Science, Technology and Development 34 (2): 94-100, 2015
ISSN 0254-6418 / DOI: 10.3923/std.2015.94.100

Effect of Alternative Nutrient Sources During Anaerobic Degradation of Potato Wastewater

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Abstract: The supplementation of nutrients on biological wastewater treatment in many cases may become a relevant part of the treatment cost. In that sense, the finding of new efficient and economic nutrient sources is very important in large scale industrial applications. This work reports on the effect of four readily available alternative nutrient sources during anaerobic degradation of potato wastewater: Peptone, soybean meal, sludge from an aerobic wastewater treatment plant (aerobic sludge) and NH_4NO_3 plus Micronutrient Supplementation (MS). The addition of alternative nutrient sources changed the rate limiting step from the last steps to hydrolysis in all the cases. While the added soybean meal allowed obtaining the higher final conversion, the degradation rate was slower than the non-supplemented control. The assays with NH_4NO_3+MS as nitrogen source presented the highest degradation rate. Moreover both NH_4NO_3 and MS are needed to improve the degradation rates. The percentage of residual COD after treatment was similar for peptone, aerobic sludge and NH_4NO_3+MS test. However, the lowest percentage of residual soluble COD was obtained when the aerobic sludge was used as supplement. The aerobic sludge presented Co and Mo deficiencies. Finally, taking into account the advantages and disadvantages discussed, among those tested, the aerobic sludge seems to be the best alternative nutrient source for industrial applications.

Key words: Anaerobic digestion, nitrogen, industrial application, micronutrients

INTRODUCTION

Potato industry wastewaters are currently subject to biological digestion, both aerobic (Lasik *et al*., 2010) and anaerobic (Fang *et al*., 2011) to reduce the organic pollution load. Although, anaerobic digestion processes have been carried out for decades, interest in the economic recovery of methane gas from industrial and agricultural wastewaters has recently increased due to the changing socio-economic situation in the world (Nishio and Nakashimada 2007). Besides, biological treatment appears to be a promising technology to attain revenue from Certified Emission Reduction (CER) credits, more commonly known as carbon credits from the Clean Development Mechanism (CDM) as methane gas is generated from anaerobic digestion and can be utilized as renewable energy (Chan *et al*., 2009).

The supplementation of nutrients on biological wastewater treatment is generally needed and usually becomes an important cost on anaerobic degradation (Grady *et al*., 1999). The lack of understanding or underestimating nutrient requirements of methanogens could be a serious problem in commercial applications of anaerobic biotechnology as well (Speece, 1983). In particular, macronutrients requirement is an important factor that affects the microbial processes since it affects directly the biomass growth (Gerardi, 2003). The lack of nitrogen and phosphorous availability could cause reactor failure. Therefore, the availability of macro nutrients is of paramount importance in biogas digester as well (Demirel and Scherer, 2008). The effect of nitrogen and phosphorous was widely studied in aerobic degradation (Krishnan *et al*., 2008). However, the studies referred to nutrients requirements during anaerobic treatment generally only fall into the influence of trace metals (Demirel and Scherer, 2011; Cresson *et al*., 2006; Lo *et al*., 2012; Zandvoort *et al*., 2006; Munk *et al*., 2010) and rarely studied the nitrogen or phosphorous requirements and its sources (Britz *et al*., 1988; Sterling *et al*., 2001; Scherer *et al*., 2009).

In that sense, the finding of new efficient and economic nutrient sources is of great importance to proper management of large scale industrial applications. This study shows the effect of alternative nutrient sources during anaerobic degradation of potato wastewater and evaluates them from a cost-efficiency point of view. Finally, deficiencies in trace metals are also tested.

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MATERIALS AND METHODS

The potato Wastewater (WW) was the same used on previous works (Durruty *et al*., 2012). Sludge from a methanogenic industrial digester was used as inoculum, it was kindly supplied by McKein SA (Balcarce, Argentina), from its anaerobic wastewater treatment plant. The inoculum-substrate ratio was fixed as 5% v/v which is the same used in the industrial digester.

Five 0.2 L batch tests were performed simultaneously in order to evaluate the effect of four alternative nutrient sources on anaerobic degradation. The experimental scheme is depicted on Fig. 1. All the tests were performed at least by duplicate and inoculated with 10% v/v of anaerobic sludge. The pH was fixed to 7.2 with buffer phosphate, ensuring the phosphorous requirement. This pH value is within the optimum range of the different strains involved on process (Gerardi, 2003).

The first reactor was filled with 0.1 L of Wastewater (WW) and 0.1 L of inoculated buffered distilled water as control. The second one was filled with 0.1 L of WW and 0.1 L of inoculated buffered solution of peptone. The peptone and yeast extract are usually used in lab scale to ensure nutrient requirements during biological growth (Gerardi, 2003). The third reactor was filled with 0.1 L of WW and 0.1 L of inoculated buffered suspension containing soybean meal. The soybean meal is a by-product of soybean industry, rich on nitrogen and minerals. It is cheap and it is readily available in agricultural zones where the potato industries are generally located. The fourth one was filled with 0.1 L of WW and 0.1 L of inoculated buffered distilled water containing 25 mL of aerobic sludge from an aerobic Wastewater Treatment Plant (WWTP). Since generally the anaerobic treatment requires an aerobic post-treatment to achieve the disposal standards (Chan *et al*., 2009) and the aerobic treatment generates an important amount of aerobic sludge (Nishio and Nakashimada, 2007), which contains nitrogen and trace metals, this aerobic sludge constitutes an economic choice for supply of nutrients on anaerobic treatment while at the same time it is stabilized. The aerobic sludge used was obtained from aerobic post-treatment plant from the same WWTP than the anaerobic activated sludge. The fifth one was filled with 0.1 L of wastewater and 0.1 L of inoculated buffered solution containing Micronutrient Supplementation (MS) and NH_4NO_3. The micronutrients were supplied adding 0.1% v/v of commercially available trace quelated elements solution composed by Mg (6.1 g L^{-1}), Mn (15 g L^{-1}), Cu (2.5 g L^{-1}), Zn (20 g L^{-1}), Fe (20 g L^{-1}), B (7.5 g L^{-1}), Mo (0.25 g L^{-1}) and Co (0.025 g L^{-1}). The commercial micronutrients solution is formulated for crops and it is available in agricultural zones where the potato industries are generally located.

In all the test the ratio COD/N/P was fixed at 200/5/1 according to Krishnan *et al*. (2008) to avoid nitrogen deficiencies. The COD and N as total nitrogen were measured via colorimetric HACH® tests. The total and volatile suspended solids were determined according to APHA (1998). Table 1 summarizes the conditions in the 5 tests.

Table 1: Experimental set point for different batch test

Batch No.	Nutrient source	Initial COD (mg L^{-1})	Soluble	Solid	Initial TN (mg L^{-1})	Additional cost (U$S m^{-3} WW)
1	None (control)	6613	2598	4015	16	-
2	Peptone	8363	4321	4042	165	1600
3	Soybean meal	8843	3432	5411	166	1
4	Aerobic sludge	79934	2623	5370	177	0
5	NH_4NO_3+MS	6493	2595	3898	169	23

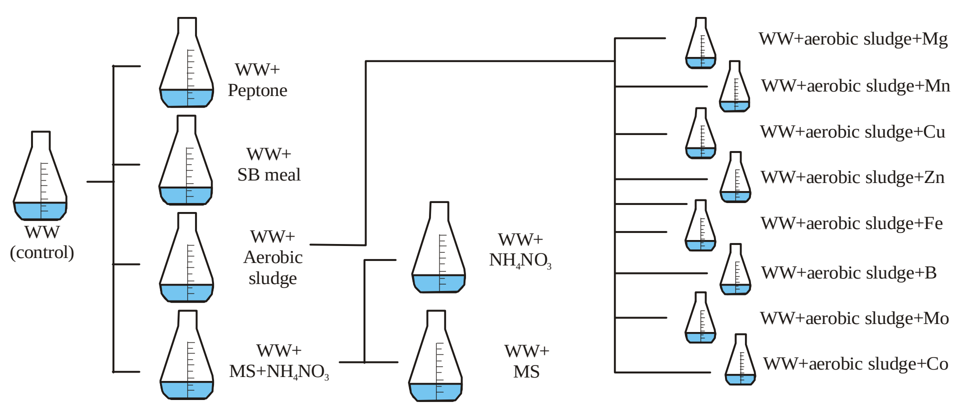

Fig. 1: Experimental scheme. Test of the 4 alternative nutrient sources, discrimination between effects due to NH_4NO_3 and MS and finally the study of micronutrient deficiencies for aerobic sludge as a nutrient source

RESULTS AND DISCUSSION

In Table 1, it can be seen that the addition of some nutrient sources implied an increase in the organic load. The peptone has a 5% w/w of nitrogen and easily degradable organic soluble load while the soybean meal has almost 10% w/w of nitrogen, mostly in a non-soluble particulate form. The aerobic sludge has a 0.2% w/v of nitrogen and led to an increase in solid organic load. The NH_4NO_3 has 35% w/w of nitrogen and did not increase the COD, however, as it did not provide any other nutrient, MS was added to supply trace elements requirements (Lo *et al*., 2012). It has been reported that the ammonia concentration has to be kept in excess of at least 40-70 mg L^{-1}, to prevent reduction of bacterial activity (Takashima and Speece, 1989). Furthermore, it was reported that the optimum conditions for *Methanosaeta concilii* which is the most ammonia-sensitive methanogen, were in the range of 195-860 mg L^{-1} (Steinhaus *et al*., 2007). The Table 1 shows that the WW without supplementation falls below the minimum concentration proposed by Takashima and Speece (1989) highlighting the need of nitrogen addition. This phenomenon was previously reported for a similar organic source (beet without leaves) (Demirel and Scherer, 2008). On the other hand, the supplemented batches meet the requirement and fall far below the maximum reported by Steinhaus *et al*. (2007).

The Fig. 2 shows the solid (particulate) and soluble COD evolution for the tests using different alternative nutrient sources. In most studies about anaerobic degradation with suspended organic matter, the rate-limiting step is the hydrolysis of solids (Vavilin *et al*., 2008). However, some researches (Siles *et al*., 2008; Neves *et al*., 2006) have reported that the degradation of the particulate organic load was not the limiting factor. The accumulation of soluble COD during the control test (Fig. 2a) demonstrates that the degradation of soluble organic load is slower than the degradation of the particulate organic load. However, the addition of alternative nutrient sources (Fig. 2b-e) avoided this accumulation indicating that the hydrolysis became the slowest step. In the particular case of soybean meal (Fig. 2c), this change is due to the slower solid degradation rate. The nutrients are captured inside the solid matrix that must be first hydrolyzed to become available to the microorganisms. Besides, the soybean meal added solid organic load different from that the potato starch which the hydrolytic bacteria should also consume. These phenomena lead to a reduction in the solid COD degradation rate. Finally, in the cases of peptone addition (Fig. 2b), aerobic sludge (Fig. 2d) or NH_4NO3 +MS (Fig. 2e), the change in relatives rates is due to the higher soluble degradation. In these cases the easily available nutrients accelerated the latest steps during anaerobic degradation biomass production and methane generation (Gerardi, 2003).

The Biochemical Methane Potential (BMP) is an important parameter for assessing design, economic and managing issues for the full-scale implementation of the anaerobic digestion process (Eiroa *et al*., 2012). However, in this study the BMP on different assays did not show differences. The value obtained for BMP was 0.35 g COD-CH_4/g $COD_{degraded}$ and the difference on methane production on every case are intimately linked to COD degradation. Figure 3 shows the normalized degradation of total organic load as fraction of the total residual COD. This figure shows that the addition of NH_4NO_3+MS accelerated the initial degradation (first two weeks) more than the addition of peptone or aerobic sludge and more than non-supplemented control. The addition of soybean meal presented a deleterious effect over the total degradation rate due to the reduction at the earlier step of the degradation discussed above. However, the final residual COD percentage was the lowest. It is relevant to note that although the addition of this nutritional source resulted in the lowest degradation rate, the percentage of removal was the highest.

The improvement of the degradation rate in NH_4NO_3+MS assay is attributed to the following factors: (a) The easily bio-available nitrogen source: Bryant and Robinson (1961) have demonstrated that none of single nitrogen compounds tested in their study would increase growth of the studied strains above that obtained with a growth-limiting concentration of ammonia. (b) The presence of micro-nutrients: the availability or lack of trace elements such as Fe, Co, Ni, Zn and Mo definitely plays a significant role in maintaining a stable and an efficient conversion process in a biogas digester (Demirel and Scherer, 2011). Besides to the bioavailability of metals, the scale of application and the type of substrate used also play a crucial role in the supply of trace metals during anaerobic digestion (Lo *et al*., 2012). With the aim to discriminate between these possible causes, an additional set of tests was performed with one batch with NH_4NO_3 and without MS and the other without NH_4NO_3 but with MS. The addition of nitrogen alone did not present differences respect to control. On the other hand, the addition of MS alone yielded a faster degradation than the control but slower than the batch with NH_4NO_3+MS. This clearly demonstrates that the improvement on degradation rates discussed above is due to both the addition of trace metals and nitrogen supply, demonstrating that the original wastewater has deficiencies in both macro and micro nutrients.

Since, the solid residual load could be removed in a further post-treatment step by mechanical means it is

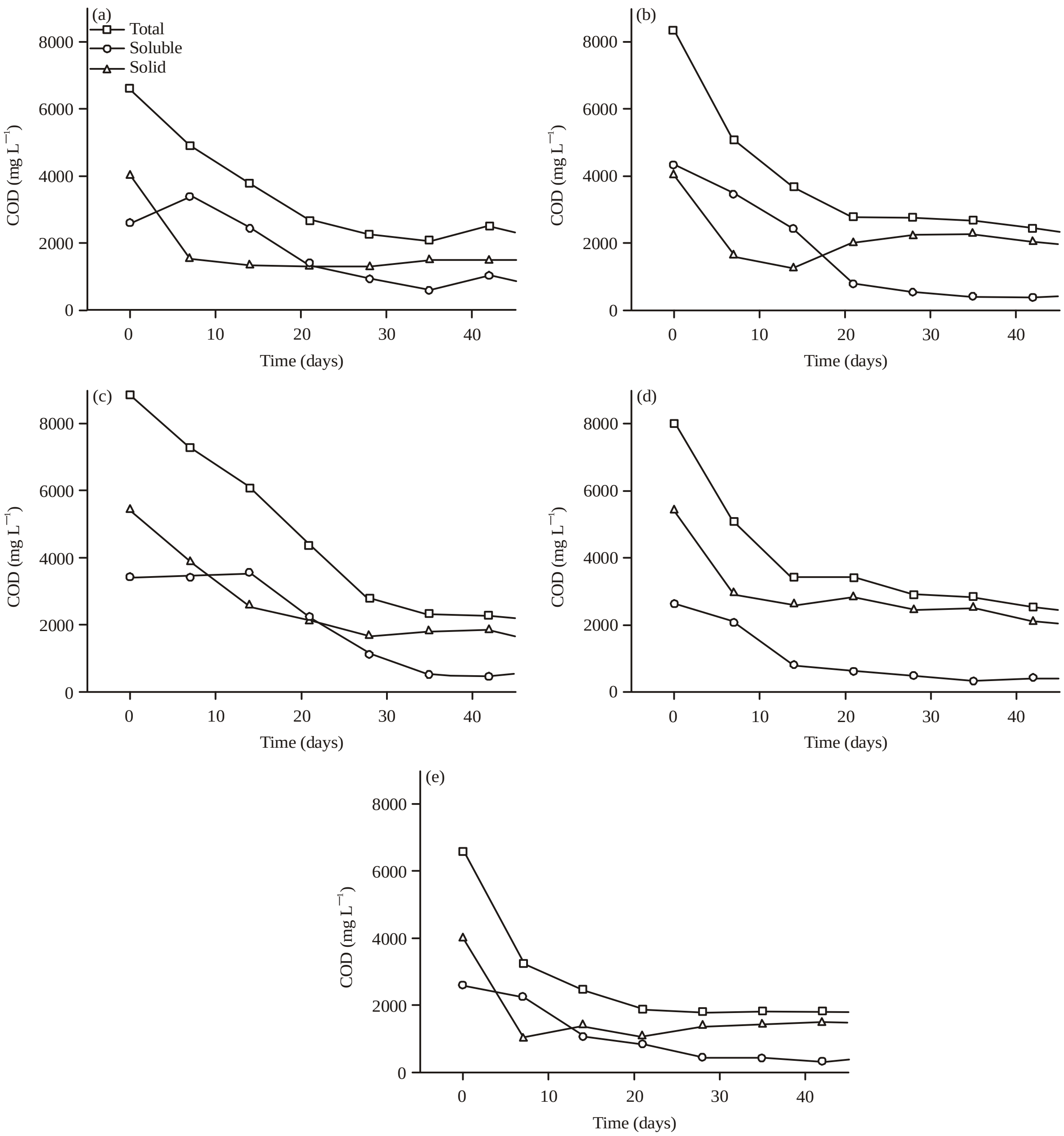

Fig. 2(a-e): COD versus time for alternative nitrogen sources and control, (a) Control, (b) Peptone, (c) Soybean meal, (d) Aerobic sludge and (e) NO_3NH_4+MS

important to study the effects of different sources in residual soluble loads. Figure 4 shows how the percentage of residual soluble COD is lower in all the supplemented tests, although is the lowest in the test supplemented with aerobic sludge.

In turn, the solid residual organic load is directly related with the biomass produced during the process, since a previous study (Durruty *et al*., 2012) has revealed that this wastewater did not have a particulate inert fraction. The highest percentage of residual solid COD is present in the aerobic sludge supplemented test. This is because a fraction of the solid COD provided by the aerobic sludge by-passed the treatment and remained in the effluent. The percentage of biomass produced (% solid residual COD) respect to the original total COD load, analogous to the conventional biomass yield coefficient, differs depending to the supplement used: in the peptone supplemented test and in the micronutrient supplemented test is around 22%, while in the soybean meal supplemented test the final biomass rounds the 16% of the

Table 2: Advantages and disadvantages from alternative nitrogen sources

Nutrient sources	Advantages	Disadvantages
Peptone	High degradation rate	Very high cost Increase in initial soluble organic load
Soybean meal	Lowest final soluble organic load Lowest final solid organic load Low cost	Low degradation rate Increase in initial particulate organic load
Aerobic sludge	Zero cost Lowest final soluble organic load High degradation rate Alternative treatment to stabilize aerobic sludge	High final solid organic load (sludge) Increase in initial particulate organic load
NH_4NO_3+Micronutrient supplement	Highest degradation rate No increment on initial organic load	High cost Complex storage- explosion risk. Needed of trace metals supplementation

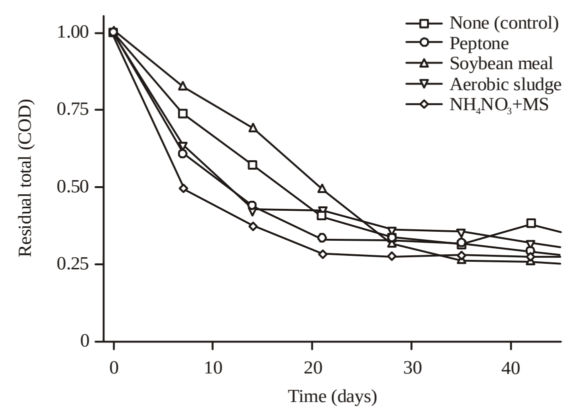

Fig. 3: Total residual COD expressed as fraction of total initial COD versus time for alternative nutrient sources and control

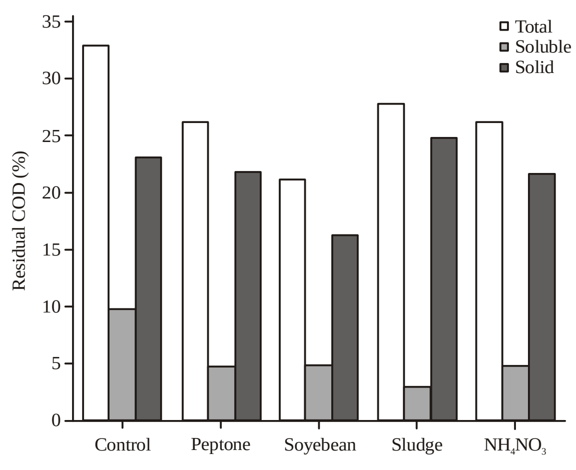

Fig. 4: Final total, solid and soluble residual COD expressed as percent of total initial COD for alternative nutrients sources and control

original COD load. This lower value of biomass yield together with the lowest hydrolysis rate explains the slow global degradation rate. The highest value of final biomass expressed as percentage of initial COD was found in the aerobic sludge supplemented test. This leads to the need for treating and stabilizing excess of biomass.

Table 2, summarizes the above discussed advantages and disadvantages of alternative nutrient sources. Among the presented choices, the activated sludge minimized the cost, allowed a good degradation rate and presented the lowest final soluble organic load. On the other hand its disadvantages were related with the high amount of particulate organic load both in the inlet and the outlet streams. However, this alternative was presented as a solution to treat and stabilize aerobic sludge, the high particulate organic load is a preexistent issue which in this way is partially solved and does not imply a new problem. In conclusion, the aerobic sludge seems to be the most suitable alternative nutrient source for industrial applications among those tested.

Finally, with the aim to evaluate micro-nutritional deficiencies when the aerobic sludge is used as nutritional supplement an extra set of tests was performed as shown (Fig. 1). Several simultaneous batch tests were carry out containing WW, aerobic sludge and each of the trace metals contained on MS individually (control has only WW plus aerobic sludge). When Mg, Mn, Zn, Cu, Fe or B were added individually the degradation rates did not present differences from the control indicating the lack of need of these trace metals. The obtained result indicates that these metals were present in nutrient supplement (aerobic sludge) or potato wastewater since they are indispensable to carry out anaerobic degradation (Demirel and Scherer, 2011). This phenomenon was previously observed in other works (Kumar *et al*., 2006; Sager, 2007).

In our tests, the additions of Mo and Co improved individually the degradation rate with respect to that of the control. This indicates the insufficiency of these elements on aerobic sludge together with a high dependence of these metals during anaerobic degradation. Several authors have studied the effect of Co on anaerobic degradation (Jarvis *et al*., 1997; Lebuhn *et al*., 2008; Pobeheim *et al*., 2011; Qiang *et al*., 2012). Furthermore Co was reported to be the most limiting element (Lebuhn *et al*., 2008). The high importance of this metal is due to the fact that cobalt is the central ion of corrinoids (vitamin B_{12} derivatives), involved in methyl transfers in methanogenesis and is present in both hydrogenotrophic and acetotrophic methanogens (Jarvis *et al*., 1997). In addition, the methyl-H4SPT: coenzyme M Methyltransferase complex contains cobalt (Pobeheim *et al*., 2011). Fewer works studied the effect of Mo on anaerobic degradation (Worm *et al*., 2009; Lebuhn *et al*., 2008). Lebuhn *et al*. (2008) reported that Mo was a limiting element, along with Co and Se. Most of methanogenic bacteria contain formate dehydrogenases. The catalytic centers of this enzyme contain molybdenum or tungsten as metal-binding cofactors that are essential for their catalytic function (Worm *et al*., 2009). Furthermore, depletion of molybdenum and tungsten was described to decrease formate dehydrogenase activity in defined syntrophic growth of the propionate oxidizing Syntrophobacter fumaroxidans and methanogenic Methanospirillum hungatei (Plugge *et al*., 2009). Therefore, the lack of molybdenum, tungsten or selenium during an anaerobic biological process could thus result in a decrease of the propionate consumption (intermediate reaction) and ultimately lead to process failure.

CONCLUSION

In this study, it has been demonstrated that the original potato processing WW presents a deficiency in nutrients and it needs to be supplemented to improve the efficiency of process. In that sense the addition of all of the alternative nutrient sources tested causes the hydrolysis to the slowest reaction instead of the last steps. The cost-efficiency analysis showed that the aerobic sludge appears as the better choice to manage properly the anaerobic degradation of potato processing wastewater. The activated sludge minimized the cost, allowed a good degradation rate and presented the lowest final soluble organic load. Furthermore, this alternative was presented as a solution to treat and stabilize aerobic sludge. Further improvement can be achieved by adding Co and Mo.

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