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Modifications of Two Palynological Processing Techniques: Ultrasonic Processing and Early-stage Sieving

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ABSTRACT

This paper describes two palynological sample enrichment techniques useful for processing samples which contain abundant chemically resistant fossil fecal pellets or which otherwise have low palynomorph productivity. Both techniques described here were used for Neogene samples, one from California and one from Abu Dhabi. The California samples were rich in fecal pellets that were resistant to chemical disaggregation. However, many of the pellets could be destroyed by ultrasonic processing, which permitted close control of the physical breakup of these pellets and other unwanted organic material until the palynomorphs could be viewed easily. The other (Abu Dhabi) samples were very small in size and sparse in palynomorphs; thus it was critical to minimize treatment, increase overall sample yield and minimize the loss of palynomorphs during processing. For these samples, the key step in processing was early-stage sieving of the post-HF residue. Palynomorphs could then be concentrated using heavy liquid separation.

INTRODUCTION

The techniques currently used for the extraction of fossil palynomorphs from rock and sediment samples are varied and extensively documented. Generally they involve mechanical cleaning and crushing of the sample, dissolution and disaggregation of the inorganic rock matrix, and separation and concentration of palynomorphs from the mineral and nonpalynomorph fractions using specific gravity and particle size differences. These procedures are modified to accommodate the characteristics of individual samples. Particular procedures often are omitted or repeated several times for best results. The order of the procedures should be modified to isolate and concentrate the fossil palynomorph assemblage most effectively. Two of these processing techniques are described below: ultrasonic processing and early stage sieving. Both were found to be useful for increasing sample yield in Neogene sediments and rocks from California and Abu Dhabi, respectively.

A PALYNOLOGICAL TECHNIQUE UTILIZING A HIGH INTENSITY ULTRASONIC PROCESSOR: NEOGENE SAMPLES FROM CALIFORNIA

Recently, samples from the Neogene of California were received in which the palynomorphs were chemically and physically bound in a matrix of fecal pellets produced by small marine invertebrates. Standard processing techniques did not recover enough palynomorphs for a meaningful analysis of these samples. Previous papers described the destruction of fecal pellets and other amorphous organic aggregates by means of nitric oxide (Ballog and Malloy, 1981) or aqua regia (Srivastava, 1984). However, the pellets in our samples were so chemically resistant that the chemical treatment necessary to destroy this inorganic

matrix also severely corroded the palynomorphs. Furthermore, screening and gravitational settling techniques alone were ineffective due to the small pellet size of 25-45 μm. High intensity ultrasonic processing was the most efficient method found to physically disaggregate the fecal pellets. The processor we used could be controlled closely to avoid destroying the palynomorphs. The equipment and the technique are as follows:

Equipment

Tekmar, High Intensity Ultrasonic Processor, 600-watt model
1/2" standard horn
1/4" tapered microtip, high intensity, 3-10 ml volume
Laboratory stand, 24", and clamp
Disposable 15 ml. centrifuge tubes and caps
Centrifuge
Deionized water
Disposable transfer pipettes
Test tube racks
Hearing protection

Methods

Ultrasonic Processing in General

The processor's settings are dependent upon the machine's horn and tip design requirements, sample volume, the vulnerability of the sample to breakup, and the chemistry of the sample solution. In our palynology laboratory we commonly work with small samples; therefore, we installed a 1/4" tapered microtip that accepts volumes ranging from 3 ml to 10 ml on the ultrasonic processor. Small plastic disposable centrifuge tubes worked efficiently with this system. Other tips and probes are available and may be selected from the manufacturer's catalog to match individual laboratory requirements. When processing, the Power Monitor rarely indicates 100% delivery of energy into the probe because the sample and container are never 100% receptive to the energy transfer. This is to be expected, but you can optimize this reading through experimentation.

Tuning

After a microtip is installed, you turn the Pulser Switch to off and set the Output Control to 5. (Caution: Never exceed an output of 5 or permit a microtip to vibrate in the air for more than 20 seconds. Otherwise, the microtip may fracture or be damaged irreparably.) Don your hearing protection and turn on the Power Switch. Depress the Tune Switch and slowly rotate the Tuner until you get a minimal reading on the Power Monitor. If the vibrating probe produces a high-pitched scream, stop and check the tip to insure that it is attached firmly to the horn.

If a minimal reading cannot be reached, remove the tip and check the reading without it. If this step produces a reading, replace the tip. When you have achieved a minimal reading, the ultrasonic processor will be tuned. After tuning your tip, you only have to tune it periodically to check for drift or when you change tips. This procedure maximizes efficient energy transfer from the processor into the sample.

Processor Settings

(1) Timer: 10 seconds. The ultrasonic treatment initially should be set at 10 second increments, and the sample checked (on a wet mount microscope slide) after each exposure, until you achieve the most optimal result. Incremental processing should protect your sample from destruction. The ultrasonic treatment can shatter palynomorphs and destroy your sample.

(2) Pulser: On, 50% duty cycle. The pulser serves two functions. The first is to inhibit heat build-up. Ultrasonic processing creates heat, and the sample's temperature and probe's effectiveness are inversely related. In other words, a cool sample is preferable for efficient cavitation. The second function of the pulser is to facilitate mixing. The conical centrifuge tubes enhance mixing also. The energy of the probe is focused directly beneath its tip and you need to expose the entire sample equally to its influence. The pulser enhances physical mixing, allowing materials to circulate between pulse cycles.

(3) Output Control: 5. The setting of 5 is the highest allowed with a 1/4" microtip. Higher settings may damage the microtip. This factory specification may change if you change tip size.

Sample Preparation

Ultrasonic processing provided best results when it followed initial treatments with hydrochloric acid and hydrofluoric acid. The ultrasonic treatment is most effective when the sample's exposure is maximized. Smaller samples were placed into 15 ml conical disposable centrifuge tubes. Larger samples could be placed into 50 ml conical centrifuge tubes. Ultrasonic treatment always is performed in a neutral pH solution. The sample size should not exceed 10 ml, due to the design requirements of the 1/4" tapered microtip. If the volume of the sample solution exceeds 10 ml there will be an appreciable dissipation of energy, resulting in longer treatment to achieve the same results. When the tip is inserted, it has to be extended at least 1/2" into the sample solution to insure delivery of the ultrasonic's vibrational energy. A tightly fitting test tube rack is adequate for holding tubes during the processing. A small squirt bottle of distilled water is useful at this point for adjusting the sample solution's level in the tube. The microtip <u>should not touch</u> the walls of the tube: the tube and probe may chip or

fracture. Allow room in the sample tube for the fine aerosol that sometimes forms during the ultrasonic process. Aerosol formation can be stopped by a decrease in the power and an increase in the processing time, or by lowering the sample's temperature. Remember, cooler temperatures enhance cavitation. After tuning the ultrasonic processor and establishing the machine's settings, it is often helpful to split your sample and process only half of it. In this way, you retain an untreated sample split if the other split is damaged by the ultrasonic processor. You may make several test sample splits and sequentially expose them to increasing 10-second time intervals of ultrasonic processing. In this manner, you would produce a test gradient of treated sample splits that would range from untreated sample to those which may be overprocessed and damaged. In this manner, you can safely develop your technique and calibrate it for your samples. In our work with Neogene samples from California, the best results were obtained between 60 seconds and 180 seconds. It was necessary to count the pollen species on the slides. It was found that after 60 seconds of ultrasonic treatment, many of the bisaccate grains lost their sacci; however, unaffected fecal pellets remained after only 60 seconds of treatment. On the other hand, angiosperm pollen still remained well preserved after as much as 180 seconds of treatment. Therefore, an initial count of 100-200 grains was made of the 60-second residue, then a second set of counts was made of angiosperm pollen species in the 180-second residue.

The ultrasonic process produces clay-sized detritus (mainly pellet materials) that may obscure the pollen; therefore, it is advisable to follow the ultrasonic processing with sieving. Heavy liquid separations will further clear the sample by removing unwanted fine mineral matter.

Remarks

The ultrasonic processor permits a controlled physical breakup of pellets and other non-palynomorph organic material and release of palynomorphs. Chemical oxidation and reduction of unwanted lignitic compounds, using nitric acid and potassium hydroxide treatment, are other useful palynological techniques that make palynomorph samples easier to study. Both ultrasonic and oxidation-reduction techniques may be necessary for the same sample. However, severe oxidation-reduction of samples physically weakens palynomorphs or makes them brittle. Therefore, it is recommended that ultrasonic processing precede the oxidation and reduction steps.

A PALYNOLOGICAL TECHNIQUE UTILIZING SIEVING AT AN EARLY STAGE IN THE PROCESSING: NEOGENE SAMPLES FROM ABU DHABI

This technique was developed to process Neogene ditch samples from Abu Dhabi, United Arab Emirates. The individual samples were small, averaging 30 grams before cleaning, and they

were found to have only low concentrations of palynomorphs; the first few slides resulting from standard processing techniques contained very few palynomorphs. Therefore, the normal processing procedures had to be modified and edited to adapt them to minimize processing steps and maximize palynomorph recovery. Size selective sieving, a method commonly utilized in a palynology laboratory, proved to be critical to processing these samples.

Equipment

Small manila envelopes (for the Lithology file)
Disposable polyurethane beakers/lids (400 ml)
HCl, 37% solution (approximately 60 ml/sample)
HF, 48-50% solution (approximately 60 ml/sample)
Deionized water
Sieving material (8 μm screen, 8 X 8 cm, and
150 μm screen, 3 X 3 cm)
Sieve frames (interlocking polyurethane collars)
Siphoning plate (base of a bell jar vacuum system)
Siphon and siphon hose (attached to a water outlet)
Sparkleen solution
Ethanol, 25% solution
Disposable centrifuge tubes/Caps (15 ml)
Centrifuge
ZnCl, 2.1 specific gravity heavy liquid
HCl, 10% solution
Slides/Cover slips
Glycerin jelly
Flint storage vials for the Residue files
Glycerol

Methods

The cuttings were received in the form of 30 gram samples. A small chip was taken, placed in a small manila envelope, labelled, and stored in the Lithology file. A rock sample would have been physically broken into 0.5 cubic centimeter fragments before processing. The samples were weighed and placed into disposable 400 ml polyurethane beakers to which was added approximately 60 ml of HCl. After approximately 48 hours or after the solution lost its reactivity, the sample was siphoned and deionized water was added. After 4 hours of settling, this was repeated until the pH of the sample was neutral. Then it was treated with HF for a 24 hour period. After this treatment, the sample was again brought back to a neutral pH by siphoning and adding deionized water.

What we did differently with these samples was that at this early stage of processing, the entire sample was sieved through a coarse 150 μm screen and a fine 8 μm screen. The 150 μm screen was formed into a funnel and hand held over the 8 μm screen. The 8 μm screen was held in a circular polyurethane frame. This

frame consisted of two 5 cm diameter collars that screwed into each other and sandwiched the screen. The siphoning plate drew the sample solution through the 8 μm screen and facilitated the sieving process that utilized washes with Sparkleen solution, 25% ethanol and deionized water. The palynomorph-bearing residue was removed from the fine screen, acidified in a disposable centrifuge tube with 10% HCl, and mixed with 10 ml. of the heavy liquid (2.1 specific gravity ZnCl). This solution was centrifuged at 500 rpm for 20 minutes and then at 1,500 rpm for 10 minutes. The palynomorph-bearing "float" was removed and rinsed with two separate 10% HCl washes. Multiple ZnCl runs have improved sample productivity. The organic "float" residue was then neutralized with deionized water washes, and glycerin jelly slide mounts were made. The remaining residue, if any, was placed into a flint vial with two drops of glycerol and a 25% solution of ethanol, labelled, and stored in the residue collection.

Remarks

The small, palynomorph-poor samples had to be processed to assure recovery of most or all the palynomorphs from each sample. The HCl and HF treatments do not appreciably alter the palynomorph exine. Because samples were found to be uncarbonized and lacking extraneous organic matter, further chemical treatment (i.e., oxidation, reduction, and ultrasonic processing) were considered unnecessary and potentially destructive. The samples' characteristic small sizes made it feasible to sieve the entire post-HF residue. This removed the clay-sized fine material (-8 μm) and the coarse fraction (+150 μm) but retained the palynomorph-bearing particle size fraction. The residue, at this point, was reduced in volume and sorted only to silt and very fine sand size. The mineral fraction therefore could be more effectively removed with centrifugation in a heavy liquid. The result was a clean, clay-free sample in which the palynomorphs were not chemically eroded or physically broken, and palynomorphs had not been lost through additional processing.

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