannot be directly transported across the bacterial membranes. Therefore, some bacteria excrete hydrolases (for example, amylase for starch and protease for protein) that degrade these polymers to small assimilable molecules. Therefore, the hydrolysis of polymers to monomers may be the rate-determining step in the overall removal (consumption) of organic compounds during the biological wastewater treatment (Ubukata, 1997).

Many of the compounds, such as humic acids and refractory organic compounds, are relatively stable under the normal treatment conditions because of the limitations of the microbial action. By using physical/chemical treatment processes to remove or break up the large/bulkier molecules, biological treatment can work more effectively.

In this study, we have used enzymatic pretreatment to possibly alter the molecular composition of the distillery effluent. Higher biomass levels were attained in the untreated batch, and the enzymatically pretreated batch yielded lower biomass levels and yet gave higher initial overall COD degradation rates. The enzymatic pretreatment of the distillery effluent led to in situ formation of the hydrolysis products, which must have had different physical properties (say for e.g. diffusion coefficients—no analysis has been carried out) and were easier to assimilate than the parent pollutant molecules by the microorganisms, which led to faster initial rates of aerobic oxidation even at lower biomass levels. This was not the case for the biomass which was fed with the untreated effluent sample, indicating that the hydrolysis of the effluent components and its subsequent assimilation were at the expense of synthesis and secretion of more hydrolytic enzymes leading to higher biomass content. In the case of the untreated batch, the macromolecular degradation governed the initial phase of aerobic oxidation, thus slowing the rates of COD removal during the initial phase of aerobic oxidation.

One of the criteria for the successful application of the enzyme for the treatment is that the enzyme must exhibit a reasonable amount of its native activity under typical subsequent bio-treatment conditions. Hence the effect of the treatment conditions on the stability of the enzyme was tested. The monitoring of the enzyme activity during the pretreatment step indicated that the enzyme was fairly stable under the set of pretreatment conditions studied in this work.

The pH was periodically monitored during the aerobic oxidation step. Different experiments indicated a peculiar evolution of the pH during the aerobic oxidation step. The pH value gradually decreased from the initial value of 7.5–7.6 in first 8 h of oxidation to a value of 6.7–6.9 and then the pH gradually rose till the end of the run (72 h) attaining a value of 9.0–9.2. No obvious explanation could be offered at this stage. The effect has been demonstrated in Fig. 6. The results were similar for the pretreated and the untreated

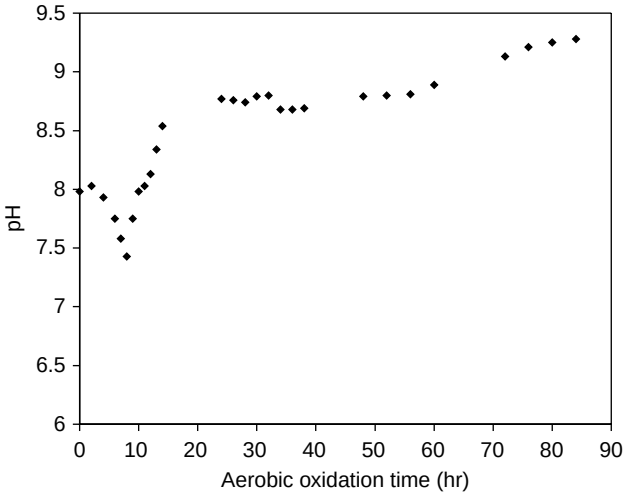

Fig. 6. Typical evolution of pH observed during the biological oxidation.

batches, indicating the role of the enzyme in only fragmenting the macromolecules present in the effluent.

A marginal increase (~100–120 mg or ~5–7%) in the sugars (estimated as reducing sugars) as hydrolysis products of the cellulosic materials was also observed during the pretreatment stage. Certain changes which are physical in nature without a corresponding increase in the reducing sugar values have also been noticed in the early stages of the enzymatic action. These physical changes may indicate an initial random disintegration of the macromolecular chains into molecules still retaining a relatively high molecular weight and without any large increase in the reducing groups (Gascoigne and Gascoigne, 1960). Another macromolecular degradation study by Haldane and Logan (1994) on dextran revealed that polyscaccharides can be released back into the solution before being completely utilized by suspended microbes, thus producing a large transformation in size (molecular weight) distribution with only minimal changes in the total carbohydrate concentrations (COD) (Haldane and Logan, 1994). The effect observed in this study is similar to that reported in the literature giving credence to such an observation. Thus it is hypothesized that the enzymatic pretreatment step led to the formation of intermediate size, partially hydrolyzed products rather than their monomeric form or complete breakdown. These intermediates must have been further relatively easily acted upon by the employed microbial system during the aerobic oxidation, resulting in enhanced aerobic oxidation rates.

5. Conclusions

The enzymatic pretreatment could be successfully used as a pretreatment step for treating the distillery spent wash. The pretreatment step did not reduce the COD of the effluent, but it did alter the metabolic value (microbial acceptability) of the effluent by the microbes used in the aerobic oxidation step by generating intermediate hydrolysis products from the parent cellulosic compounds present in the spent wash. The molecular weights of the intermediate products might have been lower than those of the parent molecules and hence must have led to an improved biodegradability of the distillery spent wash during the aerobic oxidation step (more than a 2 fold increase in the rate of degradation). The optimum pH for the pretreatment step may not always exactly correspond to the optimum pH for the maximum enzyme activity due to the complexity of the effluent. The enzyme was fairly stable under the conditions of pretreatment.

It is evident from the study that pre-hydrolyzing the effluent using enzymes enhanced the rates of the subsequent aerobic oxidation step. Care must be taken, however, when applying such a strategy in practice owing to the enormous volumes of the spent wash generated and the requirement of considerable dilution. The cost of the enzyme is also an important factor to be considered before using this technique on a large scale. The enzyme(s) may be immobilized and hence reused (as they were found to be stable) for the pretreatment step to decrease the enzyme cost to be able to integrate it or as an add on facility in a conventional treatment scheme for the spent wash.

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