

# STICKWATER MULTI-STEP TREATMENT: EFFECT ON ORGANIC MATERIAL REMOVAL

TRATAMIENTO MULTI-ETAPAS DE AGUA DE COLA:  
EFECTO EN LA REMOCIÓN DE MATERIA ORGÁNICA

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## ABSTRACT

The effect of a multi-step process (centrifugation-pH adjustment (acid/alkaline)-centrifugation-ultrafiltration) on organic material removal from stickwater was evaluated. After centrifugation and pH adjustment-centrifugation (TCA-NaOH, HCl-NaOH) protein and lipids were removed from the stickwater by 43 and 99% respectively. Also, a decrease of 81% on biochemical oxygen demand ( $BOD_5$ ) and 62% of chemical oxygen demand (COD) were observed. Final effluents with hydrodynamic radius of 0.21 and 0.19  $\mu\text{m}$  were obtained by TCA-NaOH and HCl-NaOH pH adjustment, respectively. Ultrafiltration reduced in 99 and 90% the  $BOD_5$  and COD of effluent, respectively, whereas protein content decreased by 77% for both treatments. Results suggest that pH adjustment, prior to ultrafiltration, resulted in a greater removal of larger size particles from stickwater compared to centrifugation. The significant decrease of chemical components and the composition of the final effluent, indicate that the multi-step process is an adequate alternative to reduce the concentration of organic material from stickwater.

**Key words: stickwater; pH adjustment; ultrafiltration; organic material removal.**

## RESUMEN

Se evaluó el efecto del tratamiento multi-etapas (centrifugación-ajuste de pH (ácido/alcalino)-ultrafiltración) sobre la remoción de materia orgánica contenida en agua de cola. Después de la centrifugación y ajuste de pH (ATC-NaOH, HCl-NaOH), se removieron 43 y 99% de proteínas y lípidos del agua de cola. Adicionalmente, la demanda bioquímica de oxígeno ( $DBO_5$ ) y demanda química de oxígeno (DQO) disminuyeron 81 y 62% respectivamente. El radio hidrodinámico en efluente final obtenido ajustando el pH con ATC-NaOH fue de 0.21  $\mu\text{m}$ , mientras que con HCl-NaOH fue de 0.19  $\mu\text{m}$ . Aplicando el proceso de ultrafiltración, los valores de  $DBO_5$ , DQO y proteínas disminuyeron 99, 90 y 77% respectivamente para ambos tratamientos (ATC-NaOH, HCl-NaOH). Comparando el ajuste de pH previo a la ultrafiltración con la primera centrifugación, el primero presentó mayor remoción de partículas más grandes provenientes del agua de cola. La disminución significativa de los componentes químicos y la composición del efluente final, sugieren que el proceso multi-etapas podría ser una alternativa para reducir el material orgánico contenido en el agua de cola.

**Palabras clave: Agua de cola; ajuste de pH; ultrafiltración; remoción de materia orgánica.**

## INTRODUCTION

Three main waste effluents are generated during fishmeal production: bail water, blood water and stickwater (SW). In terms of generated volume, SW is the most important, representing about 60% of the processed fish weight. SW is a complex effluent consisting of a variety of solids (6-10%), most of which should be removed before effluent can be discarded directly into the sea (Fernández *et al.*, 2003). In practice, solids obtained during the evaporation process of SW are reincorporated into the fishmeal during the drying process; however, they could also be recovered and used for human consumption as food ingredients with high nutritional value, enhancing the profit of such by-product (García-Sifuentes *et al.*, 2009).

In particular, SW usually contains suspended soluble and insoluble particles, such as proteins, fat globules and dissolved inorganic ions. Its chemical composition is highly variable and depends upon the fish species, fish storage characteristics and operating plant conditions (Guerrero *et al.*, 1998; Bechtel, 2005). There are currently several processes for SW treatment, which include physical, chemical and biological methods. However, the industry lack of capacity for SW treatment, causing SW accumulation. In addition, when SW is evaporated, the product becomes highly viscous and hard to manage, increasing the possibilities of getting fouled pipes and fluid movement interruption (Jacobsen, 1985). Besides, SW evaporation traditionally requires energy-consuming evaporation systems, resulting in an expensive process (Bechtel, 2007).

Applications of cross flow membrane technology for effluent protein recovery in the fish industry have been reported (Afonso *et al.*, 2002). Special membranes of low molecular weight cutoff (LMWCO) are employed in ultrafiltration (UF) and nanofiltration (NF) processes; however, their use in some cases is not recommended because membranes can be easily fouled (Afonso y Bórquez, 2002; Fernández *et al.*, 2003). Yet, membrane filtration is an alternative method used by the fish industry to recover solids from effluents. However,

if only UF treatment is used, membrane fouling can occur, leading to an incomplete process; consequently, a pretreatment is required in order to avoid or diminish membrane fouling problems.

Hence, the objective of the present study was to evaluate the effect of a multi-step (centrifugation-pH shift-UF) SW treatment on the removal of its organic material and evaluation of the final effluent.

## MATERIALS AND METHODS

### Stickwater

Stickwater was collected from a fishmeal plant located in Guaymas, Sonora, Mexico. Samples came from four seasonal sardine batches captured. Each batch contained a mixture of several pelagic species commonly used by the fish industry to produce fishmeal such as Monterey sardine (*Sardinops sagax caerulea*), Californian anchovy (*Engraulis mordax*), Pacific anchovy (*Cetengraulis mysticetus*) and Chub mackerel (*Scomber japonicus*). Samples were taken in 4 L containers and immediately transported on coolers with ice to the laboratory at Centro de Investigación en Alimentación y Desarrollo A.C. (C.IAD) for further analyses.

### Treatment

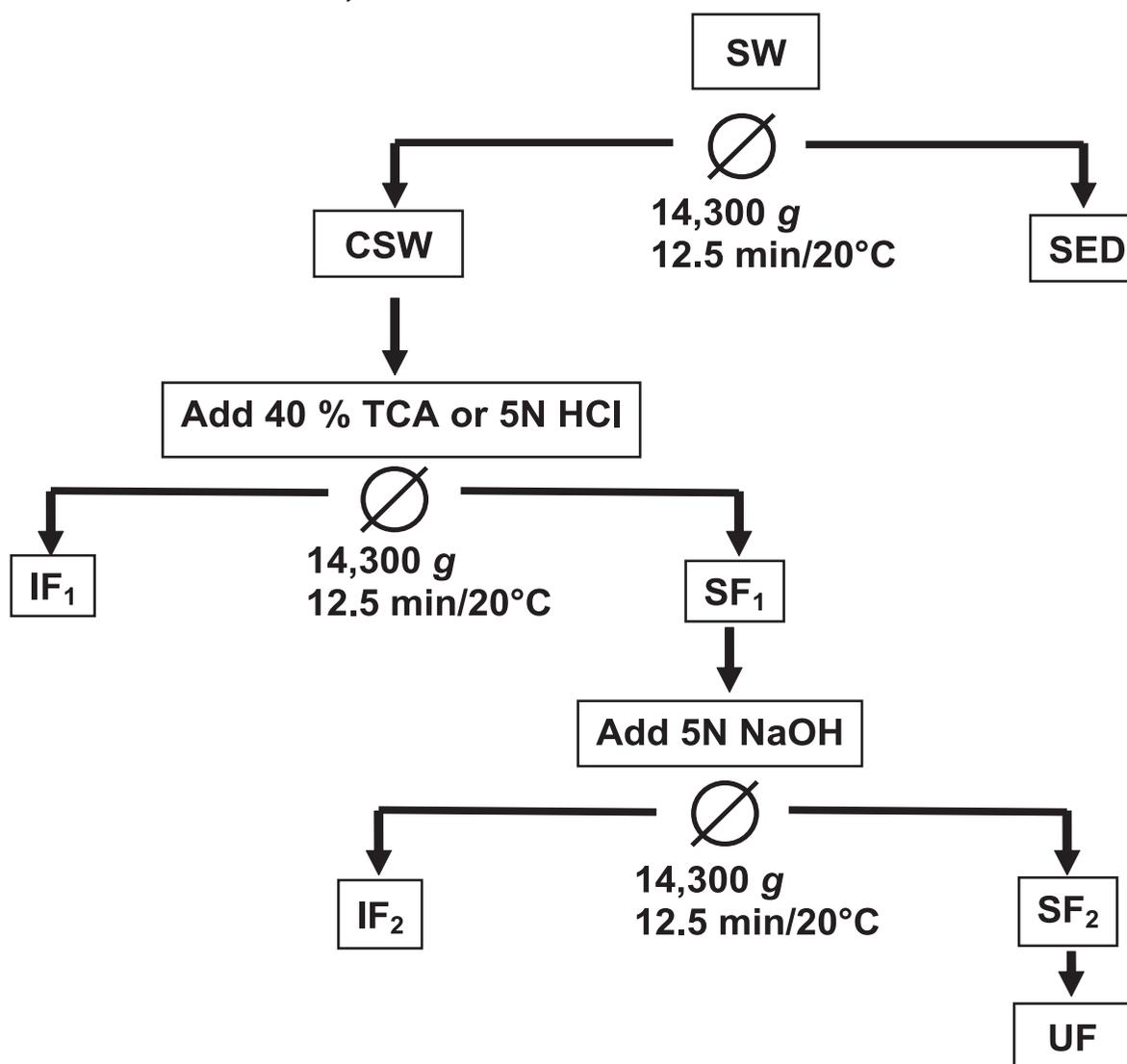
Stickwater was first centrifuged at  $14,300 \times g/12.5$  min/ $20^\circ\text{C}$  (Beckman J2-2; Beckman Inst., Palo Alto, CA) in order to precipitate its solids, obtaining the new so-called centrifuged stickwater (CSW). Thereafter, the CSW was subjected to pH adjustment using 40% trichloroacetic acid (TCA) (adjustment to pH 1.5) or 5N HCl (adjustment to pH 2.5) followed by a second centrifugation step (preliminary results showed that adjusting those pH with TCA and HCl resulted in the greatest solids removal); the precipitated solids were discarded and the resultant solution, soluble fraction 1 (SF<sub>1</sub>), was exposed to other pH adjustment (8.5) using 5N NaOH, followed by a final centrifugation; the precipitated solids were discarded and the resultant solution, soluble fraction 2 (SF<sub>2</sub>), with reduced solid concentration, was finally collected and ultrafiltered



through a UF regenerated cellulose cartridge membrane (PLAC Prep/Scale TFF 0.56 m<sup>2</sup> Unit, 1 kDa MWCO) (Millipore Corporation, Billerica, MA) at 120-130 kPa with a cross flow velocity of 20 mL min<sup>-1</sup>, pH 8.5 and 25 °C, using a Monostat Preston variable speed peristaltic pump (Barnant Co., Barrington, IL) (**Fig. 1**). The UF process was carried out in order to generate an effluent with the least organic charge. The pH and centrifugation conditions were based in preliminary studies carried out in the laboratory.

### Analytical techniques

A Corning pH meter model 240 (Corning Inc., Corning, NY) was used for all pH measurements. Total nitrogen in samples was carried out by using a Leco FP-528 nitrogen analyzer (Leco Corp., St. Joseph, MI). Protein content was calculated as nitrogen percent  $\times$  6.25. Lipids, moisture, total solids and ash contents were measured by standard methodologies (AOAC, 2000).



**Figura 1** Diagrama de flujo del tratamiento de agua de cola usado en el estudio. SW: Agua de cola. CSW: Agua de cola centrifugada. SED: Sedimentos. IF: Fracción insoluble. SF: Fracción soluble. TCA: Ácido tricloroacético.

**Figure 1** Flow diagram of the stickwater treatment used in the study. SW: Stickwater. CSW: Centrifuged stickwater. SED: Sediments. IF: Insoluble fraction. SF: Soluble fraction. TCA: Trichloroacetic acid

Biochemical oxygen demand (BOD<sub>5</sub>) and chemical oxygen demand (COD) were estimated following the procedures established by the Mexican Normative for industrial effluents to be discarded (DGN, 2001a; 2001b). For BOD<sub>5</sub> analysis, a Thermo Orion Oxymeter model 410 A, coupled with an Orion 97-08 dissolved oxygen electrode was used (Fisher Scientific, Waltham, MA). Results were reported as g of O<sub>2</sub> L<sup>-1</sup>. For COD, oxidizable matter was calculated in terms of oxygen equivalents and reported as g of O<sub>2</sub> L<sup>-1</sup>. Total volatile bases nitrogen (TVB-N) was measured following the method proposed by Woyewoda (Woyewoda *et al.*, 1986).

Transmittance (%) was measured using a spectrophotometer Perkin Elmer Lambda 3B (Perkin Elmer, Waltham, MA), at 575 nm with distilled water as a blank (Castillo *et al.*, 1987). In order to evaluate the particle size of SW, CSW and SF<sub>2</sub> during SW treatment, their hydrodynamic radius (R<sub>H</sub>) were measured by the dynamic light scattering (DLS) technique (Juarez *et al.*, 2007). Measurements were performed using an ALV-5000 digital correlate system (Langen-GmbH, Germany) at 25 ± 0.1 °C. The incident light was vertically polarized with a λ<sub>0</sub> = 632 nm argon laser (30 mW), and the scattered light was measured at 90°. Hydrodynamic radius was obtained for diluted samples using the Stokes-Einstein equation:  $D_0 = k_B T / 6\pi\eta R_H$ , where k<sub>B</sub> is the Boltzmann constant, T the absolute temperature, η the viscosity of the solvent, and D<sub>0</sub> the diffusion coefficient at infinite dilution. All measurements were conducted by triplicate, each one recorded in a time intervals of 30 s and averaged with the instrument software (ALV 5000/E/WIN Software).

### Statistical analysis

Four samplings were conducted from four seasonal sardine batches captured. All analyses were carried out by triplicate. Data was analyzed by a oneway ANOVA (NCSS version 5.1; Kaysville, UT) and differences among means compared using Duncan's multiple range test with a level of significance of P ≤ 0.05.

## RESULTS AND DISCUSSION

Chemical composition of SW and CSW, as well as BOD<sub>5</sub> and COD results are shown in Table 1. As expected, centrifugation *per se* showed a significant effect (P ≤ 0.05) on these parameters. BOD<sub>5</sub> and COD have been extensively used as pollution parameters on discarded effluents. Centrifugation decreased (p < 0.05) BOD<sub>5</sub> and COD values by 48 and 43%, respectively, indicating the removal of organic material in SW. Similar results for BOD<sub>5</sub> levels (a 42% decrease) were reported in a previous experiment on our laboratory, where SW was submitted to a centrifugation step, followed by chitosan addition (for organic matter removal) at different pH values (Pacheco-Aguilar, *et al.*, 2009). Results in the present study also showed that centrifugation removed (from SW) 40, 48 and 93% of total solids, protein and lipids, respectively. In addition, most of the organic material dissolved or suspended in either, SW or CSW was protein (Table 1). It was reported protein contents (dry weight basis) of 70 and 86% in SW derived from the processing of Alaska pollock (*Theragra chalcogramma*) (Bechtel, 2005) and salmon (*Oncorhynchus sp*) (Sathivel *et al.*, 2005), respectively.

The SW analyzed in this study was 10% lower in protein, 3% lower in fat and 3.4% higher in ash than the reported for Alaska Pollock stickwater (Bechtel, 2005). Besides, some variation on SW chemical composition parameters was observed in the present study (Table 1). Differences in SW chemical composition and variability inside each parameter can be explained in terms of fish species-composition and the whole-fish/fish by-products ratio used for fishmeal manufacturing. It is well known that fishmeal composition, as well as that of its derived stickwater varies depending on factors such as fish species, species blend used, season, fish physiological condition and processing conditions (Bandarra *et al.*, 1997; Bechtel, 2005; Sathivel *et al.*, 2005). This variation in composition confers a major technological challenge for the fish industry. In this regard, samples used in the present study were from four different seasonal sardine batches, thus the variation. However, the centrifugation step reduced SW



total solids and protein variation (Table 1), indicating that this only step achieves a more uniform effluent composition regardless the heterogeneity of SW. Thus, centrifugation (under conditions of the study) is recommended for any additional operational step implemented for SW treatment, as well as it can help on processing equipment maintenance (preventing pipe clogging).

An unexpected protein precipitation with regard to the use of extreme pH was observed. Commonly, a high or low pH used on a protein solution promotes protein solubility instead of precipitation; however, pH values used in the present study promoted protein precipitation. One possible theory about this behavior is the high ion (cations or anions) content stickwater has (García-Sifuentes *et al.*, 2009) which can interact with the pH promoting charges on proteins, thus making it less soluble (Tchobanoglous *et al.*, 2003; Kelleher *et al.*, 2004; Kim *et al.*, 2005).

The effect of centrifugation-pH shift-UF on the final effluent composition, BOD<sub>5</sub>, COD, transmittance and R<sub>H</sub> of SW is shown in Table 1. No statistical differences

( $P \geq 0.05$ ) were shown for BOD<sub>5</sub>, COD and protein levels when compared final effluents previous to UF. After applying UF, final effluents showed a significant decreased of approximately 99, 90 and 77% in BOD<sub>5</sub>, COD and protein content, respectively (Table 1).

Ultrafiltrated treatments showed BOD<sub>5</sub> levels of final effluents to fall very close to the level allowed by the Mexican Normative for discarding this type of effluent (BOD<sub>5</sub> = 0.40 g O<sub>2</sub> L<sup>-1</sup>) and similar to the final effluent collected from the evaporation system generated by the fishmeal processing plant (BOD<sub>5</sub> = 0.35 ± 0.04). For comparison, final industrial effluent resulted almost 40% higher in total volatile bases nitrogen (TVB-N) than the effluent resulted from the centrifugation-pH shift (TCA)-UF treatment (5.0 ± 2.0 vs. 3.6 ± 0.1 mg N 100g<sup>-1</sup>, respectively). On the contrary, the total nitrogen content (%) on these same samples resulted higher in the centrifugation-pH shift (TCA)-UF treatment (0.22 ± 0.01%) than the industrial effluent (0.08 ± 0.01 %). Thus, it can be hypothesized that most of the organic material used by the BOD<sub>5</sub> measurement in our experimental samples came from very low molecular weight protein, small peptides and

**Tabla 1** Composición química, demanda bioquímica y química de oxígeno del agua de cola, agua de cola centrifugada y los diferentes efluentes producidos después del tratamiento.

**Table 1** Chemical composition and chemical and biochemical oxygen demands of stickwater, centrifuged stickwater and the different effluents produced after treatments.

Sample	Protein (%)	Ash (%)	Fat (%)	BOD <sub>5</sub> (g O <sub>2</sub> L <sup>-1</sup> )	COD (g O <sub>2</sub> L <sup>-1</sup> )	Total Solids (%)	NPN (%w/w)
SW	5,8 ± 1,1 <sup>a</sup>	1,6 ± 0,0 <sup>b</sup>	1,4 ± 0,5 <sup>a</sup>	48,5 ± 6,2 <sup>a</sup>	131,2 ± 23,5 <sup>a</sup>	9,2 ± 1,9 <sup>a</sup>	0,6 ± 0,2 <sup>a</sup>
CSW	3,1 ± 0,2 <sup>b</sup>	1,3 ± 0,2 <sup>c</sup>	0,1 ± 0,1 <sup>b</sup>	25,3 ± 8,9 <sup>b</sup>	75,2 ± 27,4 <sup>b</sup>	5,5 ± 1,0 <sup>b</sup>	0,4 ± 0,1 <sup>a</sup>
SF <sub>2</sub> HCl	2,7 ± 0,1 <sup>b</sup>	1,9 ± 0,1 <sup>a</sup>	n.d	6,5 ± 2,2 <sup>c</sup>	35,7 ± 7,8 <sup>c</sup>	n.d	0,2 ± 0,0 <sup>b</sup>
SF <sub>2</sub> HCl + UF	1,6 ± 0,2 <sup>c</sup>	1,8 ± 0,0 <sup>a</sup>	n.d	0,5 ± 0,0 <sup>d</sup>	15,4 ± 3,8 <sup>d</sup>	3,2 ± 0,3 <sup>c</sup>	0,1 ± 0,0 <sup>c</sup>
SF <sub>2</sub> TCA	2,5 ± 0,3 <sup>b</sup>	1,9 ± 0,2 <sup>a</sup>	n.d	6,6 ± 2,3 <sup>c</sup>	38,9 ± 7,9 <sup>c</sup>	n.d	0,2 ± 0,0 <sup>b</sup>
SF <sub>2</sub> TCA + UF	1,3 ± 0,2 <sup>c</sup>	1,6 ± 0,0 <sup>b</sup>	n.d	0,4 ± 0,0 <sup>d</sup>	13,4 ± 3,4 <sup>d</sup>	3,7 ± 0,4 <sup>c</sup>	0,1 ± 0,0 <sup>c</sup>

Values are means ± SD of for samples taken in different month (n = 4). Different superscripts in the same column indicate significant differences ( $P \leq 0.05$ ). SW: Stickwater. CSW: Centrifuged stickwater. SF: Soluble fraction. BOD<sub>5</sub>: Biochemical oxygen demand; COD: chemical oxygen demand. NPN: Non protein nitrogen. UF: Ultrafiltration. TCA: Trichloroacetic acid

amino acids. Because samples showed high nitrogen content on the final effluents, further studies are necessary to improve its removal.

Results indicated that chemical treatments themselves were not satisfactory to remove the total organic material; thus, UF is a necessary step to reduce the organic material in the effluent. Similar results were found, protein removal up to 82% for SW when using 1 kDa MWCO ceramic membrane for UF was reported (Afonso y Bórquez, 2002). In addition, COD removal of 80% for SW when using a bubble accelerated flotation system was found (Miller *et al.*, 2001). In other research, using a chitosan matrix and pH shift for solids removal in SW from the same fishmeal industry, reported reductions of 70 and 73% in BOD<sub>5</sub> and COD levels, respectively, indicating the effectiveness of UF for solids removal on similar SW composition (Pacheco-Aguilar *et al.*, 2009) (present study showed removals of 99 and 90% for BOD<sub>5</sub> and COD levels, respectively). In the present study, ultrafiltered treatments showed no differences ( $P > 0.05$ ) in BOD<sub>5</sub>, COD and protein removals, respectively (Table 1).

Initial centrifugation resulted in a significant ( $P < 0.05$ ) ash removal (19% removal); however, addition of acid and base for the pH shift made the ashes to return to their initial levels (Table 1).

Transmittance measurement has been used as an additional pollution indicator for wastewater treatment, as the more solids present in SW, the less transmittance obtained in the measurement. In the present study, the effect of centrifugation-pH shift-UF had a significant ( $P < 0.05$ ) effect on the transmittance of samples (Table 2), going from a value of 0 to 98% for both treatments, thus improving the clearness of final effluents. On similar SW, authors reported transmittance values of 86%, similar to values obtained prior UF (Table 2) (Pacheco-Aguilar *et al.*, 2009).

Data of  $R_H$  (Table 2) shows how particle sizes from SW decreased as the different treatment protocols were applied. The acid/alkaline treatments previous

UF had a positive effect on removal of size particles from SW (compared to only centrifugation) generating particles of 0.19 to 0.21  $\mu\text{m}$ . Ultrafiltration further eliminated particles, leaving only extremely small ( $8.8\text{E}^{-06}$  to  $4.1\text{E}^{-04}$   $\mu\text{m}$ ) ones (Table 2), suggesting that the final effluents were composed by highly soluble-low molecular weight material, such as free amino acids (García-Sifuentes *et al.*, 2009). The particle size measurement has been used to understand, follow and control the removal of suspended particles in a given fluid material (Rattanakawin, 2005). Results in the present study indicates that  $R_H$  could be a useful parameter to design and control processing strategies for SW treatment; besides, it could be used to achieve a better criteria for membrane selection in future studies. However,  $R_H$  technique should be validated for this kind of process.

**Table 2** Efecto del tratamiento del agua de cola en la transmitancia y radio hidrodinámico de sus sólidos.

**Table 2** Stickwater (SW) treatment effect over the transmittance (%) and hydrodynamic radius ( $R_H$ ) of their soluble solids.

Sample	Transmittance (%)	Hydrodynamic radius ( $R_H$ ) ( $\mu\text{m}$ )
SW	0 <sup>a</sup>	$2.5 \pm 1.0$ <sup>a</sup>
CSW	$1 \pm 0.1$ <sup>b</sup>	$2.1 \pm 0.9$ <sup>a</sup>
SF <sub>2</sub> HCl	$83 \pm 6.0$ <sup>c</sup>	$0.19 \pm 0.0$ <sup>b</sup>
SF <sub>2</sub> HCl + UF	$98 \pm 0.1$ <sup>d</sup>	$8.83\text{E}^{-06} \pm 0.0$ <sup>c</sup>
SF <sub>2</sub> TCA	$84 \pm 5.0$ <sup>c</sup>	$0.21 \pm 0.1$ <sup>b</sup>
SF <sub>2</sub> TCA + UF	$98 \pm 0.3$ <sup>d</sup>	$4.1 \text{E}^{-04} \pm 0.0$ <sup>d</sup>

Different superscripts in the same column indicates significant differences ( $P \leq 0.05$ ). SW: Stickwater. CSW: Centrifuged stickwater. SF: Soluble fraction. UF: Ultrafiltration.

## CONCLUSION

Stickwater is the main polluting effluent from the fishmeal industry; this by-product can have a serious impact on marine ecosystems. The alternative treatment proposed in the present study, this is centrifugation-pH shift-UF, clearly showed its benefits,

as most solid removal was attained, thus obtaining a clearer final effluent with BOD<sub>5</sub> and COD levels near the Mexican Normative. The study provides an alternative stickwater treatment, which could be applied to remove organic and inorganic matter such as solids, proteins and lipids, generating a less contaminant effluent than the SW by itself.

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