Zero Valent Iron Significantly Enhances Methane Production from Waste Activated Sludge by Improving Biochemical Methane Potential Rather Than Hydrolysis Rate

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Anaerobic digestion has been widely applied for waste activated sludge (WAS) treatment. However, methane production from anaerobic digestion of WAS is usually limited by the slow hydrolysis rate and/or poor biochemical methane potential of WAS. This work systematically studied the effects of three different types of zero valent iron (i.e., iron powder, clean scrap and rusty scrap) on methane production from WAS in anaerobic digestion, by using both experimental and mathematical approaches. The results demonstrated that both the clean and the rusty iron scrap were more effective than the iron powder for improving methane production from WAS. Model-based analysis showed that ZVI addition significantly enhanced methane production from WAS through improving the biochemical methane potential of WAS rather than its hydrolysis rate. Economic analysis indicated that the ZVI-based technology for enhancing methane production from WAS is economically attractive, particularly considering that iron scrap can be freely acquired from industrial waste. Based on these results, the ZVI-based anaerobic digestion process of this work could be easily integrated with the conventional chemical phosphorus removal process in wastewater treatment plant to form a cost-effective and environment-friendly approach, enabling maximum resource recovery/reuse while achieving enhanced methane production in wastewater treatment system.
In this work, the impacts of three different types of ZVI (i.e., iron powder, clean scrap and rusty scrap) on methane production from WAS in anaerobic digestion were evaluated systematically using both experimental and mathematical approaches. A model-based analysis was performed to reveal the mechanism of ZVI-driven enhancement of methane production from WAS. Based on the results of economic analysis, a cost-effective integrated ZVI-based anaerobic WAS digestion process was also proposed.

Results
Effects of ZVI addition on methane production. Three types of ZVI were evaluated, i.e., iron powder, clean scrap and rusty scrap. Fig. 1 presents the methane production results from the biochemical methane potential (BMP) tests in Experiment I and II (see Methods Section). In general, ZVI addition enhanced the methane production from WAS. The increased ZVI powder addition resulted in increasing methane production (Fig. 1a). For example, 4 g/L ZVI addition increased methane production by 21% as compared to the control (0 g/L ZVI powder addition) on Day 20. As shown in Fig. 1b, 10 g/L ZVI powder, 10 g/L clean iron scrap and 10 g/L rusty iron scrap led to 11%, 22% and 30% increase on methane production, respectively, compared to that of control on Day 20. The level of methane production variation between Experimental I and II is not unexpected as real sludge from a full-scale WWTP was used, methane production variation between Experimental I and II is not unexpected as real sludge from a full-scale WWTP was used, and the confidence region moved rightward to the higher B0 direction (x-axis) in Fig. 2. In contrast, the ZVI addition had no effect on the k value and the obtained k values were constant in both Experiment I (ca. 0.083 d⁻¹) and Experiment II (ca. 0.072 d⁻¹) regardless of the amount of ZVI addition.

**Discussion**

ZVI addition improved biochemical methane potential of WAS rather than its hydrolysis rate. There are two key measures of sludge degradability that are relevant, the apparent first order degradation rate coefficient (k) and the biochemical methane potential (B0), which represent the speed and extent of sludge conversion, respectively. Model-based analysis of these two parameters and the related parameter identifiability in this work clearly showed that ZVI addition significantly enhanced methane production from WAS through improving the biochemical methane potential of WAS rather than its hydrolysis rate.

Feng et al. did not look into the mechanisms for the enhanced methane production by ZVI addition and only hypothesized that the main reason might be the improved major enzyme activities related...
to hydrolysis and acidification. Contradictorily, this study demonstrated that the ZVI addition did not accelerate the hydrolysis rate (k) in both experiments with different types of ZVI addition. On the contrary, biochemical methane potential (B₀) was significantly improved by ZVI addition, indicating that ZVI increased the extent of sludge conversion and altered the sludge property. It has been reported that VS destruction during anaerobic digestion of waste activated sludge generally increased with the increase of ferrous iron content in the sludge. Indeed, ZVI can release from Fe₀ to Fe²⁺ (Fe₀ + 2H⁺ = Fe²⁺ + H₂), and thus leading to a significant increase of iron content in the sludge. As shown in Fig. 3, in this work, the released ferrous iron concentrations from ZVI also showed a good correlation with both VS reduction and the biochemical methane potential (B₀). Therefore, the alternation of sludge property to improve biochemical methane potential by ZVI could likely be the main reason for the enhanced performance of methane production.

### Table 1 | The estimated k and B₀ as well as the calculated Y from Experiment I and II using one-substrate model (with 95% confidence intervals)

| Experiment | k (d⁻¹) | B₀ (L CH₄/kg VS) | Y     |
|------------|---------|-----------------|-------|
| **Experiment I** |         |                 |       |
| 0 g/L Fe powder | 0.083 ± 0.007 | 248 ± 12 | 0.44 ± 0.02 |
| 1 g/L Fe powder  | 0.083 ± 0.006 | 271 ± 12 | 0.48 ± 0.02 |
| 4 g/L Fe powder  | 0.083 ± 0.005 | 300 ± 11 | 0.53 ± 0.02 |
| **Experiment II** |         |                 |       |
| 0 g/L Fe scrap   | 0.073 ± 0.003 | 214 ± 6  | 0.34 ± 0.01 |
| 10 g/L Fe powder | 0.072 ± 0.003 | 240 ± 5  | 0.37 ± 0.01 |
| 10 g/L clean Fe scrap | 0.072 ± 0.003 | 262 ± 6 | 0.41 ± 0.01 |
| 10 g/L rusty Fe scrap | 0.071 ± 0.003 | 275 ± 6 | 0.44 ± 0.01 |

**Figure 2** | The 95% confidence regions of the estimated hydrolysis rate (k) and biochemical methane potential (B₀) with different ZVI additions: (a) using data from Experimental I; and (b) using data from Experiment II.

**Figure 3** | Relationships between the released ferrous iron concentrations and the percentage of VS reduction as well as the obtained B₀ value in Experiment I.

A strategy to implement ZVI-based anaerobic digestion process in wastewater treatment plant. From an integrated environmental and economic perspective, nutrients source in wastewater treatment systems should be managed such that both good nutrients removal performance and high resource recovery or reuse can be achieved. Based on the findings of this work, a new strategy could be proposed to simultaneously enhance methane production from WAS and iron resource reuse through integrating the ZVI-based anaerobic digestion process of this work with the conventional chemical phosphorus removal process in WWTPs.

As presented in Fig. 4, waste iron scrap (the most efficient ZVI as demonstrated in this work) can be freely obtained from machinery factory and then transported to the WWTP. The obtained iron scrap (ZVI) can be added to the anaerobic digester in order to enhance the methane production by increasing the biochemical methane potential. In anaerobic digester, ZVI can be released from Fe₀ to Fe²⁺, and thus eliminated the potential sulfide production/accumulation issues as well as the possible H₂S emission in the biogas in traditional anaerobic digester through iron sulfide precipitation. This in turn could further enhance the performance of WAS digestion without additional chemical cost from external ferrous/ferric iron dosing. With regard to the generation of organic sulfur odors from the dewatered sludge cakes, iron could also reduce odor-causing gases, resulting in better quality of dewatering sludge. More importantly, the Fe (II) in anaerobic digestion liquor can be recycled to bioreactor and further oxidized to Fe (III), which can be used for chemical
phosphorus removal via the generation of FePO₄. This strategy would not only represent a significant process cost reduction (further discussed below), but also improve the sludge and wastewater treatment efficiency, enabling maximum resource (iron) reuse while achieving improved methane production. In addition, from a network-wide view, commonly used ferric iron dosing in sewers for H₂S control might also be useful for CH₄ production enhancement during anaerobic digestion and phosphorus removal in the WWTP.

Potential economic feasibility of ZVI-based technology for enhancing biological methane production. It has been demonstrated that the estimated lab-scale BMP results are more conservative or comparable to full-scale test results. Thus, the estimated values obtained in the current study are used for a conservative assessment of the potential economic feasibility of the proposed ZVI-based anaerobic digestion technology. This was carried out by a desktop scaling-up study on a full-scale WWTP with a population equivalent (PE) of 400,000 and with an anaerobic sludge digester at a hydraulic retention time (HRT) of 20 days. 10 g/L rusty iron scrap was chosen for the following economic evaluations.

From the Fe²⁺ released (41 mg/L), theoretically, the iron scrap could be recycled for approximately 243 batches (10*1000/41) if the loss of iron solid through effluent is ignored. With a 29% increase in methane production at this level of ZVI addition, the net economic benefit is estimated to be around $231,000 per annum compared with the system without ZVI addition (see Table S2 in Supplementary Material). The net benefit arises from the enhanced methane production associated benefit (i.e., its conversion to heat and power) ($150,000 per annum) and decreased WAS and disposal costs ($90,000 per annum) outweighing the additional costs for ZVI transport and ZVI chamber ($9,000 per annum). The advantages of ZVI addition on sulfide control in digester, phosphorus removal through anaerobic digestion liquor recycle and better dewa-

anaerobic digestion liquor recycle and better dewa-

Figure 4 | A proposed strategy to integrate ZVI-based anaerobic digestion process of this work with the conventional chemical phosphorus removal process in wastewater treatment plant. ZVI addition in anaerobic digester can enhance methane production from WAS. The sulfide produced in anaerobic digester can be precipitated by ferrous iron that produced from ZVI addition, resulting in enhanced sulfide-free biogas (methane) production. The anaerobic digestion liquor containing Fe (II) can be reused and fed into bioreactors, in which the Fe (II) can be oxidized to Fe (III). The generated Fe (III)-containing effluent can then be used for chemical phosphorus removal process, to form a cost-effective and environment-friendly technology, enabling maximum resource recovery/reuse while achieving enhanced methane production in wastewater treatment system.

Methods

Waste activated sludge. The waste activated sludge used in this work was collected from the sludge treatment unit at a full-scale municipal wastewater treatment plant in Dalian, China. The sludge was stored at 4°C before use. The volatile solids (VS) to total chemical oxygen demand (TCOD) ratios of the sludge used for methane production ranged between 0.60 and 0.67.

ZVI sources. Three types of ZVI were evaluated, i.e., iron powder, clean scrap and rusty scrap. The ZVI powder has a diameter of 0.2 mm with BET surface area of 0.05 m²/g and purity >98%. The rusty scrap (about 8 mm * 4 mm * 0.5 mm, purity >95%) was obtained from a machinery workshop in Dalian, China. The clean scrap was acquired through a pretreatment of the rusty scrap to remove the rusty cover. The difference between the two scraps is that the rusty scrap had a corrosion layer covering the surface of the scrap.

Anaerobic biochemical methane potential tests. In order to evaluate the effect of different forms of ZVI on methane production in anaerobic digestion, methane production from the WAS with different types of ZVI addition was assessed using anaerobic batch BMP tests. The inoculum for the BMP tests was collected from an anaerobic digester. Two types of batch experiments were performed. In Experiment 1, 0, 1.0, and 4.0 g/L of ZVI powder were added into three identical sets of BMP test vials, respectively. In Experiment II, 10 g/L ZVI powder, 10 g/L clean scrap and 10 g/L rusty scrap were used as ZVI sources and dosed to three identical sets of BMP vials for comparison, with a control test in which no ZVI was added.

In each test, WAS, ZVI and the inoculum obtained from the anaerobic digester were added into serum vials for BMP tests. After that, the vials were capped with silica
gel stoppers. The oxygen was removed from the headspace by exchanging it with nitrogen gas for at least 10 min. All BMP tests were conducted at 35 ± 1 °C for 20 d. The biogas (methane) production in BMP vials was collected and monitored by using gas chromatograph (Shimadzu, GC-14C) equipped with a thermal conductivity detector. More details of the BMP tests can be found elsewhere.  

**Model-based analysis.** The hydrolysis rate (k) and biochemical methane potential (B0) are the two key parameters associated with methane production from WAS. In this work, these two parameters were used to evaluate and compare the methane production kinetics and potential of the WAS at different ZVI levels or with different types of ZVI. They were estimated by fitting the methane production data from the BMP tests to a first-order kinetic model using a modified version of Aquasim 2.1d with sum of squared errors (SSEP) as an objective function. The uncertainty surfaces of k and B0, based on a model validity statistical F-test with 95% confidence limits, were also estimated by using Aquasim 2.1d. 

Two models were applied. The first one considered a single substrate type (i.e., one-substrate model) in the first-order kinetic model, as shown in Equation (1): 

\[ B(t) = B_0(1 - e^{-kt}) \]  

where \( B(t) \) (L CH4/kg VS) is the cumulative methane production at time \( t \) (d). 

In the second model, the WAS samples comprised a rapidly biodegradable substrate type and a slowly biodegradable substrate type (i.e., two-substrate model)\(^1\). The equation of the two-substrate model is shown below: 

\[ B(t) = B_{\text{rapid}}(1 - e^{-k_{\text{rapid}} t}) + B_{\text{slow}}(1 - e^{-k_{\text{slow}} t}) \]  

where \( B_{\text{rapid}} \) and \( B_{\text{slow}} \) (L CH4/kg VS) are biochemical methane potentials of the rapidly biodegradable substrates and slowly biodegradable substrates, respectively. \( k_{\text{rapid}} \) and \( k_{\text{slow}} \) (d\(^{-1}\)) are hydrolysis rates of the rapidly biodegradable substrates and slowly biodegradable substrates, respectively. 

Based on the determined \( B_0 \), the degradation extent (Y) of WAS could then be calculated using Equation (3): 

\[ Y = \frac{B_0 - B_{380}}{808} \]  

where \( B_{380} \) (L CH4/kg TCOD) is theoretical biochemical methane potential of WAS under standard conditions (25°C, 1 atm); \( RWAS \) is the measured VS to TCOD ratio in the WAS. 

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**Acknowledgments**

This study was supported by the Australian Research Council (ARC) through Project DP130103147. Yiwen Liu gratefully received the Endeavour International Postgraduate Research Scholarship (IPRS) and The University of Queensland Centennial Scholarship (UQCents). Bing-Jie Ni acknowledges the supports of ARC Discovery Early Career Researcher Award (DE130100451) and ARC Linkage Project (LP110201095). Yaobin Zhang acknowledges the supports of National Natural Scientific Foundation of China (51378087 and 21177015).

**Author contributions**

Y.L., Q.W., Y.Z. and B.-J.N. wrote the manuscript; Y.L., Y.Z. and B.-J.N. developed the methodology; Y.L. and Q.W. performed data analysis and prepared all figures; All authors reviewed the manuscript.

**Additional information**

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

**Competing financial interests:** The authors declare no competing financial interests.

**How to cite this article:** Liu, Y., Wang, Q., Zhang, Y. & Ni, B.-J. Zero Valent Iron Significantly Enhances Methane Production from Waste Activated Sludge by Improving Biochemical Methane Potential Rather Than Hydrolysis Rate. *Sci. Rep.* **5**, 8263; DOI:10.1038/srep08263 (2015).

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