Materials Research Proceedings 29 (2023) 350-354 https://doi.org/10.21741/9781644902516-39

An integrated approach for treatment and biomass resource recovery from juice industry wastewater using purple non-sulfur bacteria

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Keywords: Purple Non-Sulfur Bacteria, Juice Wastewater, Biomass Production, Carbon Sources

Abstract. This study aims to treat juice industry wastewater and recover resources using purple non-sulfur bacteria (PNSB). Simulated juice wastewater was prepared and used as a feedstock for PNSB. Carbon sources, including sucrose, fructose, glucose, and tris-Na-citrate, were added to different bottles separately and inoculated with a mixed culture of PNSB. Tris-Na-citrate showed higher growth and biomass production (650±70 mg/L) than the other carbon sources. The PNSB growth was associated with the pH change; tris-Na-citrate showed different pH change behavior than the other carbon sources, i.e., it showed a lesser decrease in the medium pH than the others. The effect of substrate (using glucose) concentration on PNSB biomass production was determined in the following experiment. A substrate concentration of 400 mg/L (as COD) showed the highest growth, whereas 800 mg/L (as COD) returned the highest biomass production (776±1 mg/L). Total organic carbon removal (TOC) increased with an increase in substrate concentration. Inorganic carbon removal initially decreased but then increased over the growth period; however, it was also directly correlated with initial substrate concentration. Lastly, the effect of pH on TOC, IC, and COD removal was investigated. A pH of 6.0 or above (up to 8.0) was required to remove organic and inorganic carbon.

Introduction

The juice industry is widespread globally and generates a large volume of wastewater during packing, washing, and extraction. Juice industrial wastewater (JIW) is characterized by high COD, suspended solids, dissolved solids, and low pH [1]. Several treatment methods, including coagulation, flocculation, dissolved air flotation, and membrane filtration, are employed to treat JIW. Implementing these methods at an industrial scale is not currently cost-effective. In this perspective, biological treatments are desirable due to relatively low energy inputs. Recently, anaerobic treatment using PNSB has received considerable research attention [2-4]. They are considered favorable due to their unique metabolic features. They can utilize a variety of organic and inorganic carbon sources as a substrate, govern high substrate yield, and can switch their growth metabolism between photo-heterotrophy and chemo-heterotrophy, depending on environmental conditions. PNSB biomass can generate value-added bio-products such as single-cell protein, bioplastics, and photo-pigments. Hence, PNSB-based JIW treatment offers a dual benefit—wastewater treatment and biomass production [5, 6]. This study aims to utilization of JIW as a substrate for PNSB, their treatment performance, and biomass production. So far, no study has been reported that demonstrates the use of PNSB to treat JIW [7].

Methodology

Simulated juice wastewaters were prepared by dissolving glucose, fructose, sucrose, or tris-Na citrate individually to a theoretical chemical oxygen demand (COD) concentration of 1000 mg/L to create four different simulated juice wastewaters. Salts including potassium chloride (1.35 g/L), magnesium sulfate heptahydrate (9.93 g/L), sodium chloride (20 g/L), calcium chloride dihydrate (1.0 g/L), and sodium bio-carbonate (75 g/L) were added. Essential nutrients, trace metals, and vitamins were added per the ATCC-2 medium. The effect of glucose concentration as a sole substrate was also tested in a separate experiment. For this, glucose 0 to 1200 mg- as COD/L was added to the bottles. The remaining components of the simulated juice wastewater composition were the same. The solutions were transferred to 160 mL serum bottles and inoculated with a mixed microbial culture of PNSB. The initial pH of the solutions was noted but not adjusted during the treatment process. Using a crimper, the flasks were tightly sealed with a rubber stopper and an aluminum rim. The flasks were placed in an incubator shaker (Innova®44, Eppendorf), stirring continuously at 200 RPM with a temperature of 35 °C ± 2, under the illumination of 7.0 W/m^2 (Figure 1).

PNSB growth was monitored by measuring absorbance at 420 nm using a spectrophotometer (UV-3600 Plus, Shimadzu). Total suspended solids (TSS) were measured by the standard gravimetric method (APHA 2012), and COD was measured using HACH kits. Total organic carbon (TOC), total nitrogen, and inorganic carbon were measured using a TOC-L analyzer with a TNM-1 module (Shimadzu). Volatile fatty acids (VFAs) were measured by using ion chromatography (940 Professional IC Vario, Metrohm). The experimental treatments were analyzed by the student's t-test at a significance level of $p < 0.05$ using JASP (version 0.14.1) software to determine statistically significant differences between specific pairs.

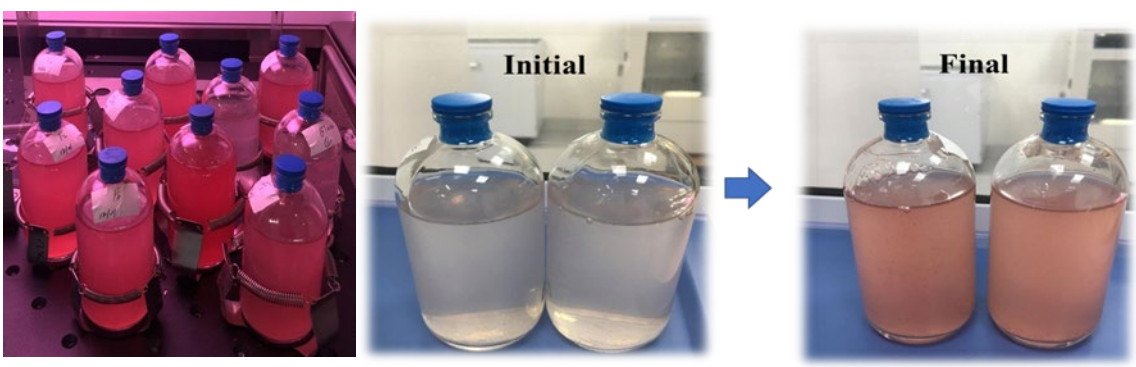

Fig. 1: An illustration of PNSB growth in a shaking incubator (left), and the initial and final growth culture of PNSB (right)

Results and Discussion

Effect of Different Carbon Sources. Juice wastewater is reported to contain one or multiple carbon sources, depending on the type of juice [8, 9]. In this experiment, PNSB growth was carried out using different carbon sources; each bottle contained only one kind of carbon source. A bottle without any carbon source was set as a control. The absorbance values indicate that the PNSB grew slowly for the first five days, and then a sharp increase in growth was observed. pH decreased significantly during the exponential growth period and then again increased. The same trend was found in all treatments except the control. Notably, PNSB fed with tris-Na-citrate showed the highest growth, and the pH drop in this treatment was relatively less than the other carbon sources. Tris-Na-citrate also returned the highest biomass production (total suspended solids, TSS). The biomass production in sucrose, fructose, glucose, tris-Na-citrate, and control were 575±35, 550±71, 600±5, 650±70, and 175±35 mg/L, respectively. Volatile fatty acids (VFAs) were also the highest (2096 ± 606 mg/L) while using tris-Na-citrate as a carbon source (Figure 2). High biomass productivity using Tris-Na-citrate can be linked to the pH changes. As Tris-Na-citrate showed less pH change than the other carbon sources, in those carbon sources, the pH was dropped to 6.0, which may not be favorable for PNSB.

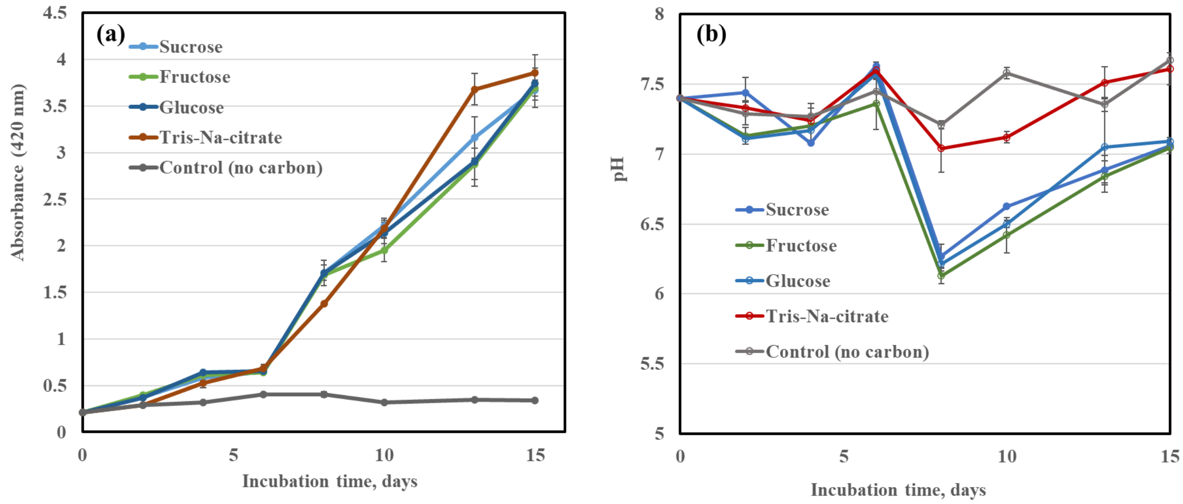

Fig. 2: (a) Effect of carbon different carbon sources on PNSB growth and (b) pH changes during the cultivation

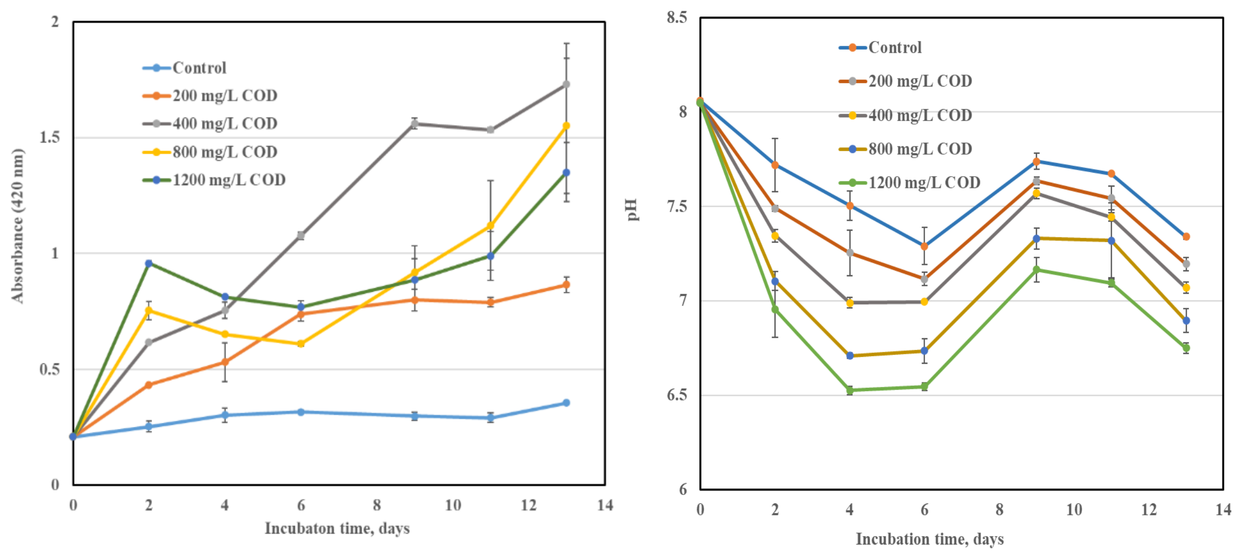

Fig. 3: (a) Effect of substrate (glucose) concentration, and (b) pH changes on PNSB cultivation

Effect of Substrate (Glucose) Concentration. The effect of substrate concentration on cell growth and medium pH was also investigated. The cells were grown under varying glucose concentrations (0-1200 mg/L as COD) at an initial pH of 8.0±0.1. Cell growth was pH-dependent, as the cells showed high growth when the pH was high, which decreased significantly as the pH dropped. However, the pH drop was larger at high substrate concentrations, linked with inorganic carbon production (Figure 3). A substrate concentration of 400 mg/L seems to show the highest growth performance; however, the absorbance values were not significantly ($p>0.05$) different than 800 mg/L and 1200 mg/L. The biomass production (TSS) was 555±21, 687±21, 729±4, 776±1 and 747±4 mg/L at 0, 200, 400, 800, 1200 mg/L substrate concentration, respectively. Based on TSS results, 800 mg/L can be considered the optimal substrate concentration. High glucose concentration showed an inhibitory effect, perhaps due to the reasons that it caused to drop in the pH, and the PNSB showed attenuated growth response.

Carbon Removal. Carbon removal is a crucial parameter in assessing the efficiency of a wastewater treatment system. TOC and IC removals were determined at different time points during the treatment process. The results indicate a continuous decrease in TOC over the treatment period. TOC removal corresponded to the initial substrate concentration. The highest TOC removal (233±46 mg/L) was found at a substrate concentration of 1200 mg/L, followed by 800, 400, 200 mg/L, and control, which were 208±39, 157±3, 103±10, and 26±11 mg/L, respectively (Figure 4). IC concentration gradually increased over the period of cultivation. This phenomenon was more pronounced at high initial substrate concentrations (800-1200 mg/L) than at the initial low substrate concentration (0-400 mg/L). IC removals were also high at higher substrate concentrations and vice versa.

COD, TOC, and IC removals were tested at different pH also in a separate experiment. The results indicate that the TOC removal at pH 6.0, 7.0, and 8.0 was almost the same, whereas, at pH 5.0, TOC removal was significantly lower than the others (data not shown). Similarly, COD removal at pH 5.0 was much lower than the other treatments. COD removal at pH 6.0, 7.0, and 8.0 was not significantly different. IC removal increased with an increase in the pH, the highest at pH 8.0 and the lowest at pH 5.0.

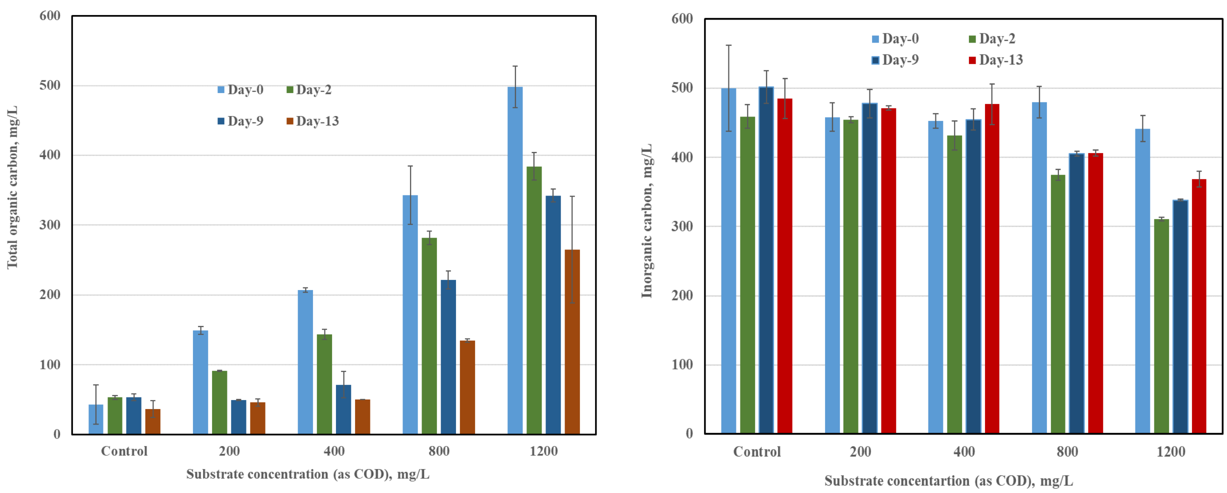

Fig. 4: (a) Total organic carbon, and (b) Inorganic carbon removal while using glucose as a substrate

Summary

The investigations of this study reveal that PNSB are suitable feedstock for the treatment of juice industry wastewater and produce a reasonable amount of biomass. The treatment performance depends on the pH, type of carbon sources, and substrate concentration. Further investigation should be carried out to characterize PNSB for producing value-added bio-products, including single-cell protein, photo-pigments, and lipids.

Acknowledgements

The authors would like to acknowledge the support of the Qatar National Research Fund (MME01-0910-190029) and Qatar Shell Research and Technology Centre for their financial and technical support, respectively.

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