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MICROBIOLOGICAL TREATMENT OF LIQUID WASTES PRODUCED DURING WET STORAGE OF SUGAR CANE BAGASSE IN PAPER PULP INDUSTRY

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ABSTRACT

Wet storage of bagasse used in the paper pulp industry produces considerable amounts of liquid wastes that can pollute the environment, particularly in the absence of the suitable treatment. Wastewater samples were analyzed for chemical oxygen demand (COD), 5-days biological oxygen demand (BOD_5), pH, total suspended solids (TSS), ash, nitrogen, phosphorus and microbial counts. Low records of microflora of *ca.* 10^3 cfu ml^{-1} and pH of 4.5 were estimated while concentration of organic substances was high (COD equals 3472 mg l^{-1}). The BOD_5 reached 2700 mgl^{-1} which corresponded to 77.8 % of the COD value. Nitrogen and phosphorus concentrations were almost 20 and 2 mg l^{-1}, respectively representing a poor COD: N: P ratio of 1736: 10: 1. Therefore, the optimal environmental conditions required for biological wastewater treatment were studied. The activity of microflora in the wastewater was enhanced which in turn stimulated the biodegradation of organic substances. The percentage of COD reduction appeared to be depended on the COD: N: P ratio and the pH. Results showed that this ratio should be modified to be suitable for nutrition and enrichment of microorganisms in the wastewater and the necessity of addition of 5% activated sludge from wastewater treatment plant as a source of acclimatized microorganisms. The best results were obtained when the COD: N: P ratio was adjusted to 1017: 16: 4, where the organic matter removal efficiency was 97%. The suspended solids in the effluent could be reduced to 40.7 mg l^{-1}, which corresponded to 8.9% of that of the influent. Maintaining the pH constant within a range of 7 during the treatment resulted in acceleration of the COD reduction *i.e* 68.2% reduction after 2 days compared to 33% without pH control. BOD_5 was reduced to 54.9 mg l^{-1}. The addition of a composite bacterial inoculant was efficient for COD reduction, since 80% of the COD was degraded within one day. However, considering the final COD content, activated sludge appeared to be more effective. The aerobic treatment of this wastewater would render it suitable to be discharged without environmental problems. These results show that there is potential for the plant to use such a treatment for treating its wastewater for discharge or reuse. The final water quality would be acceptable for dilution and pulp washing applications.

Key words: *activated sludge , bagasse, biological wastewater treatment, BOD_5, COD, paper pulp industry, wet storage.*

1. INTRODUCTION

There are 11 facilities of pulp and paper industries in Egypt producing 41 million m^3 of wastewater per year (Ezz, 2003). Thus, discharge of such high quantities without adequate treatment can pollute the environment. Among raw materials used for paper industry is the sugar cane bagasse (outer stalk sugarcane). It is the fibre left over after extracting sugar from sugarcane. The bagasse obtained from the sugar mill, having 3-4 % residual sugars is stored in the bagasse yard by wet bulk storage method (Atchison, 1971) for a period of 3-9 months. During storage, the bagasse is kept under wet conditions by spraying water over bagasse pile to preserve bagasse quality. Reclamation water, used to recover bagasse from storage, has a pH of approximately 4 and a population of acidogenic bacteria due to the acidogenic conditions under which bagasse is preserved during storage. This stream also contains suspended organic and inorganic materials (Hunt and Pretorius, 2000). With the everincreasing focus on the environmental issues and the development of more stringent laws, the prospect of minimizing water consumption and recovering wastewater for reuse has become more appealing to industry. The mill in question is investigating the possibility of eliminating wastewater discharge. To achieve this goal, it is necessary to treat a wastewater stream for reuse in the mill. One of the methods currently used for disposal of such wastewater is to discharge the effluent directly. Biological wastewater treatment includes a variety of methods which are used by pulp and paper mills for purifying their wastewater (such as the activated sludge method) in which natural microorganisms decompose the organic substances. The organisms constitute a nutrient cycle mainly consisting of bacteria and protozoa. Such method reduces the biological oxygen demand (BOD_5) by more than 95 % and removes *ca.* 50 % of the organic compounds from effluent. Preliminary investigations ruled out the possibility of using conventional biological treatment, chemical precipitation and acid induced lignin precipitation as possible wastewater treatment methods (Hunt and Pretorius, 1999). Both anaerobic and aerobic systems are used to treat wastewater (Lee *et al.*, 1989). However, Kortikaas *et al.* (1994) found that anaerobic/aerobic series treatment systems produce better final effluent at lower capital and running costs than anaerobic or aerobic processes on their own.

The major goal of this study was to determine if the bagasse wash wastewater could be biologically treated to meet both the international and local standards for discharging.

2. MATERIALS AND METHODS

2.1. Culture media

Cellulose agar (Atlas, 1997) was used for enumeration of the cellulytic bacteria, it is composed of (g l^{-1}): $(NH_4)_2SO_4$, 0.5; KH_2PO_4, 1.0; KCl, 0.5; $MgCl_2.6H_2O$, 0.5; yeast extract, 0.5; cellulose powder 10; L-asparagine, 0.5; $MgSO_4$, 0.2; $CaCl_2$, 0.1; agar, 20 and pH 7.0. The plates were inspected visually for a zone of clearing indicative of the consumption of insoluble cellulose. For enumeration of yeasts and molds, both Sabouraud Dextrose Agar (Oxoid, 1983) (g l^{-1}: peptone, 10; dextrose, 40; agar, 15 and pH 5.6) and Czapek Dox Agar (Oxoid, 1983) (g l^{-1}: $NaNO_3$, 2; KCl, 0.5; magnesium glycerol phosphate, 0.5; Fe SO_4, 0.01; K_2SO_4, 0.35; sucrose, 30; agar, 12 and pH 6.8) were used. The last medium received yeast extract, 5 g l^{-1}; streptomycin, 30 mcg ml^{-1} and aureomycin, 4 mcg ml^{-1} and the pH was 4.0 for fungi counting. Nutrient broth medium was used for growing the isolated bacteria and contained (g l^{-1}): peptone, 5; beef extract, 3 and pH 7.0.

2.2. Samples

The bagasse wash wastewater samples were collected in plastic tanks from the paper pulp plant at Aswan during the wet storage process. The process flow diagram is presented in Figure (1) (Covey *et al.*, 2006). Aerobic compost (cow dung) used as seed was obtained from the Faculty of Agriculture, Cairo University. The aerobically digested sludge was obtained from the domestic wastewater treatment plant at Zenin, Giza. Seed sludge was incubated aerobically for 24 h to oxidize any organic matter that might be present. Immobilized cells (polyseed, Polybac corporation, Belgium) were used for the BOD_5 test.

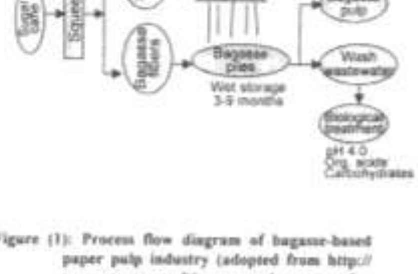

Figure (1): Process flow diagram of bagasse-based paper pulp industry (adopted from http:// www.coveyconsulting.com.au/paper_gc_ds_ potential_for_bagasse_in_aust).

2.3. Isolation and identification of bacteria from bagasse wash wastewater

A total of 21 bacterial colonies developed on agar plates enumerating of either total aerobic or cellulytic bacteria during the biological treatment of bagasse wash wastewater was picked up at random, purified and maintained on nutrient agar slants. Identification of the isolates was carried out according to their morphological, cultural, physiological and biochemical characteristics as described in Bergey's Manual of Determinative

Bacteriology (1994). Colony and cell morphology, Gram staining and catalase and oxidase reactions were determined as well.

2.4. COD reduction

Experiments were conducted either in 500-ml Erlenmeyer flasks or in a double walled glass column of 14.5 cm diameter and 46.5 cm height coupled with a pump (L/S Easy-Load Pump Heads, Model MZ 7518-00 Cole-Parmer) with an aeration velocity of 2.25 Liters min^{-1}. Flasks were shaken on a rotary shaker (Cole-Parmer, model 51704-25, USA) at 100 rpm and the column was aerated continuously. At zero time and at every alternate day for a period of 5 - 17 days, 2.5 ml were withdrawn for COD analysis. To study the removal effect of aerobic compost microbial population and aerobic sludge on COD degradation, 50 ml of seed aqueous compost solution filtrate were added to the column (wastewater initial pH 4.4). After 12 days of aerobic treatment, 20 ml of seed aerobic sludge were added and the wastewater was treated for another 5 days. The effect of phosphorus and nitrogen addition on COD reduction was investigated by supplementation of the wastewater (the initial pH was adjusted to7.3) with NH_4Cl (47.69 mg l^{-1}) and K_2HPO_4 (33.08 mg l^{-1}) and 5% (v/v) aerobic sludge. To investigate the influence of different nutrients concentrations, NH_4Cl (23.13 and 76.93 mg l^{-1}) and K_2HPO_4 (7.73 and 46.2 mg l^{-1}) were added to the wastewater supplemented with 5% aerobic sludge. The effect of pH was studied by maintaining the pH at 7.0 by automatic addition of NaOH and HCl during the treatment process and initial seed sludge was 5%. The ability of isolated bacteria to reduce the COD was monitored using a composite bacterial inoculant of members of *Pseudomonas*, *Acinetobacter*, *Micrococcus*, *Bacillus*, *Aeromonas* and 2 unidentified cultures. Cultures were grown each in 10 ml nutrient broth for 24 h, mixed together in equal portions, inoculated (5% v/v) into 120 ml wastewater supplemented with nitrogen (final conc. 40 mg l^{-1}) and phosphorus (final conc. 8 mg l^{-1}) and incubated for 7 days. To investigate the effect of different sludge concentrations, 5, 10 and 20 % aerobic sludge were added to wastewater received the same nutrients. After 7 days of treatment with the composite inoculant or 5% sludge, 10 ml of the wastewater-cell mixture were re-inoculated into 120 ml raw wastewater containing the same nutrients and incubated for another 7 days. To examine the ability of natural microflora in bagasse wash wastewater for COD reduction, wastewater supplemented with nitrogen and phosphorus (initial pH 7.0) was incubated without addition of any inoculants. All experiments were carried out at 25 °C. At the end of experiment, bacterial biomass was measured by centrifugation at 10000 rpm for 10 min and dried at 105 ºC for 12 h.

2.5. Chemical analysis

2.5.1. Determination of chemical oxygen demand

Chemical oxygen demand (COD) was measured using the closed reflux, colorimetric method (APHA, 1992). The tubes and caps were washed with 20% H_2SO_4 before use. Sample fractions (2.5 ml) were added into COD glass tubes and 1.5 ml of digestion solution (10.216 g $K_2Cr_2O_7$, 167 ml conc. H_2SO_4 and 33 g $HgSO_4$ were added to 500 ml distilled water and diluted to 1000 ml) was added. A volume of 3.5 ml of sulfuric acid reagent (5.5 g Ag_2SO_4 dissolved in 1 kg conc. H_2SO_4) was added down inside of tubes. The tubes were tightly sealed with screw caps, inverted each several times and placed in a COD block digester (model RS32, Fisher, Strasburg) preheated to 150 °C for 2 h. Blank and standard solutions were treated with the same manner as well. After digestion, the tubes were allowed to cool to room temperature and inverted several times and allowed the solids to settle before measuring absorbance at 600 nm using spectrophotometer (Model 6300 Jenway). To prepare the calibration curve, five standards from potassium hydrogen phthalate solution with COD equivalents from 20 to 900 mg O_2/l were prepared. The following linear equation was used to calculate the unknown COD: y=ax+b. The COD decrease was calculated by the difference between the initial COD of bagasse wash wastewater and the final COD after the biological treatment as a percentage (Gonzalez *et al*., 1997).

2.5.2. Measurement of 5-days biological oxygen demand

Biological oxygen demand (BOD_5) was measured according to APHA (1992) using narrow-mouth BOD_5 bottles (Nirschmann EM, Germany) with a 300 ml capacity and with glass stoppers. Using a wide-tip volumetric pipette, the wastewater samples were added to the bottles. Polyseed (Polybac corporation, Belgium) was added at the rate of 1.1 mg seed/bottle. Bottles were filled with enough dilution water (1 ml each of phosphate buffer, $MgSO_4$, $CaCl_2$ and $FeCl_3$ solutions/l of distilled water). The initial dissolved oxygen (DO) level was measured using a dissolved oxygen meter (model DO 100 series, Cole-Parmer). The bottles were then tightly sealed and incubated in the dark at 20 ºC for 5 days. After incubation, the final DO level was measured and the reduction in the amount of DO during incubation gave a measure of the BOD_5. The following equation was used for calculation:

$$BOD_5\ (mg/l) = \frac{(D1-D2) - (B1-B2)\,f}{P}$$

where: *D1* = DO of diluted sample immediately after preparation (mg l^{-1}).
D2 = DO of diluted sample after 5 d incubation at 20 °C (mg l^{-1}).
P = decimal volumetric fraction of sample used.
B1 = DO of seed control before incubation (mg l^{-1}).
B2 = DO of seed control after incubation (mg l^{-1}).
f = ratio of seed in diluted sample to seed in seed control = (% seed in diluted sample) / (% seed in seed control).

2.5.3. Nitrogen, phosphorus, ash, TSS and pH determinations

Total nitrogen was determined by Kjeldahel method (AOAC, 1995) while analysis of NO_3-N according to Cataldo's method (Cataldo *et al.*, 1975). Phosphorus was determined using photometric method according to AOAC (1995). Ash content was determined by ignition of oven-dried (105 ºC) samples at 600 ºC for 3 hrs in a temperature-controlled muffle furnace (AOAC, 1995). Measurement of pH was done using a standard pH glass electrode (Jenway, model 3020, UK). Total solids (TS) were determined by drying the sample at 105 ºC for 12 h. Total suspended solids (TSS) were estimated according to APHA (1999) as follows: a well-mixed sample was filtered, the residue retained on the filter was dried to a constant weight at 105 ºC and the TSS was calculated from the following equation: mg/l TSS = [(*A* – *B*) x 1000] / sample ml where *A* equals the weight of the filter plus the residue (mg) and *B* equals the weight of the filter (mg).

3. RESULTS AND DISCUSSION

3.1. Chemical and microbiological characterization of bagasse wash wastewater

Table (1) summarizes the chemical and microbiological analyses of the examined bagasse wash wastewater. The wastewater was characterized by low pH (4.6 ± 0.2). The high volatile fatty acid concentrations of 500-3500 mg l^{-1} and low pH of 4.5-5.5 are among the unique characteristics of bagasse wash wastewater (Chinnaraj and Venkoba, 2006). When bagasse is received from the sugar mill, it carries around 3-4% of residual sugars and during the storage these sugars are converted into organic acids by anaerobic microbial reactions that take place in the bagasse piles (Salabar and Maza, 1971). These organic acids reduce the pH in bagasse pile and preserve the bagasse quality, and get washed away during bagasse washing before pulping. Also, bagasse is stored in the wet condition by sprinkling the water over the pile to prevent degradation and fire hazard. The organic acids present in the pile get oozed out and join the bagasse wash wastewater and increase the volatile fatty acids content and reduce pH (Chinnaraj and Venkoba, 2006). Because of the fundamental relationship that exists between levels of pH, it was important to include this parameter in the standard for industrial effluents. The average value for chemical oxygen demand (COD) content recorded up to 2009 (filtered) or 3472 (unfiltered) mg l^{-1}. Low strength wastewaters are those with COD below 2000 mg l^{-1} (Kato *et al.*, 1994). Consequently, the examined bagasse wash wastewater is considered a high strength wastewater. The 5-days biological oxygen demand (BOD_5) reached 1838 (filtered) and 2700 (non filtered) mg l^{-1} representing a BOD_5/COD ratios of 0.92 and 0.78, respectively. The BOD test is very useful in determining the strength of industrial wastewater pollutants, in terms of oxygen that is required when discharged in natural water courses. It is also greatly useful in assessing the performance of industrial wastewater treatment plants. The wastewater produced from pulp mill contains suspended solids, carbohydrates and lignin with COD and BOD_5 of 76000 and 25000 mg l^{-1}, respectively (Gray, 1989). Hunt and Pretorius (2000) reported the following quality parameters of the waste water produced from bagasse-based pulp and paper mill (mg l^{-1}): total COD, 7775-15775 and TSS, 1024-5228). Lower values of suspended solids (400-1000 mg l^{-1}) and soluble COD (2000-7000 mg l^{-1}) were recorded by Chinnaraj and Venkoba (2006). With respect to growth nutrients, the bagasse wash wastewater contained 20.1 mg l^{-1} total nitrogen and 2 mg l^{-1} of phosphorus, which represent a poor COD: N: P ratio of 1004.5: 10: 1.0. No nitrate-N traces could be detected in the wastewater. The bagasse wash wastewater contained 457 mg l^{-1} total suspended solids. The amount of suspended solids determines the need for, and design of primary sealing-tanks in plants employing biological treatment processes. The numbers of microflora were relatively low (cfu ml^{-1}: total aerobic counts, 3.8 x 10^3 and cellulytic bacteria, 1.5 x 10^2). Examination of the colonies developed on the nutrient agar plates revealed that the Gram-negative short rods and Gram-positive spore-forming bacteria predominated other groups (each 35.3 %). Other groups included yeasts (17.6 %), molds (5.9 %) and Gram-positive cocci (5.9 %) as shown in Table (1).

3.2. Time course of COD reduction in bagasse wash wastewater

During the first day of treatment, no remarkable decrease in the COD content was observed (Table, 2). This might be attributed to the acidic reaction of the wastewater (pH 4.40 - 4.54). Acidification of a whey-containing wastewater was found to be the rate limiting step in the COD reduction (Kato *et al*., 1994). The microbial populations remained almost without considerable change. As the pH rose (from 4.54 to 7.24) after 3 days, the microbial population doubled around 6 times and the COD content decreased by 76.2% representing 882 mg COD removed per liter and day. Further treatment of the wastewater up to 7 days resulted in only a slight decrease (4.5%) of COD. Re-inoculation of the column with aerobic sludge led to 9.1% more COD reduction after 17 days. Up to this time, 89.8% of the COD was degraded. Aerobic treatment of bagasse wash wastewater greatly reduced its carbon content. After the aerobic treatment, the pH rose to 8.78. However, the COD decrease continued indicating that this alkaline pH was not inhibitory to the microorganisms which were able to digest more carbon. A similar trend was noticed in experiments on bagasse based pulp and paper mill carried out by Hunt and Pretorius (2000) who reported an increase of pH to 9.0 during the aerobic treatment process. This might be due to the formation of ammonium through the microbial activities which was not further oxidized to nitrate probably due to the absence or inhibition of the nitrifying bacteria since nitrate was not detected. Chinnaraj and Venkoba (2006) suggested that the increase in pH and alkalinity during anaerobic treatment of bagasse wash wastewater was good indication that most of the organic compounds were converted into CH_4 and CO_2.

3.3. Time course of COD reduction in bagasse wash wastewater after addition of phosphorus and nitrogen

The calculated COD: N: P ratio in the bagasse wash wastewater was estimated as 1004.5: 10.1: 1. The ratio required for industrial wastewater treatment varies, depending on the wastewater type and the microorganisms used. Ratios of 1000: 13: 1 (malt whisky distilleries; Brown *et al*., 1976), 100: 10: 2 (molases; Kargi and Eylisleyen, 1995) and 900: 5: 1.7 (olive mill wastewater; Dirk *et al*., 2002) were applied. In the present study, the ratio was modified through the addition of 12.5 mg l^{-1} nitrogen (total concentration 32.6 mg l^{-1}) and 6 mg l^{-1} phosphorus (total concentration 8 mg l^{-1}) to the wastewater (unfiltered COD: N: P = 1306: 12.3: 3) (filtered = 759.3: 12.3: 3) at initial pH of 7.3. Such modification accelerated the COD decrease, where more than 95% of COD reduction was achieved after 5 days (Table, 3).

This efficiency was 1.3 fold more than that obtained without addition of nitrogen and phosphorus. The biomass produced after 5 days was 0.191 g l^{-1} representing 0.073 g dry cells /g COD removed. The 5-days biological oxygen demand (BOD_5) content dropped from initial 2700 mg l^{-1} to 148.9 mg l^{-1} in the effluent, indicating that the remained COD (188 mg l^{-1}) in the supernatant after separation of the cells represented biodegradable carbon and not microbial cells. Total suspended solids (TSS) dropped to 40.7 mg l^{-1} which corresponded 8.9% of that of the influent and representing 91.1% reduction efficiency due to the entrainment and digestion of solids by the activated sludge. Of particular significance, is that TSS were reduced to 1.2% of the initial COD value as was recommended (less than 10%) for UASB reactors by Lettinga and Hulshoff (1991). Studies conducted by Hunt and Pretorius (2000) with wastewater from bagasse-based pulp and paper mill showed 93% TSS reduction in an aerobic reactor and final TSS of *ca*. 165 mg l^{-1} which corresponded to *ca*. 1.4% of the initial COD. They suggested that some tertiary treatments would be required to lower the TSS levels to the recommended 50 mg l^{-1} for spray applications. All biological treatment processes employed by environmental engineers are dependent upon the reproduction of organisms involved in the treatment process. In planning wastewater treatment facilities it becomes important to know whether the wastewater contains sufficient quantities of nitrogen and phosphorus for the

Table (1): Chemical and microbiological characteristics of bagasse wash wastewater.

Average quality parameters	Value
I. Chemical parameters (mg l^{-1}):	
COD (filtered wastewater)	2009.1*
BOD_5 (filtered wastewater)	1838
COD (non filtered wastewater)	3472.3*
BOD_5 (non filtered wastewater)	2700
Total N (Kjeldahel N)	20.1
Total P	2.0
Nitrate-N	0.0
COD: N: P (COD filtered)	1004.5 :10.1: 1.0
COD: N: P (COD non filtered)	1736.2: 10.1: 1.0
Total suspended solids (TSS)	457
Total solids (dry weight)	900
Ash content	175
AFDW**	725
pH	4.6 ± 0.2
II. Microbiological characteristics (cfu ml^{-1}):	
(100%): Total aerobic counts	3.8×10^3
Gram negative short-rods (35.3%)	
Gram positive spore-forming rods (35.3%)	
Yeasts (17.6%)	
Molds (5.9%)	
Gram positive cocci (5.9%)	
Cellulytic bacteria	1.5×10^2
Yeasts	6.0×10^2
Molds	< 10

* Mean of 8 samples: ** Ash free dry weight (a crude measurement of total organic substance).

Table (2): Time course of COD reduction in bagasse wash wastewater * .

Time (days)	COD remained (non filtered)		pH	Microbial counts (cfu ml^{-1})			
	mg l^{-1}	%		Total aerobic bacteria	Cellulytic bacteria	Yeasts	Molds
0	3472.3 (compost addition)	100	4.40	4.0×10^4	1.2×10^2	1.9×10^4	<10
1	3450.1	99.4	4.54	3.0×10^4	3.6×10^2	<10	<10
3	826.4	23.8	7.24	2.6×10^5	2.8×10^2	<10	12
5	815.3	23.5	8.27	2.0×10^6	5.8×10^3	6.1×10^2	18
7	650.7	18.7	8.78				
12	670.1 (sludge addition)	19.3					
15	470.8	13.6					
17	354.3	10.2					

*COD non filtered 3472.3 (COD: N: P 1736.2: 10.1: 1.0); total N, 20.1 mg l^{-1}; total P, 2 mg l^{-1}.

Table (3): Time course of COD reduction in bagasse wash wastewater after addition of N and P at initial pH of 7.3.

Time	COD (mg l^{-1})		BOD_5 (mg l^{-1})	TSS (mg l^{-1})	pH	NO_3^- (mg l^{-1})	Microbial counts (cfu ml^{-1})		Biomass (g l^{-1})**
	Non-filtered	Filtered					Total aerobic bacteria	Cellulytic bacteria	
0 h	3482.0 (100)*	2025 (100)	2700	457	7.3	0	9.1×10^4	4.8×10^2	-
5 h	2635.9 (75.7)	-	-	-	-	-	-	-	-
20 h	2402.6 (69.0)	1354.7 (66.9)	-	-	-	-	-	-	-
26 h	2225.0 (63.9)	1354.7 (66.9)	-	-	8.6	0	1.7×10^8	6.2×10^5	-
46 h	-	1354.7 (66.9)	-	-	9.0	0	1.1×10^7	8.4×10^4	-
3 d	454.2 (18.3)	336.2 (16.6)	-	-	9.1	0	2.2×10^7	1.2×10^5	-
4 d	550.2 (15.8)	279.5 (13.8)	-	-	9.5	0	1.6×10^7	1.1×10^5	-
5 d	188.0 (5.4)	216.7 (10.7)	148.9	40.7	9.5	0	9.2×10^7	1.2×10^5	0.191

*Values between brackets are percentages COD remained; total N, 32.6 mg l^{-1}; total P, 8.0 mg l^{-1}; COD: N: P = 435.3: 4.1: 1.0 (non filtered); COD: N: P = 253.1: 4.1: 1.0 (filtered); -, not determined.

**Dry weight.

microorganisms. The important questions as nutrient ratios, e.g. C: N and C:P ratios, have been almost totally ignored, in spite of the fact that industrial wastewaters are frequently deficient in nitrogen and/ or phosphorus as far as the microbial growth is concerned. The physiological potential of microbes depends on their ability to synthesize enzymes that mediate substrate biodegradation. Enzyme synthesis is a function of the availability of a nitrogen source.

3.4. Effect of different concentrations of phosphorus and nitrogen on COD reduction efficiency

The highest drops in COD concentration of 82.5 and 70.2 % were achieved after 2 days of treatment with 12.4 mg l^{-1} P (COD: N: P = 613: 11: 4.1) and 40.1 mg l^{-1} N (COD: N: P = 790: 20: 4), respectively (Tables 4 and 5). This coincided with the increase in total microbial population (*ca.* 10^{8} cfu ml^{-1}). At this time, a positive correlation was obtained between percentage of COD reduction and both P and N concentrations (Figure. 2). Hamer (1990) reported that a BOD_5: N: P ratio of 100: 5: 1 was ideal for growth and treatment process.

3.5. Time course of COD reduction in bagasse wash wastewater after addition of phosphorus and nitrogen at controlled neutral pH

Maintaining the pH constant within a range of 7.0 during the treatment resulted in acceleration of the COD reduction *i.e.* 68.2% reduction after 2 days (Table, 6) compared to 33.1% (Table, 3) without pH control. This indicates that neutralization of pH favored the bacterial growth since at that time the total aerobic microflora and cellulytic bacteria were 5.1 and 16.7 fold higher than those without pH control, respectively. After 5 days, the aerobic treatment reduced the COD to 59.3 mg l^{-1} (*ca.* 97% reduction) confirming the claims in the literature that aerobic treatment was required to achieve optimum COD reduction. About 3.0% of COD in the wastewater remained without degradation in the effluent and 14.7% of the initial COD was converted to biomass (0.3 g dry biomass) representing 0.15 g dry cells /g COD removed. The remaining 82.4% was apparently converted to CO_2. The BOD_5 content of the effluent dropped to 54.9 mg l^{-1}, which corresponded to 2.9% of that of the influent and 92.6% of the final COD and representing a reduction efficiency of 97.1%. The observed decrease efficiency was higher than that obtained by Hunt and Pretorius (2000) with wastewater from bagasse-based pulp and paper mill who reported 93% COD reduction in an aerobic reactor. In a wastewater, the pH has a special significance in the sense that, it must be controlled within a range favorable to particular organisms involved. The biomass formed after 5 days in the present study was 1.6 fold higher than that without pH control. This favors the proposed hypothesis that the effective wastewater biodegradation being a growth associated process.

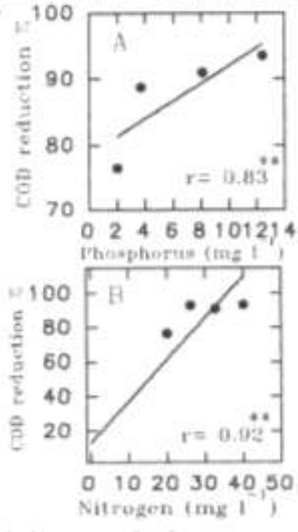

Figure (2): Linear regressions between percentage COD reduction and both phosphorus (A) and nitrogen (B) concentration.

3.6. Isolation and identification of bacteria from bagasse wash wastewater

During the time course of biological treatment of bagasse wash wastewater, different bacteria were isolated and identified to investigate the predominant candidates that are capable of growing under such conditions and to test their ability to degrade the wastewater. Isolates 1, 2, 7-11 and 13-21 were obtained from plates inoculated by dilutions of wastewater of pH 9.0. Results of the identification are summarized in Table (7). A total of 21 bacterial cultures was obtained. Thirteen (61.9 %) out of the total

Table (4): Time course of COD reduction in bagasse wash wastewater as affected by different nitrogen concentrations (26.1, 32.6 and 40.1 mg l^{-1}) (initial pH of 6.7).

Time (days)	COD (mg l^{-1}) (filtered)			pH			Bacterial counts (cfu ml^{-1})					
							Total aerobic bacteria			Cellulytic bacteria		
	26.1 N	32.6 N	40.1 N	26.1 N	32.6 N	40.1 N	26.1 N	32.6 N	40.1 N	26.1 N	32.6 N	40.1 N
0	1615 (100)	1597.7 (100)	1580.5 (100)	6.7	6.7	6.7	9.5×10^4	9.5×10^4	9.5×10^4	3.3×10^2	3.3×10^2	3.3×10^2
1	1619.3 (100.3)	1653.8 (103.5)	1481.1 (93.7)	8.1	8.0	8.1	6.1×10^7	9.5×10^7	1.1×10^8	8.0×10^5	2.4×10^6	4.2×10^6
2	773 (47.9)	980.2 (61.4)	470.7 (29.8)	8.3	7.9	8.4	2.0×10^7	3.7×10^7	7.3×10^7	1.1×10^6	1.9×10^6	1.9×10^6
3	504.2 (31.2)	240.5 (15.1)	249.6 (15.8)	8.2	8.5	8.1	1.6×10^7	7.9×10^7	4.5×10^7	2.5×10^5	7.7×10^5	2.9×10^5
4	152.9 (9.5)	295.7 (18.5)	214.4 (13.6)	8.6	8.3	8.3	1.4×10^7	2.5×10^7	4.3×10^7	2.6×10^5	6.0×10^5	2.8×10^5
5	116.6 (7.2)	143.9 (9.0)	106.3 (6.7)	8.9	8.7	8.7						
6	-	51.3 (3.2)	101.9 (6.5)	8.9	8.8	8.9						

Total phosphorus concentration in all treatments is 8.1 mg l^{-1}.

Table (5): Time course of COD reduction in bagasse wash wastewater as affected by different phosphorus concentrations (3.7, 8.1 and 12.4 mg l^{-1}) (initial pH of 6.7) .

Time (days)	COD (mg l^{-1}) (filtered)			pH			Bacterial counts (cfu ml^{-1})					
							Total aerobic bacteria			Cellulytic bacteria		
	3.7 P	8.1 P	12.4 P	3.7 P	8.1 P	12.4 P	3.7 P	8.1 P	12.4 P	3.7 P	8.1 P	12.4 P
0	2005.7 (100)	1597.7 (100)	1839.1 (100)	6.7	6.7	6.7	9.5×10^4	9.5×10^4	9.5×10^4	3.3×10^2	3.3×10^2	3.3×10^2
1	1438.1 (71.7)	1653.8 (103.5)	1679.7 (91.3)	8.07	8.0	8.14	4.9×10^7	9.5×10^7	9.0×10^7	2.2×10^6	2.4×10^6	1.4×10^6
2	874.5 (43.6)	980.2 (61.4)	522.5 (17.5)	8.28	7.9	8.04	6.2×10^7	3.7×10^7	1.0×10^8	8.0×10^5	1.9×10^6	1.4×10^6
3	417.2 (20.8)	240.5 (15.1)	351.0 (19.1)	8.50	8.47	8.29	2.1×10^8	7.9×10^7	9.6×10^7	5.5×10^5	7.7×10^5	2.4×10^5
4	439.3 (21.9)	295.7 (18.5)	202.6 (11.0)	8.83	8.26	8.67	1.2×10^8	2.5×10^7	4.4×10^7	7.0×10^5	6.0×10^5	2.1×10^5
5	226.6 (11.3)	143.9 (9.0)	118.9 (6.5)	8.90	8.73	8.89						
6	110.3 (5.5)	51.3 (3.2)	168.4 (9.2)	8.91	8.79	9.0						

Total nitrogen concentration in all treatments is 32.6 mg l^{-1}.

Table (6): Time course of COD reduction in bagasse wash wastewater after addition of nitrogen and phosphorus as affected by pH control (constant pH 7)

Time (days)	COD ($mg\ l^{-1}$) (Filtered)	BOD_5 ($mg\ l^{-1}$)	Microbial counts ($cfu\ ml^{-1}$)		Biomass**
			Total aerobic bacteria	Cellulytic bacteria	($g\ l^{-1}$)
0	2035 (100)*	1888	7.7×10^4	2.1×10^3	-
1	1818 (89.3)	-	4.5×10^7	4.5×10^5	-
2	648 (31.8)	-	5.6×10^7	1.4×10^6	-
3	193.6 (9.5)	-	7.9×10^6	7.5×10^4	-
4	154.1 (7.6)	-	-	7.5×10^4	-
5	59.3 (2.9)	54.9	1.8×10^6	1.0×10^3	0.3

*Values between brackets are percentages of COD remained; total N, 32.6 $mg\ l^{-1}$; total P, 8.0 $mg\ l^{-1}$; COD: N: P = 254.4: 4.1: 1 ; **Dry weight

Table (7): Morphological and biochemical characteristics of bacterial cultures isolated during biological treatment of bagasse wash wastewater

Isolate No.	Isolated from	Colony color	Motility	Catalase	Oxidase	Glucose		Proposed genus
						Oxidative	Fermentative	
Gram negative short rods								
1	Cel. plates	Gray	+	+	+	-	-	
2	Cel. plates	Colorless	+	+	+	-	-	
3	Cel. plates	Colorless	+	+	+	+	-	
4	Cel. plates	Colorless	+	+	+	±	-	
5	Cel. plates	Colorless	+	+	+	-	-	
6	Cel. plates	Colorless	+	+	+	-	-	
7	TC	Gray	+	+	+	+	-	
8	TC	Gray	+	+	+	+	-	
9	TC	Colorless	±	+	+	-	+	*Aeromonas**
10	TC	Gray		+	+	+	-	
11	TC	Colorless	+	+	+	+	+ (gas)	
12	TC	Colorless	-	+	-	+	+	
13	TC	Colorless	-	+	-	-	-	
Gram negative non-spore-forming thin long rods								
14	Cel. plates	Colorless	+	+	+	-	-	
15	Cel. plates	Colorless	+	+	+	+	-	
16	TC	Gray	+	+	-	-	-	
17	TC	Gray	+	+	-		-	
18	TC	Colorless	+	+	+	-	-	
Gram positive spore-forming rods								
19	TC		+	+	+	+	+	*Bacillus***
Gram positive coccus-shaped cells								
20	TC		-	+	-	+	+	*Micrococcus****
Gram positive oval-shaped cells								
21	TC			+	-	+	-	

Morphological and biochemical tests	Identification of *Pseudomonas* Characteristics of isolates				Identification of *Acinetobacter* Characteristics of isolates		
	3	4	7	8	12	13	
Cell shape	rods	rods	rods	rods	coccobacilli	coccobacilli	
Motility	+	+	+	+	-	-	
O_2 requirement	aerobic	aerobic	aerobic	aerobic	aerobic	aerobic	
Oxidase test	+	+	+	+	-	-	
O/F glucose test	+/-	±/-	+/-	+/-	+/-	-/-	
Gelatin hydrolysis	+	+	+	+	-	-	
NO_3^- reduction	+	+	+	+	-	-	
Starch hydrolysis	-	-	-	-	nd	nd	
Indol from					-	-	
tryptophan					-	-	
Acid from glucose					+	+	
Citrate utilization							

(+), positive result; (-), negative result; ±, weak result; nd, not determined; TC, total aerobic counts plates; rod-shaped cells are straight rods.
*Mannitol (+); arginin dihydrolase (+); urease (-); gelatin hydrolysis (+); NO_3^- reduction (+).
**Gelatin hydrolysis (+); starch hydrolysis (+); casein hydrolysis (+); acetyl methyl carbinol (+); citrate utilization (+).
***NO_3^- reduction (+); arginin hydrolysis (-); 7.5% NaCl not required for growth.

bacterial isolates were microscopically recognized as Gram negative rod shaped. They were further recognized into two major groups; non pigment producers and producers of diffusible gray pigment. Four (30.8 %) isolates out of 13 Gram negative rod shaped isolates were found to belong to the genus *Pseudomonas*. Two (15.4 %) isolates could be identified as *Acinetobacter*. One isolate belonged to the genus *Aeromonas*. Gram positive spore-forming rods (mainly *Bacillus*) and Gram positive coccus-shaped cells (mainly *Micrococcus*) were isolated in low frequencies.

3.7. Ability of isolated bacteria to degrade the bagasse wash wastewater

Referring to the ecological concept "the environment selects", it was decided to apply an actively growing composite inoculant of members of *Pseudomonas*, *Acinetobacter*, *Micrococcus*, *Bacillus*, *Aeromonas* and 2 unidentified cultures. The microorganisms, when grown in pure cultures utilize the same substrate constituents, while in mixed culture different constituents are used by each candidate, permitting a stable and complementary active population. Two subsequent inoculation runs were conducted in the raw wastewater. In the 1st run, the inoculant was efficient in the COD reduction, since *ca* 80% of the COD was degraded within 1 day (Table, 8). However, this inoculant did not achieve the desirable COD reduction after 6 days, since *ca.* 10% of the COD content remained. Re-inoculation (2nd run) of the growing cells into raw wastewater resulted in 1 - 5.5% more COD reduction and the reduction efficiency increased to *ca.* 97%. It is supposed that the re-inoculated cells are more acclimatized and consequently were more efficient.

3.8. Effect of activated sludge concentration on the COD reduction efficiency

To select the most appropriate sludge quantity, three concentrations were tested *i.e.* 5, 10 and 20%. The highest COD reductions efficiencies were observed with 5% sludge (Table, 8). However, after 6 days of treatment, a positive correlation between sludge concentration and the percentage of decrease was obtained (Figure, 3). Re-inoculation of the grown cells into fresh wastewater had no remarkable effect on the COD reduction efficiency. Activated sludge appeared to be more effective (97.5% COD reduction after 6 days) than the isolated composite inoculum (89.8%).

Wastewater incubated without the addition of any inoculants had a poor degradation activity towards COD. The COD decreased slowly and the reduction efficiency estimated after 6 days was 1.2 -1.3 fold lower than that with sludge or bacterial inoculant (Table,8), indicating the necessity of the addition of acclimatized microorganisms.

Finally, it could be concluded that the aerobic treatment of the bagasse wash wastewater might successfully reduce the organic load to a minimum extent (mg l^{-1}: COD, 10.8; BOD_5, 54.9 and TSS, 40.7 representing reduction efficiencies of 99.4, 97.1 and 91.1%, respectively) which guarantee a substantially purified effluent. It is strongly recommended to adopt the COD: N: P ratio of 1017: 16: 4 to secure the proper treatment. The US Environmental Protection Agency (EPA, 1998) 'dentified' best" facilities on a BOD performance criteria of achieving a 95% BOD reduction or a BOD effluent level of 40 mg l^{-1}. According to the Egyptian guidelines (1994), the discharge criteria were (mg l^{-1}): BOD_5, 60; COD, 100 and TSS, 60. Wastewater generated from bagasse washing and from the bagasse yard having relatively high COD when compared to other wastewater from bleach plant and paper machine, can be treated by anaerobic process followed by activated sludge process. The anaerobic stage is credited for removing most of the COD while producing smaller quantities of sludge than equivalent aerobic systems, while the aerobic stage is capable of more complete COD reduction. Another advantage of the aerobic over anaerobic treatment is the conversion of organic compounds to CO_2 whose global warming potential is 21 times less than CH_4(IPCCC, 1996).

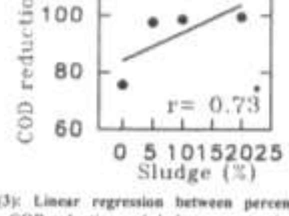

Figure (3): Linear regression between percentage COD reduction and sludge concentration.

Table (8): Time course of COD reduction in bagasse wash wastewater using composite bacterial inoculant or different sludge concentrations (initial pH 7)

Time (hours or days)	COD (mg l^{-1}) (Filtered)				
	Composite bacterial inoculant	Sludge (%)			Without sludge or bacterial inoculation
		5	10	20	
1st inoculation run:					-
0	1829.1 (100)	1725.2 (100)	1806.8 (100)	1886.1 (100)	1934.3 (100)
17 hour	368 (20.1)	447.9 (26)	672.1 (37.2)	716.7 (38)	1108.4 (57.3)
26 hour	381.7 (20.9)	290.8 (16.9)	623.3 (34.5)	805.4 (42.7)	723.4 (37.4)
42 hour	326.5 (17.9)	203.3 (11.8)	616.1 (34.1)	386.7 (20.5)	524.2 (27.1)
50 hour	247 (13.5)	168.7 (9.8)	272.8 (15.1)	452.7 (24)	729.2 (37.7)
3 days	247 (13.5)	61.6 (3.6)	209.6 (11.6)	173.5 (9.2)	468.1 (24.2)
4 days	-	-	65.0 (3.6)	62.2 (3.3)	512.6 (26.5)
6 days	167.6 (9.2)	43.3 (2.5)	-	11.3 (0.6)	433.3 (22.4)
7 days	-	32.9 (1.9)	10.8 (0.6)	-	272.7 (14.1)
2nd inoculation run:					
0	1886.6 (100)	1802.8 (100)			
1 day	469.7 (24.9)	403.8 (22.4)			
2 days	277.3 (14.7)	248.8 (13.8)			
3 days	150.9 (8.0)	176.7 (9.8)			
4 days	84.9 (4.5)	155.0 (8.6)			
5 days	113.2 (6.0)	72.1 (4.0)			
6 days	58.5 (3.1)	45.1 (2.5)			
7 days	-	45.1 (2.5)			

Values between brackets are percentages of remained COD; total N, 40 mg l^{-1} and total P, 8 mg l^{-1}.

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المعالجـة الميكروبيولوجيـة للمخلفـات السائلـة الناتجـة عن التخـزين الرطـب لمصاصـة قصـب السكـر فى مصانـع لـب الورق

رفاعـى ابراهيـم رفاعـى

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ملخص

ينتج عن التخزين الرطب لمصاصة قصب السكر المستخدمة فى صناعة لب الورق مخلفات سائلة بكميات كبيرة تسبب تلوثا للبيئة اذا لم يتم معالجتها فى الوقت المناسب نظرا لاحتوائها على العديد من المواد العضوية القابلة للتحلل. اشتملت التحليلات الكيميائية لهذة المخلفات السائلة على تقدير كل من الـ pH ، الفوسفور، النيتروجين ، المادة الصلبة المعلقة الكلية، الرماد، COD، BOD_5 ,والنيترات. واشتملت التحليلات الميكروبيولوجية على تقدير كل من الأعداد الكلية للميكروبات وأعداد البكتريا المحللة للسليلوز وأعداد كل من الفطريات والخمائر. تميزت هذه المخلفات السائلة بانخفاض درجة الـ pH (4,5) واحتوائها على أعداد منخفضة نسبيا من الكائنات الحية الدقيقة ولكنها احتوت على تركيزات مرتفعة من المواد العضوية حيث بلغت قيمة الـ COD 3472 جزء فى المليون ، قيمة الـ BOD_5 2700 جزء فى المليون، حيث مثلت الـ BOD_5 77,8 % من قيمة الـ COD. كانت نسبة كل من النيتروجين والفوسفور منخفضه نسبيا (20 ، 2 جزء فى المليون على التوالى) معطية نسبة غير متوازنة من الـ الكربون : النيتروجين : الفوسفور حوالى 1736: 10: 1 . بلغت الاعداد الكلية للكائنات الحية الدقيقة $3,8 \times 10^{3}$ مستعمرة/ مل وأعداد البكتريا المحللة للسليلوز حوالى $1,5 \times 10^{2}$ مستعمرة / مل لذلك

فقد تم دراسة أنسب الظروف البيئية لمعالجة هذه المخلفات ميكروبيولوجيا تحت الظروف الهوائية، وذلك باكثار أعداد الكائنات الحية الدقيقة التى يتصادف وجودها بها وتعظيم دورها فى تحليل المواد العضوية الموجودة والتخلص منها. اتضح أن معدل النقص فى الـ COD يعتمد بدرجة أساسية على نسبة الـ COD: N: P والـ pH. أثبتت الدراسة أنه يلزم تعديل نسبة الكربون : النيتروجين : الفوسفور بحيث تكون مناسبة لتغذية واكثار أعداد الكائنات الحية الدقيقة وكذلك اضافة 5 % من الحمأة النشطة المتأقلمة المتحصل عليها من محطات معالجة الصرف الصحى كمصدر خارجى للميكروبات. تحققت أفضل النتائج عندما كانت نسبة الكربون: النيتروجين: الفوسفور 1017: 16 : 4 حيث بلغت كفاءة تكسير المادة العضوية 97%. أمكن خفض محتوى ماء المخلف المعالج من المواد المعلقة الى 40,7 ميلليجرام/لتر ويمثل ذلك 8,9% من قيمتها فى ماء المخلف قبل المعالجة. أدى الحفاظ على درجة pH فى مدى من التعادل أثناء المعالجة الى الاسراع فى انقاص الـ COD حيث نقص 68% فى خلال يومين مقارنة بحوالى 33% بدون التحكم فى الـ pH. انخفض مستوى الـ BOD_5 الى 54,9 ميلليجرام/لتر. كان اضافة لقاح بكتيرى مختلط معزول فعالا فقد تم تكسير حوالى 80% من الـ COD خلال يوم واحد. ومع ذلك فعند الأخذ فى الاعتبار المحتوى النهائى من الـ COD فكانت الحمأة النشطة أكثر كفاءة. يتبين مما سبق أن المعالجة الهوائية لهذا المخلف السائل يجعله امنا للتصريف بدون مشاكل بيئية وأنه من الممكن تطبيق هذه المعالجة فى مصانع لب الورق وأن المواصفات النهائية للماء المخلف بعد المعالجة تطابق المواصفات المحلية والعالمية.

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