Original Research Paper

Evaluation of particle recovery from microalgae

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1. Introduction

Coccoliths are micro-structured $CaCO_3$ particles synthesized intracellularly by the algae family of Coccolithophorida. Although they are known to exist from the Triassic period, the particles have been discovered and linked to the algae clade only two centuries ago [1,2]. Since then, coccoliths have contributed immensely in biomineralization studies [3–6] and acted as a source for paleothermometry [7]. The total number of species, identified from fossil and current records, reaches above 4000 [8,9]. From them, the majority are now extinct and there are only a small number of living species that has been successfully cultivated and studied in laboratories [10–12]. One such example, the Emiliania huxleyi species, will be the main focus of this work. Emiliania huxleyi is capable of calcification in the diploid phase and produces heterococcoliths, known to be complex structures typically assembled by up to a hundred crystal units [13,14]. Each coccolith's form is speciesspecific and there are many forms, shapes and sizes to be found. This particle variability has helped to distinguish the numerous participants from each other and has been used as a basis to establish the current classification schemes [15,16]. Based on those criteria, the coccoliths observed herein have a placolith profile, are very small in terms of size classification (<3 lm), have an elliptical shape and belong to Type A from the six different morphotypes known for Emiliania huxleyi [17]. Apart from observing their morphology for the above-mentioned purposes, studies have also focused on further coccolith properties. An example of that, is the estimation of the particles' weight [18,19]. In some of the most recent studies, cross-polarized light [20] and circularly polarized light [21] estimated an approximate weight of 2.3 to 2.6 pg for one single Emiliania huxleyi coccolith [18,21].

The biomineralization patterns and the crystallographic orientation of coccoliths are well documented. However, a closer look at this impressive and still increasing literature database reveals several gaps when it comes to applicability. Current knowledge about the particle's potential and their superior features to inorganic calcium carbonate such as their high specific surface area [22], higher stability in undersaturated calcite solutions [23] and their light illuminating properties under magnetic fields [24] have yet to be translated into specific applications. The particles are seen as a key for future micro and nano devices with their structural geometry acting as a means to transport fluids and their surface charge enabling targeted ion transportation [25]. This clearly demonstrates that work needs to be done, in order to shift from hypothetical implementations to real and concrete ones. By stepping however, towards this direction, one is immediately faced with further fundamental uncertainties. What are for example, the most common methods to recover coccoliths? How is the particles' structure affected by these processes? Or, for instance, how does the particles' distribution change with treatment? It is supposed at this point, that any superiority observed for coccoliths would be traced back to their structure, their composition and to the fact that they are narrowly distributed in terms of size. Further, it is expected that individual particles offer different properties than agglomerated ones and that broken coccolith pieces will lack features caused by their unique structure. Bearing these suppositions in mind, shows that the condition of particles, meaning their intact form and their presence as individual entities, is an important factor that needs to be considered throughout their recovery.

The aforementioned uncertainties have acted as the driving force for this study. The behaviour of the particles, their intriguing characteristics and finally the establishment of an efficient yet simple recovery were some of the questions that were aimed to be addressed. Therefore, with this ultimate goal in mind, known recovery methods found in literature were tested for identical samples. It was important at this point, to conceive a clear idea of what has already been tried in the past and evaluate the outcome. To our knowledge this has not been done for freshly cultivated and calcifying algae. Besides the above, considerable attention was given to appropriate characterization methods. By expanding the list of methods that have been used in previous studies and shifting the focus from ''what can coccoliths reveal to us" to ''what can coccoliths offer us", we hope to contribute to this still young field of research.

Recovery of coccoliths over the years

Coccoliths became popular in trace element chemistry after a substantial amount of studies had been performed for other species [26,27]. Primarily, the particle's structure was only perceived as a means to extract information and was dissolved in the final step to give its elemental composition. In addition to that, the first treated samples originated from sediment traps of various geographic locations while laboratory samples were treated much later. For this large variety of coccolith samples, two main cleaning approaches have been followed. The first one being of a chemical and the second one of a mechanical approach. In this study, we will focus and evaluate methods following a chemical approach.

To that counts for example the method proposed by Boyle, where a combination of 0.1 M NaOH and 30% H_2O_2 was used to clean foraminifera species [28]. Foraminifera species and coccoliths are similar in that both produce a calcium carbonate shell and because of the material similarity this method will be reviewed here as Method A. As in the original publication, the ratio of reagents was not unequivocally provided and we followed a 1:6

Table 1

ratio for Method A. Boyle later published a modified version where the reagents' ratio was set to 1:2 [29]. This second attempt, taken again from foraminifera species, will be reviewed as Method B. Method C was presented by Jakob et al. and counts to the very few attempts to isolate and clean a large volume of coccoliths from laboratory cultivations (gL^{-1} scale) by using 12% NaOCl [30,31]. In yet another study, originally developed for sediment trap samples, 2.8% NaOCl and 35% H_2O_2 were used synergistically to remove organic remnants (Method D) [32]. Method A, B and D were reviewed for an artificial system, composed of synthetic calcium carbonate and non-calcifying Chlorella algae [7]. In this same publication, the authors added two further methods, developed for botanical samples [33]. Both used different concentrations of tetramethylammonium hydroxide (TMAH), a typically used tissue solubilizer in an effort to leach organic matter (Method E, Method F) [7]. Since most methods, particularly the ones treating sediment trap samples, had different compositions as well as objectives we adjusted the methods in a way to highlight the effect of the oxidizing agent in question. A detailed description of the six methods as used in this study can be found in the supplementary material.

The variability of samples, characterization methods and purpose in the above studies explains the current limitations in this field. However, at the same time, this lack of cohesiveness gives a clear indication about the required plan of action. With that said, the six methods will be applied to a laboratory cultivation of Emiliania huxleyi. In contrast to the study presented by Stoll et al., we here use a calcifying algae species and analyse each method by giving particular attention to the particles structure, composition and dispersibility. On top of that, it is interesting to compare our results of a calcifying species to the artificial system used in the mentioned study. The following table (Table 1) gives a summary of the used methods by also summarizing both the sample they were originally developed for, the treatment duration and the characterization method used to evaluate the results. It shows evidently the variability amongst the samples as they alternate between laboratory cultivations, fossil coccoliths and artificial systems made up by synthetic $CaCO_3$.

2. Materials and methods

2.1. Cultivation of coccoliths

Emiliania huxleyi cells (CCMP 3266) were cultivated in a 5 L carboy bottle reactor at 17 C in modified Enriched Seawater, Artificial Water (ESAW) medium [31]. The nutrients were sustained at 34.08 mgL^{-1} NO^{-}_{3}, 2.1 mgL^{-1} PO^{3-}_{4} and 350 $mg{\cdot}L^{-1}$ Ca^{2+} while the light intensity was set at a photon flux density (PFD) of 300 $\mu molm^{-2}s^{1}$ (Li-Cor LI-250 A). Through daily $NaHCO_3$ addition, the total alkalinity (TA) was retained above 230 $mg{\cdot}L^{-1}$ and the pH was sustained at 8.5.

2.2. Characterization methods

Scanning electron microscopy images were obtained using a JSM-6390LV (Jeol) at an accelerating voltage of 5 kV. The particle size distribution of the suspension was measured by laser diffraction spectroscopy (LDS). For the LDS (Helos KR; Sympatec) the sample was dispersed in 1 $g{\cdot}L^{-1}$ tetrasodium pyrophosphate decahydrate ($Na_4P_2O_710H_2O$) solution. Cells and coccoliths were estimated with a haemocytometer (Neubauer Improved, BlauBrand) under light microscopy (Axiotech 100, Zeiss). Thermogravimetric analysis (TGA) was performed under an O_2 - N_2 stream ($4\ mL{\cdot}min^{-1}$ to $16\ mL{\cdot}min^{-1}$) in a temperature range from 30 1000 C and a heating rate of 10 K per minute (TG 209 F1 Iris; Netzsch). Fourier transform infrared spectroscopic analysis (FTIR) was implemented to identify the surface chemical composition of the material (Nicolet 6700; Thermo Scientific). Prior to analysis the samples were dried at 80 C overnight and finely ground with an agate mortar and pestle to increase the penetration depth for the germanium crystal (penetration depth 0.67 μm). Light microscopy captures were made by a Carl Zeiss Axiotech 100 HD camera. The specific surface area was estimated using nitrogen adsorption by continuous flow on a NOVA-e (Quantachrome) instrument. A multipoint BET incorporating five points was used in a relative pressure range of 0.05 to 0.35.

3. Results

The algae cultivation run for 52 days and noted a cell concentration of 7.310^{6} $cellsmL^{1}$. At the end of the cultivation the particle concentration was estimated at 2.310^{8} $coccolithsmL^{1}$. The 5 L cultivation broth was concentrated to 600 mL by removing the supernatant after cells and coccoliths had deposited to the bottom of the vessel. Finally, equal samples of 20 mL were prepared by rotational sample dividers (Retch PZ and Arbo AF 14) and treated with Method A – Method F.

3.1. Reference Material (RM)

The result of the laboratory cultivation is a material mixture composed of salts, cell remnants, coccoliths bound in their original structure (coccospheres) and individual coccoliths. Scanning electron micrographs of the reference material can be seen in Fig. 1.

Fig. 1 gives a first impression of the intriguing shape of coccoliths. As can be seen, the particles appear to be in a good condition, which is a sign that the cultivation run smoothly with the right amount of nutrients, temperature and Ca^{2+} supply. In the lower magnification capture (x2500) salt remnants and partial coccospheres are visible. In the higher magnification (x5000) the disclike shape of the Emiliania huxleyi coccolith is shown. The particles are made up by two oval shaped discs, bound in the middle through a central tube with typical disc diameters of 2.4 μm for the short and 2.9 μm for the long axis. The thickness of each disc was measured at 45 nm. From a size distribution point of view,

List of cleaning methods found in literature with reference to the original sample they were developed for and characterization method used to evaluate the results.

Method	Cleaning solution	Sample	Time [min]	Characterization
Method A	0.1 M NaOH / 30% H_2O_2	Foraminifera	60	Cd/Ca ratio [28]
Method B	0.1 M NaOH / 30% H_2O_2	Synthetic	90	Mg/Ca ratio [7]
Method C	12% NaOCl	Cultivation	60	Microscopy [30]
Method D	2.8% NaOCl / 30% H_2O_2	Sediment trap	40	SEM [32]
Method E	1% TMAH / 50% EtOH	Synthetic	300	Mg/Ca ratio [7]
Method F	5% TMAH	Synthetic	300	Mg/Ca ratio [7]

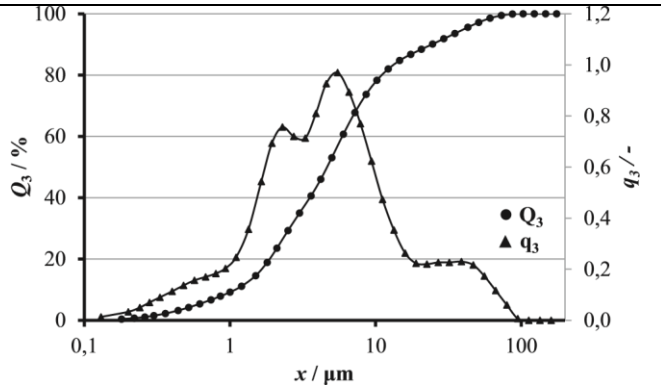

Fig. 2. Cumulative and density particle size distributions measured with laser diffraction spectroscopy for the RM.

based on Fig. 2, the reference material appears to range from 1 to 20 lm, which verifies that individual coccoliths are trapped in larger structures. The size distributions were determined from three individual measurements.

The above measurements noted a $x_{10,3}$ = 1.2 lm, $x_{50,3}$ = 4.6 lm and $x_{90,3}$ = 24.2 lm.

Next, the surface chemistry of the material and its overall composition was characterized. To reduce variability due to humidity adsorption the samples were dried and measured immediately after. Results of infrared spectrometry and thermogravimetric analysis are shown in Fig. 3.

The spectrum obtained from the FTIR analysis (Fig. 3) shows a broad band at 3600–3000 cm^{1} with two peaks at 3504 and 3351 cm^{1}. The overall existence of the band is attributed to humidity while the distinctive peaks are expected to show asymmetric and symmetric N-H stretches. The bands at 2977 and 2942 cm^{1} show the presence of aliphatic hydrocarbons. The peak at 1610 cm^{1} can be caused by N-O. The trivial bands characteristic for $CaCO_3$ can be recognized at 1415 and 873 cm^{1}.

In the thermogravimetric analysis of the reference material an abrupt change of 13.9% mass loss noted up to 300 C shows the hygroscopic nature of the material. A further removal of organic matter has also been verified to take place up to 300 C and explains the mass loss [35]. The further 6.9% mass loss up to 700 C demonstrate the low percentage of $CaCO_3$.

3.2. Comparison of methods

The effect of treating the reference material with different reagents and concentrations resulted in an improved appearance of the particles in some samples and in an increase of agglomerates and impurities in others. Fig. 4 shows the samples after treatment.

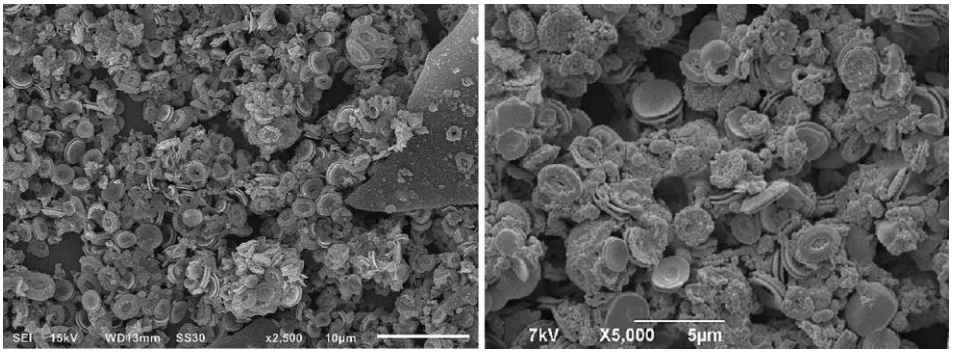

Fig. 1. Scanning electron microscopy of the reference material WD 9 mm, SEI, SS 40, 5 kV, x5000 magnification [34].

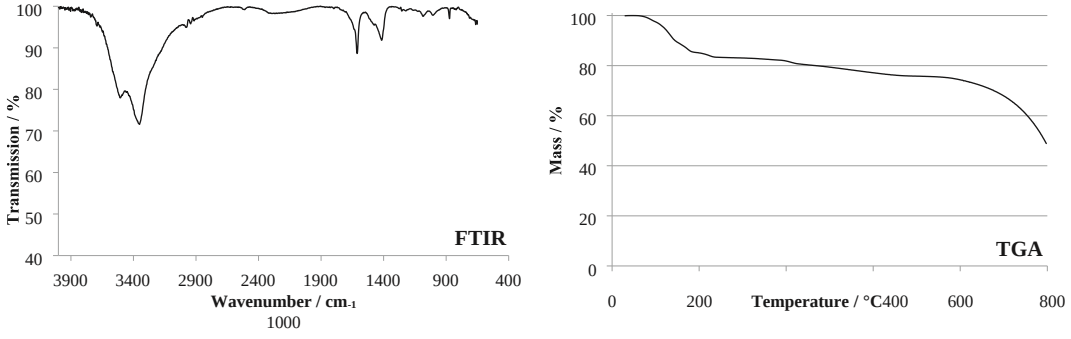

Fig. 3. Fourier-transform infrared spectroscopy (FTIR) and thermogravimetric analysis (TGA) of the reference material.

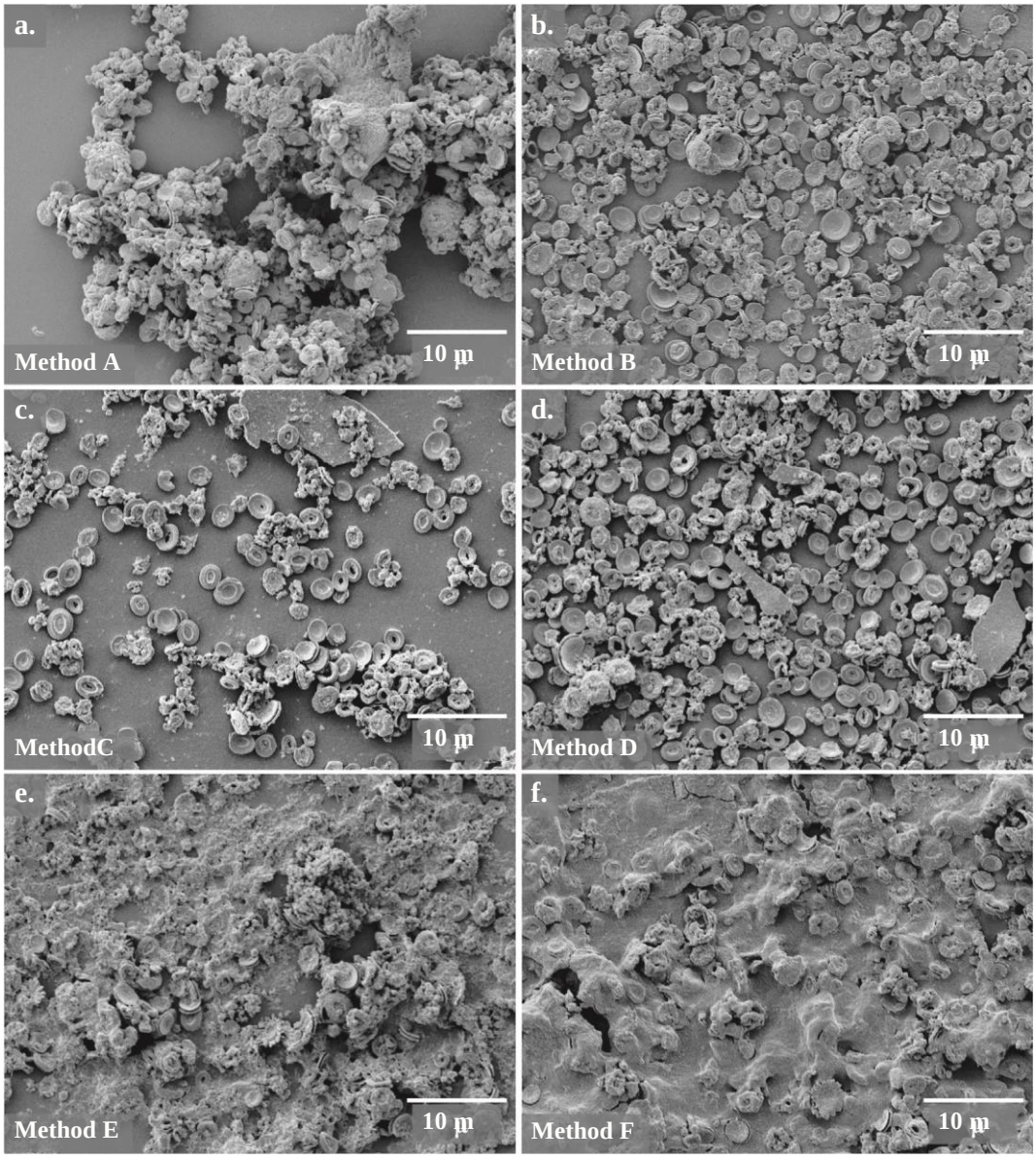

Fig. 4. Scanning electron micrographs of coccoliths after treatment. WD 9 mm, SEI, SS 40, 5 kV, x2500 magnification [34].

As would be expected, every method affected the morphology of the reference material in a different way. Due to the reactions that took place, coccoliths are shown either as individual particles or as part of larger structures. This is true for example for Method A, where coccoliths appear very similar to the reference material.

The particles seem trapped in larger agglomerates, while coccosphere remnants are also visible. The 1:6 ratio of NaOH and H_2O_2 in this method indicates an insufficient concentration of H_2O_2 to react with the present organic matter. Changing the ratio to 1:2 in Method B appears to have increased the removal efficacy but most importantly has deagglomerated the sample. The proportion of individual coccoliths appears to increase in the two following methods as well, namely in Method C and Method D. This changes radically in the last two scenarios, were the particles are trapped in agglomerates or appear coated with reagent residues. This variety in agglomerates and individual particles is mirrored in the next step, where the size distribution was estimated.

The samples were measured by laser diffraction analysis and were compared with the distribution of the reference material as well as to each other. In order to simplify the comparison of methods the reference material is shown in each graph in grey. Again, here, the results of Fig. 5 give the average of three individual measurements.

Although there are slight changes in the cumulative distributions, one can hardly draw concrete conclusions about any changes to the reference material. Treatment appears to have shifted the distribution slightly to the right in Method A, changing the median from 4.6 lm to 5.9 lm. The same slight increase in x_{50} is also observed for Method B, Method E and Method F. In Methods C the median remains almost the same and lastly in Method D it decreases slightly. Detailed characteristic values for every distribution are given in the supplementary material.

This insufficient amount of information changes however drastically if one observes the density size distributions of the samples (Fig. 6).

Fig. 6 gives a clear indication of how the material is distributed and what fractions are in the sample. The results complement what was visible in Fig. 4. In more detail, Method A seems to have increased the middle fraction of the sample. In contrast to that, Method B – Method D appear to have generated three clearly distinguishable fractions. This is a valuable information that supports the presence of individual coccoliths found in the SEM micrographs. Individual coccoliths are detected in the finer fraction, ranging from 1 to 3 lm.

Finally, Method E and Method F have both shifted the distribution to the right and sustained the structure of agglomerates. In general, the cleaning method should increase the amount of individual coccoliths and disintegrate any remaining coccospheres. The results of Fig. 6 demonstrate that particles size measurements can offer valuable information about the efficiency of the treatment and more specifically after observing the density (q_3) rather than the cumulative (Q_3) size distribution.

The effect of treating the reference material with different reagents had mixed results also in the surface of the material. As the FTIR spectra show, there were varying proportions of organic remnants and $CaCO_3$ detected in each sample (Fig. 7).

Through the spectra of Fig. 7, the surface alterations become visible. A first obvious difference to the reference material (Fig. 3) is seen in the broad band at 3500 cm^{1}, which has been transformed to one sharp peak at 3690 cm^{1}, indicating the presence of the O-H bond. This change demonstrates that any remaining water in the sample is bound to the crystal structure and is sterically hindered to form any hydrogen bonds. The higher degree of agglomeration in Method A, could explain why this phenomenon is more evident for this sample. A second radical change

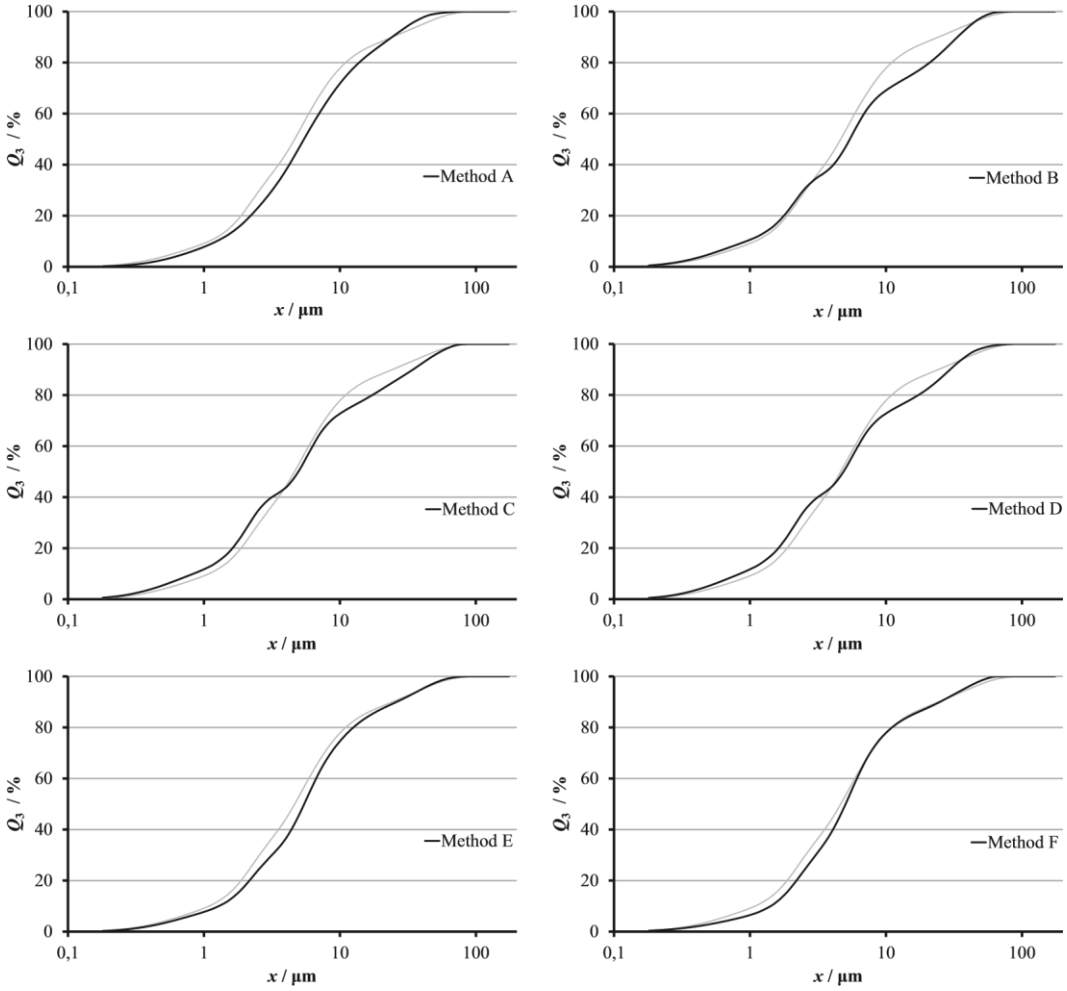

Fig. 5. Cumulative particle size distributions measured with laser diffraction spectroscopy for each method given in Table 1.

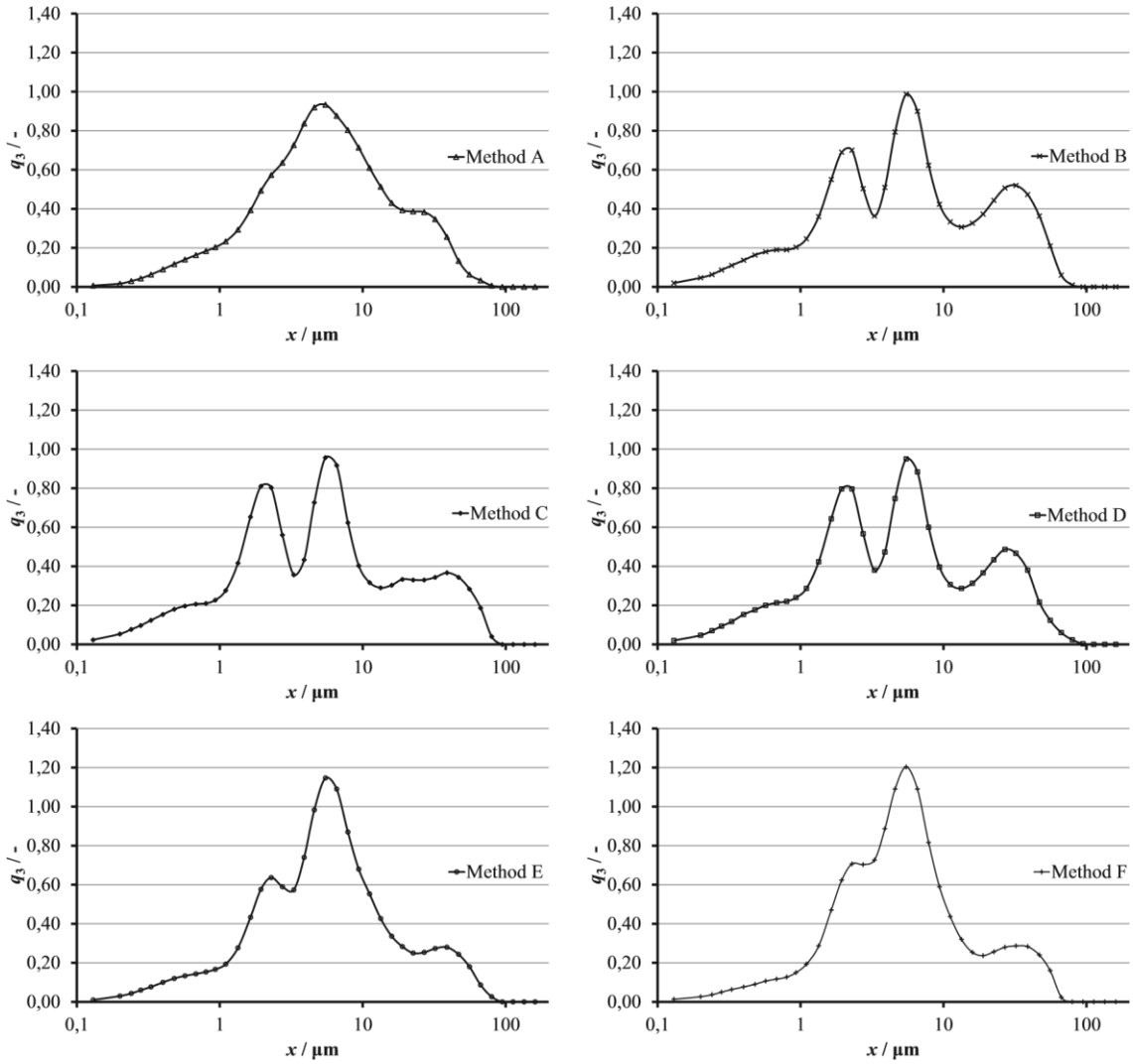

Fig. 6. Density particle size distributions measured with laser diffraction spectroscopy for each method given in Table 1.

is seen in the disappearance of the N-H peak at approximately 3500 and 3350 cm^{1}, as well as the N-O peak at 1600 cm^{1}. It appears that NO^{-}_{3}, one of the main coccolith nutrients has been efficiently replenished. This indication is further supported by our previous findings, where the absence of N from the chemical composition of coccoliths was verified by an XRF analysis [22]. In contrast to that, remnants of PO^{3-}_{4}, another basic nutrient used during cultivation, are accounted responsible for the double peak noticed at 1080 and 1000 cm^{1}. This is caused by the P-O-P stretching vibrations. The P = O stretching could further explain the peak at 1222 cm^{1}, however C=O absorb in this region as well. In all methods, the presence of $CaCO_3$ has become more evident. This is seen through the distinct peaks at the fingerprint region of the carbonate anion ($CO^{2}_{3}^{-}$) at 1415, 873 and 712 cm^{1}. Lastly, in Method F, remnants of the chemical agent complement the supposition made earlier by the SEM analysis.

To support and conclude the findings presented so far, a thermogravimetric analysis (TGA) followed next. By comparing the mass loss caused at distinct temperatures, conclusions about the samples' composition were drawn. In an effort to make the progression comparable for each sample, three temperature regions were defined and the results are given in Table 2.

As seen in Table 2, the three temperature regions ranged from 100 to 300 C, 300–500 C and from 500 to 800 C. This selection was based on previous findings of the decomposition of the reference material [35]. Changes up to 300 C were expected to represent mainly humidity and organic matter losses, then up to 500 C loss of further organic matter and finally up to 800 C the decomposition of $CaCO_3$. What is aimed, at this point, is a minimal mass loss up to 500 C and a maximal mass loss between 500 and 800 C. This would translate into a low percentage of impurities and at the same time a high percentage of pure $CaCO_3$. Once again, when the results of Table 2 are reviewed, Method B, C and D are closer to what is desired. Fig. 8 demonstrates graphically the results given in Table 2. Again, here, for comparison purposes the reference material is depicted in grey.

The first clear difference visible in all samples is noticeable in the first temperature region up to 300 C. In contrast to the reference material, the treated samples show a much lower percentage of humidity. Secondly, the curves verify the increased amount of $CaCO_3$ by the degradation step occurring at 600–700 C, showing the transformation of calcium carbonate to CaO and CO_2. The presence of organic remnants in Method F is again demonstrated here by a higher loss observed in the first temperature region.

3.3. Clean coccolith particles

The results of the previous section have thus far helped distinguish Method C and Method D for laboratory cultivations. It is however important at this point to highlight the advantages and disadvantages of both methods. Starting with the first of the two, NaOCl has been observed to cause less damages on the particles. Conversely, the repeated washing steps can become rather time consuming while also the treated quantities are dependant by the working volume of the centrifuge. Proceeding to the second method, the rapidity of the reaction was perceived positively, however the added reagent counted mostly as a disadvantage, and it has been observed on more than one occasion to cause damage to the structure. Furthermore, the rapid reaction is cancelled out in high particle concentrations by the time-consuming process of filtration. The use of a centrifuge has expedited the process; however, this is only possible after the reactants become neutralized as existing frothing interferes with the task. The use of a centrifuge in this case, would also add the time-consuming argument made for the first method. Method C has also preserved coccospheres while high concentration of reagents in Method D have disintegrated the particles' structure. An example of coccosphere remnants can be seen in the following example. Clean coccoliths were successfully recovered by both methods and are depicted in Fig. 9.

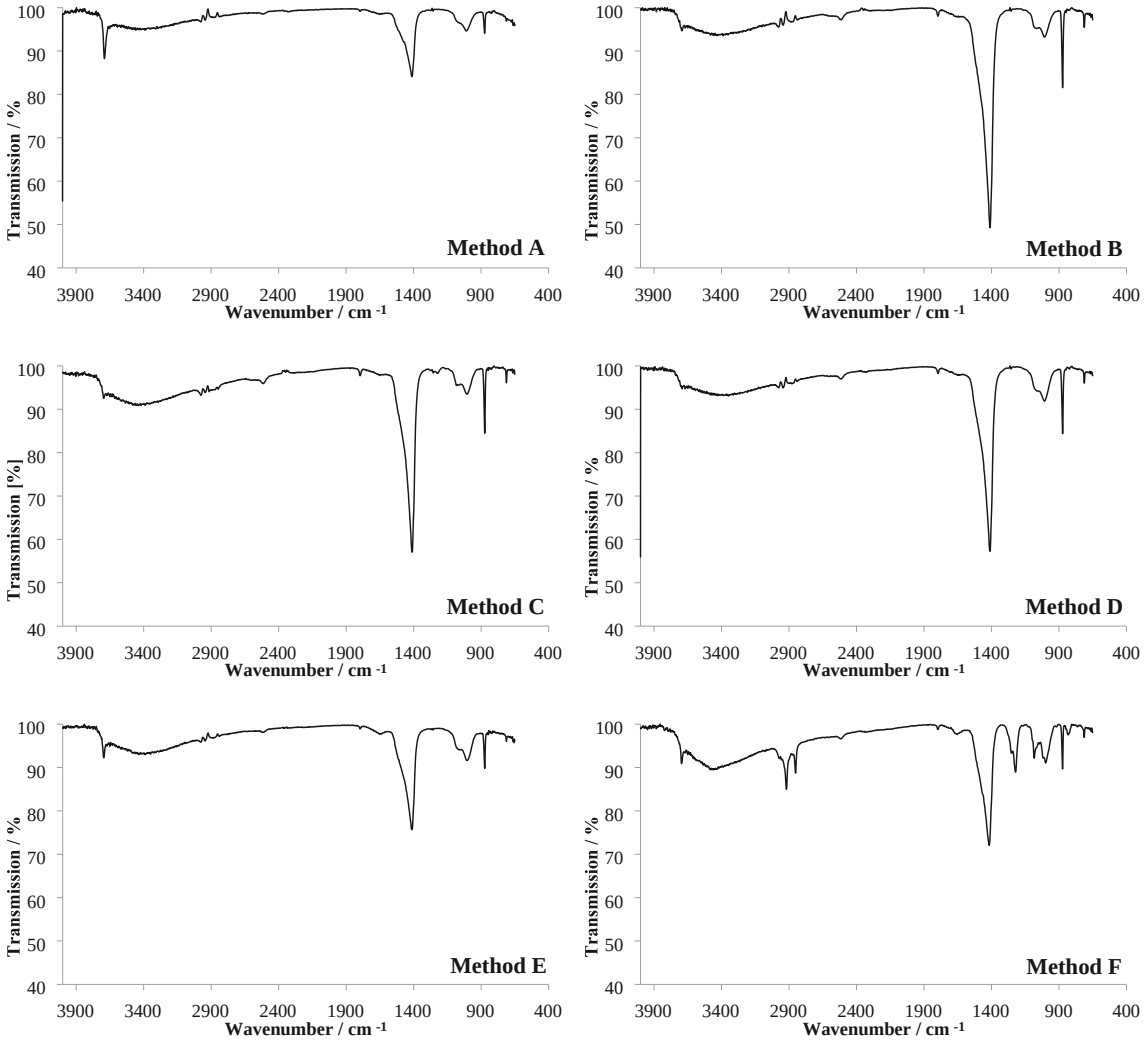

Fig. 7. FTIR transmission spectra of coccoliths treated with each method given in Table 1.

Table 2
Total mass loss and temperature specific mass loss of treated samples of each method given in Table 1.

	Mass Loss [%]			
Method	Total	100 – 300 C	300 – 500 C	500 – 800 C
RM	21.0	13.9	3.6	3.5
Method A	36.8	2.02	17.3	17.5
Method B	45.8	3.48	7.95	34.4
Method C	42.4	2.44	4.99	35.0
Method D	46.4	3.12	9.98	33.3
Method E	39.2	4.35	10.63	24.2
Method F	32.7	3.05	16.04	13.6

The coccoliths shown in Fig. 9 were treated according to Method C and Method D after first reducing the system's salinity from 33 ppt to approximately 1 ppt at pH = 8.8. Complementary to the SEM micrographs, Fig. 10 gives light microscopy captures to shed light onto the distribution of the particles.

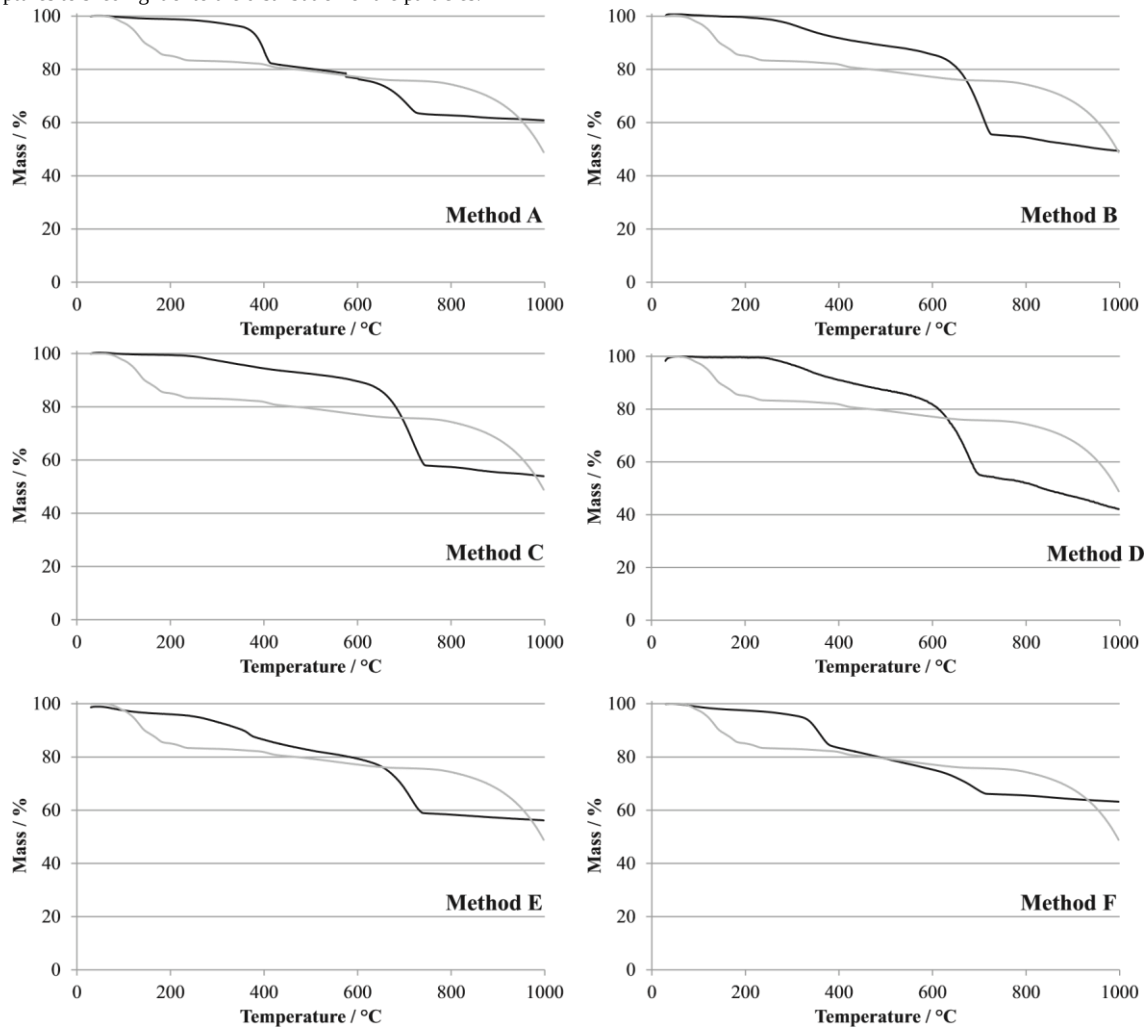

Fig. 8. Thermogravimetric analysis curves obtained for Method A - Method F. Reference material is depicted in grey.

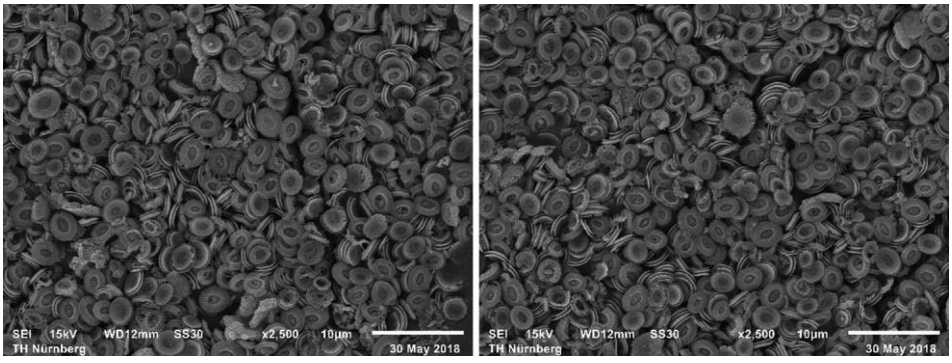

Fig. 9. SEM captures of particles treated with Method C (left) and Method D (right).

The larger structures in Method C, between 6 and 13 lm, represent partial coccosphere structures, sustained throughout the treatment. As was argued previously, treatments with NaOCl tend to have coccospheres in the final product. Light microscopy captures, as the ones of Fig. 10, were further used to estimate an image size distribution for the samples and compare it with a distribution measured by LDS. It was considered essential to identify how the particles are perceived through common size estimation techniques and to rule out any possible measuring errors. The resulted distributions are shown in Fig. 11.

Fig. 11 shows a distribution estimated through laser diffraction and image analysis. The characteristic values for the sample of Method C showed an x_{50} = 2.8 lm for the LDS distribution and an x_{50} = 2.5 lm for the image size

distribution. The same values for Method D were x_{50} = 1.9 lm for the LDS distribution and x_{50} = 1.9 lm for the image size distribution. The specific surface

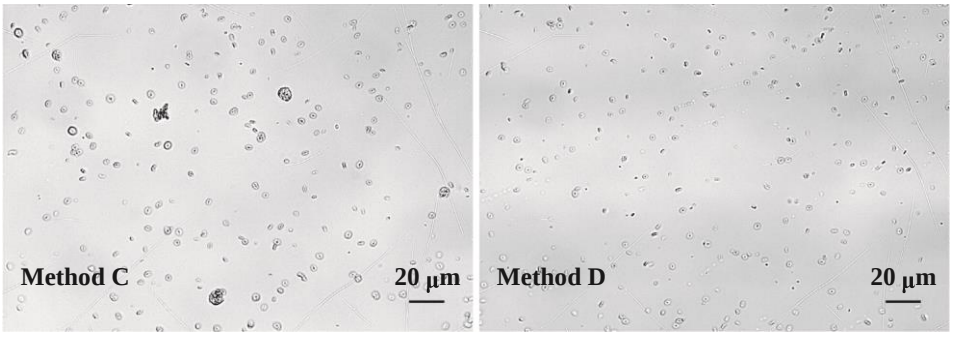

Fig. 10. Light microscopy micrographs of coccoliths treated by Method C (left) and Method D (right) x500 magnification.

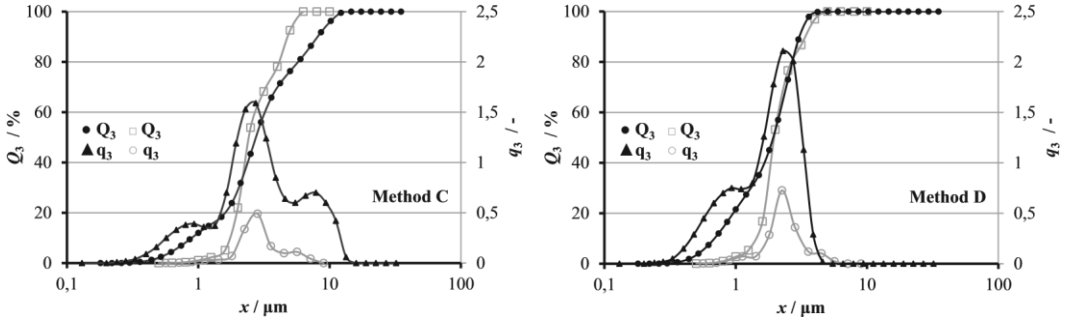

Fig. 11. Coccolith samples treated according to Method C and Method D and measured by laser diffraction spectroscopy (black) and image size analysis from light microscopy captures (grey).

area (SSA) of the samples was estimated through a 5-point BET analysis showing a SSA_C = 10.233 m^2g^1 for Method C and SSA_D = 11.603 m^2g^1 for Method D. As a comparison, a synthetic $CaCO_3$ sample reported a value of SSA = 1.8 m^2g^1 and an x_{50} = 4.2 lm after a 2 min grinding process in a planetary ball mill at 7000 rpm. For the assembly of the image size distributions an approximate of 1500 particles were visually counted and plotted by using size intervals of the Renard series R"5 (ISO 3).

Before proceeding to any of the reactions, it is advised to reduce the salinity of the broth as this has increased the reactivity of reagents. In addition to the above, abrupt pH changes should be avoided for the cultivation broth as this has led to flocculation of the material and added difficulties in recovery. The observed flocculation might result from proteins unfolding, which as they are present in the broth can be triggered by pH changes and alter their conformational stability.

4. Discussion

From the data collected in the previous section, one concludes that the most promising recovery processes are the ones using H_2O_2, NaOCl or better yet a combination of the two. This is similar to the conclusions presented by Stoll et al. for a synthetic $CaCO_3$ system, although the objective of this study is very different from theirs [7]. Yet a concrete understanding of the exact reaction pathways remains a challenge. The given concentrations in the treatment protocols are based on protocols introduced from other fields and their transferability to coccolith systems might be less than ideal. This suggests that there is room for optimizations and adjustments. At first glance, the pH of the solution, the temperature as well as the duration can expect to have a tremendous effect. Further important parameters are the size of particles or agglomerates in the solution, the use of dispersing additives and last but not least the concentration of the reactants themselves. For instance, hydrogen peroxide is regarded thermodynamically unstable and is known to self-react, with decomposition being accelerated at elevated temperatures and pH values. Furthermore, it is important to know that any excess of OH^- ions would favour the reducing properties of H_2O_2. Studies from other fields have found that removal of organic matter by H_2O_2 is more effective under low pH [36], however the dissolution rate of $CaCO_3$ renders this approach impractical. Another suggestion that could however be advantageous for coccoliths is the use of tetrasodium pyrophosphate ($Na_4P_2O_7$) as a dispersing agent in order to hinder agglomeration and improve the removal of organic matter [37]. Similar findings can also be collected for sodium hypochloride. For example, it was noted that 6% NaOCl at pH 9.5 had better organic removal properties than 30% H_2O_2 [38]. Cheshire et al. noted that under those conditions the desorption of organic matter from the mineral surfaces was enhanced and because of that the efficiency of the method was improved.

Since both reactants are fairly unstable, it is expected that when combined they will follow more than one reaction pathways. This includes for example a simultaneous decomposition, each on their own, and reaction with organics and metals present in the cultivation broth. Aside from factors discussed above, it should be kept in mind that any organic matter found in the final product does not necessarily correlate to reactant deficiency. Here, the dispersibility of the particles play a pivotal role, since organic molecules participating in agglomerates can be shielded from oxidation, remaining thus in the sample. A further explanation of such an outcome can be caused from the mineral itself, which, if capable of bonding with the organic matter in more than one sides, can hinder any reactivity occurring with either NaOCl or H_2O_2.

In view of the multiple factors that have been neglected in coccolith recovery the next step should focus on an optimization approach. In our first attempt to optimize these methods the concentration of reactants, the duration of the reaction, and the pH value were some of the first parameters we varied. Ultimately, the addition of dispersing agents with and without mechanical agitation to improve dispersibility were also taken into account. Preliminary results suggest an improvement of the results in Method C when 6% NaOCl is used and the pH is adjusted to 9.5. The same goes for Method D where the

combination of 5% NaOCl and 20% H_2O_2 improved the results. Evidence of this assumption are presented in preliminary data in the supplementary material. This last part requires still further experimental data and is therefore not presented yet in detail. We hope however that our suggestions can demonstrate the missing parts that need to be considered in future studies.

5. Conclusions

The incorporation of biobased-materials has been and will remain beneficial for many industrial sectors. Their utilization however is often associated with recovery and stabilization challenges that if not addressed in detail make the material in question inaccessible. To change and resolve some current limitations found in the recovery of coccoliths we created a coherent basis by comparing cleaning protocols and applying them for the first time to a laboratory cultivation. A considerable effort was given on the particles' distribution before and after every treatment, as this was found indicative of the cleaning efficiency. Additionally, a number of methods were also used to help identify changes in the particles' composition and morphology. Two cleaning protocols were found to have equally promising outcomes, namely the method using 12% NaOCl (Method C) and the one using 2.8 %NaOCl – 30 %H_2O_2 (Method D). Preliminary results of an optimization approach carried out showed that decreasing the concentration at 6% NaOCl in the former and changing to 5% NaOCl – 20% H_2O_2 in the latter can further improve the outcomes of each method respectively. These conditions require further investigation and are currently viewed as a basis to build upon our future coccolith studies. Attractive applications for coccoliths include nanodevices, carrier materials while promising appears their incorporation in composite materials for bone tissue engineering. Although there is still considerable scientific effort required to reach these goals and know all about coccoliths and their recovery, we are optimistic that this work will pave a pathway towards this direction. This study summarizes challenges found in coccoliths recovery and offers guidelines that would aid to overcome struggles found in this field of research.

Declaration of Competing Interest

The author declare that there is no conflict of interest.

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