



## A recycling method for LST<sup>®</sup> contaminated during heavy liquid separation in palynological processing

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### ABSTRACT

An aqueous solution of LST<sup>®</sup> is one of many heavy liquids used to concentrate palynomorphs. It and aqueous solutions of sodium polytungstate (SPT) are replacing toxic heavy liquids, such as bromoform and zinc bromide, and to a lesser extent zinc chloride, in many palynology laboratories. Both non-toxic heavy liquids can be recycled through filtering and evaporation of water and/or ethanol added to the sample during processing, and commonly are. Both media, and especially LST<sup>®</sup>, are somewhat reactive with organic matter, and in humic-acid rich samples become contaminated and discoloured. This paper presents the authors' methods for recycling LST<sup>®</sup> and removing contamination through a combination of filtration through activated charcoal and 0.45  $\mu\text{m}$  nylon membranes. Filtration through Celite<sup>®</sup> was found to be unnecessary.

### KEYWORDS

LST<sup>®</sup>; heavy liquids; recycling; discolouration; palynological processing

### 1. Introduction

Heavy liquid or dense-media separation has been an integral component of palynology preparation since the earliest stages of the discipline (Brown 2008). Over the past 20 years, a slow transition is being made away from toxic heavy liquids, including bromoform and zinc bromide, and to a lesser extent zinc chloride to less toxic sodium polytungstate (SPT; Munsterman & Kerstholt 1996; Campbell et al. 2016), sodium metatungstate (SMT; Krukowski 1988) and LST<sup>®</sup> (Patrick & Patrick 1997; O'Keefe & Eble 2012; Caffrey & Horn 2013). While initially expensive, the use of these liquids is becoming preferential because of their low toxicity and ease of recycling (Proske et al. 2015; Campbell et al. 2016).

Recycling methods for standard heavy liquids used in palynology, micropalaeontology and kerogen studies are well established in the literature, including those for clarifying media discoloured by oil and humic acids (Hanna 1927; von Bitter et al. 1978). Many palynology laboratories recycle heavy media using filtration and reconcentration (Wagoner et al. 1961; Gray 1965; Burgess 1974); however, these methods do not remove colour from samples contaminated with humic acids. Barker (1981), recognising this as a significant problem when preparing kerogen isolates, advocated treatment of contaminated zinc bromide with hydrogen peroxide and filtration through Celite<sup>®</sup>, while older works suggested decolourisation with Fuller's Earth (Hanna 1927; von Bitter et al. 1978), or a variety of re-distillation methods coupled with decolourisation with Fuller's Earth (Hauff & Airey 1980).

The use of mineral salts for heavy density separation was introduced in the mid-1980s (Callahan 1987; Gregory & Johnston 1987) and quickly spread to the micropalaeontological

community (Krukowski 1988; Savage 1988). As alternate heavy liquids became more common, several methods for recycling SMT, SPT and LST<sup>®</sup>, a proprietary heavy medium (Patrick & Patrick 1997), have been published. Patrick & Patrick (1997) outline a recycling method. The recycling method on most suppliers' websites calls for simply re-concentrating spent LST<sup>®</sup> through boiling; however, a flowchart that includes additional recycling procedures is present on some suppliers' webpages (e.g. [http://www.chem.com.au/heavy\\_liquid.html#recycle](http://www.chem.com.au/heavy_liquid.html#recycle), <http://www.heavyliquids.com/application.html#Recycle>). This flow chart generally indicates filtering at 5  $\mu\text{m}$  to remove particles, re-concentrating and carbon filtration. These directions have been recognised as insufficient from the earliest use of mineral salts in micropalaeontology.

Krukowski (1988) advocated vacuum filtering through No. 4 qualitative filter paper followed by evaporation, and if contaminated with iron and thus blueish in colour, adding a drop or two of 3% hydrogen peroxide to return SMT to its original state. Savage (1988) recommended washing all filters and apparatus that came in contact with SPT into a recovery container with distilled water, then evaporating the liquid in an oven. Six et al. (1999) advocate filtration of SPT through a complex column packed with layers of glass wool, activated carbon, glass wool, resin, glass wool, activated carbon, and more glass wool; this method, which does remove colour effectively, is more complex than necessary. More recently, Proske et al. (2015), recycled LST<sup>®</sup> by passing it through glass filters twice, then re-condensing the filtrate. This process is equivalent to the pre-recycling treatment of LST<sup>®</sup> at Morehead State University, Morehead, KY, USA, whereby particulate matter is removed as the used LST<sup>®</sup> is passed through a 0.45  $\mu\text{m}$  filter. This does not, however,

remove colour gained during heavy-density separation from the dilute LST<sup>®</sup> (Plate 1, crude LST<sup>®</sup>).

The goal of this paper was to develop and share an efficient, inexpensive method for recycling LST<sup>®</sup> contaminated by 'humic acids' in palynology laboratories. The method described below is straightforward and was easily accomplished by technicians with little chemistry or palynology experience.

## 2. Materials and methods

Discoloured LST<sup>®</sup> waste was obtained from two laboratories. Laboratory A provided three aliquots of waste LST<sup>®</sup>, one discoloured lightly brown (sample 1), one discoloured dark brown to

black (sample 2), and one discoloured pink by Safranin-O (sample 3); all had starting specific gravities of approximately 1.7. Laboratory B's waste was lightly brown discoloured and had a starting specific gravity of less than 1 (sample 4). Sample 1 was used as the experimental sample. Only method 4 was used with samples 2–4.

### 2.1. Method 1: Celite<sup>®</sup>

As an initial test (run 1), a pipette column was prepared by packing a 5-mL pipette with approximately 2 g of Celite<sup>®</sup> diatomaceous earth (enough to fill the pipette half-way). One half millilitre of contaminated LST<sup>®</sup> from sample 1 was poured



Plate 1. Filtration apparatus for method 1, run 2 showing 'tunnels' through the Celite<sup>®</sup>.

through the Celite<sup>®</sup>. The procedure was scaled up to 60 g of Celite<sup>®</sup> in a glass-fritted Buchner funnel (run 2); 20 mL of LST<sup>®</sup> was poured through the system. The density of the LST<sup>®</sup> was reduced to approximately 1.34 g/mL and the scaled-up method re-tested. All subsequent methodologies used LST<sup>®</sup> with this lower density for the initial test.

## 2.2. Method 2: hydrogen peroxide

Approximately 0.5 mL of 30% hydrogen peroxide was added to 10 g of contaminated LST<sup>®</sup> solution and vortexed. This was then filtered through activated charcoal multiple times.

## 2.3. Method 3: activated charcoal plus Celite<sup>®</sup>

Activated charcoal (Norit<sup>™</sup>, Neutral) was mixed with contaminated LST<sup>®</sup> from sample 1 using a ratio of 1 g to 40 g. The activated charcoal was filtered out by passing through P8 cellulosic paper suspended in a glass funnel. The filtrate was filtered again through a pad of Celite<sup>®</sup> formed in a 60-mL glass-fritted Buchner funnel (run 3). The final filtrate was then passed through a 0.45 µm nylon membrane.

## 2.4. Method 4: activated charcoal

Contaminated LST<sup>®</sup> from sample 1 was mixed with activated charcoal (Norit<sup>™</sup>, Neutral) in a 250-mL beaker using a ratio of 40 g to 1 g; in this test, approximately 160 g of contaminated LST<sup>®</sup> was treated. The mixture was stirred until the colour of the solution changed from brownish to black, then, while tipping the beaker to allow the suspension to settle, the liquid was observed to be colourless. This suspension was poured into a medium-sized filter funnel (7.0 cm diameter) and passed through P8 cellulosic paper. The resultant filtrate was then passed through a 0.45 µm nylon filter.

## 2.5. Reconcentration

Many methods of reconcentration of cleaned LST<sup>®</sup> are possible. At Morehead State University, the final filtrate is placed on a magnetic stirrer hot-plate set to about 125°C until reduced by 2/3, taking care not to crystallise the LST<sup>®</sup> at high solution density (>2.8 SG). Reconcentrated LST<sup>®</sup> can be stored in clean Nalgene<sup>®</sup> bottles and sealed as per laboratory practice.

## 2.6. Optical microscopy

Samples from the initial tests of methods 1, 3 and 4 were examined for (1) remnant organic matter and (2) crystalline material using both white and blue light illumination on a Leitz Ortholux II microscope with 630× total magnification. The blue light source was a CoolLED pE-100 lamp. Photomicrographs were obtained with a Leica MC170 HD camera and captured via the Leica Application Suite software package.

## 3. Results

### 3.1. Colour change

#### 3.1.1. Method 1

The resulting filtrate in method 1, run 1 was clear; however, significant problems with this method became apparent during run 2. In run 2, the filtrate maintained its brown colour. Close observation of a second trial of run 2 revealed that the high-density LST<sup>®</sup> pushed through the Celite<sup>®</sup> particles, forming 'tunnels' (Plate 1), which resulted in contaminants not being removed.

#### 3.1.2. Method 2

The sample turned neon-orange, and could not be cleared beyond light yellow-brown with multiple runs through activated charcoal (Plate 2, vials BGVN-1-7 & BGVN-1-13). The



**Plate 2.** Vials of treated and untreated aliquots of sample 1. From the left, untreated LST<sup>®</sup> as received from Laboratory A (density 1.7 g/mL); diluted LST<sup>®</sup> (density 1.34 g/mL) used for analyses; method 2, run 1 (BGVN-1-7); method 2, run 2 (BGVN-1-13); method 3 (BGVN-1-15).

density could not be increased via heating, and the sample was judged to be unusable.

### 3.1.3. Method 3

The re-condensed filtrate is nearly clear and reached its original density (Plate 2, vial BGVN-1-15).

### 3.1.4. Method 4

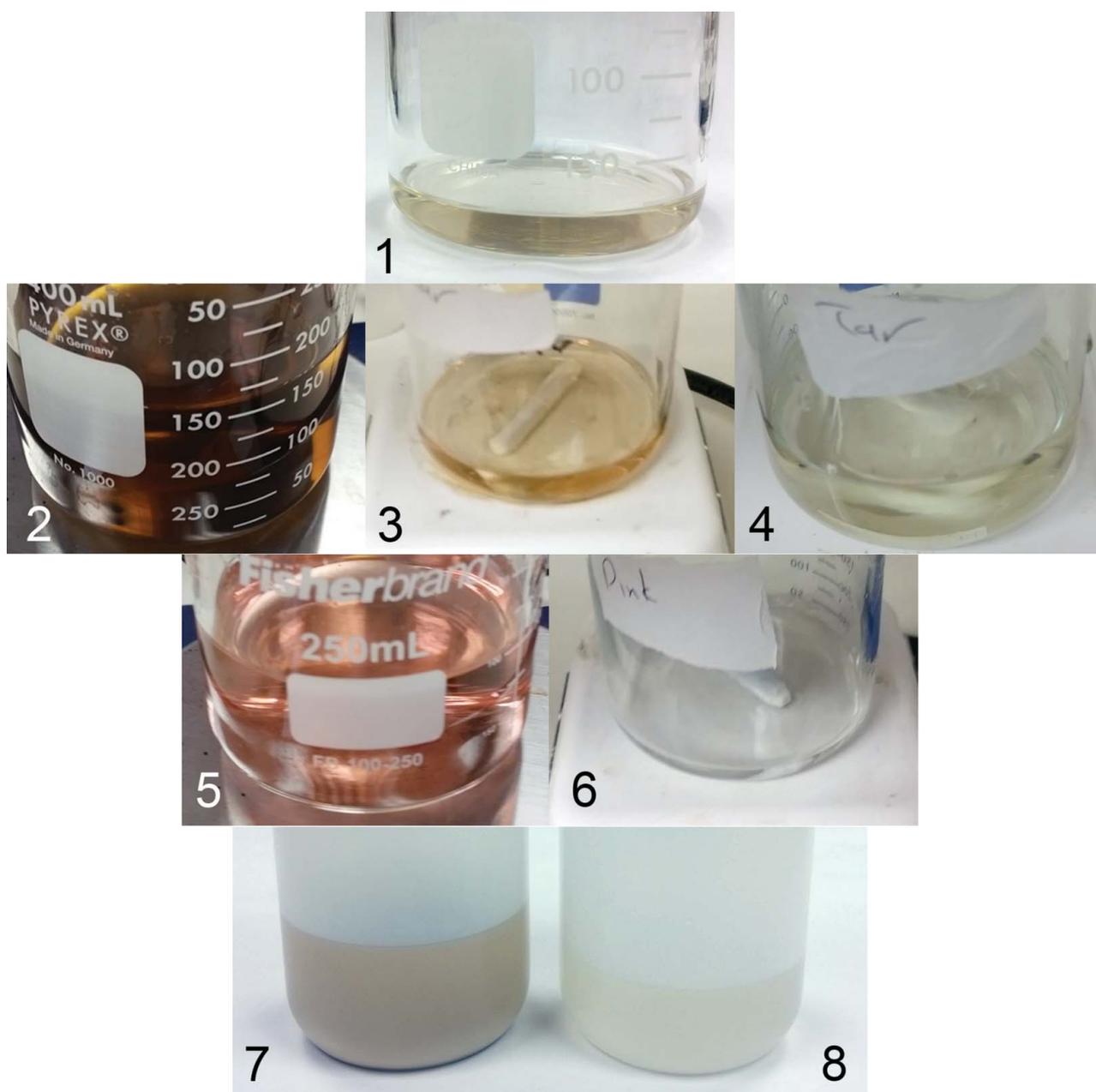
Sample 1 achieved a pale golden colour virtually identical to that of a virgin sample of LST<sup>®</sup> (Plate 3, figure 1). Sample 2 achieved a pale golden colour following multiple rounds of filtration (Plate 3, figures 2–4). Sample 3 is colourless (Plate 3, figures 4 and 5), as is sample 4 (Plate 3, figures 6 and 7).

### 3.1.5. Optical characterisation

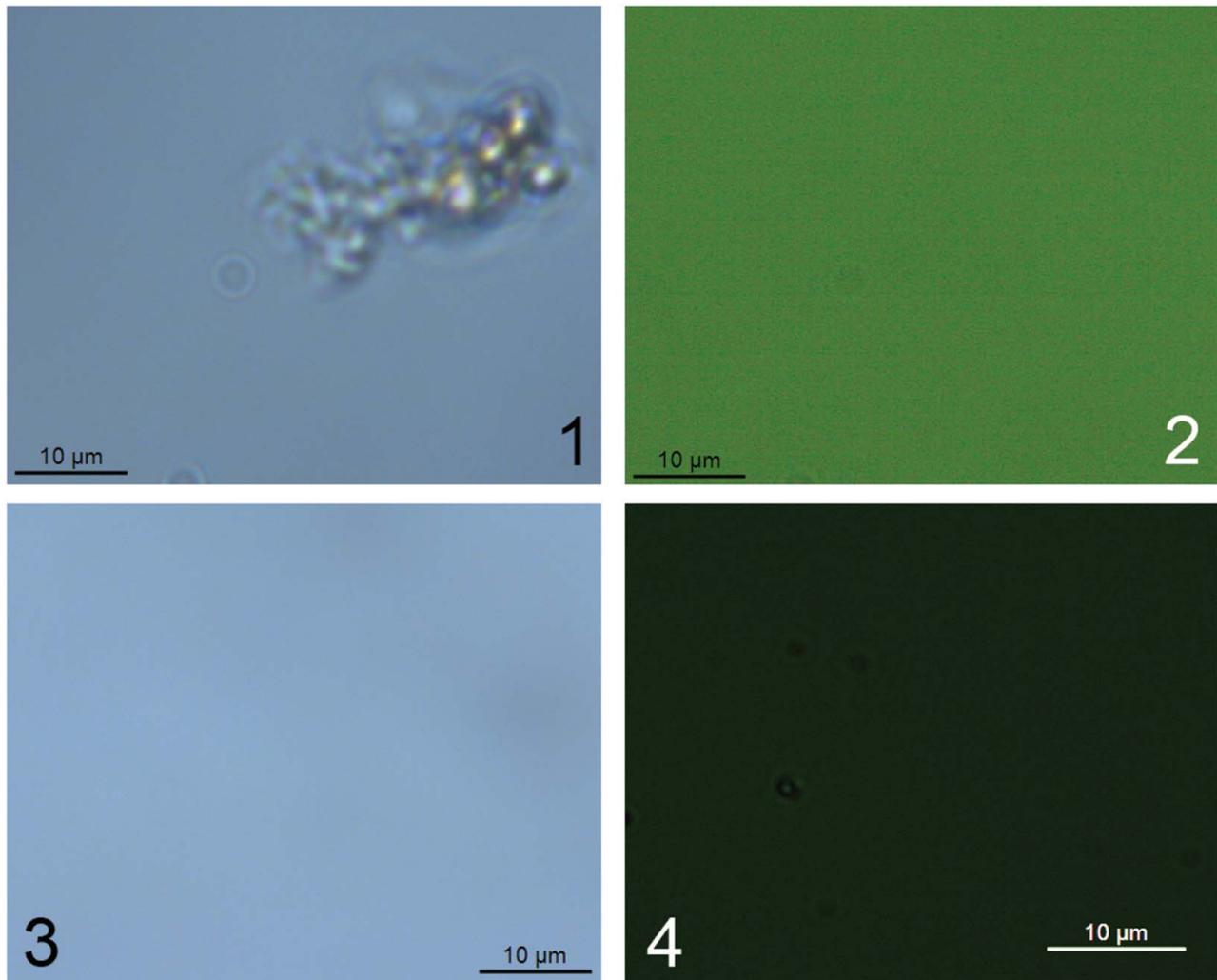
Contaminated LST<sup>®</sup> is weakly fluorescent (Plate 4, figure 2), as are samples from method 1. Samples from method 1 also contained fragments of silica (Plate 4, figure 1). LST<sup>®</sup> from methods 3 and 4 were non-fluorescing and contained no particulate matter (Plate 4, figures 3 and 4).

## 4. Conclusions and recommendations

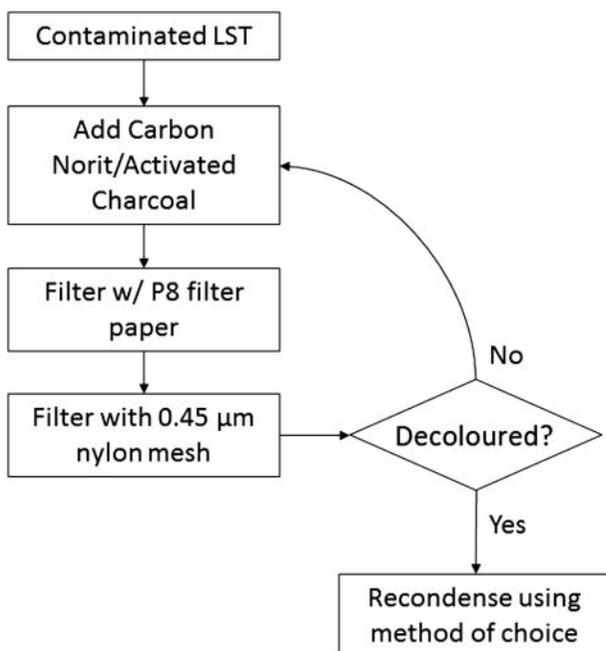
While method 3 worked well, method 4 appeared to work equally well and involves fewer steps and less waste material (Figure 1). LST<sup>®</sup> can be successfully cleaned and re-condensed using basic practices comparable to those in place in many laboratories for cleaning zinc bromide (ZnBr<sub>2</sub>) and



**Plate 3.** Results of method 4. Figure 1 is sample 1; figures 2, 3 and 4 are raw sample 2, 2× filtered sample 2 and 4× filtered sample 2, respectively; figures 5 and 6 are raw and treated sample 3, respectively; and figures 7 and 8 are raw and treated sample 4, respectively.



**Plate 4.** Figure 1, contaminated LST<sup>®</sup> showing weak yellow fluorescence; figure 2, particulate matter remaining in sample 1 following method 1. Figures 3 and 4 are results of methods 3 and 4, which are non-fluorescent and contain no particulate matter (unfortunately, dirt on a light-path mirror is present in the image).



**Figure 1.** Flowchart showing recommended decolouration method.

zinc chloride ( $ZnCl_2$ ). The process is easily followed by technicians, and rapid, provided the LST<sup>®</sup> is not allowed to crystallise. Even if it does, however, the LST<sup>®</sup> is soluble in water and rehydration merely adds another step to the process.

We cannot demonstrate that the recycled LST<sup>®</sup> achieved through this method is carbon-free and suitable for use with samples meant for radiocarbon dating using accelerator mass spectrometry (AMS); however, it is suitable for routine palynological preparations, and has been used successfully for such at Morehead State University.

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## References

- Barker CE. 1981. Decontaminating and recycling zinc bromide solution used in sink-float separation of Kerogen. *Journal of Sedimentary Petrology* 51:666–667.
- Brown C. 2008. Palynological techniques. 2nd ed. Riding JB, Warny S, editors. Dallas (TX): American Association of Stratigraphic Palynologists Foundation; p. 137.
- Burgess JD. 1974. Reclaiming zinc bromide solution used in palynology and kerogen processing as a result of skyrocketing costs. Gulf Resources Company, Unpublished report, 3 p.
- Caffrey MA, Horn SP. 2013. The use of lithium heteropolytungstate in the heavy liquid separation of samples which are sparse in pollen. *Palynology* 37:143–150.
- Callahan J. 1987. A nontoxic heavy liquid and inexpensive filters for separation of mineral grains. *Journal of Sedimentary Petrology*. 57:765–766.
- Campbell JFE, Fletcher WJ, Hughes PD, Shuttleworth EL. 2016. A comparison of pollen extraction methods confirms dense-media separation as a reliable method of pollen preparation. *Journal of Quaternary Science* 31:631–640.
- Gray J. 1965. Extraction techniques. In: Kummel B, Raup O, editors. *Handbook of paleontological techniques*. San Francisco: Freeman; p. 530–586.
- Gregory MR, Johnston KA. 1987. A nontoxic substitute for hazardous heavy liquids – aqueous sodium polytungstate ( $3\text{Na}_2\text{WO}_4 \cdot 9\text{WO}_3 \cdot \text{H}_2\text{O}$ ) solution. *New Zealand Journal of Geology and Geophysics* 30:317–320.
- Hanna MA. 1927. Clarification of oil-discolored bromoform. *Journal of Paleontology* 1:145.
- Hauff PL, Airey J. 1980. The handling, hazards, and maintenance of heavy liquids in the geologic laboratory. *United States Geological Survey Circular* 827, 24 p.
- Krukowski ST. 1988. Sodium metatungstate: a new heavy mineral separation medium for extraction of conodonts from insoluble residues. *Journal of Paleontology* 62:314–316.
- Munsterman D, Kerstholt S. 1996. Sodium polytungstate, a new non-toxic alternative to bromoform in heavy liquid separation. *Review of Palaeobotany and Palynology* 91:417–422.
- O'Keefe JMK, Eble CF. 2012. A comparison of HF-based and non-HF-based palynology processing techniques in clay-rich lignites from the Claiborne Group, upper Mississippi Embayment, United States. *Palynology* 36:116–130.
- Patrick JM, Patrick VA, inventors. 1997 May 27. Heavy Liquid for Material Separations. United States patent US 5632382.
- Proske U, Wood R, Fallon S, Stevenson J. 2015. Use of heavy liquid density separation to remove pyrite from sediment for radiocarbon dating. *Quaternary Geology* 25:66–71.
- Savage NM. 1988. The use of sodium polytungstate for conodont separations. *Journal of Micropalaeontology* 7:39–40.
- Six J, Schultz PA, Jastrow JD, Merckx R. 1999. Recycling of sodium polytungstate used in soil organic matter studies. *Soil Biology and Biochemistry* 31:1193–1196.
- von Bitter PH, Plint-Geberl HA, Miller AAL. 1978. Decolourization of heavy liquids – an economic assessment. *Canadian Journal of Earth Sciences* 15:1872–1875.
- Wagoner DL, Pruitt TL, Thompson JH. 1961. Palynological laboratory techniques and procedures. Richfield Oil Corp., Unpublished report, 14 p.