Electrostatic Separation of Cysts of the Sugar Beet Nematode

D. R. VIGLIERCHIO AND J. R. GOSS¹

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The separation of a large number of nematode cysts cannot be accomplished by repeated use of any one highly-refined separation process. Since there can be considerable overlap in the properties of cysts and accompanying debris, a higher degree of enrichment can be achieved by utilizing a series of less sensitive procedures, each based on different properties $(2, 3)^2$; hence, the investigation of the electrostatic separation of cysts from debris.

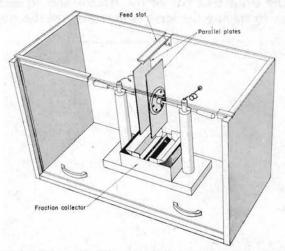


Figure 1.-Schematic diagram of the electrostatic separator.

Apparatus and Technique

The device used for this purpose (Figure 1) consisted of brass parallel plates $(100 \times 250 \times 3 \text{ mm})$ attached to brass sliding rods mounted on bakelite posts which in turn were mounted on a bakelite floor plate. The fraction collector consisted of a beta pan $(\pm\beta)$ fraction collector, $205 \times 155 \times 62$ mm with a floor sloping away from either side of a central knife edge) and 2 alpha pans $(\pm a \text{ fraction collectors}, 55 \text{ mm wide})$ constructed as shown so that the positions of the knife edges were adjustable. In practice the three fraction-collector knife edges were fixed so that the plate gap was divided into four equal sections for each gap setting, +a representing that section nearest the positive plate, $+\beta$ the

¹ Assistant Nematologist and Assistant Agricultural Engineer, respectively, University of California, Davis, California.

² Numbers in parentheses refer to literature cited.

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adjacent section on the positive side of the central knife edge, $-\beta$ the section on the negative side of the central knife edge, and -athe section nearest the negative plate. When the plates were in contact and vertically aligned with the feed slot and the beta pan knife edge, they were 90 mm above the beta pan and 10 mm below the feed slot. The plate assembly was positioned so that the plates extended 10 mm beyond both ends of the feed slot in order to reduce the edge effects of the electrostatic field. In practice only the middle portion of the slot was used since the pour time for the small samples was about 20-30 seconds.

The enclosure $(330 \times 750 \times 480 \text{ mm})$ constructed of plyboard and plexiglass reduced cyst movement by air currents and served as a shield from the high voltages applied to the plates.

The variable high voltage supply was provided by a Pt. No. HV200-102 from Plastic Capacitors Inc., Chicago, Illinois. The resistors and microammeter were precision 1% tolerance devices (Figure 2). The applied plate voltage was calculated from the 100 megohm series resistance and the measured current flow. The plates were sprayed with acrylic resin to reduce particle jumping from plate to plate at high potentials. Grounding of the feed slot improved the reproducibility of the separation.

Three samples were run at each set of conditions, cysts and debris in each fraction were hand separated for weighing and average weights then represented the results for a given set of conditions.

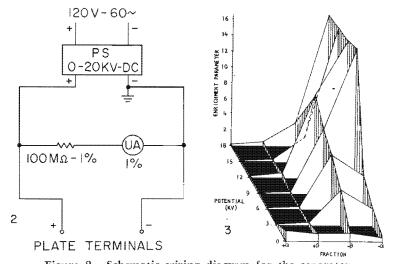


Figure 2.—Schematic wiring diagram for the separator. Figure 3.—Enrichment parameter variation with increasing potential of each fraction at constant plate gap (12 cm) and humidity (25% RH).

Results

A good separation pattern is shown in Figure 3. The fractions were arranged in sequence from positive to negative plate. The measure of degree of cyst separation and yield has been expressed as a composite relaton, EP (enrichment parameter) where

$$EP = \left(\frac{Avg. wt. of cysts in a fraction}{Avg. wt. of debris in a fraction}\right) \left(\frac{Avg. wt. of fraction}{Avg. wt. of sample}\right)$$

EP was selected in this manner as a compromise between purity and overall cyst yield. The bulk of the material was usually collected about the center of the gap, i.e., $+\beta$ and $-\beta$. The greatest purity was obtained near the plates, i.e., +a and -a. When $-\beta$ and -a had similar EP values, the $-\beta$ value resulted from a greater proportion of material at low purity whereas the -a value resulted from a low proportion of material at much higher purity. When EP values were very high >10, both purity and yield were relatively high resulting in the greatest number of pure cysts. For example at 25% RH, a plate gap of 12 cm and from 9-18 KV, the best fraction, -a, contained about 35% of the cysts and 1.9% of the debris from a sample, 75% cysts and 25% debris. Cysts in an electrostatic field therefore migrated preferentially toward the negative plate.

The electric field intensity (E) between parallel plates was essentially a function of plate potential (V) and distance between plates (d), i. e.,

$$E = \frac{V}{d}$$

when d was small with respect to the dimensions of the plates. The EP curves in Figure 4 show that enrichment was proportional to electric field intensity at the smaller plate gaps where the d assumption was valid. At larger plate gaps fringe field effects could no longer be neglected and EP was no longer proportional to $\frac{V}{d}$ -. Higher potentials than those shown for each plate gap were impractical since charge transfer caused the particles to jump from plate to plate and rendered the biodata unreliable.

In recycling the enrichment parameter for an efficient system could be expected to decrease with the removal of the more pure fraction. In Figure 5 it is evident that separation was essentially completed in two passes when about 75% of the cysts were obtained. It became increasingly more difficult to separate the remaining cysts from the more concentrated debris.

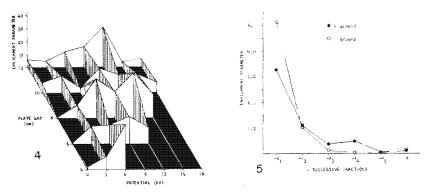


Figure 4.—Enrichment parameter variation with increasing plate gap and potential at constant humidity (45% RH) for the --a fraction.

Figure 5.—The enrichment parameter of successive -a fractions obtained by re-running the combined $-\beta$, $+\beta$ and +a fractions from the previous run. **D** is the final recombination.

Moisture, troublesome in sustaining useful static charge distributions because of increased leakage currents or charge migrations, was a factor in electrostatic separation (Figure 6). The cyst concentrate was stored at 43% RH until just before processing through the apparatus which was maintained at the RH indicated in Figure 6. With cyst concentrate stored at 90% and

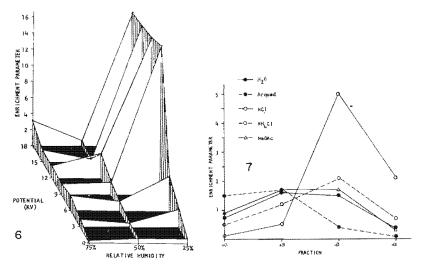


Figure 6.—Enrichment parameter variation with increasing potential and relative humidity at constant plate gap (12 cm).

Figure 7.—Enrichment parameter variation of separator fractions with different treatment of lots of cysts from a common source,

53% RH and then processed through the separator at 45% RH the EP values were of the same order observed in Figure 6 for 75% and 50% RH. Cyst separation could be achieved at a higher RH by using electric fields of greater intensity though the enrichment was never as great as that achieved in the drier atmospheres.

The effect upon cyst separation of pretreatment with dilute solutions of ionic substances is shown in Figure 7. Dilute HCl improved the normal cyst-debris separation. Ammonium chloride solution also improved the cyst separation but less markedly. Sodium acetate appeared to have no effect. Arquad, a cationic surface active agent (1), tended to reverse the normal cathode drift, i.e., the cysts migrated preferentially to the positive plate.

In view of the results (Figure 3) with a negatively grounded feed slot it might be expected that positively grounding the slot would affect the sample distribution. The polarity of the grounded feed slot was determined by the polarity of the grounded side of the high voltage supply, Figure 2. Positive grounding of the feed slot in processing impure cyst material did not necessarily improve the separation. Direct comparison of all four fractions showed that a negatively grounded feed slot was more effective as is also shown by an alternate comparison (Figure 5).

It is of interest to note that when cysts were collected on greased paper to preserve orientation, about 80% of the cysts were aligned with their major axis parallel with the electric field irrespective of feed slot grounding polarity.

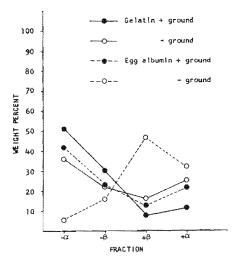


Figure 8.—Relative weight distribution in fractionation of gelatin and egg albumin with negatively and positively grounded feed slots.

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If two purified proteins were treated in the same manner the resulting distributions were altogether different. Gelatin particles migrated preferentially toward the cathode plate; the migration was increased by using a positively grounded feed slot (Figure 8). Egg albumin particles migrated toward the cathode plate with a positively grounded feed slot but toward the anode plate with a negatively grounded feed slot.

Discussion

The orientation of the cysts with the major axis parallel to the electric field indicated that there was induced axial polarization of the cysts much as would occur with any elipsoidal particle. Presumably the polarization was superimposed upon the net charge of each particle. The cyst reaction was not indigenous to proteins. Gelatin and ovalbumin with similar iso-electric points differed markedly in their reactions with the electrostatic separator used for cysts. The charge on the ovalbumin particle was determined largely by the polarity of the grounded feed slot where the charge on the gelatin particle was only slightly modified by the polarity of the feed slot. It would be difficult to predict the successful electrostatic separation of other cystforming nematodes. The outcome would need to be empirically determined in view of the electrostatic response of the two purified proteins and the effects of mineral and surface active solutions on Heterodera schachtii cysts. The mechanism of separation is uncertain; further investigation is essential for a better understanding of the underlying principles.

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