

# Development and optimization data of a tissue digestion method for the isolation of orthopedic wear particles under mesophilic conditions

Dr. Banshidhar Singh

HOD Zoology, D.C.S.K PG Collage Maunath Bhanjan Mau (U.P)

**Abstract:** The management of terrestrial weed is of great concern for the scientific community as these weeds cause adverse effect in different ecosystems like forest, agriculture and urban. The widespread of these weeds by their adaptive capability and morphological advancement is difficult to control. *Parthenium hysterophorus*, *Lantana camara*, *Saccharum spontaneum*, *Ageratum conyzoides* are the weeds that spread all over the world. There are various management practices employed for the control of this weeds. But all of these practices have some drawbacks those are neither environment friendly nor economical. In this paper a review has been done to evaluate various alternative management practices for these terrestrial weeds and to analyze their feasibility. Vermicomposting and anaerobic digestion can be viable alternative option which is cost effective as well. There are few studies regarding vermicomposting and anaerobic digestions of terrestrial weeds are done.

**Keywords:** Ultra-High Molecular Weight Polyethylene; Wear Debris; Periprosthetic Tissue; Tissue Digestion

## I. INTRODUCTION

A wild plant growing where it is not required and is competition with cultivated plant is termed as weed. There is a dynamic system that involves the interaction between weeds, crops, humans and environment. According to different definitions of weed – a plant growing where it is not wanted, a plant out of place, a plant growing where it is desired that something else growth, those plants with harmful or objectionable habits. Therefore, it can be summarized that weeds are those uninvited plants which are grown in undesirable place and period causing competition for cultivated crops and economic loss. Invasive plant species not only change the dynamics of species composition and biodiversity but also hamper the system productivity and efficiency in invaded regions [1]. Besides rapidly colonizing areas replacing the native vegetation, it is also known to cause a number of human health problems, environmental degradation including threat to tourism activities [2]. The characteristics that make a plant weed are-

- 1) long seed life in the soil,
- 2) quick murgence,
- 3) rapid early growth,
- 4) no special environmental requirements for germination,
- 5) ability to survive and prosper under disturbed condition.

Organizations engaged in invasion research defined invasive species as a species that is not native to the ecosystem under consideration whose introduction causes or is likely to cause economic or environmental harm or harm to human health,

species, subspecies or lower taxon, introduced outside its natural past or present distribution; includes any part, gametes, seeds, eggs, or propagates of such species that might survive and subsequently reproduce, animals, plants or other organisms introduced by man into places out of their natural range of distribution, where they become established and disperse, generating a negative impact on the local ecosystem and species, an alien species whose introduction does or is likely to cause economic or environmental harm or harm to human health (Invasive Species Advisory Council, 2001). In India, exotic weeds, especially *Parthenium hysterophorus* in urban areas, *Lantana camara* in forestlands, and *Ageratum conyzoides* in croplands, have assumed the proportion of noxious biological pollutants. Each of these three natives of tropical America has wide ecological amplitude. Because of ecological, agricultural, environmental, and health hazards for cattle and man, the respective governments of the states as well as the union government of India are trying hard to assess the damage and find a solution for their control [3]. Twelve sustainable weed management strategies are described as [4]: Know the weeds on your farm, Plant cropping systems to minimize open niches to weeds, keep the weed guessing, design the cropping system and select tools for effective weed control, grow vigorous, competitive crops, put the weeds out of work-grow cover crops, manage the weed seed bank, minimize deposition and maximize withdrawals, Know the weeds out at critical time, utilize biological processes to enhance weed control, bring existing weeds under control before planting sensitive crops and long term perennial crops, keep observing the weeds and adapt practices accordingly. In India, *Parthenium* it was testified

that it cause 40% damages in yield of agricultural crops [5] whereas in Australia, an yearly damage of \$16.8 million to the cattle industry was valued due to presence of this weed in grasslands [6]. Crop likes black gram (*P. mungo*), throughout first 30–45 days after crop planting, the existence of this weed caused significant yield losses, therefore management of this weed is essential [7].

Keep up on new development and practices have been done but for the developing countries, the use of organic manure on crop will not only improve the soil properties but will also cut down on the foreign exchange need for the purchase of mineral fertilizers. Weeds are available plenty but they are not wanted [8]. Weeds are unwanted plants but which can be converted into valuable resources and available free of cost growing without cultivation, irrigation and protecting the soil by giving of a warm soil cover. A farmer can produce his individual vermicompost from the biodegradable waste like weeds, made their own farm and need not spend extra money to purchase the raw material of vermicompost. Chemical fertilizers have been one of the major components of modern agriculture. Use of chemicals has now raised many questions related to the productivity of land and continuously increasing cost of cultivation. Vermicompost technology has been solved many problems. Earthworms have been known as farmer's friends for long (Darwin, 1881). Vermicompost technology is converting all biodegradable waste into plant nutrient rich organic manure with the help of composting.

## II. TISSUE DIGESTION CONDITIONS

Tissue samples were cut into 1 g tissue pieces, which were then cut into 0.25 x 0.25 cm cubes and exposed for 24 h to basic, acidic or enzymatic solutions to compare digestion efficiency. The tissues were processed by a single researcher, and all efforts were made to ensure the resultant size of each tissue was consistent by using a 0.25 cm grid. The digestion agents were prepared in 10 mL aliquots of distilled water for bases and acids, and PBS pH 7.4 for enzymes. After 24 h, each tissue digestive solution combination was diluted to a total volume of 200 mL by addition of distilled water. Dilution was necessary to avoid exposure of the polycarbonate filter to strong alkalinity (16 M NaOH), which can compromise the structural integrity of the filter. Nucleopore1 polycarbonate filters with a pore size of 1  $\mu$ m were weighed before sample filtration. Diluted samples were vacuumfiltered, transferred to Petri dishes and thoroughly dried for 1 h by convection heat lamps followed by 4–6 h at ambient temperature. Filters were reweighed and weight measurements were recorded. All samples were weighed on a calibrated scale with precision to four decimal places

## III. TISSUE DIGESTION EFFICIENCY CALCULATIONS

To evaluate digestion efficiency, initial and final filter and tissue weights were measured, and the percentage of residual

or undigested tissue was determined for each test condition. The final filter weights represented the amount of residual digested tissue that did not pass through the 1  $\mu$ m filter pores during vacuum-filtration.

## IV. TISSUE DIGESTION METHOD FOR THE ISOLATION OF ORTHOPEDIC WEAR PARTICLES UNDER MESOPHILIC CONDITIONS

For porcine hip tissue, consequences of every assimilation condition are appeared in Figure 1. The lingering tissue rates following 24 hours of processing were factually most reduced for 5M NaOH, 5M KOH and 15M KOH ( $p < 0.05$ ). These medications brought about conclusive tissue weights of  $< 1\%$  of the underlying tissue weight. Be that as it may, in view of photodocumentation (Fig. 2), just channels from the 5M NaOH processed examples demonstrated negligible measures of undigested tissue material. Every single other example, including the 5M, 10M and 15M KOH treated examples, contained undigested cell material. Channel pictures in figure 2 are illustrative of the measure of remaining tissue garbage.

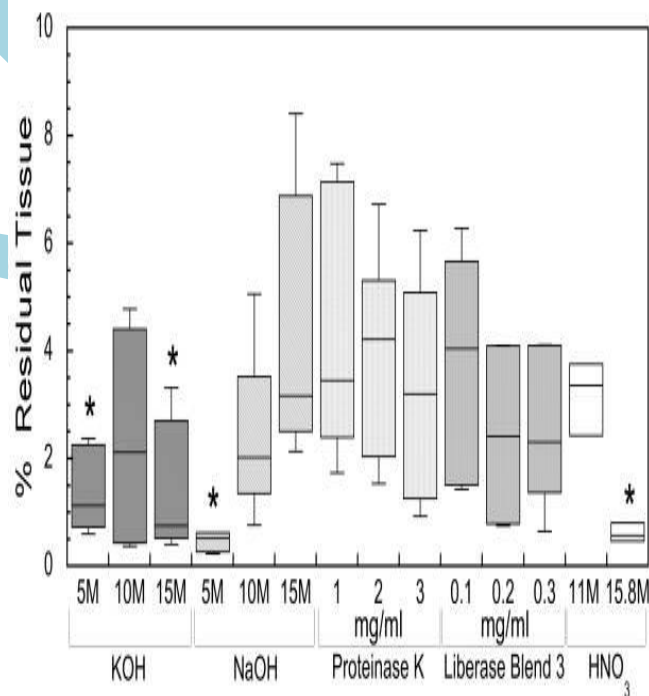


Figure 1

Assimilation results were measurably noteworthy for 5M NaOH, 5M KOH, 15M KOH ( $n=6$ ) and 15.8M HNO<sub>3</sub> ( $n=3$ ) arrangements, when contrasted with enzymatic and different arrangements ( $p < 0.05$ ). Following absorption with 5M NaOH and 15.8M HNO<sub>3</sub>, no leftover tissue clusters were watched. The diminished viability of NaOH at higher molar

fixations might be credited to the development of sodium accelerates.

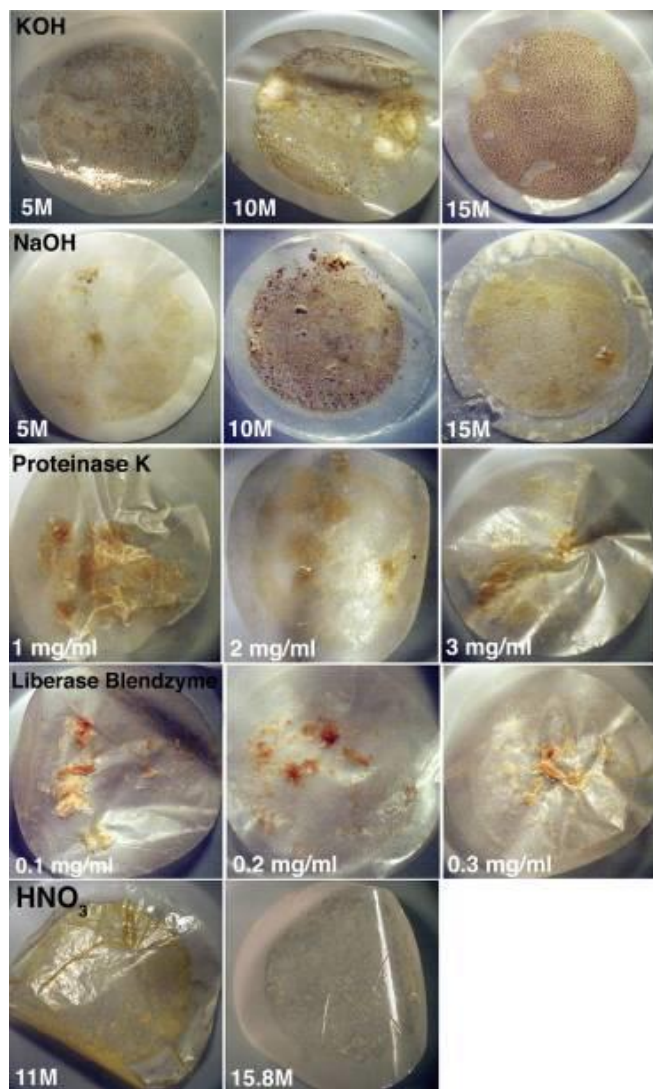


Figure 2

## V. DISCUSSION

This examination embraced a quantitative correlation of existing techniques used to process periprosthetic tissue for UHMWPE wear flotsam and jetsam investigation. Initial, a strategy for assessing and looking at the productivity of tissue absorption arrangements by evaluating leftover tissue weight was accounted for. Second, human tissues were most totally processed by 5M NaOH, 5M KOH or 15.8M HNO<sub>3</sub> arrangements. Every single other grouping of the corrosive and base stomach related operators, and also enzymatic arrangements, contained undigested bits of tissue. Third, ESEM assessment checked that UHMWPE particles and Ceridust particles presented to NaOH, KOH and HNO<sub>3</sub> arrangements don't experience morphological changes. At

long last, assimilation of formalin-settled tissue was most entire with 15.8M HNO<sub>3</sub>.

The present investigation results demonstrate that, over a time of 24 hours, neither Proteinase K nor Liberase Blendzyme 3 adequately processed tissue. Anyway effective enzymatic tissue absorption has been accounted for by various examiners (Table 1). Campbell et al. (1994) detailed that fruitful absorption was accomplished utilizing bacterial collagenase over a 24-hour time span, or, in other words enzymatic technique introduced in the writing. 38 Successful assimilation depended on a visual evaluation of the process after ultracentrifugation. Different specialists have utilized papain, which required the every day expansion of new protein more than 3 days to extricate particles. 34– 37 In the present investigation, absorption utilizing Liberase Blendzyme 3 and Proteinase K fixations was not stretched out past 24 hrs, since the requirement for steady checking and broadened time association and in addition the mind-boggling expense of materials make this methodology significantly less engaging.

Like different examinations, bring down molar arrangements of NaOH or KOH were observed to be compelling stomach related arrangements. Concentrates by Campbell et al. (1995) demonstrated that 5M NaOH adequately solubilized tissue inside a 24 hour era. 16,23,24 Shanbhag et al. (1994) utilized 4M KOH at 56°C for 48 hr to process tissue. 28 Additionally, concentrated HNO<sub>3</sub> was a successful processing arrangement. This is in concurrence with Slouf et al. (2007) who utilized concentrated HNO<sub>3</sub> over a 48 hour day and age. 30,32 Overall, the present examination demonstrates that utilizing 5M NaOH or KOH at 65°C or 15.8M HNO<sub>3</sub> at room temperature brought about ≥99% of tissue assimilation following 24 hours.

Since a considerable lot of the periprosthetic tissues are put in formalin, the present investigation assessed absorption productivity of the corrosive and construct arrangements in light of formalin-settled porcine tissues. Processing utilizing concentrated HNO<sub>3</sub> was altogether more productive than 5M NaOH or KOH arrangements. Dissimilar to the fundamental arrangements, the stomach related activity of 15.8M HNO<sub>3</sub> was not influenced by formalin obsession as contrasted and non-settled tissues (Figs. 3, ,4). Margevicius et al. (1994) and Hirakawa et al. (1996) utilized concentrated nitric corrosive for 48+ hours to process formalin-settled tissue. 30 The present finding that formalin fixative reductions processing proficiency is in concurrence with strategies utilized by Hahn et al. (1997) and Wolfarth et al. (1997) who utilized 5M NaOH for 24hr, and by Tipper et al. (2000) and Howling et al. (2001) who utilized 12M KOH for 2– 5 days to accomplish finish assimilation. 27,29 The creators don't wish to propose formalin obsession as a general standard for gathering tissue recovery, as this strategy for protection can force confines on corresponding examinations of tissue tests by histological or immunohistochemical strategies. In any case, this information recommends that intensive tissue processing can be accomplished utilizing concentrated

HNO<sub>3</sub> for occurrences where formalin obsession is the current convention of tissue accumulation.

## VI. CONCLUSION

The aftereffects of the current quantitative examination feature the fluctuation of current tissue processing techniques. Of the fourteen conditions tried, three stomach related arrangements brought about remaining tissue weight under 1% of the underlying example tissue weight: 5M NaOH, 5M KOH and 15.8M HNO<sub>3</sub>. A few points of interest of utilizing HNO<sub>3</sub> are that example weakening and along these lines the last example volume can be decreased in light of the fact that concentrated HNO<sub>3</sub> does not influence the polycarbonate sift used to channel through the UHMWPE wear trash, and it doesn't impact the UHMWPE molecule morphology. Furthermore, concentrated nitric corrosive has been utilized to seclude metal particles bringing about <1% of molecule disintegration and no adjustment in morphology following 48 hours, as affirmed by nuclear assimilation spectroscopy and ESEM, individually. 30 interestingly, soluble base arrangements running from 2– 2M have been appeared to influence the size and particle creation of metal particles as right on time as 2 hours after introduction. 41,42 Based on the present assessment of processing productivity, and past wear flotsam and jetsam examination, concentrated HNO<sub>3</sub> is both successful and the minimum dangerous stomach related arrangement. These outcomes give an essential system to sped up and effective tissue assimilation to remove UHMWPE or metal wear garbage.

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