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基于磷酸盐富集回收的生物膜培养研究进展

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摘要：与基于生物强化除磷的侧流回收工艺相比,生物膜法磷富集回收工艺以磷富集效果好、磷回收率高等优点在污水资源回收领域极具应用潜力.富集培养出以聚磷菌为优势菌群的生物膜是该工艺运行的关键.然而,聚磷生物膜的富集培养受多种因素影响,导致其挂膜缓慢.因此,为实现聚磷生物膜的快速培养,从聚磷生物膜富集培养的运行控制条件、环境影响因素及聚磷生物膜的形成机制等方面进行了梳理与总结.发现好氧曝气量、排泥时间以及碳源种类和浓度是培养聚磷生物膜的关键控制条件.展望了进一步实现聚磷生物膜快速培养的研究方向,以期为聚磷生物膜反应器的快速启动提供理论依据.

关键词：聚磷生物膜；快速培养；磷回收
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Research progress of biofilm culture based on phosphate enrichment and recovery. DING Yan-yan[1,2], JIANG Lei[1], HUANG Yong[1,2], LI Lu[1,2], PAN Yang[1,2*] (1.School of Environmental Science and Engineering, Suzhou University of Science and Technology, Suzhou 215009, China；2.National and Local Joint Engineering Laboratory of Municipal Sewage Resource Utilization Technology, Suzhou 215009, China). *China Environmental Science*, 2025,45(8)：4303~4310

Abstract：Compared with the side-stream phosphorus recovery process based on enhanced biological phosphorus removal, biofilm phosphorus enrichment-recovery process has great application potential in the field of wastewater resource recovery due to the advantages of high phosphorus enrichment effect and phosphorus recovery rate. Enrichment and cultivation of biofilm predominated by phosphate-accumulating bacteria is the key to the operation of the process. However, the culture of phosphate-accumulating biofilms is affected by multiple factors, resulting in slow biofilm formation. Therefore, in order to realize the rapid cultivation of phosphate-accumulating biofilm, the operation control conditions, environmental influencing factors and formation mechanism of phosphate-accumulating biofilm was summarized in this paper. It was found that the aerobic aeration volume, sludge discharge time, as well as the type and concentration of carbon source are the key control conditions for the cultivation of phosphate-accumulating biofilm. The research direction for further achieving the rapid culture of phosphate-accumulating biofilm was prospected, with the expectation of providing a theoretical basis for the rapid start-up of phosphate-accumulating biofilm reactor.

Key words：phosphate-accumulating biofilm；rapid culture；phosphorus recovery

磷资源是一种兼具资源稀缺性和污染性双重特性的不可再生资源[1].目前,全球 84%~90%的磷肥供应来自于磷矿的开采,然而全球磷矿面临日益匮乏的危机,预计将在 50~100a 内耗尽[2].从污水中回收磷可以满足全球磷需求的 15%~20%[3-4].因此,从污水中去除、回收磷成为了防治水体富营养化、实现污水全面资源化的核心环节之一[5].值得注意的是,从污水中回收磷首先要将进水当中低浓度的磷酸盐富集形成高浓度的磷酸盐才具有技术经济可行性[6-8].

目前城市污水处理厂一般先通过生物强化除磷工艺(EBPR)把污水中的磷富集至悬浮生长的活性污泥中[9],然后再从污泥浓缩发酵上清液或者压滤液中回收磷,但存在磷回收率低、回收工艺复杂等问题[10-11],增加了城市污水磷回收成本.与 EBPR 法不同的是,生物膜法磷回收工艺是一种利用特定生物膜技术从污水中回收磷的主流回收工艺,能够直接从污水中实现磷酸盐的去除与同步富集,具有磷富集效果好、磷损失少等优点,同时还能够降低工艺以及污泥处理的成本[12].目前已有研究证明生物膜

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序批式反应器(BSBR)工艺可以通过简单的操作实现高达 245mg/L 的磷富集浓度,并且能够保持极低的污泥产率[13-16],在面向未来城市污水全面资源化的背景下,生物膜法将更具有应用潜力.

生物膜法磷回收工艺的核心在于利用附着生长在填料上的聚磷菌(PAOs)好氧条件下超量吸磷、厌氧条件下释磷的特性来实现污水中磷酸盐的富集.在此过程中所培养形成的生物膜被称为聚磷生物膜,是由 PAOs 为优势菌群的微生物以及微生物分泌的胞外聚和物(EPS)构成的微生物聚集体膜状物[17].传统的生物膜多用于有机物的去除,进水中往往含有一定的有机物,因此异养菌能够较快的在填料上附着生长完成挂膜,但不利于 PAOs 的定向富集.PAOs 生长遵循的是“厌氧吸收-好氧利用”,即在厌氧条件下吸收碳源,然后在好氧条件下分解利用碳源用于细胞增殖[18].鉴于此,采用厌氧好氧交替运行的培养模式并在厌氧阶段投加碳源、好氧阶段不提供碳源,更有利于 PAOs 的生长,同时还能削弱其他异养菌的影响,促进 PAOs 的定向富集.因此聚磷生物膜的培养驯化条件与传统生物膜有较大的差别.研究发现,PAOs 富集形成聚磷生物膜的周期较长,易与反硝化细菌等异养菌的生长形成竞争,且容易受反应器运行控制条件以及温度等环境因素影响,导致挂膜缓慢甚至挂膜失败.以往的挂膜结果表明聚磷生物膜的培养时间多为 20~40d[19-22],需要较大的时间成本.

因此,快速培养聚磷生物膜并持留高丰度的 PAOs 以提高磷回收效率是目前亟待解决的问题.本文从聚磷生物膜富集培养的运行控制条件、环境影响因素及聚磷生物膜的形成机制等方面对聚磷生物膜快速培养进行梳理与总结,以期为生物膜法磷回收工艺的富集培养提供参考.

1 聚磷生物膜富集培养的运行控制条件

1.1 填料类型

填料类型的选择对聚磷生物膜的快速形成及菌种的高效富集至关重要.诸如石英砂填料[23]、尼龙悬挂填料[24]、悬浮填料[25]等已被广泛研究,同时新型填料以及组合填料也在不断被研究.具有多孔性和大比表面积的填料会更有利于微生物的粘附.张成等[26]发现火山岩-石英砂组合填料 25d 即可挂膜成功,而单一的石英砂填料则需要 30d 以上,两者的除磷率分别为 80.21%和 75.38%. Massoompour 等[27]采用典型的移动床生物膜反应器填料和由活性炭颗粒包被的新型碳基移动填料(CBMC)分别进行聚磷生物膜的培养,发现 CBMC 因多孔结构使生物质粘附增加约 15%.廖烜弘等[28]采用尼龙悬挂填料(使用寿命 3~4a)和悬浮填料(使用寿命 10a 以上)分别进行聚磷生物膜的培养,发现比表面积更大的尼龙悬挂填料 10d 即可完成挂膜,优于悬浮填料的 35d,除磷率分别为 97.23%和 83.6%.但随着后期反应器的运行,生物膜越来越厚,尼龙悬挂填料的传质效果变差导致性能也逐渐恶化.虽然尼龙悬挂填料在前期取得了较好的挂膜效果,但在后期的传质效果与使用寿命上却不如悬浮填料.因此,培养聚磷生物膜时不仅要选取孔隙率高和比表面积大的填料,还要考虑其传质效果和使用寿命.

1.2 好氧曝气量

在聚磷生物膜的培养过程中,曝气可以调节好氧阶段水中的溶解氧(DO)含量,同时也会改变水流剪切力的大小.Zheng 等[29]发现排泥前 DO 为 4mg/L 更有利于 PAOs 的培养,排泥后 DO 为 6mg/L 更有利于生物膜生长,而 DO 为 2 和 8mg/L 挂膜效果不理想.同样,Bi 等[12]对比了 DO 为 6 和 2mg/L 对 PAOs 的影响,发现 DO 为 6mg/L 会迫使 PAOs 优先进行聚磷代谢(PAM),有利于其建立优势生态位.这是由于 DO 过小会使 PAOs 活性下降从而导致代谢的迁移,而 DO 过高会加速 PHAs 的消耗从而分解 Gly 提供能量,减弱厌氧合成 PHAs 能力,丧失碳源竞争优势.此外,排泥前后的最适 DO 也不同.DO 为 4mg/L 更适合排泥前悬浮状态的 PAOs 生长,而排泥后 DO 为 6mg/L 更有利于氧的传质从而促进生物膜生长.水流剪切力虽然能够诱导 EPS 的产生,但在排泥前过大会导致污泥絮体结构受到影响,不利于微生物地聚集和附着[30-31],而在排泥后则会促进生物膜的更新,使除磷更加稳定[32-33].

1.3 排泥时间

聚磷生物膜挂膜启动常用接种排泥法.接种污泥会提供原始菌群,排泥时间会影响生物膜生长.Zheng 等[29]以生物膜吸磷性能不低于活性污泥为排泥指标,第 41d 排泥后,生物膜厚度和生物量激增.Zhao 等[22]同样以此为排泥指标,第 18d 排泥后,

生物膜厚度和生物量迅速增至 38.5μm 和 145.69g,增长速率也达到峰值 2.26μm/d 和 6.26g/d.章豪等[34]以生物膜厚度增长速率下降为排泥指标,第 16d 排泥后,生物膜厚度增长速率上升至 0.8μm/d.由于没有统一的排泥标准,排泥时间存在较大的差异,详见表(1).过早地排泥会影响微生物的丰度以及群落稳定性,同时还会中断微生物的附着,减少体系内 EPS 的含量,从而影响生物膜的形成.但过晚地排泥也不利于生物膜的培养.悬浮污泥会与生物膜微生物产生竞争,导致生物膜生物量降低、形成速率下降[35].因此,在适宜的时间排泥才有利于聚磷生物膜的培养,但对于排泥时间的选择还需要进一步研究.

表 1　不同运行控制条件下的聚磷生物膜培养

Table 1　Cultivation of phosphate-accumulating biofilm under different operating control conditions

厌氧阶段		回收液运转模式	好氧阶段			排泥时间	培养期	磷去除率	回收液浓	参考
碳源投加浓度(mg/L)	HRT(h)		进水磷浓度(mg/L)	DO(mg/L)	HRT(h)	(d)	(d)	(%)	度(mg/L)	文献
2000	6	不循环	5	3±0.5	6	–	55	95	58	[36]
200	4	不循环	15	3±0.5	4	–	65	95	50	[37]
200	2	循环	0.52~3.35	6	2	41	80	85	140	[29]
300	6	不循环	30	6	6	10	52	90	85	[20]
200	3	循环	10	4~6	3	16	49	95	179.5	[34]
231.75	2	循环	43.8	3.5	3	–	20	85	225.5	[38]
200±20	2	循环	10	6	4	25	25	95	90.62	[32]
200	2	循环	10	6±0.5	4	18	40	95	154.08	[22]
6000	4	循环	70	6±0.5	4	16	30	90	60	[19]
400	2	循环	5	8	4	–	24	50	125	[39]

1.4　碳源种类及浓度

碳源种类会影响聚磷生物膜培养,调控碳源可筛选优势菌群并改变微生物菌群结构[40–41].鲍林林等[42]发现乙酸、丙酸作碳源时反硝化除磷系统效率无差别,而葡萄糖作碳源时系统除磷性能下降.但葡萄糖在培养发酵型聚磷菌时却能取得良好效果[43].因此,不同 PAOs 培养应采用不同碳源.潘婷等[44]和李薇等[45]均发现以乙酸为碳源,PAOs 在与聚糖菌(GAOs)竞争时能占据绝对优势,有利于 PAOs 的生长.而蔡曼莎等[46]发现,乙酸和丙酸作碳源时,丙酸系统中 PAOs 丰度更高.综上,以乙酸、丙酸为碳源,对聚磷菌的培养都能够取得良好的效果.

还有研究表明不同碳源浓度也会影响反应器性能及微生物菌群.冉治霖等[47]发现低碳源浓度(COD=200mg/L)磷的去除率为 17%左右,荧光原位杂交实验(FISH)结果表明 GAOs 是优势菌群,而在正常碳源浓度(COD=400mg/L)磷的去除率在 80%以上,除磷效果较好,富集出了聚磷菌.另外,单捷等[38]发现当厌氧碳源投加浓度分别为 200,600 和 1000mg/L,2h 内对应的磷释放量分别为 24.7,33.18 和 43.33mg/L.说明厌氧阶段投加高碳源可以刺激磷的释放,有利于 PAOs 吸收碳源储存能量用于好氧阶段的生长.

1.5　回收液运转模式

目前的研究表明在 PAOs 在培养过程中回收液的运转模式分为回收液不循环和回收液循环两种运转模式(表 1).回收液不循环的运转模式是将厌氧出水直接排放;回收液循环的运转模式是将厌氧出水排回至回收罐中,作为下一阶段的厌氧进水.回收液不循环的运转模式能够避免外界液相中磷酸盐浓度过高而与胞内形成较大的渗透压阻碍 PAOs 释磷,有利于 PAOs 在好氧阶段处于“磷饥饿”状态,提高 PAOs 的好氧主动吸磷能力[48].而回收液循环的运转模式能够引起微生物菌群形态与组成发生变化,促进 PAOs 成为优势菌群.张顺等[49]发现在回收液循环过程中生物滤池底部生物膜中聚磷菌所占比例由 43%提高至 70%,且随滤池高度增加 PAOs 比例不断提高.虽然两种回收液运转模式各有优势,但哪种更有利于聚磷生物膜培养尚需进一步研究.

2　聚磷生物膜富集培养的环境影响因素

2.1　温度

温度也会影响 PAOs 的生长.当温度低于 5℃会抑制糖原分解所需酶的活性,影响糖原分解供能[50].Zheng 等[51]发现在 10℃左右,丙酸盐系统中 PAOs 会发生代谢迁移.王建辉等[52]发现在 15~20℃时,PAOs 可以发挥优势功能.而当温度从 20℃上升到 35.5℃

时,PAOs 会消耗大量的能量维持自身生长代谢,GAOs 以及普通异养菌会取代 PAOs 的优势地位[53-55].从对碳源的利用来看,当温度高于 20℃时 GAOs 具有优先吸收有机物的优势[56].因此,室温(15~20℃)更有利于 PAOs 生长.但又有研究表明高温条件下 PAOs 也能保持较好的除磷效果.张玲等[57]在 SBR 反应器中以 30℃高温培养 PAOs,磷的去除率高达 99%以上,PAO 占总细菌的比例高达(86%±3%),而 GAO 的比例仅占 4%左右,可能是由于 PAOs 的菌种不同所导致.随后,Liu 等[58]发现在 10°C 时,*Tetrasphaera* 的生物活性受到明显抑制,EBPR 效率仅 73%,而在 20~30°C 时,EBPR 效率达到 99%,*Tetrasphaera* 的相对丰度高达 90%,更加证实了不同菌种的 PAOs 的最适温度不同.

2.2 进水磷浓度

进水磷浓度对聚磷生物膜的培养也有较大的影响.当进水磷浓度过低时,PAOs 会从 PAM 迁移到聚糖代谢(GAM),难以长期保持优势地位[59].Willie F 等[60]发现磷酸盐浓度从 16mg/L 上升至 160mg/L,污泥中的磷含量从 50.1mg-P/g-SS 上升至 200.4mg-P/g-SS,表明 PAOs 吸磷量随磷酸盐浓度增加而增多[61].孟璇等[62]发现高磷浓度下培养的聚磷生物膜的最大吸磷速率是低磷浓度的 6.7 倍以上,释磷速率是 2.8 倍以上,且专一性更强,详见表(2),高磷浓度下培养出来的聚磷生物膜在进水磷浓度为 5mg/L 的条件下仅用 6d 即可富集到 50mg/L,富集性能较好.同样,Tian 等[63]发现当进水磷浓度从 10mg/L 上升至 15mg/L 时,生物滤池中 PAOs 丰度从 14.5%上升至 98%.因此,高进水磷浓度有利于聚磷生物膜培养,增强 PAOs 竞争优势,且培养出的生物膜展现出较好磷富集性能[64].

表 2 不同进水磷浓度挂膜效果[62]

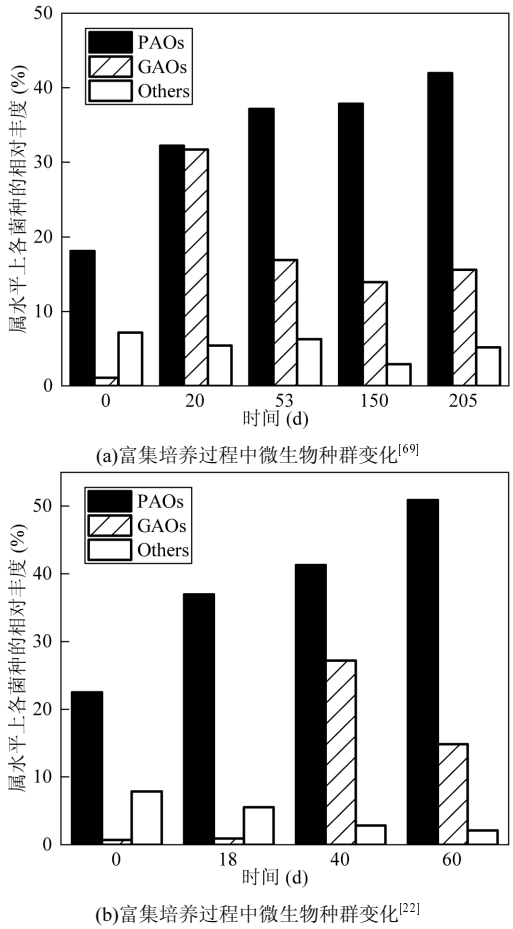
Table 2 Effect of different influent phosphorus concentration on biofilm formation[62]

进水磷浓度 (mg/L)	吸磷速率 [mg/(L·h)]	释磷速率 [mg/(L·h)]	Shannon 指数	培养期 (d)
5	6.45	4.2	7.66 下降至 7.30	50
15	43.23	11.76	6.84 下降至 5.83	

2.3 金属离子

金属离子是微生物生长所必需的微量元素,其浓度和种类的不同会产生不同的效果[65].有研究表明,低浓度的 Mn^{2+}和 Fe^{3+}对 PAOs 的生长有一定的促进作用,高浓度则抑制;Pb^{2+}和 Cu^{2+}则无论浓度如何,均抑制 PAOs 生长甚至致其死亡[66].但金属离子对 PAOs 的作用不仅限于此.金属离子会刺激信号分子酰基高丝氨酸内酯(AHLs)的释放,AHLs 引导的群体感应会增强磷的摄取[7].但随着进水 Ca^{2+}浓度的升高,胞外磷酸钙沉淀的趋势和程度也会增加.Zhang 等[67]发现当 Ca^{2+}浓度为 30~50mg/L 时,胞内会合成惰性钙结合态的多聚磷,胞外磷酸钙沉淀以及胞内惰性钙结合态多聚磷的过度合成会使反应器中部分 PAOs 从 PAM 向 GAM 转移,丧失优势地位.还有研究表明,多种共存的金属离子会增加 EPS 含量,有利于生物膜的形成[68].综上,金属离子对于聚磷生物膜的影响是复杂的.因此,培养生物膜时需监测进水金属离子种类和浓度,以防不利影响.

3 聚磷生物膜的形成机制



(a)富集培养过程中微生物种群变化[69]

(b)富集培养过程中微生物种群变化[22]

图 1 聚磷生物膜富集培养过程中微生物种群变化

Fig.1 Changes of microbial population during enrichment culture of phosphate-accumulating biofilm

富集培养具有高效磷代谢能力的微生物是聚磷生物膜形成的关键.Ni 等[69]在好氧进水磷浓度为0.34~3.36mg/L,好氧 DO 为(2.5±0.5)mg/L,厌氧 COD 为 200mg/L,总水力停留时间为 6h(4h 好氧、2h 厌氧)的条件下对聚磷生物膜进行培养,在 20d 排泥并在第 47d 完成了生物膜的富集培养,而后稳定运行 163d.通过对培养过程中微生物种群进行监测,发现排泥后 PAOs 和 GAOs 的占比大幅增长,并且基本持平,而在后期运行过程中,PAOs 开始占据优势地位,其占比约为 GAOs 的 2~3 倍(图 1(a)).此外,Zhao 等[22]在好氧进水 P 浓度为 10mg/L,好氧 DO 为(6±0.5)mg/L,厌氧 COD 为 200mg/L,总水力停留时间为 6h(4h 好氧、2h 厌氧)的条件下对聚磷生物膜进行

培养,在第 18d 排泥并在第 40d 完成生物膜的富集培养,而后稳定运行.其培养过程中的微生物种群变化情况与 Ni 等基本一致(图 1(b)).排泥促进了优势微生物群落结构的演替,并且随着培养时间的延续,逐渐向 PAOs 集中.

研究表明,在聚磷生物膜形成过程中,微生物的粘附能力是生物膜挂膜和快速生长的重要前提,与 EPS 含量呈正相关[70].附着在填料上的大分子会更有利于微生物利用鞭毛、纤毛和菌丝等胞外细胞器和外层膜蛋白粘附于填料表面.孔隙率小、表面粗糙的填料会提高 PAOs 与填料的接触概率,有利于微生物的附着[71-72].微生物分泌 EPS 越多、PN 与多糖(PS)的比值越高越有利于微生物的团聚,从而促进生物膜的形成[73].反应器启动时内部曝气引起的流动剪切力会诱导 EPS 的产生,但曝气量过大也会导致 DO 过高、流动剪切力过强从而影响 PAOs 的代谢以及微生物的附着.所以将好氧曝气量控制在适宜的范围内更有利于聚磷生物膜的形成.研究表明,信号分子 AHLs 和环二鸟苷酸(C-di-GMP)可以刺激 EPS 的分泌从而促进生物膜的形成[74-76].通过对系统内微生物种群、信号分子以及 EPS 的相关性分析发现 GAOs 在成膜过程中也发挥了重要作用.AHLs 的分泌主要来自于 GAOs 中丰度较高的 *Candidatus_Competibacter*,其不仅不产生群体淬灭,还会表现出比 PAOs 更强的 AHLs 合成、EPS 分泌和 C 补偿能力,有利于生物膜的形成[69].而在适当的时间排泥不仅可以刺激 GAOs 分泌信号分子 AHLs,促进 EPS 的分泌、增强生物膜粘附力进而有利于生物膜的形成,还可以刺激 PAOs 大量分泌同时 GAOs 少量分泌 C-di-GMP,对生物膜的形成有利,同时实现培养过程中磷酸盐富集能力的提升[22](图 2).

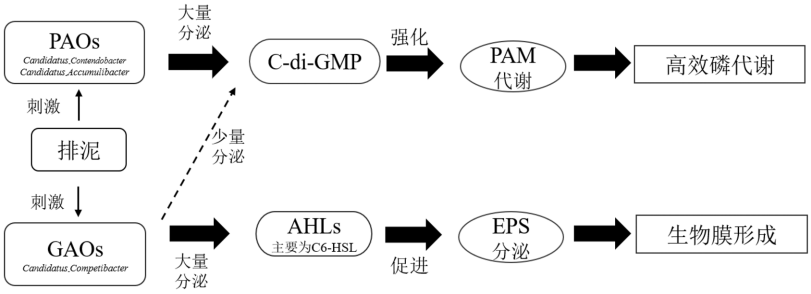


图 2　聚磷生物膜富集培养调控模型[22]

Fig.2　Regulation model of phosphate-accumulating biofilm cultivation[22]

但关于 GAOs 与 PAOs 之间协同作用的机制,目前的研究尚不清楚.另外,金属离子也会促进信号分子 AHLs 的释放以及 EPS 的分泌,但浓度过高或毒性过强也会抑制 PAOs 的活性,导致生物膜结构的解体.

4　结语

生物膜中的聚磷微生物是生物膜对进水磷酸盐同步去除与高倍富集的主要载体.聚磷生物膜形成的关键在于增大粘附能力并保持 PAOs 的 PAM 代谢行为,以保证以 PAOs 为优势菌群的微生物能够快速粘附在填料上.在培养聚磷生物膜的过程中,好氧曝气量、排泥时间以及碳源种类和浓度是关键控制条件.合适的好氧曝气量以及排泥时间能够加速泥膜混合系统向生物膜系统的演替,而适宜的碳源种类及浓度能够促进生物膜的生长,使微生物群落结构发生变化,进而 PAOs 获得优势生态位.研究发现生物膜中的 GAOs 通过分泌 EPS 促进了聚磷生物

膜的形成,但其与 PAOs 在生物膜形成过程中的协同作用尚需进一步研究.

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