

Biosolid quality evaluated by its stability (FDA, CO₂) and maturity (GI)**Calidad de un biosólido evaluada por su estabilidad (DAF, CO₂) y madurez (GI)**

JIMAREZ-ORTIZ, Dulce Rosario†, TRUJILLO-TAPIA, Ma. Nieves and RAMÍREZ-FUENTES, Eustacio*

Universidad del Mar, campus Puerto Ángel; San Pedro Pochutla, Oaxaca. México

ID 1° Author: *Dulce Rosario, Jimarez-Ortiz* / CVU CONACYT-ID: 722412

ID 1° Coauthor: *Ma Nieves, Trujillo-Tapia* / ORC ID: 0000-0003-1160-9260, CVU CONACYT-ID: 201401

ID 2° Coauthor: *Eustacio, Ramírez-Fuentes* / ORC ID: 0000-0001-9601-0083, CVU CONACYT-ID: 121193

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Abstract

The aims of the work were: i) to obtain a biosolid of good quality with desirable characteristics of stability and maturity; ii) provide alternative use of sewage sludge mixed with sawdust and contribute to reduce the environmental impact generated by the inadequate disposal of these wastes. In an open pile system with turning (daily), sewage sludge was mixed with sawdust (1:1) keeping the humidity between 50-60%; after 42 days the biosolid was obtained and the stability and maturity was evaluated by means of the enzymatic assay with fluorescein diacetate, the static respirometry and the germination index. Based on the results and Mexican regulations, biosolid quality is of type A and excellent class. In addition, by stability and maturity tests, it is classified as Very stable. The contribution of the work is to present a stable and mature biosolid, which can be used in agriculture as a soil improver, or alternatively, to prove it as an attenuator in soils contaminated with agrochemicals. On the other hand, present the alternative to reduce the amount of waste generated, eliminate them properly and reduce the environmental impact and human health.

Sewage sludge, Sawdust, Compost

Resumen

Los objetivos del trabajo fueron: i) obtener un biosólido de buena calidad con las características deseables de estabilidad y madurez; ii) presentar una alternativa de uso de los lodos sewagees mezclados con el serrín, y contribuir a disminuir el impacto ambiental generado por la inadecuada disposición de dichos residuos. En un sistema abierto en pila con volteo diario, se mezcló el lodo sewage con serrín (1:1) manteniendo la humedad entre el 50-60%; transcurridos 42 días se obtuvo el biosólido y se evaluó la estabilidad y madurez mediante el ensayo enzimático con diacetato de fluoresceína, la respirometría estática, y el índice de germinación. Con base en los resultados y la normatividad mexicana, la calidad del biosólido es de tipo A y clase Excelente. Además, por las pruebas de estabilidad y madurez, es clasificado como Muy estable. La contribución del trabajo es presentar un biosólido estable y maduro, el cual puede ser utilizado en la agricultura, como mejorador de suelo, o bien, probarlo como atenuador en suelo contaminado con agroquímicos. Por otro parte, presentar la alternativa para disminuir la cantidad de residuos generada, su adecuada disposición y aminorar el impacto al ambiente y a la salud humana.

Lodo sewage, Serrín, Compost

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* Correspondence to Author (email: eustacio@angel.umar.mx)

† Researcher contributing first author.

Introduction

The generation of wastewater as a product of human activities is increasing rapidly, and worldwide sanitation and water treatment works are carried out for this type of water. The wastewater treatment is a process by which its characteristics are modified, with the aim of giving it a later use; according to Salgot and Folch (2018), the main use of this water is in agriculture (irrigation), urban use (not for drinking), in industry (cooling, cleaning, in processes), recovery of water bodies (increase in the volume of water) recharging aquifers, drinking water (direct use or for drinking) and in recreational areas (golf courses, parks, swimming pools).

During the wastewater treatment train, solid waste called sewage sludge is generated and accumulated, which in most cases is deposited in open dumps, causing public health and environmental problems, contaminating soil, water and air. To overcome this concern, effective management strategies must be adopted for the disposal of sludge from a wastewater treatment plant (WWTP); among the management options, composting is gaining great popularity due to the benefits that are obtained as: the sanitation of waste (reduction of pathogens), profitability and the conversion of waste to a value-added product, known as biosolids (Amuda et al., 2008).

Composting is defined as an aerobic digestion, it is a heterogeneous biochemical process that involves the mineralization of organic matter, its transformation into CO₂, NH₃, H₂O, and in incomplete humification; the result is a stabilized and mature final product with less toxicity and pathogenic organisms than the original material (Das et al., 2011). The decomposition process occurs under aerobic conditions in the presence of a substrate and is influenced by physicochemical parameters such as aeration, temperature, pH, particle size, moisture content, electrical conductivity and type of substrate, among others (Li et al., 2013; Juárez et al., 2015).

Once the transformation has been carried out, the resulting product (compost) has stability, a term related to the resistance of compost organic matter to degradation or greater microbial activity (Hachicha et al., 2009); on the other hand, the degree of maturity of the compost describes the capacity that it has to be used effectively in agriculture, that is, it does not present problems of phytotoxicity and does not inhibit the growth of the plants.

Oviedo-Ocaña et al., (2015) establish that the stability of a compost is not necessarily an indicator of maturity; therefore, for the assessment of stability and maturity are considered physicochemical parameters such as the ratio C: N, NH₄: NH₃, evolution of CO₂, pH, temperature, electrical conductivity (EC), moisture content, carbon (C) soluble in water, cation exchange capacity (CEC), Oxygen absorption rate, total organic C, volatile organic C, production of humic and fulvic acids; to evaluate the degree of phytotoxicity of mature biosolids, the germination index (GI) and the plant growth index are used, among others (Onwosi et al., 2017). In the present work we rely on the enzymatic assay with fluorescein diacetate (FDA) and static respirometry (CO₂ production) as an indicator of stability; and the germination index (GI) as an indicator of maturity.

A key element in the composting process is the substrate that is used. Negro et al., (2000) list 33 possible materials, all of them with different physical and chemical characteristics. However, it has been proposed that the material to be used should have at least three desirable properties: 1) have a C:N ratio between 20-40, 2) moisture content of 40 to 65%, and 3) a pH of 6.5 to 8.5. From the above, an option for composting is sawdust, a waste derived from carpentry and the manufacture of furniture; it is characterized by being a good adsorbent of humidity, its degradability is moderate to poor, it confers porosity and it is used to give volume (agent bulking, in English) providing excellent structure to the compost¹ (Negro et al., 2000). Sawdust is generally available wherever there is human activity and it can be used, and it has a relatively low cost compared to other materials.

¹ Del fr. **compost**, y este del lat. **compositus** 'compuesto'. Real Academia Española.

In Bahías de Huatulco, Oaxaca, the government company FONATUR is in charge of wastewater treatment (WWTP), where 6 treatment plants operate and use 100% of the treated wastewater; however, the generation of sewage sludge is approximately one ton per day and its final disposal is the open-air municipal dump, generating environmental and public health problems. On the other hand, in the Universidad del Mar (Umar), in the area of carpentry, solid waste (sawdust) is generated whose final disposal is the rubbish bin.

In view of this panorama, in the present work the following objectives have been proposed: i) obtain a biosolid of good quality with the desirable characteristics of stability and maturity; ii) present an alternative use of both waste (sewage sludge generated in the WWTP and sawdust), and contribute to reduce the environmental impact generated by the inadequate disposal of such waste.

Methodology

Characterization of sewage sludge

The sewage sludge was obtained directly from the Chahué wastewater treatment plant (PTAR), located in Bahías de Huatulco, Oaxaca; whose final process consists of a mesophilic anaerobic digestion. Sufficient sludge was collected to fill two containers with capacity of 200 L each and was transferred to the laboratory of Environmental Biotechnology of Umar (Oaxaca) for its characterization (Table 2). The sludge was dried at room temperature, then a sample of 2 kg was taken to characterize it according to section five of the official Mexican standard 004 of the secretary of environment and natural resources (NOM-021-RECNAT-2000): fecal coliforms (CF) (most likely number), Salmonella spp, and helminth eggs (HH); heavy metals (Ar, Cd, Cu, Cr, Ni, Pb and Zn)). The physical and chemical properties of the sludge were determined using the techniques established in the official Mexican standard 021 of the Secretary of Environment and Natural Resources (NOM-021-RECNAT-200).0): pH, electrical conductivity (EC), real density (Dr), bulk density (Da), porous space (Ep), relative humidity, water retention capacity (WHC), texture, organic matter (OM), total nitrogen (TN).

Composting of sewage sludge

Due to its compactness and high water content (> 11% humidity), sludge can not be composted alone, must be mixed with bulking agents to provide structural support and create voids between particles (Eftoda and McCartney, 2004). Sawdust is a filler and waste material that is a good moisture adsorbent, have a high C:N ratio (> 30), pH between 6-8, among other characteristics (Table 1), It is generally used for composting.

The aerobic digestion of the sludge was carried out in an open system in pile with turning, placing sawdust as substrate and filling agent; it was mixed in a 1:1 ratio (sewage sludge: sawdust, w / w) as it is an adequate ratio for composting anaerobic sewage sludge (Banegas et al., 2007); a first layer of substrate (5 cm) was placed followed by a layer of 5 cm of sewage sludge previously dehydrated to form a pile; each layer was moistened using approximately twelve liters of water and thus maintain humidity around 50-60%.

To avoid anaerobic conditions, the mixture was turned daily with a shovel to keep the system oxygenated and alternately temperature and humidity were monitored. Once the digestion period was completed, the stability and maturity of the biosolids was evaluated using the enzymatic assay with fluorescein diacetate (FDA), the static respirometry with the production of CO₂ and the germination test (GI).

Characteristic	Igbal et al. (2010)	Zorpas and Loizidou (2008)
pH	6.3	8.0
O.M. (%)	91.0	90.1
N (%)	0.49	1.8
C (%)	45.5	55.5
C/N	92.3	30.5
NH ₄ (mg g ⁻¹)	NR	19.7
PO ₄ (%)	NR	2.6
EC (mS cm ⁻¹)	NR	1.1
Lignin (%)	NR	30.5
Cellulose (%)	NR	52.1
NR (Not reported).		

Table 1 Reported characteristics of sawdust and its use as agent bulking

Source: Self Made

Enzymatic assay with DAF

The FDA test is a test of enzymatic activity used as an indicator of soil microbial activity (Adam and Duncan, 2001), has recently been used as an indicator for the stability of compost (Komilis et al., 2011). The test is based on the hydrolysis of fluorescein by extracellular enzymes and membrane, releasing the fluorescence, which can be read at a wavelength of 490 nm; the greater the enzymatic activity, the higher the fluorescein release rate and the readings obtained.

In the stability and maturity tests, a previous incubation of the biosolids was carried out for 7 days, using 30 g of the biosolide on a humid basis (humidity was adjusted to 50% of the water retention capacity) and introducing it in glass jars of 1 L next to a vial with NaOH (respirometry analysis). Later for the FDA analysis, 6 g of the biosolid was taken in wet base placing it in 50 ml Falcon tube, 50 ml of 60 mM phosphate buffer at pH 7.6 was added and agitated until a homogeneous sample was obtained; subsequently, 0.50 ml of the DAF solution (20 mg FDA substrate ® Sigma in 10 ml of acetone) were added, shaken and placed in a water bath at 37 ° C for 30 min, after which time, 2 ml of acetone were added to stop the hydrolysis of the diacetate; 30 ml of the solution were transferred into 50 ml Falcon tubes and centrifuged at 6000 rpm for 5 min, then filtered using Whatman # 2 paper.

Finally they were transferred to a spectrophotometry tube to measure the absorbance at 490 nm in a spectrophotometer (Beckman Du 530). The samples were analyzed on day 1, 3 and 7 of the incubation period, in triplicate. The standard curve is linear ($R^2 = 0.982$, $y = 0.893x$) in the range of 0.03 to 0.5 mg of fluorescein and covers the concentration of FDA in different types of soil (Green et al., 2006). The fluorescein concentration was calculated according to equation 1:

$$FDA_t = CNF * V_{fs} / t * MS \quad (1)$$

Where: DAF_t: net fluorescein release rate (mg fluorescein kg⁻¹ h⁻¹); CNF: net concentration of fluorescein; V_{fs}: volume (L) of the soluble phase; t: incubation period in hours (h); and MS: dry weight of the biosolide (kg).

Static respirometry test

The stability test of the compost measured through the quantification of microbial respiration, is based on the production of carbon dioxide in the sample and expressed as the amount accumulated during a specific period of time (mg C-CO₂ / g C day). Samples were taken in triplicate on days 1, 3 and 7. The analysis consisted in taking each vial with 20 ml of 1 M NaOH and quantifying the CO₂ by titration with hydrochloric acid (0.5 N). The total CO₂ produced in the biosolid after 7 days of incubation was calculated according to equation 2 (Oviedo-Ocaña et al., 2015):

$$C-CO_2 = (B-M) * N_{HCl} * 4 * 12 / P_{soil} * d \quad (2)$$

Where: B: consumed volume of acid used in the titration in the blank (mL); M: consumed volume of acid in the sample (mL); NHCl: normality of HCl (0.5 N), 4 is the dilution factor (20/5), 12 is the atomic weight of C; P_{soil}: weight of the biosolid on a wet basis (mg); d: days of incubation (7 d).

Maturity test

To demonstrate the maturity of the biosolids, it was evaluated using the germination index (GI) (Komilis and Tziouvaras, 2009) using certified red tomato seeds (Optimax). The biosolids extract was obtained by weighing 15 g (wet basis), placing it in plastic bottles of 100 ml capacity and adding distilled water in a ratio of 10:1 (water ml:g of biosolids), stirring for 30 minutes at 100 rpm in a reciprocating shaker (Eberbach) and allowed to stand for 15 minutes; of the supernatant 5 ml were taken and placed in petri dishes with 25 seeds of red tomato (Optimax), the incubation was for 7 days at a temperature of 22 ° C in the dark. The same procedure was carried out for the control (distilled water). The germination index (expressed as a percentage) was calculated as follows (Komilis and Tziouvaras, 2009), equation 3:

$$GI = \frac{\frac{\text{Germinated seeds (sample)}}{N}}{\frac{\text{Germinated seeds (control)}}{N}} \times \frac{\frac{\text{TLRGS (sample)}}{\text{NGS (control)}}}{\frac{\text{TLRGS (sample)}}{\text{NGS (control)}}} \quad (3)$$

GI: germination index; TLRGS: Total length of the root of germinated seeds; NGS: number of germinated seeds; N: number of seeds placed in the petri dish.

Results

Composting of sewage sludge

The effectiveness of the composting process is influenced by factors such as temperature, oxygen supply (aeration), moisture content, pH, C:N ratio, particle size and the degree of compaction (Juárez et al., 2015). Temperature is one of the main parameters to monitor the composting process and can vary according to the process phase. Chen et al., (2015) divide the composting process into four phases in relation to temperature: mesophilic (20-40 ° C), thermophilic (40-65 ° C), cooling (<40 ° C) and maturity (room temperature). However, Lazcano et al., (2008) propose only two phases: thermophilic (active phase) and mature (decreased temperature). In both cases, the common factor -independent of the number of phases-, is the change in the initial temperature of the composting process and its increase due to the biodegradation of organic matter by microorganisms (Raut et al., 2008). The temperature interval for each phase is particular in each composting process and will depend basically on the mixture used. Figure 1 shows the temperature profile of the sewage sludge composting (WWTP, Huatulco) mixed with sawdust (1:1 w / w).

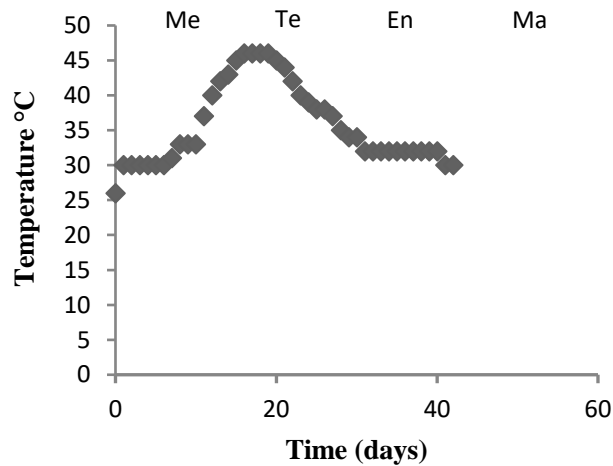
The initial temperature of the process registered a value of 25 ° C and increased to 32 ° C in the first 10 days (mesophilic phase), then from day 11 to 20 the temperature increased until reaching the maximum of 45 ° C (thermophilic phase); the decrease in temperature (cooling phase) was recorded on day 21 to 30, and from day 31 the recorded temperature was 30 ° C, remaining constant and unchanged (maturity phase) until day 42. As it is observed in the temperature curve of the composting process (Fig. 1) it comprises a period of approximately 10 days between each phase, and compared with that reported by Banegas et al., (2007) the interval of days between phases is similar to the generated in this work (8 to 10 days). The thermophilic phase reached the maximum temperature of 45 ° C,

The presence of easily biodegradable material from the sewage sludge promoted greater microbial activity (Oviedo-Ocaña et al., 2015; Hassen et al., 2001). The temperature of 45 ° C recorded in the present work is reported for composting piles with anaerobic sewage sludge and sawdust (Banegas et al., 2007), and is below the reported temperature for the composting of aerobic waste sludge (> 50 ° C); the maximum temperature difference between the types of sewage sludge, may be due to the low organic matter content of the sewage sludge used in this experiment (3.0%), and therefore, a lower substrate availability and lower microbial activity; In addition, the anaerobic sludge contains more stable organic matter (lignin and cellulose) and a lower content of easily biodegradable compounds (Manios 2004), which are used by the microbial population (as a carbon source) present in the composting mass.

As in the thermophilic phase, differences in time have been reported to reach the maturity phase. Alidadi et al., (2008) reported that after 100 days the maturity stage was reached in the aerobic sewage sludge mixed with sawdust; and in the case of anaerobic and sawdust sewage sludge, the maturity phase was reached at approximately 60 days (Banegas et al., 2007). In our compost the maturity phase was obtained from day 31, less than half the time reported by Banegas et al. (2007); this was due to the proper handling of the compost pile.

The frequency of (daily) turning of the compost mass in the early stages of the degradation of organic matter can result in a shorter time to reach the stage of maturity (Awasthi et al., 2014); the daily turning together with the application of water, allowed to maintain the moisture content by 50-60%, this percentage is necessary for biological activity during composting (Luangwilai et al., 2011); The above was achieved with the mixture of sludge and sawdust (1:1 v / v), since the latter has a high water adsorption capacity (Iqbal et al., 2010). In addition to the aforementioned, the environmental temperature is another factor that allowed the maturity phase to be reached more quickly; unlike cold and temperate climates where the increase in temperature is slower for the composting processes to take place (Margesin et al., 2006), the tropical climate seems to be more favorable in maintaining the proper temperature during composting (Nguyen and Shima, 2018).

In the present work, the average daily temperature was 30 ° C (dry tropical climate), therefore, the time to obtain the compost was lower.



Graph 1 Monitoring of the temperature recorded during the composting process of the sewage sludge, and the stages of the process: Me (mesophilic), Te (Thermophila), En (cooling), Ma (maturity)

Source: self made

Physical-chemical and microbiological characterization of biosolids

In the Mexican regulations (NOM-004-SEMARNAT-2002), a biosolid is defined as "sludge that has been subjected to stabilization processes and that due to its content of organic matter, nutrients and characteristics acquired after its stabilization, can be susceptible to exploitation". In the same standard 004, criteria for the classification of the use of biosolids are established according to the minimum and maximum limits based on the content of heavy metals, and the content of pathogens and parasites.

In the present work, the content of heavy metals Cd, Pb, Ni and Zn registered in the biosolid (Table 2) is well below the minimum allowable limit according to the norm 004, therefore, the biosolid is classified as **excellent**. With respect to the bacteriological content of pathogens and parasites in the biosolids, the value is below the maximum limit allowed by NOM-004-SEMARNAT-2002 (Table 2), therefore, it is classified as **class A** biosolids.

According to the obtained results and the quality (class A) of the biosolids, the same norm 004 establishes that the biosolids can be used for urban use with direct public contact, forestry and agricultural use, as well as soil improver-degraded soils. However, in the environmental aspect it can be used for the remediation of soils contaminated with hydrocarbons, heavy metals, saline soils, among others. In general, the biosolids presented better characteristics in relation to the initial characteristics of the sewage sludge. The content of organic matter, from 3.0 to 3.7%, is considered a high content (NOM-021-RECNAT-2000); the water holding capacity in the biosolid was increased 1.3 times; the C:N ratio and the pore space increased by 2.6 and 1.1 times, respectively. The real and apparent density decreased by 3.5 and 15 times (respectively), which is reflected in the highest percentage of porous space in the biosolid (94.3%).

The content of helminth eggs and fecal coliforms decreased with composting (Table 2); however, according to the regulations, the sewage sludge did not present problems in this characteristic. The metals analyzed decreased or remained at the same concentration, with the exception of the Cd content in the biosolids, which increased 1.7 times, remaining below the permissible limits (Table 2).

The pH was maintained without significant change, step from 5.7 to 5.2, both classified as moderately acidic (NOM-021-RECNAT-2000). The electrical conductivity of the biosolid was 2.7 (dS m⁻¹) and according to NOM-021-RECNAT-2000 it is classified as moderately saline. In this last characteristic the biosolide must be used taking the pertinent precautions; although it did not present phytotoxicity (see results of GI).

characteristics	Sewage skudge	Biosolid	NOM-004
pH	5.7	5.2	NA
EC (dS m ⁻¹)	3.0	2.7	NA
OM (%)	2.32	3.7	NA
WHC (mL g ⁻¹)	4.21	3.07	NA
TN (%)	1.75	2.05	NA
TOC (%)	0.41	2.2	NA
C:N	ND	1.07	NA
Dr (g cm ³)	1.4	0.4	NA
Da (g cm ³)	0.30	0.02	NA
EP (%)	78.8	94.3	NA
HH	1	0.0	< 1
CF	3	2	< 1000
Cd	1.8	3.6	39-85
Pb	15.3	13.9	300-840
Ni	3.8	3.8	420
Zn	62.9	44.9	2800-7500
Ar	ND	ND	41-75
Cr	ND	ND	1200-3000

NA (not applicable). ND (not determined). The value of heavy metals is expressed in mg Kg⁻¹ on a dry basis.

Table 2 Physical, chemical and microbiological characteristics of the sewage sludge (from the WWTP in Bahías de Huatulco) and the biosolids (final product of the composting process)

Source: *Self Made*

Stability and maturity of biosolids

Stability is a term related to the resistance of the organic matter of a product (compost) against degradation or greater microbiological activity (Hachicha et al., 2009); In the present work, it was determined by two tests: the fluorescein diacetate enzymatic assay (FDA) and the static respirometry (CO₂ production). The low microbial activity in both tests (Table 3), is explained by the fact that the compost went through an active degradation stage and the majority of degradable carbon has already been eliminated, leaving only the recalcitrant material (Komillis et al., 2011).

For the average values obtained in the aforementioned tests: FDA 8.4 and C-CO₂ 0.28 mg kg⁻¹ (for both), the biosolid is classified as Very stable (Komillis et al., 2011). The stability in a compost is very important; an unstable or immature organic product prevents the growth of plants and negatively affects soil quality (Oviedo-Ocaña et al., 2015) if used for this purpose.

The result of the germination test (GI) with the application of the biosolids extract in the tomato seed was 84.15%.

According to the interpretation criterion of the GI, they are classified into three phytotoxic categories (Zucconi et al., 1981): i) severe, GI values ≤ 50% indicate that there is a strong presence of phytotoxic substances; ii) moderate, if a value between 50 and 80% is obtained, it is interpreted as the moderate presence of phytotoxic substances; and iii) mild, if the GI ≥ 80%, which indicates that there are no phytotoxic substances such as volatile organic acids or the content of phenols, or are in very low concentration (Zubillaga and Lavado, 2006). The biosolid in the described conditions, has the characteristic of desirable maturity to be used effectively in agriculture, as a soil improver or to test it as an attenuator of some type of contaminant in soil (eg. herbicides).

Sampling day	FDA mg kg ⁻¹ h ⁻¹	C-CO ₂ g kg ⁻¹	Classification of biosolids
1	7.7	0.45	
3	8.1	0.19	Very stable*
7	9.6	0.22	

Table 3 Classification of biosolids with reference to their stability, calculated by enzymatic assay (FDA) and static respirometry (CO₂ production). * Classification according to Komillis et al. (2011). Values are reported on dry biosolid basis.

Source: *Self Made*

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Conclusions

At the end of the composting process, a final product (biosolid) of good quality was obtained with the desired physical-chemical and microbiological characteristics, and according to NOM-004-SEMARNAT-2002, the biosolid obtained is **type A of excellent** class. According to Oviedo-Ocaña et al. (2015), it is difficult to obtain a biosolid that complies with both stability and maturity properties; however, and based on the stability index and biosolid maturity index, which is classified as **Very stable**, and **excellent** class according to NOM-004-SEMARNAT-2002.

We conclude that biosolids are suitable for use in different ways: 1) as a soil improver, it could be applied to agricultural crops in the coastal area of Oaxaca, including jamaica, papaya, corn, tomato and coffee; 2) in bioremediation of contaminated soils, as an attenuator of some type of contaminant (eg, herbicides, insecticides, among others), and 3) for urban, forestry and agricultural use.

In addition, we can say that by mixing both waste: sewage sludge and sawdust - a biosolid with the aforementioned quality - is an excellent alternative to reduce the amount of waste generated, its proper disposal and reduce the impact on the environment and human health.

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