

# Ciência e Tecnologia

## Para o Desenvolvimento Ambiental, Cultural e Socioeconômico

Xosé Somoza Medina  
(organizador)

VOL III

 EDITORA  
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## PRÓLOGO

Por tercera vez, la editorial Artemis organiza un volumen para promover la difusión de investigaciones originales que desde diferentes ámbitos pretenden promover el desarrollo ambiental, cultural y socioeconómico. En esta ocasión, se trata de catorce trabajos estructurados en dos bloques, Ciencia y Tecnología, como en el volumen precedente, para de esta manera percibir con claridad como desde ambos campos del saber se puede proyectar un mundo mejor.

La ciencia y la tecnología en el siglo XXI deben orientar sus esfuerzos a ofrecer soluciones a los grandes problemas presentes de la humanidad y de nuestro planeta. Las Naciones Unidas iniciaron el camino en el año 2000 con los Objetivos del Milenio, reformulados y ampliados en 2015 con los ahora denominados Objetivos de Desarrollo Sostenible, ODS. Más allá de una simple declaración, los ODS deberían convertirse en el faro guía de todo avance científico o técnico. Lo ideal sería que cada persona científica o tecnóloga, independientemente de su origen o vinculación profesional, pensara en la fase de diseño de la investigación cuál de los ODS contribuye a alcanzar la consecución de su proyecto, para de esta manera orientar los esfuerzos de millones de seres humanos en todo el mundo a resolver el futuro de las próximas generaciones y no al contrario, que el progreso de nuestra civilización suponga una amenaza real para la Tierra, como parece que hemos estado haciendo hasta ahora. Todavía estamos a tiempo de cambiar nuestro destino, pero debemos concienciarnos y actuar en consecuencia.

Con este pensamiento en la mente, los trabajos que presentamos en este volumen adquieren una dimensión mayor. En el primer bloque, Ciencia, se agrupan siete trabajos que desde las ciencias de la educación y las ciencias económicas y empresariales contribuyen a alcanzar esos objetivos enunciados, bien a través de encuestas a una muestra de estudiantes de diferentes carreras universitarias o bien a través del análisis local de casos concretos. Así se pueden desarrollar temas de gran actualidad como la responsabilidad social, la incertidumbre de las políticas monetarias, la importancia de las microempresas en contextos determinados, las redes sociales, la internacionalización del sector turístico, la sostenibilidad en las empresas o la ansiedad provocada por la pandemia.

En el segundo bloque, Tecnología, se agrupan siete investigaciones con aportaciones igual de interesantes y novedosas, como los avances en teledetección de incendios, los tratamientos con bacterias para eliminar los residuos de aceites, la evaluación de antioxidantes en el desarrollo “in vitro” de plantas de caña de azúcar, los análisis informáticos para la predicción de plagas en los cultivos, las técnicas kinésicas para el tratamiento de la incontinencia urinaria femenina, la inteligencia aumentada de usuario o el estudio de un megaproyecto urbanístico como el de Saemangeum en Corea del Sur.

Xosé Somoza Medina  
Universidad de León, España

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# CAPÍTULO 9

## BACTERIAL OPTIMIZATION OF BIODETERGENT SYNTHESIS AND LIPOLYTIC ACTIVITY INDUCED BY WASTE RESIDUAL OIL

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**ABSTRACT:** In soil contaminated by waste motor oil. It's possible to isolate genera and species of bacteria with the capacity to synthesize biodetergents and lipolytic activity to emulsify and hydrolyze WMO. Of interesting

<sup>1</sup> Corresponding author.

potential value for use in the bioremediation of environments impacted by hydrocarbons. The objectives of this work were: The objectives of this work were: 1) to analyze that WMO and lubricating oil are inducers of biodetergent synthesis and lipolytic activity 2) to optimize the synthesis of crude biodetergents and lipolytic activity in these genera and bacterial species 3) to partially characterize the type of biodetergent synthesized by this bacterial group. In this sense, *Achromobacter denitrificans*, *Bacillus horneckiae*, *B. safensis*, *B. subtilis* and *Gordonia amicalis* isolated from soil impacted by WMO were inoculated in a mineral medium with lubricating oil and WMO as a carbon source, cultivated at 30°C and 200 rpm for 144 h compared to *Xanthobacter autotrophicus*. To detect and partially characterize anionic biodetergents and lipolytic activity, in a mineral medium with WMO. The optimization of the synthesis of biodetergents and lipolytic activity were used two sources of inorganic nitrogen  $\text{NH}_4\text{NO}_3$ , and  $\text{NaNO}_3$  under agitation. For which, every 48 h / 6 days, the anionic glycolipid biodetergents, their emulsification capacity, lipolytic activity and quantification of WMO consumption as an inducer of biodetergents and lipolytic activity were detected in the crude extracts, all experimental data were analyzed by ANOVA Tukey. The results showed that *A. denitrificans* reached the highest lipolytic activity of 280.3 U/mL in mineral medium with WMO and 210.07 3 U/mL in mineral medium with lubricating oil.

Compared with *X. autotrophicus*, that reached 275.53 U/mL induced by WMO and 165.6 U/mL induced by lubricating oil. *G. amicalis* reached the maximum production of anionic biode detergent with 1.13 ml/100 ml of mineral medium and WMO. The WMO was the best inducer for the optimization of biode detergent synthesis and lipolytic activity, thus in *A. denitrificans* with NaNO<sub>3</sub>, at pH 7 and 250 rpm reached 0.3147 mg of crude biode detergents/mL, and 0.654 mL of anionics/100 mL of mineral medium compared to *X. autotrophicus* that induced by WMO, NaNO<sub>3</sub>, pH 6.5 and 250 rpm registered the highest concentration of crude biode detergent with 0.352 mg/mL, 0.7156 mL of anionic biode detergent/100 mL of mineral medium at 144 h of incubation. *A. denitrificans* induced WMO with NaNO<sub>3</sub>, pH 6.5 and 250 rpm reached 94.23 U/ml. 197.63 U/mL of lipolytic activity compared to *X. autotrophicus* with and 45.16 U/mL of lipolytic activity. The highest emulsification rate of 100% was registered in the crude biode detergents of *A. denitrificans* with NaNO<sub>3</sub>, at pH 6 and 250 rpm. Qualitatively, the glycolipids were the most synthesized for those genera. The WMO with NH<sub>4</sub>NO<sub>3</sub> pH 6, 250 rpm, was the greatest inducer of biode detergents synthesis and lipolytic activity by *A. denitrificans*. This research showed the potential capacity of genera and species isolated from soil impacted by WMO to synthesize biode detergents and induction of lipolytic activity for an efficient application in the bioremediation of soil impacted by hydrocarbon mixtures.

**KEYWORDS:** Soil. Heterotrophic aerobic bacteria. Emulsification. Hydrolytic enzymes. Hydrocarbons metabolism.

## 1 INTRODUCTION

Biology explains that heterotrophic cells require biode detergents to emulsify nonpolar compounds like waste motor oil and then lipases to hydrolyze them for use as a carbon and energy source. Hydrocarbon mixtures are one of the main agricultural soil contaminants, such as used motor oil (WMO), that can select genera and bacterial heterotrophic species capable of emulsifying and hydrolyzing WMO. Consequently, genera and heterotrophic species of bacteria such as *Achromobacter denitrificans*, *Bacillus horneckiae*, *B. safensis*, *B. subtilis*, *G. amicalis* isolated from soil impacted by WMO have the potential ability to synthesize biode detergents to emulsify and lipase activity to hydrolyze WMO, as essential mechanisms to mineralize it (Rabbani et al., 2013; Karlapudi et al., 2018; Soumeya et al., 2022).. This implies that the synthesis of biode detergents and lipolytic activity could be artificially induced with lubricating oil or with WMO (Alam et al., 2018). The objectives of this work were: 1) to analyze that WMO and lubricating oil are inducers of biode detergent synthesis and lipolytic activity 2) to optimize the synthesis of crude biode detergents and lipolytic activity in these genera and bacterial species 3) to partially characterize the type of biode detergent synthesized by this bacterial group.

## 2 MATERIALS AND METHODS

### 2.1 ANALYSIS OF SYNTHESIS OF BIODETERGENTS AND ACTIVITY LIPOLYTIC IN GENERA AND SPECIES ISOLATED FROM SOIL IMPACTED BY WMO

Genera and species of bacteria classified as: *Achromobacter* sp, *B. horneckiae*, *B. safensis*, *B. subtilis*, *G. amicalis*, were grown in 5 ml of mineral medium with WMO and lubricating oil at 1% v/v as sole carbon and energy sources for the synthesis of biodetergents and lipolytic activity compared to *X. autotrophicus* isolated from agricultural soil not polluted by WMO. The WMO was obtained from a mechanical workshop in the city of Morelia, Michoacán, Mexico, while the lubricating oil was Mobil Super 15w40 Multigrade Trc-pro 946 MI. All strains were activated on the following chemical composition (g/L): WMO or lubricating oil 10.0 g/L of  $\text{NH}_4\text{NO}_3$  or  $\text{NaNO}_3$ ; 1 g/L of  $\text{K}_2\text{HPO}_4$ ,  $\text{KH}_2\text{PO}_4$ ,  $\text{MgSO}_4$ ,  $\text{NaCl}$ , 0.5 g/L of  $\text{FeSO}_4$  and  $\text{CuSO}_4$ . Bacterial cultures were incubated at 30 °C for 48 h at 250 rpm, then 1 ml aliquots were taken and filtered on 0.22-micron membranes to obtain the cell-free supernatant in that the crude secreted enzymes were found. Crude extracellular lipases were quantified spectrophotometrically, using p-nitrophenyl palmitate (p-NPP) (Sigma-Aldrich) as substrate according to Li *et al.*, 2013. In this method, the hydrolysis of p-NPP to release p-nitrophenol (p-NP) was measured. Thirty mg of p-NPP was dissolved in 10 ml of isopropanol and 90 ml of 100 mM Tris-HCl at pH 8.0. From this mixture 150  $\mu\text{L}$  was taken and the reaction was initiated by adding 100  $\mu\text{L}$  of the cell-free filtrate grown in mineral medium with WMO and lubricating oil at 1% v/v. After 1 h incubation at 37°C, absorbance was measured at 405 nm in a spectrophotometer against a control containing the same components except the cell-free filtrate. One unit of enzyme activity was defined as the amount of enzyme releasing 1  $\mu\text{mol}$  of p-nitrophenol per minute under the assay conditions (Pedroza-Padilla *et al.*, 2017). While anionic biodetergents were measured by the spectrophotometric method with methylene blue (Selberg *et al.*, 2007).

### 2.2 OPTIMIZATION OF THE SYNTHESIS OF BIODETERGENTS AND LIPOLYTIC ACTIVITY BY *A. DENITRIFICANS* COMPARED TO *XANTHOBACTER AUTOTROPHICUS*

To test the enhanced synthesis of biodetergents, an experiment was performed in 250 ml flasks in triplicate with mineral medium, previously, WMO was selected as the best source of carbon and energy and inducer of biodetergents and lipases activity. As a source of nitrogen, 5 g/L of  $\text{NH}_4\text{NO}_3$  or  $\text{NaNO}_3$ , of yeast extract as a growth factor that was kept for all treatments, as well as 1 g/L of  $\text{K}_2\text{HPO}_4$ ,  $\text{KH}_2\text{PO}_4$ ,  $\text{MgSO}_4$ ,  $\text{NaCl}$ , 0.5 g/L of  $\text{FeSO}_4$  and  $\text{CuSO}_4$ . Then the mineral mediums were inoculated with 10 ml of *A.*

*denitrificans* to compare with *X. autotrophicus* in detergent saline solution individually with an inoculum density according to McFarland's number 4. The incubation temperature was 30°C, the pH used were: 6.5 and 7, in agitation at 200 and 250 rpm. To analyze the maximum concentration of biodetergents, samples were taken at 48, 94 and 144 h in plastic Falcon-type tubes with lids. Then, the possible biodetergents and lipases were extracted by centrifugation of the mineral medium at 8000 rpm for 15 minutes at 4 ° C. From the extracted supernatants, 3 ml were taken to perform the quantification of lipolytic activity by spectrophotometry, described by Li *et al.*, 2013, as well as the measurement of anionic biodetergents by the spectrophotometric method by Selberg *et al.*, 2007. The remaining supernatants were acidified to pH 2 with 6 M HCl and kept overnight for precipitation of biodetergents (Bharali *et al.*, 2011). Final extraction of the biodetergents was performed with 2: 1 (v/v) organic solvents of chloroform and methanol according to Satpute *et al.*, 2010. The solvents were evaporated at room temperature and the resulting residue was the crude biodetergents. The weight or dry weigh of the biodetergents was obtained in mg/ml (Khan *et al.*, 2017).

### 2.3 EMULSIFICATION INDEX

The emulsification index (E24) of the crude biodetergents extracted at 144 h was determined by the method of Alvarez *et al.*, 2015 modified, the cell-free supernatant was added in WMO with a ratio of 3:2 (crude biodetergents: WMO). The mixture was gently agitated and the height in cm of the emulsion layer and the total solution height after 24 h were calculated. The emulsion index was calculated using the equation:

$$\text{Emulsification index (E24)} = \frac{\text{Emulsified height}}{\text{Total solution height}} \times 100$$

### 2.4 DETECTION OF GLYCOLIPID BIODETERGENTS

To 1 ml of cell-free supernatant, 1 ml of 5% (w/v) phenol was added. To this mixture, 2-5 ml of concentrated sulfuric acid was added dropwise until the characteristic color developed. The development of orange color indicated the presence of glycolipids according to Ellaiah *et al.*, 2002.

### 2.5 BIURET TEST FOR DETECTION OF LIPOPEPTIDES AND GLYCOLIPIDS

The Biuret qualitative test was used to detect the presence of biodetergents lipopeptides and glycolipids. 2 ml of biosurfactant crude extract solution were first heated

to 70 °C before mixing with 2 ml of 1M NaOH solution. Then, a drop of two milliliters of 1% CuSO<sub>4</sub> was slowly added to observe any color change; green color for glycolipid and violet color for lipopeptide (Kadhun & Haydar, 2020).

## 2.6 STATISTICAL ANALYSIS

The experimental data were analyzed by ANOVA Tukey HSD at 0.05 % with the program Statgraphics Centurion XVI.II (Walpole *et al.*, 2007).

## 3 RESULTS AND DISCUSSION

### 3.1 ANALYSIS OF THE SYNTHESIS OF BIODETERGENTS AND LIPASES ACTIVITY IN BACTERIAL GENERA AND SPECIES ISOLATED FROM SOIL IMPACTED BY WMO

Table 1 shows the potential capacity of biodetergents and lipases activity synthesis induced by WMO and lubricating oil. All five genera and species of bacteria synthesized biodetergents of anionic nature. *B. safensis* registered the highest value of 1.18 ml of anionic biodetergents/100 ml of medium, compared to *X. autotrophicus* registered 0.62 ml of anionic biodetergents/100 ml of medium and *A. denitrificans* with 0.65 ml of anionic biodetergents/100 ml of medium. These results show that *X. autotrophicus* synthesizes biodetergents of anionic nature compared to *G. amicalis* and *A. denitrificans* that synthesize biodetergents of the glycolipid type which are anionic kind that could be rhamnolipids or sophorolipids, composed of a hydrophobic part of lipids and a hydrophilic part composed of carbohydrates (Fenibo *et al.*, 2019; Joy *et al.*, 2019; Zargar *et al.*, 2022). While in the case of *B. subtilis* it is suggested that it synthesized Surfactin type biodetergent that is an anionic cyclic lipopeptide composed of an amino acid chain linked to a fatty acid chain (Moutinho *et al.*, 2021), that has been reported as one of the most common biodetergents synthesized by this genus and species of bacteria (Zhao *et al.*, 2020). In the case of lipolytic activity *A. denitrificans* reached the highest activity of 280.3 U/mL induced by WMO and 210.07 U/mL in mineral medium induced by lubricating oil, while *G. amicalis* registered lipolytic activity of 205.2 U/mL induced by WMO, and 195.6 U/mL with lubricating oil. Compared to, *X. autotrophicus* that registered a lipolytic activity of 275.53 U/mL in mineral medium induced by WMO and 165.7 U/mL in mineral medium induced by lubricating oil, numerical values statistically different from the uninoculated mineral medium where no lipolytic activity was registered.

The results show that these genera and species of bacteria synthesized a greater diversity of biodetergents and specific lipolytic activity in demand for the emulsification and hydrolysis of the wide diversity of WMO hydrocarbons such as aliphatic and aromatic

hydrocarbons from 15 to 50 carbons of WMO, as well as 34 to 90 times more aromatic hydrocarbons than lubricating oil (Dominguez Rosado and Pichtel, 2003), that had registered an average hydrocarbon composition of 7 to 26 aliphatic and aromatic carbons (Yao *et al.*, 2020). These results confirm that *A. denitrificans*, *B. horneckiae*, *B. safensis*, *B. subtilis*, *G. amicalis* compared to *X. autotrophicus*, could synthesize biodetergents and lipases activity induced by WMO including an adequate temperature and oxygenation level. The results showed that these genera and species could have a potential to synthesis and application of crude extracts of biodetergents and lipases for environmental impacted by hydrocarbons mixtures to emulsify and hydrolyze them (Noro *et al.*, 2020). In that sense extract crude of biodetergents and lipases as part of the strategies for soil bioremediation polluted by WMO. They are a promising action to solve this type of problem of environmental contamination caused by mixtures of hydrocarbons (Jacob *et al.*, 2022).

Table 1. Concentration of biodetergents and lipolytic activity from *Achromobacter denitrificans*, *Bacillus horneckiae*, *B. safensis*, *B. subtilis*, *Gordonia amicalis* compared to *Xanthobacter autotrophicus* induced by lubricating oil and waste motor oil.

Bacteria	Mineral medium with lubricating oil		Mineral medium with waste motor oil	
	Concentration of anionic biodetergent (mL/100 ml of medium)	Lipolytic activity (U/ml)	Concentration of anionic biodetergent (mL/100 ml of medium)	Lipolytic activity (U/ml)
Control	0 <sup>e</sup>	0 <sup>c</sup>	0 <sup>e</sup>	0 <sup>c</sup>
<i>A. denitrificans</i>	0.55 <sup>b</sup>	210.07 <sup>b</sup>	0.62 <sup>b</sup>	<b>280.3<sup>a</sup></b>
<i>B. horneckiae</i>	0.39 <sup>d</sup>	124.1 <sup>f</sup>	0.42 <sup>d</sup>	190.4 <sup>e</sup>
<i>B. safensis</i>	0.44 <sup>c</sup>	<b>223.5<sup>a</sup></b>	<b>1.18<sup>a</sup></b>	255.4 <sup>b</sup>
<i>B. subtilis</i>	0.41 <sup>d</sup>	179.2 <sup>d</sup>	0.45 <sup>c</sup>	243.3 <sup>c</sup>
<i>G. amicalis</i>	<b>1.11<sup>a</sup></b>	195.6 <sup>c</sup>	<b>1.13<sup>a</sup></b>	205.2 <sup>d</sup>
<i>X. autotrophicus</i>	0.55 <sup>b</sup>	165.7 <sup>e</sup>	0.65 <sup>b</sup>	<b>275.53<sup>a</sup></b>

\*Different letters are statistically different according to ANOVA-Tukey HSD at 0.05 %.

Table 2 shows the selection of the best results of the optimization of the synthesis of biodetergents and specific lipolytic activity of *A. denitrificans* at 48 h in mineral medium induced by WMO, with NaNO<sub>3</sub>, pH 6.5 and 250 rpm reached 0.227 mg of crude biodetergents /mL, 0.436 mL of anionic biodetergents /100 mL of mineral medium and 194.23 U/mL of lipolytic activity. Those numerical values were statistically different compared to *A. denitrificans* using NH<sub>4</sub>NO<sub>3</sub> at pH 7 at 200 rpm, that reached 0.018 mg crude biodetergents /mL, 1.044 mL anionic biodetergents /100 mL mineral medium and 132.44 U/mL lipolytic activity.

Table 3 shows the optimization of synthesis of biodetergents and lipases activity of *A. denitrificans* at 96 h in mineral medium induced by WMO, using NaNO<sub>3</sub>, pH 6.5



and 250 rpm that reached 0.2804 mg of crude biodetergents /mL, 0.610 mL of anionic biodetergents /100 mL of mineral medium and 127.03 U/mL of lipolytic activity. Those numerical values were statistically different compared to those of *A. denitrificans* with  $\text{NaNO}_3$ , pH 7 at 200 rpm, that registered 0.2624 mg of crude biodetergents /mL, 0.5174 mL of anionic biodetergents /100 mL of mineral medium and 48.415 U/mL of lipolytic activity.

Table 4, shows the optimization of synthesis of biodetergents and lipases activity of *A. denitrificans* at 144 h in mineral medium WMO, using  $\text{NaNO}_3$ , pH 7 and 250 rpm registered 0.3147 mg of crude biodetergents /mL, 0.6548 mL of anionic biodetergents /100 mL of medium and 45.165 U/mL of lipolytic activity, these numerical values had not statistical difference compared to  $\text{NH}_4\text{NO}_3$ , pH 7 and 250 rpm, when *A. denitrificans* reached 0.2977 mg of crude biodetergents /mL, 0.8425 mL of anionic biodetergents /100 mL of mineral medium and 42.695 U/mL of lipolytic activity. The results were similar when hydrocarbon mixtures were used as carbon source for the synthesis of biodetergents (Wang *et al.*, 2014; Jimoh & Lin, 2018). While for inducing lipases activity vegetable oils were very usefully such as olive oil it has been reported by Llesanmi *et al.*, 2020. The synthesis of biodetergents by *A. denitrificans* had maximum at 144 h, in opposite way that reported in other research with *Paenibacillus* sp D9 that registered the highest synthesis after 72h of incubation (Jimoh & Lin, 2018). In that sense the highest lipases activity was detected at 48 h, then decreased, other reports also point out the maximum lipase activity in the first 12 h until 48h. These data suggest that after this time lipases activity decreases due to nutrient consumption of hydrocarbons. In the case of inorganic and organic of compounds of nitrogen source, are necessary for cell growth as well as biodetergent synthesis (Chakraborty *et al.*, 2015). In this work inorganic source of nitrogen as well as  $\text{NaNO}_3$  was better one for the synthesis of biodetergents and lipases activity than  $\text{NH}_4\text{NO}_3$ , as other research reported indicated that  $\text{NaNO}_3$  is one of the best inorganic compounds for the synthesis of biodetergents (Onwosi & Odibo, 2012; Amin *et al.*, 2021). While of pH, the highest lipolytic activity was detected in a pH narrow range of 6.5-7, that coincides with other studies that neutral pH 7 could be optimal for synthesis of biodetergents and lipases activity, compared to alkaline or acidic pH values, that cause drastic decrease in synthesis of biodetergents and lipases activity (Jimoh & Lin, 2018; Llesanmi *et al.*, 2020). Finally, agitation of 250 rpm affecting  $\text{O}_2$  distribution inside of mineral medium due that biodetergents and lipases depends on aerobic metabolism, compared to lower levels of agitation values, that reduces the concentration of oxygen which inhibits the synthesis of biodetergents and lipases activity (A'yuni *et al.*, 2021; Zambry *et al.*, 2021).

Table 2. Optimization of the synthesis of biodetergents and lipolytic activity from *Achromobacter denitrificans*, at 30°C at 48 h.

Run	Source inorganic nitrogen	pH	Agitation (rpm)	48 h		
				Biodetergent weight(mg/ml)	Anionic biodetergent (ml/100 ml of medium)	Lipolytic activity (U/ml)
1	NH <sub>4</sub> NO <sub>3</sub>	7	200	0.0187g*	<b>1.0441a</b>	132.446e
2	NaNO <sub>3</sub>	7	200	0.1336e	0.5882b	116.198f
4	NaNO <sub>3</sub>	6.5	200	0.2054b	0.4558e	160.346c
9	NH <sub>4</sub> NO <sub>3</sub>	6.5	0	0.0039h	0.5617c	153.781d
11	NH <sub>4</sub> NO <sub>3</sub>	6	0	0.1668d	0.58b	53.5036g
15	NH <sub>4</sub> NO <sub>3</sub>	6.5	250	0.125f	0.5219d	178.263b
16	NaNO <sub>3</sub>	6.5	250	<b>0.2277a</b>	0.436f	<b>194.236a</b>
18	NaNO <sub>3</sub>	6	250	0.1802c	0.5126d	155.356d

\*Different letters are statistically different according to ANOVA-Tukey HSD at 0.05 %.

Table 3. Optimization of biodetergent synthesis and lipolytic activity from *Achromobacter denitrificans*, in mineral medium and waste motor oil at 30°C at 96 h.

Run	Inorganic source of nitrogen	pH	Agitation (rpm)	96 h		
				Biodetergent weight(mg/ml)	Anionic biodetergent (ml/100 ml of medium)	Lipolytic activity (U/ml)
2	NaNO <sub>3</sub>	7	200	<b>0.2624a*</b>	0.5174c	48.415f
4	NaNO <sub>3</sub>	6.5	200	<b>0.2682a</b>	0.4776d	42.671g
13	NH <sub>4</sub> NO <sub>3</sub>	7	250	0.2022c	0.5705b	<b>165.762a</b>
14	NaNO <sub>3</sub>	7	250	<b>0.2685a</b>	<b>0.6369a</b>	155.91b
15	NH <sub>4</sub> NO <sub>3</sub>	6.5	250	0.2475b	<b>0.6192a</b>	74.65d
16	NaNO <sub>3</sub>	6.5	250	<b>0.2804a</b>	<b>0.6104a</b>	127.03c
17	NH <sub>4</sub> NO <sub>3</sub>	6	250	0.2287b	0.579b	73.854d
18	NaNO <sub>3</sub>	6	250	0.2354b	0.5201c	68.612e

\*Different letters are statistically different according to ANOVA-Tukey HSD at 0.05 %.

Table 4. Optimization of biodetergent synthesis and lipolytic activity from *Achromobacter denitrificans*, in mineral medium waste motor oil at 30°C at 144 h

Run	Inorganic source of nitrogen	pH	Agitation (rpm)	144 h		
				Biodetergent weight(mg/ml)	Anionic biodetergent (mL/100 ml of medium)	Lipolytic activity (U/ml)
1	NH <sub>4</sub> NO <sub>3</sub>	7	200	<b>0.2826a*</b>	0.4865c	76.644b
2	NaNO <sub>3</sub>	7	200	<b>0.2855a</b>	0.4776d	54.16d
4	NaNO <sub>3</sub>	6.5	200	0.2742b	0.4953c	65.812c
6	NaNO <sub>3</sub>	6	200	0.2122c	0.497c	<b>110.522a</b>
13	NH <sub>4</sub> NO <sub>3</sub>	7	250	<b>0.2977a</b>	<b>0.8425a</b>	42.695g

Run	Inorganic source of nitrogen	pH	Agitation (rpm)	144 h		
				Biodetergent weight(mg/ml)	Anionic biodetergent (mL/100 ml of medium)	Lipolytic activity (U/ml)
14	NaNO <sub>3</sub>	7	250	<b>0.3147a</b>	0.6548b	45.165f
16	NaNO <sub>3</sub>	6.5	250	<b>0.2998a</b>	0.6538b	46.285f
17	NH <sub>4</sub> NO <sub>3</sub>	6	250	0.2714b	0.6712b	51.256e

\*Different letters are statistically different according to ANOVA- Tukey HSD at 0.05 %.

Table 5 shows the selection of the best results of the optimization of synthesis of biodetergents and lipolytic activity of *X. autotrophicus* at 48 h in mineral medium using WMO, NH<sub>4</sub>NO<sub>3</sub>, pH 6 and 250 rpm reached 0.1452 mg of crude biodetergents / mL, 0.7869 mL of anionic biodetergents /100 mL of mineral medium and 174.233 U/ mL of lipolytic activity. These numerical values had statistically different from those of *X. autotrophicus* growing in NH<sub>4</sub>NO<sub>3</sub>, pH 6 at 200 rpm, that reached 0.0923 mg crude biodetergents /mL, 0.4466 mL anionic biodetergents /100 mL mineral medium and 154.43 U/mL of lipolytic activity.

Table 6 shows the optimization of synthesis of biodetergents and lipolytic activity of *X. autotrophicus* at 96 h in mineral medium with WMO, using NaNO<sub>3</sub>, pH 6.5 and 250 rpm reached 0.2755 mg of crude biodetergents /mL, 0.8945 mL of anionic biodetergents /100 mL of mineral medium and 115.699 U/mL of lipolytic activity. Those numerical values were statistically different compared to *X. autotrophicus* grown in NaNO<sub>3</sub>, pH 7 at 200 rpm, that reached 0.2351 mg crude biodetergents /mL, 0.7269 mL anionic biodetergents /100 mL of mineral medium and 82.489 U/mL lipolytic activity.

Table 7, shows the optimization of synthesis of biodetergents and lipases activity of *X. autotrophicus* at 144 h in mineral medium plus WMO, with NH<sub>4</sub>NO<sub>3</sub>, pH 6 and 250 rpm reached 0.2986 mg of biodetergents /ml, 0.7569 mL of anionic biodetergents /100 ml of mineral medium and 245.742 U/mL of lipolytic activity, these numerical values had statistical difference when grown in NH<sub>4</sub>NO<sub>3</sub>, pH 7 and 250 rpm, there *X. autotrophicus* reached 0.3466 mg of biodetergents /ml, 0.6426 ml of anionic biodetergents /100 ml of mineral medium and 108.544 U/mL of lipolytic activity. The synthesis of biodetergents and lipolytic activity by *X. autotrophicus* reached the maximum at 144 h. These values were similar to those reported by Khademolhosseini *et al.*, 2019, when *Pseudomonas aeruginosa* release highest biodetergent synthesis at 144 h of growth. In the case NaNO<sub>3</sub> induced the maximum synthesis of biodetergents and lipolytic activity, same as well as other studies reported that NaNO<sub>3</sub> is the best inorganic source of nitrogen to increase biodetergents and lipolytic activity (Cheirsilp *et al.*, 2021; Krishna & Chandran, 2022). While the effect

of pH on the highest lipolytic activity was detected in at pH range between 6-6.5, similar data to those reported by Nema *et al.*, 2019, when *Aspergillus niger* reached the highest lipolytic activity at pH 6. In biodetergent synthesis most research point to pH 7 as the optimum (Kumar *et al.*, 2021). While in terms of agitation at 250 rpm *A. denitrificans*, *X. autotrophicus* reached the highest synthesis of biodetergents, that with higher agitation there is greater oxygenation, that allows aerobic synthesis of biodetergents and lipolytic activity (Adetunji *et al.*, 2021; Li *et al.*, 2022).

Table 5. Optimization of biodetergent synthesis and lipolytic activity from *Xanthobacter autotrophicus* in mineral medium and waste motor oil at 30°C at 48 h.

Run	Inorganic source of nitrogen	pH	Agitation (rpm)	48 h		
				Biodetergent weight(mg/ml)	Anionic biodetergent (ml/100 ml of medium)	Lipolytic activity (U/ml)
5	NH <sub>4</sub> NO <sub>3</sub>	6	200	0.0923b*	0.4466d	154.43b
13	NH <sub>4</sub> NO <sub>3</sub>	7	250	0.08562c	0.6348c	69.485e
14	NaNO <sub>3</sub>	7	250	<b>0.1422a</b>	0.7458b	68.478e
15	NH <sub>4</sub> NO <sub>3</sub>	6.5	250	<b>0.1365a</b>	0.7655b	78.463d
16	NaNO <sub>3</sub>	6.5	250	0.0952b	<b>0.8756a</b>	88.478c
17	NH <sub>4</sub> NO <sub>3</sub>	6	250	<b>0.1452a</b>	0.7869b	<b>174.233a</b>
18	NaNO <sub>3</sub>	6	250	0.0753d	0.6421c	66.662f

\*Different letters are statistically different according to ANOVA-Tukey HSD at 0.05 %.

Table 6. Optimization of the synthesis of biodetergents and lipolytic activity from *Xanthobacter autotrophicus* in waste motor oil at 30°C at 96h.

Run	Inorganic source of nitrogen	pH	Agitation (rpm)	96 h		
				Biodetergent weight(mg/ml)	Anionic biodetergent (ml/100 ml of medium)	Lipolytic activity (U/ml)
4	NaNO <sub>3</sub>	6.5	200	0.2156c*	0.4555e	101.755e
5	NH <sub>4</sub> NO <sub>3</sub>	6	200	0.2016d	0.4326e	164.361b
13	NH <sub>4</sub> NO <sub>3</sub>	7	250	0.2485b	0.7129c	84.157g
14	NaNO <sub>3</sub>	7	250	0.2351b	0.7269c	82.489g
15	NH <sub>4</sub> NO <sub>3</sub>	6.5	250	0.2214c	0.7556b	108.236d
16	NaNO <sub>3</sub>	6.5	250	<b>0.2755a</b>	<b>0.8945a</b>	115.699c
17	NH <sub>4</sub> NO <sub>3</sub>	6	250	<b>0.2662a</b>	0.7645b	<b>201.455a</b>
18	NaNO <sub>3</sub>	6	250	0.2365b	0.6148d	89.266f

\*Different letters are statistically different according to ANOVA Tukey HSD at 0.05 %.

Table 7. Optimization of biodetergent synthesis and lipolytic activity from *Xanthobacter autotrophicus* in mineral medium and waste motor oil at 30°C at 144 h.

Run	Inorganic source of nitrogen	pH	Agitation (rpm)	144 h		
				Biodetergent weight(mg/ml)	Anionic biodetergent (mL/100 ml of medium)	Lipolytic activity (U/ml)
1	NH <sub>4</sub> NO <sub>3</sub>	7	200	0.3165c*	0.5086e	94.369g
4	NaNO <sub>3</sub>	6.5	200	<b>0.3563a</b>	0.377g	89.938h
5	NH <sub>4</sub> NO <sub>3</sub>	6	200	0.3248c	0.4126f	178.452d
13	NH <sub>4</sub> NO <sub>3</sub>	7	250	0.3466b	0.6426d	108.544f
14	NaNO <sub>3</sub>	7	250	0.3356b	0.6849c	145.336e
15	NH <sub>4</sub> NO <sub>3</sub>	6.5	250	0.2799d	0.6452d	186.266c
16	NaNO <sub>3</sub>	6.5	250	<b>0.3526a</b>	0.7156b	197.636b
17	NH <sub>4</sub> NO <sub>3</sub>	6	250	0.2986d	<b>0.7569a</b>	<b>245.742a</b>

\*Different letters are statistically different according to ANOVA Tukey HSD at 0.05 %.

Table 8 shows the best results of the emulsification index of the crude biodetergents of *A. denitrificans*, in mineral medium and waste motor oil at 30°C at 144 h. There, *A. denitrificans* grown with NaNO<sub>3</sub>, at pH 6 and 250 rpm, the crude biodetergents reached 100% emulsification of WMO. Numerical per cent values were statistically different compared to crude biodetergents of *A. denitrificans* using NH<sub>4</sub>NO<sub>3</sub> at pH 6 and 250 rpm reached 95% emulsification of WMO. These numerical values were statistically different compared to those detected of the control tube of water and WMO, registered 0% emulsification of WMO.

Table 9 shows the best results of the emulsification index of the crude biodetergents of *X. autotrophicus*, using NaNO<sub>3</sub> as inorganic source of nitrogen, pH 6 and 7, 200 rpm, as well as with NaNO<sub>3</sub> pH 7 and 250 rpm, the crude biodetergents registered 100% emulsification of WMO. These values were statistically higher than the WMO water control tube, with 0% WMO emulsification. These results support the results of the optimization of the synthesis of crude biodetergents due to high speed of agitation, maximum production and therefore the best rate of WMO emulsification.

Table 8. Emulsification index of *Achromobacter denitrificans* biodetergents in mineral medium and waste motor oil at 30°C at 144 h.

Run	Source of inorganic nitrogen	pH	Agitation (rpm)	Emulsification index (%)
Control (water)	-	-	-	0d*
3	NH <sub>4</sub> NO <sub>3</sub>	6.5	200	91c
4	NaNO <sub>3</sub>	6.5	200	91c
6	NaNO <sub>3</sub>	6	200	90c

Run	Source of inorganic nitrogen	pH	Agitation (rpm)	Emulsification index (%)
13	NH <sub>4</sub> NO <sub>3</sub>	7	250	95b
14	NaNO <sub>3</sub>	7	250	90c
17	NH <sub>4</sub> NO <sub>3</sub>	6	250	95b
18	NaNO <sub>3</sub>	6	250	<b>100a</b>

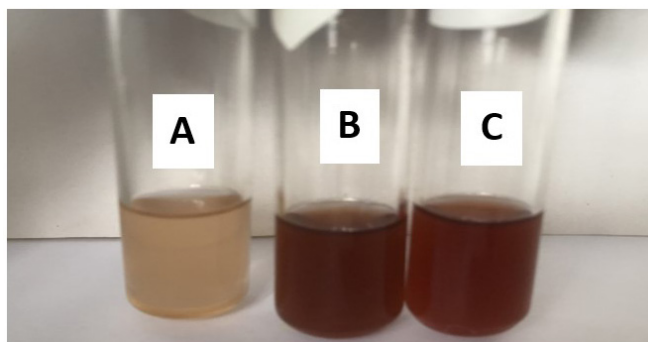
\*Different letters are statistically different according to ANOVA-Tukey HSD at 0.05.

Table 9. Emulsification index of *Xanthobacter autotrophicus* biodetergents in mineral medium and waste motor oil at 30°C at 144 h.

Run+	Inorganic source of nitrogen	pH	Emulsification index (%)
Control (water)	-	-	0c*
2	NaNO <sub>3</sub>	7	<b>100a</b>
6	NaNO <sub>3</sub>	6	<b>100a</b>
13	NH <sub>4</sub> NO <sub>3</sub>	7	95b
14	NaNO <sub>3</sub>	7	<b>100a</b>
15	NH <sub>4</sub> NO <sub>3</sub>	6.5	98a
17	NH <sub>4</sub> NO <sub>3</sub>	6	95b
18	NaNO <sub>3</sub>	6	95b

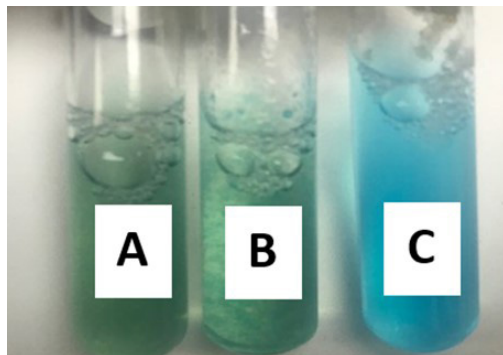
+ = agitation 250 rpm \*Different letters are statistically different according to ANOVA Tukey HSD at 0.05 %.

Figure 3. Detection of glycolipid biodetergents from *A. denitrificans*, in mineral medium and waste motor oil at 30°C at 144 h.



**A:** Control (Water + WMO), **B:** biodetergents with NH<sub>4</sub>NO<sub>3</sub>, pH 6, 250 rpm+ WMO, **C:** biodetergents with NaNO<sub>3</sub>, pH 6, 250 rpm + WMO.

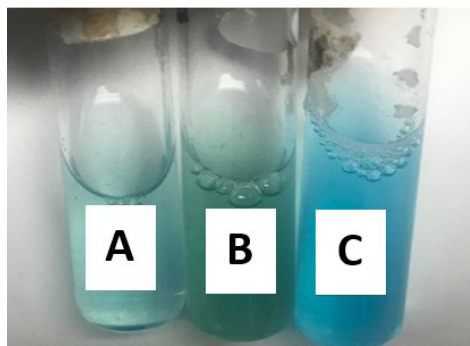
Figure 5. Biuret test for detection of glycolipids and lipopeptides of *X. autotrophicus*, in mineral medium plus waste motor oil at 30°C at 144 h.



**A:** water + WMO, **B:** biodetergents with  $\text{NaNO}_3$ , pH 6.5, 200 rpm + WMO, **C:** biodetergents with  $\text{NaNO}_3$ , pH 6, 250 rpm + WMO.

Figure 3 shows the detection of glycolipid-type biodetergents of *A. denitrificans*, in mineral medium plus WMO at 30°C at 144 h, in all the runs of the mineral medium plus WMO *A. denitrificans* releasing glycolipids due to the dark orange coloration, in the mineral medium with WMO (B) *Achromobacter* grown with  $\text{NH}_4\text{NO}_3$ , pH 6, 250 rpm, and in the mineral medium with WMO (C) *A. denitrificans* grown on  $\text{NaNO}_3$ , pH 6, 250 rpm, compared with the control tube (A it was not detected). In Figure 4, the detection of glycolipids was also evident that in mineral medium with WMO (B) *X. autotrophicus* grown in  $\text{NaNO}_3$ , pH 6.5, 200 rpm, and in mineral medium with WMO (C) *X. autotrophicus* grown with  $\text{NaNO}_3$ , pH 6, 200 rpm, compared to control or water, there the presence of glycolipids was not detected. These results indicated that *X. autotrophicus* synthesizes biodetergents of glycolipid, induced by WMO. Also *A. denitrificans* synthesize biodetergents of the glycolipid type that are anionic composed of a hydrophobic lipid part and a hydrophilic part composed of a carbohydrate (Fenibo et al., 2019; Joy et al., 2019; Zargar et al., 2022).

**Figure 5.** Biuret test for detection of glycolipids and lipopeptides by *X. autotrophicus*, in mineral medium and waste motor oil at 30°C at 144 h.



**A:** biodetergents on  $\text{NaNO}_3$ , pH 6, 250 rpm + WMO. **B:** biodetergents on  $\text{NH}_4\text{NO}_3$ , pH 6, 250 rpm + WMO. **C:** Control (water + WMO).

*A. denitrificans* in mineral medium with WMO, at 30°C at 144 h, released glycolipids due the green coloration, except in the mineral induced by WMO (A) *A. denitrificans* using NaNO<sub>3</sub>, pH 6, at 250 rpm the light blue coloration was light blue, compared to WMO mineral medium (B) *A. denitrificans* using NH<sub>4</sub>NO<sub>3</sub>, pH 6, at 250 rpm green coloration was detected. In that sense Kadhum & Haydar in 2020, isolated 6 bacterial genera that were positive for the synthesis of glycolipid biodetergents by Biuret assay. These results support the ability of these bacteria to synthesize glycolipid-type biodetergents induced by WMO. The consumption of WMO in ml, after the synthesis of biodetergents and lipolytic activity of *Achromobacter* sp, in mineral medium induced by WMO, at 30°C after 144 h. The highest WMO consumption was registered in mineral medium with NH<sub>4</sub>NO<sub>3</sub>, pH 6, 250 rpm, *A. denitrificans* was able to consume 7.35 ml of WMO out of the initial 10 ml, this support that these conditions inducing to synthesize the maximum amount of biodetergents and lipolytic activity to mineralize of WMO, which numerical values were statistically different compared to the consumption of 0.55 ml of WMO out of the initial 10 ml, in mineral medium with WMO, using NaNO<sub>3</sub> pH 6.5, at 250 rpm.

## 4 CONCLUSIONS

This research demonstrated the potential ability of genera and species isolated from soil polluted by WMO, to synthesize biodetergents and lipolytic activity induced by WMO for its mineralization.

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## SOBRE O ORGANIZADOR

**Xosé Somoza Medina** (1969, Ourense, España) Licenciado con Grado y premio extraordinario em Geografía e Historia por la Universidad de Santiago de Compostela (1994). Doctor en Geografía e Historia por la misma universidad (2001) y premio extraordinario de doctorado por su Tesis “Desarrollo urbano en Ourense 1895-2000”. Profesor Titular en la Universidad de León, donde imparte clases desde 1997. En la Universidad de León fue Director del Departamento de Geografía entre 2004 y 2008 y Director Académico de la Escuela de Turismo entre 2005 y 2008. Entre 2008 y 2009 ejerció como Director del Centro de Innovación y Servicios de la Xunta de Galicia en Ferrol. Entre 2007 y 2009 fue vocal del comité “Monitoring cities of tomorrow” de la Unión Geográfica Internacional. En 2012 fue Director General de Rehabilitación Urbana del Ayuntamiento de Ourense y ha sido vocal del Consejo Rector del Instituto Ourenseño de Desarrollo Local entre 2011 y 2015. Ha participado en diversos proyectos y contratos de investigación, en algunos de ellos como investigador principal, con temática relacionada con la planificación urbana, la ordenación del territorio, las nuevas tecnologías de la información geográfica, el turismo o las cuestiones demográficas. Autor de más de 100 publicaciones relacionadas con sus líneas de investigación preferentes: urbanismo, turismo, gobernanza, desarrollo, demografía, globalización y ordenación del territorio. Sus contribuciones científicas más importantes se refieren a la geografía urbana de las ciudades medias, la crisis del medio rural y sus posibilidades de desarrollo, la evolución del turismo cultural como generador de transformaciones territoriales y más recientemente las posibilidades de reindustrialización de Europa ante una nueva etapa posglobalización. Ha participado como docente en masters y cursos de especialización universitaria en Brasil, Bolivia, Colombia, Paraguay y Venezuela y como docente invitado en la convocatoria Erasmus en universidades de Bulgaria (Sofía), Rumanía (Bucarest) y Portugal (Porto, Guimarães, Coimbra, Aveiro y Lisboa). Ha sido evaluador de proyectos de investigación en la Agencia Estatal de Investigación de España y en la Organización de Estados Iberoamericanos (OEI). Como experto europeo en Geografía ha participado en reuniones de la Comisión Europea en Italia y Bélgica. Impulsor y primer coordinador del proyecto europeo URBACT, “come Ourense”, dentro del Programa de la Unión Europea “Sostenibilidad alimentaria en comunidades urbanas” (2012-2014). Dentro de la experiencia en organización de actividades de I+D+i se pueden destacar la organización de diferentes reuniones científicas desarrolladas dentro de la Asociación de Geógrafos Españoles (en 2002, 2004, 2012 y 2018).

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