

Reduction of Organic Matter and Bacteria in Surface Waters of the Huatanay River using Chitosan Nanoparticles

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The progress in the contamination of rivers makes it impossible to use surface water from these sources or constitutes a risk to the health of those who do so. The high presence of organic matter in rivers can cause its decomposition to cause a decrease in dissolved oxygen in the water, reaching septic conditions, eutrophication of water, and methane emission if there are anaerobic processes; Likewise, the presence of pathogenic organisms (bacteria, viruses) makes the water unfit for human consumption and may be a route of disease transmission. The objective of the research was to reduce organic matter and bacteria in river water using chitosan nanoparticles. The treatment was verified by taking into account the Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) as indicators of organic matter and contamination by pathogenic organisms by the level of total coliforms. The results obtained were a reduction of BOD by 73.541% with a dose of 12 g, COD by 67.75% with a dose of 10 g, and Fecal Coliforms 95.6%, with a dose of 8 grams. The methodology is viable to improve the surface water quality of rivers in an easy way.

1. Introduction

For the United Nations Organization, (2019) currently "two billion people live in countries with a high level of water stress and some four billion people suffer severe water shortages for at least once a year", the main problem is population growth (Richter et al., 2020) and water pollution affecting the quality and quantity (Fitria Rini et al., 2022), putting in trouble the fulfilment of the UN Sustainable Development Goal 6 (Dalstein and Naqvi, 2022), facing this problem many countries with water stress have started to implement management mechanisms such as limiting the idea of agricultural use of water and adapting a virtual water strategy (Khaneiki et al., 2022), other societies have sought to adapt through the socio-hydrological approach seeking to improve the water-human interaction (Kumar et al., 2020); the truth is that all of them refer to improvements in the field of water treatment. In Peru, there is uncertainty regarding the treatment capacity of surface water for human consumption because there is no current diagnosis, only general data on drinking water consumption and chlorine levels, but there is no consensus on water quality (Defensoría del Pueblo, 2022). The waters of the Huatanay River, located in the province of Cusco and Quispicanchi, are used for both rural and urban purposes, so water treatment is essential for the health of the Cusco population. Conventional treatments (polymers and biopolymers) are inefficient and use higher doses, so a nanomaterial would improve costs and is more environmentally friendly because there are no secondary contaminants.

Research has already been carried out on the use of chitosan for wastewater treatment, taking advantage of the fact that this nanomaterial has good flexibility because it contains hydroxyl (-OH) and amine (-NH₂) groups in its nanostructure, which gives it good efficiency for the treatment of surface water (Benettayeb et al., 2023). Silver nanoparticles have also been used to evaluate industrial waters (Real and Benites, 2021), magnetic nanoparticles to treat wastewater (Barozzi et al., 2021) and especially nano chitosan to remediate soils (Nakum and Bhattacharya, 2022) or as a heavy metal adsorbent (Mohammad et al., 2021); employing this natural polymer (chitosan) has a high potential to separate contaminants from water by adsorption, the stability of the material and its environmental friendliness (Lee and Patel, 2022). According to this scientific literature, the research responds to the objective of improving the physicochemical and biological parameters of the waters of

the Huatanay River with chitosan nanoparticles, for which the physicochemical and biological characteristics were previously determined before treatment and to evaluate how these parameters are reduced after treatment.

2. Methodology

The research was experimental and consisted of treating a sample of water from the Huatanay River using nano chitosan and evaluating the variation of physicochemical properties (pH, electrical conductivity (EC), turbidity, total dissolved solids (TDS), dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD)), and microbiological properties (total coliforms) of the water.

2.1 Water sample

The water sampling point of the Huatanay river was in the Kayra area with latitude: -13.5529645 and longitude: -71.8724257, at an altitude of 3195.6541904 m. This place was considered to be the midpoint between the upper and lower part of the Huatanay River Basin that crosses the city of Cuzco, where the wastewater from the city is discharged.



Figure 1: Appearance of the water sample before treatment

2.2 Obtaining chitosan

Commercial chitosan was obtained from Xi'an Complex Bio-Tech Co. Ltd., with Lot Code No. HL220915N and date of manufacture 2022-09-22, with the following characteristics as shown in Table 1.

Table 1: Characteristics according to chitosan analysis

Item	Specification	Results	Item	Specification	Results
D.A.C. %	≥ 95	96.7	Arsenic mg/kg	≤ 0.5	0.02
Viscosity cps	≤100	21.5	Mercury mg/kg	≤ 0.5	0.001
Insoluble %	≤ 1	0.03	Cadmium mg/kg	≤ 0.5	0.01
Ash %	≤1	0.69	Chromium Cr ⁺⁶ mg/kg	≤ 0.2	0.02
Moisture %	≤ 10	9.19	Density g/ml	≥ 0.28	0.32
Finesse nm	60-600	Pass	Appearance	White Powder	Pass
Lead mg/kg	≤ 2	0.37			

Fuente: Xi'an Complex Bio-Tech Co. Ltd (Wang Zunhua)

2.3 Experimental Procedure

For research purposes, the flocculation, coagulation and precipitation processes were carried out in the Biotechnology laboratory of the Universidad César Vallejo, using the jugs test equipment, with the design shown in Figure 2. Figure 3 shows the water samples after treatment with the different doses of nano chitosan.

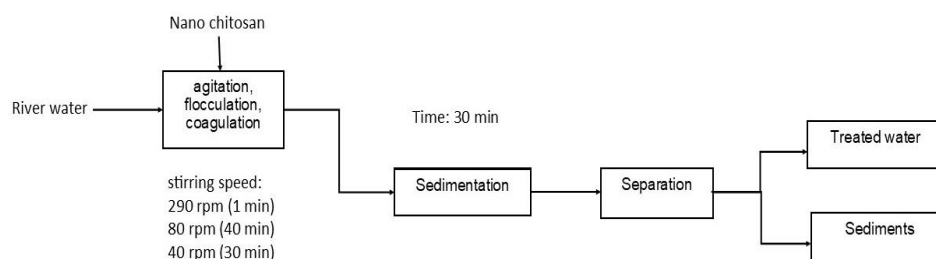


Figure 2: Experimental treatment process

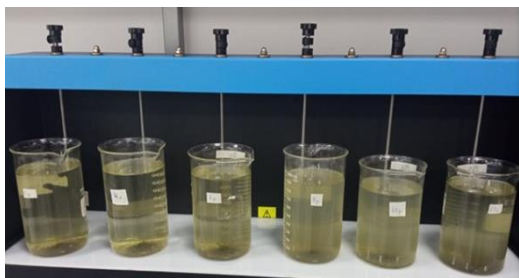


Figure 3: Water after nano chitosan treatment

2.4 Analysis of physicochemical and microbiological properties of the water

The physicochemical analysis was carried out according to the following methods:

The pH, Temperature, Electrical Conductivity (EC), Turbidity, Total Dissolved Solids (TDS): With Multiparameter.

Dissolved Oxygen (OD): Winkler Method

Biochemical Oxygen Demand (BOD): APHA 5210-B, 21Th Edition 2005. 5-Day BOD Test.

Chemical Oxygen Demand (COD): APHA 5220 D, 21Th Edition 2005, Closed Reflux, Colorimetric Method.

Total Coliforms: APHA 9222 B, 21Th Edition 2005, Standard Total Coliform Membrane Filter Procedure.

The evaluated parameters were compared with the Environmental Quality Standards (EQS) for Category 3, waters (Vegetable irrigation and animal drinking) of the Peruvian regulations (MINAN, 2017) because these waters are used for agricultural and livestock activity of the Huatanay River basin. Table 2 presents these EQS.

Table 2: Environmental Quality Standards, Water of Category 3

Parameters	Units	Value for irrigation and animal water
pH		6.5 – 8.5
Temperature (T)	Celsius	Δ 3
Electrical conductivity (EC)	μS/cm	2,500
Turbidity* (T)	NTU	(n. c.)
Total Dissolved Solids (TDS)	mg/L	500
Dissolved Oxygen (DO)	mg/L	≥ 4
Biochemical Oxygen Demand (BOD)	mg/L	< 15
Chemical Oxygen Demand (COD)	mg/L	40
Thermotolerant Coliforms	NMP/100mL	1,000

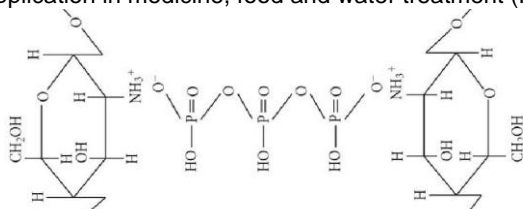
* Parameters for category 3, not considered (n.c.)

Source: MINAN (2017)

3. Results and Discussion

3.1 Nano chitosan Characteristics

Based on the characteristics already noted in Table 1, the nano chitosan purchased from Xi'an Complex Bio-Tech Co. Ltd, presented a size between 60 to 600 nm, with a density of 0.32 g/mL and white colour. The main characteristics of chitosan are its non-toxicity and biodegradability and many researchers have verified its application in medicine, food and water treatment (Islam et al., 2017). Figure 3 shows the chemical structure.



Source: Rao et al., (2002)

Figure 3: Nano chitosan structure

3.2 Physical characteristics of water treated with nano chitosan

In jug 0 was the water where nanochitosan was not added for the treatment, presenting characteristics of the original river water used in the investigation, which is presented in Table 3. The water after treatment with doses of 2, 4, 6, 8, 10 and 12 g of nanochitosan, the temperature increased from 17.4 to 20.3 °C; that is, there is a variation from 1.7 to 2.9 °C, as presented in Table 3, complying with the quality standards Environmental (Peru) that indicates a maximum of $\Delta 3$ °C. (MINAM, 2017). Electrical conductivity was reduced from 3.043 to 2.93 $\mu\text{S}/\text{cm}$, remaining within the quality standards Environmental. Turbidity, despite not being within controlled parameters, was drastically reduced from 130 to 4.13 NTU in jug 1 with 2 g of nanochitosan; with the same dose, the dissolved solids are reduced from 324 to 14.24 mg/L, a significant reduction that is maintained within the quality standards Environmental (QSE). These reductions in Turbidity, CE and SDT are due to the presence of the important cationic charge and the primary amino groups that nanochitosan presents. (Elgadir et al., 2015).

Table 3: Physical characteristics of the water after treatment

Water sample	Added nanochitosan dose (g)	Temperature (°C)	Electrical conductivity ($\mu\text{S}/\text{cm}$)	Turbidity (NTU)	Total Dissolved Solids (mg/L)
jug 0	0	17.4	3043	130	324
jug 1	2	19.1	2.93	4.13	14.24
jug 2	4	19.1	3.08	4.65	18.03
jug 3	6	19.1	3.09	7.75	18.29
jug 4	8	20.1	3.08	15.16	22.16
jug 5	10	20.1	3.09	21.15	22.56
jug 6	12	20.3	3.07	28.19	23.11

Note: Pitcher 0, corresponds to river water without nanochitosan.

Of all the doses tested, the results indicate that 2 g of chitosan improved the physical properties of the treated water, with important percentages such as 99.9 % for electrical conductivity, 96.42 % for turbidity and 95.6 % for TDS, see Table 4.

Table 4: Variation of the physical characteristics of water after treatment

Water sample	Added nanochitosan dose (g)	Temperature (% decrease)	Electrical conductivity (% decrease)	Turbidity (% decrease)	Total Dissolved Solids (% decrease)
jug 1	2	9.77	99.90	96.42	94.44
jug 2	4	9.77	99.90	94.04	94.35
jug 3	6	15.52	99.90	88.34	93.16
jug 4	8	15.52	99.90	83.73	93.04
jug 5	10	16.67	99.90	78.32	92.87
jug 6	12	9.77	99.90	96.82	95.60

3.3 Chemical characteristics of water treated with nano chitosan

The pH after treatment is reduced to values within the EQS when using the tested doses of nano chitosan; Dissolved Oxygen improves and increases to values required by the EQS. BOD5 and COD decrease but do not reach the EQS level (see Table 5). BOD and COD as indicators of organic matter decrease due to the adsorption capacity of nano chitosan for having amino (-NH₂), hydroxyl (-OH), (-COOH), (-SH) groups that fix water pollutants (metals, and others) which in turn gives reactivity to most pollutants (Nghah and Ferdosh, 2015); also due to the nanometric size that allows increasing the surface area, giving preferential surface adsorption (Ghosh et al., 2022).

Table 5: Chemical characteristics of the water after treatment

Water sample	Added nanochitosan dose (g)	pH	Dissolved Oxygen (mg/L)	Biochemical Oxygen Demand (BOD)	Chemical Oxygen Demand (mg/L)
jug 0	0	8.8	2.05	721	1228
jug 1	2	7.8	4.16	283	453
jug 2	4	7.78	4.84	255	438
jug 3	6	7.71	5.05	220	414
jug 4	8	7.52	5.27	197	406
jug 5	10	7.75	5.96	192	394.6
jug 6	12	8.15	5.14	191	404

From the above results and from Table 6, the most convenient dose that improved the chemical parameters was 10 g of chitosan; it highlights the significant improvement percentage of the DO (190.73 %), which means that the treatment reduces the contamination caused by organic or inorganic material (Posada et al., 2013); a fact that is corroborated by having also reduced the BOD₅ by 73.37 % and COD by 67.87 %.

Table 6: Variation of the chemical characteristics of water after treatment

Water sample	Added nanochitosan dose (g)	pH (% decrease)	Dissolved Oxygen (% increase)	Biochemical Oxygen Demand (BOD) (% decrease)	Chemical Oxygen Demand (% decrease)
jug 1	2	11.36	102.93	60.75	63.11
jug 2	4	11.59	136.10	64.63	64.33
jug 3	6	12.39	146.34	69.49	66.29
jug 4	8	14.55	157.07	72.68	66.94
jug 5	10	11.93	190.73	73.37	67.87
jug 6	12	7.39	150.73	73.51	67.10

Temperature, pH and dissolved oxygen are important parameters that influence water treatment; High temperatures can reduce the level of dissolved oxygen in the water, which would harm the growth of beneficial aerobic microorganisms for aquatic ecosystems. On the other hand, the pH intervenes in nitrification and biological processes (De Francisco, 2003).

3.4 Microbiological characteristics of water treated with nano chitosan

The parameter of thermotolerant coliforms before treatment was at a very high level, the treatment with nano chitosan reduced this concentration to values very close to the maximum level of the EQS. A reduction of around 99.5% is observed for any of the doses tested; this is due to the presence of oxydryl radicals that act with their oxidizing power and give antibacterial properties that complement its use as an adsorbent for water treatment (Benettayeb et al., 2023).

Table 7: Thermotolerant (fecal) coliforms in water after treatment

Water sample	Added nanochitosan dose (g)	Thermotolerant Coliforms (NMP/100mL)	Percentage reduction (%)
jug 0	0	240000	0
jug 1	2	1500	99.4
jug 2	4	1200	99.5
jug 3	6	1200	99.5
jug 4	8	1100	99.5
jug 5	10	1100	99.5
jug 6	12	1100	99.5

4. Conclusion

The treatment of water with nano chitosan improved in a good percentage the physical, chemical and microbiological property, reaching in some cases up to the level of the environmental quality standards of the Peruvian norm, increasing the quality of the water resource, in such a way that it is verified that it is one of the best nano bio adsorbents of water pollutants. This methodology can be used because of its economic advantage, easy application and, above all because it is environmentally friendly since it is non-toxic and biodegradable.

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