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《环境科学》征稿简则(1092) 《环境科学》征订启事(1158) 信息(1166,1261,1271)

# 全氟辛酸对大肠杆菌的氧化胁迫和膜损伤

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**摘要:** 全氟辛酸(perfluorooctanoic acid, PFOA)因其具有极高的化学稳定性和良好的疏水疏油性而在工业生产中广泛使用,但近年来被认为是一种在环境中广泛分布的持久性有机污染物. 利用流式细胞术等检测技术,研究了 PFOA 对大肠杆菌(*Escherichia coli*, *E. coli*)的氧化胁迫和膜损伤,并对其毒性作用机制进行了初步的探索. 结果表明,在 PFOA 胁迫下,大肠杆菌胞内活性氧(reactive oxygen species, ROS)含量增加,膜脂肪酸饱和度降低,丙二醛(malondialdehyde, MDA)浓度升高、细胞膜通透性增大、跨膜电位降低,而细胞膜上的  $\text{Na}^+ \text{K}^+ \text{-ATPase}$ 、 $\text{Ca}^{2+} \text{Mg}^{2+} \text{-ATPase}$  活性随时间的延长代偿性地先上升后降低. 由此说明,在 PFOA 胁迫下,大肠杆菌细胞内升高的 ROS 与细胞膜不饱和脂肪酸发生过氧化反应,降低膜脂肪酸的饱和度,使得 MDA 在细胞内积累,进一步引起细胞膜损伤及其上相关 ATPase 活性降低,最终导致大肠杆菌细胞失活或死亡. 实验结果对研究 PFOA 胁迫下环境生态毒理提供更多依据.

**关键词:** 全氟辛酸; 大肠杆菌; 流式细胞术; 胞内活性氧; 丙二醛; ATPase

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## Effect of PFOA on Oxidative Stress and Membrane Damage of *Escherichia coli*

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**Abstract:** Perfluorooctanoic acid (PFOA) is widely used in industrial production because of its strong chemical stabilities and good hydrophobic and oleophobic properties. It was considered to be a widespread persistent organic pollutant in environment in recent years. The oxidative stress and membrane damage of *Escherichia coli* exposed to PFOA were measured by flow cytometry (FCM) and the toxic mechanism of PFOA was also preliminarily explored. The results showed that, under the stress of PFOA, the intracellular reactive oxygen species (ROS) content of *E. coli* increased, the unsaturation degree of fatty acid decreased, the malondialdehyde (MDA) content increased, the membrane permeability increased, the membrane potential decreased, and the activities of  $\text{Na}^+ \text{K}^+ \text{-ATPase}$  and  $\text{Ca}^{2+} \text{Mg}^{2+} \text{-ATPase}$  showed a compensatory increase first and then decreased. Therefore, owing to the stress of PFOA, the higher intracellular ROS in *E. coli* reacted with membrane unsaturated fatty acids by peroxidation, and then reduced cell membrane fatty acid saturation, accumulated MDA in cells, and further caused damage to cell membrane, reduced the ATPase activity, and eventually resulted in inactivation or apoptosis of *E. coli*. This study provided more evidence for the further study on environmental ecological toxicology of PFOA.

**Key words:** perfluorooctanoic acid (PFOA); *Escherichia coli*; flow cytometry (FCM); intracellular reactive oxygen species (ROS); malondialdehyde (MDA); ATPase

全氟辛酸(PFOA)是将辛酸烷基以及碳链中所含氢原子取代为氟原子形成的具有 15 个氟原子、8 个碳原子组成的烃链,其末端连接一个羧基. 由于 C—F 共价键键能极高,使得 PFOA 具有极强的热稳定性,同时 PFOA 独特的结构使之兼具疏水性和疏油性,因此被大量应用于造纸、纺织、制革等工业. 但同时也具有难降解性、生物蓄积性和沿食物链在生物体内富集作用,被认为是一种在环境中分布广泛的持久性有机污染物<sup>[1]</sup>.

有关 PFOA 的生物毒性大多以人体细胞<sup>[2~6]</sup>、动物细胞<sup>[7]</sup>以及原生动物<sup>[8]</sup>为对象,获得了许多研

究成果. 然而, PFOA 对环境微生物的影响的研究却非常少,对 PFOA 的微生物细胞毒性作用及相关机制还没有形成系统完善的认识. 大肠杆菌作为一种原核模式微生物,是环境中最常见的微生物之一. 研究 PFOA 对大肠杆菌的毒性作用,对 PFOA 的环境影响具有重要的评估意义.

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流式细胞术(flow cytometry, FCM)由于能从微角度了解单细胞的生理状态而逐渐被应用到微生物领域<sup>[9,10]</sup>. 本研究以大肠杆菌为实验对象,通过流式细胞术等检测技术,考察在不同浓度的暴露过程中,PFOA对大肠杆菌的氧化胁迫与细胞膜特性的影响,以期PFOA的环境生态风险提供更多理论依据.

## 1 材料与方法

### 1.1 实验菌株及试剂

实验菌株:大肠杆菌(*Escherichia coli* ATCC 9739).

培养基:肉汤蛋白胨培养基(牛肉膏 3.0 g,蛋白胨 10.0 g,氯化钠 5.0 g 于 1 L 去离子水中).

培养条件:温度为 37℃, 160 r·min<sup>-1</sup> 恒温振荡培养至对数期待用.

PFOA(纯度 99%)、0.9% 生理盐水、pH = 7 磷酸盐缓冲液、1% 多聚甲醛溶液(Sigma 公司),超微量 ATP 酶试剂盒、丙二醛 MDA 试剂盒(南京建成生物工程研究所),JC-1 染液(Tetraphenylarsonium ethylbenzimidazol carbocyanine iodide)、碘化丙啉(Propidium iodide, PI)染液、2',7'-二氯荧光素二乙酸酯(2',7'-Dichlorodihydrofluorescein diacetate, DCFH-DA)染液(碧云天生物技术公司).

### 1.2 方法

#### 1.2.1 实验方案设计

配制 10 倍母液的无机盐培养基(K<sub>2</sub>HPO<sub>4</sub> 4.0 g, KH<sub>2</sub>PO<sub>4</sub> 2.0 g, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 20.0 g, MgSO<sub>4</sub> 1.0 g, KCl 2.0 g 于 1 L 去离子水中). 实验时分别向含有 0.1、1.0、10.0 mg·L<sup>-1</sup> PFOA 的培养基中加入 1 mL 培养至对数期的大肠杆菌菌悬液(20.0 g·L<sup>-1</sup>),使培养体系总体积为 20 mL,于 37℃、160 r·min<sup>-1</sup> 条件下振荡培养,以不添加 PFOA 的无机盐培养基体系为对照. 分别于培养开始后 0 h(本文中 0 h 是指与污染物接触后迅速离心得到的菌体,测定相关指标)、12 h、36 h 收集大肠杆菌细胞进行分析.

#### 1.2.2 细胞膜通透性、ROS、跨膜电位的测定

定时取样,离心收集细胞,对菌体细胞进行前处理(在无机盐培养体系中定时收集细胞,6 000 r·min<sup>-1</sup> 离心 10 min,收集大肠杆菌细胞后,用 PBS 洗涤 2 次,每次洗涤后 6 000 r·min<sup>-1</sup> 离心 5 min,倾倒入上清液)后,采用流式细胞仪 BD FACS Aria III (Becton, Dickinson and Company, Franklin Lakes, NJ, USA)测定大肠杆菌细胞膜通透性、ROS、跨膜

电位<sup>[11~13]</sup>.

#### 1.2.3 菌体丙二醛 MDA 浓度测定

收集细胞,用 PBS 洗涤 2 次后,加入 1 mL 生理盐水混匀,收集上清液,按照试剂盒说明书进行测定.

#### 1.2.4 ATP 酶活性测定

收集细胞,用生理盐水洗涤 2 次后,采用与 MDA 测定相同的条件进行超声破碎,超声破碎(4℃,40% 功率,开启 5 s,停止 7 s,共计 10 min),然后在 1 000 r·min<sup>-1</sup> 离心 5 min 收集上清液,按照试剂盒说明书进行测定.

#### 1.2.5 细胞膜磷脂脂肪酸含量测定

定时收集细胞,用 PBS 洗涤 2 次,洗涤后倾倒入上层液体,保留底部菌体. 加入 2 mL 的 2% H<sub>2</sub>SO<sub>4</sub> 甲醇溶液,充入氮气排空离心管中空气,将螺口玻璃试管放入 80℃ 水浴锅中水浴 1 h,冷却至室温,分别加入 2 mL 的蒸馏水和 2 mL 的正己烷,充分振荡,4 000 r·min<sup>-1</sup> 离心 10 min,取出上层有机相放置到新的离心管中,氮气吹干,加入 9.9 mL 正己烷和 10 μL 内标十九酸甲酯,振荡后转移至上机瓶中,封口待测<sup>[14]</sup>.

## 2 结果与分析

### 2.1 PFOA 胁迫对大肠杆菌胞内 ROS 的影响

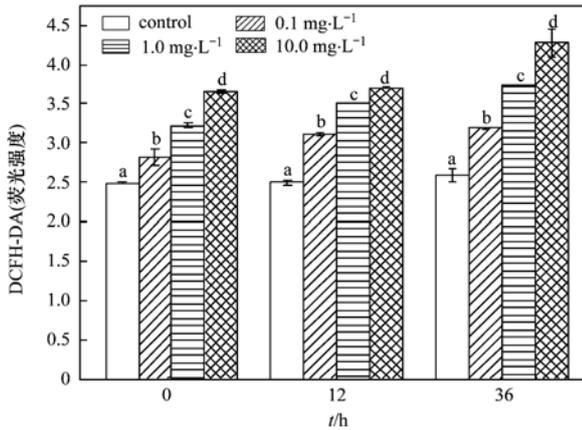
活性氧(ROS)主要包括氧自由基(O<sub>2</sub><sup>-</sup>)、过氧化氢(H<sub>2</sub>O<sub>2</sub>)、羟自由基(OH<sup>-</sup>)等,是生物细胞内有氧代谢的产物<sup>[15]</sup>. 利用荧光探针 DCFH-DA 通过流式细胞仪可以检测细胞内活性氧的水平. 图 1 为 PFOA 对大肠杆菌胞内 ROS 含量的影响. 结果显示,在 0 h,各 PFOA 浓度处理下,胞内 ROS 发生了显著升高,说明 PFOA 在极短时间的接触即对大肠杆菌 ROS 代谢产生了显著影响. 在处理 12 h 与 36 h,变化趋势与 0 h 类似.

### 2.2 PFOA 胁迫对大肠杆菌膜不饱和脂肪酸含量的影响

图 2 中 0 h 的结果表明,在 PFOA 处理下,胞内不饱和脂肪酸比率发生了显著下降. 与 0 h 的结果比较,12 h 与 36 h 胞内不饱和脂肪酸比率只略有降低. 有研究表明,生物细胞在受到外界胁迫时,胞内过量 ROS 会攻击膜<sup>[16]</sup>,与细胞膜中不饱和脂肪酸发生氧化反应. 这与本研究结果有相似之处:当胞内 ROS 升高(图 1),其迅速与膜上的不饱和脂肪酸反应,从而降低了细胞膜的不饱和脂肪酸度.

### 2.3 PFOA 对大肠杆菌胞内 MDA 浓度的影响

生物胞内含量过高的 ROS 会攻击细胞膜使膜



不同小写字母代表差异显著,  $P < 0.05$ , 下同

图1 PFOA对大肠杆菌细胞内活性氧含量的影响

Fig. 1 Effect of PFOA on intracellular ROS of *Escherichia coli*

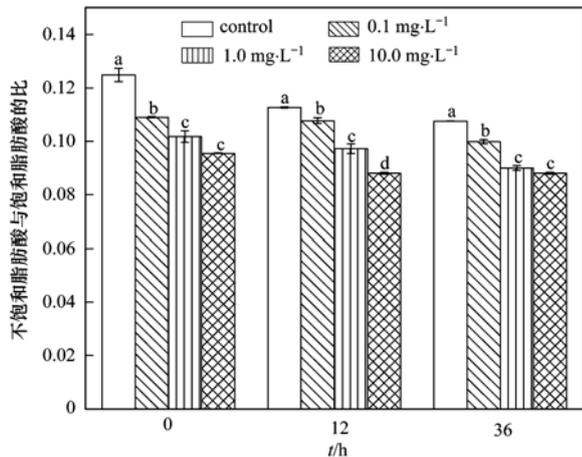


图2 PFOA对大肠杆菌细胞膜不饱和脂肪酸比率的影响

Fig. 2 Effect of PFOA on the ratio of cell membrane unsaturated fatty acid of *Escherichia coli*

发生过氧化作用进而影响膜的流动性与稳定性<sup>[17]</sup>. MDA是膜脂过氧化的主要产物之一,是评价膜脂过氧化作用强弱的一个重要标志.由图3可知,PFOA可以很快导致细胞内MDA的增加.加入PFOA 0 h时,各浓度PFOA体系中大肠杆菌细胞MDA浓度均有显著增高.当处理时间延长,菌体细胞内MDA上升,与空白对照相比,在处理12 h与36 h,在PFOA浓度为0.1、1.0和10.0 mg·L<sup>-1</sup>时,MDA浓度分别升高了1.91%、3.71%、9.13%与3.61%、12.47%、28.91%,说明菌体内MDA与PFOA胁迫时间和浓度也呈正相关.

#### 2.4 PFOA胁迫对大肠杆菌细胞膜通透性的影响

PI可以用来进行细胞膜通透性的检测<sup>[18]</sup>.图4为PFOA胁迫对大肠杆菌细胞膜通透性的影响.结果表明,在加入12 h后,与对照相比,10.0 mg·L<sup>-1</sup>的PFOA对细胞膜通透性产生了显著影响.36 h,各

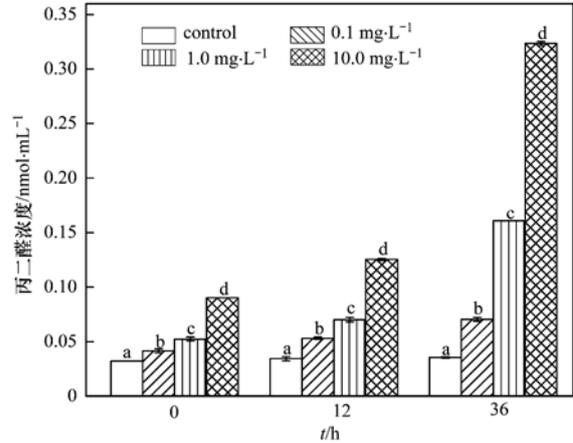


图3 PFOA对大肠杆菌细胞MDA浓度的影响

Fig. 3 Effect of PFOA concentration on MDA content of *Escherichia coli*

浓度PFOA体系中染色细胞比例有显著性变化,说明在此时,PFOA已经对大肠杆菌细胞膜通透性产生了显著影响.然而,尽管如此,总的染色细胞的比例仍然不高,最高浓度10.0 mg·L<sup>-1</sup>的PFOA处理下,染色细胞比例只有23%,说明大肠杆菌对PFOA具有较强的抗性或耐受性.

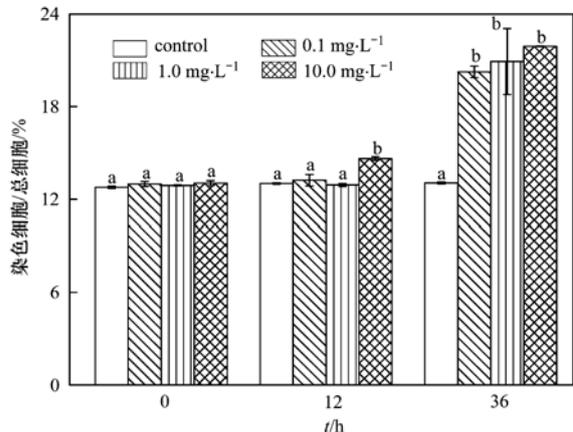


图4 PFOA对大肠杆菌细胞膜通透性的影响

Fig. 4 Effect of PFOA on membrane permeability of *Escherichia coli*

#### 2.5 PFOA胁迫对大肠杆菌跨膜电位的影响

图5是以相对荧光强度(relative fluorescence intensity, RFI)表示PFOA对大肠杆菌膜电位的影响.从中可知,与对照相比,在0 h,0.1 mg·L<sup>-1</sup>和1.0 mg·L<sup>-1</sup>的PFOA处理下,RFI并未明显下降,此结果与前面PFOA对细胞膜系统通透性的影响相一致.在12 h和36 h,0.1 mg·L<sup>-1</sup>和1.0 mg·L<sup>-1</sup>体系中的RFI并未明显下降,10.0 mg·L<sup>-1</sup> PFOA处理体系中的RFI则出现显著下降,降幅分别达到25.77%和11.97%.研究结果表明低于1.0 mg·L<sup>-1</sup>的PFOA对大肠杆菌细胞膜跨膜电位没有影响,10.0

$\text{mg}\cdot\text{L}^{-1}$  PFOA 处理对大肠杆菌细胞膜跨膜电位有显著影响。

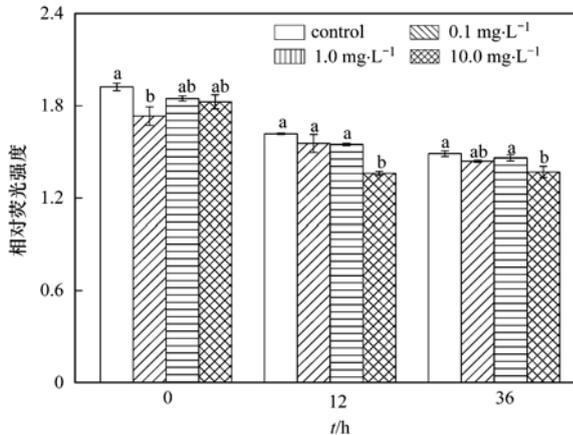


图5 PFOA 对大肠杆菌跨膜电位(相对荧光强度)的影响

Fig. 5 Effect of PFOA concentration on transmembrane potential (RFI) of *Escherichia coli*

## 2.6 PFOA 胁迫对大肠杆菌 ATP 酶活性的影响

在细胞受到胁迫时,原核生物细胞膜上的 ATP 酶活性会发生变化<sup>[19~22]</sup>. 图 5 表明 PFOA 对大肠杆菌  $\text{Na}^+\text{K}^+$ -ATPase [图 6 (a)] 和  $\text{Ca}^{2+}\text{Mg}^{2+}$ -ATPase [图 6 (b)] 酶活性产生的影响. 其中,  $0.1\text{ mg}\cdot\text{L}^{-1}$  和  $1.0\text{ mg}\cdot\text{L}^{-1}$  的 PFOA 对大肠杆菌  $\text{Na}^+\text{K}^+$ -ATPase、 $\text{Ca}^{2+}\text{Mg}^{2+}$ -ATPase 有相似的规律性: 0 h 时  $10.0\text{ mg}\cdot\text{L}^{-1}$  的 PFOA 对大肠杆菌  $\text{Na}^+\text{K}^+$ -ATPase、 $\text{Ca}^{2+}\text{Mg}^{2+}$ -ATPase 就有显著性影响, 12 h 时各处理组活性有显著增高, 但趋势却耐人寻味: PFOA 浓度越高, ATPase 活性越低. 但在 36 h 后, 3 个 PFOA 处理组的  $\text{Na}^+\text{K}^+$ -ATPase、 $\text{Ca}^{2+}\text{Mg}^{2+}$ -ATPase 均已显著低于对照组, 且 PFOA 浓度越高, ATPase 活性越低. 说明在 PFOA 胁迫下, 大肠杆菌细胞在一定时间内自身通过代偿性的增高 ATPase 的活性来抵御

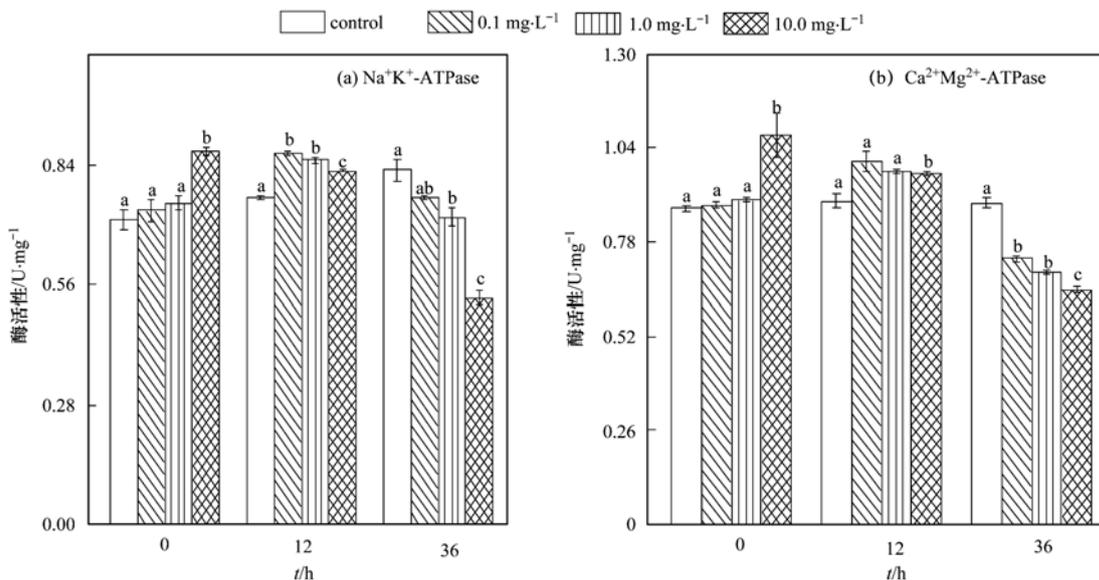


图6 PFOA 对大肠杆菌  $\text{Na}^+\text{K}^+$ -ATPase、 $\text{Ca}^{2+}\text{Mg}^{2+}$ -ATPase 酶活性的影响

Fig. 6 Effect of PFOA concentration on  $\text{Na}^+\text{K}^+$ -ATPase and  $\text{Ca}^{2+}\text{Mg}^{2+}$ -ATPase activities of *Escherichia coli*

PFOA 的胁迫, 但是随着 PFOA 胁迫时间的延长, 大肠杆菌细胞膜的完整性遭到破坏, ATP 酶受攻击, 催化环境受影响, 导致 ATP 酶活性下降。

## 3 讨论

在细胞受到外界胁迫时, 细胞内 ROS 含量升高, 过量 ROS 会攻击膜和蛋白质<sup>[6]</sup>, 与细胞膜中不饱和脂肪酸发生氧化反应, 导致细胞质膜的过氧化而引起膜氧化损伤<sup>[23]</sup>, 进而影响膜的流动性与稳定性<sup>[7]</sup>. 丙二醛 (MDA) 不仅是细胞膜脂过氧化作用的产物之一, 它还可以通过与细胞内一些成分发生反应, 放大 ROS 作用, 进一步造成细胞膜损伤, 使得

菌体细胞膜的通透性、流动性、稳定性、完整性受损, 正常生理活动与物质能量代谢受阻, 导致细胞生理活性受到抑制<sup>[24]</sup>, 进而导致细胞损伤与凋亡<sup>[25]</sup>. 此外, ROS 还可以降低膜电位, 影响能量代谢酶系统的活性. 而受到外界刺激胁迫导致生物细胞内高浓度的  $\text{Ca}^{2+}$  也会干扰细胞正常能量代谢和很多生理功能<sup>[26]</sup>. 在细胞内部, 包括  $\text{Na}^+\text{K}^+$ -ATPase、 $\text{Ca}^{2+}\text{Mg}^{2+}$ -ATPase 等在内的 ATP 酶是广泛存在于细胞膜及细胞器膜上的一种十分重要的酶系统, 对于细胞的能量代谢、细胞内外渗透压的平衡、离子的运输与平衡和细胞膜完整性起到了重要的作用<sup>[27~29]</sup>, 并参与多种代谢活动, 维持细胞正常的生

理活性。

本研究中,在 PFOA 胁迫下,大肠杆菌细胞内的 ROS 水平升高,与细胞膜中不饱和脂肪酸发生氧化反应,使膜脂肪酸饱和度降低,引起细胞膜过氧化作用,MDA 含量增高,膜通透性改变,同时降低跨膜电位,使呼吸链受抑,能量代谢功能被削弱。此时包括  $\text{Na}^+ \text{K}^+ \text{-ATPase}$ 、 $\text{Ca}^{2+} \text{Mg}^{2+} \text{-ATPase}$  等在内的 ATP 酶对外界胁迫环境做出反应,表现出代偿性的活性升高,通过提高酶的活性来影响物质运输,维持细胞膜两侧的膜电位、胞内离子水平以及细胞内外渗透压的平衡。但随着时间的延长,菌体细胞体内产生了过多的 ROS,同时由于膜结构完整性遭到破坏,能量代谢功能受阻,又会进一步使细胞产生更多的 ROS,发生的过氧化反应使细胞膜系统完整性受损更严重,进一步增大大肠杆菌细胞膜通透性,膜上的 ATP 酶也受攻击,酶催化环境受影响,表现出 ATP 酶活性下降,物质运输受影响,更多的 PFOA 得以进入细胞中,出现恶性循环,最终使得大肠杆菌细胞出现失活和衰亡。

#### 4 结论

(1)在 PFOA 胁迫下,大肠杆菌细胞内 ROS 和 MDA 含量上升,膜脂肪酸饱和度降低,通透性增大,跨膜电位降低,膜上  $\text{Na}^+ \text{K}^+ \text{-ATPase}$  酶活性与  $\text{Ca}^{2+} \text{Mg}^{2+} \text{-ATPase}$  酶活性随着处理时间的延长呈先上升后下降的趋势。

(2)PFOA 对大肠杆菌产生了氧化胁迫,对其细胞膜有损害作用。

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