Biodegradation studies of sapropel-based composite materials

V. Obuka¹, O. Muter², M. Sinka³, M. Klavins¹

¹ Department of Environmental Science, University of Latvia, Jelgavas street 1, LV 1004, Riga, Latvia
² Institute of Microbiology and Biotechnology, University of Latvia, Jelgavas street 1, LV 1004, Riga, Latvia
³ Riga Technical University, Faculty of Civil Engineering, Institute of Materials and Structures, Kalku Str. 1, Riga, LV-1658, Latvia

E-mail: vaira.obuka@lu.lv

Abstract. Traditional composite materials used for construction purposes currently face many questions regarding their sustainability – mainly because they do not come from renewable sources and due to the problems related to their end-of-life management. These challenges motivate companies and researchers to look at the natural fiber composite materials with increased interest. Usually natural fiber materials in construction are used together with mineral binders, but in this research new organic binder – sapropel (organic rich lake sediments) is used as a binder for natural fiber composite materials with various fillers. In previous research these composite materials have proven their applicability in construction industry due to their sufficient mechanical strength and low thermal conductivity. Thus, in this research evaluation of the biodegradability of composite materials were done, comparing them to natural fiber materials with mineral binders. As a methods respiration intensity of microorganisms in soil and enzyme activity of microorganisms were used. For studied materials biodegradability potential have been examined, depending on the filler properties and presence of mineral matter content in the obtained composite materials.

Key words: Sapropel, Composite materials, Biodegradation, Environmental applications

1. Introduction

One of the key questions regarding development of new construction materials is their sustainability - mainly because industrial construction materials do not come from renewable sources and due to the problems related to their end-of-life management [1;2]. Traditional natural fiber materials partially answer these challenges as they use mineral binders and natural fiber fillers. In this research a new kind of organic binder - sapropel (organic rich lake sediments) is used.

Sapropel-based composite materials can be considered to be a new natural fiber composite materials which are cost effective, recyclable and locally produced, as sapropel is a byproduct of lake recultivation
processes and various filler materials, which are byproducts from such industries as agriculture and wood processing, can be used. Natural fiber composite materials should not only have properties required by the industry, but also be biodegradable after the end of their life cycle.

Sapropel can be used in the production of composite materials for building industry, interior functional design objects with both cold compaction techniques and hot–glue press at elevated temperature and pressure [3, 4]. In the previous research of sapropel-based materials prospective area of their application included creation of different composite materials: Sapropel – wood fiber, sapropel – wood sanding dust, sapropel – hemp shives [5], sapropel – wood-chips [6]. In these composite materials sapropel was used as a binder [5, 7, 8]. The research results showed that the use of sapropel as a binder and various byproducts as a filler allows the finished product to be included in the thermal insulation material category according to the technically qualitative indicators. In addition, the results of compressive and flexural strength resistance show that the strength of the composite materials is sufficient to be used in the industry.

Evaluation of biodegradability of various materials in soil is based mainly on the measuring evolved carbon dioxide as a function of time [9]. Microorganisms play an important role in maintaining nutrient cycles in soil (C, N, P, S) by recycling organic matter [10]. The activity of microorganisms depends on soil characteristics, climatic conditions and soil tillage technologies.

Conditions with different humidity were modelled in experiments. Biotechnology often uses so-called slurry systems [11, 12]. Slurry systems with high humidity and water content have a beneficial effect on the activity of biodegradable microorganisms due to the better transfer of substances.

Our study was aimed at comparative evaluating of the biodegradability of ten composite materials, which have been newly-developed from different types of sapropel and wood, hemp derived fillers. Enzyme and respiration activity of microorganisms in soil in the presence of composite materials was chosen as the evaluation criteria of biodegradability.

2. Materials and methods

2.1. Sapropel (binder for composite materials) sampling.

Sapropel sediments were sampled from three lakes in Latvia – Padelis (further: carbonate sapropel (CS)), Veiveru (further: green algae sapropel (GAS)) and Pilvelu (further: cyanobacteria sapropel (CBS)), located in Rezekne District, Latgale Region. Loss on ignition (LOI) method was applied in order to estimate moisture content, content of carbonate matter and organic matter of sediments [13]. Moisture content of sapropel was determined after drying at 105°C, followed by organic matter estimation at 550°C for 4 h. The content of mineral substances was determined after heating at 900°C for 2 h.

2.1.1. Fillers for composite materials

Birch wood sanding dust (also known as wood dust) and fiber (also known as wood fibers), and hemp shives were selected as fillers for production of composite materials. Birch wood sanding dust and fiber are industrial by-products coming from JSC “Latvijas finieris” – a plywood manufacturing company. Birch wood fiber was up to 15 mm long and up to 0.1 mm thick (diameter). In addition, hemp shives ("Bialobrezeskie") were taken from "Zalers" Ltd. Hemp shive slices were up to 5.5 cm long and up to 0.6 cm thick.

2.1.2. Composite material preparation and curing

For the developed composite materials three types of raw sapropel have been used as a binder, i.e., green algae sapropel (GAS); cyanobacteria sapropel (CBS) and carbonatic sapropel (CS). Sapropel was mechanically treated by mixing together with electrical hand mixer until smooth and homogeneous material was formed. Mixing of sapropel–filler mass was done manually until homogeneous and smooth mixture was reached at the stage, where filler was fully covered with sapropel. Binder-filler mass ratio was 6:1. Metal molds with dimensions of 30×30 cm and with adjustable height were used for composite material production. The mixture of raw materials was laid in by layers in molds for more dense composite material structure, higher mechanical strength and for minimizing final product shrinkage. Sapropel-filler specimens were cured at the temperature of 80 – 105°C for 36 – 72 hours until the constant weight was reached.
For a reference of biodegradation tests a biocomposite materials with the same filler (hem peat) were used. Mineral binders developed in previous studies were used for these materials - dolomitic lime consisting of 100% DL60 lime (Dolomite) and hydraulic lime consisting of 60% DL60 lime and 40% calcinated kaolin clay (Clay) [14]. Binder-filler mass ratio was 2:1. Block peat ("Laflora") was also used for composite materials biodegradation studies as a control material.

2.2. Evaluation of the biodegradability of the tested materials

In order to compare the potential biodegradability of the composite materials tested, an experimental scheme was developed: soil microbial enzyme activity and respiration were used as the main indicators [15]. Considering the need for microorganisms to adapt to the substrate, 7-day incubation period was included in the process requiring appropriate physicochemical conditions, growth factors (macro- and microelements, nutrients, vitamins). To accelerate the biodegradation process, the composite materials were placed in the soil amended with a substrate (molasses and a source of vitamins (plant extract)), as well as a consortium of soil-derived microorganisms with a high hydrolytic activity [16]. Altogether 10 composite materials were evaluated.

Incubation of 0.25 mg specimen for evaluation of the biodegradability of the tested materials was performed in the sealed 100 mL vessels containing 10 g soil at 37 ± 2 °C for 7 days. The composition of the substrate added to the specimens was the following: 10 g clay loam soil, 2 mL mineral broth, 100 μL 30% molasses, 200 μL cabbage leaf extract, 100 μL inoculum (2.0x10^10 CFU/mL) and 50 mL sterile distilled water [16]. The composition of mineral broth was the following, g/L: MgSO\(_4\) – 0.2, CaCl\(_2\) – 0.02, KH\(_2\)PO\(_4\) – 1.0, K\(_2\)HPO\(_4\) – 1.0, NH\(_4\)NO\(_3\) – 1.0, FeCl\(_3\) – 0.05). Specimens were prepared in three replicates. Soil moisture was 60% of the maximum water capacity. The physicochemical characteristics of clay loam soil are summarized in Table 1.

2.2.1. Respiration intensity of microorganisms

The microbial respiration was tested according to [15] with some modifications. The glass with 5 mL 0.05 M NaOH was placed in the sealed 100 mL vessel as described in 2.5. The respiration was estimated by back-titration of the unreacted NaOH using 0.05 HCl (adding 0.1% phenolphthalein indicator to the NaOH prior to titration). Two measurements of respiration have been made for each vessel with a sample, i.e., at the beginning of incubation and after 7 day of incubation period. Respiration assay was performed at 23 °C for 24h in the dark.

The intensity of the SIE was calculated by the formula [17]:

\[
SIE = \frac{(A - B) \cdot 1.1 \cdot 60}{m \cdot h}
\]

where,
- A - titrated, control sample, mL;
- B - titrated for the test sample, mL;
- 1.1 - coefficient (depends on molarity);
- 60 - coefficient (for conversion into hours);
- m - sample weight, g;
- h - trial time, min.

2.2.2. Enzyme activity of microorganisms: fluorescein diacetate hydrolysis

After 7 day incubation period, as described in 2.5., the fluorescein diacetate (FDA) hydrolytic activity of microorganisms was tested. 100 μL specimen was transferred to 1 mL tube containing 400 μL reaction mixture (4 mg FDA, 2 mL acetone, 48 mL 0.06M phosphate buffer, pH 7.6). The mixture was incubated for 60 min at + 37±2 °C [18, 19]. After incubation, 500 μL acetone was added to the specimens to stop the hydrolysis reaction. The optical density was measured at 490 nm using a microplate reader Infinite f50 (TECAN, Swirtzeland). Calibration curve was prepared using the thermally hydrolyzed FDA.
Table 1. Characteristics of the soil used

| Parameter            | Values | Parameter                          | Values |
|----------------------|--------|------------------------------------|--------|
| N, %                 | 0.20   | Electrical conductivity, mS/cm      | 0.162  |
| C, %                 | 2.06   | Water capacity, %                  | 149.47 |
| PO₄³⁻, μg/g          | 47.8   | Na, mg/kg                          | 14.3   |
| P, μg/g              | 15.6   | Mg, mg/kg                          | 218.5  |
| pH (1M KCl)          | 6.72   | K, mg/kg                           | 146.5  |
| Redox potential, mV | -24.9mV| Ca, mg/kg                          | 1802.6 |

The soil characteristics of the clay loam used in the experiment was determined (Table 1) using standard soil analysis methods. The Na, Mg, K, Ca content of the specimens was determined using a PerkinElmer AAnalyst 200 atomic absorption spectrometer. A non-electrode discharge lamp (Perkin Elmer) was used as a source, Na measurements were made at 589 nm wavelength, Mg at 285.2 nm, K measurements at 766.5 nm and Ca at 422.7 nm using flame atomization. N₂O, acetylene was used as the oxidizing gas. ANOVA (Anova: Single factor analysis) was used for statistical data processing.

3. Results and discussion

3.1. Composite materials (sapropel sampling)

Sapropel samples differed from one another in terms of moisture, organic matter and carbonates.

Table 2. Characteristics of the sapropel samples

| Lake                        | Moisture, % | Organic matter, % | Carbonates, % |
|-----------------------------|-------------|-------------------|---------------|
| Padelis (carbonatic sapropel; CS) | 85.97       | 15.27             | 35.57         |
| Pilvelu (cyanobacteria sapropel; CBS) | 94.99       | 84.51             | 1.26          |
| Veveru (green algae sapropel; GAS) | 97.66       | 86.25             | 1.18          |

Characteristics of the sapropel samples are shown in Table 2. For example, Lake Padelis sapropel sample contained 35.57% carbonates, moisture – 85.97%, and color – pale gray-pink. Lake Pilvelis sapropel sample was dark greenish brown with homogeneous and jelly-like structure. Lake Veveru sapropel sample moisture level was comparatively high, i.e. 97.66% and organic matter – 86.25%.

3.2. Evaluation of the biodegradability

Biodegradation experiments were performed by adding the composite materials to the soil amended with nutrients and a consortium of microorganisms with a high hydrolytic activity [16] in order to provide favorable conditions for degradation processes.

3.2.1. Soil microbial enzyme activity after incubation in the presence of composite materials

One of criteria for evaluating the biodegradability of the tested materials could be an increase of microbial enzyme activity, which responded to the presence of bioavailable nutrients. FDA hydrolysis involves the activity of various enzyme groups of microorganisms, i.e., hydrolases, proteases, esterases, lipases, etc. [20]. As shown in Fig. 1, after 7-day incubation period all composite materials added to the soil stimulated FDA hydrolysis activity, comparing with the control set. After 7-day incubation period in the batch system, the lowest FDA hydrolysis activity was observed in the non-composite control (Fig. 1). All tested composite materials showed a stimulating effect on the enzyme activity of the microorganisms, with the highest mean value for [GAS-CS/Hemp shives], i.e., 2.01 ± 0.75 μM FDA h^{-1} g dw^{-1} (Fig. 1). Comparison of the FDA hydrolysis activity showed a statistically significant (p<0.05) difference between control and composite materials, except CS/wood fibers. Greater FDA hydrolysis activity may indirectly indicate more intense biodegradation processes, as it depends on the availability of nutrients, the concentration of microorganisms, and their physical, chemical and environmental properties [20, 21].
Figure 1. Fluorescein diacetate hydrolysis activity of microorganisms in a clay loam soil amended with nutrients, microbial consortium and composite materials. The ratio of a composite material to the substrate was 0.25:10.0. The substrate was prepared as described in Materials and Methods. FDA activity was measured after 7 day incubation period of a composite material with soil at 37 °C. GAS – green algae sapropel; CBS – cyanobacteria sapropel; CS – carbonatic sapropel. Control – the soil substrate without composite materials.

3.2.2. Respiration intensity of soil microorganisms in the presence of composite materials
The respiration intensity of microorganisms in the experimental batches was observed before and after 7 day incubation period at 37 °C. An increase of respiration intensity in the composite materials has been observed. The amended batches at the beginning of incubation showed statistically significant difference (p<0.05) and varied in the range from 31% to 70%, as compared to the control batch (Fig.2A). The highest respiration intensity was in the soil containing CS/Wood fibers, while the lowest – CS/Wood sanding dust, i.e., 7.68 ± 0.35 and 5.92 ± 0.43 µg C-CO\(_2\) h\(^{-1}\) g dw\(^{-1}\), respectively (Fig.2A). Among the types of composite materials, no statistically significant differences in respiration stimulating effect were found.

Figure 2B summarizes a second test carried out after 7 day incubation period, when readily available substrates have been used by microorganisms. The respiration intensity of microorganisms after 7 day incubation period was considerably lower than that at the beginning of the experiment. This can be explained by the fact that microorganisms have already degraded easily degradable substances. Subsequently, this data indicate the degradation state of comparatively hardly biodegradable substances (cellulose, hemicellulose, lignin) resulting in the original material fractionation in respect to polymer stability. No respiration was detected in the control soil. The highest respiration intensity was detected in CBS-CS/Hemp shives, while the lowest – in CBS/Wood sanding dust, i.e., 2.70 ±0.89 and 0.70 ±0.87 µg C-CO\(_2\) h\(^{-1}\) g dw\(^{-1}\), respectively (Fig.2B).

The obtained data can be interpreted as the potential biodegradability of the tested composite materials under given test conditions. It shows that the materials used are biodegradable but with varying rate. It can be seen that it is mostly dependent on the used filler, the wood sanding dust has the highest biodegradability ratio as it shows the lowest respiration after 7 days, wood fibers and hemp shives have lower biodegradability as they have higher respiration after 7 days. Sapropel binder shows similar reparation as the reference lime binders, as the used sapropel is with high carbonate percentage. The used materials demonstrate that with varying ratio all of the materials are biodegradable and can be used to decrease the overall environmental impact of construction materials.
Figure 2. Respiration intensity of microorganisms in a clay loam soil amended with nutrients, microbial consortium and composite materials. The ratio of a composite material to the substrate was 0.25:10.0. The substrate was prepared as described in Materials and Methods. Respiration intensity was measured before incubation (A) and after 7 day incubation period (B) of a composite material with soil at 37 °C. GAS – green algae sapropel; CBS – cyanobacteria sapropel; CS – carbonatic sapropel. Control – the soil substrate without composite materials.

Previous studies of composite materials from peat and cellulose fibers [22] demonstrated reasonably good resistance in respect to destruction, as after a month of incubation their biodegradability reached 20-30%. Overall, in the experiment described above, the highest biodegradation intensity was in materials consisting of peat, 85% cellulose fiber and grape processing by-products. It is explained by the fact that the grape processing residues are an additional filler between the fibers, reducing the fiber content. However, the smallest biodegradation activity was shown in the mixture of peat and 100% cellulose fiber. In turn, the results of biological activity expressed in mg CO₂ per 100 g of substrate
indicate the lowest activity in studied composites with increased mineral content [22]. Also our study demonstrate similar trend.

Patnaik and colleagues [23] have explored the biodegradation potential of thermal insulation and sound insulation materials, from wool waste and recycled polyester. Sheep wool showed the highest level of biodegradation over 50 days followed by sheep wool products and a control sample of cellulose fiber. At the same time, recycled polyester showed the lowest biodegradation level. Thus, it can be concluded that composite material, just as those developed in our study at the end of its life cycle, can be used on agricultural land as a nitrogen fixator for plants, thus reducing the fertilizer utilization rate [23].

4. Conclusions

Organic rich lake sediments (sapropel) – a byproduct of the lake cleaning – can be efficiently used to develop composite materials together with other production waste materials. Sapropel of different origin (green algae sapropel, cyanobacteria sapropel and carbonatic sapropel) at the development of composites serves as binder, but birch wood fiber, sanding dust, hemp shives as a filler material. The biodegradability of the obtained composites has been studied and major differences of the biodegradability potential have been found, mostly depending on the filler properties, but also on the presence of mineral matter content in the obtained composites. The obtained results demonstrate potential to use sapropel as a raw material for composites in combination with other waste materials with potential application as construction materials and design products, to extend the life of natural materials and achieve aims of reduction of waste streams.

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