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# Bioproducts for Sludge Reduction in Activated Sludge Systems Treating Oil Refinery Wastewater

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***Abstract*** *— The use of bioproducts that change the cellular metabolism and reduce microbial growth without affecting the organic matter removal is very promising for reducing the amount of sludge in wastewater treatment systems. In this study, two bioproducts were evaluated and compared with a well-known chemical (2,4-DiNitroPhenol – DNP) in activated sludge treating petroleum refinery wastewater. In batch experiments, 10 mg/L of DNP, 0.8 mg/L of a bioproduct based on Folic Acid (FA) and 10 mg/L of a bioproduct based on Stress Proteins (SP) led to 30.6%, 43.2% and 29.8% lower disposal of total solids, respectively. Operating on a continuous regimen, the addition of 10 mg/L of the bioproduct based on SP led to 45.7% lower disposal for 50 days. In all cases, no loss of efficiency in the Chemical Oxygen Demand (COD) removal was observed.*

**Résumé — Bioproduits pour la réduction de boue dans des systèmes de boues actives pour le traitement d'eaux résiduelles en raffinerie de pétrole** — L'application de bioproduits qui modifient le métabolisme cellulaire et réduisent la croissance microbienne sans affecter I'enlèvement de matière organique, est très prometteuse pour réduire la quantité de boue rejetée dans les systèmes de traitement d'effluents. Dans cette étude, deux bioproduits ont été évalués, en comparaison avec un produit chimique bien connu, 2,4-DiNitriPhenol (DNP), dans des boues activées lors de traitement d'effluents dans une raffinerie de pétrole. Lors des expériences en *batch*, 10 mg/L de produit connu (2,4-dinitriphenol), 0,8 mg/L d'un bioproduit à base d'acide folique (FA, *Folic Acid*) et 10 mg/L d'un bioproduit à base de protéines de stress ont eu comme conséquence un rejet moindre de solides totaux de 30,6 %, 43,2 % et 29,8 %, respectivement. En opération continue, I'addition de 10 mg/L du bioproduit à base de protéines de stress a eu comme conséquence un rejet inférieur de 45,7 % à celui obtenu lors d'essai *batch* de 50 jours. Dans tous les cas, il n'a pas été observé de perte d'efficacité pour la demande chimique d'oxygine (COD, *Chemical Oxygen Demand*).

## ABBREVIATIONS

| | |
|---|---|
| BOD | Biochemical Oxygen Demand |
| COD | Chemical Oxygen Demand |
| DCP | 2,6-DiChloroPhenol |
| DNP | 2,4-DiNitroPhenol |
| FA | Folic Acid |
| HRT | Hydraulic Retention Time |
| SVI | Sludge Volume Index |
| SP | Stress Protein |
| TSS | Total Suspended Solids |
| TVS | Total Volatile Solids |
| VSS | Volatile Suspended Solids |

## INTRODUCTION

Despite the development of new technologies and products such as biofuels, oil still has great commercial importance and will continue to play a critical role in the global energy industry for many decades, especially with the discovery of the pre-salt layer [1].

Oil refineries process extremely high volumes (in 2012 the refining capacity in operation worldwide was 89 million barrels/day) and are major contributors to pollution, both in volume and in the concentration and toxicity of contaminants [1, 2].

Among the solid wastes generated in an oil refinery, oily residues, sediments from the bottom of storage tanks, treatment clays, biological sludge, residues arising from the cleaning of heat exchangers and solid materials contaminated with oil stand out [3]. However, in studies evaluating in detail the management of solid waste in oil refineries, biological sludge is little discussed. Although representing a problem due to new environmental regulations that limit the disposal of solid waste in landfills and the opening of new landfills [4], these residues are less of a concern because they cause much less environmental impact than oily residues.

Wastewaters generated by oil refining plants have variable composition, depending on the units in operation and type of oil processed [1, 5]. However, regardless of their characteristics, the activated sludge system is one of the most used in the treatment of these wastewaters. The main disadvantage of these treatment systems is the generation of excess sludge, which is proportional to the amount of biomass generated by growth [6]. Even accounting for only 1-2% of the total volume of treated wastewater, the cost of managing the excess sludge can reach 60% of the total operating cost of the refining plant [4].

In oil refineries that use activated sludge systems with extended aeration, the operating conditions already favor reduced growth; however, the high volumes of processed wastewaters still generate a significant amount of sludge to be disposed of. The management of this residue can be a complex problem for engineers working in the area of wastewater treatment and is as important as the removal of organic matter and nutrients [4, 7, 8].

There are two basic strategies for reducing sludge: treatment to reduce the amount to be disposed of and changes in the process to reduce the amount generated. The choice of the most appropriate technique for each case depends on many factors, but cost and environmental impacts are the most important [9]. In a sustainable development model, the second alternative is more environmentally friendly. Among the available technologies, the addition of chemical compounds or bioproducts capable of changing the cellular metabolism without affecting the treatment efficiency stands out. The ease of operation is one of the main advantages, as it is only necessary to implement a system for dosing the product in the aeration tank [4].

There are several studies in the literature using chemicals that act as metabolic uncouplers (2,4-DiNitroPhenol – DNP) to reduce excess sludge in activated sludge systems [10-13], but their implementation is still restricted to the laboratory scale [4], mainly because only short times and synthetic wastewaters are assessed. Regarding bioproducts based on Folic Acid (FA) [14, 15] and Stress Proteins (SP) [16, 17], there are studies on laboratory, pilot and industrial scales, but the results are variable, depending on the system evaluated.

Thus, the aim of this study was to evaluate the effect of the addition of three products, DNP (as a reference product) and two bioproducts, one based on FA and another based on SP, on the sludge disposal reduction and treatment efficiency in bench activated sludge systems treating oil refinery wastewater.

## 1 METHODS

### 1.1 Origin of the Wastewater and Sludge

The wastewater and sludge used in this study were obtained from Brazilian oil refineries. Batch bioreactors operated with wastewater (sour water) containing COD of 1 087 ± 435 mg/L, while bioreactors operated in a continuous regimen were fed with two different wastewaters:

- sour water (COD 1 245 ± 314 mg/L),
- oily water (COD 452 ± 213 mg/L).

The aerobic sludge used as an inoculum was collected after dewatering and had 71% (dry weight) of volatile solids.

### 1.2 Products Used and Mechanisms of Action

In this study, three commercial products were used: DNP (*Vetec*, Brazil), one bioproduct based on FA (*Neotex*, Brazil) and one bioproduct based on chemical surfactants and SP produced by *Saccharomyces cerevisiae* (*Advanced Biocatalytics Corporation*, USA).

The DNP is a metabolic uncoupler well studied and described in the literature. It carries protons across the cytoplasmic membrane of prokaryotic organisms, dissipating the proton motive force created by the transfer of electrons from the substrate to the final acceptor (oxygen). Thus, it uncouples the oxidative phosphorylation of the electron transport chain, reducing the production of Adenosine TriPhosphate (ATP) and hence biomass growth [18]. On the first day of operation, the product was added directly into the bioreactor at a concentration of 1 mg/L. Thereafter, the product was added into the feed so that it presented the desired concentration, ranging from 1 to 15 mg/L of DNP. Therefore, considering that the product is biodegradable and completely consumed in 5.5 h of reaction time, the concentrations evaluated inside the bioreactor ranged from 0.26 to 3.9 mg/L of mixed liquor.

The stabilized product based on FA, a B complex vitamin, helps growth, optimizes the metabolic pathways of microorganisms and makes the cells become more active, leading to more rapid substrate consumption. Without changing the treatment characteristics (decreasing Hydraulic Retention Time – HRT, for example), the cells come into endogenous respiration, leading to sludge reduction [15]. The concentration of 0.8 mg/L of product was evaluated. On the first day of operation, the product was added directly into the bioreactor at a concentration of 0.8 mg/L. Thereafter, the product was added into the feed. The product's biodegradability was estimated based on the COD and Biochemical Oxygen Demand ($BOD_5$) values for the dilute product solution ($BOD_5$/COD 0.54). Therefore, considering that the product is completely consumed in 5.5 h of reaction time, the concentration evaluated inside the bioreactor was 0.21 mg/L of mixed liquor.

The product based on SP is a complex biodegradable product essentially consisting of a mixture of chemical surfactants and SP produced by *Saccharomyces cerevisiae*. The synergistic effect of the mixture leads to a metabolic uncoupling similar to that observed with chemical uncouplers, and SP are the most responsible for this effect [17]. On the first day of operation, the product was directly added into the reactor at a concentration of 5 mg/L, as recommended by the sales representative. From the second medium exchange, the product was added into the feed to present the desired concentration. Two lots of the product provided by the *Advanced Biocatalytics Corporation* (USA) at concentrations of 5, 10 and 20 mg/L were assessed. The product's biodegradability was estimated based on the COD and $BOD_5$ values for the dilute product solution ($BOD_5$/COD 0.49). Therefore, considering that the product is completely consumed in 5.5 h of reaction time, the concentration evaluated inside the bioreactor ranged from 1.3 to 5.2 mg/L of mixed liquor.

The effect of each product on the sludge reduction was evaluated in experiments with bioreactors operating in sequential batches and in a continuous regimen, as described below.

## 1.3 Batch Bioreactors

Experiments were performed on batch bioreactors of 1 L with a working volume of 500 mL (240 mL of sludge and 260 mL of effluent) operated at room temperature (23 ± 2°C). The system homogeneity and adequate oxygen supply were obtained by magnetic stirring and introducing air through a diffuser installed on the bottom of bioreactors. For each product tested, two bioreactors operated simultaneously: in the test bioreactor, the bioproduct was added to the feed, while the control bioreactor operated without any addition.

The wastewater samples used in this study had nitrogen concentrations sufficient for the metabolic activity of microorganisms; therefore, the feeding of bioreactors consisted of wastewater supplemented with phosphorus only ($KH_2PO_4$) for a COD:P ratio of 100:1 and pH adjusted to values close to 7.0. In test bioreactors, products were added into the feed so that the desired concentration was achieved.

The experiments were conducted in order to maintain the following operation conditions of the oil refinery wastewater treatment station: Volatile Suspended Solids (VSS) 2 800 mg/L, reaction time of 5.5 h, sludge age 20 days and recycle ratio 1 (exchange of 50% of the supernatant volume), analyzing pH, COD, settleable solids and Total Suspended Solids (TSS).

## 1.4 Bioreactors in Continuous Mode

Due to the greater complexity of the operation, only the product based on SP was tested in a continuous bioreactor because of the promising results in batch studies. Two bench systems were used with an aeration tank of 3.4 L and sedimentation of 0.3 L. The content of the aeration tanks was kept homogeneous by mechanical stirring and introducing air through a porous diffuser installed on the bottom of the tank. Feed and recycle flow rates were maintained through Masterflex® pumps. The sludge age (20 days) was obtained by daily collections of a representative sample of mixed liquor from the aeration tank, considering the VSS concentration to be negligible in the output of the reactors. Two bioreactors operated simultaneously, a control bioreactor (without addition of the bioproduct) and a test bioreactor (with addition of the bioproduct). In the test bioreactor, the product was initially added into the feed, and then the product was added directly to the aeration tank through a metering pump so that the entire volume of the aeration tank showed the desired concentration.

### 1.5 Analytical Methods

All analyses were performed according to standard methods [19]. However, the volume of settleable solids used to calculate the Sludge Volume Index (SVI) was obtained by sedimentation in a 25-mL graduated cylinder for 40 minutes. Thus, the SVI values obtained served only for comparison between the control and test bioreactors. All results were statistically analyzed by the *t*-test with 95% confidence using the Statistica 7.0 software.

## 2 RESULTS

### 2.1 Effect of DNP on Bioreactors Operating in Sequential Batches

The mean values and respective standard deviations of variables controlled during the operation of control (no addition of product) and test bioreactors operating in sequential batches with the addition of 1, 2, 5, 10 and 15 mg/L of DNP into the feed with a reaction time of 5.5 h and sludge age of 20 days are shown in Table 1.

Despite the variability of the results due to the operation conditions of bioreactors and variations in the composition of the industrial wastewater, it is possible to compare different operation periods of the control and test bioreactors. It was observed that the addition of DNP up to 10 mg/L did not change the effluent pH of batches. By adding 15 mg/L of DNP, the effluent pH increased slightly to 7.3 ± 0.3, but still remained within appropriate values for aerobic biodegradation.

The COD in the feed of both bioreactors varied considerably during the experiment because the wastewater was collected at different operating conditions in the industrial plant. Despite this variation, the effluent COD remained at moderate levels, resulting in COD removal almost always higher than 60%. The COD removal in the control bioreactor increased proportionally with the operation time, indicating an adaptation of the biomass to the wastewater constituents. In the test bioreactor, it was found that the addition of DNP interfered with the biomass adaptation and COD removal throughout the batches. The addition of 15 mg/L DNP led to a greater effluent COD, and consequently lower COD removal in the test bioreactor. This result was probably due to an inhibitory effect observed at the highest DNP concentration [12].

There was an increase in the VSS concentration throughout the batches, but without significant differences between bioreactors, with the exception of the period in which 10 mg/L DNP was added, where the VSS concentration in the test bioreactor was 19.2% lower than in the control bioreactor.

The similarity observed between the VSS concentrations of the bioreactors when the product concentration was increased to 15 mg/L is probably due to the adaptation of cells to DNP after a long time in contact with the product (153 days). Tian *et al.* [10] studied the effect of 2,6-DiChloroPhenol (DCP) in an activated sludge system for a period of 90 days. After 40 days of operation, the authors observed that the cells had adapted to the compound due to an increase in the EPS matrix (extracellular polymeric substances), which acts as a protective barrier, allowing cells to survive the stress caused by the presence of the compound.

DNP and DCP are similar molecules. Both feature a phenol molecule linked to two groups with a similar inductive effect [20]. Moreover, both are weak lipophilic acids, which is a basic feature for a molecule to have an uncoupling function [21, 22]. Thus, the hypothesis proposed by Tian *et al.* [10] to explain the behavior observed with DCP can also be applied to DNP. However, as the test bioreactor in the last study period showed a higher effluent COD than the control bioreactor (effluent COD 257 ± 31 mg/L in the test bioreactor against 179 ± 25 mg/L in the control bioreactor), the results observed may not only be due to the adaptation, but also to an inhibitory effect observed at the highest DNP concentration.

Chen *et al.* [12] evaluated DNP concentrations from 1 to 20 mg/L in activated sludge batch systems. The DNP concentration of 10 mg/L led to a 20.5% loss in system efficiency (compared with control); however, approximately 44% reduction in the yield coefficient ($Y_{\text{obs}}$) was observed. They found that the yield coefficient decreases with increasing concentration, but at concentrations greater than 7.5 mg/L, the COD removal in the test reactor was lower than that of the control reactor. Therefore, the result of this study is in agreement with data obtained by Chen *et al.* [12], since higher DNP concentrations resulted in lower COD removal in the test bioreactor compared with the control bioreactor. The difference in results in terms of growth observed in both studies is probably due to different operation times, and therefore, different contact times of the biomass with the compound.

Another experiment with 10 mg/L of DNP in the feed was conducted for a total operation period of 130 days. Within the first 60 days of operation (data not shown), the operation was not stable and no significant difference in the sludge disposal was obtained. Steady state was achieved in the remaining 70 days (Tab. 1), resulting in 30.6% lower sludge disposal in the test bioreactor. This result remained for almost 70 days of operation without impairing the COD removal efficiency or sludge sedimentation, confirming that the contact time of the product with the biomass is of paramount importance in the results observed.

TABLE 1

Effect of different DNP concentrations on batch experiments with 5.5 h of reaction time

| Parameters | Bioreactor | Experiment 1 | | | | | Experiment 2 |
|---|---|---|---|---|---|---|---|
| | | Period 1 | Period 2 | Period 3 | Period 4 | Period 5 | |
| Time of operation (days) | C and T | 40 | 21 | 25 | 67 | 13 | 70* |
| DNP – feed (mg/L) | T | 1 | 2 | 5 | 10 | 15 | 10 |
| DNP – mixed liquor (mg/L) | T | 0.26 | 0.52 | 1.3 | 2.6 | 3.9 | 2.6 |
| DNP/VSS (mg DNP/g VSS) | T | 0.8 ± 0.2 | 1.5 ± 0.3 | 2.9 ± 0.4 | 5.8 ± 0.9 | 6.3 ± 0.7 | 13.0 ± 6.1 |
| Effluent pH | C | 8.0 ± 0.7 | 7.7 ± 0.3 | 7.8 ± 0.1 | 7.2 ± 0.3 | 7.0 ± 0.2 | 7.5 ± 0.4 |
| | T | 7.9 ± 0.7 | 7.8 ± 0.3 | 7.8 ± 0.2 | 7.1 ± 0.5 | *7.3 ± 0.3* | 7.4 ± 0.4 |
| COD – feed (mg/L) | C and T | 458 ± 288 | 1 338 ± 175 | 1 705 ± 197 | 1 371 ± 139 | 1 353 ± 126 | 1 166 ± 458 |
| Effluent COD (mg/L) | C | 147 ± 99 | 411 ± 55 | 507 ± 102 | 294 ± 126 | 179 ± 25 | 247 ± 125 |
| | T | 161 ± 119 | 435 ± 76 | *608 ± 81* | 331 ± 131 | *257 ± 31* | 270 ± 107 |
| COD removal (%) | C | 67.2 ± 14.2 | 68.9 ± 4.7 | 70.3 ± 5.2 | 78.4 ± 9.1 | 86.6 ± 2.8 | 78.6 ± 6.7 |
| | T | 65.1 ± 14.2 | 67.2 ± 5.3 | *63.8 ± 6.7* | 75.7 ± 9.6 | *80.7 ± 3.7* | 75.4 ± 9.3 |
| TSS (mg/L) | C | 2 301 ± 575 | 1 820 ± 213 | 2 069 ± 212 | 2 371 ± 278 | 2 766 ± 218 | 1 460 ± 438 |
| | T | 2 144 ± 561 | 1 694 ± 227 | 1 959 ± 174 | *1 912 ± 325* | 2 574 ± 248 | *1 002 ± 323* |
| VSS/TSS | C | 0.60 ± 0.04 | 0.80 ± 0.06 | 0.90 ± 0.02 | 0.93 ± 0.03 | 0.91 ± 0.04 | 0.91 ± 0.06 |
| | T | 0.60 ± 0.04 | 0.79 ± 0.06 | 0.88 ± 0.04 | 0.93 ± 0.03 | *0.94 ± 0.02* | 0.92 ± 0.06 |
| SVI (mL/g) | C | 101 ± 33 | 185 ± 23 | 135 ± 16 | 71 ± 25 | 56 ± 14 | 84 ± 46 |
| | T | 95 ± 25 | *133 ± 12* | *101 ± 11* | *58 ± 10* | 52 ± 7 | 80 ± 28 |
| Sludge disposal (mg TSS/day) | C | 58 ± 14 | 45 ± 5 | 52 ± 5 | 59 ± 7 | 69 ± 5 | 36 ± 11 |
| | T | 54 ± 14 | 42 ± 6 | 49 ± 4 | *48 ± 8* | 64 ± 6 | *25 ± 8* |
| Sludge reduction (%) | T/C | – | – | – | 18.6 | – | 30.6 |

Mean and standard deviation of at least 13 sequential batches.
In ital: values statistically different from control (95% confidence).
C = control, T = test. * Operation time in steady state regimen.

One of the causes of lower sludge disposal in the first experiment could be the adaptation to the compound, as previously explained. Before achieving sludge reduction, the biomass was in contact with the product at lower concentrations for a total period of 86 days. In the second experiment, the biomass was in contact with the product already at the desired concentration of 10 mg/L for a period of 60 days before showing the expected effect. This DNP concentration is relatively high, which may lead to increased stress on the system cells [12].

In addition to increased operating time with the addition of 10 mg/L DNP, the ratio between the mass of the product and biomass ($C_{\text{product}}$/VSS) in both experiments conducted with this concentration may also explain the different sludge reductions achieved. According to Liu [13], this ratio shows the actual force that a metabolic uncoupler exerts on the system biomass. This ratio in the first experiment with 10 mg/L, that led to 18.6% lower sludge disposal in the test bioreactor, was 5.8 mg DNP/g VSS, while in the second experiment, which led to 30.6% lower sludge disposal in the test bioreactor, it was 13.0 mg DNP/g VSS. That is, an increase in the DNP mg/g VSS ratio resulted in significantly lower sludge disposal.

The addition of DNP did not compromise the sludge settleability, and lower SVI values were found in the test bioreactor. Figure 1 shows the means and standard deviations of sludge disposal (mg TSS/d) obtained during the operation of bioreactors in sequential batches. The disposal of solids was

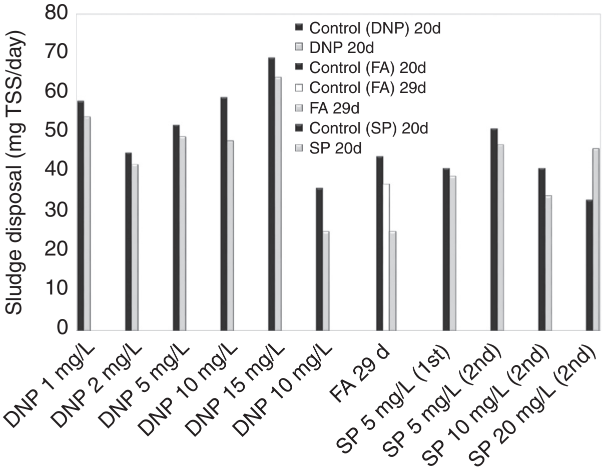

Figure 1

Effect of the addition of different DNP concentrations and bioproducts based on FA and SP on the sludge disposal in bioreactors operating in sequential batches; ■ = sludge disposal in control bioreactor with sludge age of 20 days, □ = sludge disposal in control bioreactor with sludge age of 29 days, ▨ = sludge disposal in test bioreactor with sludge age of 20 days (DNP and SP) and 29 days (FA).

lower in the test bioreactor at all concentrations evaluated. However, only at a concentration of 10 mg/L of DNP was the sludge disposal difference observed between the control and test bioreactors statistically significant (95% confidence), with 18.6% lower sludge disposal in the test bioreactor (59 ± 7 mg TSS/d in control and 48 ± 8 mg TSS/d in test bioreactors), a result that was maintained for a period of 67 days. According to the sludge disposal results and the fact that there was no loss of COD removal efficiency in the system, a concentration of DNP 10 mg/L (in the feed) would be the most suitable to achieve sludge disposal reduction.

## 2.2 Effect of the Bioproduct Based on FA on Bioreactors Operating in Sequential Batches

The mean values and respective standard deviations of variables monitored during the operation of bioreactors for 44 days in sequential batches with sludge ages of 20 and 29 days without and with addition of 0.8 mg/L (recommended as the shock dosage by the manufacturer) of the product based on FA stabilized in the feed are shown in Table 2.

The increased sludge age from 20 to 29 days increased the VSS concentration in the reaction medium, resulting in 15.9% lower sludge disposal, as expected. However, despite no change in the COD removal efficiency, the sludge settleability was impaired, as shown by the SVI values shown in Table 2. Such a result with greater sludge ages is widely reported in the literature [8].

TABLE 2

Effect of bioproduct based on stabilized FA on batch experiments with 5.5 h of reaction time

| Parameters | Control (20 days) | Control (29 days) | Test (29 days) |
|---|---|---|---|
| Time of operation (days) | 44 | 44 | 44 |
| FA – feed (mg/L) | – | – | 0.8 |
| FA – mixed liquor (mg/L) | – | – | 0.21 |
| Bioproduct/VSS (mg FA/g VSS) | – | – | 0.63 ± 0.12 |
| Effluent pH | 7.6 ± 0.4 | 7.4 ± 0.4 | *7.4 ± 0.3* |
| COD – feed (mg/L) | 1 352 ± 359 | 1 352 ± 359 | 1 352 ± 359 |
| Effluent COD (mg/L) | 296 ± 115 | 319 ± 122 | 295 ± 87 |
| COD removal (%) | 78.4 ± 4.9 | 76.4 ± 6.0 | 78.1 ± 3.7 |
| TSS (mg/L) | 1 771 ± 234 | *2 172 ± 210* | *1 432 ± 195* |
| VSS/TSS | 0.94 ± 0.03 | 0.94 ± 0.04 | 0.92 ± 0.08 |
| SVI (mL/g) | 60 ± 16 | *90 ± 22* | *76 ± 12* |
| Sludge disposal (mg TSS/day) | 44 ± 6 | *37 ± 4* | *25 ± 3* |
| Sludge reduction (%) | – | 15.9 | 43.2 |

Mean and standard deviation of at least 13 sequential batches.
In ital: values statistically different from control 20 days (95% confidence).

The combination of greater sludge age and the addition of the bioproduct based on FA results in sludge reductions much higher than those obtained with increased sludge age only, and without impairing COD removal or sedimentation. When the discard of the test bioreactor with sludge aged 29 days was compared with the discard of the control bioreactor with sludge aged 20 and 29 days, the reduction was 43.2% and 32.4%, respectively, confirming the effect of the bioproduct under increased sludge age (Fig. 1).

The increase in sludge age to 35 days (data not shown) resulted in a considerable decrease in the effect of the product: the discard reduction was only 8.3% and the SVI of the test bioreactor was 17.2% higher than in the control bioreactor with the same sludge age. The loss of effect of the FA may have been due to the adaptation and biodegradation of microorganisms in the system, since it is a biodegradable product ($BOD_5$/COD 0.54). However, the only difference between the periods with sludge ages of 29 and 35 days (32.4% and 8.3% disposal reduction, respectively) was the sludge age, and it could be inferred that this parameter affects the biomass response to the tested product [23].

These results indicate that stabilized FA shows the desired effect under certain conditions of the bioreactor concentration and operation, which vary with the type of wastewater and treatment system used. However, even in ideal conditions, the effect of the bioproduct remains only for a certain period of operation, which indicates loss of activity. This loss of activity may occur due to adaptation and biodegradation of the microbial flora or loss of stability during storage.

It is noteworthy that although the effect was not maintained for a long period (44 days) and because there are still questions about the mechanism of action of FA in reducing sludge production, in no period of this study was a significant difference in the system efficiency in terms of COD removal observed.

## 2.3 Effect of the Bioproduct Based on SP on Bioreactors Operating in Sequential Batches

To evaluate the effect of the bioproduct based on SP and chemical surfactants, the results were followed for 282 days, divided into four periods according to the product concentration and product lot. The results were highly variable due to the change in the composition of the wastewater collected in different operating situations of the industrial plant. Nonetheless, steady state was achieved at 95 days and significant differences in the results obtained in each bioreactor were observed. The mean values and respective standard deviations of variables monitored during the operation of bioreactors without and with the addition of 5, 10 and 20 mg/L of bioproduct into the feed are shown in Table 3.

Except for the first operation period (5 mg/L), when the biomass was still not fully adapted, the SVI showed low and statistically similar values in both bioreactors. Adding the product at this concentration also led to a reduction in the effluent pH of batches, even though the value was within the acceptable range for aerobic biodegradation.

The product is used for several purposes, so it has several formulations, which vary in the percentage of SP and protein fraction used. When 5 mg/L of product was added into the feed, in the test bioreactor statistically equal disposal to the control bioreactor was obtained and 7.8% smaller than the control, differing only in the product lot used. This shows that to achieve disposal reduction, the product lot (or its purpose) is as important as the operation conditions.

The relationship between the metabolic uncoupler and biomass concentrations in the system is the real force that the uncoupler exerts on microorganisms. The greater this relationship, the lower the growth factor observed [13]. As the test product shows a protein fraction with an effect similar to a metabolic uncoupler [16, 17], the relationship between the product and biomass concentrations is important to evaluate the results obtained. Increasing the product concentration to 10 mg/L in the feed, the disposal in the test bioreactor was on average 17.1% lower than in the control bioreactor, a satisfactory result that could be caused by an increase of 2.8 times in the relationship between the product and biomass concentrations. However, considering only the initial 33 days of this condition, the maximum discard reduction was 29.8% (47 ± 7 mg TSS/d in control and 33 ± 7 mg TSS/d in test), with a loss of effect in the final 27 days.

Doubling the bioproduct concentration in the feed (20 mg/L) or increasing the ratio between biomass and bioproduct concentrations from 8.0 ± 1.2 to 11.7 ± 1.4 mg bioproduct/g VSS, the result was the opposite of that expected: 39.4% increase in sludge disposal. The result is quite evident in the sludge disposal chart shown in Figure 1. By introducing changes into the system, which stress the microbial ecosystem, there may be changes in the population or adaptation [24]. The loss of effect of the product may have been, therefore, due to adaptation, followed by biodegradation of microorganisms in the consortium due to the high level of bioproduct biodegradability ($BOD_5$/COD 0.49).

Although the result was the opposite of that expected in sludge disposal for a limited period of time (33 days), in no period of this study was a significant difference in the system efficiency in terms of COD removal observed. That is, the addition of up to 20 mg/L of bioproduct into the feed obtained at least 77% COD removal.

## 2.4 Effect of the Bioproduct Based on SP on Bioreactors Operating in a Continuous Regimen

Bioreactors were operated in a continuous regimen for a period of 70 days for biomass adaptation and stabilization of results. From this point, the bioproduct based on SP and chemical surfactants began to be added to one of the bioreactors. Table 4 shows the mean values and standard deviations of the parameters evaluated and operating conditions. The total operating time with the addition of the bioproduct was 159 days divided into five operation periods, which differ in terms of wastewater flow used, HRT and bioproduct concentrations, and form of addition.

Despite the great COD variability in the feed, the results in terms of total and VSS were relatively constant within each operation period. In period 2, the concentration of solids and consequently the sludge disposal in the test bioreactor were higher than in the control bioreactor. However, this difference is due to the accumulation of solids in the settler tank of the control bioreactor and consequent loss of biomass in the system output, reducing the concentration of solids in the control bioreactor.

TABLE 3

Effect of different concentrations of bioproduct based on SP on batch experiments with 5.5 h of reaction time

| Parameters | Bioreactor | Period 1 | Period 2 | Period 3 | Period 4 |
|---|---|---|---|---|---|
| Time of operation (days) | | 134 | 53 | 60 | 35 |
| SP – feed (mg/L) | T | 5 (1st lot) | 5 (2nd lot) | 10 (2nd lot) | 20 (2nd lot) |
| SP – mixed liquor (mg/L) | T | 1.3 | 1.3 | 2.6 | 5.2 |
| SP/VSS (mg SP/g VSS) | T | 4.1 ± 1.6 | 2.9 ± 0.2 | 8.0 ± 1.2 | 11.7 ± 1.4 |
| Effluent pH | C | 7.5 ± 0.4 | 7.3 ± 0.3 | 7.4 ± 0.2 | 7.4 ± 0.2 |
| | T | *7.3 ± 0.3* | *7.1 ± 0.3* | 7.3 ± 0.2 | 7.4 ± 0.2 |
| COD – feed (mg/L) | C and T | 982 ± 479 | 1 311 ± 221 | 995 ± 97 | 992 ± 36 |
| Effluent COD (mg/L) | C | 187 ± 126 | 273 ± 65 | 221 ± 43 | 206 ± 18 |
| | T | 205 ± 166 | 283 ± 58 | 227 ± 33 | 211 ± 20 |
| COD removal (%) | C | 81.7 ± 6.8 | 79.0 ± 4.3 | 77.7 ± 4.0 | 79.2 ± 1.8 |
| | T | 80.9 ± 9.4 | 78.2 ± 3.6 | 77.1 ± 3.4 | 78.7 ± 1.9 |
| TSS (mg/L) | C | 1 649 ± 666 | 2 054 ± 198 | 1 631 ± 330 | 1 319 ± 251 |
| | T | 1 561 ± 595 | *1 865 ± 149* | *1 353 ± 211* | *1 847 ± 196* |
| VSS/TSS | C | 0.89 ± 0.07 | 0.95 ± 0.02 | 0.95 ± 0.06 | 0.94 ± 0.04 |
| | T | 0.89 ± 0.06 | 0.94 ± 0.03 | 0.94 ± 0.04 | 0.94 ± 0.03 |
| SVI (mL/g) | C | 110 ± 54 | 42 ± 5 | 55 ± 11 | 57 ± 7 |
| | T | *164 ± 73* | 42 ± 7 | 52 ± 20 | 54 ± 7 |
| Sludge disposal (mg TSS/day) | C | 41 ± 17 | 51 ± 5 | 41 ± 8 | 33 ± 6 |
| | T | 39 ± 15 | *47 ± 4* | *34 ± 5* | *46 ± 5* |
| Sludge reduction (%) | | – | 7.8 | 17.1 | – |

Mean and standard deviation of at least 13 sequential batches.
In ital: values statistically different from control (95% confidence).
C = control, T = test.

In the first 53 days of operation (periods 1 and 2), no significant differences were observed in the COD removal of bioreactors, keeping mean removals from 60 to 63% without and with the addition of bioproduct in the feed.

The feeding change from sour water to oily water with lower organic matter concentration, keeping all other operating conditions constant, resulted in a lower concentration of solids in both bioreactors. However, the VSS concentration in the test bioreactor was still greater than that observed in the control bioreactor, due to the loss of many solids in the settler tank of this bioreactor.

With relatively stable values at the end of the third feeding period with lower COD, it was decided to reduce the HRT to 11 h in period 4. The lower HRT reduced the contribution of the endogenous respiration in the metabolism of microorganisms, allowing their growth in both bioreactors and equating the concentrations of solids (2 311 ± 916 mg TSS/L in control and 1 985 ± 454 mg TSS/L in test).

By adding the bioproduct into the feed, the effect cannot be observed because the dilution occurs when the feed enters the reactor, and also due to the product losses by adsorption to the walls of the feeding bottle. In addition, the product is biodegradable ($BOD_5$/COD 0.49). Therefore, from period 5, the bioproduct was directly added into the reactor through a metering pump so that the aeration tank showed the desired concentration. With this change of operation, the sludge disposal in the test bioreactor was 45.7% lower than in the control bioreactor. Figure 2 clearly shows the great difference between solids and sludge disposal curves of the control and test bioreactors.

During the 159 days of operation with addition of the bioproduct based on SP, no statistical difference in COD

TABLE 4

Evaluation of different operating conditions of bioreactor in continuous regimen (with sludge aged of 68 days) without and with addition of bioproduct based on SP

| Parameters | Bioreactor | Period 1 | Period 2 | Period 3 | Period 4 | Period 5 |
|---|---|---|---|---|---|---|
| Time of operation (days) | | 37 | 16 | 28 | 28 | 50 |
| Influent stream | | Acid water | Acid water | Oily water | Oily water | Oily water |
| HRT (h) | | 22 | 22 | 22 | 11 | 11 |
| Bioproduct conc. (mg/L) | T | 5 (feed) | 10 (feed) | 10 (feed) | 10 (feed) | 10 (aeration tank) |
| F/M (kg COD/kg VSS · day) | C | 0.98 ± 0.19 | 0.53 ± 0.09 | 0.28 ± 0.14 | 0.29 ± 0.15 | 0.20 ± 0.08 |
| | T | 0.85 ± 0.24 | 0.44 ± 0.08 | *0.18 ± 0.10* | 0.35 ± 0.13 | *0.37 ± 0.13* |
| VOL (kg COD/m$^3$ · day) | C | 1.51 ± 0.32 | 0.98 ± 0.10 | 0.38 ± 0.22 | 0.77 ± 0.17 | 0.72 ± 0.24 |
| | T | 1.54 ± 0.25 | 0.99 ± 0.06 | 0.39 ± 0.24 | 0.80 ± 0.16 | 0.79 ± 0.27 |
| COD – feed (mg/L) | C | 1 371 ± 291 | 887 ± 91 | 339 ± 185 | 703 ± 157 | 330 ± 111 |
| | T | 1 400 ± 229 | 901 ± 57 | 348 ± 205 | 728 ± 148 | 358 ± 122 |
| Effluent COD (mg/L) | C | 509 ± 154 | 345 ± 81 | 125 ± 80 | 131 ± 25 | 89 ± 29 |
| | T | 572 ± 240 | 332 ± 60 | 142 ± 79 | 138 ± 27 | 102 ± 26 |
| COD removal (%) | C | 62.8 ± 8.0 | 61.0 ± 8.9 | 63.9 ± 8.8 | 80.9 ± 3.3 | 72.0 ± 6.7 |
| | T | 60.1 ± 11.5 | 63.1 ± 7.1 | 57.5 ± 16.9 | 80.6 ± 4.3 | 69.3 ± 10.4 |
| Effluent pH | C | 7.5 ± 0.3 | 7.6 ± 0.2 | 7.8 ± 0.1 | 7.8 ± 0.3 | 7.7 ± 0.4 |
| | T | 7.2 ± 0.6 | 7.8 ± 0.2 | 7.9 ± 0.1 | 8.0 ± 0.3 | 7.8 ± 0.4 |
| TSS (mg/L) | C | 1 149 ± 215 | 1 186 ± 115 | 880 ± 169 | 2 311 ± 916 | 3 178 ± 299 |
| | T | 1 264 ± 282 | *1 534 ± 116* | *1 421 ± 154* | 1 985 ± 454 | *1 715 ± 334* |
| VSS/TSS | C | 0.93 ± 0.04 | 0.95 ± 0.05 | 0.91 ± 0.04 | 0.87 ± 0.03 | 0.84 ± 0.02 |
| | T | 0.93 ± 0.03 | 0.93 ± 0.04 | 0.94 ± 0.02 | 0.89 ± 0.03 | 0.85 ± 0.03 |
| SVI (mL/g) | C | 198 ± 71 | 275 ± 16 | 231 ± 20 | 165 ± 24 | 143 ± 12 |
| | T | 210 ± 55 | *225 ± 37* | *186 ± 15* | *138 ± 26* | 144 ± 20 |
| Sludge disposal (mg TSS/day) | C | 57 ± 11 | 59 ± 6 | 44 ± 8 | 116 ± 46 | 127 ± 12 |
| | T | 63 ± 14 | *77 ± 6* | *71 ± 8* | 99 ± 23 | *69 ± 13* |
| Sludge reduction (%) | | – | – | – | – | 45.7 |

Mean and standard deviation of at least 16 days of operation.
In ital: values statistically different from control (95% confidence).
C = control, T = test.

removal of the bioreactors was observed, pointing out that the product reduces growth without affecting the system efficiency.

The SVI of both bioreactors was higher than in batch experiments, but this is due to the greater age of the sludge used. Furthermore, in most of the operating time, the test bioreactor showed SVI values lower than in the control bioreactor.

Based on the results obtained in studies evaluating the effect of (bio) products, the operation of bioreactors should be as stable as possible. Even on an industrial scale, variations occur with great intensity and have to be taken into account; to understand the effect of the products and their responses one must eliminate as many variables as possible. Methodologies to assess the cellular metabolism, to assist in analyzing the results and to understand the mechanism of action of products must be

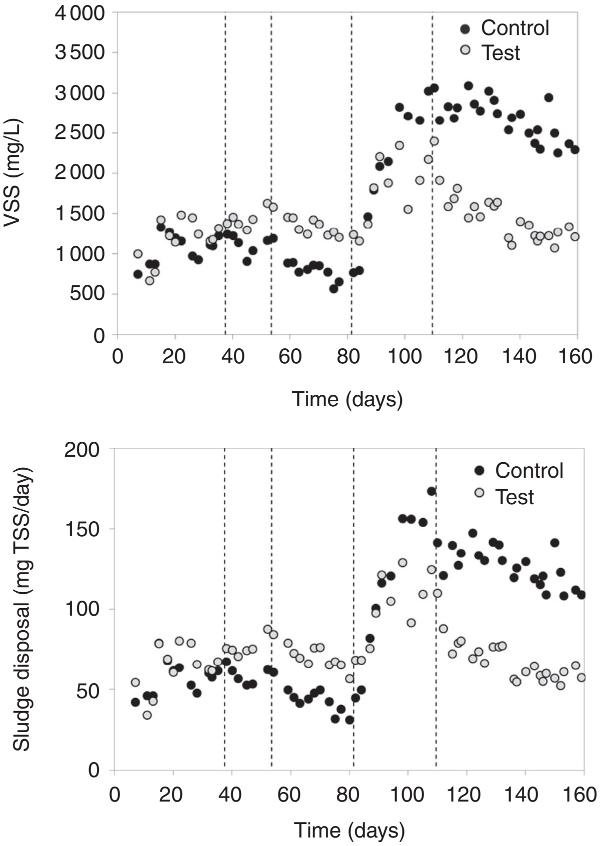

Figure 2

Evolution of the VSS concentration and sludge disposal under different operating conditions in bioreactors operating in a continuous regimen without and with the addition of the bioproduct based on SP.

used, as well as specific methods of quantification of products to assess their loss of activity and/or biodegradability better for long-term applications.

## CONCLUSION

The three products evaluated showed satisfactory results (30-46% sludge disposal reduction). However, though efficient, DNP is toxic and the bioproducts based on FA and SP showed unstable results, indicating a loss of effect throughout operation. Thus, studies conducted for longer operation times and evaluating the mechanism of action of bioproducts should be performed to evaluate their effects better under real treatment conditions.

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