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Hydrodynamic Cavitation for Vinasse Treatment: Optimization using the Taguchi Method and Phytotoxicity Evaluation

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The operational factors that maximize the removal of color, polyphenols and chemical oxygen demand in vinasse were evaluated by hydrodynamic cavitation with orifice plates, and the phytotoxicity of the treated effluent was analyzed under optimal conditions. A Taguchi experimental design with three operating factors was used: treatment time (20 and 60 min), orifice plate diameter (1 and 1.5 mm) and number of orifices (6 and 9) with the signal-to-noise (S/N) ratio of 'Larger is better'. The experiments were carried out at an inlet pressure of 4 bar and a controlled pH of 2. Phytotoxicity was evaluated with optimal factors in *Raphanus sativus L. (*radish*)* seeds. The results showed that optimal conditions were achieved with a treatment time of 20 min and the use of a 6-orifice plate of 1.5 mm diameter each orifice. These conditions allowed a reduction in COD by 44.19%, color intensity by 33.96% and polyphenol content by 90.25%. Under these conditions, the germination rate of radish seeds in cavitated vinasse reached 104.10%, indicating a significant decrease in toxicity. Hydrodynamic cavitation has been proven to be effective in vinasse treatment, combining high removal of contaminants and improvement in the environmental viability of effluent.

1. Introduction

Vinasse is a dark brown effluent containing high biochemical oxygen demand and chemical oxygen demand (COD), respectively, acidic pH, presence of total solids, phenolic compounds, and melanoidins (Singh et al., 2020). Untreated discharge into aquatic ecosystems compromises ecosystem dynamics by reducing light penetration, inhibiting photosynthetic activity, and decreasing dissolved oxygen. In the agricultural field, its untreated application can inhibit germination, affect phytotoxicity, and alter soil properties, decreasing alkalinity and the bioavailability of essential nutrients (Chowdhary et al., 2018). Various processes have been evaluated to mitigate the environmental impacts of vinasse (Chowdhary et al., 2018; Souza et al., 2013). However, its implementation has involved high costs, the use of chemicals, and post-treatment processes, evidencing the need to develop sustainable and economic alternatives. The hydrodynamic cavitation (HC) consists of the formation of vapor cavities within the liquid, experiencing low local pressures, growth and collapse (Nagarajan & Ranade, 2020). This process occurs when a wastewater passes through a geometric constriction that causes an increase in fluid velocity due to a reduction in pressure gradient (Zampeta et al., 2022). The cavitation generation mainly depends on the shape, size, thickness, orifice design and arrangement of orifices in the plate (Yi et al., 2021). Several investigations have addressed improving biodegradability and reducing toxicity in vinasses (Bocanegra et al., 2024; Poblete et al., 2020), however, there is still a research gap regarding the optimization of the operating conditions of HC. The objective of this study was to optimize the operational factors of HC using orifice plates for the removal of color, polyphenols, and COD from vinasse with a three-factor Taguchi L8 experimental design with the signal-to-noise (S/N) ratio of 'Larger is better'. The optimal parameters are then applied to evaluate the phytotoxicity of treated vinasse on *Raphanus sativus L. (*radish) seeds.

2. Materials and methods

2.1 Physicochemical analysis of the vinasse

Vinasse was collected in 20 L plastic containers from the effluent of a distillation column located in the Lambayeque region, Peru, and was refrigerated at 4 °C with pH adjustment to 2 for conservation and later use. Physicochemical analysis was performed with raw vinasse diluted in distilled water in a volumetric ratio of 1:10 (España-Gamboa et al., 2017) with pH adjustment to 2. Quantification of COD, total solids and volatile solids was carried out according to APHA standard methods 5220 D, 2540 B and 2540 E, respectively (Rice, 2022). The color was measured at 475 nm, and the total polyphenol content was quantified at 765 nm using the Folin-Ciocalteu method (Correa-Mahecha et al., 2022). Both measurements were recorded with a visible spectrophotometer (Thermo Scientific GENESYS 30). pH and electrical conductivity were determined with the HANNA HI5221 and HANNA HI2300 instruments, respectively. The removal efficiency was calculated with equations 1 and 2:

$$\text{Color removal efficiency (\%)} = \frac{\text{Abs}_0 - \text{Abs}_f}{\text{Abs}_0} * 100 \quad (1)$$

$$\text{Polyphenol (or COD) removal efficiency (\%)} = \frac{C_0 - C_f}{C_0} * 100 \quad (2)$$

Abs_0 and Abs_f are the initial and final absorbances; C_0 and C_f are the initial and final concentrations in mg L^{-1}.

2.2 Experimental procedure

The preparation of the sample and the configuration of the experimental module used in this study were described in a previous investigation (Bocanegra et al., 2024). In the present investigation, the same procedure and experimental configuration were used, with an adjustment in the inlet pressure, set at 4 bar. For the tests, stainless steel orifice plates with a thickness of 2 mm were used, with configurations of 6 and 9 orifices (1.0 and 1.5 mm in diameter, respectively) arranged in a regular grid pattern with equidistant spacing. Figure 1a shows the scheme of the experimental module, and Figure 1b shows the configuration of an orifice plate.

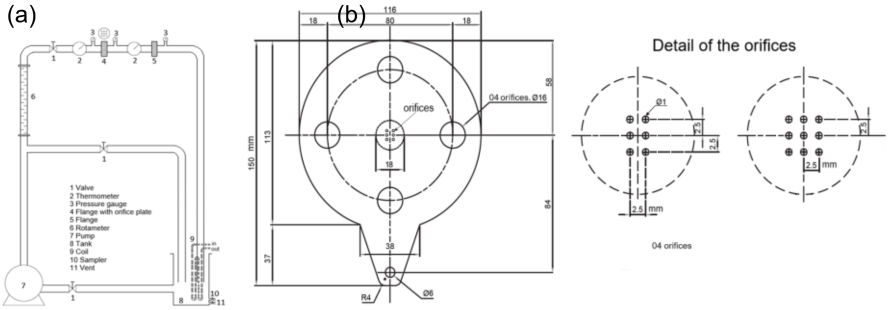

Figure 1: (a) schematic representation of the experimental module, (b) orifice plate configuration

2.3 Phytotoxicity analysis

The phytotoxicity analysis was performed on seeds of *Raphanus sativus L.* River water was used as substrate, washed twice with distilled water, and sterilized in an autoclave at 121 °C and 15 psi for 15 min (Maquen-Perleche et al., 2023). Five experimental treatments were established: T0 (control: distilled water), T1 (raw vinasse), T2 (diluted vinasse), T3 (cavitated vinasse: 20 min, 6 orifices, 1 mm diameter) y T4 (cavitated vinasse: 20 min, 6 orifices, 1.5 mm diameter), each with three replicates. In each Petri dish, 5 g of river sand, 5 ml of the assigned treatment, and 25 seeds were placed and covered with kraft paper to maintain optimal germination conditions. Germinated seeds were counted, and radicle length was measured at 48–96 h intervals. RSG corresponds to the seed germination rate, RRE to root elongation, and GI to the germination index, all expressed as % and calculated using Equations 3, 4 and 5, respectively (Ravindran et al., 2016). A one-way ANOVA (95% confidence level) was applied, followed by Duncan's test to identify significant differences between treatments.

$$\text{RSG (\%)} = \frac{\text{Number of seeds germinated in the sample}}{\text{Number of seeds germinated in the control}} * 100 \quad (3)$$

$$\text{RRE (\%)} = \frac{\text{Mean root elongation in the sample}}{\text{Mean root elongation in the control}} * 100 \quad (4)$$

$$\text{GI (\%)} = \frac{(\text{RSG\%}) * (\text{RRE\%})}{100} \quad (5)$$

2.4 Design of experiments

A Taguchi L8 experimental design was used with three factors (treatment time, number of orifices in the plate and diameter of the orifices) with a signal-to-noise (S/N) ratio of 'Larger is better'. Additionally, an analysis of variance (ANOVA) was performed with a confidence level of 95% to quantify the contribution of each factor. The experimental data processing was performed using Minitab® 21.1.

3. Results and discussion

3.1 Physicochemical analysis of diluted vinasse

The physicochemical analysis of diluted sugarcane molasses ethanol vinasse is presented in Table 1.

Table 1: Physicochemical analysis of sugarcane molasses ethanol vinasse

Properties	Units	Average value
COD	mg L^{-1}	17616 ± 68.7
Total solids	mg L^{-1}	8363 ± 344
Total volatile solids	mg L^{-1}	2683.3 ± 105
pH	–	4.83 ± 0.0025
Electrical conductivity (EC)	dS m^{-1}	4.25 ± 0.031
Polyphenols	mg GAE L^{-1}	346.03 ± 7.21
Color	absorbance	2.391 ± 0.0015

3.2 Geometry of orifice plates

Table 2 presents the orifice plate geometry used for removing color, polyphenols and COD from vinasse.

Table 2: Geometric parameters of orifice plates

Orifices		Flow rate	Flow area	Total perimeter	Velocity	Cavitation Number
number	diameter, mm	L min^{-1}	mm^2	mm	m s^{-1}	
6	1	6.5	4.7124	18.8496	22.99	0.34
6	1.5	16	10.6029	28.2744	25.15	0.23
9	1	7	7.0686	28.2744	16.50	0.56
9	1.5	20	15.9044	42.4116	20.96	0.30

3.3 Optimum conditions by Taguchi L8

Table 3 shows the results of the experimental Taguchi L8 design applied to the removal of color, polyphenols and COD in ethanol vinasse from sugarcane molasses.

Table 3: Experimental design matrix using Taguchi L8 with S/N ratio, 'Larger is better'

Run	Time (min)	Orifices		Color		Polyphenols		COD	
		Number	Diameter (mm)	% Removal	S/N ratio	% Removal	S/N ratio	% Removal	S/N ratio
1	20	6	1	30.67 ± 0.10	29.73	89.63 ± 0.26	39.05	54.64 ± 2.07	34.75
2	20	6	1.5	33.96 ± 0.15	30.62	90.25 ± 0.07	39.11	44.19 ± 2.27	32.91
3	20	9	1	26.91 ± 0.11	28.60	90.74 ± 0.58	39.15	40.72 ± 0.26	32.20
4	20	9	1.5	13.90 ± 0.06	22.86	87.03 ± 0.20	38.79	35.96 ± 0.40	31.12
5	60	6	1	19.18 ± 0.02	25.65	88.01 ± 0.02	38.89	59.42 ± 0.95	35.48
6	60	6	1.5	27.84 ± 0.10	28.89	87.60 ± 0.50	38.85	56.78 ± 2.43	35.08
7	60	9	1	16.24 ± 0.09	24.21	86.88 ± 0.88	38.78	46.96 ± 0.95	33.43
8	60	9	1.5	11.03 ± 0.06	20.85	84.17 ± 0.18	38.50	46.14 ± 0.40	33.28

The optimal configuration with a 1.5 mm 6-orifice plate and 20 min of treatment, was found to result in removals of 33.96% (S/N of 30.62) for color, 90.25% (S/N of 39.11) for polyphenols, and 44.19% (S/N of 32.91) for COD. Intensification of hydrodynamic cavitation (HC), as evidenced by a cavitation number of 0.23 (Table 2), promoted greater formation of hydroxyl radicals, thus facilitating advanced oxidation of contaminants, which is consistent with previous studies (Gogate & Patil, 2015). In comparison, a plate with 9 orifices under 3.6 bar and pH 2 conditions showed removal of color and polyphenols of 32.71% and 88.62%, respectively (Bocanegra et al., 2024). Simultaneous reduction in COD and color is associated with fragmentation of melanoidins and phenolic compounds, which are mainly responsible for the color and toxicity of wastewater (España-Gamboa et al., 2017). The greater removal of polyphenols at 20 min is attributed to their reactivity with hydroxyl radicals, which directly attack phenolic structures and specific chromophores (Saharan et al., 2013). A positive correlation between polyphenol removal and color reduction suggests that their degradation contributes to the reduction of the color intensity of wastewater (Poblete et al., 2020). In contrast, COD removal using the 6-orifice plate reached maximum values (59.42% and 56.78%) after 60 min, whereas color and polyphenols removal were optimized at 20 min. This is explained by the rapid degradation of pigments and dyes during early cavitation stages, while extended treatment may lead to the accumulation of colored by-products or stable intermediate (Nagarajan & Ranade, 2020). Organic compounds associated with COD exhibit greater structural complexity and require longer exposures times. In previous studies, COD removal in industrial wastewater by HC required up to 180 min at 6 bar and 50% dilution (Joshi & Gogate, 2019), attributed to the persistence of refractory compounds in vinasse (Nagarajan & Ranade, 2020). Furthermore, the higher degradation of polyphenols (90.25%) compared to color (33.96%) and COD (44.19%) can be explained by the higher affinity of phenolic compounds for the gas-liquid interface, enhancing their exposure to OH radicals and thermal effects from cavity collapse (Saharan et al., 2013). These findings underscore the importance of optimizing HC conditions according to target pollutant characteristics.

3.4 Analysis of variance (ANOVA)

The results of the analysis of variance of the S/N ratios with a confidence level of 95% and the response table for the S/N ratios are presented in Table 4 and Table 5, respectively.

Table 4: Analysis of variance for SN ratios for the removal of color, polyphenols and COD

Source	Color				Polyphenols				COD			
	df	Sum of squares	F-value	P	df	Sum of squares	F-value	P	df	Sum of squares	F-value	P
Time (A)	1	18.5961	255003.77	0.001	1	0.147300	33.64	0.109	1	4.9761	145.96	0.053
Orifice number (B)	1	42.2594	579494.54	0.001	1	0.055959	12.78	0.174	1	8.3857	245.98	0.041
Orifice diameter (C)	1	3.1000	42509.40	0.003	1	0.047871	10.93	0.187	1	1.5062	44.18	0.095
A*B	1	0.0439	602.62	0.026	1	0.007873	1.80	0.408	1	0.0310	0.91	0.515
A*C	1	2.7899	38257.85	0.003	1	0.000020	0.00	0.957	1	0.7055	20.69	0.138
B*C	1	21.8478	299593.50	0.001	1	0.053954	12.32	0.177	1	0.1264	3.71	0.305
Residual error	1	0.0001			1	0.004398			1	0.0341		
Total	7	88.6372			7	0.317357			7	15.7650		

Table 5: Response table for S/N ratios (Larger is better) for removal of color, polyphenols and COD

Factors	Levels	Color			Polyphenols			COD		
		S/N	Rank	% C	S/N	Rank	% C	S/N	Rank	% C
Time (min)	20	27.95	2	20.98	39.03	1	46.41	32.74	2	31.56
	60	24.90			38.76			34.32		
Orifice number	6	28.73	1	47.68	38.97	2	17.63	34.55	1	53.19
	9	24.13			38.81			32.51		
Orifice diameter (mm)	1	27.05	3	3.50	38.97	3	15.08	33.96	3	9.55
	1.5	25.81			38.81			33.10		

The statistical analysis presented in Table 4 confirmed that the removal of color was significantly affected by the number of orifices, the treatment time, and the diameter of the orifice and the interactions between them ($p<0.05$), while the number of orifices was the only factor that had a significant effect ($p<0.05$) on the removal of COD. However, for the removal of polyphenols, it was observed that the main effects and the interactions between them did not have a significant effect ($p>0.05$). Regarding the percentage factor contribution (%C), the number of orifices was found to contribute 47.68% and 53.19% to the removal of color and COD, respectively, followed by the treatment time with 20.98% and 31.56%, respectively. However, for polyphenol removal, treatment time was the main factor, followed by the number of orifices. The diameter of the orifices showed low effectiveness in removing the three contaminants analyzed (Table 5). It has been observed that fewer orifices and an optimal cavitation number improve cavitation performance (Saharan et al., 2013). On the contrary, when the number of orifices is increased, the pressure in each orifice decreases, resulting in a reduction in the intensity of the hydrodynamic cavitation effect (Yi et al., 2021). The results obtained in this study are consistent with previous studies on the removal of color and polyphenols (Bocanegra et al., 2024), and with similar behaviors observed in the removal of norfloxacin (Yi et al., 2021). On the contrary, an increase in the exposure time to

COD removal intensifies the interaction between cavitation bubbles and contaminants, facilitating their decomposition. Previous studies have shown that increasing the number of passes through the cavitation device significantly improves the biogas yield, indicating improved COD removal (Nagarajan & Ranade, 2020).

3.5 Phytotoxicity analysis

Figure 2a shows the effect of vinasse treatments on phytotoxicity parameters (RSG, RRE y GI) in *Raphanus sativus L.*, while Figure 2b shows the germination of radish seeds in Petri dishes.

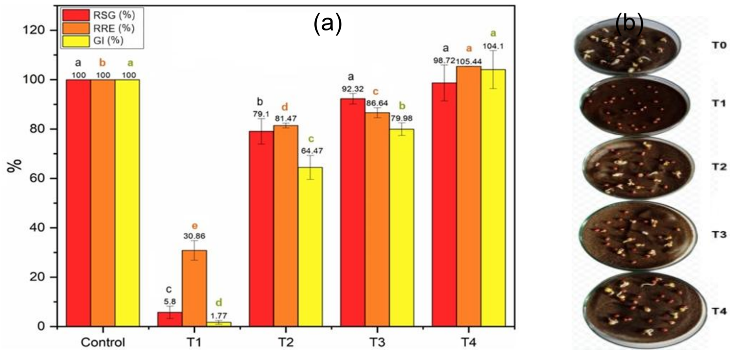

Figure 2: (a) Effect of vinasse treatments on germination and root grow of radish seeds, (b) Germination index

The phytotoxicity analysis was conducted using vinasse treated under the optimized cavitation conditions, which involved a 6-orifice plate (1.5 mm diameter) and a treatment time of 20 minutes. Statistical analysis showed that treatments T0 (control), T3, and T4 did not show significant differences in RSG ($p>0.05$). On the contrary, treatments T1 and T2 showed significant differences ($p<0.05$) with reduced efficacy. Regarding RRE, all treatments showed significant differences between them ($p<0.05$). The RRE values of T3 and T4 were higher than those observed in T2 and T1, which is due to the reduction of inhibitory xenobiotics in the treated vinasse along with the optimization of water and nutrient content (Vilar et al., 2018). In terms of GI, T4 and T0 were statistically similar, while T1, T2 and T3 showed GI values below the critical threshold of 80%, indicating phytotoxic effects that limit their agricultural use (Ravindran et al., 2016). The greatest efficacy was observed with T4 treatment (98.7%±7.3 for RSG, 105.4%±0.06 for REE and 104.1%±7.7 for GI).

The results obtained in this study are consistent with previous studies on phytotoxicity reduction. The application of hydrodynamic cavitation significantly reduced phytotoxicity in *Lepidium sativum*, *Sinapis alba*, and *Sorghum saccharatum*, as manifested by an increase in root growth and a reduction in the RRE and RSG indices. The GI index values were significantly higher than those observed in untreated printing ink effluents, indicating a reduction in the effectiveness of the treatment in reducing phytotoxicity by removing chromophores and COD (Zampeta et al., 2022). Another study (Ravindran et al., 2016) showed that GI values higher than 100% in *Lycopersicon esculentum*, *Raphanus sativus*, *Daucus carota*, and *Allium cepa* suggest not only the absence of phytotoxicity but also the presence of biostimulant properties due to the removal of toxic organic compounds and the consequent reduction of cytotoxicity in the treated effluents. In comparative studies on the biological treatment of sugarcane vinasse, the raw effluent showed a strong phytotoxic effect on *Triticum aestivum L.* and *Raphanus sativus* even in the diluted state. On the contrary, treated vinasse dilutions (12.5%, 25%, and 50%) improved root development, attributed to a reduction in COD and phenolic compounds (Rulli et al., 2020).

4. Conclusions

Hydrodynamic cavitation has proven to be an effective method for the treatment of industrial wastewater, representing an environmentally sustainable alternative with high efficiency in the degradation of organic compounds. The configuration with a 1.5 mm 6-orifice plate and a treatment time of 20 min optimized the removal of color (33.96%) and polyphenols (90.25%), while the removal of COD was 44.19%. The number of orifices and the treatment time were the factors with the greatest influence on the removal of color and COD, while for the removal of polyphenols, it was the treatment time followed by the number of orifices. Under optimal conditions (6 orifices of 1.5 mm and 20 minutes), the treated vinasse does not represent any environmental risk, evidenced by a germination index of 104.10% in radish seeds. These results confirm the potential of orifice-based hydrodynamic cavitation as a sustainable alternative to mitigate the environmental impacts of the alcohol distillation industry and its potential agricultural applicability.

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