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Research Article

Microbial Proteolytic and Hydrolytic Enzyme Kinetics in Postmortem Liquefaction and Tissue Degradation: A Systematic Review for PMI Mapping

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ABSTRACT

Background: Estimating the postmortem interval (PMI) beyond the early algor-mortis window remains one of the least standardized areas of forensic pathology. As decomposition proceeds, host autolytic enzymes and a succession of microbial proteases, lipases, and other hydrolases drive the progressive liquefaction of soft tissue, and the kinetics of this enzymatic activity have been proposed as quantitative PMI markers.

Objective: This systematic review synthesizes evidence on microbial proteolytic and hydrolytic enzyme activity during postmortem tissue liquefaction and degradation, and evaluates its utility, alongside host-derived enzymatic and omics markers, for PMI mapping.

Methods: A structured search of PubMed-, Scopus-, Web of Science-, and Google Scholar-indexed literature was conducted for studies published between July 2000 and April 2026 addressing protein, lipid, or other macromolecule degradation kinetics in postmortem tissue, with emphasis on microbial enzymatic drivers. Eligible study types included animal and human cadaveric studies, proteomic/metabolomic PMI studies, and systematic reviews; entomological and pure skeletal-isotope PMI methods without an enzymatic/biochemical component were excluded.

Key findings: Twenty-eight studies met inclusion criteria. Host calpain-mediated proteolysis dominates the first hours to days postmortem in skeletal muscle, while microbial hydrolasesgenerated as gut and environmental bacteria proliferate and disseminatebecome the principal driver of tissue liquefaction from roughly the second day onward, producing characteristic byproducts such as hydrogen sulfide, ammonia, and biogenic amines. Multi-omics and machine-learning approaches integrating these markers now predict PMI to within approximately one to two days across a multi-week range, substantially outperforming classical methods beyond the first 72 hours.

Conclusion: Microbial enzyme kinetics offer a biologically grounded, decomposition-resistant complement to host-protein degradation and entomological methods for PMI mapping, but standardization of sampling, enzyme assay, and statistical modelling is required before broad forensic validation. Practical and research implications are discussed.

Keywords: Postmortem Interval, Forensic Enzymology, Proteolysis, Hydrolytic Enzymes, Thanatomicrobiome, Tissue Liquefaction, Forensic Proteomics, Putrefaction.

INTRODUCTION

Postmortem interval (PMI) estimation underpins much of forensic death investigation, yet the precision of available methods declines sharply once the early window of body cooling and rigor mortis has passed [1,9]. Beyond approximately 48–72 hours, classical physical methods (rectal temperature, vitreous potassium) lose reliability, and forensic practitioners must rely on biochemical and biological proxies for elapsed time since death [11,12]. Among these, the enzymatic breakdown of tissue macromolecules has long been recognized as a fundamentally time-dependent process: host-derived enzymes (notably the calcium-dependent calpains) initiate autolysis within hours of circulatory arrest, while an expanding consortium of bacterial proteases, lipases, nucleases, and other hydrolases progressively takes over as

the principal agent of tissue breakdown, ultimately producing the grossly observable liquefaction of soft tissue characteristic of the putrefactive stage of decomposition [2,3,14].

The rationale for this review arises from a persistent fragmentation in the literature: studies of host protein degradation (proteomics, Western blotting, zymography) have developed largely within forensic biochemistry groups [1–3], while studies of the bacterial communities that drive later-stage liquefaction have developed within forensic microbiology and thanatomicrobiome research [14–18,21], and multi-omics/metabolomic PMI studies have emerged as a third, increasingly dominant strand [4,5,8,13]. Each strand has produced credible, time-correlated markers, but no existing synthesis has examined them jointly through the specific lens of enzyme kineticsthe rate and pattern of catalytic activityas the unifying mechanistic thread connecting autolysis, putrefaction, and the biomolecular signatures now exploited by proteomic and metabolomic PMI models.

Accordingly, this review asks: What is known about the kinetics of microbial proteolytic and hydrolytic enzyme activity during postmortem tissue liquefaction, how does this activity interact with host-derived autolytic enzymes, and how have these processes been operationalized for PMI mapping? The scope is bounded to studies published between July 2000 and April 2026; to animal and human postmortem/cadaveric studies addressing protein, lipid, or related macromolecule degradation with an explicit or inferable enzymatic or microbial mechanism; and to systematic reviews synthesizing this literature. Pure entomological PMI methods, skeletal radiocarbon dating, and studies of living-tissue enzymology without a postmortem or decomposition component were considered out of scope, though several are referenced for context where they inform interpretation of the early-PMI window against which microbial-hydrolase-driven liquefaction must be distinguished.

This review is intended for forensic pathologists, forensic biochemists, and forensic microbiologists seeking a single point of reference connecting host autolysis, bacterial putrefactive enzymology, and the modern proteomic/metabolomic/machine-learning PMI literature, and for researchers seeking to identify methodological gaps where enzyme-kinetic modelling of microbial hydrolases remains underdeveloped relative to host-protein degradation modelling.

LITERATURE SEARCH STRATEGY

Searches were conducted iteratively across PubMed-, Scopus-, Web of Science-, MDPI-, Springer-, Nature-, eLife-, and Google Scholar-indexed literature, supplemented by hand-searching reference lists of retrieved systematic reviews and forensic-biochemistry primary studies, a recognized supplementary strategy given inconsistent indexing of "enzyme kinetics" terminology across forensic pathology, microbiology, and proteomics literatures.

Search terms combined three concept blocks with Boolean logic: (1) process terms"postmortem interval", "time since death", "putrefaction", "tissue liquefaction", "autolysis"; (2) enzyme/mechanism terms"proteolytic enzyme", "hydrolytic enzyme", "calpain", "protease", "lipase", "protein degradation", "microbial enzyme", "thanatomicrobiome"; and (3) application terms"forensic", "PMI estimation", "proteomics", "metabolomics". Searches were restricted to records published between July 2000 and April 2026 in English.

Inclusion Criteria Were: (a) original research (animal or human, in vivo, ex vivo, or cadaveric) or systematic/literature review reporting enzymatic, proteomic, or metabolomic degradation data with a postmortem time component; (b) an explicit or inferable mechanistic link to proteolytic or hydrolytic enzyme activity (host or microbial); (c) peer-reviewed publication.

Exclusion Criteria Were: studies confined to entomological succession or skeletal isotope dating without a biochemical/enzymatic component; studies of living-tissue enzymology without a postmortem application; conference abstracts without full text; non-English sources.

Across search iterations, approximately 95 unique records were identified. Title/abstract screening removed records on unrelated topics (e.g., industrial collagenase biotechnology, non-forensic hydrogen sulfide toxicology, clinical wound enzymology). Approximately 38 full texts were assessed in detail, of which 28 m*et al*l inclusion criteria. The included set comprises one dedicated systematic review of host protein-degradation PMI markers [1], three primary calpain/zymography studies [2,3], one multi-omics bone study [4], one large-cohort metabolomics/machine-learning study [5], three hydrogen-sulfide/metabolomics PMI studies [6–8], three

additional PMI-method reviews [9–11], two vitreous-humor/potassium studies [12,13], and fourteen thanatomicrobiome/postmortem-microbiology studies addressing the bacterial-enzyme-driven side of liquefaction [14–28]. No single quality-appraisal tool was applied uniformly across this methodologically heterogeneous set; instead, study design, sample size, and risk-of-bias considerations reported within the included primary systematic review [1] were adopted as a reference standard, and remaining studies were tabulated narratively (Table 1) for qualitative weighting.

Study Selection Flow (PRISMA-informed)

- Records identified through database and supplementary searching: ~95
- Full texts assessed after duplicate removal and title/abstract screening: ~38
- Full texts excluded (no enzymatic/postmortem component, wrong outcome, non-English, abstract only): ~10
- Studies included in qualitative synthesis: 28

TYPE OF REVIEW

This article is a systematic review with narrative thematic synthesis rather than a meta-analysis. A quantitative meta-analytic pooling of effect sizes (e.g., a single pooled degradation rate constant) was judged infeasible because the 28 included studies measure fundamentally different outcome metricsimmunoblot band-intensity decay, zymographic enzyme-activity units, mass-spectrometric peptide abundance, 16S rRNA relative taxon abundance, and machine-learning prediction erroracross non-commensurable tissue matrices (skeletal muscle, bone, blood, vitreous humor, gravesoil-adjacent skin). Forcing these into a single pooled estimate would violate basic assumptions of meta-analytic homogeneity.

Instead, this review follows the systematic methodology described in Section 2 (explicit search strategy, prospectively defined inclusion/exclusion criteria, PRISMA-informed selection flow) combined with thematic narrative synthesis (Section 4), mirroring the design adopted by the primary systematic review this work builds most directly upon, Zissler *et al.*'s PRISMA-guided synthesis of postmortem protein degradation [1], as well as by other recent PMI-method reviews in this space [9–11].

The review is systematic rather than purely narrative because: (i) the search strategy, databases, and date range are fully specified and reproducible (Section 2); (ii) eligibility criteria were defined prospectively; (iii) a defined screening and selection flow is reported; and (iv) included studies are tabulated with design, sample size, enzyme/method, and finding (Table 1), allowing the reader to weigh evidence strength independently of narrative interpretation. It is not a meta-analysis because no pooled quantitative degradation-rate or diagnostic-accuracy estimate is calculated across studies, and it is not a pure scoping review because explicit eligibility criteria, rather than open topic-mapping, governed selection.

This design has direct interpretive consequences: conclusions in this review describe consistent qualitative kinetic patterns (e.g., "calpain-mediated proteolysis dominates the first 24–48 hours, after which microbial hydrolase activity becomes the principal driver of further degradation") rather than a single validated rate equation applicable across tissue types and environmental conditions. Readers seeking a specific, validated quantitative PMI model for casework should consult the primary studies directlyfor example, the calpain-zymography time courses in Pittner *et al.* [2,3] or the neural-network metabolomic model in Magnusson *et al.* [5]rather than treating this synthesis as itself a calibrated forensic tool.

MAIN BODY

Host-Driven Autolysis: The Early Enzymatic Baseline

In the first hours to days after death, tissue degradation is dominated by host-intrinsic, calcium-activated proteases rather than microbial activity. Pittner *et al.*'s porcine model tracked the inactive-to-active transition of calpain 1 and calpain 2 via casein zymography across 240 hours postmortem and found that titin, nebulin, desmin, cardiac troponin T, and SERCA1 degraded in a regular, accumulated-degree-day-dependent sequence [2]. This pattern was subsequently confirmed in 40 human forensic cases, with degradation kinetics distinctly associated with both temperature and PMI [3]. Zissler *et al.*'s systematic review of 36 studies spanning 130 proteins across 11 tissues confirmed this general principle of time-dependent, tissue-specific proteolysis, while noting that consistent cross-study evidence existed for only a minority of method-tissue-protein combinations, reflecting substantial methodological heterogeneity across the field [1].

Microbial Hydrolase Succession and the Liquefactive Transition

As host autolytic processes exhaust available substrate and immune containment fails, gut- and environment-derived bacteria proliferate and disseminate, contributing an expanding repertoire of extracellular proteases, lipases, and other hydrolases. Heimesaat*et al.*'s murine model demonstrated that intestinal bacterial translocation into extra-intestinal compartments begins within five minutes of death, with enterobacterial and enterococcal populationstaxa rich in extracellular proteolytic and hydrolytic enzymesrising three to five log-orders by 72 hours [19]. Javan *et al.*'s thanatomicrobiome work showed a parallel rise in Clostridium spp. dominance with increasing PMI in human cadaveric organs [14], and Metcalf *et al.*'s mouse-cadaver model went further, directly linking microbial community succession to shifts in metabolic and enzymatic function rather than taxonomy alone, providing a function-based rather than purely compositional decomposition clock [16]. The downstream byproducts of this microbial hydrolytic activityhydrogen sulfide, ammonia, and biogenic amines such as putrescine and cadaverineare themselves measurable and time-correlated, as demonstrated in Wang *et al.*'s controlled kinetic study of postmortem hydrogen sulfide production [6], though Suzuka *et al.* caution that this same putrefactive production confounds toxicological interpretation of antemortem poisoning in decomposed casework [7].

Integration: Multi-Omics and Machine-Learning PMI Mapping

The most methodologically advanced recent studies integrate host-enzymatic and microbial-hydrolytic signatures within multi-omics or machine-learning frameworks rather than treating them as competing markers. Bonicelli *et al.*'s ForensOMICS approach combined metabolomics, lipidomics, and proteomics in human bone, finding that bone-matrix-protected proteins persisted far longer than blood/plasma proteins and that combined panels improved discrimination across decomposition stages spanning up to 834 days [4]. Most strikingly, Magnusson *et al.*'s 2026 neural-network model, trained on metabolomic profiles from 4,876 human femoral blood samples, predicted PMI across a 1–67-day range with a mean absolute error of only 1.45 days, with the most informative features clustering around markers of proteolysis, lipid degradation, and mitochondrial dysfunctiondirectly validating enzyme-kinetic byproducts as machine-learning-ready PMI features at a scale far exceeding any single-enzyme study [5]. Agreement across studies is strongest on the general two-phase kinetic model (host calpain-dominant phase giving way to microbial hydrolase-dominant phase); disagreement is most evident in the precise transition timing, which varies with ambient temperature, tissue type, and whether muscle, bone, blood, or vitreous humor is sampled [1,9,12,13].

Table 1: Summary of Key Included Studies

Author, Year	Design / n	Enzyme / Method	Key Finding Relevant to Enzyme Kinetics & PMI Mapping
Zissler *et al.*, 2020	Systematic review, 36 studies	Western blot, MS, IHC130+ proteins, 11 tissues	First systematic synthesis of protein-degradation kinetics for PMI; confirms time-dependent, tissue-specific fragmentation but high methodological heterogeneity and risk of bias.
Pittner *et al.*, 2016 (porcine)	Animal model, biceps femoris, 240 hpm	SDS-PAGE, Western blot, casein zymography	Calpain 1/2 activation tracked via zymography showed a predictable inactive→active transition; titin, nebulin, desmin, cTnT, SERCA1 degraded in regular, ADD-dependent sequence.
Pittner *et al.*, 2016 (human)	Cadaveric, 40 forensic cases	Western blot, casein zymography	Confirmed porcine calpain-driven degradation pattern in human skeletal muscle; degradation strongly temperature- and ADD-dependent, supporting forensic translatability.
Bonicelli *et al.*, 2022	Human bone, taphonomic	Multi-omics: metabolomics, lipidomics, proteomics	Bone-matrix-protected proteins persisted far longer than blood/plasma proteins; combined omics panels

	facility, n = 4 donors		improved PMI discrimination across decomposition stages up to 834 days.
Magnusson *et al.*, 2026	Retrospective cohort, n = 4876	Untargeted metabolomics, neural network	Markers of proteolysis, lipid degradation, and mitochondrial dysfunction predicted PMI (1–67 days) with 1.0–1.45-day mean error, outperforming six other ML architectures.
Wang *et al.*, 2022	Spiked-blood kinetic model	GC-MS / LC-MS, H2S and thiosulfate	Demonstrated that hydrogen sulfidea hydrolytic/putrefactive byproduct of bacterial sulfur-amino-acid catabolismis generated postmortem at rates dependent on storage temperature, confounding toxicological interpretation.
Steuer *et al.*, 2024	Human femoral blood cohort	Targeted/untargeted metabolomics	Endogenous compound levels (amino acids, amines) shifted measurably with time since death, reflecting ongoing proteolytic/hydrolytic turnover even in stored postmortem blood.
Javan *et al.*, 2016	Cross-sectional, n = 27 corpses	16S rRNA, thanatomicrobiome, PMI 3.5–240 h	Clostridium spp. dominance increased with PMI, consistent with proliferation of organisms expressing potent extracellular proteases/hydrolases driving tissue liquefaction.
Metcalf *et al.*, 2016	Mouse cadaver model, controlled decomposition	16S rRNA + metabolomics, network modelling	Linked microbial community succession directly to metabolic/enzymatic function during decomposition, providing a predictive, function-based PMI clock rather than taxonomy alone.
Heimesaat*et al.*, 2012	Murine model	Culture + molecular, gut/extra-intestinal sites	Intestinal bacterial translocation began within 5 minutes of death; enterobacteria/enterococci (carrying proteolytic and hydrolytic enzyme repertoires) rose 3–5 log-orders by 72 h, confounding tissue-of-origin attribution of enzymatic activity.

DISCUSSION

Synthesizing across the included evidence, three trends emerge. First, a coherent two-phase enzyme-kinetic model of postmortem tissue degradation is well supported: an early, host-driven calpain/autolytic phase (roughly the first 24–72 hours, temperature-dependent) gives way to a microbial hydrolase-driven liquefactive phase that dominates from approximately the second day onward and continues for weeks [1–3,14,16,19]. Second, the field has moved decisively toward multi-marker, machine-learning-integrated approaches, with the most recent and largest studies [4,5] substantially outperforming single-enzyme or single-metabolite models, suggesting the future of PMI mapping lies in integrated panels rather than any single proteolytic or hydrolytic marker.

Inconsistencies across the literature concern the precise transition timing between host- and microbially-driven phases, which is confounded by postmortem bacterial translocation beginning within minutes of death [19] and by environmental temperature variation not uniformly reported across studies [1,2]. Clinically and forensically, these findings suggest that enzyme-kinetic and microbial-hydrolase markers are most useful as components of integrated multi-omics PMI models rather than as standalone tools, particularly beyond the 72-hour window where classical physical methods fail [9,11]. Compared with existing forensic guidance, which still relies primarily on temperature-based and entomological methods for early-to-mid PMI and is largely without a standardized biochemical method for late PMI [9], this review

supports continued development of integrated enzyme-kinetic/metabolomic panels as the most promising path toward a validated late-PMI biochemical standard.

LIMITATIONS OF THE REVIEW

Several limitations should be considered. The evidence base directly modelling microbial hydrolytic enzyme kinetics (as opposed to host protein degradation or downstream metabolomic byproducts) remains comparatively thin, with much of the mechanistic inference in this review drawn from murine translocation models [19] and indirect metabolomic byproduct studies [5,6] rather than direct microbial enzyme-activity assays in human postmortem tissue. Publication bias likely favors studies reporting statistically significant, well-correlated degradation markers over null results, and the dominance of a small number of forensic-biochemistry research groups (e.g., the Salzburg calpain/zymography group [1–3]) in the host-protein literature may limit independent replication.

Considerable heterogeneity exists across studies in tissue type, sampling site, assay method (zymography, Western blot, mass spectrometry, 16S rRNA sequencing, untargeted metabolomics), and environmental control, precluding quantitative pooling. This review was restricted to English-language, peer-reviewed sources accessed via systematic search engines spanning PubMed-, Scopus-, and Web of Science-indexed content rather than native proprietary database query interfaces, and a small number of relevant non-English or database-exclusive records may have been missed. Finally, several included sources were themselves reviews rather than primary data; where possible, primary studies were traced and cited directly, but this was not exhaustive given the scope of this synthesis.

CONCLUSION

This systematic review synthesized 28 studies addressing microbial proteolytic and hydrolytic enzyme kinetics in postmortem tissue liquefaction and their application to PMI mapping. The evidence supports a two-phase kinetic model: host calpain-mediated autolysis dominates the early postmortem window, while an expanding consortium of bacterial proteases, lipases, and other hydrolasesdisseminated via translocation beginning within minutes of death and proliferating over subsequent daysbecomes the principal driver of macroscopic tissue liquefaction from approximately the second day onward, producing measurable byproducts (hydrogen sulfide, biogenic amines, degraded protein/lipid fragments) that now form the feature basis of state-of-the-art multi-omics and machine-learning PMI models.

In answer to the review question, current evidence supports microbial enzyme-kinetic markers as a mechanistically coherent and increasingly well-validated component of late-PMI estimation, particularly when integrated into multi-omics panels, but not yet as a standalone, independently calibrated forensic tool outside research settings. For practice, forensic pathologists and laboratories should consider integrated proteomic/metabolomic panels, rather than single-marker assays, when classical temperature- and entomology-based methods are no longer applicable. For policy, forensic science accrediting bodies should prioritize development of standardized sampling, assay, and statistical-reporting protocols for enzyme-kinetic and microbial-hydrolase PMI markers before such evidence is introduced into casework or courtroom testimony, given the current heterogeneity documented in this review. For future research, priority should be given to: (i) direct enzyme-activity assays of microbial proteases/hydrolases in human (not only murine or porcine) postmortem tissue; (ii) explicit modelling of the host-to-microbial kinetic transition under varying environmental conditions; (iii) large-cohort replication of multi-omics machine-learning models such as Magnusson *et al.*'s [5] across diverse populations and PMI ranges; and (iv) harmonized reporting standards to enable future quantitative meta-analysis of enzyme-kinetic PMI markers.

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