Effect of Cation on Efficiency of Aspegillus Flavus Bioflocculant Produced from Chicken Viscera Hydrolysate

https://doi.org/10.3991/ijoe.v16i01.12169

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Abstract—The cations are needed as stimulants for effective flocculation by the cation-dependent bioflocculants. Addition of metal ions (cations) can counterbalance anionic functional groups of both bioflocculant and solid particles thereby increasing the bioflocculant adsorption to suspended particles. In the present study, addition of all dose of both Ca^{2+} and Mg^{2+} stimulated the efficiency of the bioflocculant with optimum flocculation efficiency of 95% recorded with 5 mL of 1% Ca^{2+}. While lower dose (1 – 2 mL) of Al^{3+} also stimulated the bioflocculant to about 94%, Na^{+} and Fe^{3+} inhibited flocculation at all doses tested. K^{+} slightly enhanced the flocculation at 4 - 10mL of 1%. The present cationic bioflocculant can be suggested as a substitute for chemical flocculants.

Keywords—Cationization; bioflocculant; Aspergillus flavus; chicken viscera

1 Introduction

Generally chemical flocculants are cost-effective and efficient in flocculation, they are however associated with generation of secondary pollutants since they are not completely degraded. To address this limitation, ongoing research efforts are focusing on extra cellular polymers (products of microbial fermentations called bioflocculant) that have flocculation ability. These microbial based polymers can aggregate solid particles and cells from solutions to facilitate their sedimentation and removal. They are easily degradable to non-toxic residues that are not pollutant in nature (Sun et al., 2015). However, their application is hinder by high cost of production arising from cost of fermentation substrate and low efficiency (Mohammed and Dagang, 2019a).

Compositional characterization of biopolymer flocculants revealed existence of anionic functional moieties including uronic acids and proteins which contained mostly carboxylic functional groups and proteins whose amino acids mostly glutamic and aspartic acid. Both uronic acid and proteins contains carboxylic functional groups

(Seviour et al., 2010). Moreover, the polysaccharides components of bioflocculants are also deprotonated at some pH peculiar to most activated sludge systems. Majority of the solid particles found in the wastewater are also negatively charged. These limits the efficacy of the microbial based flocculants (Lin et al., 2013).

Cations play a significant role in stimulation of cation dependent bioflocculant through neutralization and stabilization of the lingering negative charges mainly of the active moieties of the biopolymer flocculants and thus bridge between particles and the biopolymer. In this process, the cations lessen the distance between the particles and the biopolymer flocculants through increase in electrostatic attraction between the duo (Okaiyeto et al., 2016, Ndjiko and Dagang, 2019b). The added metal ions also increases the floc size thereby facilitating sedimentation of flocculated particles (Murugesan et al., 2017). The present study focuses on hybridization of bioflocculant produced with chicken viscera hydrolysate (a very low-cost fermentation substrate) with cations to increase cost of production and concurrently increase the flocculation efficiency of the bioflocculant.

2 Methodology/Materials

2.1 Bioflocculant production

The bioflocculant was produced by growing A. flavus in a liquid viscera hydrolysate made up of crude protein 5.40, sugar 3.20, carbon 5.86, nitrogen 1.27, sulphur 0.83 and hydrogen 10 all in %w/w as the production medium. The culture conditions used included temperature 35°C, agitation 150 rpm, incubation time 72 h, inoculum 4% and pH 7 as optimized in our previous studies. Subsequently, the 72h culture broth was dispensed in to 50mL centrifuge tubes and spined at 10,000 rpm with the aid of a centrifuge (KUBOTA 5922) to remove the biomass. The bioflocculant rich culture supernatant was collected into sterile glass beaker and used as the crude bioflocculant in subsequent experiment.

2.2 Determination of flocculation efficiency and cationization

The bioflocculant efficiency was estimated in accordance with the methods demonstrated by More et al. (2015), Czemierska et al. (2017) and Xia et al. (2018). Briefly, 4mL (optimum dose) crude bioflocculant was dispensed in to 100 mL suspended Kaolin clay (4g/L, pH 7) in 500 mL glass beaker. Different doses (1 – 10mL) of 1% of the cation of interest were added as the bioflocculant aid. The cations considered include Na^+, K^+, Ca^{2+}, Mg^{2+}, Al^{3+} and Fe^{3+} and were all added as chloride salts. The suspension containing the bioflocculant and the cations were stirred in a flocculator tester (JLT6, VELP) at 200 rpm for 1 min, 80 rpm for 5 min, and then held motionless for 5 min to sediment. The optical density reading of clarified top solution at 550 nm estimated with T60 spectrophotometer was recorded. The efficiency was finally calculated using the following equation (Wang et al., 2015)

$$\text{Flocculation efficiency} = [(A - B/A) \times 100\%] \quad (1)$$

Where A represent the optical density of the control (in which sterilized viscera medium was used in place of the bioflocculant) and B, the optical density of the sample at 550 nm. The control experiment in which no cation was added was carried out and calculated following the same procedure above.

2.3 Measurement of zeta potential

The zeta potential studies were conducted with the aid of Zeta potential analyser, Zeecom (ZC-3000 series). Samples were prepared for zeta potential measurement by dissolving 5mg of the flocculated particles in10mL deionized water. The samples were loaded in to a clean measurement cell and mounted to Zeecom main unit. All measurements were conducted for at least 200 particles using automatic tracking measurement mode using scattered light source

3 Results and Findings

3.1 Effect of N^+ on the bioflocculant efficiency

The effect of Na^+ on the bioflocculant is presented in Figure 1. The results indicated that all the doses tested inhibited flocculation of the Kaolin suspension. The maximum flocculation efficiencies recorded were 54 and 53.4% at the cation dose of 1 and 2 mL 1% $NaCl_2$ respectively as compared to the flocculation efficiency (60.3%) of the control in which no cation was added. Interestingly the inhibition of flocculation increases as the cation dose increases to the lowest flocculation efficiency of 26.5% at the cation dose of 10mL 1% $NaCl_2$. The zeta potential of the flocculated particles at pH 7 also increases to the maximum of -29.3mV as the cation dose increases thereby consolidating the flocculation efficiencies. The zeta potential value has a direct relationship with stability of suspended particles and is popularly used to predict flocculation. Particles with ZP values of ±(0 –10mV) are extremely unstable, ± (10–20mV) is discreetly stable, ±(20–30mV) is moderately stable and more than 30mV is highly stable (Bhattacharjee, 2016; Freitas and Müller, 1998). Thus, the closer the zeta potential of particles to zero, the higher their tendency to aggregate and vice versa.

The binding ability of the cations to the biopolymers has direct relationship with the ionic strength, size and radius of the hydration shell of the cations. Increase in ionic size triggers decrease in the hydration shell radius. Therefore, cations that has high valency, size, and tinny hydration shell could move nearer to the negative charge spots of the biopolymers to form bonds with them (Kara et al., 2008). Though potassium and sodium possess same charge, the hydration radius of potassium (0.53 nm) is smaller than that of sodium (0.79 nm) (Kiyohara and Minami, 2018). As such potassium can easily loses its hydration shell when it is in proximity with the functional groups of the extracellular polymeric substances while the water molecules around the sodium prevents it approach to the surface (Goddard, 2017). Thus, sodium's poor stimulation of bioflocculants is linked to its monovalency, small size and higher hydration radius.

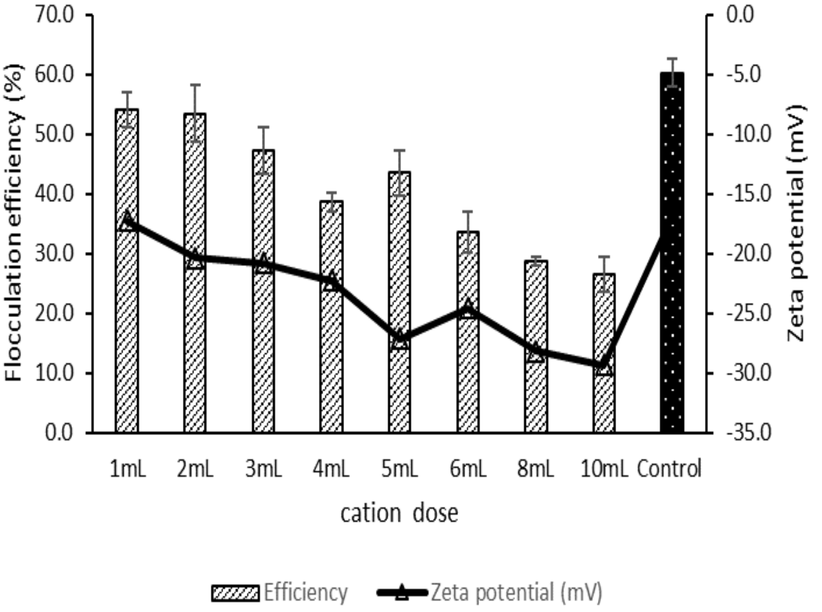

Fig. 1. Effect of Na+ on bioflocculation efficiency of A. flavus bioflocculant produced from chicken viscera hydrolysate

3.2 Effect of K^+ on the bioflocculant efficiency

The effect of K^+ is as display in Figure 2. It shows very little stimulation on the efficiency of the bioflocculant. The maximum flocculation of about 77.6 % was achieved at the cation dose of 4 mL. As the cation dose increases beyond 5 mL, the efficiency dropped to minimum of 69.2% at 10mL.

The minimum zeta potential recorded was -5.6mV at 4mL cation dose while the highest of 23.2 was recorded at 2 mL cation dose. K^+, a monovalent cation has a lone valency on its exterior electron arrangement and thus can only form a single bond with the bioflocculant (Mohammed and Dagang, 2019b). This limits it capacity for further complex formation because it needs a higher ionization energy to do that (Ueyama et al., 2002)

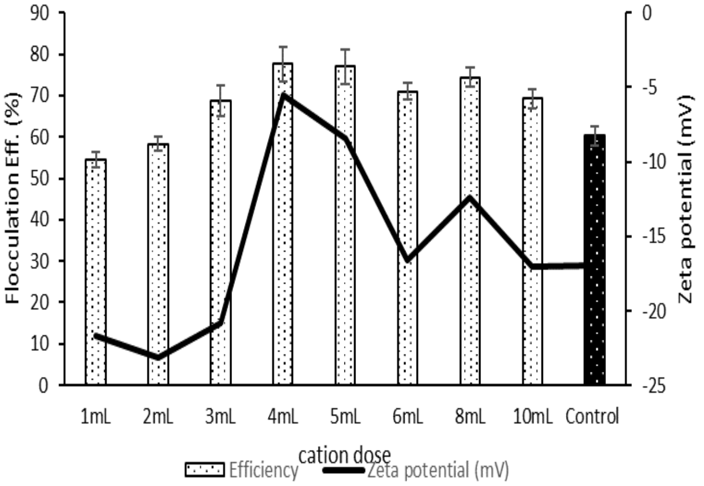

Fig. 2. Effect of K+ on bioflocculation efficiency of A. flavus bioflocculant produced from chicken viscera hydrolysate

3.3 Effect of Ca^{2+} and Mg^{2+} on the bioflocculant

The stimulation activity of the Ca^{2+} on the bioflocculant is as shown on Figure 3. All the doses tested showed remarkable stimulation on the bioflocculant as compared to the control but peaked to about 95% at 5mL. Meanwhile, the efficiency dropped to minimum of 76.2% at 10mL cation dose. Lowest efficiency of 84.9% was also recorded at the lowest dose of 1mL indicating the importance optimum cation dose in bioflocculant stimulation by the metal ions. Like the Ca^{2+}, Mg^{2+} highly stimulated the bioflocculant at all doses tested (Figure 4) however maximum efficiency of about 91 – 93% was recorded with 3 – 5 mL MgCl2. The lowest efficiency of 75.9% was recorded at 10 mL of 1% MgCl2. In agreement with these findings, the efficiency of biopolymer scretted by a haloalkaliphilic Bacillus sp. was greatly stimulated by divalent cations including Ca^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+} and Fe^{2+}(Kumar et al., 2004). Many other studies (Abu-Elreesh et al., 2011;Cosa and Okoh, 2014; Makapela et al., 2016; Wang et al., 2014) have demonstrated enhanced bioflocculation with divalent cations.

The divalent cations have valency of 2^{+} on its exterior conformation and can form two bonds with the bioflocculant and suspended Kaolin particles. These bonds held the bioflocculant and the particles nearer and firmer together (Khiew et al., 2016). Though in the present study, Ca^{2+} only show a slight stimulation capacity (95%) over Mg2+ (93%), Ca^{2+} have been widely applied and reported to be most effective cation in terms of bioflocculant stimulation as compare to other divalent cations such as Mg^{2+} and Mn^{2+}. This is for the reason that Ca^{2+} has less stability. Its electron conformation and atomic radius is 4s2 and 197 pm respectively in comparison with Mg^{2+} with only an electron conformation and radius of 3s2 and 160 pm. Thus binding between Ca^{2+} and the carboxylate group in the biopolymer is easier (Khiew et al., 2016). The lower zeta potentials recorded for both Ca^{2+} and Mg^{2+} collaborated their stimulatory effect. Increase in ionic strength leads to compression of the electric double layer (EDL) thereby lowering the zeta potential and vicky-verky (Bhattacharjee, 2016). Thus, the EDL of the bioflocculant stimulated with Ca^{2+} and Mg^{2+} become more compress as compared to those stimulated with monovalent cations thereby lowering the zeta potential.

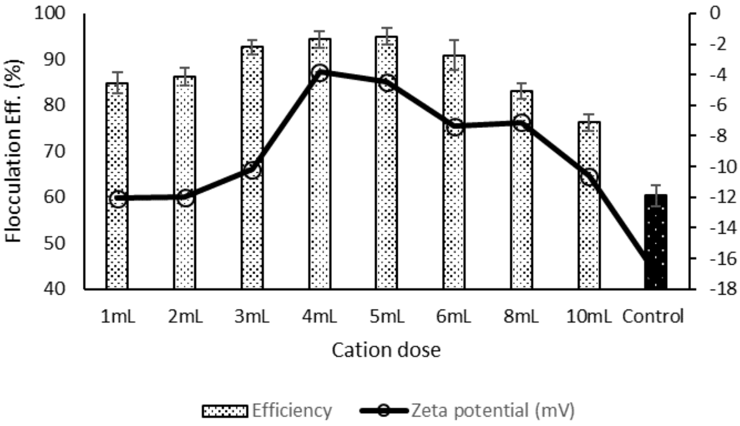

Fig. 3. Effect of Ca^{2+} on bioflocculation efficiency of A. flavus bioflocculant produced from chicken viscera hydrolysate

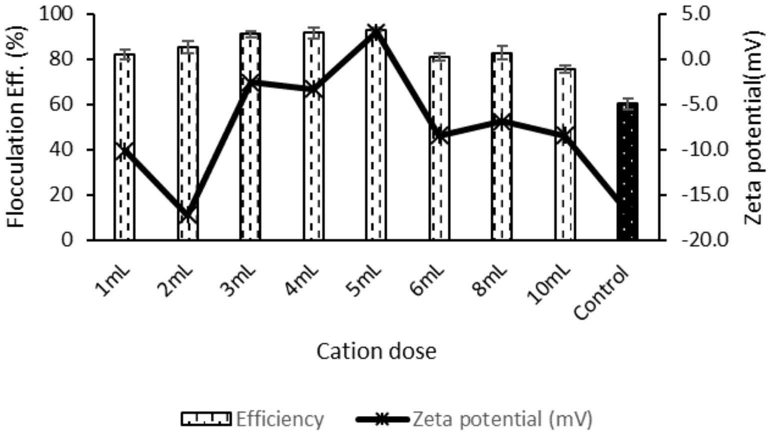

Fig. 4. Effect of Mg^{2+c} on bioflocculation efficiency of A. flavus bioflocculant produced from chicken viscera hydrolysate

3.4 Effect of Fe^{3+} on the bioflocculant

The Fe^{3+} has high inhibition effect on the bioflocculant at all the doses (Figure 5). The highest efficiency recorded was 45.5% at 2 mL of 1% $FeCl_3$. The inhibition of the bioflocculation became more pronounced as the cation dose increases with only about 33% efficiency at 10 mL of 1% $FeCl_3$. This inhibition is consolidated by the high zeta potential (-21 - -29.88 mV) recorded for all the cation doses. This results agrees with the work of Zheng et al. (2008) who demonstrated the inhibitory effect of Fe3+ on the ability of Bacillus sp. F19 bioflocculant to flocculate Kaolin, activated carbon and fly coal. Many other studies (Elkady et al., 2011; Gomaa, 2012; Liu et al., 2010; Makapela et al., 2016; Ugbenyen et al., 2014) reported inhibitory effect of Fe3+, Lu et al. (2005) and Liu et al. (2015) centrally reported its stimulatory effect.

The inhibitory effect of Fe3+ is because addition of trivalent ion does not only add to the cationic concentration of the bioflocculant, but likewise increase the cationic thickness over the surface of the particles with its extra electron. This alters the stabilization of the system and prevents flocs formation between hybridized bioflocculant and the particles. The remaining ion in the system may also replace the H^+ in Kaolin suspension.

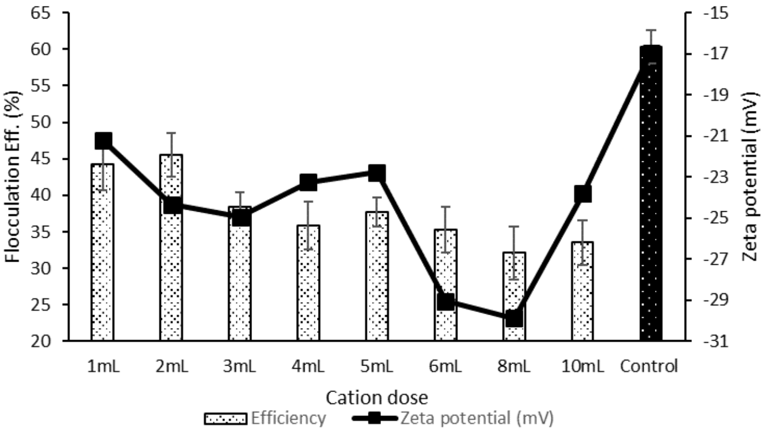

Fig. 5. Effect of Fe3+ on bioflocculation efficiency of A. flavus bioflocculant produced from chicken viscera hydrolysate

3.5 Effect of Al^{3+} on the bioflocculant

The stimulatory effect of Al^{3+} on the bioflocculant is shown in Figure 6. Interestingly, significant stimulatory effect was recorded at lower doses of 1 – 3mL with highest efficiency of 94.6% at 1mL. No significant effect was observed as the cation dose increases to 4 – 10mL. This result indicated that the wide reported inhibition by the Al3+ is due to use of higher dose. This findings agree with Salehizadeh et al. (2000) who reported highest flocculation activity for bioflocculant As-101 stimulated with a 0.2 mM concentration of Al^{3+}.

They also demonstrated a rapid dropped in flocculation activity as the concentration increased to 0.8Mm. Further, Al^{3+} stimulated the flocculation activity of pH and cation dependent bioflocculant produced by a Consortium of Halomonas sp. Okoh and Micrococcus sp. Leo (Okaiyeto et al., 2013)

Generally, addition of cations augment flocculation by neutralizing and stabilizing the lingering negative ions of uronic acid and pyruvic acid found in the bioflocculants through bridge formation thereby binding the Kaolin particles together (Wong et al., 2012).

Thus, the presence of the cations more than the residual negative groups of the bioflocculants will add to the residual metal ions within the system. These diffused residual ions could compete with the cation hybridized bioflocculant by creating a bridge amid the metal ion and the suspended particles. These phenomenon inhibit floc formation between the cationized bioflocculant and the particles (Khiew et al., 2016) The important of using the appropriate cation dose for bioflocculant stimulation is demonstrated in Figure 7

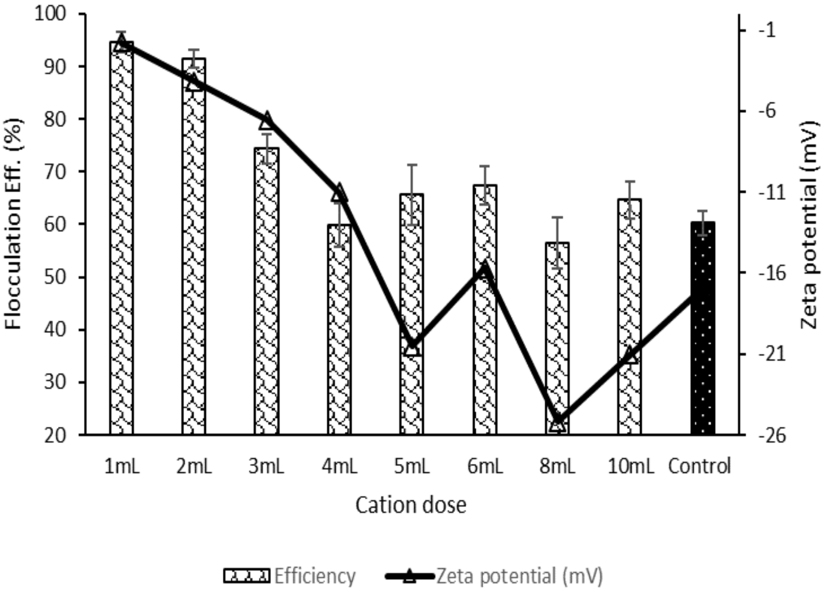

Fig. 6. Effect of Al3+ on bioflocculation efficiency of A. flavus bioflocculant produced from chicken viscera hydrolysate

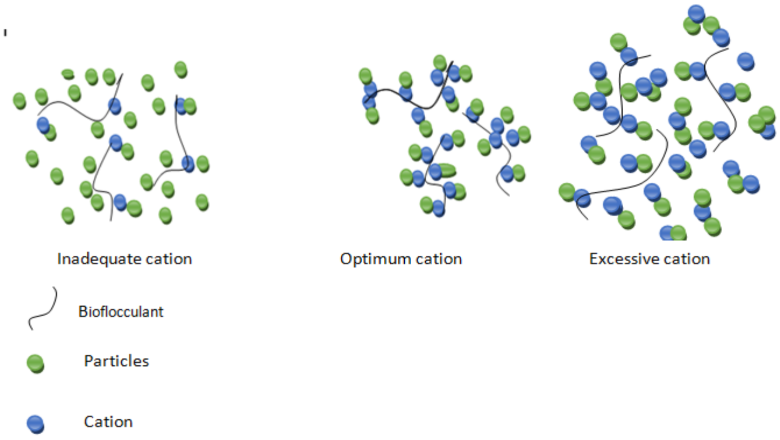

Fig. 7. Schematic effect of cation dosage on bioflocculant stimulation

3.6 Mechanism of bioflocculant stimulation by the metal ions

Cation stimulated bioflocculation occurs through neutralization and stabilization of lingering negative charges found on the active sites of the bioflocculants thereby bridging between the particles and the bioflocculant. this subsequently improves the flocculation capacity of the bioflocculant (Tang et al., 2014). In the present study, the zeta potentials were generally low at higher flocculation efficiencies recorded with multivalent cations such as Ca^{2+}, Mg^{2+} and Al^{3+} while higher zeta potential values were recorded at low flocculation efficiencies with cations such as Na^{+}, Fe^{3+} and higher doses of Al^{3+}. For example, when flocculation efficiency of 95% was achieved with 5mL 1% $CaCl_{2,}$ the zeta potential of only -4.5mV was recorded. When the flocculation efficiency (32.1%) was inhibited at 8mL 1% $FeCl_3$, the zeta potential rises to -29.88mV. The lower zeta potentials recorded were due to the ability of the cations to neutralize and bridge between the bioflocculants and the particles while lack of neutralization and bridging resulted in low flocculation efficiencies and higher zeta potential values**.**

4 Conclusion

The present study achieved a bioflocculant production from a bioflocculant producing fungus; A. flavus using hydrolysed chicken viscera as medium. The flocculant secreted has good flocculating efficiency promoted by hybridization with divalent cations (Ca^{2+}, Mg^{2+}) and a trivalent cation Al^{3+} in Kaolin suspension. While K^{+} only slightly promoted the flocculation efficiency flocculation was inhibited by Na^{+} and Fe^{3+}. The stimulatory effects of Ca^{2+}, Mg^{2+} were visible at 1 – 10mL of 1% of both cations while Al^{3+} was at 1- 3mL of 1%. The zeta potentials of the flocculated particles were in most cases correspondent to the flocculation efficiencies. Overall, bridging mediated by the cations is suggested as the mechanism of bioflocculation for the present bioflocculant**.**

5 Acknowledgement

This research work is supported by Universiti Teknologi Malaysia, GUP Tier1 (Q.J130000.2545.13H22) and Demand-Driven Innovation grant (R.J130000.7845. 4L190)

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Article submitted 2019-11-01. Resubmitted 2019-12-03. Final acceptance 2019-12-17. Final version published as submitted by the authors.