Simultaneous treatment of fruit juice industry wastewater and single-cell protein synthesis using purple non-sulfur bacteria

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Background and problem statement

The fruit juice industry generates excessive wastewater through various processes such as extraction, packing, soaking, and washing. Fruit juice wastewater (FJW) is characterized by low pH, high chemical oxygen demand (COD), suspended and dissolved solids. It requires an appropriate treatment before discharging into the environment to safeguard human and ecological health (Amor et al., 2012). Conventional methods of FJW treatment such as coagulation, membrane filtration, and adsorption are not applicable at a large scale due to the high cost.

Recently, anaerobic treatment of wastewater using purple non-sulfur bacteria (PNSB) is being realized as an economical and sustainable approach. This approach offers dual benefits—wastewater treatment and single-cell protein (SCP) synthesis. PNSB present unique metabolic characteristics; they can grow in imbalanced nutrient conditions, demonstrate high substrate yield, and do not require strict sterilization. PNSB-based SCP can be used as a feed for humans, animals, and aquaculture. PNSB-based SCP contains amino acids, coenzyme Q10, carotenoids, and bacteriochlorophyll, which enhance the nutritional quality of SCP.

The purpose of this study is to investigate the potential of treating FJW and producing SCP using PNSB. Two types of FJW including citrus (orange) juice wastewater (CJW) and mixed fruit juice wastewater (MJW) have been tested. CJW and MJW are the first and third most consumed juice flavors (FONA, 2017) and present significant differences in their compositions. The effect of pH on COD removal, biomass productivity, and volatile fatty acids (VFAs) was investigated. SCP, bacteriochlorophyll, and carotenoid contents were also measured in the biomass. To the best of the author's knowledge, this is the first study involving the treatment of FJW and SCP production using PNSB.

Experimental

CJW and MJW wastewater samples were collected from a local juice industry located in Qatar. FJW was diluted with fresh water and additional nutrients including NH_4Cl, KH_2PO_4, vitamins, and trace metals as per media composition (ATCC-2672) were added. Initial COD values of CJW and MJW working solutions were 1950±14 mg.L^{-1}and 1703±3 mg.L^{-1}, respectively. The solutions were transferred to 160 mL serum bottles, then inoculated with a mixed culture of PNSB and their pH was adjusted to 5.0, 6.0, 7.0, and 8.0. The flasks were tightly sealed with a rubber stopper and aluminum rim by using a crimper. The flasks were placed in an incubator shaker (Innova®44, Eppendorf) stirring continuously at 150 RPM with a temperature of 35°C ± 1, under the illumination of 7.0 W.m$^{-2.}$

VSS were measured by the standard gravimetric method. 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). VFAs were measured by using ion chromatography (940 Professional IC Vario, Metrohm). Crude protein contents were estimated by using a 6.25-multiplication factor with total nitrogen contents measured through an elemental analyzer (EA 3000, Eurovector). Protein content was also analyzed using Modified Lowry Protein Assay Kit. Bacteriochlorophyll and carotenoids were extracted using acetone, and measured by adopting the method described in the literature (Zhou et al., 2014). The experimental treatments were analyzed by 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.

Finding and results

PNSB grown in CJW showed the highest VSS (852 ± 115 mg.L^{-1}) at pH 8.0 followed by 760 ± 96 mg.L^{-1} , 557 ± 125 mg.L^{-1} , and 278 ± 5 mg.L^{-1} at pH 7.0, 6.0, and 5.0, respectively. In MJW, VSS at pH 8.0, 7.0, 6.0, and 5.0 were 625 ± 35 mg.L^{-1}, 450 ± 141 mg.L^{-1}, 175 ± 35 mg.L^{-1}, and 225 ± 35 mg.L^{-1}, respectively. It inidated that for PNSB biomass production, neutral or alkali pH conditions were more favorable. COD removal was also pH-dependent. COD removals in CJW at pH 5.0, 6.0, 7.0, and 8.0 were 709 ± 65 mg.L^{-1}, 1501 ± 25 mg.L^{-1}, 1665 ± 8 mg.L^{-1}, and 1729 ± 3 mg.L^{-1}, respectively (**Figure-1**). Slightly lower COD removals were achieved in MJW: 661 ± 48 mg.L^{-1}, 593 ± 14 mg.L^{-1}, 1599 ± 11 mg.L^{-1}, and 1575 ± 17 mg.L^{-1} at pH 5.0, 6.0, 7.0, and 8.0, respectively. TOC consumption also showed similar a trend as expected. The biomass yields in CJW at pH 5.0, 6.0, 7.0, and 8.0 were 0.393 ± 0.01, 0.371 ± 0.08 ± 1, 0.456 ± 0.06, and 0.493 ± 0.07 g-VSS.g-COD^{-1}. These were higher than the biomass yields in MJW, which were 0.340 ± 0.05, 0.295 ± 0.06, 0.281 ± 0.09, and 0.397 ± 0.02 g-VSS.g-COD^{-1} at pH 5.0, 6.0, 7.0, and 8.0 (**Figure-2**). It was notable that the biomass yield increased with an increase in pH implying that not only the activity of the PNSB is enhanced at slightly alkali conditions but cellular metabolism becomes more efficient also. In this study, the biomass yield using FJW as a substrate is lower than the other reported substrates used for PNSB, but is still comparable (Hulsen et al., 2014).

Determination of VFAs revealed that their concentrations in the medium increased after anaerobic treatment. The highest VFAs in CJW were 485 ± 58 mg.L^{-1} at pH 8.0, whereas initial VFAs were 371 mg.L^{-1} only. In MJW also, VFAs concentration increased from 405 mg.L^{-1} to 811 ± 30 mg.L^{-1} by the end of the test. Generally, PNSB utilize VFAs to develop their biomass. However, in this study, PNSB did not utilize VFAs, perhaps due to a low concentration of acetate that leads to deficiency of acetyl-CoA and reduction in VFAs utilization (Little et al., 2008).

Protein contents were determined at the optimized pH (8.0) only. Lowry assay showed 48 ± 1 % and 40 ± 3 % protein in CJW and MJW, respectively. Crude protein estimates (at pH 8.0) in CJW and MJW were 64.5 ± 2.1%, and 60 ± 3.8 %, respectively. These are very comparable to other SCP studies using PNSB. Pigments analysis revealed that, in CJW, bacteriochlorophyll and carotenoid contents were 2097 ± 110 and 1354 ± 64 µg.g^{-1}. In comparison, the PNSB in MJW showed bacteriochlorophyll of 3100 ± 614, and carotenoids 1558 ± 218 µg.g^{-1}, respectively. Carotenoid contents in MJW and CJW were not statistically different ($p>0.05$).

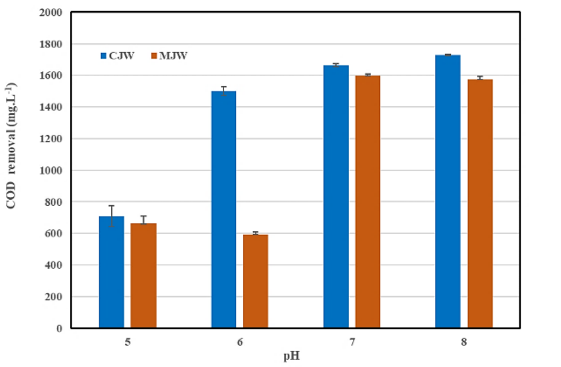

Figure 1: COD removal by PNSB in CJW and MJW at different pH

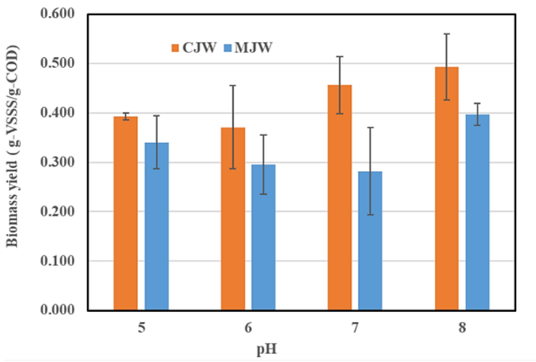

Figure 2: Biomass yields of PNSB in CJW and MJW at different pH

Conclusions

The results demonstrate that PNSB efficiently treat FJW and produce a moderate level of SCP. The treatment performance was pH-dependent—high pH supported biomass production, organics removal, and biomass yield. PNSB biomass contained a reasonable amount of carotenoids and bacteriochlorophyll, which can be useful to enhance the quality of SCP. As compared to other substrates used for PNSB growth, the biomass yield using FJW is still low. However, the biomass yield can be improved by optimizing process parameters such as light supplement, carbon to nitrogen ratio, mixing, trace elements, and vitamins concentration. Future studies should focus on improving SCP contents of biomass and demonstrating the effect of PNSB-SCP feeding on animals or aquaculture health.

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References

Amor, C., Lucas, M., Pirra, A., Peres, J. 2012. Treatment of concentrated fruit juice wastewater by the combination of biological and chemical processes. *Journal of environmental science and health. Part A, Toxic/hazardous substances & environmental engineering*, **47**, 1809-17.

ATCC-2672. https://www.atcc.org/~/media/A457A0AE8C604A12B5597E5EB1A6583A.ashx, Vol. 2021.

FONA. 2017. https://www.fona.com/wp-content/themes/fona/migrated-files/Juice%20Category%20Report%200917.pdf. in: *FONA International*, Vol. 2021, 2017. Geneva.

Hulsen, T., Batstone, D.J., Keller, J. 2014. Phototrophic bacteria for nutrient recovery from domestic wastewater. *Water Res*, **50**, 18-26.

Little, A.E.F., Robinson, C.J., Peterson, S.B., Raffa, K.F., Handelsman, J. 2008. Rules of Engagement: Interspecies Interactions that Regulate Microbial Communities. *Annual Review of Microbiology*, **62**(1), 375-401.

Zhou, Q., Zhang, P., Zhang, G. 2014. Biomass and Pigments Production in Photosynthetic Bacteria Wastewater Treatment: Effects of Light Sources. *Bioresource technology*, **179C**, 505-509.