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TOPIC

Bacterial Biodegradation of Paracetamol (*Bacillus atrophaeus* and *Bacillus .amyloliquefaciens*)

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الملخص

تُعدّ الملوّثات الصيدلانية الدقيقة من بين الملوّثات الناشئة التي تثير اهتمامًا متزايدًا بسبب آثارها السلبية المحتملة على البيئة والصحة العامة. ويُعتبر الباراسيتامول (Paracetamol) من أكثر الأدوية استهلاكًا على مستوى العالم، حيث يؤدي طرحه المستمر في الأوساط المائية إلى مخاطر بيئية متنامية، خاصةً في ظل محدودية كفاءة محطات معالجة مياه الصرف الصحي التقليدية في إزالة هذا النوع من الملوثات بشكل كامل في هذه الدراسة، تم تقييم قدرة سلالتين بكتيريتين، هما *Bacillus atrophaeus* B16و*Bacillus amyloliquefaciens* B18، على التحلل البيولوجي للباراسيتامول. وقد أُجريت التجارب باستخدام وسط غذائي أدنى مُدعّم بالباراسيتامول بتركيزين مختلفين (200 و400 ملغ/لتر)، مع حضن المزارع البكتيرية لمدة سبعة أيام عند درجة حرارة 35°م. وتمت متابعة عملية التحلل باستعمال طريقة فولين–سيوكالتو (Folin–Ciocalteu)، في حين تم تقييم النمو البكتيري من خلال قياس الكثافة الضوئية عند طول موجي 600 نانومتر (OD600). أظهرت النتائج قدرة السلالتين على إزالة الباراسيتامول بكفاءة مرتفعة، ويُعزى ذلك إلى نشاط مجموعة من الإنزيمات، ولا سيما إنزيمات الأميداز (Amidases) والأوكسيجيناز(Oxygenases) ، التي تسهم في تفكيك المركب عبر تكوين نواتج وسيطة مثل 4-أمينوفينول والهيدروكينون، يليها تكسير الحلقة الأروماتية وتحويلها إلى مركبات أبسط قابلة للتمعدن الحيوي. كما بينت النتائج أن سلالة *Bacillus atrophaeus* B16 حققت أعلى نسبة إزالة للباراسيتامول بلغت 98.92% عند أعلى تركيز مدروس، مقارنةً بنسبة 93.31% المسجلة لدى سلالة *Bacillus amyloliquefaciens* B18.وتؤكد هذه النتائج الإمكانات الواعدة لهاتين السلالتين، *وخاصةً* *Bacillus atrophaeus* B16 ، في تطبيقات المعالجة الحيوية لمياه الصرف الملوثة بالمركبات الصيدلانية، مما يجعلها مرشحًا فعالًا لتطوير تقنيات صديقة للبيئة لإزالة الملوثات الدوائية.

الكلمات المفتاحية: الباراسيتامول، الملوثات الصيدلانية، التحلل البيولوجي، المعالجة الحيوية، *Bacillus atrophaeus*، *Bacillus amyloliquefaciens*.

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Abstract

Pharmaceutical micropollutants are among the emerging contaminants that pose an increasing threat to environmental sustainability. Paracetamol (acetaminophen) is one of the most widely consumed pharmaceuticals worldwide, and its continuous release into aquatic environments has raised growing concerns due to the limited efficiency of conventional wastewater treatment plants in completely removing this compound. This study investigated the biodegradation potential of two bacterial strains, *Bacillus atrophaeus* B16 and *Bacillus amyloliquefaciens* B18, for paracetamol removal. Experiments were conducted in a minimal medium supplemented with paracetamol at two concentrations (200 and 400 mg/L). The bacterial cultures were incubated at 35°C for seven days. Paracetamol degradation was assessed using the Folin–Ciocalteu method, while bacterial growth was monitored by measuring the optical density at 600 nm (OD600). The results demonstrated that both strains were capable of efficiently degrading paracetamol. This biodegradation process is likely associated with the activity of amidase and oxygenase enzymes, which catalyze the transformation of paracetamol into intermediate metabolites such as 4-aminophenol and hydroquinone, followed by aromatic ring cleavage and further mineralization. These findings highlight the potential of these bacterial strains for the bioremediation of pharmaceutical contaminants. Moreover, *Bacillus atrophaeus* B16 and *Bacillus amyloliquefaciens* B18 achieved high biodegradation efficiencies, reaching 98.92% and 93.31%, respectively, at the highest concentration tested. *Bacillus atrophaeus* B16 exhibited superior degradation performance at both concentrations, making it a particularly promising candidate for the biological treatment of wastewater contaminated with pharmaceutical compounds.

Keywords: Paracetamol, pharmaceutical pollutants, biodegradation, bioremediation, *Bacillus atrophaeus*, *Bacillus amyloliquefaciens*.

Résumé

Les micropolluants pharmaceutiques figurent parmi les contaminants émergents qui suscitent une préoccupation croissante en raison de leurs effets potentiels sur les écosystèmes et la santé publique. Le paracétamol, l'un des médicaments les plus consommés à l'échelle mondiale, est fréquemment détecté dans les milieux aquatiques. Son rejet continu dans l'environnement constitue un risque environnemental important, notamment en raison de l'efficacité limitée des stations d'épuration conventionnelles à l'éliminer totalement. Cette étude a porté sur l'évaluation du potentiel de biodégradation du paracétamol par deux souches bactériennes : *Bacillus atrophaeus* B16 et *Bacillus amyloliquefaciens* B18. Les expérimentations ont été réalisées dans un milieu minimum supplémenté en paracétamol à deux concentrations (200 et 400 mg/L). Les cultures bactériennes ont été incubées pendant sept jours à 35 °C. La dégradation du paracétamol a été suivie à l'aide de la méthode de Folin-Ciocalteu, tandis que la croissance bactérienne a été évaluée par la mesure de la densité optique à 600 nm (DO600). Les résultats obtenus ont montré que les deux souches étaient capables de dégrader efficacement le paracétamol. Ce processus de biodégradation serait associé à l'activité d'enzymes telles que les amidases et les oxygénases, qui transforment le composé en métabolites intermédiaires, notamment le 4-aminophénol et l'hydroquinone, avant la rupture du cycle aromatique et la minéralisation progressive de la molécule. Ces observations confirment le potentiel de ces bactéries dans les stratégies de bioremédiation des polluants pharmaceutiques. Par ailleurs, les souches *Bacillus atrophaeus* B16 et *Bacillus amyloliquefaciens* B18 ont présenté des taux de dégradation élevés, atteignant respectivement 98,92 % et 93,31 % à la concentration la plus élevée testée. La souche *Bacillus atrophaeus* B16 a montré une efficacité supérieure à celle de *Bacillus amyloliquefaciens* B18 pour les deux concentrations étudiées, ce qui en fait un candidat particulièrement prometteur pour le traitement biologique des eaux usées contaminées par des résidus pharmaceutiques.

Mots-clés : Paracétamol, micropolluants pharmaceutiques, biodégradation, bioremédiation, *Bacillus atrophaeus*, *Bacillus amyloliquefaciens*.

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Introduction

Introduction

Pharmaceutical compounds are widely used to treat, prevent, or alleviate various diseases in both humans and animals. However, their extensive production and consumption have led to their continuous release into the environment. Nowadays, these substances are recognized as emerging contaminants, particularly in aquatic ecosystems, where they contribute to water quality deterioration and pose potential risks to aquatic organisms and human health (Estrada-Almeida et al., 2024).

In recent decades, rapid population growth—now exceeding 8 billion people—has intensified the demand for healthcare services and pharmaceutical products. Consequently, the pharmaceutical industry has experienced significant expansion, which has been accompanied by an increased environmental burden. Large quantities of pharmaceutical residues are discharged into the environment through multiple pathways, including human excretion, improper disposal of unused medications, and industrial effluents. These compounds are frequently detected in wastewater and surface waters worldwide.

Pharmaceutical pollutants are classified as emerging contaminants due to several key factors: (i) their increasing occurrence in aquatic environments, (ii) the limited efficiency of conventional wastewater treatment plants in removing them, (iii) the insufficient understanding of their long-term ecological and health impacts, and (iv) the lack of well-established regulations regarding their discharge and acceptable environmental concentrations (Estrada-Almeida et al., 2024). These challenges highlight the urgent need for effective and sustainable removal strategies.

Among the various pharmaceutical compounds, paracetamol (acetaminophen) is one of the most widely consumed drugs globally due to its analgesic and antipyretic properties. Its widespread use has resulted in its frequent detection in wastewater treatment plants and natural water bodies (Wu et al., 2012). Conventional treatment processes often fail to completely eliminate this compound, leading to its persistence and accumulation in aquatic environments (Makulec et al., 2026). Moreover, paracetamol and its degradation by-products may exert toxic effects on aquatic organisms and disturb ecosystem balance.

To address this issue, several treatment methods—physical, chemical, and biological—have been explored for the removal of pharmaceutical pollutants from water.

Among these approaches, biodegradation using microorganisms has emerged as a promising, cost-effective, and environmentally friendly alternative. Various bacteria and fungi are capable of transforming or mineralizing pharmaceutical compounds under suitable conditions. In particular, bacterial genera such as *Bacillus* have attracted increasing attention due to their metabolic versatility, resilience, and ability to degrade a wide range of organic pollutants.

Recent advances in environmental biotechnology have also emphasized the importance of understanding biodegradation pathways and identifying intermediate metabolites, as some transformation products may still exhibit toxicity (Wang et al., 2023). Furthermore, microbial interactions—either within single strains or consortia—can significantly influence degradation efficiency and process stability (Yisau et al., 2024).

In this context, the present study aims to investigate the biodegradation of paracetamol by selected bacterial strains belonging to the genus *Bacillus*, namely *Bacillus atrophaeus* and *Bacillus amyloliquefaciens*, under controlled experimental conditions. The study focuses on evaluating the biodegradation efficiency, monitoring bacterial growth, and analyzing the relationship between microbial activity and pollutant removal, as well as the kinetics of the biodegradation process.

This work is structured into two main parts. The first part provides a theoretical overview of pharmaceutical pollution, including definitions, sources, environmental occurrence, and ecotoxicological impacts, with a particular focus on paracetamol. The second part presents the experimental study, which examines the biodegradation potential of the selected *Bacillus* strains and assesses their applicability in the treatment of contaminated aquatic systems.