Biodegradation of Natural Rubber Wastewater in the Submerged Membrane Bioreactor by Pichia Guilliermondii and Yarrowia Lipolytica

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M.N. Nsoe[1,2,*], E.V. Amba[2], N.M. Kameni[2], G.P. Kofa[2], K.S. Ndi[2], M. Heran[3] and G.J. Kayem[2]

[1]*Laboratory of Chemical Engineering and Environment, University Institute of Technology (IUT), P.O. Box: 455 University of Ngaoundéré, Cameroon*

[2]*Water Treatment and Filtration Research (Chem. Eng.) Group, Department of Process Engineering, ENSAI, University of Ngaoundere, P.O. Box 455, Ngaoundere, Cameroon*

[3]*Institute europeen of membrane UMR 5635(CNRS-ENSCM-UMII) CC047 Place Eugène Bataillon 34095 Montpellier CEDEX5*

Abstracts: Despite the many services that natural rubber provides to humanity, its production generates significant quantities of polluted effluents which have a negative impact on health and the environment. In order to reduce this pollution and allow water to be reused after treatment, a synthetic effluent treatment trial was carried out in a submerged membrane bioreactor in the presence of two strains of yeast (Yarrowia lipolytica, Pichia guilliermondii) and biodegradation parameters (COD, NH_4^+, NO_2^-, NO_3^-) were followed. The Mohlman Index (MI), the particle size distribution, the Lowry method for proteins and the Dubois method for sugars have made it possible to characterize the sludge generated during biodegradation. It appears from these experiments that the reduction rate of COD was 98% and that of nitrification and denitrification 90%. There is a decreasing linear relationship between the MI and SS (Suspended Solids) with an R^2 of 95%. The distribution of the particle size of the sludge is tri-modal with a maximum of sludge having an average size of 1000 µm. The sludge formed resists filtration with a polysaccharide/protein ratio of 0.45.

Keywords: Biodegradation, Membrane bioreactor, Pichia guilliermondii, Rubber effluent, Yarrowia lipolytica.

INTRODUCTION

The increase in population and increasing use of industrial and agricultural resources are causing water shortage, which are leading to a decline in the quality and quantity of freshwater [1]. According to statistics, water consumption has increased by 20% over the last century, and 90% of wastewater is discharged without any treatment [2].

In the industrial sector, 70% of factory wastewater is untreated and discharged into the environment, which is more than 22% of surface water pollution [3]. According to WHO statistics in 2014, 2.3 million people die every year due to water contamination from industrial water waste. In addition, more than half of the world's wetland and 20% of aquatic living things have also disappeared. In full expansion with rubber production increasing from 1.3 million tons in 1985 to over 9.9 million tons in 2012. Production of 1 kg of rubber generated 22.7 L of wastewater: which corresponds to a volume of 247.5 million cubic meters

of water used [4], these studies by the following authors show that this wastewater has a negative effect on the health of the local population, who get their drinking water from rivers and streams where these effluents are discharged [5-8]. At the environmental level, we are seeing the eutrophication of mangroves and the reduction of oxygen in rivers. This is causing the disappearance of more than 200 aquatic species in the Niger Delta [7, 9]. Given the health and environmental concerns related to this compound, it's important to be aware of its potential risks. It is important to treat the effluents resulting from the coagulation of rubber latex before they are discharged into nature.

Lagooning and oxidation pits are the most commonly used processes to treat rubber industry effluents [10, 11]. There are limits to these processes. In particular, it has a large footprint, requires a long residence time for contact between microorganisms and contaminant loads, emits odors, and lacks compliance with legal restrictions on waste. To overcome these problems, other processes such as biofiltration [12], electrolysis [13], coagulation/ aggregation [14], membrane bioreactor [6] and

*Address correspondence to this author at the Laboratory of Chemical Engineering and Environment, University Institute of Technology (IUT), P.O. Box: 455 University of Ngaoundéré, Cameroon;
Tel: (+237) 677660466/699776841; E-mail: nsoemenguenestor@yahoo.fr

ultrafiltration [15] are also being studied in the laboratory.

Among the many processes still under study, processes using strains of microorganisms with a high purifying power are promising. Studies focus on testing the biodegradation of wastewater by bacteria [9, 16-19], but very little on yeast.

However, the use of yeast for wastewater treatment shows that the latter achieve better results than bacteria. In fact, the use of yeast allows to reduce sludge production, increase nitrification yield and increase resistance to inhibition with nitrogen, metals and phenolic compounds [20]. In the treatment of rubber effluents, Ndi *et al.* [12] used two yeast strains isolated from rubber effluents with yields in excess of 80% in total nitrogen removal. However, the main disadvantage of using yeast is the source of additional contamination [21-23]. To separate this filamentous sludge from the treated water, membrane bioreactors could be used satisfactorily, which would limit the pressure on water resources, comply with increasingly restrictive norms and laws, and most importantly, reuse this water. For this reason, the biological degradation tests of a rubber waste water as well as the characterization of the sludge obtained are examined in this work.

2. MATERIEL AND METHODS

2.1. Yeast Inoculum

The yeast strains used in this work were isolated from the effluents of a rubber industry thanks to Nsoe protocol [24, 25]. The yeast characteristics are shown in Table **1**. Yeast were cultivated on the following growing medium [26]: meat extract 1 g L^{-1} (Sherlan réf 07-075, Spain), yeast extract 2 g L^{-1} (OXOID code L21, England), peptone 5 g L^{-1} (Liofilchem réf 610038, Italy), and sodium chloride 5 g L^{-1} (Jeulin réf 107 115, France) at pH = 6.5.

2.2. Experimental Set-up: Membrane Bioreactor

The submerged membrane bioreactor pilot (Figure **1**) studied consists of a plexiglass feed tank with a capacity of 40 L and 35 L of useful volume. A RENA brand compressor is used to inject air at the bottom of the reactor through a thin cylindrical PVC bubble diffuser; 8 cm diameter. The aeration cycles were fixed using a COGEX brand timer in order to have a maximum oxygen concentration of 6 mg.L^{-1} in the medium. The pilot is powered by a peristaltic pump (GILSON™ Model minipuls 2) connected to a feed tank with a capacity of 30 L. The treated water is suctioned with a peristaltic pump (CEBILON ModelReverse Osmosis System) through a polyvinylidene fluoride (PVDF) microfiltration membrane from A3 GSmbH Germany. The Membrane Module contains 8 parallel plates spaced 12 mm apart. The plates were connected together and act as a single membrane module with a membrane effective area of 0.2 m^2 and a cut-off of 0.14 µm. A flowmeter (PLATON ModelU-3270350), is used to control flow at the exit of the membrane. A pressure sensor (KELLER MANNO 200 Model LEO 2) with an accuracy of 0.01 bar is used to measure the transmembrane pressure (TMP).

1. Storage tank, 2. Feed pump, 3. Bioreactor, 4.Membrane module, 5. Manometer, 6. Permeate pump, 7. Flowmeter, 8. Perméate, 9. Compresor, 10. Evacuation valve, 11. Air diffuser, 12. Recirculation channel.

2.3. Preparation of Synthetic Rubber Wastewater

In order to get closer to the real rubber effluent, the preparation was made using the latex taken from a rubber tree field, and kept at pH 9-10 with ammonia to prevent its coagulation. The latex is coagulated in a reactor (1L) in which it is kept under mechanical stirring using a stirrer (EUROSTAR, digital IKA-WERKE, Allemagne). Latex pH is determined using a portable pH meter (Fisher Bioblock Scientific type pH 330i WTW82362 Wellhem,Allemagne). 2% formic acid is

Table 1: Characteristics of Yeast Strains [26]

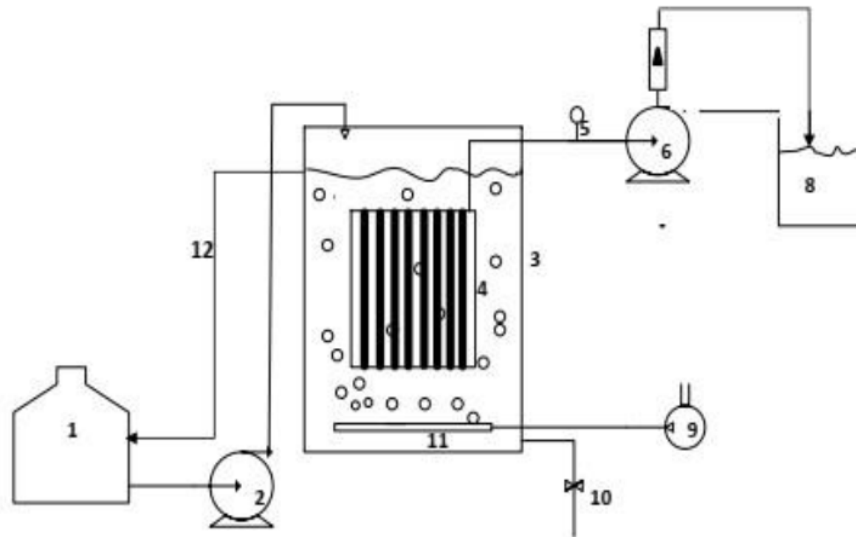| Code	Yeast color	Potentiel Zêta pH (5,7-7)	Yeast shape	Yeast size (µm)	Growth in selective media	
					Acid medium	**Alkaline medium**
Yarrowia lipolytica	Red	-25,3 à -37,8	Oval	0,3-150	yes	no
Pichia guilliermondi	White	-27,8 à -30,5	Oval	0,2-100	yes	no

Figure 1: Experimental set-up: Membrane bioreactor [27].

introduced at 5min intervals until rubber coagulation. The coagulated rubber is then removed and then dried. The yellowish colored filtrate obtained represents the serum which is very concentrated. A dilution with a dilution factor of 9 is carried out in order to obtain pollutant concentrations close to those found in industrial effluents [24]. The physicochemical parameters of the synthetic effluent are presented in Table **2**.

2.4. Analytical Methods

Samples for analysis were taken on a regular basis at three locations. On entry into membrane bioreactor, the mixed liquor (The effluent inside the bioreactor during biodegradation) is taken halfway up the reactor and centrifuged at 5000 rpm, 4°C, 10 min to each sample and finally the treated effluent (permeate). The wastewater was analyzed for Biochemical Oxygen

Demand (BOD), Chemical Oxygen Demand (COD), using the standard techniques described by [26] (APHA, 1998). The turbidimeter used is of brand HACH RATIO/XR TURBIDIMETER (0-2000 NTU) de HACH Inc. (USA). Ammonium ions (NH4+) where titrated by a portable mini-photometer of mark HANNA Checker HC ® HI 733 Woonsocket RIUSE ROMANIE. Nitrates were measured using a brand HORIBA LAQUAtwin B-743. Nitrites using a branded mini portable photometer HANNA Checker HC[®] HI 764 Woonsocket RIUSE ROMANIE. The SS and EMS are thus calculated from the following equations 1 and 2 [29]:

$$SS = \frac{M^A 105° - M^A}{V\ sludge} \quad \textbf{Equation 1}$$

$$EMS = \frac{M_A 550° - M_A 105}{V\ slugge} \quad \textbf{Equation 2}$$

Table 2:   Physico-chemical Characteristics of Synthetic Rubber Effluent

Parameters	Values	Cameroonian standard, 2005 [28]
	5.9±2	5.5 pH 9.5
Turbidity(UNT)	27.4	<10
ConductivitY (µS/ cm)	1300	< 400
organic matter (%)	13.26	/
COD (mg O_2/L)	3050	< 400
Ammonium (mg/L)	153	< 20
Color (EBC)	8.325	/
BOD (mg/O_2 /L)	1087	<100

The proteins are assayed according to the method of Lowry [30] modified by Frolund. The assay of the polysaccharides was carried out by the colorimetric method proposed by Dubois *et al.* [31]. The volume of sludge and the Mohlman index were measured according to the standard methods protocol 2700 D [32]. A MASTERSIZER type laser particle sizer (HYDRO 2000SM) from MALVERN Instruments (England) is used to determine the particle size distribution of the sludge.

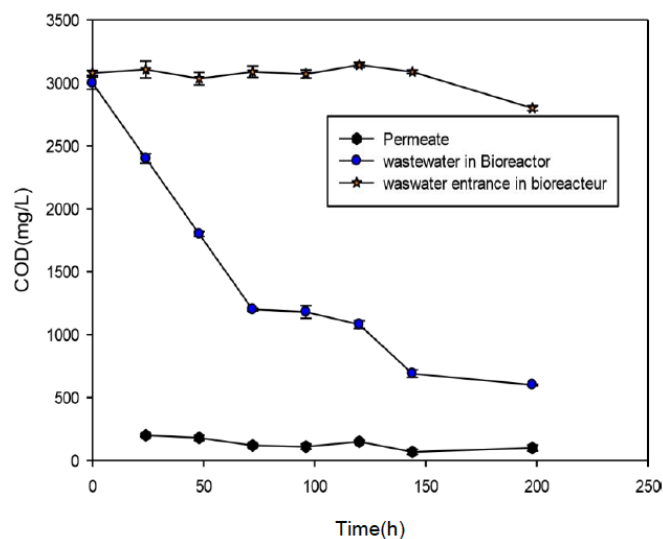## 3. RESULTS AND DISCUSSIONS

3.1. COD Reduction

Figure 2: Variation of the COD of the wastewater at the inlet of the bioreactor, inside the bioreactor and of the permeate as a function of time during biodegradation.

Figure **2** presents the measurements of the chemical oxygen demand (COD) at the reactor inlet, in the bioreactor and at the outlet (after membrane filtration). Throughout the period of monitoring the functioning of the membrane bioreactor, the COD concentrations at the BRM inlet fluctuated between 3080 mg.L^{-1} and 2800 mg.L^{-1}. On the other hand, in the supernatant inside the bioreactor, a reduction in COD is observed around 500 mg.L^{-1}. COD reduction is caused by the activity of yeasts which degrade organic matter. The residual concentrations of COD at the outlet of the BRM are also presented. An almost total COD reduction was observed (≥ 98%), regardless of the initial concentration at the bioreactor inlet. These results are similar to those found by Nik [6] who studied the treatment of a rubber effluent in a submerged membrane bioreactor with a COD reduction of almost 96%. This shows us that the yeast strains studied are capable of improving the treatment of rubber effluents.

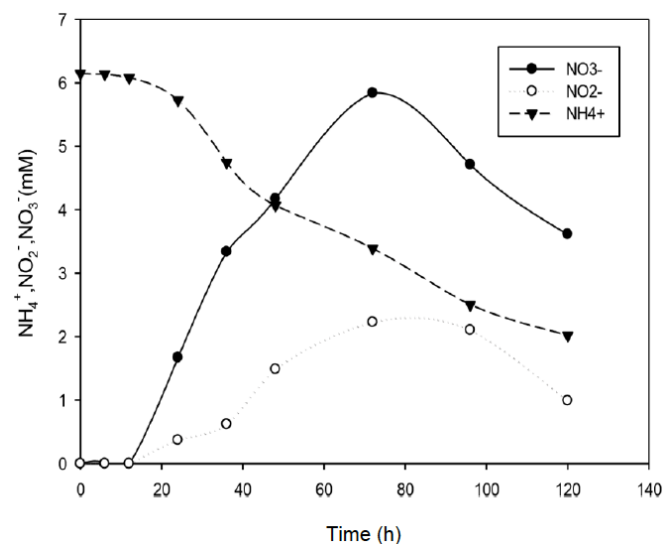## 3.2. Reduction of Ammonium ions, Nitrites and Nitrates in the Membrane Bioreactor

Figure 3: Variation of NH$_4^+$, NO$_2^-$, NO$_3^-$ as a function of time at (pH 6.28 ± 2°C).

The elimination of nitrogenous compounds in wastewater by microorganisms goes through two main steps: nitrification and denitrification. Nitrification takes place in the presence of oxygen and denitrification takes place in the absence of oxygen. But in our system all the reactions take place in the presence of oxygen with high yields of more than 90%. This leads us to believe that the yeast strains used metabolize nitrogen compounds in the presence of oxygen without an anoxia phase. But we notice a low production of nitrite: This is in agreement with the work of Kurakov and Popov [33] who demonstrated that the use of yeasts in the degradation of nitrogenous compounds led to a reduction or absence of the production of nitrites. On the other hand, a high production of nitrates is observed [34,35,36].

3.3. Evolution of pH

Observation of the curves in Figure **4** shows the evolution of the pH in the bioreactor. And at the output of the filter present. These curves show two phases: a first phase during which the pH decreases by 6 and reaches a minimum value at pH 5.6. The drop in pH may be the consequence of the assimilation of organic matter linked to the growth of biomass and the consumption of the nitrogen contained in the effluent. The assimilation by the latter of nitrogen in the form of ammonium ion is known to cause a drop in pH during the process [37], the cause being the expulsion of protons into the medium for each mole of NH$_4^+$ consumed because according to Metahri. [38]

nitrification leads to the production of H^+ protons which leads to a decrease in pH. On the other hand, in the second phase during which the pH increases from 5 to pH 7, the opposite phenomenon occurs: the H^+ protons are used by the microorganisms causing an increase in the pH due to the phenomenon of denitrification. These results allow us once again to affirm that the yeasts used are capable of assimilating formate and certainly of carrying out nitrification and denitrification in aerobiosis.
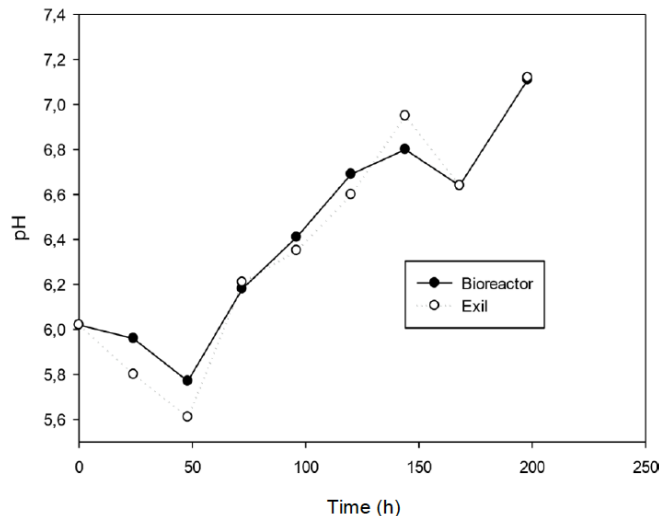

Figure 4: Variation of pH as a function of time during the biodegradation of the synthetic effluent in the bioreactor and at the outlet of the membrane filter at 28 ± 2°C.

3.4. Relationship Between Turbidity and Suspended Matter

During operation of the pilot, the SS concentration and turbidity in the bioreactor were monitored.
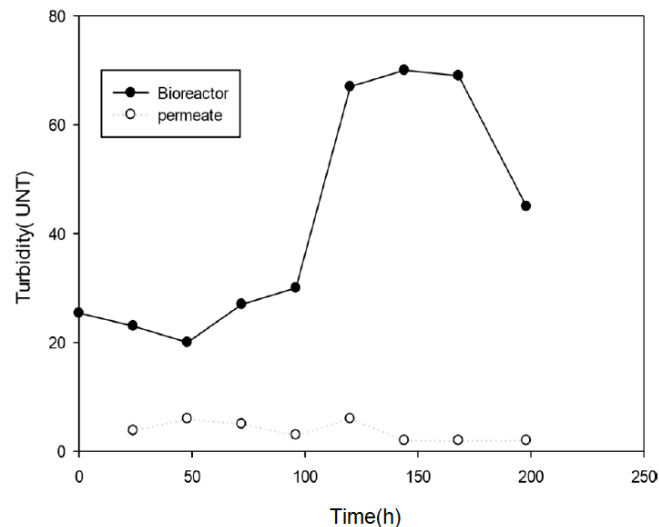

Figure 5: Variation of turbidity as a function of time during biodegradation in the bioreactor and after filtration (permeate).
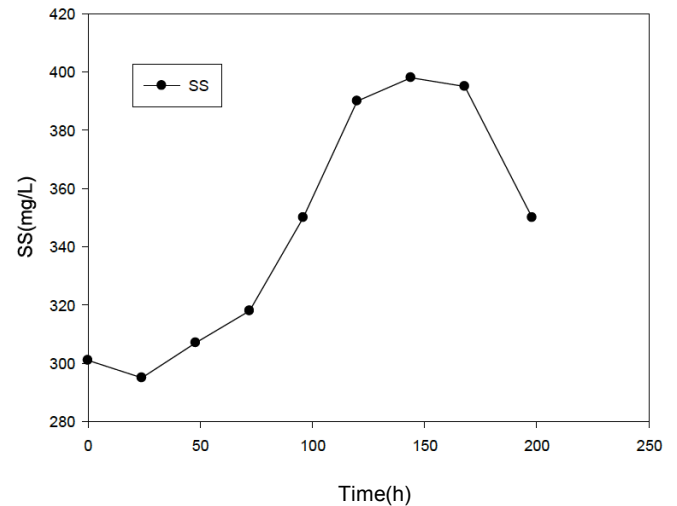

Figure 6: Variation of SS in the bioreactor as a function of time during biodegradation.

Figure **5** above shows us the evolution of turbidity as a function of time in the bioreactor and at the outlet of the bioreactor. In the bioreactor, we observe three phases. The first phase is characterized by the decrease in turbidity from 25 NTU to 20 NTU during the first 25 hours. The second phase characterized by the increase in turbidity in the bioreactor until the 150th hour then drops to a value of 47 NTU at the end of the treatment. On the other hand, at the outlet of the bioreactor after filtration, the turbidity is almost constant throughout the biodegradation up to a value of 1 NTU. These turbidity trends in the bioreactor are also observed for SS as can be seen in Figure **6**. According to Delrue, [39] and Massé, [40], the decrease in turbidity or SS during the first phase can be explained by a period of adaptation of the yeasts to the new environment. As for the increase in turbidity and SS, this is caused by the development of biomass with secretion of exopolymers. With regard to the decrease in turbidity up to 47 NTU and SS 350 mg/L, this phenomenon can be explained by the fact that the molecular weight of the biomass becomes increasingly high. This will cause it to settle at the bottom of the reactor. In order to find a relationship between SS and turbidity, we plotted the graph of SS versus turbidity as shown in Figure **7**.

Figure **7** shows that there is a linear relationship between suspended matter and turbidity during biodegradation, the equation of which is: NTU = 0.52SS -136.27 with an R^2 of 0.98. In order to determine if our sludge is settleable, we studied the biomass sludge index during biodegradation.

3.5. Relationship between Mohlman Index and Suspended Matter

Figure **8** shows the relationship between the Mohlman Index and suspended solids after 30 minutes of settling at each sample during biodegradation.
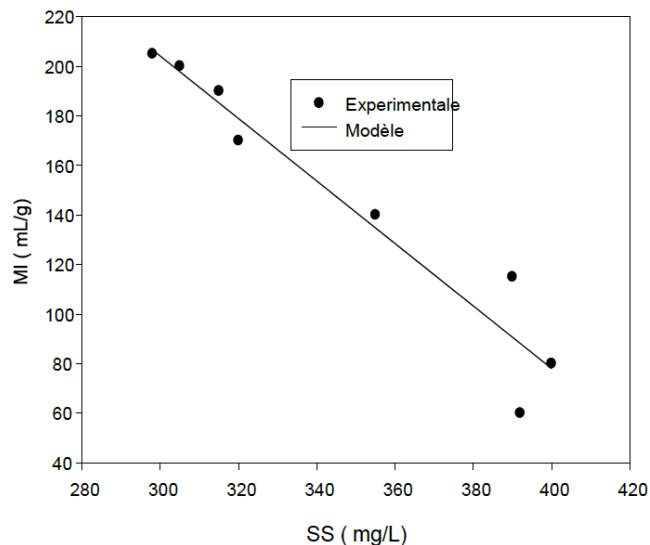

Figure 8: Relationship between suspended solids (SS) and Mohlman Index (MI).

We observed that the sludge index decreases with the increase in SS concentration according to a linear law (MI = -3.78*SS –582) with an R^2 of 0.96. The decrease in the sludge index, which is the volume occupied by one gram of sludge, translates the densification of the flocs and leads to a better capacity for thickening and better settleability of the sludge. This allows us to conclude that the sludge resulting from the combination of the two strains does not float. This is why knowledge of sludge particle size is necessary.

3.6. Floc Particle Size

The size distribution of the flocs formed was analyzed at the end of the experiment (Figure **9**).
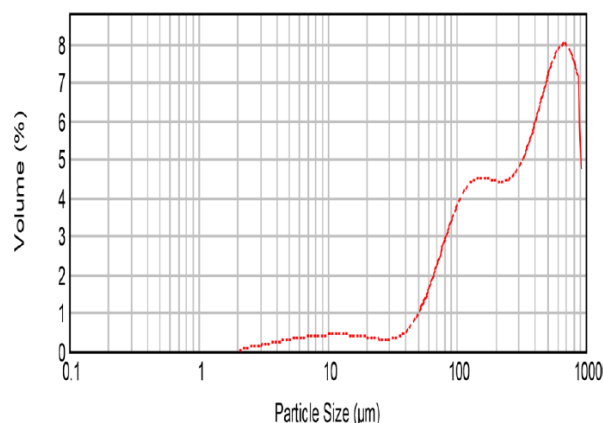

Figure 9: Floc size distribution.

The sludge resulting from the biodegradation of the synthetic rubber effluent shows a trimodal distribution with average sizes of 10 µm, 100 µm and 700 µm. This explains the observed settling of sludge in the study of the sludge index. In an expanded biomass process, the size of the flocs is an important parameter which conditions the nitrifying activity of the yeasts [41]. When the (hydrodynamic) conditions allow it, too high an increase in aggregates can simultaneously cause nitrification and denitrification in the same reactor. This may explain the reduction of nitrites and nitrates observed in the bioreactor. Similarly, in a study of the distribution of flocs and the influence of their size on the activity of microorganisms, in a membrane bioreactor, Boran *et al*. [42] concluded that the specific nitrification rate increases with size.

3.6. Determination of some physico-chemical parameters of sludge

Proteins and polysaccharides (Table **3**) are analyzed at the end of the operation to assess their influence on membrane resistance

Table 3: Composition of Sludge (Protein and Polysaccharide)

Polysaccharide (mg/mg DM)	94 ± 3
Protein (mg/mg DM)	210 ± 7
Rapport polysaccharide / Protein	0.45

From Table **3**, the protein concentration is higher than the polysaccharide concentration by 2.23 times. These results are in agreement with those of Mikkelsen and Keiding [43] who based themselves on sludge samples from different purification systems to demonstrate the influence of the quantity of proteins in the resistance to filtration. They explained that a high concentration of proteins in the flocs is essential for the formation of well-structured flocs, and therefore for a low resistance to sludge filtration. They concluded that the amount of protein is important for the formation of a well-structured floc. Along the same lines, Petros *et al*., [44], linked sludge filterability and sludge resistance to the protein/polysaccharide ratio compared to the total concentration of EPS or the concentration of individual compounds. This better correlation is explained by the neutralization of charges between proteins and polysaccharides. Affecting the surface characteristics. This neutralization of the charges will lead to a reduction in the surface charge and consequently the flocculation capacity [45].

3.7. Characterization of the Effluent after Treatment

The effluent parameters obtained allow us to comment on the physicochemical quality of the water obtained and to conclude whether this water is treated and perhaps can either be reused for specific uses or be discharged into nature.

Figure 10: showing the color of the effluent before (**A**) and after treatment (**B**).

The results obtained were compared to Cameroonian standards for industrial effluent discharges and to WHO and FAO standards for use in irrigation. With regard to Cameroonian discharge standards, all the parameters followed comply with Cameroonian standards, which proves that the use of submerged membrane bioreactors makes it possible to have water that can be discharged into nature without any risk of pollution. Moreover, by comparing the values obtained with the standards for the use of treated wastewater for irrigation and watering [45, 1], we find that this water can be reused in irrigation and watering. fields with a nutrient supplement.

CONCLUSION

Rubber wastewater discharged without treatment pollute the receiving waterways and the soil. The objective of our work was to follow some physicochemical parameters during the biodegradation kinetics of a synthetic rubber effluent in the presence of yeast strains. The COD was abated to more than 98%. Nitrification and denitrification proceeded in the presence of oxygen with a purification efficiency of more than 90%. There is a linear relationship between the sludge index and TSS with an R^2 of 0.96. The sludge granulometry is tri-modal with a maximum sludge size of 1000 μm. The sludge formed is resistant to filtration with a polysaccharide/protein ratio of 0.45. According to the physicochemical data obtained, these waters can be reused in the rubber washing line; which makes membrane bioreactors an avenue to explore for the treatment of rubber effluents.

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Table 4:   Physico-Chemical Characteristics of the Effluent before and after Treatment

Parameters	Unit	Waste water before treatment	Waste water after treatment	[28]	[1, 45]
Turbidité	(NTU)	21	12	< 10	/
pH	/	6.1	8.5	5.5<pH<9.5	6.5-8.6
NH4+	(mg/L)	66.13	38	10	<100
NO_3^-	(mg/L)	8.5	12,5	20	<50
BOD	(mg /L)	1087	17	<100	28
COD	(mg/L)	3050	69	<400	30

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