LEACHATE CONDUCTIVITY AS AN INDEX FOR QUANTIFYING LEVEL OF FORAGE CONDITIONING

T. J. Kraus, R. G. Koegel, R. J. Straub, K. J. Shinners

ABSTRACT. *A method based upon measuring the conductivity of the leachate (LC) from mechanically conditioned forage was evaluated and used as an index for quantifying the extent of mechanical damage caused by various mechanical treatments. Relative to a Surface Area Index (SAI) method previously used, the LC method was simple, fast, and could be completed with readily available laboratory equipment (orbital shaker table, Waring® blender, and conductivity meter). The LC method differentiated various mechanical conditioning treatments more often and with greater sensitivity than the SAI method. The consistency of different Waring blender treatments was assessed to determine if it could be used as a standard treatment for normalizing leachate conductivity values.*
Keywords. *Alfalfa, Forage, Cell rupture, Maceration.*

In many studies, severe mechanical conditioning of plant material (maceration) has increased forage drying rates (Shinners et al., 1987b; Savoie et al., 1992), improved forage digestibility and utilization (Koegel et al., 1992; Hong et al., 1988; Yang et al., 1993) and improved forage ensilability (Shinners et al., 1988; Savoie et al., 1994; Muck et al., 1989). Other studies have shown maceration to have little or no effect on these characteristics (Cowan et al., 1957; Baxter et al., 1966; Chiquette et al., 1993). These conflicting results could be attributed to different levels of conditioning. It is possible that in those studies where improved digestion and fermentation properties were found, the conditioning level was greater than in those studies where no such differences were found. However, the level of conditioning was not measured in any of these studies. Therefore, a significant new tool would be a method to quantify level of forage conditioning.

Several methods have been developed to quantify level of conditioning. Savoie et al (1996) developed a method to measure the bulk density of mechanically conditioned forage. Average bulk densities of unconditioned first cut alfalfa was 223 kg/m^3 compared to 687 kg/m^3 for severely conditioned crop. Although a correlation between conditioning level and final bulk density of the forage was found, further assessment was not conducted because the method could be affected by confounding variables such as material length and randomness of material orientation. It is evident that material cut into shorter lengths will tend to fill more of the void spaces in a forage mass. Therefore, material with small particle-size may have bulk densities similar to that of highly conditioned material having a longer particle-size. Although the final bulk densities of these two materials would be similar in this case, their physical characteristics—such as drying rate, fermentation rate, and digestibility rate—might not be similar.

Shinners et al. (1987c) proposed the surface area index (SAI) as an indication of the degree of maceration. The extent to which dried plant material absorbs water is related to its specific surface area and can be correlated to the extent of physical damage of the plant. Kraus et al. (1993) found SAI values ranged from 0.9 to 1.8 for slightly conditioned and severely conditioned material, respectively. This method has shown considerable promise, but its sensitivity and accuracy are unknown.

Locus et al. (1994) reported using the diffusion of K^+ ions from wounded perennial ryegrass into water as a measure of the intensity of conditioning. Although there was a relationship between the degree of conditioning and this diffusion for some types of mechanical treatments, the method was considered to be too complex and time consuming. In a similar approach, Emetarom (1976) used the electrical conductivity of the leachate from severely macerated plant material as an index of the extent of cellular rupture. With this method, it was assumed that damaged cells leached more rapidly than intact cells. Since the amount of leached electrolytes (ions) changes the conductivity of the leachate, measurement of the electrical conductivity of the leachate was used as an indicator of the extent of cell damage.

This procedure, though promising, had several drawbacks. First, the method was too complex and time consuming. Second, the forage samples were exposed to water for a relatively long time which may allow cells to imbibe water resulting in rupture and/or transfer of ions across cell membranes via exosmosis (Duke et al., 1983). In this case, the concentration of ions in the leachate would

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The authors are **Timothy J. Kraus**, ***ASAE Member Engineer***, Graduate Student, Biological Systems Engineering Dept., University of Wisconsin-Madison (presently Project Engineer, New Holland North America, Inc., New Holland, Pa.), **Richard G. Koegel**, ***ASAE Member Engineer***, Research Engineer, U.S. Dairy Forage Research Center, USDA-ARS, **Richard J. Straub**, ***ASAE Member Engineer***, Professor, and **Kevin J. Shinners**, ***ASAE Member Engineer***, Professor, Biological Systems Engineering Dept., University of Wisconsin-Madison. **Corresponding author:** Timothy J. Kraus, New Holland North America, Inc., 500 Diller Ave., PO Box 1895, New Holland, PA 17557-0903; voice: (717) 355-3345; fax: (717) 355-1939; e-mail: tkraus@newholland.com.

be the sum total of ions leached from mechanically ruptured cells and exosmosis, resulting in an overestimation of the level of crop damage. Third, absolute LC values give no indication of the ratio of damaged and undamaged cells within a sample. LC is not only dependent upon the extent of cellular damage, but also upon plant chemistry. Since forages may vary in chemical composition and physiological activity depending on the time of day, weather conditions, stage of growth, and soil fertility, absolute LC values cannot be compared across days, cuttings and/or regions.

It was proposed that if absolute LC values were reported as a fraction of LC values of a standard mechanical treatment that ruptures the vast majority of cells within a forage sample, absolute LC values would be normalized with respect to changes in plant chemistry. The rationale behind this approach is that if the standard treatment ruptured nearly every cell within a sample, changes in absolute LC of the standard treatment would be due to changes in plant chemistry. Consequently, the ratio (Treatment LC:Standard Treatment LC) would be normalized with respect to plant chemistry.

Therefore, a two part study was conducted. The purpose of the first part was to develop a simple and reliable method for measuring (LC), and to assess and compare the precision and accuracy of the LC and SAI methods. The purpose of the second part was to measure the extent of cell rupture of different blender treatments across a range of crop maturities and to determine if a blender treatment could be used as a standard treatment for normalizing LC values.

PROCEDURES

PART 1

The procedure to determine the LC of a forage sample was: (1) a relatively large sample of conditioned forage was cut with a scissors into lengths of approximately 5 cm and thoroughly mixed; (2) a 25 g sub-sample of the forage was placed into a 450 mL glass jar; (3) 300 mL of distilled water was added; (4) the mixture was shaken on an orbital shaker table at 200 cycles/min for 2 min; (5) the contents were filtered through two layers of cheese cloth; and (6) the conductivity of the leachate was measured using a Cole-Parmer model 1481-60 temperature compensated conductivity meter. The LC was determined for 10 replicate samples for each treatment.

The procedure to determine the SAI of forage samples (Kraus et al., 1993) was: (1) sub-samples, approximately 100 g each, from each treatment were oven-dried at 103°C for 24 h (ASAE standard: S358.1); (2) once dried, each sample was placed into a pre-weighed screened cylindrical canister; (3) each canister and contents were weighed to determine the initial mass of dried sample; (4) each canister and sample was immersed in tap water at room temperature for 1 min, removed from the water and tipped at 45° to drain for 45 s, and centrifuged at an acceleration rate of 12 g for 1 min; (5) once centrifuged, each canister and contents were weighed to determine the amount of moisture absorbed; (6) SAI was defined as the ratio of the mass of water absorbed by the sample to the oven dried mass. The SAI was determined for 10 replicate samples for each treatment.

To assess the sensitivity of the LC and SAI methods, four experiments were conducted across a variety of crop conditions. For Experiment 1, alfalfa (*Medicago sativa*) was hand harvested and conditioned to four different levels on three different days. For level 1, the alfalfa was not conditioned. For level 2, the alfalfa was intermittently crimped by passing it between two intermeshing rubber cover rolls typical of those used in conventional mower-conditioners. For level 3, the alfalfa was conditioned using a crushing-impact macerating device described by Kraus et al. (1993). This mechanism severely crushed and shredded the alfalfa stems into long fibrous pieces. For level 4, the alfalfa was conditioned using a rotary impact macerator (Kraus, 1997). This unit had four blunt blades in a plane attached to a high speed electric motor which was mounted centrally inside a cylindrical tube. As plant herbage was metered into the center of the rotating blades, it was impacted numerous times by the blunt blades causing the herbage to be extremely disrupted or macerated.

For Experiment 2, alfalfa was hand-harvested and conditioned to four different levels on two different days. For level 1, the alfalfa was not conditioned. For levels 2 and 3, the alfalfa was chopped into relatively short lengths using a Fox pull-type forage harvester with a six-knife cylindrical-type cutter-head with theoretical lengths of cuts of 0.95 and 2.54 cm, respectively. For treatment 4, the forage was conditioned using the rotary impact macerator.

In both Experiments 1 and 2, the extent of mechanical damage for each treatment was sufficiently different to be clearly visible. However, it was desired to assess the sensitivity of the LC and SAI methods further. Therefore, for Experiment 3, alfalfa was conditioned to six different levels using the crushing-impact macerator (Kraus et al., 1993). The force applied to the crushing rolls and the impact rotor speed of this device was increased with each treatment thereby incrementally increasing the severity of each successive treatment. In this experiment, physical differences between consecutive treatments could not be visually discerned; however, differences between the least and most severe treatments were visually apparent.

A mixture of bromegrass, quackgrass, and orchardgrass was conditioned using four different treatments, unconditioned, intermittently crimped with intermeshing rubber-covered rolls, severely crushed and fiberized with the crushing impact macerator, and extremely macerated with the rotary impact macerator, for Experiment 4. Further details of each experiment can be found in Kraus (1997).

The variances of the SAI data within treatments were relatively equal; therefore, daily SAI data within each experiment were pooled and analyzed using a one way analysis of variance. Differences between treatment means were determined by calculating a least squared difference (LSD). The variances of the LC data within treatments were not equal; therefore, the LC data were analyzed using a paired-t test for populations having unequal variances (Snedecor and Cochran, 1989).

PART 2

It was believed that macerating a sample using a Waring blender would serve as a suitable standard mechanical treatment because: (1) it is believed to rupture the vast majority of cells within a sample; (2) the process may be severe enough such that changes in crop strength would not

adversely affect the extent of cellular damage (conditioning level); and (3) the blender could be readily purchased and thereby available to laboratories. Experience has shown, however, that the severity of a constant mechanical treatment typically decreases as a crop matures and gains mechanical strength.

To measure the consistency of blender treatments, the fraction of damaged and intact cells within a sample was quantified by measuring the amount of chlorophyll leached from damaged cells as well as that remaining within intact cells. Total chlorophyll content was calculated as sum of each fraction. The ratio of chlorophyll leached from damaged cells to total chlorophyll within the sample was used as an index of extent of cellular rupture.

First and second cutting alfalfa were hand harvested at the University of Wisconsin West Madison Experimental Station in Madison, Wisconsin. Each cutting was harvested at three stages of growth. First cutting was harvested at late vegetative, late bud, and mid-flower; and second cutting was harvested at early bud, late flower, and late seed pod. The stage of growth of each cutting was determined using the mean stage by weight (MSW) method described by Fick and Mueller (1989). With this method, stems of alfalfa are separated according to their different morphological stages of development. Each stage has a pre-assigned number from 0 to 9. Once separated, the dry matter content of each sample is determined by oven drying at 103°C for 24 h (ASAE standard: S358.1). The MSW is calculated as the weighted average of the individual stage categories present in the herbage sample using equation 1.

$$MSW = \sum \frac{S \times D}{W} \quad (1)$$

where

S = stage number (0-9)
D = dry weight of stems in stage S
W = total dry weight of stems in herbage sample

Blender Treatments

A fresh sample from each cutting was cut with a scissors into pieces approximately 5 cm in length and thoroughly mixed. Once mixed, a 25 g sub-sample was placed into a 500 mL blender jar with 300 mL of distilled water and severely macerated with a Waring blender Model CB-5 using one of three constant blender treatments. The three treatments involved blending a sample for: (1) 1 min at 18,300 rpm; (2) 2 min at 22,000 rpm; and (3) 5 min at 22,000 rpm. In the latter treatment, the sample was alternately blended for 1 min and cooled in an ice-bath for 5 min until the sample had been blended for a total of 5 min. Cooling was required to prevent the mixture from overheating. Each treatment was replicated five times.

Chlorophyll Extraction

Once blended, each mixture was filtered through a 22 to 25-μm mesh filter paper. The residue of each sample was flushed with approximately 2500 mL of distilled water to remove chlorophyll from damaged cells. The filtrate (liquid fraction) was collected and diluted to a constant volume of 3000 mL with distilled water. A 20-mL sub-sample was collected from each filtrate sample and placed in an opaque vial. The residue fraction from each sample was placed in an opaque plastic container. Both the residue and filtrate samples were frozen for subsequent chlorophyll determination.

The chlorophyll content of each residue sample was determined using spectrophotometric methods described in the *Official Methods of Analysis of the Association of Official Analytical Chemists* (AOAC) handbook (Williams, 1984). This procedure is based upon extracting chlorophyll from the residue using acetone and determining the chlorophyll concentration by measuring light absorbency of the chlorophyll solution at two different wavelengths 660 and 642.5 nm.

The chlorophyll content of each filtrate sample was determined using a method based on filtering the water-chlorophyll mixture through a reverse phase column. The rationale behind this technique was that non-polar and mid-polar molecules (chlorophyll molecules) bind to the column whereas polar molecules (water molecules) pass through the column.

Prior to filtering, each 6 mL, 500 mg reverse phase column was washed with approximately 3 to 5 mL of methanol followed by 3 to 5 mL of milli-Q distilled water. Next, 5 mL of each filtrate was filtered through each column. After filtering, the chlorophyll bound to each column was removed by flushing approximately 1 mL of ether through each column three times. The ether-chlorophyll extract was collected and diluted with ether to a constant volume of 5 mL. The absorbency of this solution was measured at two wavelengths, 660 and 642.5 nm, using a Beckman DU-50 Series spectrophotometer according to the methods described in the *Official Methods of Analysis of the Association of Official Analytical Chemists* (AOAC) handbook (Williams, 1984). The total chlorophyll content of each filtrate sample was calculated by multiplying the chlorophyll concentration by the appropriate dilution ratios.

The total chlorophyll content of each fresh alfalfa sample was estimated as the sum of the residue and filtrate chlorophyll extracts. A chlorophyll ratio (CR), defined as the ratio of filtrate/total chlorophyll content, was used as an index of the extent of cellular damage of each treatment. The data for each treatment were pooled and analyzed using a one-way analysis of variance. The least squared difference (LSD) at P = 0.05 was used to determine statistical differences between treatment means.

Results

Part 1

Because each experiment was replicated across several days, statistical comparisons of treatment means listed in each table were made within rows, not between rows.

Table 1. Mean SAI values for alfalfa conditioned to four different levels (Experiment 1)*

Day	Uncond.	Mower-conditioner	Crushing-impact	Rotary-impact
1	0.78 a	1.07 b	1.76 c	1.73 c
2	0.84 a	0.93 a	1.74 c	1.49 b
3	1.00 a	1.13 b	1.59 c	1.88 d

* Different alphabetic designations denote statistical difference @ P = 0.05 within rows.

Table 2. Mean LC (μS/cm) values for alfalfa conditioned to four different levels (Experiment 1)*

Day	Uncond.	Mower-conditioner	Crushing-impact	Rotary-impact
1	13 a	67 b	674 c	899 d
2	28 a	52 b	550 c	880 d
3	28 a	60 b	518 c	922 d

* Different alphabetic designations denote statistical difference @ P = 0.05 within rows.

Tables 1 and 2 list the mean SAI and LC values for Experiment 1. Table 1 illustrates that there was no statistical difference between SAI values in a number of instances. On day 1, there was no statistical difference between treatments 3 and 4. Likewise, on day 2 there was no statistically difference between treatments 1 and 2. Furthermore, on day 2 the SAI value of treatment 3 was greater than treatment 4. On the other hand, table 2 illustrates that on each day there was a statistical difference between the mean LC values of each treatment. Moreover, the LC always increased as the severity of treatment increased.

Table 3 lists the SAI and LC values for Experiment 2. It can be seen that on both days, the LC increased as the severity of treatment increased and there was a statistical difference between treatment means. The SAI method, on the other hand, indicated there was no statistical difference between treatments 1 and 2 on day 2.

Table 4 lists the SAI and LC values for Experiment 3. In this experiment, a visual difference was apparent between the least and most severe treatments. However, the extent of mechanical damage should have increased with each treatment because the crushing roll force and impact rotor speed was increased with each successive treatment.

It can be seen that there was a statistical difference between five out of six treatments as measured by LC. Although treatment 4 was not statistically different than treatments 3 and 5, the mean LC values increased with each incremental treatment. Similarly, the mean SAI value increased incrementally as the severity of each treatment increased. However, there was only a significant difference between three out of six treatments as measured by SAI method, indicating that the LC method was more sensitive than the SAI method.

Table 3. LC (μS/cm) and SAI values of alfalfa with four different mechanical treatments (Experiment 2)*

Day	Method	Uncond.	Course Chopped	Fine Chopped	Rotary-impact
1	SAI	0.78 a	1.05 b	1.11 c	1.65 d
1	LC	11 a	65 b	122 c	932 d
2	SAI	0.95 a	0.95 a	1.21 b	1.68 c
2	LC	16 a	67 b	147 c	828 d

* Different alphabetic designations denote statistical difference @ P = 0.05 within rows.

Table 4. Mean LC (μS/cm) and SAI values for alfalfa conditioned to six different levels (Experiment 3)*

Method	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6
SAI	1.33 a	1.36 a	1.44 b	1.45 b	1.47 b	1.56 c
LC	237 a	288 b	399 c	440 cd	474 d	544 e

* Different alphabetic designations denote statistical difference @ P = 0.05 within rows.

Table 5. Mean SAI and LC values (μS/cm) of a grass mixture conditioned to four different levels (Experiment 4)*

Method	Uncond.	Mower-conditioner	Crushing-impact	Rotary-impact
SAI	0.81 a	1.08 b	1.17 b	1.71 c
LC	46 a	70 b	245 c	770 d

* Different alphabetic designations denote statistical difference @ P = 0.05 within rows.

Table 5 lists the SAI and LC values of grass conditioned using four different mechanical treatments. Again, there was a statistical difference between each treatment as measured by LC, but there was only a significant difference between three out of four treatments as measured by the SAI method.

PART 2

Figures 1, 2, and 3 are plots of average CR versus MSW for blender treatments 1, 2, and 3, respectively. The error bars represent the LSD at P = 0.05. If the difference between any two treatment means is greater than LSD, the two means are considered to be statistically different.

On average, the three treatments released 83 to 88% of the total chlorophyll. For treatment 1, there were no statistical differences between mean CR values for both cuttings (fig. 1). For treatment 2, there was a statistical difference between the mean CR values for the least

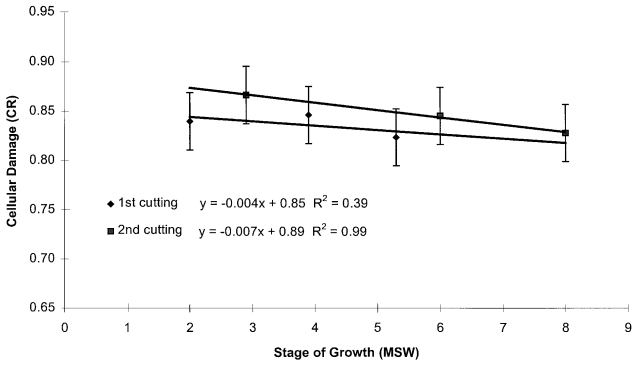

Figure 1–Cellular damage (based on chlorophyll release) vs stage of growth of alfalfa processed in Waring blender treatment 1 (1 min @ 18,300 rpm).

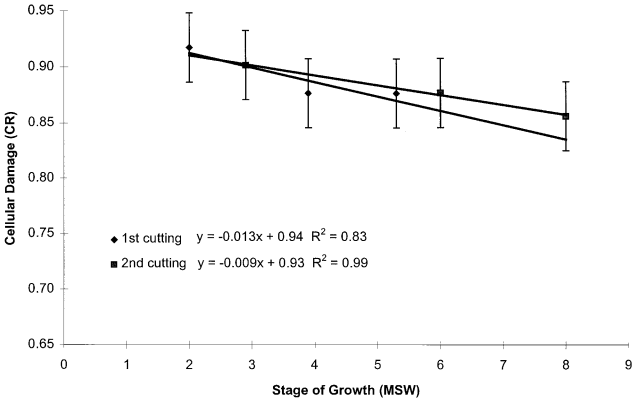

Figure 2–Cellular damage (based on chlorophyll release) vs stage of growth of alfalfa processed in Waring blender treatment 2 (2 min @ 22,000 rpm).

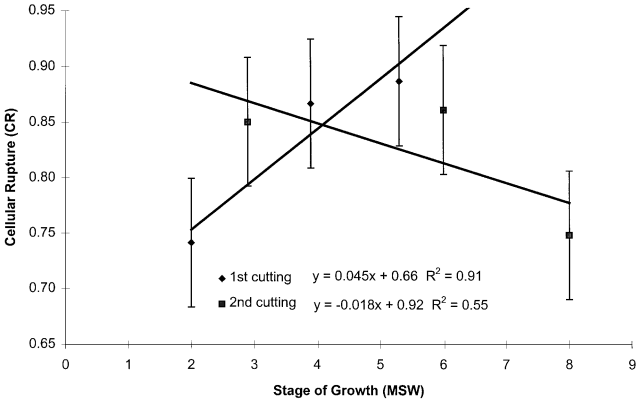

Figure 3–Cellular damage (based on chlorophyll release) vs stage of growth of alfalfa processed in Waring blender treatment 3 (5 min @ 22,000 rpm).

mature and most mature harvests. For both treatments, the extent of cell rupture appeared to decrease slightly as the alfalfa matured.

Treatment 3 was not as consistent as treatments 1 and 2 (fig. 3). The inconsistency may have been due to overheating the sample during the blending process. It was found when the sample was blended at 22,000 rpm continuously for 5 min, the temperature of the mixture increased causing much of the green particulate matter to precipitate out of solution. In an effort to minimize this effect, each sample was iteratively blended for 1 min then cooled in an ice bath for 5 min until it was blended for a total of 5 min. It is possible that the sample may still have over-heated, however. Longer cooling times were considered to be impractical and therefore, were not tested.

The results of part 1 indicate that the LC method consistently discerned differences in crop damage between various mechanical treatments. It was more sensitive than the previously developed SAI method which may improve the ability to quantify level of conditioning. In addition, the LC method consistently discerned differences between mechanical treatments in alfalfa and a mixture of grasses illustrating that it can be used across a wide variety of crop conditions and crop species.

The results of part 2 illustrate that blending a sample at 18,300 rpm for 1 min or at 22,000 rpm for 2 min could be used as standard treatment based on the consistency of cellular rupture. Although the extent of cell rupture appeared to decrease slightly as the crop matured, the extent of cell rupture remained nearly constant for the alfalfa harvested between late bud (MSW = 4) and late flower (MSW = 6); which is when most alfalfa is harvested for livestock feed. It is recommended that a blender speed of 18,300 rpm for 1 min be used as a standard mechanical treatment for normalizing LC values because there was no difference in consistency between treatments 1 and 2. The shorter blending, however, reduces the time to perform the procedure, and reduces the potential for overheating the mixture.

CONCLUSIONS

A method based upon measuring the conductivity of the leachate from mechanically conditioned forage was tested and used as an index for quantifying the extent of mechanical damage caused by various mechanical treatments. Relative to the SAI method, the LC method was simple, fast, and could be completed with readily available laboratory equipment (orbital shaker table and conductivity meter). The LC method quantified differences between various mechanical conditioning treatments more often and with greater sensitivity than the SAI method.

Blending times of 1 or 2 min with blender speeds of approximately 18,000 and 22,000 rpm, respectively, consistently ruptured 83 to 88% of cells in alfalfa harvested between early bud and late flower stages of growth. There was no difference in consistency of cell rupture between these two treatments.

The following method is recommended for measuring the level of conditioning using the leachate conductivity method and normalizing the LC values using a standard Waring Blender treatment so that values can be compared across a wide variety of crop conditions. It is recommended that the conductivity of at least five sub-samples from each treatment (including the Waring blender treatment) be measured.

Step 1: (1) Collect a relatively large sample of the conditioned forage, cut into lengths of approximately 5 cm, and thoroughly mix; (2) place a 25-g sub-sample into a 450-mL glass jar; (3) add 300 mL of distilled water; (4) shake the mixture on an orbital shaker table at 200 cycles/min for 2 min; (5) filter the contents through two layers of cheesecloth; and (6) immediately measure the conductivity of the leachate using a temperature compensated conductivity meter.

Step 2: (1) Place a 25-g sub-sample of the fresh material, that was cut into lengths of approximately 5 cm, into a 500-mL blender jar; (2) add 300 mL of distilled water; (3) blend the mixture for 1 min at a speed of 18,000 rpm using a Waring blender Model CB-5; (4) filter the mixture through two layers of cheesecloth; and (4) immediately measure the conductivity of the leachate using a temperature-compensated conductivity meter.

Step 3: Calculate the conditioning index by dividing the mechanical treatment conductivity by the Waring blender conductivity (eq. 2).

$$CI = \frac{\text{Treatment Leachate Conductivity}}{\text{Waring Blender Leachate Conductivity}} \times 100 \quad (2)$$

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