J. Lake Sci.(湖泊科学), 2009, **21**(3): 345-350
http://www.jlakes.org. E-mail: jlakes@niglas.ac.cn

浅水湖泊沉积物脱氢酶活性的测定及其生态学意义*

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摘 要: 以武汉市浅水湖泊(月湖)为实验对象确定了沉积物脱氢酶活性(DHA)测定的最佳条件: 以经甲醛灭菌的沉积物为对照, 以氯化三苯基四氮唑为电子受体(0.4%), 沉积物用量为 0.5g, pH 值为 7.5, 培养时间为 3h. 据上述条件测定的 DHA 比未优化条件下的相应值高 3–5 倍, 且表现出与沉积物有机质含量更为显著的正相关关系. 武汉东湖 3 个子湖共 23 个采样点沉积物样品的测试结果进一步证实了上述方法的可行性, 同时初步揭示了湖泊沉积物脱氢酶反映微生物活性与有机质状态的生态学意义.
关键词: 沉积物; 脱氢酶; TTC; 微生物活性; 有机质; 湖泊

Determination of dehydrogenase activity in sediment of shallow lakes and its ecological significance

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Abstract: The samples were taken from the shallow urban lakes(Lake Yuehu) in Wuhan city to optimize the determination of dehydrogenase activity (DHA) in sediment, which gave the following suitable conditions: the sediment sterilized by formaldehyde was used as the control, the concentration of electron acceptor (2,3,5-triphenyl-2H-tetrazolium chloride) was 0.4%, the sediment amount used was 0.5g, pH value was 7.5 and the reaction time was 3h. Under these conditions, the DHA assayed was 3–5 times higher, and its positive relation to the content of organic matter in sediment was more significant, compared to those determined by the unmodified method. The data based on the 23 sites from 3 basins of Lake Donghu demonstrated the feasibility of the method. In the same time, the ecological significance of DHA was further shown in terms of the measurements of microbial activities and organic matter status in lake sediment.
Keywords: Sediment; dehydrogenase; TTC; microbial activities; organic matter; lake

湖泊沉积物有机质具有重要的生态学意义. 富营养化湖泊沉积物积累的大量有机质可为微生物生长提供充足的碳源, 微生物对有机质的分解将导致底层缺氧. 作为电子受体的 Fe^{3+}被还原成 Fe^{2+}, 与之结合的磷酸盐将向水中释放, 从而加速富营养化过程, 甚至引起水华[1]. 脱氢酶(dehydrogenase)是普遍存在于活体微生物中的一种催化底物去掉氢的酶. 作为电子传递体系中催化有机质脱氢作用的第一个酶, 它在有机质的分解过程中具有关键作用. 国内外大量研究结果表明, 脱氢酶活性(DHA)与微生物活动密切相关[2-5], 故已广泛用于重金属[6-7]与农药污染及其修复研究[8-9], 但多集中于土壤和污水, 关于湖泊沉积物

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* 国家“973”计划(2008CB418005)、中国科学院知识创新工程重要方向项目(KZCX2-YW-426-01)和中国科学院知识创新工程重大交叉项目(KZCX1-YW-14-1)联合资助. 2008–08–25 收稿; 2009–02–19 收修改稿. 黄代中, 女, 1984 年生, 硕士研究生; E-mail: hdz1023@163.com.
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脱氢酶的研究极少. 此外, 沉积物 DHA 与有机质的关系亦有歧见.

1962 年 Lenhard 发现, 沉积物中 DHA 与有机碳含量显著正相关(r=0.89, P=0.01)[10], 1973 年 Mario 等指出, 华盛顿河沉积物中脱氢酶活性与有机碳含量并无密切关系(r=0.42)[11]. 本项研究以武汉市不同营养水平的浅水湖泊沉积物为对象, 系统讨论了不同物理化学因素对沉积物 DHA 的影响, 并据此提出脱氢酶测定的优化条件, 同时依据相对大量的样本探讨了沉积物 DHA 与有机质含量的关系, 其目的在于将沉积物脱氢酶研究系统拓展至淡水湖泊, 进而深入揭示湖泊(尤其是富营养化湖泊)沉积物有机质分解过程的生物化学机制及其生态学意义.

1 材料与方法

1.1 实验湖泊

月湖(114°15′E, 30°33′N)位于武汉市中心, 面积 0.66km^2, 平均水深 142.4cm; 湖水透明度 22cm; 沉积物有机质平均含量为 8.34%. 采样点分布如图 1a 所示. 东湖(114°23′E, 30°33′N)位于武汉市武昌东北部, 面积约 28km^2, 平均水深约 1.8m. 东湖由郭郑湖、团湖、汤菱湖、喻家湖、庙湖等若干子湖组成. 采样点分布于水质优良的团湖与污染较为严重的庙湖和喻家湖(图 1b).

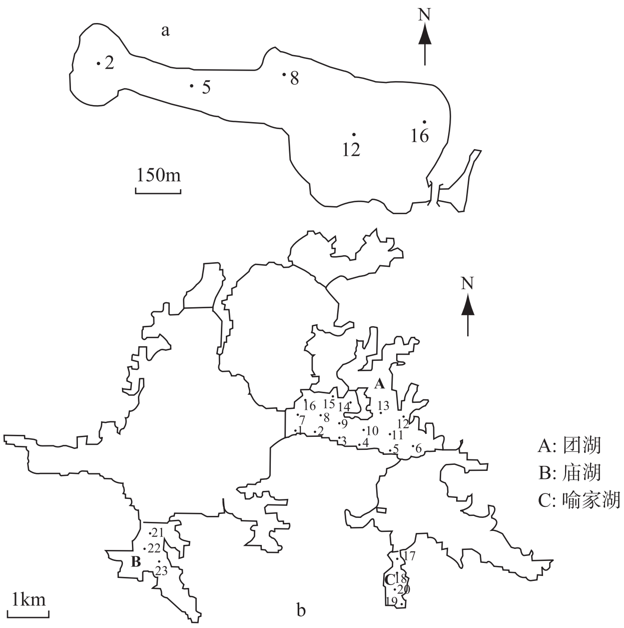

图 1 月湖(a)和东湖(b)采样点分布

Fig.1 Sampling sites of Lake Yuehu(a) and Lake Donghu(b)

1.2 样品采集

2007 年 9 月和 2007 年 11 月用彼得森采泥器分别采集月湖和东湖沉积物样品, 将其过筛(<2mm), 并置 4℃下保存.

1.3 测定方法

根据 Neto M[12]等建立的方法测定 DHA. 取过筛沉积物 0.5g 置 50ml 塑料离心管中, 加入 2ml Tris-HCl 缓冲液(pH=8.4)与 2ml 0.1%的氯化三苯基四氮唑(TTC), 置 37℃水浴锅振荡反应 1h, 离心 5min(4000r/min), 弃上清液, 用 20ml 丙酮于暗处萃取反应产物 TF(10min), 离心 5min(4000r/min)后用分光光度法(490nm)测定上清液的吸光度, 并据标准曲线换算 TF 的浓度;

高温对照: 高温(121℃, 20min)处理待测沉积物, 后续步骤同上;

甲醛对照: 用 2ml 甲醛处理离心管中的待测沉积物, 2h 之后加入 TTC, 其余步骤同上.

用烧失量法测定沉积物有机质含量. 取混匀沉积物(干重 1–2g)至坩埚中, 烘烤 2d(110℃), 称重后置马弗炉中烘烤 3h(550℃), 冷却后称重、计算有机质含量[13].

用软件 SPSS 13.0, 据方差分析法检验不同处理间差异的显著性.

2 结果与讨论

2.1 DHA 测定的对照设置

以甲醛处理为对照测得的 DHA 显著高于以高温处理为对照的相应值(P<0.05, ANOVA)(图 2). 除微生物的呼吸作用之外, 沉积物中的还原性物质亦能将电子传至 TTC, 使之生成 TF. 高温处理可破坏沉积物的结构[14], 并产生还原性物质[12,15], 从而增大对照值, 导致 DHA 的低谷.

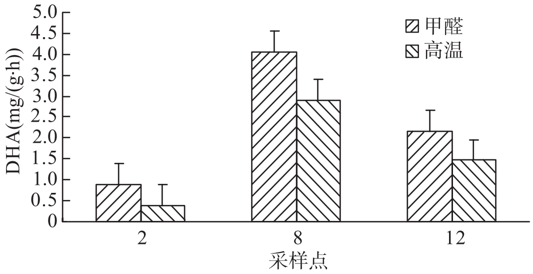

图 2 不同对照条件下 DHA 的变化

Fig.2 Changes in DHA at different control conditions

2.2 DHA 测定的沉积物用量

DHA 测定所选用的沉积物或土壤量多为 1g[16-19], 酶活性与沉积物用量呈正比, 后者升至一定程度时, DHA 增幅减小[11]. 沉积物用量为 0.5g 时, DHA 最高, 用量增大, DHA 反而降低(图 3a), 其原因可能在于沉积物富含的有机质将吸附酶促反应产物 TF[17]. 因此, DHA 测定的适宜沉积物用量为 0.5g.

2.3 DHA 测定的 TTC 浓度

TTC 可能因吸附于有机质而失去活性(不能被还原生成 TF). 有机质亦能吸附反应产物(TF), 使之不能被丙酮所完全萃取. 因此沉积物 DHA 的测定需用相对较高的 TTC 浓度. 实验选取的 5 号和 8 号采样点沉积物有机质含量均高(分别为 9.92%和 7.75%). DHA 随 TTC 浓度的增加而逐渐升高, 其峰值对应的 TTC 浓度为 0.4%. 当 TTC 浓度超过 0.4%时, 5 号采样点沉积物 DHA 基本恒定, 8 号采样点沉积物 DHA 略显下降趋势, 故湖泊沉积物 DHA 测定的适宜 TTC 浓度为 0.4% (图 3b).

2.4 DHA 测定的 pH 值

脱氢酶的适宜 pH 值范围为 7–9[20-21] . 当 pH 小于 7.5 时, 不同采样点沉积物 DHA 均随缓冲液 pH 值的增加而升高. 当 pH 值高于 7.5 时, DHA 下降(图 3c). 过高的 pH 将抑制脱氢酶的活性[16]. 因此, DHA 测定的适宜 pH 值为 7.5.

2.5 DHA 测定的培养时间

不同采样点沉积物 DHA 均随培养时间的增加而升高, 其峰值对应的培养时间均为 3h, 培养时间继续增加时, DHA 趋于恒定或略降(图 3d), 其原因在于反应底物的减少和产物的增加. 因此, DHA 测定的适宜培养时间为 3h.

2.6 优化条件下湖泊沉积物 DHA 的测定及其生态学意义

东湖 3 个子湖共 23 个采样点沉积物样品的测试结果表明, 不同条件下测定的沉积物 DHA 依不同采样点的大小排序基本相同(团湖 6 号除外). 而优化条件下测得的 DHA 明显较高, 其数量一般为未优化条件下测定值的 2–3 倍(图 4), 且表现出与沉积物有机质含量更为明显的正相关关系(图 5). 因此, 经优化的湖泊沉积物 DHA 测定方法更加灵敏和准确.

湖泊沉积物 DHA 与有机质含量显著正相关, 这种现象亦可见诸土壤[22-23]. 作为催化有机质分解的关键酶[24], 脱氢酶的生态学意义主要体现在两个方面. 首先, 它能基本反映沉积物微生物的数量与活性. 德国 Bostalsee 水库夏季沉积物氧化还原电位通常较低, 这种厌氧状态源于异养细菌对高含量有机碳的分解, 而细菌数量与脱氢酶活性表现出相同的变化趋势[25]. 巴西 **N**itero´i 港沉积物重金属含量明显高于自然背景值, 且达中度至极度污染水平, 但脱氢酶活性未受抑制, 据此可以推测, 相关重金属基本不具备生物可利用性[26]. 换言之, DHA 可基本表征微生物的活性. 与贫瘠土壤相比, 森林土壤有机质含量明显

较高，且与脱氢酶活性正相关，即使微生物生物量极低，脱氢酶活性仍可作为反映森林土壤与贫瘠土壤有机质含量与微生物活性差异的敏感指标[27]；其次，DHA 的变化可反映有机质的状态. 它是指示有机堆肥成熟与稳定性的最佳参数之一[28-29].

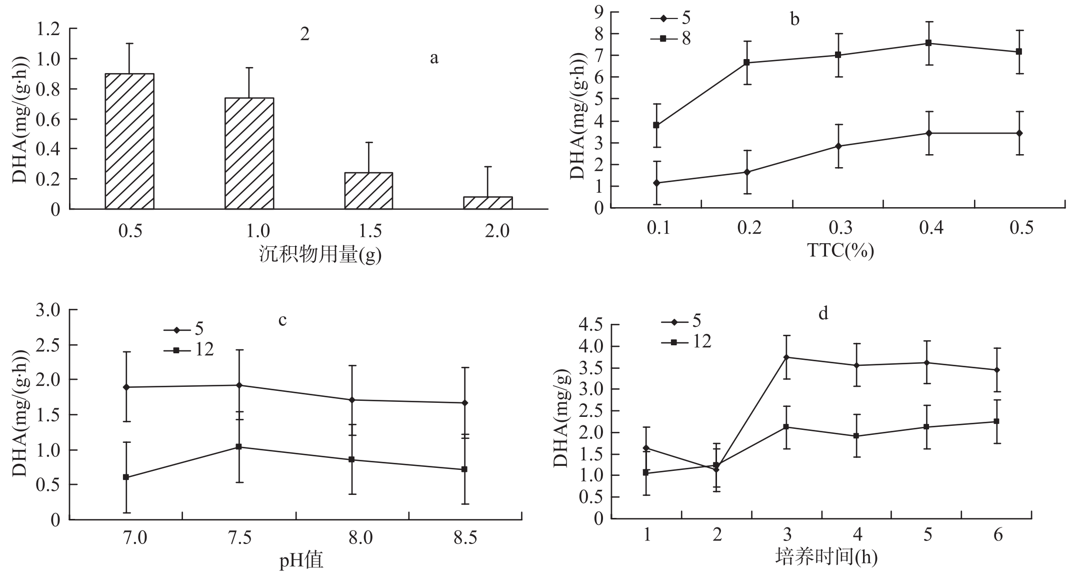

图 3 沉积物用量(a)、TTC 浓度(b)、pH 值(c)和培养时间(d)对脱氢酶活性的影响

Fig.3 Effect of sediment amounts used(a), substrate concentrations(b), pH values(c), and incubation time(d) on dehydrogenase activity

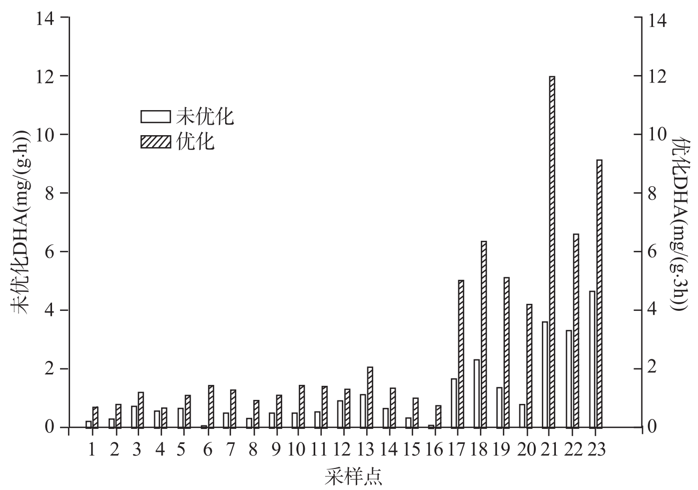

图 4 优化与未优化条件下不同湖泊沉积物 DHA 测定值的比较

Fig.4 Comparison between Dehydrogenase activities in sediment of different lakes determined under optimized and unoptimized conditions

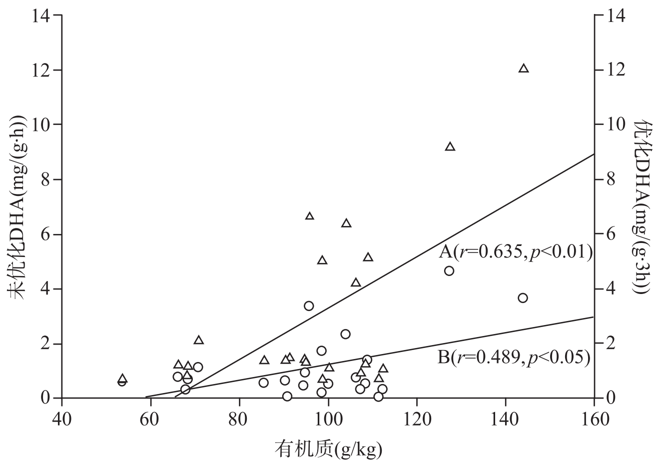

图 5 不同湖泊沉积物有机质含量与优化(A)和未优化(B)条件下脱氢酶活性测定值的关系

Fig.5 Relationships between content of organic matter and dehydrogenase activity determined under the optimized(A) as well as unoptimized(B) conditions in different lakes

3 结论

湖泊沉积物中 DHA 测定的最佳条件如下：沉积物重量为 0.5g、TTC 浓度为 0.4%、pH 值为 7.5、培养时间为 3h，并以经甲醛灭菌的沉积物为对照．上述经优化的沉积物 DHA 测定方法更加灵敏与准确．湖泊沉积物 DHA 与有机质含量显著正相关，故能反映沉积物微生物的数量与活性以及有机质的状态，因而具有重要的生态学意义．

致谢：姜苹红、纪磊等同学参与采样工作，谨此致谢．

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