RESEARCH NOTE

BIOLOGICAL PRETREATMENT OF A BEVERAGE WASTE USING YEAST ISOLATED FROM THE FACTORY SLUDGE

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Abstract The beverage industry has been a heavy producer of organic pollution. The major contaminants found in waste are biodegradable organic compounds, volatile organic compounds, toxic metals, and recalcitrant xenobiotics, suspend solids, nutrients (Nitrogen and Phosphor) Microbial pathogens and parasites. Activated sludge flocs contain a wide range of microorganisms such as bacteria, Fungi, yeast, viruses, and protozoa. In this study, we isolated some strains of yeasts from the factory sludge. More than 50 strains of yeast were selected. Among them 12 strains had a high removal of TOC.Yeast No. 11 (Y-11) has shown an overall efficiency of 87.5% in terms of TOC removal.

Key Words Beverage Wastewater, Activated Sludge, Total Organic Carbon (TOC), Microorganisms, Yeasts, Bioagumentation

چکیده کارخانه های نوشابه سازی بیشترین سهم را در آلوده کردن محیط زیست دارا می‌باشند. بیشترین بار آلودگی پساب این کارخانه‌ها شامل ترکیبات آلی، ترکیبات آلی فرار، سموم، فلزات، مواد مغذی (مانند نیتروژن و فسفر) و میکروارگانیسمهای بیماریزا می‌باشند. در این مطالعه بیش از ۵۰ گونه مخمر در کشت اولیه لجن از قسمتهای مختلف کارخانه، انتخاب شدند. دوازده گونه از این مخمرها بیشترین کاهش TOC از خود نشان دادند. مخمر شماره ۱۱ (Y-۱۱) بعد از بهینه سازی شرایط تا حد ۸۷/۵ درصد کاهش کل کربن آلی را از خود نشان داده است.

1. INTRODUCTION

The major contaminants found in wastewater are biodegradable organic compounds, volatile organic compounds, recalcitrant xenobiotics, toxic metals, suspended solids, nutrients (Nitrogen and phosphor), microbial pathogens and parasites. The requirements of plant were to remove organic matter and suspended solids. Research efforts are now being focused on the removal of nutrients (N,P), odors, volatiles organic compounds, metals and toxic organic after their passage through wastewater treatment plants. Activated sludge flocs contain a wide range of microorganisms such as bacteria, fungi, Viruses, Yeast, and protozoa [1]. Bacteria, particularly the gram-negative bacteria, constitute the major part of activated sludge flocs. Hundreds of bacterial strains thrive inactivated sludge but only a relatively small fraction can be detected by culture-based techniques.

Activated sludge does not usually favor the growth of fungi, although some fungal filaments are occasionally observed in activated sludge flocs. Fungi may grow under specific condition of low pH, toxicity, and nitrogen-deficient waste [2]. Yeasts are able to grow in high concentration of carbohydrate and high pH (~11) conditions. [3]. The early attempts to use microorganisms in the pollution control field have focused anaerobic digestion. Later on, microorganisms capable of degrading herbicides and other chemicals in industrial waste were isolated and used in commercial preparations designed for pollution control. Microbial strains for enhancing biodegradation of specific chemicals are generally

isolated from environmental sample (Wastewater, sludge, compost, soil) and selected by conventional enrichment techniques [4]. They are grown in nutrient media that contain a specific organic chemical as the sole source of nitrogen. Some of the microbial strains may be subsequently irradiated to obtain a desirable mutation [5]. One of the major problems affecting biological waste treatment is sludge bulking. Jell or viscose bulking is associated with microorganisms that are present in large amount of extra cellular slim [6]. Therefore to effect of this problem, should reduced setting and compaction rates by bioaugmentation process [7]. Based on suitable condition for growing yeast in beverage wastewater, we decided to isolate some strains of yeasts from the activated sludge of the factory. The TOC removal of the sample by bioaugmentation techniques prior to activated sludge process was the main propose of this study. TPC represents the total organic carbon in a given sample and is independent of the oxidation state of the organic matter. TOC is determined via oxidation of the organic carbon with heat and oxygen or chemical oxidants, followed by the measurement of the CO_2 liberated with an infrared analyzer [8].

2. METHODS AND MATERIALS

Fresh wastewater was obtained from different parts of a beverage factory. The pH as received was 11-12 and adjusted to 6-7 on experiments. Potato Dextrose Agar (PDA) and sabro dextrose broth were obtained from Merk Co.

3. MICROORGANISMS ISOLATION AND SCREENING

More than 50 microbial stains were isolated from the activated sludge of the factory and inoculated on a plate containing PDA. The plate was incubated at 30°C for 48 to 72 hours. These were prepared for TOC measurements.

4. PRE-CULTURE CONDITION

One loop from each slant was inoculated into 5 ml of sterilized sabro dextrose broth as medium, and incubated at 30°C for 24 h. After centrifugation and discarding the liquid, the yeast was precipitated and some sterilized distilled water was added to the test tub. The amount of water was varied due to cellular mass remaining in test tub after centrifugation. The OD was adjusted to 1 at 640 nm. Then 4 ml of this suspension was added to 50 ml pre-culture media as sabro dextrose broth and was shaken in a 30°C incubator at 130 rpm for 24 h. After recentrifugation and readjusted the OD to 1, 4 ml of this suspension was added to a 50 ml wastewater (pH 6-7), and the shaker was operated at 30°C, at 130 rpm for 48 h. Then the TOC was determined using a TOC analyzer (Skalar TOC Analyzer CA-10).

5. RESULT AND DISCUSSION

Table 1 shows the TOC reduction by different isolated yeasts. Among these, yeast No. 11(Y-11), showed the highest rate of removal of TOC up to 61.1%. Therefore it was selected for further experimental performance in order to optimize the efficiency of this test organism. The effect of pH, temperature and shaking rate (rpm) on cell growth

TABLE 1. TOC Reduction by 12 Isolated Yeasts.

Yeast's No.	*TOC=1669.68 mg/L*	*TOC reduction*
Y-1	860.59	48%
Y-2	768.64	64%
Y-3	1091.94	34.6%
Y-4	941.67	43%
Y-5	1089.86	30%
Y-6	1036.75	37%
Y-7	1093.08	34%
Y-8	1071.74	35%
Y-9	1041.14	37%
Y-10	745.37	55%
Y-11	639.95	61.1%
Y-12	1250.23	25%

pH = 8 Temperature = 25°C

of Y-11 and the TOC reduction were studied.

6. OPTIMUM pH

The cell growth of Y-11 at various pH levels was examined at 30°C. The results are shown in Figure 1. At pH 9 maximum OD value observed after 46 hours, which is related to maximum cell growth. The increasing of the cell growth at this pH value after 8 hours is considerable, because the effect of time on reducing of the concentration of organic load in wastewater in short time is very important. Figure 2 shows the TOC reduction by Y-11 at different pH levels. This reduction is significant at pH 9 (from 1669.68 to 407.73 mg/l) after 8 hours. This is enough time to prevent further formation of jell in the wastewater.

7. OPTIMUM TEMPERATURE

The optimal temperature for Y-11 was determined

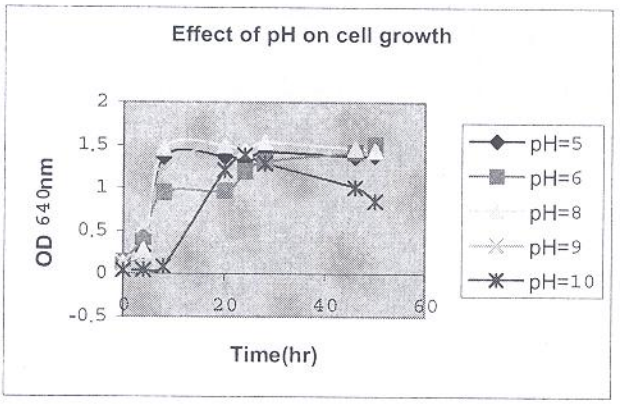
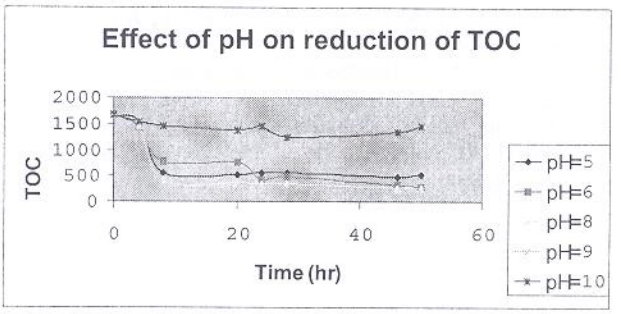


Figure 1. Effect of pH on cell growth.



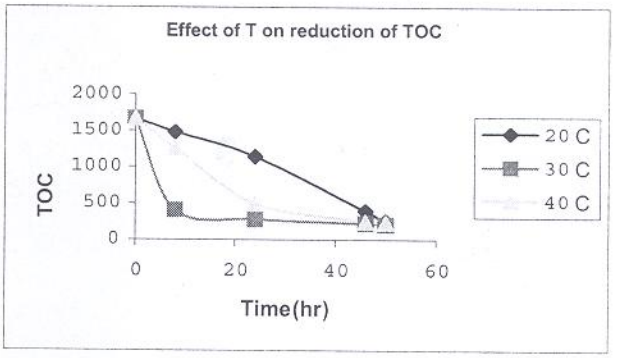


Figure 3. Effect of Temperature on reduction of TOC.

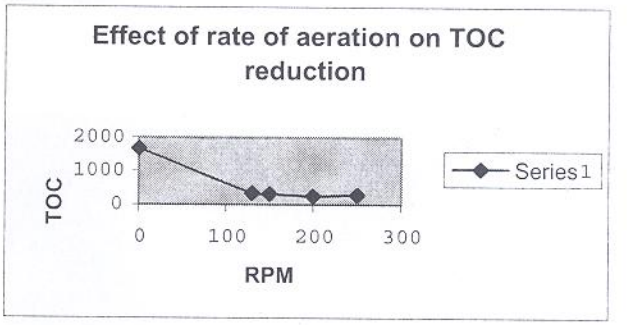


Figure 4. Effect of rate of aeration on TOC reduction.

at pH 9. Figure 3 depicts these results. At 30°C the highest reduction of TOC observed after 46 hours (from 1669.68 to 222.85 mg/l). Increasing the temperature results in decrease in activity of Y-11.

8. OPTIMUM SHAKING RATE (RPM)

The shaking rate of the sample was measured at pH 9 and at 30°C. The maximum reduction of TOC was obtained at 200 rpm. Increasing rpm value results in increase in activity of this test organism to reduce the TOC concentration.

Taxonomical studies of the strain Y-11 was carried out according to References 9 and 10. The results indicated that this test organism is pichia. Figures 5 and 6 show this test's organism at growth and germination state. The magnifications of the pictures are 23000X.This organism was added to the biological treatment system of the mentioned factory and they claimed that there are no more

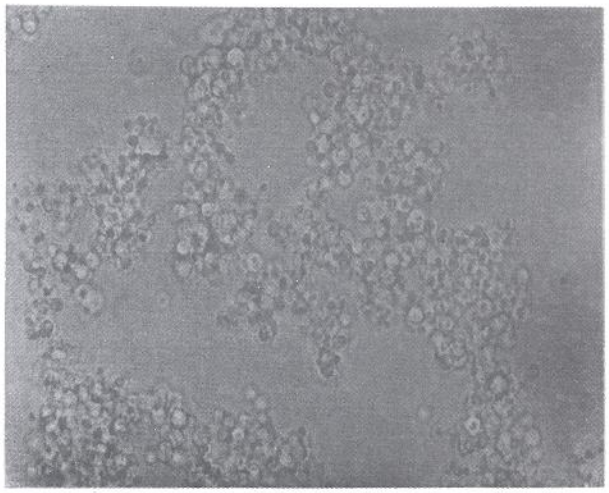


Figure 5. Y-11 grown in (PDA) (23000 magnification).

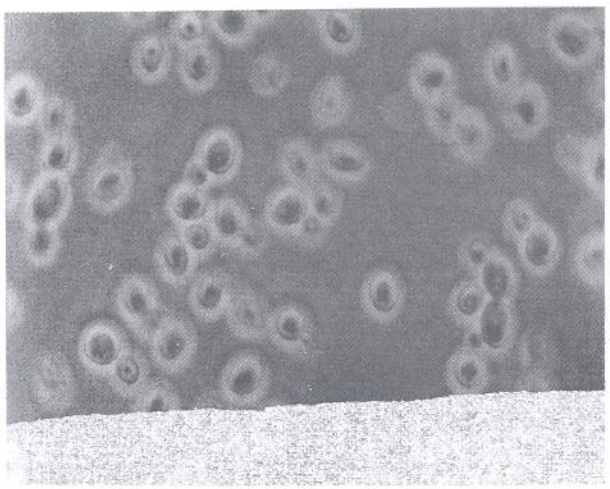


Figure 6. Y-11 in germination state (23000 magnification).

bulking problems left.

9. CONCLUSION

Results of this study support the effectiveness of a pretreatment system using an isolated microorganism from the sludge prior to the activated sludge process, to remove TOC. This pretreatment overcomes the bulking problems of the activated sludge by preventing the load of the organic matter in wastewater from becoming too high and forming a jell. We suggest that further work is needed to improve the efficiency of this test organism.

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